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Sofía Noemí Chulze *Editors*

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Preface

Fusarium Head Blight (FHB) is one of the most severe disease affecting wheat crops worldwide. The main pathogen associated to the disease, *Fusarium graminearum* sensu stricto (Schwabe), can survive on stubble, and under favorable environmental conditions can infect wheat spikes. Different strategies have been proposed for managing FHB in order to reduce the losses on yield and quality and food safety due to the accumulation of mycotoxins. Among these strategies we can mention cultural practices (type of tillage, crop rotation), fungicides application, use of less susceptible cultivar, identification of rotations least conducive to the built up of inoculum, and biocontrol. The possible control of the pathogen and reduction in toxin accumulation can be achieved through Integrated Pest Management (IPM).

Latin American countries mainly Brazil and Argentina are good wheat producers and exporters. FHB epidemics have occurred in different years, and reduction in yield and deoxynivalenol contamination were observed. These situations cause severe economic losses due to commercial restrictions in the domestic and international markets.

During the last decade, changes in the cultural practices have been done mainly in relation to tillage type. No tillage or reduced tillage are in use in many Latin American countries. This condition can modify the inoculum potential in areas of wheat cultivation.

This book reviews the recent progress in the research on Fusarium Head Blight (FHB) in Latin America, and the information was recompiled from a South America perspective. The book has been organized in six different parts. Part I is devoted to the *Fusarium* populations associated to FHB and includes four chapters which provide description on *Fusarium graminearum* population structure and genotypes and chemotypes in relation to trichothecenes production. Also a chapter about the advances on the knowledge on the eco-physiology of *Fusarium graminearum* isolated from Latin America is included.

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Part II includes two chapters and outlines the mycotoxins produced by *Fusarium* species associated with FHB in South America and the methodology for their detection.

Part III deals with the interaction plant pathogen aspects and includes two chapters. One of them describes the infection progress and the effect of the enzymes in the disease progression and the other chapter provides a general description of proteomic as a tool for studying the pathogen-host interaction.

Part IV provides the advances in the knowledge on the epidemiology of *Fusarium graminearum* and how we can control the disease with the control of the inoculum through the management of the residues.

Part V includes four chapters. The first chapter describes the integrated disease management as a tool to control Fusarium Head Blight. The second chapter deals with the use of fungicides and their application to control FHB. The third chapter presents the advances on biological control as part of an integrated disease management to control both the disease and deoxynivalenol accumulation. This part also includes a chapter dealing with modeling and forecasting systems as a tool to reduce the impact of FHB and DON accumulation from a South American experience.

Part VI summarizes the advances in South America on selection of cultivars less susceptible to Fusarium Head Blight and deoxynivalenol accumulation including field screening, marker assisted selection, and sources of resistance to FHB from alien species.

We hope that this book will provide useful information for researchers, graduate students and professionals that are dealing with the pathogen in the area of plant pathology, food industry and mycotoxicology.

We thank the authors who have contributed with their knowledge and shared the results of their research activities, and for their patience during the book preparation.

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Abbreviations

°C	Celsius degree
15 ADON	15-Acetyldeoxynivalenol
1-AN	1-Anthroylnitrile
1D	One-dimensional electrophoresis
1-NC	1-Naphthoyl chloride
2D-DIGE	Two-dimensional fluorescence difference gel electrophoresis
2DE	Two-dimensional electrophoresis
2-NC	2-Naphthoyl chloride
3 ADON	3-Acetyldeoxynivalenol
4 ANIV	4-Acetylnivalenol
ADON	Acetyldeoxynivalenol
AFB	Aflatoxins B
AFG	Aflatoxins G
AFLP	Amplified fragment length polymorphism
Agph or Dgph	Absolute or differences between values of gph at 1,000 hPa
Anther	Proportion of heads with anthers (values from 0 to 1)
APCI	Positive ion atmospheric pressure chemical ionization
AVHRR	Advanced Very High Resolution Radiometer
a_W	Water activity
BC	Backcross generation
BCA	Biological control agents
cAMP	Cyclic adenosine-3′, 5′-monophosphate
CENEB	Centro Experimental de Norman E. Borlaug
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (in Spanish)
CIMMYT	International Center of Maize and Wheat
CWDEs	Cell wall degrading enzymes
d	Day
DAD	Diode array detector
DAS	Diacetoxyscirpenol
DD	Degree days (base temperature: 0 °C)

DD_{10}	Daily accumulation of Td >= 10 °C
DD_{12}	Daily accumulation of Td >= 12 °C
DH	Doubled haploid
DIEA	Direction of agricultural statistics
DNA	Deoxyribonucleic acid
DON (µg/kg)	Deoxynivalenol grain content in micrograms per kilogram
DON	Deoxynivalenol
DTT	Dithiothreitol
DVIF	Daily values for infection favorability
EB	El Batan, Mexico
EC	Enzyme Commission
EC_{50}	Effective concentration at which 50 % of mycelial growth is reduced
ECD	Electron capture detection
EEA	Estación Experimental Agropecuaria (in Spanish)
EFSA	European Food Safety Authority
ELEM	Equine leukoencephalomalacia
ELISA	Enzyme-linked immunosorbent assay
ENSO	El Niño Southern Oscillation
Ent.	Entry or row number of the plot
ESI	Electrospray ionization
ESI-MS	Electrospray ionisation coupled with mass spectrometry
ESI-Q-TOF-MS	Electro spray ionisation time of flight mass spectrometry
Exp	Base-e exponential function (e = 2.71828)
F. graminearums.s.	*F. graminearum sensu stricto*
FAO	Food and Agriculture Organization of the United Nations
FBs	Fumonisins
FDK	Fusarium damaged kernel
FEA	Fully exserted anthers
FGSC	*Fusarium graminearum* species complex
FHB	Fusarium Head Blight
FHBSN	Fusarium Head Blight Screening Nursery
FI %	Observed Fusarium index (FI % = I %*S %/100)
FIESWN	Fusarium International Elite Spring Wheat Nursery
FIPSWN	Fusarium International Preliminary Spring Wheat Nursery
FLD	Fluorescence detection
FP	Fluorescence polarization
FPIA	Fluorescence polarization immunoassay
FT-IR	Fourier transform-infrared
FUS-X	Fusarenone X
FW	Facultative wheat
$\hat{G}$	Genotypic diversity
G_0/N	Genotypical diversity
GC/MS	Gas chromatography/mass spectrometry
GC	Gas chromatography

GCPSR	Genealogical concordance phylogenetic species recognition
GFP	Green fluorescent protein
gph	Geopotential height (meters)
G_{ST}	Fixation index
h	Hour
H	Nie's gene diversity
HFBs	Hydrolyzed derivatives of fumonisins
HMWG	High molecular weight species
hPa	Hectopascal
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
I %	Observed FHB incidence
I	FHB incidence (values from 0 to 1)
IAC	Immunoaffinity column
IARC	International Agency for Research on Cancer
I_d	Index of Dominance
INIA	National Institute for Agricultural Research
INTA	Instituto Nacional de Tecnología Agropecuaria (in Spanish)
IPCC A2 scenario	Intergovernmental Panel on Climate Change A2 medium emission scenario
IR	Infrared spectroscopy
IRB	Instituto de Recusos Biológicos (in Spanish)
iTRAQ	Isobaric tags for relative and absolute quantification
kg/ha	Kilograms/hectare
LC	Liquid chromatography
LC-ESI-MS^2	Liquid chromatograph-tandem mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
LE	La Estanzuela, Uruguay
LFD	Lateral flow devices
LMWG	Low molecular weight species
LogitAnther	Natural logarithm of (Anther/1-Anther)
LogitS	Natural logarithm of (S/1-S)
MALDI TOF	Matrix-assisted laser desorption/ionization time-of-flight
MAP-kinase	Mitogen activated protein kinase
MAS	Marker assisted selection
MeOH	Methanol
MGAP	Ministry of Livestock, Agriculture and Fisheries
MIC	Minimal inhibitory concentration
MPa	Megapascal
MS	Mass spectrometry
MSP	Ministry of Public Health
MudPIT	Multidimensional protein identification technology
NCC-Australia	National Climate Centre-Australia
NCEP/NCAR	National Center for Environmental Prediction/National Center for Atmospheric Research

ND	Number of days with simultaneous occurrence of Pr and thermal amplitude (TA = xT − nT) <7°C
nDD	Daily accumulation of the residuals resulting from subtracting 9 to the nT values (<9 °C) and the exceeding amounts of xT from 26 °C
NEO	Neosolaniol
NERC-UK	Natural Environment Research Council-United Kingdom
NHLF	Normal human lung fibroblasts
NIR	Near infrared radiation
NIV	Nivalenol
Nm	Effective migration rate
NOAA-USA	National Oceanic and Atmospheric Administration-United States of America
NOI	Niche overlap index
NP	Number of two-day periods with Pr (≥0.2mm) and RH>81 % during the first day and RH ≥ 78 % during the second one
nT	Minimum temperature
OENI	Oceanic El Niño index
OPA	*o*-Phthaldialdehyde
PA	Prediction accuracy
PCC	Pyrene-1-carbonyl cyanide
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pDD	Results of accumulating residuals >9 °C in nT, in those days where xT and nT are <25 °C and >=9 °C respectively
PEA	Partially exerted anthers
PEG	Polyethylene glycol
PFI %	Predicted *Fusarium* index
PFI_f%	Predicted *Fusarium* index adjusted to field conditions
PI %	Predicted FHB incidence
PMF	Peptide mass fingerprinting
PR proteins	Defense-related proteins
Pr	Precipitation
PRECIS	Providing Regional Climates for Impact Studies
PrL	Probability of having a light to nil epidemic
PrM	Probability of having a moderate epidemic
PrMc	Cumulative probability of an epidemic => to moderate
PrSv	Probability of having a severe epidemic
PS %	Predicted FHB severity
PTMs	Post translational modifications
qPCR	Quantitative real-time polymerase chain reaction
QTL	Quantitative trait locus
r	Pearson correlation coefficient
R^2	Determination coefficient
RAPD	Random amplification of polymorphic DNA

RBN	Bromatologic national regulation
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RIL	Recombinant inbred line
r_K	Kendall correlation coefficient
RNA	Ribonucleic acid
RP	Reversed phase
RPTEC	Renal proximal tubule epithelial cells
RS	Rio Grande do Sul
RT-PCR	Reverse transcription polymerase chain reaction
RYT	Regional yield trial
S %	Observed FHB severity
S	FHB severity (values from 0 to 1)
Sa	Sphinganine
SAM	Southern Annular Mode or Antarctic Oscillation
SCAR	Sequence characterized amplified region
SDS PAGE	Sodium dodecyl sulfate polyarylamide gel electrophoresis
SDVIF	Sum of daily values for infection favorability
SI	Spike incidence
SNPs	Single nucleotide polymorphisms
SO	Southern Oscillation
So	Sphingosine
SOI	Southern Oscillation index
SPI	Susceptible period for infection
SPR	Surface plasmon resonance
SRES	Special Report Emissions Scenarios
SRSN	Scab resistance screening nursery
SSRs	Simple sequence repeats
SW	Spring wheat
SWD	Spike wetness duration
TA	Thermal amplitude
TCA	Trichloroacetic acid
Td	Mean daily temperature (Td = xT+nT / 2)
TDI	Tolerable daily intake
TEF	Translation elongation factor
TKW	Thousand kernel weight
TLC	Thin layer chromatography
TRMM	Tropical Rainfall Measurement Mission
T_W	Temperature during the wetness period
U	Zonal wind at 500 hPa
UFRGS	Universidade Federal do Rio Grande do Sul
UPGMA	Unweighted pair group method with arithmetic mean
USA	United States of America
USDA	United States Department of Agriculture
USWBSI	United States Wheat and Barley Scab Initiative

UV	Ultraviolet
UV–vis	Ultraviolet–visible range
VCG	Vegetative compatibility group
VNTR	Variable number of tandem repeats
W	Wetness period
WHO	World Health Organization
WSRSN	Wheat Scab Resistance Screening Nursery
xT	Maximum temperature
Z61	Zadoks 61
Z65	Zadoks 65
ZEA	Zearalenone
ZI	Zonal index
ZOL	Zearalenol

Part I
Fusarium Populations Associated with Fusarium Head Blight in Latin America

Chapter 1
Population Structure of *Fusarium graminearum* Species Complex Genotypes and Chemotypes in Relation to Trichothecenes Production

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Abstract Argentina is the fourth largest exporter of wheat in the world. Fusarium Head Blight (FHB) is one of the most important fungal diseases of wheat worldwide. The main pathogen associated with FHB in Argentina is *Fusarium graminearum sensu stricto* (teleomorph *Gibberella zeae*) within the *Fusarium graminearum* species complex. This specie can produce the type B trichothecenes usually deoxynivalenol (DON) and its acetylated forms 3-ADON and 15-ADON or nivalenol (NIV). Also DON/NIV genotypes have been observed which chemotype was DON. The *G. zeae* populations from Argentina are genetically and genotypically diverse.

1.1 Introduction

Wheat production in Argentina, reached around 15 million tons, ranking the country as the fourth largest wheat exporter in the world. The wheat cultivation area in Argentina is divided in five regions designated I to V according to agro-meteorological conditions (Fig. 1.1).

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is the most common causal agent of Fusarium Head Blight (FHB), a reemergent disease for wheat (*Triticum aestivum* L) worldwide. The pathogen belongs to the *Fusarium graminearum* species complex (FGSC) which, based on DNA sequences of 12 genes, was resolved in 15 phylogenetic species *Fusarium acacia-mearnsii,*

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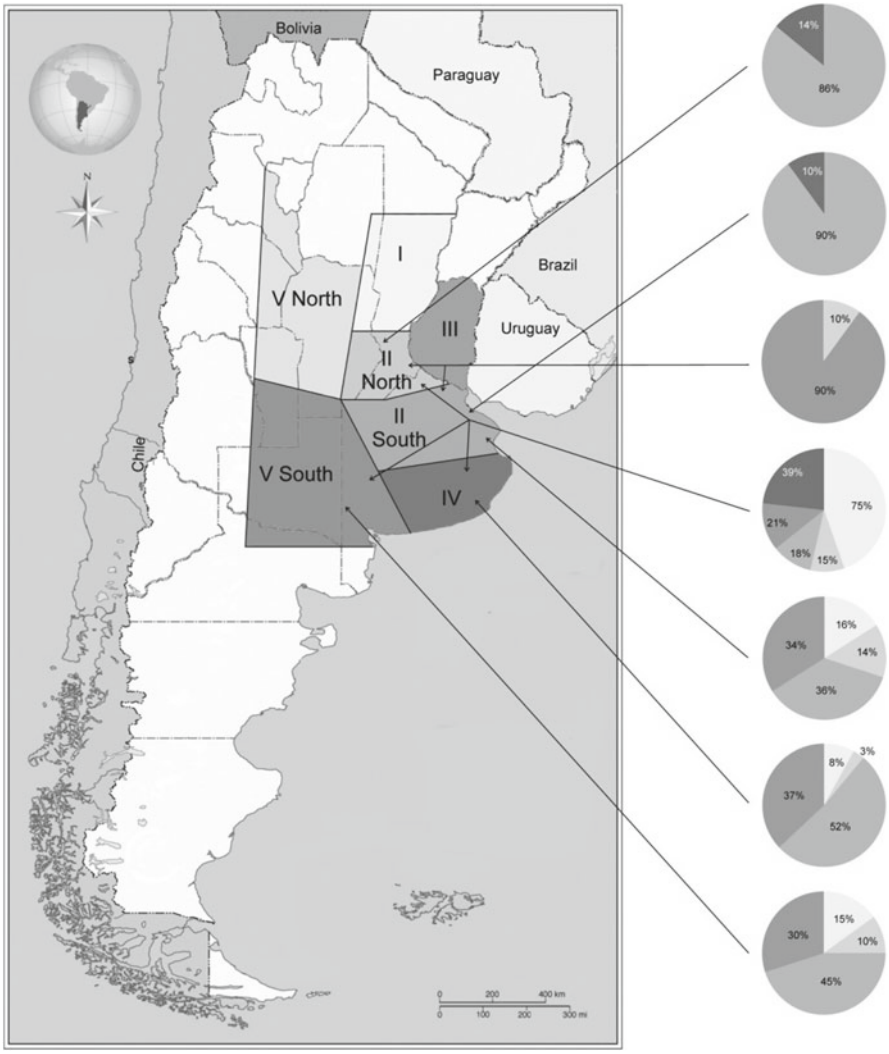

Fig. 1.1 Wheat cultivation regions in Argentina showing the proportions of *Fusarium graminearum* species complex genotypes (Reynoso et al. 2011) and chemotypes (Ramirez et al. 2006; Fernandez Pinto et al. 2008; Alvarez et al. 2011)

F. aethiopicum, F. asiaticum, F. austroamericanum, *F. boothii, F. brasilicum, F. cortaderiae, F. gerlachii, F. graminearum sensu strict, F. meridionale, F. mesoamericanum, F. ussurianum, F. vorosii, F. nepalense* and *F. lousianense* (O'Donnell et al. 2000, 2004, 2008; Starkey et al. 2007; Yli-Mattila et al. 2009; Sarver et al. 2011).

Some of the effects of FHB infection include bleached and shrunken kernels, decrease seed quality and vigor, losses of yield but the main impact from the food safety point of view of is the accumulation of mycotoxins mainly deoxynivalenol

(DON) and its acetylated derivatives 3 acetyl-deoxynivalenol (3-ADON) and 15 acetyl-deoxynivalenol (15-ADON) and nivalenol (NIV). FHB epidemics occurred in Argentina in 17 of the last 50 years, causing yield losses and price discounts due to reduced grain quality (Galich 1997).

The population structure of *F. graminearum* in South America is poorly understood compared to other production regions. Chemotype and genotype characterization has been used to characterize *F. graminearum* for their toxigenic potential. Chemical analyses using chromatographic methods have been used to determine the chemotype for the strains (Faifer et al. 1990; Lori et al. 1992; Ramirez et al. 2006; Fernandez Pinto et al. 2008; Sampietro et al. 2011) while molecular methods based on polymorphism on coding genes and introns (such as *Tri3Tri7Tri12* and *Tri13*) of the trichothecene biosynthesis pathway (TRI cluster) have been used to determine the strain genotype (Reynoso et al. 2011; Sampietro et al. 2011) (Fig. 1.1). Strains of *F. graminearum* usually express one of three sets of trichothecene metabolites either: (i) nivalenol and its acetylated derivatives (NIV chemotype), (ii) deoxynivalenol and 3-acetyl-deoxynivalenol (3-ADON chemotype), or (iii) deoxynivalenol and 15-acetyl-deoxynivalenol (15-ADON chemotype) (Ward et al. 2002). *Fusarium* isolates that produce both NIV and DON (NIV/DON chemotype) have been described as "unknown" chemotypes (Ward et al. 2002; Quarta et al. 2006). The 15-ADON chemotype dominates in North America and the 3-ADON chemotype dominates in some parts of Asia, Australia and New Zealand (Guo et al. 2008). The newly emerging 3-ADON population appears to be more aggressive and produces a higher level of DON than the 15-ADON populations (Ward et al. 2008; Puri and Zhong 2010; von der Ohe et al. 2010).

DON is associated with feed refusal, vomiting and suppressed immune functions, and NIV is more toxic to humans and domestic animals than is DON (Ryu et al. 1988). Due to their toxicity, international regulations limit the content of DON in food chains (FAO 2004; Verstraete 2008). Trichothecenes also are potent phytotoxins (Eudes et al. 2000), with DON being more phytotoxic than NIV (Desjardins 2006).

Due to the toxicological differences between NIV and DON (Desjardins and Proctor 2007), it is important to determine the chemotypes of strains present in any given geographic region.

1.2 Population Structure

Genetic diversity in populations of *G. zeae* has been assessed with various molecular markers including AFLPs (Zeller et al. 2003, 2004; Schmale et al. 2006; Ramirez et al. 2007; Lee et al. 2009), RAPDs (Ouellet and Seifert 1993; Dusabenyagasani et al. 1999; Fernando et al. 2006), RFLPs (Gale et al. 2002; Tóth et al. 2005), VNTR (Zhang et al. 2010a, b, 2012; Sampietro et al. 2011). Virtually all studies have identified high levels of genetic and genotypic diversity. Initially this result seems surprising since homothallic sexual reproduction, of which this fungus is capable, is the genetic equivalent of self-replication. High levels of genotypic diversity, with relatively few

clones, suggest that *G. zeae* outcrosses frequently enough to maintain a great deal of genetic heterogeneity in the population. Effective assessment of genetic and genotypic variation requires many markers that are randomly distributed across the genome. AFLPs are relatively easy to generate in sufficiently large numbers and are well-enough distributed across the genome that they can be used to generate a genetic map of *G. zeae* (Jurgenson et al. 2002). The genetic map demonstrates that out-crossing and recombination can occur under laboratory conditions and by inference under field conditions as well.

A study on a population of 113 *F. graminearum* strains isolated from San Antonio de Areco and from Alberti, two localities from the main wheat growing area from Argentina were evaluated using AFLP markers (Ramirez et al. 2007). Two hundred and sixteen AFLP bands were identified in the 200-500 bp range from the 113 analyzed isolates when using the three primer pair combinations *EcoR*I + AA/*MseI* + AT, *EcoR*I + CC/*MseI* + CG and *EcoR*I + TG/*MseI* + TT. Of these 216 AFLP loci, 91 % were polymorphic in the San Antonio de Areco population and 88 % were polymorphic in the Alberti population. All 113 isolates had AFLP profiles typical of *G. zeae, F. graminearum sensu strict* (*F. graminearum s.s.*). Isolates with the same AFLP genotype (clones) were rare, and only nine of the 113 strains had an AFLP genotype that was the same as that of one of the other strains examined – two pairs of two strains each in the Alberti population and one pair of two and a set of three in the San Antonio de Areco population. No strains with the same AFLP haplotype were found in different locations. Normalized genotypic diversity ($\hat{G}$) was high (≥98 % of the count) in both populations, with the highest number of clonal isolates found in the San Antonio de Areco population.

Allele frequencies were generally very similar between these two populations as were the mean gene diversities. There were 23 loci with private alleles (both allelic forms present in one population but not in the other) in the San Antonio de Areco population of which 13 had both alleles at a frequency of >5 %. In the Alberti population there were 17 private alleles with both of the alleles at four of these loci present at a frequency of >5 %. The mean frequency of the 40 private alleles across both populations was ~5.3 %. For the full set of 199 polymorphic loci, the average gene diversity was 0.25 for the combined population, 0.27 for the isolates from San Antonio de Areco, and 0.24 for isolates from Alberti (Table 1.1). The higher gene diversity in the San Antonio de Areco population might be due to the larger number of polymorphic loci (182 loci) observed in that population relative to the population from Alberti (176 loci). When we removed the 51 loci with rare polymorphic alleles (frequency of the rarer allele <5 % in both populations) from the analysis the mean gene diversity estimates for the combined populations increased from ~0.25 to 0.32.

Values of G_{ST} (fixation index or differentiation among populations as a result of population subdivision) for individual loci ranged from zero, i.e. either no divergence or equal allele frequencies, to 0.185 (Table 1.2). The mean G_{ST} across all 199 loci was 0.033 [*Nm* (effective migration rate) >15 across all 199 loci] (Table 1.2). Similar results were obtained when analysing a subset of 148 loci for which the frequency of the rarer allele was >5 % (mean $G_{ST} = 0.034$ and $Nm > 14$) (Table 1.2).

Table 1.1 Basic parameters and haplotype diversity from populations of *Fusarium graminearum* isolated from Argentina

Population	San Antonio de Areco	Alberti
Sample size	69	44
Percent polymorphic loci	91	88
Haplotype diversity[a]	96	95
Number of private alleles	23	17
Genotypical diversity G_0/N	0.99	0.99
Nie's gene diversity H[b,c]		
199 loci (all loci)	0267	0238
148 loci (highly polymorphic loci)[d]	0341	0299

[a]Percentage of unique haplotypes
[b]Estimated for clone-censored populations. Clones were defined as isolates with ≥98 % similarity in AFLP banding pattern. Only one representative of each clone was retained for subsequent analyses
[c]Calculated as in Nei (1973)
[d]Both alleles present at a frequency of >5 %

Table 1.2 Population-genetic parameters describing populations of *Fusarium graminearum* isolated from Argentina

Population	199 loci[a]	148 loci[b]
Nie's gene diversity H[c] (range)	0.252 (0.015–0.499)	0.320 (0.053–0.499)
Population subdivision G_{ST}	0.033 (0–0.185)	0.034 (0–0.185)
Gen flow N_m[d]	15 (2.2–2000)	14 (2.2–2000)
Genetic identity[e]	0.98	0.97

[a]All loci
[b]Highly polymorphic loci
[c]Calculated as in Nei (1973)
[d]Calculated as in McDermott and McDonald (1993)
[e]Calculated as in Nei (1978)

For calculation of two locus linkage disequilibria in *G. zeae* populations, there were 19,701 possible pair-wise comparisons for the 199 AFLP loci. We rejected the null hypothesis of two-locus linkage equilibrium ($P<0.01$) in favor of the alternative hypothesis of two-locus linkage disequilibrium for 1,479 pairs of loci (7.5 %) for the Alberti population and for 1,811 pairs of loci (9.2 %) for the San Antonio de Areco population. At the $P<0.05$ significance level, we rejected the null hypothesis of two-locus linkage equilibrium for 2,422 pairs of loci (12 %) from the Alberti population, for 3,041 pairs of loci (15 %) from the San Antonio de Areco population, and for 19,701 pairs of loci (19.7 %) from the combined populations.

Previous studies of *G. zeae* populations from North America with AFLPs (Zeller et al. 2003, 2004; Schmale et al. 2006) found that G_{ST} ranged from 0 to 0.167. These values are similar to those observed in the population from Argentina of 0 to 0.185 (Table 1.2). Thus our results are consistent with the hypothesis that the two fields evaluated are part of a larger population that includes, perhaps the entire north central region of Argentina. This conclusion is tempered, however, by the relatively large

number of loci (40/199; Table 1.1) identified with private alleles in the studied populations. The relatively high frequency of some of these private alleles, up to 12 %,suggests that these populations have been isolated from one another long enough for these private alleles to accumulate in the populations and that the observed migration levels could reflect historic, rather than contemporary, gene flow of population origins. Determining whether these private alleles are reflective of population subdivision or sampling artifacts will require analysis of additional populations.

Linkage disequilibrium is another character that can be used to assess genetic exchange within and between populations. Taking $P < 0.01$ as a cutoff, 12.4 % of the locus pairs from the Alberti population, 17.4 % of the locus pairs from the San Antonio de Areco population, and 12.9 % of the loci in the combined populations were in linkage disequilibrium. These values are substantially larger than those found in the North America populations (Schmale et al. 2006; Zeller et al. 2003, 2004) in which no more than 10 % and usually less than 5 % of the locus pairs examined were in linkage disequilibrium. Linkage disequilibrium has many possible causes, including inbreeding, and the relatively recent mixture of two (or more) different populations. Discerning the cause of the observed linkage disequilibrium will require further studies of more populations from more locations and collected at different times, but these results are consistent with the relatively recent introduction of a substantial amount of new genetic material into these populations or with the populations passing through a bottleneck from which they have mostly, but not completely recovered.

The data from our Argentinian populations, both in this study with AFLPs and in an earlier, more limited study with VCGs (Ramirez et al. 2006) as with the data from the North American populations (Schmale et al. 2006; Zeller et al. 2003, 2004) is consistent with a high amount of outcrossing in these populations. We cannot estimate the relative amounts of heterothallic and homothallic sexual reproduction, but the laboratory estimate of 35 % heterothallic crossing made by Bowden and Leslie (1999) would seem an upper bound. Heterothallic reproduction has been occurring for some time or the linkage disequilibrium values would be much higher than we observed. The lack of one, or a few, dominant genotypes suggests that the alleles for pathogenicity are either fixed, and thus are invariant, or that there are many ways to be an effective pathogen and that there is no intense selection for any of these individual multigenic phenotypes. Relatively little recombination is thought to be required to sustain relatively high levels of genotype diversity and to result in populations that appear to be randomly mating (Leslie and Klein 1996). Clearly there is sufficient recombination in populations of *G. zeae* for the pathogen to be able to rapidly synthesize a multi-locus response to changes in selection pressures resulting from changes in host variety or the introduction of a novel biological or chemical control method.

Other study using AFLP analysis to evaluate species identity and genetic diversity was done on 183 strains isolated from different locations across the areas of wheat producing region of Argentina. Sequence analysis of the translation elongation factor 1-α and β-tubulin genes as well as AFLP analyses confirm that *F. graminenarum s.s.*

is the predominant species of the FGSC in the temperate wheat region of Argentina (Alvarez et al. 2011).

Fusarium graminearum populations from wheat in Argentina are genotypically diverse, and belong to *F. graminearum s.s.* (O'Donnell et al. 2000, 2004). These geographically diverse populations are genetically similar and may be part of a larger, randomly mating, meta-population with significant genetic exchange probably occurring between the various subpopulations (Ramirez et al. 2006, 2007). These populations from Argentina were similar to those from Brazil were isolates with the same haplotype were rare and genotypic diversity was uniformly high (>98 % of the count) suggesting that also recombination has played a significant role. The number of migrants was estimated between 5 and 6 across all loci and all populations but the high frequency of private alleles (up 30 %) suggests a historical rather than contemporary gene flow (Astolfi et al. 2012).

1.3 *Fusarium graminearum* Species Complex Genotypes-Chemotypes

Studies on trichothecene production by species within the FGSC isolated from wheat in Argentina are controversial. Ramirez et al. (2006) reported that all the strains produced DON and a few of them produced 3-ADON using chemical analysis by strains isolated from wheat. Lori et al. (1992) reported that the strains produced DON, 3-ADON and NIV, finally Fernández Pinto et al. (2008) reported the production of DON, 3-ADON, NIV and mainly 15-ADON (Fig. 1.1).

A study was done using PCR to assess the genotype and chemical analysis to determine the chemotype of the strains on a population of *F. graminearum* isolated during the 2002 harvest season from the wheat growing region in Buenos Aires and Córdoba (Reynoso et al. 2011) (Fig. 1.1). This study showed that 12 strains were 15-ADON genotype and nine strains have the DON/NIV genotype, with no DNA fragments amplified at all from 5 strains. Neither the NIV nor the 3-ADON genotypes amongst the strains evaluated was detected. Genotype frequencies in all three populations were similar. Strains with either the 15-ADON or DON/NIV genotype in the Quarta et al. (2006) assay all had the DON genotype in the Lee et al. (2001, 2002) assays. All strains with the 15-ADON genotype produced 15-ADON and DON as assessed by chemical analyses. The nine strains with the DON/NIV genotype also produced 15-ADON and DON. The five strains that produced none of the diagnostic fragments in the PCR assays produced no detectable trichothecenes.

The trichothecene profiles of *F. graminearum s.s.* from Argentina were similar to those seen for *F. graminearum s.s.* from Europe and North America, where *F. graminearum s.s.* dominate in areas that grow both maize and wheat (Waalwijk et al. 2003; Zeller et al. 2003, 2004; Jennings et al. 2004; Tóth et al. 2005; Schmale et al. 2006; Gale et al. 2007; Audenaert et al. 2009). In Korea, Japan, China and other parts of Asia, where strains of lineage 6 dominate, the NIV genotype is the most common and both DON genotypes are rare. In South America, *Fusarium graminearum*

s.s. from Brazil all had the 15-ADON genotype while those from lineage 2 had the NIV genotype (Scoz et al. 2009; Astolfi et al. 2012). As DON is more phytotoxic than NIV towards wheat, the cropping system could be a selective factor in the trichothecene genotype/chemotype composition of the fungal population analyzed.

Alvarez et al. (2009) evaluated *F. graminearum s.s.* (144 isolates) from 3 subregions from the main wheat production area collected in different harvest season 2001 (epidemic), 2003 and 2004 (non epidemic) cropping seasons (Fig. 1.1). According to their toxin profile the isolates were grouped in 4 chemotypes: DON chemotypes for isolates that produced DON and no acetylated derivatives production, the 3-ADON chemotype for isolates producing DON and 3-ADON, the 15-ADON chemotype for isolates producing DON and 15-ADON and the 3 and 15-ADON chemotype for isolates with simultaneous production of DON and both acetyl-derivatives. The chemotype 15-ADON was the most common type. The co-occurrence of DON and NIV in the same isolates is surprising since the genetic basis of DON and NIV chemotypes has been established to be due to differences in TRI13 and TRI7 (Lee et al. 2002). These genes are non-functional in DON-producing isolates so it is unclear how DON and NIV could both be produced by a single isolate. Similarly, the co-occurrence of 3-ADON and 15-ADON in the same isolates is surprising since the genetic basis of 3-ADON and 15-ADON chemotypes is due to different forms of TRI8 in 3-ADON and 15-ADON-producing strains (Alexander et al. 2011).

Global variation in DON/NIV production by isolates of *F. graminearum* and the distribution of these isolates geographically and by host are important questions in plant pathology, plant breeding, and food security. Argentinean strains of *F. graminearum* are similar to those from wheat elsewhere, as all of these strains produce DON and belong *F. graminearum s.s.*

1.4 Conclusions

The present review adds new information on populations of *G. zeae* from Argentina to the growing list of population studies of this fungus worldwide. These populations are genetically and genotypically diverse and there is a significant amount of genetic exchange occurring between genetically proximate populations.

References

Alexander NJ, McCormick SP, Waalwijk C, van der Lee T, Proctor RH (2011) The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. Fungal Genet Biol 48:485–495

Alvarez CL, Azcarate MP, Fernandez Pinto V (2009) Toxigenic potential of *Fusarium graminearum sensu stricto* isolates from wheat in Argentina. Int J Food Microbiol 135:131–135

Alvarez CL, Somma S, Proctor RH, Stea G, Mule G, Logrieco A, Fernandez Pinto V, Moretti A (2011) Genetic diversity in *Fusarium graminearum* from a major wheat-producing region of Argentina. Toxins 3:1294–1309

Astolfi P, Reynoso MM, Ramirez ML, Chulze SN, Alves TCA, Tessmann DJ, Del Ponte EM (2012) Genetic population structure and trichothecene genotypes of *Fusarium graminearum* isolated from wheat in southern Brazil. Plant Pathol 61:289–295

Audenaert K, van Broeck R, Bekaert B, De Witte F, Heremans B, Messens K, Höfte M, Haesaert G (2009) Fusarium head blight (FHB) in Flanders: population diversity inter-species associations and DON contamination in commercial winter wheat varieties. Eur J Plant Pathol 125:445–458

Bowden RL, Leslie JF (1999) Sexual recombination in *Gibberella zeae*. Phytopathology 89:182–188

Desjardins AE (2006) *Fusarium* mycotoxins: chemistry, genetics and biology. The American Phytopathological Society (APS) Press, St. Paul

Desjardins AE, Proctor RH (2007) Molecular biology of *Fusarium* mycotoxins. Int J Food Microbiol 119:47–50

Dusabenyagasani M, Dostaler D, Hamelin RC (1999) Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Can J Plant Pathol 21:308–314

Eudes F, Comeau A, Rioux S, Collin J (2000) Phytotoxicity of eight mycotoxins associated with *Fusarium* in wheat head blight. Can J Plant Pathol 22:286–292

Faifer GC, de Sala Miguel M, Godoy HM (1990) Patterns of mycotoxin production by *Fusarium graminearum* isolated from Argentina wheat. Mycopathologia 109:165–170

FAO (2004) Worldwide regulations for mycotoxins in food and feed in 2003. FAO food and nutrition paper 81. Rome, Italy, 165 pp

Fernandez Pinto V, Terminiello LA, Basilico JC, Rittieni A (2008) Natural occurrence of nivalenol and mycotoxigenic potential of *Fusarium graminearum* strains in wheat affected by head blight in Argentina. Braz J Microbiol 39:157–162

Fernando WGD, Zhang JX, Dusabenyagasani M, Guo XW, Ahmed H, McCallum B (2006) Genetic diversity of *Gibberella zeae* Isolates from Manitoba. Plant Dis 90:1337–1342

Gale LR, Chen LF, Hernick CA, Takamura K, Kistler HC (2002) Population analysis of *Fusarium graminearum* from wheat fields in eastern China. Phytopathology 92:1315–1322

Gale LR, Ward TJ, Balmas V, Kistler HC (2007) Population subdivision of *Fusarium graminearum sensu stricto* in the upper Midwestern United states. Phytopathology 97:1434–1439

Galich MT (1997) Fusarium head blight in Argentina. In: Duvin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. Centro Internacional de Mejoramiento de Maiz y Trigo, Mexico, pp 19–28

Guo XW, Fernando WGD, Seow-Brock HY (2008) Population structure chemotype diversity and potential chemotype shifting of *Fusarium graminearum* in wheat fields of Manitoba. Plant Dis 92:756–762

Jennings P, Coates ME, Walsh K, Turner JA, Nicholson P (2004) Determination of deoxynivalenol and nivalenol producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathol 53:643–652

Jurgenson JE, Bowden RL, Zeller KA, Leslie JF, Alexander NA, Plattner RD (2002) A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics 160:1452–1460

Lee T, Oh DW, Kim HS, Lee J, Kim YH, Yun S-H, Lee Y-W (2001) Identification of deoxynivalenol and nivalenol producing chemotypes of *Gibberella zeae* by using PCR. Appl Environ Microbiol 67:2966–2972

Lee T, Han Y-K, Kim K-H, Yun SH, Lee Y-W (2002) Tri 13 and Tri7 determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl Environ Microbiol 68:2148–2154

Lee J, Chang IY, Kim H, Yun SH, Leslie J, Lee Y-W (2009) Genetic diversity and fitness of *Fusarium graiminearum* populations from rice in Korea. Appl Environ Microbiol 75:3289–3295

Leslie JF, Klein KK (1996) Female fertility and mating-type effects on effective population size and evolution in filamentous fungi. Genetics 144:557–567

Lori GA, Carranza ML, Violante A, Rizzo I, Alippi HE (1992) *Fusarium* spp. en trigo capacidad toxicogénica y quimiotaxonomía de las cepas aisladas en la Argentina. Agronomie 12:459–467

McDermott JM, McDonald BA (1993) Gene flow in plant pathosystems. Annu Rev Phytopathol 31:353–373

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U S A 70:3321–3323

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

O'Donnell K, Kistler HC, Tacke BK, Casper HH (2000) Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum* the fungus causing wheat scab. Proc Natl Acad Sci U S A 97:7905–7910

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T (2004) Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet Biol 41:600–623

O'Donnell K, Ward TJ, Aberra D, Kistler HC, Aoki T, Orwing N, Kimura M, Bjornstad A, Klemsdal SS (2008) Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within *Fusarium graminearum* species complex from Ethiopia. Fungal Genet Biol 45:1514–1522

Ouellet T, Seifert KA (1993) Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. Phytopathology 83:1003–1007

Puri KD, Zhong S (2010) The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level and DON than the prevalent 15ADON population in spring wheat. Phytopathology 100:1007–1014

Quarta A, Mita G, Haidukowski M, Logrieco A, Mule G, Visconti A (2006) Multiplex PCR assay for the identification of nivalenol 3- and 15-acetyl deoxynivalenol chemotypes in *Fusarium*. FEMS Microbiol Lett 259:7–13

Ramirez ML, Reynoso MM, Farnochi MC, Chulze S (2006) Vegetative compatibility among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat spikes in Argentina. Eur J Plant Pathol 115:129–138

Ramirez ML, Reynoso MM, Farnochi MC, Torres AM, Leslie JF, Chulze S (2007) Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. Food Addit Contam 24:1115–1120

Reynoso MM, Ramirez ML, Torres AM, Chulze SN (2011) Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. Int J Food Microbiol 145:444–448

Ryu JC, Ohtsubo K, Izumiyama N, Nakamura K, Tanaka T, Yamamura H, Ueno Y (1988) The acute and chronic toxicities of nivalenol in mice. Fundam Appl Toxicol 11:38–47

Sampietro DA, Diaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CA, Ward TJ (2011) Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. Int J Food Microbiol 145:359–364

Sarver B, Ward T, Gale L, Broz K, Kistler HC, Aoki T, Nicholson P, Carter J, O'Donnell K (2011) Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet Biol 48:1096–1107

Schmale DG, Leslie JF, Zeller KA, Saleh AA, Shields EJ, Bergstrom GC (2006) Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021–1026

Scoz LB, Astolfi P, Reartes DS, Schmale DG, Moraes MG, Del Ponte EM (2009) Trichothecene mycotoxin genotypes of *Fusarium graminearum sensu strict* and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathol 58:344–351

Starkey DE, Ward TJ, Aoki T, Gale LG, Kistler HC, Geiser MD, Suga H, Tóth B, Vara J, O'Donnell K (2007) Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet Biol 12:1745–1776

Tóth B, Mesterházy A, Horváth Z, Bartók T, Varga M, Varga J (2005) Genetic variability of central European isolates of the *Fusarium graminearum* species complex. Eur J Plant Pathol 13:35–45

Verstraete F (2008) European Union legislation on mycotoxins in food and feed: overview of the decision-making process and recent and future development. In: Leslie JF, Bandyopadhyay R, Visconti A (eds) Mycotoxins. Detection methods management public health and agricultural trade. CABI, Wallingford, pp 77–99. ISBN 1845930827

Von der Ohe C, Gauthier V, Tamburic-Ilincic L, Brule-Babel A, Fernando WGD, Clear R, Ward TJ, Miedaner T (2010) A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur J Plant Pathol 127:407–417

Waalwijk C, Kastelein P, De Vries I, Kerenyi Z, van der Lee T, Hesselink T, Köhl J, Kema GHJ (2003) Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur J Plant Pathol 109:743–754

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K (2002) Ancestral polymorphism and adaptive evolution in trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc Natl Acad Sci U S A 99:9278–9283

Ward TD, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Gilbert J, Geiser DM, Nowicki TW (2008) An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–484

Yli-Mattila T, Gagkaeva T, Ward TJ, Aoki T, Kistler HC, O'Donnell K (2009) Anovel Asian clade within the *Fusarium graminearum* complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia 101:841–852

Zeller KA, Bowden RL, Leslie JF (2003) Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology 93:874–880

Zeller KA, Bowden RL, Leslie JF (2004) Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from USA. Mol Ecol 13:63–571

Zhang H, Zhang Z, van der Lee T, Chen WQ, Xu J, Xu JS, Yang L, Yu D, Waalwijk C, Feng J (2010a) Population genetic analyses of *Fusarium asiaticum* populations from barley suggest a recent shift favoring 3ADON producers in southern China. Phytopathology 100:328–336

Zhang Z, Zhang H, van der Lee T, Chen WQ, Arens P, Xu J, Xu JS, Yang LJ, Yu DZ, Waalwijk C, Feng J (2010b) Geographic substructure of *Fusarium asiaticum* isolates collected from barley in China. Eur J Plant Pathol 127:239–248

Zhang H, van der Lee T, Waalwijk C, Chen W, Xu J, Xu J, Zhang Y, Feng J (2012) Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. PLoS One 7:e31722

Chapter 2
Species Identification, Genetic Diversity and Phenotypic Variation Studies on the *Fusarium graminearum* Complex Populations from Brazil

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Abstract *Fusarium graminearum* sensu lato, the cause of Fusarium head blight (FHB), has been reported for more than 50 years in Brazil. However, it was only during the mid-1980s to late 1990s that FHB resurged as a primary concern of wheat production. More than a dozen studies in Brazil or elsewhere have been conducted since the year 2000 employing novel and accurate methods to explore genetic variability and identify the strains to phylogenetic species. Thus far, six species of the *F. graminearum* species complex (FGSC) have been reported in association with wheat, barley, oat, soybean, maize, rice and ryegrass in Brazil, and their prevalence rates appears to be dependent on the region and host surveyed. Although *F. graminearum* sensu stricto (s. str.) is dominant in wheat, *F. meridionale* is the most frequently found pathogen in maize, and *F. asiaticum* is the only species found in rice. Most of them preferentially exhibit specific trichothecene genotypes: *F. graminearum* s. str. is of the 15-acetyl-deoxynivalenol (DON) genotype, *F. meridionale* and *F. asiaticum* are of the nivalenol (NIV) genotype, and *F. austroamericanum* is of the 3-ADON genotype; an exception is that *F. cortaderiae* exhibits either the 3-ADON or NIV genotype. They have also exhibited differences in relation to their reproductive fitness, fungicide sensitivity and pathogenicity toward wheat and maize. Continuous monitoring of the genotypic and phenotypic traits of the FGSC populations will help to improve local disease management and contribute to the global knowledge of the biology of the pathogen.

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2.1 Introduction

Fusarium graminearum sensu lato (teleomorph *Gibberella zeae*) is the main cause of Fusarium head blight (FHB), a devastating and resurgent disease of wheat both in Brazil and worldwide (Goswami and Kistler 2004). The fungus also causes stalk and ear rot of maize (Sutton 1982) and may survive in the seeds or residues of several other cereal and non-cereal crops grown in succession or rotation with wheat and barley, such as rice, soybean and several grasses (Parry et al. 1995). In Brazil, two factors are key determinants of FHB risk: intensive no-till systems that contribute to the maintenance of high levels of inoculum and a wet subtropical climate that usually favors FHB in most years (Del Ponte et al. 2009). The fungus is known to produce relatively large amounts of deoxynivalenol (DON) and, ultimately, nivalenol (NIV), two trichothecene mycotoxins that may accumulate in grains at levels considered unsafe for both human and animal consumption (Creppy 2002).

In spite of the resurgence of FHB as a major threat to wheat production in Brazil in the early 1990s, it was only in 2011 that maximum tolerance limits were promulgated for *Fusarium* trichothecene mycotoxins, more specifically for DON, in the grains and by-products of wheat, barley, rice and other crops grown in the country. However, published data for the mycotoxin contamination of commercial wheat grains in Brazil is scarce, and the most recent reports have shown the co-occurrence of DON and NIV mycotoxins in commercial grains at levels that exceed the 2 ppm threshold. Furthermore, it was found that the trichothecene content in grains can vary from 1 year to the next and also according to the region surveyed, demonstrating that the local populations are able to produce both trichothecenes (Del Ponte et al. 2012).

Based on phylogenetic species recognition, with genealogical concordance (Taylor et al. 2000), taxonomic studies in the last decade have provided evidence that *F. graminearum* sensu lato comprises at least 15 phylogenetically distinct species, also known as the *F. graminearum* species complex (FGSC) (O'Donnell et al. 2004; Sarver et al. 2011). Additionally, chemical and/or molecular analyses have been performed in some of these studies to determine the trichothecene chemotype and/or genotype composition of FGSC (Ward et al. 2002; de Kuppler et al. 2011; Reynoso et al. 2011). In addition, the genetic diversity and population structure across various spatial and temporal scales have been assessed in the FGSC populations worldwide using a variety of molecular markers, as recently reviewed (Wang et al. 2011). Phenotypic traits, such as reproductive fitness, fungicide sensitivity and pathogenicity, have also been assessed comparatively among FGSC members (Goswami and Kistler 2005; Lamprecht et al. 2011; Spolti et al. 2012a; Shen et al. 2012).

Studies in both North and South America have shown that *F. graminearum* sensu stricto (s. str), formerly known as lineage 7, mainly a DON producer, appears to be the dominant species associated with the head blight of wheat and barley (Ward et al. 2008; Alvarez et al. 2011; Schmale et al. 2011; Astolfi et al. 2012a). Other species of the complex are either limited geographically or appear to exhibit a possible host preference (O'Donnell et al. 2004; Sarver et al. 2011; Astolfi et al. 2012b).

In this chapter, we review the literature and summarize the data from studies conducted in Brazil, or elsewhere with strains from Brazil, to determine the species

diversity, genetic diversity and phenotypic traits of the FGSC populations. Our focus is not limited to the strains causing head blight of wheat and barley but also include those infecting or surviving on the seeds and residues of other economically important crops, such as maize, soybean, and rice, which may serve as inoculum sources for epidemics in winter cereal crops. It is expected that such information can provide valuable insight into the ecological and evolutionary aspects of the FGSC populations in Brazil and contribute to the global knowledge of the pathogen.

2.2 Species Identification Based on Morphological and Reproductive Traits

In Brazil, the first FHB studies date from the 1960s in Rio Grande do Sul (RS) State where the disease caused by *F. graminearum* was first recorded, reportedly during the early 1940s (Costa Neto 1947). The plant pathological herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS) contains more than 50 records of specimens of *F. graminearum* associated with wheat FHB, which were collected and deposited by Prof. Costa Neto, a plant pathologist and professor at UFRGS from 1941 to the early 1970s. The initial research began in 1960 at the Faculdade de Agronomia Eliseu Maciel and the Instituto de Pesquisas e Experimentação Agropecuária do Sul (further incorporated by Embrapa) by means of a Brazil-Japan collaboration that included exchange visits and the introduction of resistant varieties and breeding with local materials (Silva 1966; Luzzardi et al. 1972, 1974).

When Embrapa Trigo, the national wheat research center was founded in Passo Fundo, RS during the early 1970s, further studies were conducted with regard to the epidemiology and management of FHB (Reis 1986a b, 1990; Lima et al. 1998, 2000; Picinini and Fernandes 1998). Although there are records of *F. avenaceum* (Reis 1988) and *F. tricinctum* (Pierobom et al. 1977) associated with head blight symptoms, *F. graminearum* appears to be the principal causal agent of the head blight of wheat and barley in Brazil. During surveys conducted in the 1980s, *F. graminearum* Group 1, now recognized as a separate species, *F. pseudograminearum* (Aoki and O'Donnel 1999), had not been found (Reis 1986a, 1990), and no further study has confirmed its occurrence in Brazil to date.

During the mid to late 1990s, a time when FHB was no longer considered a secondary disease of wheat in Brazil, as it was in the two previous decades, researchers became more interested in exploring the variability of the putative *F. graminearum* populations causing head blight of wheat and barley or associated with other hosts. By 2012, a variety of methods had been used in 16 published studies, including scientific articles and theses, which were aimed at identifying species in the complex or to within the complex level, in addition to the genetic diversity and population structure (Fig. 2.1).

The earliest and most comprehensive study for the identification of *Fusarium* spp. associated with FHB and other hosts was based on morphology and reproduction and included 85 isolates from Embrapa Trigo collected in Rio Grande do Sul

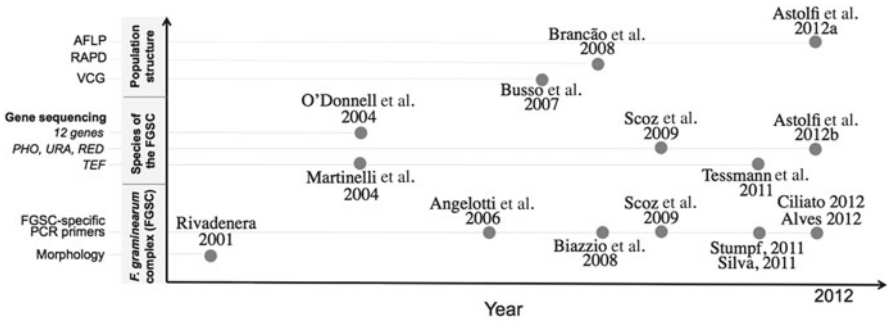

Fig. 2.1 Timeline of 16 studies conducted within a decade (2001–2012) by year of publication and the different methods used for the species identification and genetic diversity analysis of *Fusarium graminearum* species complex strains associated with agricultural crops in Brazil

State during the mid- to late 1990s. Based on morphological (reverse and surface colony color, abundance of mycelia and presence of macro- and microconidia), reproductive (mycelial growth rate and perithecia production) and pathogenic traits, all but ten isolates were identified as *F. graminearum* (Rivadenera 2001).

2.3 Molecular Identification and Genetic Diversity

The first Brazilian study using PCR (polymerase chain reaction) for the identification of *Fusarium* spp. associated with FHB was published in the mid-2000s using a limited number of isolates (n = 16) obtained from symptomatic wheat kernels and four isolates from triticale crops grown in southern Brazil (Angelotti et al. 2006). All the isolates amplified the expected fragment size for the UBC85 primer that targets the *F. graminearum* complex. In a follow-up study from the same group, Biazio et al. (2008) identified a sub-set of 14 *F. graminearum* isolates, previously identified with the UBC85 F/R primer, using a new PCR assay developed by the authors for the identification of *F. graminearum*. The new pair of primers, named GO F/R, targeted a segment of the 3′ coding region of the gaoA gene that codes for the enzyme galactose oxidase (GO) and proved to be specific to *F. graminearum* complex strains. The GO primer pair, in addition to the UBC85 and Fg16 primers, were used in PCR assays for detecting *F. graminearum* in spiked or naturally infected grains of bulgur wheat, but only one *F. graminearum* strain was found in commercial grain samples (Faria et al. 2012).

In Southern Paraná (PR) State, a small collection of nine monosporic isolates obtained from symptomatic wheat heads of six cultivars grown at an experimental station during the 2004 epidemic year was studied using VCG (vegetative compatibility groups based on nitrate non-utilizing mutants) and RAPD analysis. Although VCG separated the isolates into three groups, the UPGMA analysis using profiles of eight RAPD primers separated the isolates into two groups containing seven and

two isolates each, leading the authors to conclude that the population in their study was fairly clonal (Busso et al. 2007).

In RS State, 20 strains obtained from fields at a different municipalities of the northern region of the state during the 2005 growing season were identified as *F. graminearum* using PCR-based assays with TOX5 and GaoA-V2 primers that target potential trichothecene-producing strains of *Fusarium* and galactose oxidase-producing *F. graminearum*, respectively (Brancão et al. 2008). Additionally, the analysis based on 102 polymorphic RAPD markers separated the isolates into two distinct groups, which were, on average, 52 % similar. The first and larger group, which included 17 isolates, showed >80 % similarity, and the second group of three strains showed 60–75 % similarity among them (Brancão et al. 2008).

All the above studies conducted by Brazilian researchers have confirmed the identification of isolates at the *F. graminearum* species complex level, though not at the lineage or phylogenetic species levels, as defined by O'Donnell et al. (2004). However, there are a few recent reports of the occurrence of those lineages or species of the *F. graminearum* complex in Brazil being associated with several hosts. O'Donnell et al. (2004) first recognized nine phylogenetic species among the named *F. graminearum* isolates obtained from oat seeds in southern Brazil as *F. brasilicum*. According to their sampling of isolates from Brazil, other species would be endemic to the country, such as *F. graminearum* s. str., *F. austroamericanum*, *F. meridionale*, *F. cortaderiae* and *F. asiaticum* (O'Donnell et al. 2004).

Martinelli et al. (2004) identified to the lineage level a small collection of six isolates obtained from soybean seeds grown in PR and RS States, and one isolate obtained from wheat grown in PR State by amplifying a portion of the gene encoding translation elongation factor (TEF) and comparing the DNA sequences to those previously published from strains of known lineages. Although the only isolate from wheat was identified as lineage 8 (*F. cortaderiae*), one isolate from soybean was classified as lineage 1 (*F. austroamericanum*), three as lineage 2 (*F. meridionale*) and two as lineage 8 (*F. cortaderiae*) (Martinelli et al. 2004).

The first comprehensive molecular survey of isolates obtained at 20 locations in the major wheat-producing regions in southern Brazil was conducted during the 2006 growing season and resulted in the analysis of 82 *F. graminearum* monosporic isolates obtained from damaged wheat kernels (Scoz et al. 2009). The data from PCR assays using the Fg16 F/R, a primer pair that produces a polymorphic band for *F. graminearum* complex isolates, showed the presence of two SCAR groups: a larger group of 76 isolates produced a PCR amplicon of 450 bp, and six isolates generated an amplicon of 490 bp. To assist in the identification to the phylogenetic species level of representative isolates of these two groups, portions of the PHO, RED and URA genes from seven isolates of these groups were analyzed. Blast queries for individual gene sequences and pairwise comparisons of percentage identity based on 1676 bp of concatenated DNA sequence showed that the three representatives of the larger group were 98.7 % similar to reference isolates of *F. graminearum* s. str. and that the other four isolates were 98.43 % similar to reference isolates of *F. meridionale*. In a follow-up study, 140 isolates obtained in northern RS State during the 2007 growing season were split into three populations, each from wheat

fields at least 150 km from the others, and examined using the Fg16F/R primer pair and AFLP markers (Astolfi et al. 2012a). Based on the PCR SCAR groups, *F. graminearum* s. str. was the dominant population, followed by *F. meridionale*, with frequency levels ranging from 2 to 18 % across the fields. The genetic diversity assessed in a sample of 103 *F. graminearum* s. str. isolates using amplified fragment length polymorphism (AFLP) markers showed that the isolates with the same haplotype were rare and that the genotypic diversity was uniformly high (≥98 %), suggesting that sexual recombination has played a significant role in this group. The number of migrants was estimated between 5 and 6 across all loci and all populations, but the high frequency of private alleles (up to 30 %) suggested a historical, rather than a contemporary, gene flow. Regarding linkage disequilibrium, 0.8, 1.5 and 2.2 % of the locus pairs from the three populations were in disequilibrium, which is lower than the values reported in other regions of the world (Astolfi et al. 2012a).

The first survey of *F. graminearum* strains associated with barley kernels produced in Brazil was conducted during 3 years (2007, 2008 and 2009) using samples obtained from several fields in both southern and northern production regions of Rio Grande do Sul State, Brazil. Evidence based on the PCR SCAR groups for 92 isolates and portions of the PHO, RED and URA genes for a subsample of 8 isolates of the different groups and trichothecene genotypes showed the occurrence of *F. graminearum* s. str. as the dominant species, followed by *F. meridionale*, with a frequency higher than that found for the strains from wheat, and *F. austroamericanum* at a very low frequency (Astolfi et al. 2012b).

In maize, *F. graminearum* sensu lato has more commonly been found in South Brazil, a subtropical region, and less commonly in the tropical highlands in the Central Brazil, a region where *F. verticilloides* is more prevalent (Silva 2011; Tessmann et al. 2011). The identification of 105 monosporic isolates obtained from maize kernels from fields in both Central and South Brazil based on the partial sequence of the TEF gene revealed different species of FGSC: *F. meridionale*, the dominant species (73 %), *F. graminearum* s. str. (14 %), and *F. cortaderiae* (13 %), which is limited geographically to the southernmost part of the country (Tessmann et al. personal communication).

Thus far, six species of the *F. graminearum* species complex (FGSC) have been reported in association with important agricultural crops such as wheat, barley, oat, soybean, maize, rice and ryegrass in Brazil, and the predominance of these species appears to be dependent on the region and host surveyed (Table 2.1).

2.4 Trichothecene-Producing Potential

The FGSC populations from Brazil have also been studied with regard to their mycotoxin potential. The first study on mycotoxin occurrence in wheat grains produced in Brazil has provided indirect evidence that *F. graminearum* populations, which are the main trichothecene-producing fungi infecting wheat, were capable of producing DON and NIV, as based on the detection of these mycotoxins in wheat

Table 2.1 Phylogenetic species (former lineage) and the respective trichothecene genotype determined by molecular analyses or the chemotype* determined by chemical analysis of isolates of the *Fusarium graminearum* species complex associated with agricultural crops grown in Brazil

FGSC species	Host	Trichothecene	Reference
F. austroamericanum (Lineage 1)	Soybean	DON*	Martinelli et al. (2004)
	Maize kernel	3-ADON*	O'Donnell et al. (2004)
	Barley	3-ADON	Astolfi et al. (2012b)
F. meridionale (Lineage 2)	Soybean	NIV*	Martinelli et al. (2004)
	Wheat	NIV	Scoz et al. (2009)
	Maize kernel	NIV	Silva (2011)
	Maize kernel	NIV	Stumpf (2011)
	Maize stubble	NIV	Kuhnem et al.*
F. asiaticum (Lineage 6)	Oat	NIV	O'Donnell et al.*
	Rice	NIV	Gomes et al.*
	Ryegrass	NIV	Del Ponte et al.*
	Wheat	NIV	Del Ponte et al.*
F. graminearum (Lineage 7)	Wheat, oat, barley, soybean	15-ADON	O'Donnell et al.*
	Wheat	15-ADON	Scoz et al. (2009)
	Wheat	15-ADON	Astolfi et al. (2012a)
	Barley	15-ADON	Astolfi et al. (2012b)
	Maize	15-ADON	Silva et al.*
	Maize stubble	15-ADON	Kuhnem et al.*
	Ryegrass	15-ADON	Del Ponte et al.*
F. cortaderiae (Lineage 8)	Barley, wheat	3-ADON	O'Donnell et al.*
	Wheat, soybean	NIV*	Martinelli et al. (2004)
	Maize kernel	NIV, 3-ADON	Silva (2011)
	Maize stubble	NIV, 3-ADON	Kuhnem et al.*
	Ryegrass	NIV	Del Ponte et al.*
F. brasilicum	Oat	NIV*	O'Donnell et al. (2004)

*Personal communication

grains (Furlong et al. 1995). This result has been confirmed recently with the detection of both DON and NIV at similar levels in commercial wheat grain from RS State (Del Ponte et al. 2012).

Strains of FGSC can be classified as possessing a specific trichothecene chemotype (chemical phenotype) according to the chemical analysis of artificial or natural substrates, a method based on which trichothecene is predominantly produced and its abundance (Desjardins et al. 1993). Trichothecene genotypes have been identified with the aid of PCR assays employing a suite of primers directed to portions of genes (*TRI3, TRI5, TRI7, TRI12,* and *TRI13*) that discriminate the genotypes of NIV, DON and its two acetylates: 3-ADON and 15-ADON (Chandler et al. 2003; Ward et al. 2002; Quarta et al. 2006).

Chemotypes and/or genotypes of the FGSC strains from Brazil have been determined concomitantly with species identification or for a set of known species. In a multi-toxin production study, thin-layer chromatography was used to detect and identify trichothecene (DON, NIV, diacetoxyscirpenol, fusarenon-X, neosolaniol

and NIV), zearalenone and zearalenol production on a rice substrate by 24 FGSC isolates that were obtained from wheat (n = 19), barley (n = 1) and triticale (n = 4) grown in southern Brazil. The authors observed that 67 % of the isolates produced zearalenone and 33 % produced DON. In addition, a few isolates also produced zearalenol, diacetoxyscirpenol and fusarenon-X, though no isolate produced nivalenol or neosolaniol (Geraldo et al. 2006).

For five strains from soybean and one from wheat identified as species of FGSC, a GC/MS (gas chromatography/mass spectrometry) analysis showed that all the isolates were capable of producing DON and/or NIV, which were detected 2 weeks after spraying the anthers of the wheat heads with a spore suspension. Strains of lineages 2 (*F. meridionale*) and 8 (*F. cortaderiae*) were essentially NIV producers but also produced small amounts of DON, whereas the lineage 1 strain (*F. austroamericanum*) produced only DON in very large amounts. It was also observed that the nivalenol chemotypes were also capable of producing 3-acetylnivalenol (Martinelli et al. 2004).

Trichothecene genotypes have been determined mostly based on the amplification of portions of the *TRI3* and *TRI12* genes in a multiplex PCR assay and some using the *TRI13* gene that differentiates the DON and NIV genotypes. The first report of trichothecene genotypes for a large number of strains (n = 82) surveyed across wheat regions in South Brazil was conducted by Scoz et al. (2009), and the authors identified six NIV and 76 15-ADON genotypes based on primers targeting the *TRI3*, *TRI12* and *TRI13* genes. In an ensuing study with higher number of strains from the same field, Astolfi et al. (2012a) also identified the 15-ADON and NIV genotypes in wheat field populations surveyed in 2007 at three locations in Northern RS State. A large and variable predominance of 15-ADON across the locations was found. A fine-scale survey of the isolates obtained from several georeferenced locations within a field was conducted in 2008, and three genotypes were found, with a tendency for the less frequent genotypes (NIV and 3-ADON) to aggregate spatially (Astolfi et al. 2010). Three genotypes were also found for the FGSC strains from barley, but, differently from wheat, the NIV genotype was found at 3× higher frequency in barley, along with the detection of a few 3-ADON isolates (Astolfi et al. 2012b). The trichothecene genotype diversity among strains examined has been associated with species differences: the *F. graminearum* s. str. strains were all of the 15-ADON genotype; the *F. meridionale* strains were of the NIV genotype; and the *F. austroamericanum* strains were of the 3-ADON genotype (Table 2.1). In Brazil, the FGSC populations from maize have also been under investigation and, as in wheat, the species were also correlated to specific genotypes: *F. meridionale* were of the NIV genotype, and *F. graminearum* s. str. strains were of the 15-ADON genotype. An exception was the *F. cortaderiae* strains from maize that were either of the NIV or 3-ADON genotype (Table 2.1).

Trichothecene genotypes were also identified in a collection of 202 FGSC strains from maize stalks collected in two states, Goiás and Paraná, showing the high prevalence (96 %) of the NIV genotype compared to the 15-ADON (3 %) and 3-ADON (1 %) genotypes (Ciliato 2012). A study with 115 FGSC strains obtained from soybean seeds from both Central and South Brazil also reported that the NIV

genotype was more frequent (55 %), with a higher frequency of the 15-ADON (42 %) and a very low frequency of the 3-ADON (3 %) genotypes (Alves 2012). These strains are yet to be identified to the phylogenetic species level.

2.5 Fitness-Related and Aggressiveness Traits

Strains representative of the FGSC populations from Brazil have been examined with regard to asexual (germination, mycelial growth and sporulation) and sexual (perithecia and ascospores formation) reproductive and pathogenicity traits in a small number of studies. In an early study by Rivadenera (2001), 85 *Fusarium* spp. isolates showed variation in relation to their mycelial growth rate and pathogenicity, a result that could be due to not all of the isolates belonging to the FGSC complex or to different species of FGSC that had not yet been identified at the time. A few isolates that were not obtained from wheat heads did not produce perithecia, in contrast to the putative FGSC isolates that produced abundant perithecia in less than 2 weeks in carnation leaf agar. For the three groups discriminated by their mycelial growth rate in PDA under dark conditions, most isolates grew an average of 1.7 cm/day and 1.38 cm/day, whereas a few isolates grew at a rate of 2.26 cm/day (Rivadenera 2001).

More recently, isolates representative of different phylogenetic species of the complex and obtained from different hosts have been studied comparatively with regard to fitness-related traits and pathogenicity. Using two pairs of FGSC isolates obtained from wheat and barley, each belonging to *F. graminearum* s. str. or *F. meridionale*, Spolti et al. (2012a) examined the mycelial growth rate, sporulation and germination rates, sensitivity to tebuconazole and aggressiveness of the isolates. The values of all the variables examined, excepting the germination rate, differed significantly between the two species: on average, the *Fusarium graminearum* s. str. isolates grew 1.3× faster, sporulated 3× more, and exhibited lower sensitivity to tebuconazole than the *F. meridionale* isolates. In a pathogenicity assay based on *in vitro* glume infection and *in planta* central-floret inoculation methods, the *F. graminearum* s. str. isolates were capable of infecting and colonizing at higher rates than the *F. meridionale* isolates, a result only observed for the less susceptible (cv. Guamirim) and not the more susceptible (cv. BRS 194) variety. The authors discussed that such differences may explain the greater predominance of *F. graminearum* s. str. in wheat, as previously observed (Scoz et al. 2009; Astolfi et al. 2012b).

The aggressiveness toward maize seedlings of two *F. meridionale* and two *F. graminearum* s. str. obtained from maize kernels was evaluated using a substrate-inoculation method. Although no significant difference was found between the species, a large variation was observed for plant height (18.6–20.5 cm) and dry weight (1.8–2.3 g) (Silva 2011). In another study, strains of these two species were also compared in relation to their aggressiveness by the inoculation of maize stalks at 60 days after emergence, but no significant difference was found (Ciliato 2012). Lastly, the mycelial growth and aggressiveness of these two species was compared

based on the inoculation of stalks of 21-day-old seedlings (Kuhnem et al. 2013). Contrary to a previous study (Spolti et al. 2012a) that tested strains from wheat and barley, the mycelial growth rate did not differ significantly between these species from maize, which may be due to the low number of isolates studied and the large variation of isolates from the same species (Kuhnem et al. 2013). Furthermore, all the isolates were pathogenic toward the stalks of maize seedlings (21 days old) using a toothpick-inoculation method. Although the *F. graminearum* s. str. strains produced lesions that were, on average, 10 mm larger than those produced by *F. meridionale* at 14 days after inoculation, no significant difference was found when the isolates were grouped by species, in agreement with two other studies (Silva 2011; Ciliato 2012). Such behavior may be due to the greater variability between the isolates (6.3–53.5 mm) and, as in the previous study with isolates from wheat, also suggests that a higher sample size is necessary to better determine the potential differences in aggressiveness between these species.

2.6 Fungicide Sensitivity

In Brazil and in other regions of the world, fungicides are becoming a key component in integrated FHB management (Casa et al. 2007; Spolti et al. 2013). Chemical control has been used more intensively since the early 1990s when FHB epidemics became more frequent and research data showed the economic benefits of the use of fungicides in some situations (Reis et al. 1996; Picinini and Fernandes 1998). Triazol fungicides, such as tebuconazole and metconazole, are those most recommended, though commercial mixtures of triazol and strobilurins are also labeled and used in Brazil. In Europe and China, declines in fungicide sensitivity and increases in resistance have been detected and are suspected to be due to the intensive use of fungicides (Klix et al. 2007; Yin et al. 2009). However, the information in the literature was incomplete with regard to triazol fungicide sensitivity at the FGSC population level in Brazil until Spolti et al. (2012b) observed variability in the levels of sensitivity in 50 FGSC strains, with EC_{50} (effective concentration at which 50 % of mycelial growth is reduced) median values of 0.001 and 0.037 mg/L for tebuconazole and metconazole, respectively. Additionally, cross-resistance was detected, but no fitness-related cost was identified based on the fact that the less and more resistant groups of strains did not differ in their mycelial growth rates. In a previous study in which the sensitivity to tebuconazole of only four isolates was examined, two *F. meridionale* isolates exhibited a higher sensitivity than *F. graminearum* s. str. (Spolti et al. 2012b). Further preliminary data from the comparison of 10 strains from each species showed that the EC_{50} values of the *F. graminearum* s. str. strains were an average of 4.5-fold higher than *F. meridionale* (Spolti et al. 2013). Although variation in sensitivity levels has been observed when comparing these two species in different experiments, possibly due to a low sample size or other factors, *F. meridionale* has consistently shown a higher sensitivity to triazol in comparison to *F. graminearum* s. str. strains.

Another small-scale study about fungicide sensitivity was conducted using five FGSC strains obtained from either maize stalk or wheat and barley grains grown in PR and RS states, but that were not identified to the FGSC species level (Avozani et al. 2011). Results showed significant variation among the strains with regards to EC_{50} values, which were relatively low for triazoles (<0.01 for metconazole, prochloraz, prothioconazole and tebuconazole and 0.12 for cyproconazole) and benzimidazole (0.07 for carbendazim) fungicides and higher for strobilurin (0.21 for azoxystrobin and 1.33 for trifloxystrobin) fungicides.

2.7 Conclusions

As in other regions of the world, plant disease epidemics and the contamination of cereal grains as a result of infection with FGSC have received considerable attention in recent years by Brazilian researchers working alone or in collaboration with researchers from abroad. Advances in global research and the availability of novel tools to facilitate a thorough examination of the genotypic variability of the populations are leading to a better characterization of the regional populations and understanding of risk factors that were heretofore unknown.

The results found to date through molecular surveys conducted in important cereal crops indicate that South-Central Brazil is one of the few regions in the world that possesses an exceptionally great diversity of FGSC species. Additionally, there is evidence that fixation for specific trichothecene genotypes has occurred in some species, in contrast with other regions where there appears to be much less species diversity but the presence of dominant species possessing a variety of trichothecene genotypes. Moreover, we have not found evidence in surveys conducted over 5 years of any potential shift toward any specific trichothecene genotype or species of the complex, as reported in other regions. In fact, surveys on multiple cereals have indicated that the species may be more adapted to specific hosts or niches, which deserves further investigation. For example, it is not know why the composition and frequency of species change dramatically from one crop to another in the same growing region, e.g., wheat and maize. This observation may be due to a true host preference or some bias in the sampling either with regard to the number of samples or the niche from which the samples are obtained.

A better understanding of the relationship between the trichothecene genotypes and chemotypes is needed to understand why, for example, the NIV mycotoxin is found in grains at concentrations that does not reflect the frequency of the NIV genotypes found in symptomatic kernels. This could be related either to the ability of the populations to produce a single trichothecene, as expected from the genotypic analysis, or to differences in species compositions, which can vary from field to field or due to selection pressure by management practices or the environment. Further investigations on the exploration of phenotypic traits that include pathogenicity, fitness and fungicide sensitivity will help in the understanding of and propose hypotheses about the ecology of the pathogen and the epidemiology of the diseases

caused by the *F. graminearum* complex in Brazil. The recent promulgation of tolerance limits for *Fusarium* mycotoxins in several cereal crops in Brazil will surely contribute to increased awareness and the consideration of *F. graminearum* as a major threat to cereal production and public health, a problem that will only be solved by continued monitoring and multi-disciplinary research.

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References

Alvarez CL, Somma S, Proctor RH, Stea G, Mulè G, Logrieco AF, Pinto VF, Moretti A (2011) Genetic diversity in *Fusarium graminearum* from a major wheat-producing region of Argentina. Toxins 10:1294–1309

Alves (2012) Levantamento e identificação de espécies e genótipos tricotecenos de *Fusarium graminearum* associados a sementes de soja em regiões produtoras do centro do sul do Brasil. Thesis Universidade Estadual de Maringá, Brazil

Angelotti F, Tessmann DJ, Alves TCA, Vida JB, Jaccoud Filho DS, Harakava R (2006) Caracterização morfológica e identificação molecular de isolados de *Fusarium graminearum* associados à giberela do trigo e triticale no sul do Brasil. Summa Phytopathol 32:177–179

Aoki T, O'Donnel K (1999) Morphological and molecular characterization of *Fusarium pseudograminearum* sp nov., formerly recognized as the Group 1 population of *F. graminearum*. Mycologia 91:597–609

Astolfi P, Simon LL, Schneider L, Alves TCA, Tessmann DJ, Del Ponte EM (2010) Within-field patterns of B-trichothecene genotypes in the *Fusarium graminearum* complex affecting Wheat in Southern Brazil. In: Canty S, Clark A, Mundell J, Walton E, Ellis D, Van Sanford D (eds) Proceedings of the national fusarium head blight forum, University of Kentucky, Orlando/ Lexington, 7–9 December 2009, pp 171

Astolfi P, Reynoso M, Ramirez ML, Chulze SN, Alves TCA, Tessmann DJ, Del Ponte EM (2012a) Genetic population structure and trichothecene genotypes of *Fusarium graminearum* isolated from wheat in southern Brazil. Plant Pathol 61:289–295

Astolfi P, Dos Santos J, Schneider L, Gomes LB, Silva CN, Tessmann DJ, Del Ponte EM (2012b) Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in southern Brazil. Int J Food Microbiol 148:197–201

Avozani A, Tonin RB, Reis EM, Camera J, Ranzi C (2011) Sensibilidade de *Fusarium graminearum* a fungicidas, in vitro. In: Reis EM (ed) Seminário sobre giberela em cereais de inverno – coletânea de trabalhos. Berthier, Passo Fundo, pp 235–252

Biazio GR, Leite GGS, Tessmann DJ, Barbosa-Tessmann IP (2008) A new PCR approach for the identification of *Fusarium graminearum*. Braz J Microbiol 39:554–560

Brancão MF, Bianchi VJ, Farias CRJ, Santos J, Rossetto EA (2008) Caracterização genética de *Fusarium graminearum* Schwabe através de técnicas moleculares. Rev Bras de Agroc 14:67–76

Busso C, Kaneshima EN, Franco FA, Querol CB, Castro-Prado MAA (2007) Vegetative compatibility and molecular characterization of *Fusarium graminearum* isolates from the State of Paraná, Brazil. Cienc Rural 37:1813–1816

Casa RT, Bogo A, Moreira EM, Kuhnem PR (2007) Época de aplicação e desempenho de fungicidas no controle da giberela em trigo. Cienc Rural 37:1558–1563

Chandler EA, Duncan RS, Thomsett MA, Nicholson P (2003) Development of PCR assays to Tri7 and Tri13 and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium cerealis*. Physiol Mol Plant Pathol 62:355–367

Ciliato ML (2012) Ocorrência de espécies de *Fusarium* em colmos de milho. Thesis, Universidade Estadual de Maringá

Costa Neto KP (1947) Parasitas de plantas cultivadas no Rio Grande do Sul. Porto Alegre. Secretaria de Estado dos Negócios da Agricultura, Indústria e Comércio

Creppy EE (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol Lett 127:19–28

de Kuppler AL, Steiner U, Sulyok M, Krska R, Oerke EC (2011) Genotyping and phenotyping of *Fusarium graminearum* isolates from Germany related to their mycotoxin biosynthesis. Int J Food Microbiol 151:78–86

Del Ponte EM, Fernandes JMC, Pavan W, Baethgen WE (2009) A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. J Phytopathol 157:675–681

Del Ponte EM, Garda-Buffon J, Badiale-Furlong E (2012) Deoxynivalenol and nivalenol in commercial wheat grain related to Fusarium head blight epidemics in southern Brazil. Food Chem 132:1087–1091

Desjardins AE, Hohn TM, McCormick SP (1993) Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. Microbiol Mol Biol Rev 57:595–604

Faria CB, Almeida-Ferreira GC, Gagliardi KB, Alves TCA, Tessmann DJ, Machinski M Jr, Barbosa-Tessmann IP (2012) Use of the polymerase chain reaction for detection of *Fusarium graminearum* in bulgur wheat. Cien Tecn de Alim 32:201–208

Furlong EB, Soares LMV, Lasca CC, Kohara EY (1995) Mycotoxins and fungi in wheat harvested during 1990 in test plots in the state of Sao Paulo Brazil. Mycopathologia 131:185–190

Geraldo MRF, Tessmann DJ, Kemmelmeier C (2006) Production of mycotoxins by *Fusarium graminearum* isolated from small cereals affected with scab disease in Southern Brazil. Braz J Microbiol 37:58–63

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol 5:515–525

Goswami RS, Kistler HC (2005) Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology 95:1397–1404

Klix MB, Verreet J-A, Beyer M (2007) Comparison of the declining triazole sensitivity of *Gibberella zeae* and increased sensitivity achieved by advances in triazole fungicide development. Crop Prot 26:683–690

Kuhnem PR, Stumpf R, Spolti P, Del Ponte EM (2013) Características patogênicas de isolados do complexo *Fusarium graminearum* e de *Fusarium verticillioides* em sementes e plântulas de milho. Cienc Rural 43:583–588

Lamprecht SC, Tewoldemedhin YT, Botha WJ, Calitz FJ (2011) *Fusarium graminearum* species complex associated with maize crowns and roots in the KwaZulu-Natal province of South Africa. Plant Dis 95:1153–1158

Lima MIPM, Sousa CNA, De Fernandes JMC (1998) Informações de cultivares de trigo de origem distintas quanto ao espigamento, ao florescimento e à reação a giberela. Fitopatol Bras 23:253

Lima MIPM, Fernandes JMC, Picinini EC (2000) Avaliação da resistência a giberela em trigo. Fitopatol Bras 25:30–35

Luzzardi GC, Wetzel MMVS, Pierobom CR (1972) Meio de cultura para multiplicação de *Gibberella zeae* (Schw.), Petch. *Fusarium graminearum* Schw., agente da giberela do trigo. Fitopatol Bras 5:182–183

Luzzardi GC, Pierobom CR, Osório EA, Moreira JCS, Wetzel MMVS, Dias JC (1974) Melhoramento de trigo para resistência à giberela. In: Reunião Latino Americana de Trigo. Anais Porto Alegre, pp 117–121

Martinelli JA, Bocchese CAC, Xie W, O'Donnell K, Kistler HC (2004) Soybean pod blight and root rot caused by lineages of the *Fusarium graminearum* and the production of mycotoxins. Fitopatol Bras 29:492–498

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T (2004) Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine

phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet Biol 41:600–623
Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathol 44:207–238
Picinini EC, Fernandes JMC (1998) Avaliação de fungicidas no controle de giberela em trigo. Fitopatol Bras 23:270
Pierobom CR, Prestes AM, Luzzardi GC, Flechtmann CHW, Caetano VR (1977) Ocorrência de *Fusarium tricinctum* associado com ácaro *Tyrophagus*, em trigo. In: Reunião Anual conjunta de Pesquisa de Trigo. Londrina, PR (Brazil)
Quarta A, Mita G, Haidukowski M, Logrieco A, Mulè G, Viscontiet A (2006) Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes. FEMS Microbiol Lett 259:7–13
Reis EM (1986a) Caracterização da população de *Fusarium graminearum* ocorrente no sul do Brasil. Fitopatol Bras 11:527–533
Reis EM (1986b) Metodologia para determinação de perdas causadas em trigo por *Gibberella zeae*. Fitopatol Bras 11:951–955
Reis EM (1988) Doenças do trigo III – Giberela, 2ed. Passo Fundo, RS (Brazil)
Reis EM (1990) Perithecial formation of *Gibberella zeae* on senescente stems of grasses under natural conditions. Fitopatol Bras 15:52–53
Reis EM, Blum MMC, Casa RT, Medeiros CA (1996) Grain losses caused by the infection of wheat heads by *Gibberella zeae* in southern Brazil, from 1984 to 1994. Summa Phytopathol 22:134–137
Reynoso MM, Ramirez ML, Torres AM, Chulze SN (2011) Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. Int J Food Microbiol 45:444–448
Rivadenera M (2001) Variabilidade de *Fusarium* spp. agente etiológico de *Gibberella* em trigo e identificação de fontes de resistência à fusariose da espiga em trigos sintéticos. Dissertação (mestrado) Universidade de Passo Fundo, Brazil
Sarver BAJ, Ward TJ, Gale LR, Karen B, Kistler HC, Takayuki A, Nicholson P, Carter J, O'Donnell K (2011) Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet Biol 48:1096–1107
Schmale DG, Wood-Jones AK, Cowger C, Bergstrom GC, Arellano C (2011) Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. Plant Pathol 60:909–917
Scoz LB, Del Ponte EM, Astolfi P, Reartes DS, Schmale DG, Moraes MG (2009) Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathol 58:344–351
Shen CM, Hu YC, Sun HY, Li W, Guo JH, Chen HG (2012) Geographic distribution of trichothecene chemotypes of the *Fusarium graminearum* species complex in major winter wheat production areas of China. Plant Dis 96:1172–1178
Silva AR (1966) Melhoramento das variedades de trigo destinadas à diferentes regiões do Brasil. Estudos Técnicos 33 Ministério da Agricultura 82 p
Silva CN (2011) Identificação molecular de espécies de *Fusarium* e genes preditivos de micotoxinas associadas a grãos de milho e trigo no centro sul do Brasil. Thesis Universidade Estadual de Maringá, Brazil
Spolti P, Barros NC, Gomes LB, dos Santos J, Del Ponte EM (2012a) Phenotypic and pathogenic traits of two species of the *Fusarium graminearum* complex possessing either 15-ADON or NIV genotype. Eur J Plant Pathol 133:621–629
Spolti P, de Jorge BC, Del Ponte EM (2012b) Sensitivity of *Fusarium graminearum* causing head blight of wheat in Brazil to tebuconazole and metconazole fungicides. Trop Plant Pathol 37:419–423
Spolti P, Guerra DS, Badiale-Furlong E, Del Ponte EM (2013) Single and sequential applications of metconazole alone or in mixture with pyraclostrobin to improve Fusarium head blight control and wheat yield in Brazil. Trop Plant Pathol 38:85–96

Stumpf R (2011) Prevalência, perfil toxigênico e agressividade de espécies de *Fusarium* associados aos grãos de milho do Estado do Rio Grande do Sul. Thesis Universidade Federal do Rio Grande do Sul, Brazil

Sutton JC (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:195–209

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31:21–32

Tessmann DJ, Silva CN, Gomes LB, Faria CB, Melo MP, Barbosa-Tessmann IP, Lima CS, Del Ponte (2011) EM Molecular survey of toxigenic *Fusarium* species affecting maize kernels. In: Ramirez ML, Barros GG, Chulze S (Org.) Book of abstracts of the Mycored Argentina ISM 2011 conference: strategies to reduce the impact of mycotoxins in Latin America in a global context. Universidade Nacional de Rio Cuarto, Rio Cuarto, 2011, p 198

Wang JH, Ndoye M, Zhang JB, Li HP, Liao YC (2011) Population structure and genetic diversity of the *Fusarium graminearum* species complex. Toxins 3:1020–1037

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K (2002) Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc Natl Acad Sci U S A 99:9278–9283

Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Geiser DM, Nowicki TW (2008) An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–484

Yin Y, Liu X, Li B, Ma Z (2009) Characterization of steroldemethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. Phytopathology 99:487–497

Chapter 3
Diversity of Pathogen Populations Causing Fusarium Head Blight of Wheat in Uruguay

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Abstract Members of the *Fusarium graminearum* species complex (FGSC) are the primary pathogens causing *Fusarium* head blight (FHB), *Fusarium graminearum* is the main pathogen of FHB and can produce various mycotoxins in wheat, in particular type B trichothecenes. This review presents information on the *Fusarium* species and chemotypes diversity associated to FHB in wheat in Uruguay. In surveys performed in 2001 and 2002, *Fusarium graminearum* was the most frequently isolated species (76 %), while other non FGSC species were also identified. Among species from FGSC *Fusarium graminearum sensu stricto* was the most frequently isolated (97 %), while *F. cortaderiae* and *F. austroamericanum* were also identified. The predominant chemotype was 15ADON (95 %), followed by 3ADON (3 %) and NIV (2 %). Isolates identified as *F. graminearum sensu stricto* were characterized according to the levels of DON production, the aggressiveness on different wheat cultivars, the ability to form perithecia on wheat straw. The sensitiveness to the fungicide tebuconazole of FGSC isolates was evaluated. Most isolates showed high sensitivity levels, but a few resisted higher levels of fungicide. Lower sensitivity seemed to be tied to species since *F. cortaderiae* isolates showed the highest MIC values.

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3.1 Introduction

Fusarium head blight (FHB) is a destructive disease of wheat worldwide. It is mainly caused by the *Fusarium graminearum* species complex (FGSC) which encompasses at least 14 phylogenetic species (Sarver et al. 2011). In particular, FHB represents one of the main constraints for wheat production in Uruguay where moderate to severe outbreaks have occurred once every 4 years over the past decades (Díaz de Ackerman and Pereyra 2011). During the last decade, the most severe outbreaks occurred, in 2001, 2002, and 2012. In 2001 and 2002, *Fusarium* caused extensive damage through direct losses in grain yield and quality, particularly because of the presence of mycotoxins in harvested grain (Pereyra 2003). Grain contamination with deoxynivalenol (DON) was high, so flour had to be imported to fulfill the internal demand. Since then, the quantification of DON levels in wheat flour and by-products has been mandatory (MSP 2001).

Many *Fusarium* species have been reported as causal agents of FHB, being *F. graminearum sensu lato* the main pathogen. *Fusarium graminearum* can produce various mycotoxins in wheat, however, those from the trichothecene-B group are usually found in larger quantities. In fact, three chemotypes have been recognized within the *F. graminearum* clade, according to the trichothecenes produced: NIV chemotype which includes nivalenol and 4-acetylnivalenol producers, 3ADON and 15ADON chemotypes which includes those isolates that produce deoxynivalenol and 3-acetyldeoxynivalenol or 15-acetyldeoxynivalenol, respectively (Ward et al. 2002).

Most species and also chemotypes appear to be restricted to specific geographic regions (Xu and Nicholson 2009). In Europe, the 15ADON chemotype is dominant in various countries (Talas et al. 2011; Jennings et al. 2004) but in Asia NIV chemotype is the most commonly found (Gale et al. 2007; Zhang et al. 2007). In South America, 15ADON has been reported as the dominant chemotype from wheat in Argentina and Brazil (Alvarez et al. 2009; Astolfi et al. 2012). However, NIV chemotype has also been detected, corresponding to *F. meridionale* isolates in Brazil and Argentina (Sampietro et al. 2011; Astolfi et al. 2012). Shifts in chemotypes prevalence have occurred. In North America, where for many years 15ADON was the most prevalent chemotype found in wheat (Schmale et al. 2012), a shift from 15ADON to 3ADON occurred in the last decade (Ward et al. 2008).

Toxins different from type B trichothecenes could be present in wheat grain since many *Fusarium* species other than *F. graminearum* have been found. Some of them, like *F. acuminatum* and *F. poae*, are recognized type A trichotecenes (*i.e.* T2 toxin) producers (Bottalico and Perrone 2002). Moreover, most species causing FHB are recognized as potential zearalenone producers.

Toxic effects have been demonstrated for trichothecenes and zearalenone (Sundstøl Eriksen et al. 2004). General toxicity and immunotoxicity in experimental animals, and also haematotoxicity in case of nivalenol and T2 toxin, are considered to be the critical effects of trichothecenes (Schlatter 2004). The Scientific Committee on Food of the European Union, accepted values for a tolerable daily intake (TDI) of 1 μg/kg body weight for DON, and provisional TDI of 0.7 μg/kg body weight for

nivalenol and of 0.06 µg/kg body weight for the combination of T2 and HT2 (Commission of European Communities 2006). The critical effects of zearalenone result from its oestrogenic activity. Based on recent data in the most sensitive animal species (swine) and taking into account comparisons between pigs and humans, the Panel on Contaminants in the Food Chain established a tolerable daily intake (TDI) for zearalenone of 0.25 µg/kg body weight (EFSA 2011).

Only maximum levels for DON contamination in wheat and its by-products for human consumption have been established in Uruguay at 2,000 and 1,000 µg/kg, respectively (MSP 2001, 2002) and 10,000 µg/kg for ruminants and poultry and 5,000 µg/kg for swine (MGAP 2001). Maximum levels for zearalenone contamination have been set for corn and barley at 200 µg/kg, but not for wheat (RBN 1994).

Wheat is a main cereal crop in Uruguay (Castiglioni and Navia 2010), with an average yield of 3,220 kg/ha (DIEA 2011). For many years wheat growing area in Uruguay has been restricted mainly to the western border of the country, next to Argentina. However, recently, it has expanded to eastern and northeastern regions of the country, near Brazil (Ernst 2011). Since knowledge on *Fusarium* population diversity in a region is critical to determine the risk of contamination with different toxins, the study of wheat pathogens causing FHB from those new production areas emerged as a priority.

This review presents information on the *Fusarium* species and chemotypes diversity associated to FHB in wheat in Uruguay. Studies on DON production, aggressiveness and tebuconazole sensitivity of isolates are also presented as necessary information for effective management strategies.

3.2 Species and Chemotype Diversity in Uruguay

In 2001 and 2002, grain samples of wheat from different regions of the major wheat production zone in Uruguay were examined at the National Institute for Agricultural Research (INIA) at La Estanzuela for the presence of *Fusarium* species associated with FHB. Monosporic cultures obtained from grain were phenotypically identified to species level, based on phenotypic characteristics (Pereyra and Dill-Macky 2010). *Fusarium graminearum* was the most frequently isolated species (76 %), while *F. avenaceum*, *F. culmorum, F. poae, F. acuminatum* and *F. equiseti* were also identified.

At the same time, the laboratory of the Department of Microbiology, (Facultad de Química Universidad de la Repúlica, Uruguay), analyzed wheat grain samples to isolate and identify by molecular methods, the prevalent species within the FGSC associated with FHB in Uruguay. Grain samples from diverse wheat production areas from the major wheat production zone in Uruguay (Fig. 3.1), provided by the Ministry of Livestock, Agriculture and Fisheries were used in that study.

From each monosporic culture identified macro and microscopically as *Fusarium* sp, DNA was extracted and purified according to O'Donnell et al. (1997). After that, a PCR with specific primers Fg11F/Fg11R (Nicholson et al. 1998) was performed, to

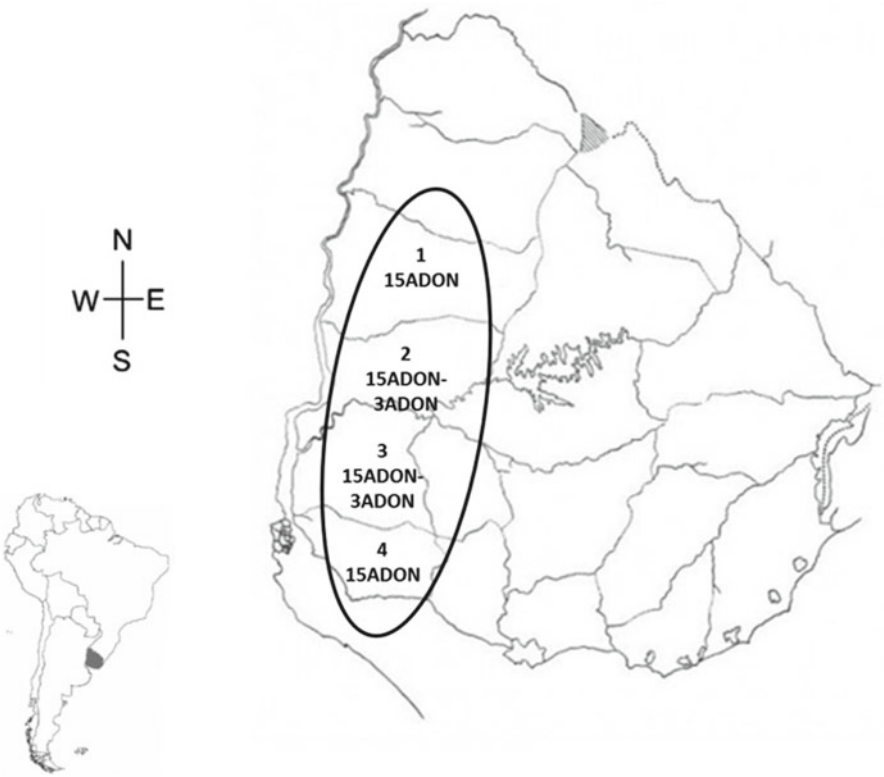

Fig. 3.1 Geographical locations of wheat production zones surveyed for *Fusarium* isolation in Uruguay, showing the chemotypes found in each zone. Numbers indicate sampling locations: (*1*) Paysandú, (*2*) Río Negro, (*3*) Soriano, (*4*) Colonia

determine if isolates belonged to the FGSC. In such conditions, an amplification product of approximately 400 bp was considered confirmatory of *F. graminearum sensu lato*. Isolates belonging to that group, were identified to species level by amplifying and sequencing the translation elongation factor 1-α (TEF) gene (Geiser et al. 2004). The obtained sequences were compared with data from *Fusarium* ID database (http://isolate.fusariumdb.org/index.php). *Fusarium graminearum sensu stricto* was the most frequently isolated species (97 %), while *F. cortaderiae* and *F. austroamericanum* were also identified (Fig. 3.2).

The chemotypes of FGSC isolates were determined using a multiplex PCR (Ward et al. 2008), based on differences in tri3 gene sequences (Fig. 3.3), which codifies for an enzyme that catalyzes acetylation of trichothecenes at carbon atom 15 (C-15) during trichothecene biosynthesis (McCormick et al. 1996). The three chemotypes were found (Fig. 3.3). The predominant chemotype was 15ADON (95 %), followed by 3ADON (3 %) and NIV (2 %) (Fig. 3.4). Most *F. graminearum sensu stricto* were 15ADON chemotype, but a minor percentage was characterized as potential 3ADON producers. *F. austroamericanum* and *F. cortaderiae* isolates were of 3ADON and NIV chemotypes respectively.

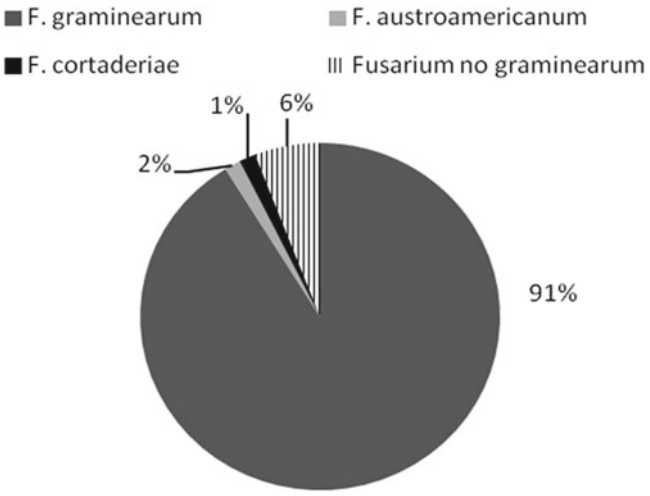

Fig. 3.2 Species distribution of *Fusarium* associated with FHB in 2001–2002 in Uruguay

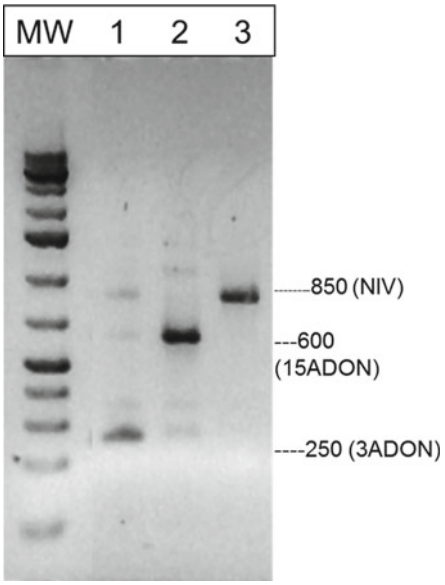

Fig. 3.3 Chemotype-specific multiplex PCR. The figure shows the band pattern in a 1 % agarose gel of the three different chemotypes. *MW* Molecular weight marker (GeneRuler™1Kb DNA ladder, Fermentas), *1*: *F. austroamericanum* 3ADON, *2*: *F. graminearum* 15ADON, *3*: *F. cortaderiae* NIV

According to the results, and to get an easier method to identify the prevalent species of the FGSC found in our country a method based on RFLP of the TEF gene was developed (Umpiérrez et al. 2011). The RFLP method was designed *in silico* comparing sequences of TEF gene for each species obtained from FusariumID database. With the aid of the software Webcutter 2.0 (http://rna.lundberg.gu.se/cutter2/), restriction profiles were obtained and enzymes that allowed differentiating the species found in Uruguay from other ones previously found in America, were selected. The method was validated experimentally confirming the expected profiles

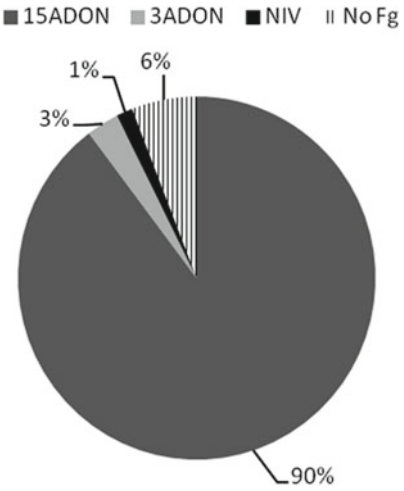

Fig. 3.4 Chemotype distribution of *Fusarium* associated with FHB in 2001–2002 in Uruguay

for different species, using type strains from USDA culture collection. Figure 3.5 shows restriction profiles obtained for different species after a digestion of PCR products with the selected enzymes: *Bsa*HI and *Tru*I. Unique profiles for *Fusarium graminearum sensu stricto* and *Fusarium cortaderiae* were obtained. This method will be useful in future surveys, since its use will allow the identification of isolates belonging to the most common species of FGSC found in Uruguay, without sequencing.

3.3 Characterization of *Fusarium graminearum* Species Complex Isolates

3.3.1 In Vitro *Deoxynivalenol Production*

Quantification of DON production was assessed for all *F. graminearum sensu stricto* isolates obtained in 2011–2002 survey, as described by Gimeno et al. (1992) with modifications. Ten grams of rice were placed in 100 mL Erlenmeyer flasks, moistened with 5 mL of distilled water and then autoclaved for 30 min at 121 °C (Garmendia 2003). After cooling, each flask was inoculated with 100 µL of a conidial suspension (10^4 conidia per mL) of a determined isolate. The cultures were incubated at 25 °C in the dark for 30 days. After incubation, culture was divided into two halves. DON was extracted from one of the halves, by agitation of cultures with 50 mL of methanol in orbital shaker at 150 rpm. After filtration DON was quantified using Veratox® DON 5/5 Quantitative DON Test (Neogen). The other half of the culture was homogenized with sterile 0.1 % aqueous and the concentration of fungal propagules in the suspension was determined by plate count in PDA. Experiments were repeated twice.

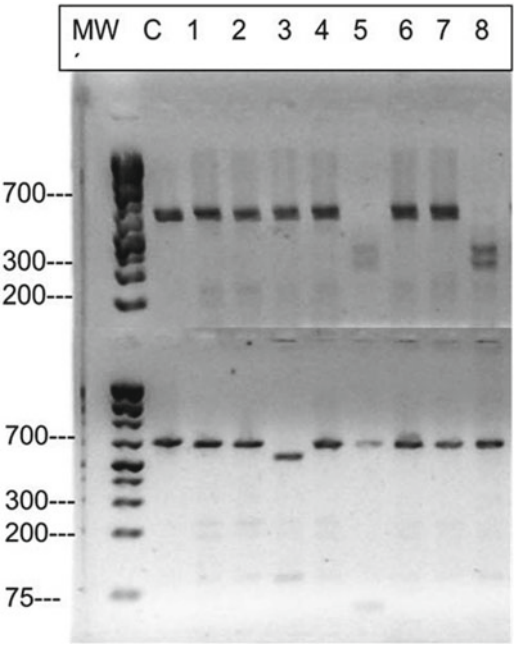

Fig. 3.5 RFLP assay for identification of species of *F. graminearum* species complex. The picture shows the band pattern of an RFLP experiment on an 1.5 % agarose-metaphor gel, after restriction. *Upper panel* shows BsaHI restriction assay (287 pb and 356 bp fragments only for *F. graminearum*). *Lower panel*: Tru1I restriction assay (26, 89, 528 pb fragments only for *F. cortaderiae*). *MW* Molecular weight marker (GeneRuler™1Kb DNA ladder, Fermentas); C PCR product tef-1 alpha no subject to restriction, 1: *F. austroamericanum,*2: *F. boothii,* 3: *F. cortaderiae,* 4: *F. brasilicum,* 5: *F.graminearum,* 6: *F. meridionale,* 7: *F. asiaticum,* 8: *F. graminearum* native isolate

Figure 3.6 shows the percentage of isolates producing different levels of deoxynivalenol. Results are expressed in μg of DON per gram of rice. Ninety percent of the isolates produced DON levels higher than 10 ppm. Propagules concentration obtained from different cultures were not significantly different, indicating that differences in DON production were not due to different levels of growth.

3.3.2 Aggressiveness

Aggressiveness of 14 *F. graminearum* isolates that produced highest levels of DON in the previous assay, was assessed by single-floret inoculation on seven wheat genotypes with a differential reaction to FHB. All isolates caused visible symptoms of FHB however; some isolates did not cause detectable disease on some resistant wheat genotypes. There were no significant isolate-cultivar interactions. The effect of the isolate on FHB severity was significant ($P = 0.0001$). The average range of FHB severities on inoculated spikes 21 days after inoculation was from 10 to 58 %.

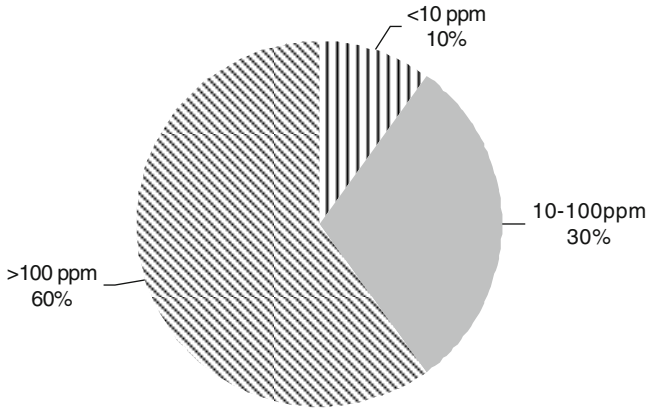

Fig. 3.6 Percentage of isolates that produced different levels of deoxynivalenol on rice grains. Results are expressed in μg of DON per gram of rice

Mean severities at 21 dpi of the different isolates on resistant wheat cultivars was low and similar, indicating that resistance is still the most effective measure of reducing the risk associated with FHB in wheat.

3.3.3 Perithecia Production on Wheat Straw

The ability to form perithecia on wheat straw was determined for the 14 isolates included in the previous assay. Sterilized wheat stem tissues (with one node per 3 cm piece) were inoculated with 100 μL of conidial suspension of the selected *F. graminearum* isolates. The inoculated tissues were placed in Petri dishes containing 3 g sterile vermiculite. The treatments were replicated three times. Plates containing residues were placed at 20–22 °C under black light (F40T12/BLB TL40W/08; Phillips, Sommerset, NJ) and cool-white light (F40/CW Worklite 25; Phillips, Sommerset, NJ) for 3 weeks to induce perithecia development (Cabrera 2009). After incubation perithecial units were counted under optical microscopy (×50). Figure 3.7 shows the number of perithecia formed by each isolate on wheat straw. Mean of three repetitions are shown. Significant differences among isolates were observed, according to ANOVA and Tukey test.

3.3.4 Quantitative Assessment of Fungicide Sensitivity

Minimal inhibitory concentration (MIC) of tebuconazole was determined for each isolate using agar dilution plate method according to Cabañas et al. (2009) with

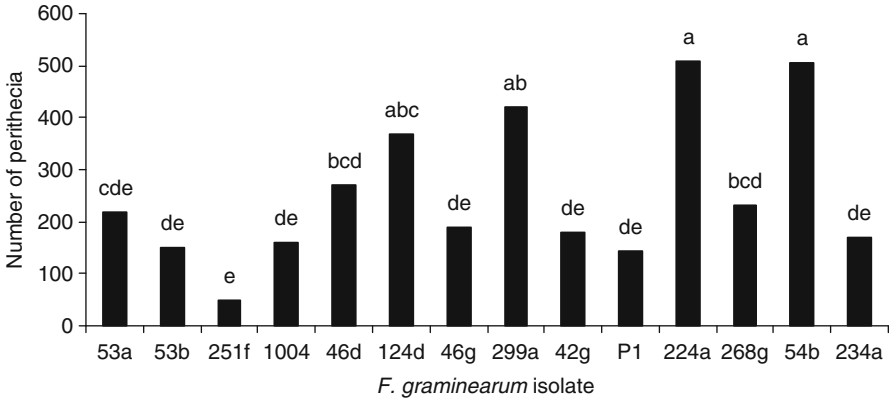

Fig. 3.7 Number of perithecia formed by each isolate on wheat straw. Mean of three repetitions are shown. Significant differences among isolates were observed, according to ANOVA and Tukey test. Values of the bars with the same letter are not significantly different according to the Tukey test ($P < 0.05$)

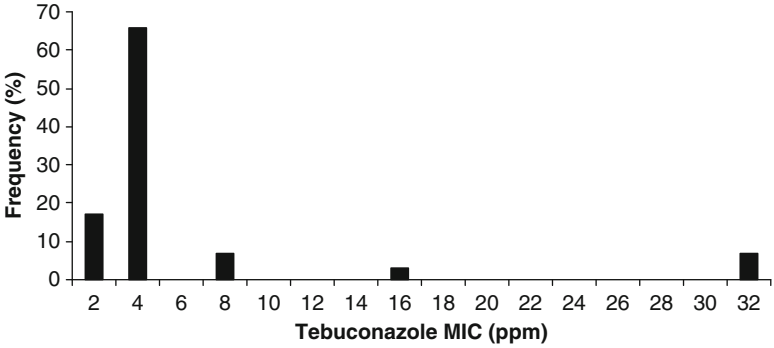

Fig. 3.8 Percentage of isolates which exhibited different MIC of tebuconazole

modifications. Conidia of 4–5 day-old PDA slant cultures were suspended in 5 mL of sterile saline containing 0.1 % Tween 80, agitated 5–10s and filtered through double layered cheesecloth to remove hyphal fragments. The suspension was adjusted to 10^4 conidia per mL and 10 µL were inoculated onto PDA plates amended with different fungicide concentrations. After 72 h of incubation at 25ºC in darkness, fungal growth was determined visually. MIC was defined as the lowest concentration showing 100 % growth inhibition. Two repetitions per treatment were performed. Fungicide concentrations assayed were 0, 2, 4, 8, 16, 32 and 64 ppm.

Figure 3.8 shows the percentage of isolates which exhibited different MIC of tebuconazole. Most isolates showed high sensitivity levels, but a few resisted higher levels of fungicide. Lower sensitivity seemed to be tied to species since *F. cortaderiae* isolates showed the highest MIC values.

3.4 Discussion

The main species associated with FHB in wheat in Uruguay is *F. graminearum* as suggested by previous studies (Boasso 1961; Pereyra and Dill-Macky 2010; Umpiérrez et al. 2011). Similar results were reported for North America (McMullen et al. 1997; Salas et al. 1999; Clear and Patrick 2000), some regions of Europe (Parry et al. 1995) and other countries in South America (Reis 1988; Lori et al. 2003). In fact, *Fusarium graminearum* is widespread in the southern cone of South America and has been isolated from a wide range of hosts, not only wheat (Reis 1988; Pereyra and Dill-Macky 2008).

Our studies showed that diversity within *Fusarium graminearum* species complex (FGSC) was not very high in wheat grains from the main production region in Uruguay. *Fusarium graminearum sensu stricto* was the predominant species, but a few isolates of *F. cortaderiae* and *F. austroamericanum* were also found. Those results were not surprising since *F. graminearum sensu stricto* is distributed panglobally and represents the most important species documented as causal agents of FHB throughout the world (Sarver et al. 2011). The presence of *F. austroamericanum* and *F. cortaderiae*, has also already been documented in South America, in fact, both species together with *F. meridionale*, and *F. brasilicum* appear to be endemic to South America (Wang et al. 2011). However, those species has not been reported in Uruguay before.

According to Sarver et al. (2011) species within FGSC are biogeographically structured. The underlying factors for geographical species distribution are still unknown but climate seems to be of great importance. Different species have been isolated from different regions in the same country. In China and Japan, *F. graminearum sensu stricto* was mainly obtained from cool regions (annual average temperature of 15 °C or lower) whereas *F. asiaticum* isolates were collected from warmer zones at the south of the country (Zhang et al. 2007; Suga et al. 2008). Until now, in Uruguay, studies on *Fusarium* diversity on wheat have been restricted to the main and traditional wheat production region, located in the west of the country, next to Argentina. Since new production areas have appeared in the eastern part of the country, next to Brazil, studies on *Fusarium* species associated with FHB in those zones are mandatory. To perform such studies, the identification method based on RFLP of tef1α gene, described in this work, will be very useful, since its use will save time and reduce costs during next surveys. With that method, unique restriction profiles for *F. graminearum sensu stricto* and *F. cortaderiae* are obtained. Since those are the most frequent species of the FGSC found as causative agents of FHB in Uruguay, its use will reduce the number of sequencing reactions needed to characterize *F. graminearum* population.

Trichothecene chemotype diversity among Uruguay an isolates seems tied to species differences as reported by Sampietro et al. (2012) for FGSC isolates from maize in Argentina. In our study, *F. cortaderiae* isolates were of the NIV chemotype, while the *F. austroamericanum* isolate was a 3ADON chemotype. Almost all *F. graminearum sensu stricto* isolates were of the 15ADON chemotype, although a

few 3ADON producers were also detected. These results are consistent with those reported by Boutigny et al. (2011) in South Africa and Monds et al. (2005) in New Zealand who found *F. cortaderiae* with NIV chemotype. Moreover, chemotype polymorphism in *F. graminearum sensu stricto* has already been reported (Shen et al. 2012; Suga et al. 2008; Zhang et al. 2011).

As discussed for species, *Fusarium* chemotypes appear to be restricted to specific geographic regions, even into the same country (Xu and Nicholson 2009). Differences in the geographic distribution of FGSC chemotypes were reported in China, Japan, Korea and also in USA (Wang et al. 2011). Gale et al. (2011) reported that most isolates from the southern Louisiana population were of the NIV type, while the majority of the isolates from 11 other U.S. states from the Midwest were of the 15ADON type. Those data emphasizes the need to study *Fusarium* chemotype diversity in the different wheat production zones in our country, to determine if trichotecenes other than DON should be analyzed in Uruguayan wheat. Such surveys have not been currently conducted in Uruguay.

The diversity of the *F. graminearum sensu stricto* population was assessed. Differences in DON production, aggressiveness and perithecia formation on wheat straw were verified. Variation in DON production among *F. graminearum* isolates has been already reported by Gilbert et al. (2002). Most Uruguay an isolates produced large quantities of DON and it was remarkable that those with a 3ADON chemotype were among the highest producers. Recently von der Ohe et al. (2010) in accordance with Ward et al. (2008) demonstrated that average DON production by the 3ADON isolates was significantly higher than for the 15ADON isolates, which indicates that 3ADON isolates could pose a greater risk to food safety.

Different levels of sensitivity to tebuconazole were detected. The lowest levels of sensitivity corresponded to *F. cortaderiae*. For most isolates, MIC of tebuconazole was 4 ppm. Although resistance problems have not been detected in the field, some isolates showed lower *in vitro* sensitivity to the fungicide. Those results justify the need for regular surveys to follow the evolution of the sensitivity of *F. graminearum* population in order to develop appropriate management strategies.

References

Alvarez CL, Azcarate MP, Pinto VF (2009) Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. Int J Food Microbiol 135:131–135

Astolfi P, Reynoso MM, Ramírez ML, Chulze SN, Alves TC, Tessmann DJ, Del Ponte EM (2012) Genetic population structure and trichothecene genotypes of *Fusarium graminearum* isolated from wheat in southern Brazil. Plant Pathol 61:289–295

Boasso C (1961) Estado fitosanitario de los cultivos de trigo de la reciente cosecha. Boletín Informativo 854:7

Bottalico A, Perrone G (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol 108:611–624

Boutigny A, Ward TJ, Van Coller GJ, Flett B, Lamprecht S, O'Donnell K, Viljoen A (2011) Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in

South Africa provides evidence of species-specific differences in host preference. Fungal Genet Biol 48:914–920
Cabañas R, Abarca ML, Bragulat MR, Cabañes FJ (2009) *In vitro* activity of imazalil against *Penicillium expansum*: comparison of the CLSI M38-A broth microdilution method with traditional techniques. Int J Food Microbiol 129:26–29
Cabrera M (2009) Control biológico de fusariosis de trigo. MSc thesis, Facultad de Ciencias Universidad de la República Uruguay
Castiglioni E, Navia D (2010) Presence of the Wheat Curl Mite, *Aceria tosichella* Keifer (Prostigmata: Eriophyidae) in Uruguay. Agrociencia Uruguay 14:19–26
Clear RM, Patrick SK (2000) *Fusarium* head blight pathogens from *Fusarium*-damaged kernels of wheat in western Canada, 1993 to 1998. Can J Plant Pathol 22:51–60
Commission of European Communities (2006) Commission regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union Legis 364:5–23
Díaz de Ackermann M, Pereyra S (2011) Fusariosis de la espiga de trigo y cebada. In: Pereyra S, Díaz de Ackermann M, Germán S, Cabrera K (eds) Manejo de enfermedades en trigo y cebada. Hemisferio Sur SRL, Montevideo
DIEA (2011) Anuario Estadístico Agropecuario. Ministerio de Ganadería, Agricultura y Pesca, Uruguay
EFSA (2011) Scientific Opinion on the risks for public health related to the presence of zearalenone in food. EFSA J 9:2197, http://www.efsa.europa.eu/en/efsajournal/doc/2197.pdf. Accessed 24 Nov 2012
Ernst O (2011) Cambios en la agricultura, situación actual y demandas de investigación. In: Castro A, Hoffman E, Viega L (eds) Limitaciones para la productividad de trigo y cebada. CYTED Departamento de Publicaciones de la Facultad de Agronomía, Montevideo
Gale LR, Ward TJ, Balmas V, Kistler HC (2007) Population subdivision of *Fusarium graminearum sensu stricto* in the upper midwestern United States. Phytopathology 97:1434–1439
Gale LR, Harrison SA, Ward TJ, O'Donnell K, Milus EA, Gale SW, Kistler HC (2011) Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana. Phytopathology 101:124–134
Garmendia G (2003) Aislamiento, identificaicón y caracterización de cepas nativas de *Fusarium* productoras de Deoxynivalenol. Tesina de grado Facultad de Química Universidad de la República Uruguay
Geiser DM, Jimenez-Gasco MDM, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K (2004) FUSARIM-ID 1.0: a DNA sequence database for identifying *Fusarium*. Eur J Plant Pathol 110:473–479
Gilbert J, Abramson D, Mc Callum B, Clear R (2002) Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. Mycopathologia 153:209–215
Gimeno A, Ramos AJ, Hernández E (1992) Estudio de la Producción de Deoxinivalenol (DON) por *Fusarium graminearum*. Rev Iberoam Micol 9:55–57
Jennings P, Coates ME, Walsh K, Turner JA, Nicholson P (2004) Determination of deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathol 53:643–652
Lori GA, Sistema MN, Haidukowski M, Rizzo I (2003) *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area in Argentina. Microbiol Res 158:29–35
McCormick SP, Hohn TM, Desjardins AE (1996) Isolation and characterization of Tri3, a gene encoding 15-O-acetyltransferase from *Fusarium sporotrichioides*. Appl Environ Microbiol 62:353–359
McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348
MGAP (2001) Resolución ministerial sobre límites máximos de DON (toxina de *Fusarium*) en alimentos para animales. http://www.mgap.gub.uy/dgssaa/Normativa/Archivos/ALIM_ANIM/Fusarium.pdf. Accessed 30 Nov 2012

Monds RD, Cromey MG, Lauren DR, di Menna M, Marshall J (2005) *Fusarium graminearum, F. cortaderiae* and *F. pseudograminearum* in New Zealand: molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. Mycol Res 109:410–420

MSP (2001) Decreto Ministerio de Salud Pública República Oriental del Uruguay. Decreto 533/01

MSP (2002) Decreto Ministerio de Salud Pública República Oriental del Uruguay. Decreto 470/02

Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW, Joyce D (1998) Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol Mol Plant Pathol 53:17–37

O'Donnell K, Cigelnik E, Weber NS, Trappe JM (1997) Phylogenetic relationships among ascomycetons truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89:48–65

Parry DW, Jenkinson P, McLeod L (1995) *Fusarium* ear blight (scab) in small grain cereals. A review. Plant Pathol 44:207–238

Pereyra S (2003) Prácticas culturales para el manejo de la fusariosis de la espiga. In Serie Actividades de Difusión Nº 312, Jornada Técnica de Cultivos de Invierno. INIA Uruguay

Pereyra SA, Dill-Macky R (2008) Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to *Fusarium* head blight inoculum. Plant Dis 92(5):800–807

Pereyra S, Dill-Macky R (2010) *Fusarium* species recovered from wheat and barley grains in Uruguay, pathogenicity and deoxynivalenol content. Agrociencia Uruguay 14(2):33–44

RBN (1994) Reglamento Bromatológico Nacional. Decreto Nº 315/994. IMPO Uruguay

Reis EM (1988) Doenças do trigo III. Giberela. Segunda ediçao. Du Pont do Brasil/Iharabras/Industrias Químicas MSD AGUET/Merck Sharp & Dohme – Química e Farmaceutica, Sao Paulo

Salas B, Steffenson BJ, Casper HH, Tacke B, Prom LK, Fetch TG, Schwarz PB (1999) *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis 83:667–674

Sampietro DA, Díaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CA, Ward TJ (2011) Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. Int J Food Microbiol 145:359–364

Sampietro DA, Aristimuño Ficoseco ME, Jimenez C, Vattuone MA, Catalán CA (2012) Trichothecene genotypes and chemotypes in *Fusarium graminearum* complex strains isolated from maize fields of northwest Argentina. Int J Food Microbiol 153:229–233

Sarver BA, Ward TJ, Gale LR, Broz K, Corby Kistler H, Aoki T, Nicholson P, O'Donnell K (2011) Novel *Fusarium* head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet Biol 48(12):1096–1107

Schlatter J (2004) Toxicity data relevant for hazard characterization. Toxicol Lett 153:83–89

Schmale DG, Ross SD, Fetters TL, Tallapragada P, Wood-Jones AK, Dingus B (2012) Isolates of *Fusarium graminearum* collected 40–320 meters above ground level cause *Fusarium* head blight in wheat and produce trichothecene mycotoxins. Aerobiologia 28:1–11

Shen C, Hu Y, Sun HY, Li W, Guo J, Chen H (2012) Geographic distribution of trichothecene chemotypes of the *Fusarium graminearum* species complex in major winter wheat production areas of China. Plant Dis 96(8):1172–1178

Suga H, Karugia GW, Ward T, Gale LR, Tomimura T, Nakajima T, Miyasaka A, Koizumi S, Kageyama K, Hyakumachi M (2008) Molecular characterization of the *Fusarium graminearum* species complex in Japan. Phytopathology 98:159–166

Sundstol Eriksen G, Pettersson H, Lundh T (2004) Comparative cytotoxicity of deoxynivalenol, nivalenol, their acetylated derivatives and de-epoxy metabolites. Food Chem Toxicol 42(4):619–624

Talas F, Parzies HK, Miedaner T (2011) Diversity in genetic structure and chemotype composition of *Fusarium graminearum sensu stricto* populations causing wheat head blight in individual fields in Germany. Eur J Plant Pathol 131(1):39–48

Umpiérrez M, Garmendia G, Pereyra S, Rodríguez A, Vero S (2011) Las técnicas moleculares en el estudio de los patógenos: ejemplos en patógenos de trigo. In: Pereyra S, Díaz de Ackermann M, Germán S, Cabrera K (eds) Manejo de enfermedades en trigo y cebada. Hemisferio Sur SRL, Montevideo

von der Ohe C, Gauthier V, Tamburic-Ilincic L, Brule-Babel AWG, Dilantha Fernando Clear R, Ward TJ, Miedaner T (2010) A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur J Plant Pathol 127:407–417

Wang J, Ndoye M, Zhang J, Li H, Liao Y (2011) Population structure and genetic diversity of the *Fusarium graminearum* species complex. Toxins 3:1020–1037

Ward TJ, Bielawski JP, Corby Kistler H, Sullivan E, O'Donnell K (2002) Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc Natl Acad Sci U S A 99(14):9278–9283

Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Nowicki TW (2008) An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–484

Xu XM, Nicholson P (2009) Community ecology of fungal pathogens causing wheat head blight. Annu Rev Phytopathol 47:83–103

Zhang JB, Li HP, Dang FJ, Qu B, Xu YB, Zhao CS, Liao YC (2007) Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycol Res 111:967–975

Zhang L, Luo P, Ren Z, Zhang H (2011) Controlling *Fusarium* head blight of wheat (*Triticum aestivum* L.) with genetics. Adv Biosci Biotechnol 2:263–270

Chapter 4
Ecophysiology of *Fusarium graminearum* Main Pathogen Associated to Fusarium Head Blight in Latin America

María Laura Ramírez, María Cecilia Farnochi, and Sofía Noemí Chulze

Abstract In Argentina, the main pathogen associated with Fusarium Head Blight (FHB) is *Fusarium graminearum* Schw, perfect stage *Gibberella zeae* (Schw.) Petch. Prevention of mycotoxin contamination of food raw materials is now considered more important than subsequent cure. Accurate information is therefore needed on the impact of key environmental factors such as water availability and temperature and their interactions, and the identification of marginal and optimum conditions for growth and toxin production. Studies done in Argentina were focused on the impact of different abiotic factors (a_W, temperature and fungicides) on *F. graminearum* growth rates and deoxynivalenol production. The impact of 5 fungicides (prochloraz, propioconazole, epoxiconazole, tebuconazole and azoxystrobin, 0.5–50 mg/ ml) on growth of *F. graminearum* isolated from wheat in Argentina were evaluated in relation to water activity (a_W; 0.99, 0.97, 0.95) and temperature (15 and 25 °C) on wheat-based media (*in vitro*) and on wheat grains (*in situ*). Also the effect of osmotic (NaCl, glycerol) and matric (PEG8000) water stress on temporal germination and growth by *F. graminearum* strains isolated from wheat in Argentina over the water potential range of −0.7 to −14.0 MPa at 15 and 25 °C. The effect on endogenous water potentials and accumulation of sugars and sugar alcohols were also evaluated. The results showed that water potential and solute type have a

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significant effect on germination, germ tube extension, growth rates, germination, internal cell water potential and endogenous accumulation of sugars and sugar alcohols in *F. graminearum.*

4.1 Introduction

In Argentina, the main pathogen associated with *Fusarium* Head Blight (FHB) is *Fusarium graminearum* Schw, whose perfect stage is *Gibberella zeae* (Schw.) Petch (Ramirez et al. 2006b, 2007) and has been known in the country since 1928. Several epidemics of varying severity occurred in the central-north area. In one of the most serious outbreaks (1993) the highest estimated losses reached 50 % in areas of no-till over maize stubble. The extent of the damage was magnified by a considerable loss in trading value of the grain resulting from low grain weight, presence of scabby grains, and mycotoxin contamination (Galich 1996). During this severe epidemic, deoxynivalenol (DON) was the only toxin reported (Dalcero et al. 1997). Deoxynivalenol, a member of the trichothecene group of mycotoxins, is primarily produced by the genus *Fusarium* (Desjardins 2006). The occurrence of DON in cereal grains is of great concern for human health, since this toxin results in food refusal, vomiting and depressed immune functions (WHO 2001).

Fungal growth and mycotoxin production results from the complex interaction of several factors and, therefore, an understanding of each factor involved is essential to understand the overall process and to predict and prevent mycotoxin development (Chamley et al. 1994). Environmental conditions have a major impact on the fungal growth and play a critical role in the mycotoxicosis epidemiology. In addition, mycotoxin production is genetically regulated in response to environmental conditions (Holliger and Ekperigin 1999). Temperature and water availability are the primary environmental factors that influence growth and mycotoxin production by several *Fusarium* species (Magan and Lacey 1984; Marín et al. 1995a, b, 1996; Hope and Magan 2003). It has been demonstrated that, within the same species under the same culture conditions, toxin production by *Fusarium* strains may vary markedly; some strains produce higher amounts of trichothecenes, whereas others produce small or undetectable amounts (Bakan et al. 2001). It is thus necessary to carry out studies to determine whether the geographical origin of the strain can determine the optimal and marginal a_W and temperature ranges for growth and mycotoxin production. This could help to explain the variability in the results reported in the literature concerning optimal environmental conditions for DON production (Llorens et al. 2004).

Prevention of mycotoxin contamination of food raw materials is now considered more important than subsequent cure. Thus Hazard Analysis Critical Control Point (HACCP) approaches are being developed to examine the critical controls points at which mycotoxicogenic moulds and mycotoxins may enter to a range of food chains (Aldred and Magan 2004). Accurate information is therefore needed on the impact of key environmental factors such as water availability and temperature and their

interactions, and identify marginal and optimum conditions for growth and toxin production (Sanchis and Magan 2004).

The effects of environmental biotic and fungicides on germination, growth and DON production by *F. graminearum* are review. An understanding of the factors involved in deoxynivalenol production is the first step preventing the toxin accumulation.

4.2 Environmental Abiotic Factors Affecting *Fusarium graminearum* Growth and Deoxynivalenol Production

Abiotic factors are nonliving variables within the ecosystem that affect the lives organisms. The abiotic factors that have the most important impact on the activity of *Fusarium* spp. are water activity (a_W) and temperature (Sanchis and Magan 2004). Since *F. graminearum* may be present on a substrate for a long periods during which a_W may change, it is important to know both the optimal a_W range for growth and that permitting sub- optimal growth.

Studies done in Argentina were focused on the impact of different abiotic factors (a_W, temperature and fungicides) on *F. graminearum* growth rates and deoxynivalenol production. Ramirez et al. (2006a) have compared the impact of a_W X temperature regimes on growth and DON production by two strains of *F. graminearum* isolates from wheat ears in Argentina on irradiated wheat grain. Taking into account that nutrient source may also influence the minimum a_W for growth (Griffin 1972) and, consequently, studies on artificial substrates may not accurately reflect capabilities for growth on natural substrates this study was carried out on gamma irradiated wheat grain, which had retained germinative capacity without the natural mycobiota being present to gain information specifically on the relationship between colonisation patterns and DON production (Lacey and Magan 1991). Generally, growth was reduced by up 70 % at $\leq$0.93 a_W. Optimal a_W levels for growth were in the range 0.995–0.95 with an optimal temperature of 25 °C. *F. graminearum* strains were able to growth slowly at the minimum aw level (0.90 a_W) at 15–25 °C.

Previous studies done by Cook and Christen (1976) and Wearing and Burgess (1979) on *F. graminearum* from root and stalk rot of cereals have shown optimum water content and temperature for growth were −0.1 to −2.8 MPa (0.995–0.98 a_W) at 20–30 °C changing to −2.8 to −5.5 MPa (0.95–0.96 a_W) at 35 °C. However, these isolates only grew if the water potential was >−8.3 to −11.3 MPa (equivalent to 0.94–0.92 a_W at 25 °C) although incubation times were short.

Studies by Hope et al. (2005) suggest that growth of UK strains of *F. graminearum* do not grow below 0.90 a_w although germination may occur at about 0.87–0.88 a_w. Also, Brennan et al. (2003) showed that the optimum temperature for *in vitro* growth of *F. graminearum* was 25 °C, although this studied was carried out in media with freely available water only (=0.995 a_w) which makes direct comparisons difficult.

Ramirez et al. (2006a) have also demonstrated that maximum amounts of DON were produced at the highest a_W treatment (0.995) after 6 weeks (42 days)

incubation at 30 °C. These conditions were not optimal for growth of the two isolates. The range of DON concentration varied considerably depending on a_W treatment and temperature interactions. Overall, production of DON occurred over a narrower a_W range (0.995–0.95) than those for growth.

Previous studies with *F. graminearum* have also demonstrated the effect of either temperature or a_W on DON production but the interactions between these two parameters were not investigated (Vesonder et al. 1982; Greenhalgh et al. 1983; Comerio et al. 1999; Ryu and Bullerman 1999; Martins and Martins 2002; Llorens et al. 2004). Also, most of the work, except Comerio et al. (1999), in the literature has been done on strains of *F. graminearum* isolated from corn and using corn or rice as substrate for DON production. Comerio et al. (1999) studied the influence of water activity (0.98, 0.945, 0.925 and 0.90) on DON accumulation at 25 ° on irradiated wheat grain by one strain of *F. graminearum* isolate from wheat in Argentina. They obtained the maximum amounts of DON at 0.98 a_W.

The knowledge of interacting environmental conditions provides very useful information for predicting the possible risk factor for DON contamination of wheat. The a_W and temperature range evaluated by Ramirez et al. (2006a) simulated those occurring in ripening grain (Magan and Lacey 1985) and harvested grain in a wet year (19–30 %; 0.90–0.995 a_W). Also the authors were able to demonstrate the contrasting impact of a_W, temperature and incubation time on growth and DON production by the *F. graminearum* strains examined. The most important findings were that the optimum temperature conditions for DON production are generally very different from those for growth. It has been proven that, among the tested values, the most suitable temperature for DON production was 30 °C. Knowledge of DON production under marginal or sub-optimal temperature and a_W conditions for growth can be important since improper storage accompanied by elevated temperature and moisture content in the grain can favour further mycotoxin production and lead to reduction in grain quality. The contour maps reported by Ramirez et al. (2006a) for growth and DON production may provide very useful guidelines for facilitating effective management of predicting risk for growth and DON production during ripening, harvesting and storage of wheat.

4.3 Environmental Abiotic Factors and Fungicides on Growth and Deoxynivalenol Production by *Fusarium graminearum*

The combination of the epidemiological characteristics of the FHB pathogen, scarce genetic resistance in the host and strong environmental influence on FHB development, make it necessary to adopt several integrated measures to decrease disease damage. The use of fungicides is a complementary control measure when weather conditions are conducive to infection from anthesis to harvest (Paul et al. 2008). Although there are a number of compounds with *in vitro* activity against FHB pathogens, control of this disease in the field has often been difficult (Milus and

Parson 1994). In both laboratory studies with pure cultures of phytopathogens and field trials with crop plants, the overall evidence concerning the effectiveness of fungicide is contradictory. There is also growing concern that sub-lethal doses of some fungicides may lead to an increase in mycotoxin production by *Fusarium* species (Milus and Parson 1994; D'Mello et al. 1998).

The impact of five fungicides (prochloraz, propioconazole, epoxiconazole, tebuconazole and azoxystrobin, 0.5–50 mgg^{1}) on growth of *F. graminearum* strains isolated from wheat in Argentina were evaluated in relation to water activity (a_W; 0.99, 0.97, 0.95) and temperature (15 and 25 °C) on wheat-based media (*in vitro*). All fungicides reduce growth rates when compared to the control, and this reduction increased as the fungicide concentration increased. In general, none of the isolates was able to grow in the presence of any fungicide treatments at concentrations >15 mg/ml, regardless of the a_W/temperature regime. The same fungicides were used in the second study on wheat grain (*in situ*), in order to evaluate the effect of two concentration (0.5, 5 mg/ml), three a_W levels (0.995, 0.99 and 0.97) and two temperatures (15 and 25 °C) and their interaction on growth rate and DON production by *F. graminearum*. All fungicides showed inhibition of growth at both concentrations in most conditions. The fungicides tested were less effective on grain in controlling growth than in *in vitro* studies. All fungicides showed DON stimulation or reduction in at least one of the conditions assayed. The results showed that stimulation or reduction in DON production in the presence of fungicides was influenced by complex interactions between a_W; temperature, fungicide concentration and time of incubation of the strains of *F. graminearum* studied. Such information is critical for effective fungicide control of Fusarium head blight of wheat (Ramirez et al. 2004a). Also the authors remark the differences between the behaviour of fungicides under *in vitro* (wheat based media) or on *in situ* (irradiated wheat grains). The use of irradiated wheat seeds that have retained viability may be a more useful system for fungicide screening.

In vitro studies with *F. graminearum*, *F. sporotrichoides* and *F. culmorum* have shown an increased production of toxin after exposure to sub-lethal concentrations of fungicides (Matthies et al. 1999; Moss and Frank 1985; Placinta et al. 1996; D'Mello et al. 1997). Unfortunately, all these studies were carried out only in media (solid and liquid) with freely available water (0.995 a_W), which makes direct comparison with studies done by Ramirez et al. (2006a) more difficult. Also, most of the studies done took no account of the interactions between the efficacy of the fungicides examined and a_W or temperature, which have been demonstrated to be key parameters determining germination, growth and mycotoxin production by *F. graminearum*.

There is only one comparable study on the effect of environmental factors and fungicides on growth of *F. culmorum* and DON production (Hope et al. 2002). The authors reported that DON production by isolates of *F. culmorum* from different parts of Europe was significantly increased at reduced a_W in the presence of epoxiconazole and propioconazole.

The mechanisms by which the fungicides stimulate toxin production in *Fusarium* spp. are not completely known. It may be assumed that in the presence of sub-lethal concentrations of certain fungicides the fungal strains respond to this

stress by increased production of secondary metabolites including mycotoxins, as a possible mechanism. Recently Audenaert et al. (2010) have hypothesized that hydrogen peroxide (H_2O_2) can induce DON accumulation biosynthesis but also they suggest that DON accumulation induced by sub-lethal triazole application is mediated through an increased production or release of H_2O_2 into the medium rendering a physiological interface of H_2O_2 influencing DON production. The authors also speculate that during the inhibition of ergosterol biosynthesis by the application of triazole fungicides, an increased cell permeability release H_2O_2 in the medium which turns activates the trichothecene biosynthesis machinery.

4.4 Comparison Between Osmotic and Matric Water Stress on Germination, Growth of *Fusarium graminearum*

Fusarium graminearum persists and multiplies on infected crop residues of small grains and maize. The chaff, light-weight kernels and other infected head debris of wheat and barley are returned to the soil surface during harvest and serve as important sites for overwintering of the fungus. Continued moist weather during the crop-growing season favours development of the fungus, and spores are windblown or water-splashed onto heads of cereal crops. Wheat and barley are susceptible to head infection from anthesis to the soft-dough stage of kernel development. Spores of the causal fungus may land on the exposed anthers of the flower and grow into kernels, glumes or other head parts (McMullen et al. 1997). Parry et al. (1994) considered that soil and crop debris infected with *F. graminearum* serve as the main reservoir of inoculum leading to wheat ear infection. In most cases, inoculum may take the form of conidia, chlamydospores, hyphal fragments or ascospores (Parry et al. 1995).

Studies have been done to investigate the influence of water potential on spore germination, growth and sporulation of *F. graminearum*, but much of the work has been done using osmotically controlled systems with salts, sugars or glycerol (Cook and Christen 1976; Sung and Cook 1981). *Fusarium* species survive on crop residues. In soil and cereal crop residues, matric potential is the major component of the total water potential (Magan and Lynch 1986). Indeed, Griffin (1981) suggested that matric potential would affect growth of soil fungi more than osmotic potential. Willcock and Magan (2001) showed that fungal colonization of crop residues, including that by *F. culmorum*, is rapid over a range of moisture and temperature regimes.

To overcome water stress, most fungi produce compatible solutes. These have been defined by Jennings and Burke (1990) as compounds that are able to change in concentration in the cell in response to a change in external water potential, thus maintaining turgidity while having no significant effect on enzyme activity. The compatible solutes are accumulated within the mycelium and then translocated to the conidia during conidiation. In the higher fungi, low molecular weight sugar alcohols (glycerol and erythritol) are accumulated at the expense of high molecular weight mannitol under water stress (Jennings 1995). This selective accumulation

probably decreases the internal osmotic potential in conidia because glycerol and erythritol molecules are smaller and more polar than mannitol (Hallsworth and Magan 1994; Jennings 1995).

Ramirez et al. (2004b) showed the effect of osmotic (NaCl, glycerol) and matric (PEG8000) water stress on temporal germination and growth of *F. graminearum* strains isolated from wheat in Argentina over the water potential range of −0.7 to −14.0 MPa at 15 and 25 °C. The effect on endogenous water potentials and accumulation of sugars and sugar alcohols also were measured. Germination occurred rapidly over the same range of osmotic or matric potential of −0.7 to −5.6 MPa after 4–6 h incubation. At lower osmotic and matric potentials (−7.0 to −8.4 MPa), there was a lag of up to 24 h before germination. Optimum germ-tube extension occurred between −0.7 and −1.4 MPa for both strains but varied with the solute used. Growth was optimal at −1.4 MPa and 25 °C in response to matric stress, with the minimum being about −8.0 and −11.2 MPa at 15 and 25 °C, respectively. In contrast, *F. graminearum* grew fastest at −0.7 MPa and was more tolerant of solute stress modified with either glycerol or NaCl with a minimum of about −14.0 MPa at 15 and 25 °C. A decrease in the osmotic/matric water potential of the media caused a large decrease in the mycelia water potential (Ψc) as measured by thermocouple psychrometry. In general, the concentration of total sugar alcohols in mycelia increased as osmotic and matric potential were reduced to −1.2 MPa. However, this increase was more evident in mycelia from glycerol-amended media. The quality of the major sugar alcohol accumulated depended on the solute used to generate the water stress. The major compounds accumulated were glycerol and arabitol on osmotically modified media and arabitol on matrically modified media. In response to matric stress, the concentration of trehalose in colonies generally was higher in the case of osmotic stress. In each water stress treatment, there was a good correlation between Ψc and total sugar alcohol content.

The above results demonstrated that water potential and solute type have a significant effect on germination, germ tune extension, growth rates, germination, internal cell water potential and endogenous accumulation of sugars and sugar alcohols of *F. graminearum*. The results suggest that matric stress inhibits germination more than osmotic stress. Studies on germination and tube extension of a range of soil fungi found that most were more sensitive to changes in matric than osmotic potential, with the exception of *Gliocladium roseum* and *G. virens* (Magan 1988).

Sung and Cook (1981) also found that many kinds of living plant tissues in dryland wheat, the water potential ranged between −1.0 and −5.0 MPa and that condition will be in the ideal range for sporulation, spore germination, perithecial production and hyphal extension of *F. graminearum* according to Ramirez et al. (2004b). However, in soil and cereal crop residues, matric potential is the major component of the total water potential (Magan and Lynch 1986).

The total cell water potential (Ψc) is the sum of the solute potential (Ψp) and the turgor pressure (Ψp) of the cell wall. When cells are exposed to water stress, low molecular mass compounds often are synthesized or accumulated intracellularly to equilibrate the cytoplasm Ψc with that of the surrounding environment. The work done by Ramirez et al. (2004b) was the first detailed study that have measure the Ψc

levels of *F. graminearum* mycelia as effected by osmotic and matric water stress. The data showed that mycelial Ψc decreased with decreasing medium water potential, regardless whether osmotic or matric stress was imposed. The decrease in total cellular water potential is necessary for the extraction of water from the substrate and its translocation to the growing mycelial front. This can be done effectively only by maintaining a water potential gradient from the substrate into the hyphal cells, which also facilitates the functioning of enzyme systems (Jennings 1995).

Ramirez et al. (2004b) showed that the patterns of accumulation of sugar alcohols and sugars by *F. graminearum* were modified significantly by osmotic and matric water stress. Also different responses were obtained according to the solute used to modify the osmotic potential of the media. When glycerol was used to modify osmotic potential, there was a significant increase in glycerol content of the colonies, perhaps via passive diffusion and some endogenous synthesis. When NaCl was used to modify osmotic potential, there was a marked increase in glycerol and arabitol content in the mycelia, suggesting endogenous synthesis to overcome the imposed water stress. The increase in glycerol content was expected because it is a better internal water potential adaptation solute than other high molecular weight sugar alcohols (Magan 1997). It was interesting to note that, although Griffin (1981) suggested that matric imposed water stress is more difficult to overcome, *F. graminearum* accumulated increased levels of arabitol under extreme matric stress (−11.4 MPa), which is not as effective a compatible solute in allowing enzyme systems to function as glycerol or erythritol (Chirife et al. 1984). The authors suggest that matric stress may not be as imposing for pathogens such as *F. graminearum*, which are adapted for effective and efficient survival and growth on cereal residues.

Ramirez et al. (2004b) also showed changes in the content of trehalose and glucose in mycelia of *F. graminearum* in relation to osmotic and matric potential modification. With matrically imposed water stress, the concentration of trehalose in colonies generally was higher than on osmotically modified media. Synthesis of higher trehalose concentrations improves desiccation tolerance of the conidia, this condition may contribute to survival in the environment and improve potential for subsequent infection. Trehalose has been shown to interact with hydrated cell components in relation to heat shock and desiccation (Crowe et al. 1984). The authors suggest that the osmotic effect of trehalose accumulation, therefore, may be of secondary value due to its other cellular functions (Davis et al. 2000; Feofilova et al. 2012).

4.5 Conclusions

All the information presented in this chapters enable a better understanding of the survival and growth strategy employed by *F. graminearum* for survival, growth and establishment in natural ecosystems. Also data on ecophysiology of *F. graminearum* is relevant from the point of view of food safety, since mycotoxins are natural contaminants and their presence is unavoidable. It is important to reduce their presence and optimized prevention strategies.

References

Aldred D, Magan N (2004) Prevention strategies for tricothecenes. Toxicol Lett 153:165–171

Audenaert K, Callewaert E, Höfte M, De Saeger S, Haesaert G (2010) Hydrogen peroxide induced by fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. BMC Microbiol 10:112

Bakan B, Pinson L, Cahagnier B, Melcion D, Semon E, Richard-Molard D (2001) Toxigenic potential of *Fusarium culmorum* strains isolated from French wheat. Food Addit Contam 18:998–1003

Brennan JM, Fagan B, van Maanen A, Cooke BM, Doohan FM (2003) Studies on *in vitro* growth and pathogenicity of European *Fusarium* fungi. Eur J Plant Pathol 109:577–587

Chamley LL, Rosenberg A, Trenholom HL (1994) Factors responsible for economics losses due to *Fusarium* mycotoxin contamination of grains, food and feedstuffs. In: Miller JD, Trenholom HL (eds) Mycotoxins in grain compounds other than aflatoxin. Egan Press, St. Paul, pp 471–486

Chirife J, Favetto G, Fontan CF (1984) Microbial growth at reduced water activities: some physicochemical properties of compatible solutes. J Appl Bacteriol 56:259–268

Comerio RM, Fernandez Pinto VE, Vaamonde G (1999) Influence of water activity on deoxynivalenol accumulation on wheat. Mycotoxin Res 15:24–31

Cook RJ, Christen AA (1976) Growth of cereal root rot fungi as affected by temperature-water potential interactions. Phytopathology 66:193–197

Crowe JH, Crowe LM, Chapman D (1984) Preservation of membranes in an hydrobiotic organisms: the role of trehalose. Science 223:701–703

Desjardins A (2006) Fusarium mycotoxins: chemistry, genetics and biology. APS Press, St. Paul, pp 79–108

D'Mello JPF, Macdonald AMC, Bonte L (1997) The effects of difenoconazole on 3-acetyl deoxynivalenol synthesis by *Fusarium culmorum*: implications for cereal quality. In: Crop protection & food quality: meeting customers needs, proceedings of BCPC and ANPP conference, Kent, UK, pp 463–466

D'Mello JPF, Macdonald AMC, Postel D, Dijksma WTP, Dujardin A, Placinta CM (1998) Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. Eur J Plant Pathol 104:741–751

Dalcero A, Torres A, Etcheverry M, Chulze S, Varsavsky E (1997) Occurrence of deoxynivalenol and *Fusarium graminearum* in Argentinian wheat. Food Addit Contam 14:11–14

Davis DJ, Burlak C, Money N (2000) Osmotic pressure of fungal compatible osmolytes. Mycol Res 104:800–880

Feofilova EP, Ivashechkin AA, Alekhin A, Sergeeva YE (2012) Fungal spores: dormancy, germination, chemical composition, and role in biotechnology (review). Appl Biochem Microbiol 48:1–11

Galich MT (1996) Fusarium head blight in Argentina. In: Duvin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. Proceedings of a workshop held at CIMMYT. CIMMYT, Mexico

Greenhalgh R, Neish GA, Miller JD (1983) Deoxynivalenol, acetyl deoxynivalenol, and zearalenone formation by Canadian isolates of *Fusarium graminearum* on solid substrates. Appl Environ Microbiol 46:625–629

Griffin DM (1972) Ecology of soil fungi. Chapman and Hall, London

Griffin DM (1981) Water and microbial stress. In: Alexander A (ed) Advances in microbial ecology 5. Plenum Publishing Co, New York, pp 91–136

Hallsworth JE, Magan N (1994) Effects of KCl concentration on accumulation of a cyclic sugar alcohols and trehalose in conidia of three entomopathogenic fungi. Lett Appl Microbiol 18:8–11

Holliger K, Ekperigin HE (1999) Mycotoxins in food producing animals. Vet Clin N Am 15:133–165

Hope RJ, Magan N (2003) Two-dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates on wheat-based substrate. Lett Appl Microbiol 37:70–74

Hope RJ, Colleate A, Baxster ES, Magan N (2002) Interactions between environmental stress and fungicides effect growth and mycotoxin production by *Fusarium culmorum* isolates from wheat grain. Eur J Plant Pathol 108:685–690

Hope RJ, Aldred D, Magan N (2005) Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett Appl Microbiol 40:295–300

Jennings DH (1995) The physiology of fungal nutrition. Cambridge University Press, Cambridge

Jennings DH, Burke RM (1990) Compatible solute the mycological dimension and their role as physiological buffering agents. New Phytol 116:277–283

Lacey J, Magan N (1991) Fungi in cereal grain: their occurrence and water and temperature relationships. In: Chelkowsky J (ed) Trichotecenes and other mycotoxins. Wiley, New York, pp 243–256

Llorens A, Mateo R, Hinojo MJ, Valle-Algarra FM, Jimenez M (2004) Influence of environmental factors on biosynthesis of type B trichothecenes by isolates of *Fusarium* spp. from Spanish crops. Int J Food Microbiol 94:43–54

Magan N (1988) Effects of water potential and temperature on spore germination and germ-tube growth in vitro and on straw leaf sheaths. Trans Br Mycol Soc 90:97–107

Magan N (1997) Fungi in extreme environments. In: Wicklow D, Soderstrom B (eds) The mycota, vol IV, Environmental and microbial relationships. Springer, Berlin, pp 99–113

Magan N, Lacey J (1984) Water relations of some *Fusarium* species from infected wheat ears and grain. Trans Br Mycol Soc 83:281–285

Magan N, Lacey J (1985) The effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. In: Lacey J (ed) Cereal grain mycotoxins, fungi and quality in drying and storage. Elsevier, Amsterdam, pp 77–118

Magan N, Lynch JM (1986) Water potential, growth and cellulolysis of fungi involved in decomposition of cereal residues. J Gen Microbiol 132:1181–1187

Marín S, Sanchis V, Viñas I, Canela R, Magan N (1995a) Effect of water activity and temperature on growth and fumonisin B_1 and B_2 production by *Fusarium proliferatum* and *F. moniliforme* on maize grain. Lett Appl Microbiol 21:298–301

Marín S, Sanchis V, Magan N (1995b) Water activity, temperature and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Can J Microbiol 41:1063–1070

Marín S, Sanchis V, Teixeido R, Saenz AJ, Ramos AJ, Magan N (1996) Water activity, temperature relationships and microconidial germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. Can J Microbiol 42:1045–1050

Martins ML, Martins HM (2002) Effect of water activity, temperature and incubation time on the simultaneous production of deoxynivalenol and zearalenone in corn (*Zea mays*) by *Fusarium graminearum*. Food Chem 79:315–318

Matthies A, Walker F, Buchenauer H (1999) Interference of select fungicides, plant growth retardants as well as piperonyl butoxide and 1-aminobenzotriazole in trichothecene production of *Fusarium graminearum* (strain 4528) *in vitro*. Z Pflanzenkr Pflanzenschutz 106:198–212

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Milus EA, Parson CE (1994) Evaluation of foliar fungicides for controlling Fusarium head blight of wheat. Plant Dis 78:697–699

Moss MO, Frank JM (1985) Influence of the fungicide tridemorh on T-2 toxin production by *Fusarium sporotrichioides*. Trans Br Mycol Soc 84:585–590

Parry DW, Pettit TR, Jenkinson P, Lees AK (1994) The cereal *Fusarium* complex. In: Blakeman JP, Williamson B (eds) Ecology of plant pathogens. CABI International, Wallingford, pp 301–320

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathol 44:207–238

Paul PA, Lipps PE, Hershman DE, McMullen MP, Draper MA, Madden LV (2008) Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: a multivariate meta-analysis. Phytopathology 98:999–1011

Placinta CM, Macdonald AMC, D'Mello JPF, Harling R (1996) The influence of carbendazim on mycotoxin production in *Fusarium sporotrichioides*. In: Proceedings of The Brighton crop protection conference British Crop Protection Council, Farnham, UK, pp 415–416

Ramirez ML, Chulze S, Magan N (2004a) Impact of environmental factors and fungicides on growth and deoxynivalenol production by *Fusarium graminearum* isolates from Argentinian wheat. Crop Prot 23:117–125

Ramirez ML, Chulze S, Magan N (2004b) Impact of osmotic and matric water stress on germination, growth, mycelial water potentials and endogenous accumulation of sugars and sugar alcohols on *Fusarium graminearum*. Mycologia 96:470–478

Ramirez ML, Chulze S, Magan N (2006a) Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. Int J Food Microbiol 106:291–296

Ramirez ML, Reynoso MM, Farnochi MC, Chulze SN (2006b) Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. Eur J Plant Pathol 115:129–138

Ramirez ML, Reynoso MM, Farnochi MC, Torres AM, Leslie JF, Chulze SN (2007) Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. Food Addit Contam 24:1115–1120

Ryu D, Bullerman LB (1999) Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. J Food Prot 62:1451–1455

Sanchis V, Magan N (2004) Environmental conditions affecting mycotoxins. In: Magan N, Olsen M (eds) Mycotoxins in food: detection and control. Woodhead Publishing Ltd, Oxford, pp 174–189

Sung JM, Cook RJ (1981) Effect of water potential on reproduction and spore germination by *Fusariun roseum* "Graminearum", "Culmorum" and "Avenaceum". Phytopathology 71:499–504

Vesonder RF, Ellis JJ, Kwolek WF, DeMarini DJ (1982) Production of vomitoxin on corn by *Fusarium graminearum* NRRL 5883 and *Fusarium roseum* NRRL 6101. Appl Environ Microbiol 43:967–970

Wearing AH, Burgess LW (1979) Water potential and the saprophytic growth of *Fusarium roseum* 'graminearum'. Soil Biol Biochem 11:661–667

Willcock J, Magan N (2001) Impact of environmental factors on fungal respiration and dry matter losses in wheat straw. J Stored Prod Res 37:35–45

World Health Organization (2001) Deoxynivalenol. In: WHO food additives series 47. Food and nutrition paper 74. International programme on chemical safety. World Health Organization, Geneva, pp 419–556

Part II
Mycotoxins

Chapter 5
Mycotoxins Associated to *Fusarium* Species that Caused Fusarium Head Blight in Wheat in Latin-America

Virginia Fernández Pinto, Andrea Patriarca, and Graciela Pose

Abstract Fusarium Head Blight (FHB) is an important disease affecting the production of wheat worldwide. *Fusarium* species are causal agents of Fusarium Head Blight (FHB) in cereals and *Fusarium graminearum* (Schwabe) (teleomorph *Gibberella zeae* (Schwein.)) is considered the main cause of the disease. FHB incidence reduces grain yields and also produces fungal toxins, primarily trichothecenes, that contaminate grains used for human and animal consumption. The most common trichothecenes produced by *F. graminearum* are Deoxynivalenol (DON), its acetyl derivatives 3- acetyl-deoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON), nivalenol (NIV), and its acetylated derivative 4-acetyl-nivalenol (4ANIV or fusarenone X). Another point of remarkable interest is the increase in the presence of other casual agents of FHB as *F. poae*, a relatively weak pathogen compared with *F. graminearum*, but capable of produce a large number of mycotoxins, including trichothecenes of type A and B, beauvericin and enniatins. Several toxins were identified in wheat in years of epidemic FHB development. All reports showed the preponderance of DON. Surveys on *Fusarium* mycotoxins in small-grain cereals and their by-products are frequently conducted in the major production regions of the world such as North America and Europe, but information in South America is scarce and previous evidence has placed DON as the main *Fusarium* toxin detected in wheat and by-products in Argentina, Brazil and Uruguay.

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5.1 Occurrence of Mycotoxins in Fusarium Head Blight Events in Wheat in Latin America

5.1.1 Introduction

Fusarium Head Blight (FHB) is an important disease affecting the production of wheat worldwide. *Fusarium* species are causal agents of Fusarium Head Blight (FHB) in cereals and *Fusarium graminearum* (Schwabe) (teleomorph *Gibberella zeae* (Schwein.)) is considered the main cause of the disease. FHB severity depends on climatic, agronomic and genetic factors. Frequent rainfall, high humidity and prolonged dew, coinciding with the flowering and early kernel-fill periods of the crop, favor disease infection and development. Infection levels were variable, showing the highest FHB incidence in the most humid regions of the cropping area. FHB incidence reduces grain yields and produces fungal toxins, primarily trichothecenes, which contaminate grains used for human and animal consumption (Desjardins 2006). The most common trichothecenes produced by *F. graminearum* are Deoxynivalenol (DON), its acetyl derivatives 3- acetyldeoxynivalenol (3ADON) and 15-acetyl-deoxynivalenol (15ADON), nivalenol (NIV), and its acetylated derivative 4-acetyl-nivalenol (4ANIV or fusarenone X). Another point of remarkable interest is the increase in the presence of other casual agents of FHB as *F. poae*, a relatively weak pathogen compared with *F. graminearum*, but capable of producing a large number of mycotoxins, including trichothecenes of type A and B, beauvericin and enniatins (Stenglein et al. 2012).

Several toxins were identified in wheat in years of epidemic FHB development. All reports showed the preponderance of DON. This is to be expected since *F. graminearum* is the most important *Fusarium* species known to produce this toxin (Marasas 1991).

Surveys on *Fusarium* spp. mycotoxins in small-grain cereals and their by-products are frequently conducted in the major production regions of the world such as North America and Europe (van Egmond et al. 2007). However, information in South America is scarce and previous evidence has placed DON as the main toxin from the genus *Fusarium* detected in wheat and by-products in Argentina (Dalcero et al. 1997; Lori et al. 2003) and Uruguay (Piñeiro et al. 1996). In Brazil, DON is the main toxin found in analyses of commercial wheat grain, flour and by-products (Furlong et al. 1995; Malmann et al. 2003; Calori-Domingues et al. 2007). Genetic profile of the regional populations, especially the type of toxin produced by the fungus (fungal chemotype), is critical for assessing the regional risk of *Fusarium* spp. mycotoxins in the food chain (Goswami and Kistler 2004; Ward et al. 2008).

5.1.2 Argentina

The first toxin survey in Argentina was undertaken after the epidemic of 1985, based on 84 injured non-random grain samples. Several *Fusarium* species were isolated and all the samples were found to be infected by *F. graminearum.* All of them were

contaminated with DON, with levels ranging from 0.1 to 25 ppm; however, almost half of the samples had levels ranging between 0.3 and 0.5 ppm. In 8 % of samples, the toxin T2 was present at levels of 0.3–0.5 ppm. No zearalenone (ZEA) was found in these analysis (Banchero et al. 1987).

Twenty-four isolates of *F.* graminearum were obtained from 82 wheat samples of the 1985/1986 crop from several localities in the Buenos Aires and Santa Fe provinces. Their toxigenic potential was determined by Thin-Layer Chromatography. Eight out of the 24 isolates produced toxins, 6 out of 8 were able to produce DON together with either 3 or 15-ADON, two of them were also able to produce ZEA. One out of 8 produced only ZEA and another produced only 15-ADON. No evidence for the presence of NIV or fusarenone X (FUS-X) could be found (Faifer et al. 1990).

Quiroga et al. (1995) analyzed a great number of wheat samples from Buenos Aires and Santa Fe provinces from the 1986 to 1992 crop seasons. In this work, 521 of 1,056 samples examined were contaminated with DON in a range of 50–2,400 µg/kg. ADON was found in 22 of 933 samples with a range of 147–192 µg/kg and ZEA was found in 78 of 933 samples in a range of 241–441 µg/kg. T-2, HT-2, neosolaniol (NEO) and (diacetoxyscirpenol) DAS were only determined in 1986 with 20, 18, 16 and 26 positive samples respectively of 261 analyzed ranging from 165 to 792 µg/ kg.

During the 1993 harvest season, there was a high incidence of FHB in Argentina. *F. graminearum* was the main *Fusarium* species isolated. From 40 samples analyzed, 80 % showed DON contamination. The levels of DON found ranged between 300 and 4,500 ppm. Only five samples showed levels of DON higher than those established in the guidelines in Canada and the USA for food and feedstuffs (Dalcero et al. 1997). In the same harvest, DON and contaminant mycota was determined on wheat heads from the main production area of Argentina. *F graminearum* was again the predominant *Fusarium* species. An analysis of DON natural contamination was performed on 44 samples. DON contamination levels in positive samples ranged from 0.2 to 30 ppb (González et al. 1996). Regarding durum wheat (*Triticum durum*), a mycological survey was carried out on a limited number of samples (60) obtained during 1996 crop season from the main production area of Argentina. *Alternaria alternata* and *Fusarium graminearum* were the predominant fungal species. DON contamination levels in positive samples ranged from 26 to 6,400 ppb (González et al. 1999).

A survey for mycotoxins on 100 samples of durum wheat from 5 locations of the major cropping area (Southern Buenos Aires Province) during two consecutive harvests were conducted by Lori et al. in 2003. In the first harvest, 22 from 40 samples (55 %) showed DON contamination, but in low levels. Only 4 samples showed DON contamination higher than 2 ppm. In the second harvest, a crop year conducive to scab development, DON was detected in 47 samples (78.2 %) and 19 samples showed levels of DON higher than those established in the Canada and USA guidelines for food and feedstuff (2 ppm). In both years all locations situated in the humid area showed levels ranging from 0 to >8 ppm. Other mycotoxins, such as NIV, NEO and T-2 toxin, were investigated but not detected. ZEA was found only in one sample and its precursor zearalenol (ZOL) was present in 6 samples from the first harvest.

During 2001, early severe symptoms were observed in the Argentinean southeast wheat cropping region (Southern Buenos Aires and La Pampa province) and

reports of severe FHB infections were registered in the central northern area too (southeastern of Córdoba, southern Santa Fe and northern Buenos Aires) (Carranza et al. 2008).

The ability of the 33 isolates of *F. graminearum* obtained from 19 wheat samples of the 2001/2002 crop to produce trichothecenes on autoclaved rice was determined by Gas Chromatography and High Pressure Liquid Chromatography (HPLC) by Fernández Pinto et al. (2008). Ten out of 33 isolates produced DON (0.1–29 mg/kg), 13 produced both DON (1.0–708 mg/kg) and NIV (0.1–6.2 mg/kg), 12 produced 3-ADON (0.1–14 mg/kg), 13 produced 15-ADON (0.1–1.9 mg/kg), 10 produced FUS-X (0.1–2.4 mg/kg) and 7 produced ZEA (0.1–0.6 mg/kg). Natural occurrence of mycotoxins showed that 13 out of 19 samples were contaminated with DON in a range of 0.3–70 mg/kg and 2 samples with both DON (7.5 and 6.7 mg/kg) and NIV (0.05 and 0.1 mg/kg), respectively. This was the first time NIV was reported in wheat cultivated in Argentina.

A total of 120 freshly harvested wheat samples from the 2004 season in nine locations from Northern Buenos Aires Province, Argentina, were analyzed by González et al. (2008) for trichothecenes natural occurrence. T-2 tetraol, T-2 triol, HT-2 and T-2 toxin, DAS, NIV, DON, 3-ADON and 15-ADON were analyzed by gas chromatography and electron capture detection. The trichothecenes type A detected were HT-2 and T-2 triol toxins and the type B were DON, NIV and 3-ADON. In this study DON was the predominant trichothecene present in all locations (85 % samples) with a minimum over positive samples of 7 μg/kg, a maximum of 2,788 μg/kg and a media of 450.7 μg/kg. Based on 120, samples the incidences were 21.7 % for 3-ADON, 22.5 % for HT-2, 27.5 % for T-2 triol and 85 % for DON. NIV was confirmed in one sample.

During 2005 and 2006, a total of 56 samples of wheat were collected either from fields or from trucks at the store plants in Southeastern Buenos Aires Province, Argentina and were analyzed for the most important mycotoxins (Roigé et al. 2009). DON and ZEA were the most common mycotoxins being detected in 49 % of the wheat samples. T-2 toxin was only detected in one sample (2 %).

In another study (Zapata Basílico et al. 2010), 53 moldy wheat samples collected from farms of 15 departments of Santa Fe province in Argentina during the 2006 harvest season were analyzed for *Fusarium* spp. mycotoxins. The wheat cultivated area in Santa Fe province is divided according to agrometeorological conditions into two zones: Zone I (North Central) and Zone II (South). DON was found in 13 out of 32 samples in Zone I in the range 0.43–3.60 mg/kg, and NIV in 6 out of 32 in the range 0.11–0.40 mg/kg. In Zone II, DON was found in 11 out of 21 samples (range 0.57–9.50 mg/kg) and NIV in 4 out of 21 (0.10–0.60 mg/kg).

Alvarez et al. (2009) studied the toxigenic potential of 144 *Fusarium graminearum sensu stricto* strains isolated from wheat in different subregions of the main production area during 2001, 2003 and 2004 harvests. Analysis of the trichothecene chemotype distribution across the Argentinean wheat cropping area revealed that 15-ADON was the most common chemotype. Two other chemotypes,

the 3-ADON and 3- and 15-ADON, were present. They also reported that the number of isolates with simultaneous production of both acetylderivatives had increased along the years.

In Argentina, investigations on the structure of the *F. graminearum* species complex have led to the identification of only one predominant species, *F. graminearum sensu stricto* (*Fusarium graminearum* Lineage 7) (Ramírez et al. 2007; Alvarez et al. 2011). Reynoso et al. (2011) concluded that the Argentinean populations of *F. graminearum* produced DON and 15-ADON and belonged to Lineage 7; therefore, they are similar to those isolated from wheat elsewhere in the world.

5.1.3 Brazil

In Brazil, current information also places DON as the main target toxin in analyses of commercial wheat grain, flour and by-products (Furlong et al. 1995; Malmann et al. 2003; Calori-Domingues et al. 2007).

Sabino et al. (1989) reported a low contamination of wheat grains from different regions of Brazil with DON. From a total of 120 samples, only two were contaminated at a level of 183 µg/kg.

A large analytical survey conducted between the years 2000 and 2003 showed that approximately 25 % of 297 samples of commercial wheat from southern Brazil were contaminated with DON with mean and maximum levels of 603 and 8,504 µg/kg, respectively (Malmann et al. 2003).

In a study carried out by Calori-Domingues et al. (2007), a total of 100 wheat samples were evaluated for DON contamination, 50 of which were from national production (Sao Paulo, Paraná and Rio Grande do Sul states) and 50 were imported from Argentina and Paraguay. The samples were collected between May and December 2005, from commercializing or processing wheat companies. The results showed that 94 % of Brazil samples and 88 % of the imported ones were contaminated with DON. The mean level of Brazil wheat samples was 332 µg/kg and from imported samples 90 µg/kg. Two of the Brazil samples showed contamination levels higher than the maximum established by European Community Regulation (1,250 µg/kg).

A survey was conducted during the 2006–2008 period on 66 samples from 38 municipalities across the northern portion of the state of Rio Grande do Sul where wheat is mostly grown. Both DON and NIV were detected and co-occurrence was predominantly observed. DON was found in 65 samples and NIV in 57 out of 65 samples. In only one of the samples NIV but not DON was found and in six samples, DON but not NIV was detected. Mean concentrations of DON and NIV were 540 and 337 µg/kg, respectively; however, 12 samples had DON concentrations exceeding 1,000 µg/kg and 9 samples showed NIV levels of 527–781 µg/kg (Del Ponte et al. 2012).

5.1.4 Uruguay

In a survey carried out by Piñeiro et al. (1996) in Uruguay from the 1993–1995 cropping seasons, a contamination of DON was found in 64 of the 116 samples analyzed in a range from 80 to >1,000 μg/kg. ZEA was also investigated and levels between 100 and >200 μg/kg were detected in 5 of the 106 samples analyzed.

5.2 Toxins

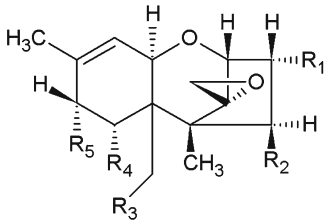
5.2.1 Trichothecenes

Certain *Fusarium* species cause Head Blight of wheat and other small grains worldwide and produce trichothecene mycotoxins. These mycotoxins can induce toxicoses in animals and humans and can contribute to the ability of some *Fusarium* species to cause plant disease.

Trichothecenes are one of the major classes of mycotoxins, causing a significant economic impact on cereal and grain crops each year (Parry et al. 1995; McMullen et al. 1997).

Early toxicity studies showed that trichothecenes inhibit eukaryotic protein synthesis, specifically by preventing peptide bond formation at the peptidyl transferase center of the 60S ribosomal subunit. This inhibition typically affects polypeptide chain initiation or elongation, although polypeptide chain termination may also be inhibited (Ueno 1985). Trichothecenes were later shown to inhibit mitochondrial protein synthesis (McLaughlin et al. 2009). Exposure to these toxins can cause feed refusal, immunological problems, vomiting, skin dermatitis, and hemorrhagic lesions (Ueno 1985). They are also phytotoxic and can cause chlorosis, inhibition of root elongation, and act as a virulence factor in wheat head scab (Desjardins et al. 1996). Trichothecenes are a family of over 200 toxins with a common tricyclic 12,13-epoxytrichothec-9- ene core structure (Cole et al. 2003). All studies confirm that the 12,13-epoxide ring is required for activity, and the number and position of hydroxyl groups and the number and position and type of esterifications can affect whether trichothecenes inhibit protein synthesis at the initiating step or at elongation termination steps (Desjardins 2006). They have been classified into four groups (Types A, B, C, and D) based on the substitution pattern of the trichothecene ring. Types A (Fig. 5.1), which are the most commonly produced in cereals, could be differentiated based on the substitution at the C-8 position. Type A trichothecenes include compounds that have a hydroxyl group at C-8 (e.g., neosolaniol), an ester function at C-8 (e.g., T-2 toxin), or no oxygen substitution at C-8 (e.g., trichodermin).

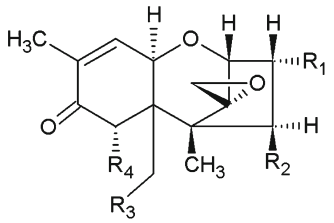
Type B trichothecenes (Fig. 5.2) have a carbonyl at C-8 (e.g., nivalenol, deoxynivalenol) and typically have a C-7 hydroxyl group (McCormick et al. 2011).



Toxin	Abbrev.	R1	R2	R3	R4	R5
Neosolaniol	NEO	OH	OAc	OAc	H	OH
Diacetoxyscirpenol	DAS	OH	OAc	OAc	H	H
T-2	T-2	OH	OAc	OAc	H	OIval
HT-2	HT-2	OH	OH	OAc	H	OIval
T-2 triol	-	OH	OH	OH	H	OIval
T-2 tetraol	-	OH	OH	OH	H	OH

OAc: O-acetyl
OIval: O-isovaleryl

Fig. 5.1 Type A trichothecenes



Toxin	Abbrev.	R1	R2	R3	R4
Nivalenol	NIV	OH	OH	OH	OH
Deoxynivalenol	DON	OH	H	OH	OH
3acetyl-DON	3-ADON	OAc	H	OH	OH
15acetyl-DON	15-ADON	OH	H	OAc	OH
Fusarenone X	FUS-X	OH	OAc	OH	OH

OAc: O-acetyl

Fig. 5.2 Type B trichothecenes

5.2.1.1 Deoxynivalenol, 3- and 15-Acetyldeoxynivalenol

Deoxynivalenol (DON) is a mycotoxin produced by the plant pathogenic fungi *Fusarium graminearum* and *F. culmorum*. These and other closely related fungi cause a disease known as Fusarium Head Blight (FHB) in small grain cereals. Increasing awareness of *Fusarium* spp. mycotoxins, especially those from the trichothecene group, such as deoxynivalenol (DON), occurred in recent years being FHB one of the major threats to food security (Goswami and Kistler 2004;

van Egmond et al. 2007). The World Health Organization (WHO) regards DON as teratogen, neurotoxin, and immunosuppressant, and trichothecenes in general have been associated with chronic and fatal intoxication of humans and animals through consumption of contaminated food and feed. Ingestion of mycotoxin-contaminated food and feed can lead to toxicoses in humans and animals, respectively. DON is the predominant and most economically important of these mycotoxins in the majority of small grain-producing regions of the world. In humans, food poisoning characterized by diarrhea, nausea, vomiting, abdominal pain, headache, dizziness, and fever has been associated with consumption of *Fusarium* infested cereals or food products (Desjardins 2006; Pestka 2007). DON is the least toxic of the trichothecene mycotoxins produced by *Fusarium* spp. in small grains, but it can cause significant harm to humans and animals when ingested in large quantities. DON has been shown to inhibit the absorption of certain nutrients by human intestinal epithelial cells (Maresca et al. 2002). In animals, clinical signs of trichothecene toxicosis include feed refusal and weight loss, emesis, hemorrhage, and cellular necrosis of mitotically active tissues such as the intestinal mucosa, skin, and bone marrow (Wegulo 2012). Chronic exposure of farm animals to DON is also an important issue in Canada, USA and continental Europe. Studies with pigs indicate that DON is a potent feed intake and growth inhibitor, the levels of reduction typically being of the order of 20 and 13 % respectively, for a dietary concentration of 4 mg DON/kg. The feed rejection and emetic syndromes are aptly embodied in the alternative term for DON, namely 'vomitoxin'. Ruminants, on the other hand, are considerably more tolerant to DON, as exemplified by the lack of effect on feed intake and milk output in dairy cows (Charmley et al. 1993). Microorganisms of the rumen are believed to play an important role in the metabolism and detoxification of DON and other trichothecenes (Desjardins 2006).

In plants, DON and 3-ADON have been shown to be phytotoxic. Wang and Miller (1988) showed that of several *F. graminearum* metabolites, DON and 3-ADON most strongly inhibited growth of wheat coleoptile tissue. Growth of the coleoptile tissue of most of the 14 spring wheat cultivars tested was inhibited at a DON and 3-ADON concentration of 10^{-6} M, and inhibition was much stronger at higher concentrations. Bruins et al. (1993) exposed seedlings, coleoptile segments, anther-derived callus, and anther-derived embryos to DON and 3-ADON. They found that DON inhibited growth of all four types of plant material. Shimada and Otani (1990) found DON to strongly inhibit root growth in seedlings of seven wheat cultivars and suggested, based on their observation, that DON might be useful in selecting cells resistant to *F. graminearum* in cell cultures. As DON is water soluble it can be translocated to other parts of the plant where it can exert physiological effects. Kang and Buchenauer (1999) found DON and 3-ADON in mycelium-free wheat plant tissues distant from *F. culmorum*-inoculated spikelets. They concluded that the toxins can be translocated upwards via xylem vessels and phloem sieve tubes, and downward via phloem sieve tubes.

5.2.1.2 Nivalenol

Nivalenol, characterized as 3,4,7,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one is one of the naturally occurring mycotoxins among trichothecenes. *Fusarium cerealis* and *F. poae* are the main producers of NIV, but isolates of *F. culmorum* and *F. graminearum* are also able to produce this toxin (Yazar and Omurtag 2008).

In mice, NIV is embryotoxic and fetotoxic but not teratogenic and it inhibits Ig production. NIV slightly increased the frequencies of chromosomal aberrations and sister chromatid exchange in Chinese hamster cells. In addition, NIV is a weak inducer of chromosomal aberrations in mammalian cells *in vitro*, and from tests results, it seems possible that NIV could cause DNA-damage. However, the available information is quite limited to evaluate its genotoxic potential. Acute/chronic toxicity analysis showed that 6 ppm or more of NIV ingestion for 1 year exhibit a characteristic toxic effect in mice. In acute doses, NIV can induce bone marrow toxicity; chronic toxicity exposure may also cause leucopenia. There is insufficient evident of carcinogenicity of NIV in experimental animals and no human data are available at present (Yazar and Omurtag 2008).

5.2.1.3 Fusarenone X

FUS-X or 4-acetylnivalenol is a type B trichothecene. It was isolated in 1968, and characterized in 1969 as 3a,7, 15-trihydroxy-4f-acetoxy-8-oxo-12,13-epoxytrichothec-9-ene. FUS-X is produced by different species of the genus *Fusarium*, such as *F. graminearum, F. oxysporum, F. semitectum, F. sporotrichioides, F. sambucinum* (Masuda et al. 1982; Yazar and Omurtag 2008).

FUS-X is immunosuppressive, carcinogenic, cytotoxic, emetic, and causes diarrhea, hypothermia, and decreased respiratory rate in experimental animals. FUS-X is highly cytotoxic to cultured cells and is known to be cytotoxic to many kinds of mammalian cells. Like other trichothecenes, FUS-X inhibits lymphocyte blastogenesis. It is toxic to murine thymocytes, lymphocytes, gastric epithelial cells and human hepatoblastoma cells. By its effects to thymocytes and lymphocytes, it can be classified as immunotoxic. Besides, the existence of a genotoxic potential at low exposure levels has been shown. According to the IARC (International Agency or Research on Cancer), not enough evidence is available to determine the carcinogenicity of FUS-X in experimental animals, and as no epidemiological data on humans data are available, FUS-X is not classifiable as to its carcinogenicity to humans (Group 3) (Yazar and Omurtag 2008).

5.2.1.4 T-2 and HT-2 Toxins

These two related trichothecenes are type A trichothecene mycotoxins, which are closely related epoxy-sesquiterpenoids. Type A trichothecenes are considered to be

much more toxic than type B trichothecenes such as DON. In the literature, T-2 has been referred to as the most toxic trichothecene (Kong et al. 2012). T-2 and HT-2 are often found together (HT-2 is a deacetylated form of T-2) and the European Commission is currently considering their legislation (Edwards et al. 2012).

T-2 toxin has been reported to be produced by different *Fusarium* species. *F. sporotrichioides* and *F. langsethiae* have been reported as potential producers of HT-2 and T-2 toxins. Some isolates of *F. poae* can produce HT-2 and T-2 in culture, but when compared to other HT-2 and T-2 producers the levels are much lower (Thrane et al. 2004; Yli-Mattila et al. 2011). *Fusarium armeniacum* (syn. *F. acuminatum* subsp. *armeniacum*) is a close relative of *F. sporotrichioides* and also a producer of T-2 (Altomare et al. 1997; Burgess and Summerell 2000). Recently, Yli-Mattila et al. (2011) reported a newly described species, *F. sibiricum*, which is closely related to *F. sporotrichioides* and *F. langsethiae*, and is able to produce HT-2 and T-2.

With regard to the metabolism of T-2 toxin in animals, various *in vivo* as well as *in vitro* studies are described in the literature showing a thorough metabolism of T-2 toxin strongly depending on the analyzed species. After absorption, trichothecenes are not accumulated in high doses in specific organs but distributed in different tissues and organs due to their relatively high water solubility (Weidner et al. 2012).

T-2 toxin is readily metabolized by the gut microflora of mammals to several metabolites. HT-2 toxin is a primary metabolite in the gut and is absorbed into the blood after ingestion of T-2 toxin. Metabolism continues in the liver (with biliary excretion), resulting in a substantial, combined first-pass effect in the gut and liver (WHO 2002).

In vivo studies in animals showed that T-2 toxin and formed metabolites were rapidly excreted via urine and feces. During metabolism, T-2 toxin is mainly subjected to reactions resulting in more polar substances. Deacetylation, hydrolysis, and hydroxylation are among the common metabolic reactions leading to a variety of metabolites (Weidner et al. 2012).

HT-2 toxin and T-2 tetraol were detected as the major metabolites in studies with human skin samples. Incubations of human liver homogenates with T-2 toxin and cell culture experiments with human fibroblasts identified HT-2 toxin as the sole metabolite. In human blood cells, HT-2 toxin and neosolaniol were detected, and carboxylesterase activity was shown to be responsible for the hydrolysis of T-2 toxin to these two metabolites. A study of T-2 toxin applied to human renal proximal tubule epithelial cells (RPTEC) and normal human lung fibroblasts (NHLF) revealed HT-2 toxin as the main metabolite besides small amounts of neosolaniol in renal proximal tubule epithelial cells (Weidner et al. 2012).

For the toxicity of T-2 toxin, the inhibition of eukaryotic protein synthesis and the induction of apoptosis in various cell lines *in vitro* as well as *in vivo* were described as the main effects. In human primary tubule epithelial cells, T-2 toxin induced apoptosis, whereas its metabolites (HT-2 toxin, T-2 triol, T-2 tetraol) showed lower cytotoxic effects but still induced apoptosis at higher concentrations (Weidner et al. 2012).

Toxicological studies on animals have shown that the immune system is the primary target of T-2, causing changes in leukocyte counts, depletion of selective

blood cell progenitors and depression of antibody formation. Exposure to the toxin results in skin pain, pruritis, redness, vesicles, necrosis, epidermal sloughing, nausea, weight loss, vomiting and diarrhea. Severe poisoning results in prostration, weakness, ataxia, collapse, reduced cardiac output, shock, and death (WHO 2001).

T-2 is a potent inhibitor of protein synthesis and, at higher concentrations, of DNA and RNA synthesis. Tests for genotoxicity in microorganisms gave uniformly negative results with T-2 toxin. In cultured mammalian cells, however, low concentrations of T-2 toxin induced DNA strand breaks, unscheduled DNA synthesis, gene mutations, chromosomal aberrations and inhibition of intercellular communication across gap junctions. There was also evidence that T-2 toxin induced DNA strand breaks and chromosomal aberrations in vivo. It was unclear whether these effects were a consequence of interaction of T-2 toxin with genetic material or were secondary to inhibition of protein synthesis by this mycotoxin (WHO 2002).

Acute toxicity of T-2 is quite high, with LD50 values for rodents in the range 5–10 mg/kg body weight. Long-term studies on poultry showed that T-2 causes mouth and intestine lesions (Pascale et al. 2003). Strain and sex differences in susceptibility to the toxicity of T-2 toxin have been observed in mice given single oral doses by gavage in studies designed to evaluate such variation. A sex difference was also observed after administration by inhalation (WHO 2002).

In a study in which cats received T-2 toxin orally in gelatin capsules at a dose of 0.06 mg/kg of body weight per day, severe toxic effects were observed, including hemorrhage in the intestinal tract, lymph nodes and heart, that led to death within 1–7 weeks. In contrast, relatively mild effects were observed in 7-weekold pigs given T-2 toxin in the diet at doses of up to 0.13 mg/kg of body weight per day for 3 weeks and in mice given a dose of 0.22 mg/kg of body weight per day in the diet for 71 weeks (WHO 2002).

The toxicity of HT-2 has been less investigated; nevertheless, due to the fact that T-2 is rapidly metabolized to HT-2 *in vivo*, it is widely accepted that the toxicity of T-2 *in vivo* includes that of HT-2 (Pascale et al. 2012).

The available studies of adverse health effects in human populations were limited to a few investigations of outbreaks of acute poisoning, in which the reported effects included nausea, vomiting, pharyngeal irritation, abdominal pain and distension, diarrhea, bloody stools, dizziness and chills. In subsequent investigations, analyses of limited numbers of suspected food or grain samples indirectly linked the outbreaks to T-2 toxin. The concomitant presence of T-2 toxin, DON, A-DON and NIV was reported in one of these outbreaks, and the presence of these or other trichothecenes in other incidents could not be ruled out. A series of episodes of food-related poisoning referred to as alimentary toxic aleukia that occurred in 1931–1947 in the former Soviet Union was associated with the ingestion of grain infected with moulds, in particular *F. poae* and *F. sporotrichioides*. The dominant pathological changes were necrotic lesions of the oral cavity, esophagus and stomach and, in particular, pronounced leukopenia consisting primarily of bone-marrow hypoplasia and aplasia. The disease was lethal in a high proportion of cases. In investigations conducted three decades later, cultures implicated in the outbreak were shown to produce T-2 toxin (WHO 2002).

5.2.2 Zearalenone

ZEA was discovered as the cause of a reproductive disorder in pigs known as vulvovaginitis, and characterized as (3S,11E)-14,16-dihydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione. ZEA is a non-steroidal, estrogenic lactone produced by *Fusarium* species, including *F. culmorum, F. graminearum, F. semitectum* and *F. equiseti* (Vaamonde et al. 1987; Yazar and Omurtag 2008; Atoui et al. 2012).

It is frequently implicated in reproductive disorders of farm animals and occasionally in hyperestrogenic syndromes in humans. Fertility problems have been observed in animals such as swine and sheep. ZEA can be transmitted to piglets in milk, causing estrogenism in pigs. The most important effects of ZEA primarily include the urogenital system. Swine are the most commonly affected animals. Cattle, poultry and laboratory rodents are also affected by this toxin. Any compound with hormonal activity may be genotoxic and/or carcinogenic and there is evidence that ZEA may show both types of activity in some animal species. The potential for ZEA to stimulate growth of human breast cancer cells containing estrogen response receptors has been demonstrated (Zinedine et al. 2007). ZEA was evaluated by the IARC in 1993 and, based on inadequate data in humans and limited evidence in experimental animals, was allocated in group 3 (not classifiable as to their carcinogenicity to humans) (Zinedine et al. 2007; Yazar and Omurtag 2008).

References

Altomare C, Petrini O, Logrieco A, Bottalico A (1997) Taxonomic relationships among the toxigenic species *Fusarium acuminatum*, *Fusarium sporotrichioides* and *Fusarium tricinctum* by isozyme analysis and RAPD assay. Can J Bot 75:1674–1684

Alvarez C, Azcarate M, Fernández Pinto V (2009) Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. Int J Food Microbiol 135:131–135

Alvarez C, Somma S, Proctor R, Stea G, Mulè G, Logrieco A, Fernández Pinto V, Moretti A (2011) Genetic diversity in *Fusarium graminearum* from a major wheat-producing region of Argentina. Toxins 3:1294–1309

Atoui A, El Khoury A, Kallassy M, Lebrihi A (2012) Quantification of *Fusarium graminearum* and *Fusarium culmorum* by real-time PCR system and zearalenone assessment in maize. Int J Food Microbiol 154:59–65

Banchero E, Miguel M, Godoy H, Di Giulio A, Dubois M, Souto G, Gomez M, Resnik F, Torres M (1987) Evaluación de la presencia de *Fusarium* y de micotoxinas en muestras de trigo de la campaña 1985/86 y su relación con la calidad comercial. Publicación Gerencia Técnica, Junta Nacional de Granos

Bruins M, Karsai I, Schepers J, Snijders C (1993) Phytotoxicity of deoxynivalenol to wheat tissue with regard to *in vitro* selection for *Fusarium* head blight resistance. Plant Sci 94:195–206

Burgess L, Summerell B (2000) Taxonomy of *Fusarium*: *Fusarium armeniacum* stat & comb. nov. Mycotaxon 75:347–348

Calori-Domingues M, De Almeida R, Tomiwaka M, Gallo C, Da Gloria E, Santos Dias C (2007) Occurrence of deoxynivalenol in national and imported wheat used in Brazil. Ciência e Tecnologia de Alimentos 27:181–185

Carranza M, Lori G, Sisterna M (2008) Wheat *Fusarium* head blight 2001 epidemic in the southern Argentinian pampas. Summa Phytopathol 34(1):93–94
Charmley E, Trenholm H, Thompson B (1993) Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production and its composition. J Dairy Sci 76:3580–3587
Cole R, Jarvis B, Schweikert M (2003) Handbook of secondary metabolites. Academic, New York, pp 199–560
Dalcero A, Torres A, Etcheverry M, Chulze S, Varsavsky E (1997) Occurrence of deoxynivalenol and *Fusarium graminearum* in Argentinian wheat. Food Addit Contam 14:11–14
Del Ponte E, Garda-Buffon J, Badiale-Furlong E (2012) Deoxynivalenol and nivalenol in commercial wheat grain related to *Fusarium* head blight epidemics in southern Brazil. Food Chem 132:1087–1091
Desjardins A (2006) *Fusarium* mycotoxins. Chemistry, genetics, and biology. American Phytopathological Society, St. Paul, 268 pp
Desjardins A, Proctor R, Bai G, McCormick S, Shaner G, Beuchley G, Hohn M (1996) Reduced virulence of trichothecene-non-producing mutants of *Gibberella zeae* in wheat field tests. Mol Plant Microbe Interact 9:1996–1023
Edwards S, Imathiu S, Ray R, Back M, Hare M (2012) Molecular studies to identify the *Fusarium* species responsible for HT-2 and T-2 mycotoxins in UK oats. Int J Food Microbiol 156:168–175
Faifer GC, De Miguel MS, Godoy HM (1990) Patterns of mycotoxin production by *Fusarium graminearum* isolated from Argentine wheat. Mycopathologia 109:165–170
Fernández Pinto V, Terminiello L, Basilico J, Ritieni A (2008) Natural occurrence of nivalenol and mycotoxigenic potential of *Fusarium graminearum* strains in wheat affected by Head Blight in Argentina. Braz J Microbiol 39:157–162
Furlong E, Soares L, Lasca C, Kohara E (1995) Mycotoxins and fungi in wheat harvested during 1990 in test plots in the state of São Paulo, Brazil. Mycopathologia 131:185–190
González H, Pacin A, Resnik S, Martinez E (1996) Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinian wheat in 1993. Mycopathologia 135:129–134
González H, Martínez E, Pacin A, Resnik S (1999) Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinian durum wheat. Mycopathologia 144:97–102
González H, Moltó G, Pacin A, Resnik S, Zelaya M, Masana M, Martínez E (2008) Trichothecenes and mycoflora in wheat harvested in nine locations in Buenos Aires province, Argentina. Mycopathologia 165:105–114
Goswami R, Kistler H (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol 5(6):515–525
Kang Z, Buchenauer H (1999) Immunocytochemical localization of *Fusarium* toxins in infected wheat spikes by *Fusarium culmorum*. Physiol Mol Plant Pathol 55:275–288
Kong W, Zhang S, Shen H, Ou-Yang Z, Yang M (2012) Validation of a gas chromatography-electron capture detection of T-2 and HT-2 toxins in Chinese herbal medicines and related products after immunoaffinity column clean-up and pre-column derivatization. Food Chem 132:574–581
Lori G, Sisterna M, Haidukowski M, Rizzo I (2003) *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area of Argentina. Microbiol Res 158:29–35
Malmann C, Dilkin M, Mürman L, Dilkin P, Almeida C (2003) Avaliação da contaminação por desoxinivalenol em trigo utilizado na alimentação humana. In: Congresso Brasileiro de Farmácia 1. São Paulo. Abstract available online at: http://www.lamic.ufsm.br/papers/2a.pdf
Marasas WFO (1991) Toxigenic *Fusaria*. In: Smith JC, Henderson RS (eds) Mycotoxins and animal food. CRC Press, Boca Raton, pp 119–139
Maresca M, Mahfoud R, Garmy N, Fantini J (2002) The mycotoxin deoxynivalenol affects nutrient absorption in human intestinal epithelial cells. J Nutr 132:2723–2731
Masuda E, Takemoto T, Tatsuno T, Obara T (1982) Immunosuppressive effect of a trichothecene mycotoxin Fusarenon-X in mice. Immunology 45:743–749
McCormick S, Stanley A, Stover N, Alexander N (2011) Trichothecenes: from simple to complex mycotoxins. Toxins 3:802–814

McLaughlin J, Bin-Umer M, Tortora A, Mendeze N, McCormick S, Tumer N (2009) A genome wide screen in *Saccharomyces cerevisiae* reveals a critical role for the mitochondria in the toxicity of a trichothecene mycotoxin. Proc Natl Acad Sci U S A 106:21883–21888

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Parry D, Jenkinson P, McLeod L (1995) *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol 44:207–238

Pascale M, Haidukowski M, Visconti A (2003) Determination of T-2 toxin in cereal grains by liquid chromatography with fluorescence detection after immunoaffinity column clean-up and derivatization with 1-anthroylnitrile. J Chromatogr A 989:257–264

Pestka J (2007) Deoxynivalenol: toxicity, mechanisms and animal health risks. Anim Feed Sci Technol 137:283–298

Piñeiro M, Dawson R, Costarrica M (1996) Monitoring program for mycotoxin contamination in Uruguayan food and feeds. Nat Toxins 4:242–245

Quiroga N, Resnik S, Pacin A, Martínez E, Pagano A, Riccobene I, Neira S (1995) Natural occurrence of trichothecenes and zearalenone in Argentinean wheat. Food Control 6:201–204

Ramírez M, Reynoso M, Farnochi M, Torres A, Leslie J, Chulze S (2007) Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. Food Addit Contam 24:1115–1120

Reynoso M, Ramirez M, Torres A, Chulze S (2011) Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. Int J Food Microbiol 4:444–448

Roigé M, Aranguren S, Riccio M, Pereyra S, Soraci A, Tapia M (2009) Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. Rev Iberoam Micol 26:233–237

Sabino M, Ichikawa A, Inomata E, Lamardo L (1989) Determinação de deoxinivalenol em trigo e milho em grão por cromatografia em camada delgada. Rev Inst Adolfo Lutz 49:155–159

Shimada T, Otani M (1990) Effects of *Fusarium* mycotoxins on the growth of shoots and roots at germination in some Japanese wheat cultivars. Cereal Res Commun 18:229–232

Stenglein S, Dinolfo M, Bongiorno F, Moreno MV (2012) Response of wheat (*Triticum* spp.) and barley (*Hordeo vulgare*) to *Fusarium poae*. Agrociencia 46:299–306

Thrane U, Adler A, Clasen P, Galvano F, Langseth W, Lew H, Logrieco A, Nielsen KF, Ritieni A (2004) Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. Int J Food Microbiol 95:257–266

Ueno Y (1985) The toxicology of mycotoxins. Crit Rev Toxicol 14:99–132

Vaamonde G, Scarmato G, Bonera N (1987) Zearalenone production by *Fusarium* species isolated from soybeans. Int J Food Microbiol 4:129–133

van Egmond HP, Schothorst RC, Jonker MA (2007) Regulations relating to mycotoxins in food: perspectives in a global and European context. Anal Bioanal Chem 389:147–157

Wang Y, Miller J (1988) Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to *Fusarium* head blight resistance. J Phytopathol 122:118–125

Ward T, Clear R, Rooney A, O'Donnel K, Gaba D, Patrick S (2008) An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–484

Wegulo SN (2012) Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins 4:1157–1180

Weidner M, Welsch T, Hübner F, Schwerdt G, Gekle M, Humpf H (2012) Identification and apoptotic potential of T-2 toxin metabolites in human cells. J Agric Food Chem 60:5676–5684

WHO (2001) Safety evaluation of certain mycotoxins in food. WHO food additives series 47, FAO food and nutrition paper 7, Presented at the 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO, Geneva, 557 pp

WHO (2002) Evaluation of certain mycotoxins in food. WHO technical report series 906, Joint FAO/WHO Expert Committee on Food Additives. Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva

Yazar S, Omurtag G (2008) Fumonisins, trichothecenes and zearalenone in cereals. Int J Mol Sci 9:2062–2090

Yli-Mattila T, Ward TJ, O'Donnell K, Proctor RH, Burkin AA, Kononenko GP, Gavrilova OP, Aoki T, McCormick S, Gagkaeva T (2011) *Fusarium sibiricum* sp. nov, a novel type A trichothecene-producing *Fusarium* from northern Asia closely related to *F. sporotrichioides* and *F. langsethiae*. Int J Food Microbiol 147:58–68

Zapata Basílico ML, Pose G, Ludemann V, Fernández Pinto V, Aríngoli E, Ritieni A, Basílico JC (2010) Fungal diversity and natural occurrence of fusaproliferin, beauvericin, deoxynivalenol and nivalenol in wheat cultivated in Santa Fe Province, Argentina. Mycotoxin Res 26:85–91

Zinedine A, Soriano JM, Moltó JC, Mañes J (2007) Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chem Toxicol 45:1–18

Chapter 6
Fusarium Mycotoxins. An Overview of Chemical Characterization and Techniques for its Determination from Agricultural Products

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Abstract The requirement to apply regulatory limits (or at least recommendations) to detect mycotoxins presence in several samples, such as food, feed, and other biological matrices has prompted the development of a vast number of analytical methods for the detection, quantification and confirmation of these metabolites. The present chapter describes several methods developed for the determination of mycotoxins produces by *Fusarium* species associated with Fusarium Head Blight worldwide. The chemical diversity of *Fusarium* mycotoxins and their varying concentration ranges in a wide range of agricultural commodities, foods and biological samples poses a great challenge to analytical chemists. The different chemical and physicochemical properties of the mentioned mycotoxins require specific extraction, cleanup, separation and detection methods. Advantages and disadvantages of each method depend on its capability to separate impurities from the analytes, the time of sample preparation and economic aspects. The *Fusarium* mycotoxicology had its beginnings in 1809 with the identification of this genus by Johann Link, who characterized this group of fungi by the typical shape of their macroconidia. In 1903 the first indication that *Fusarium graminearum* and related species were associated with mycotoxicosis in farm animals appeared, producing hemorrhagic and estrogen syndromes, and rejection of food animals, especially pigs. Between 1961 and 1991, a group of researchers discovered the three most important mycotoxins produced by *Fusarium* genus: fumonisins, trichothecenes and zearalenone, as well as other emergent mycotoxins such us: beauvericin, fusaproliferin, and moniliformin.

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6.1 Fumonisins (FBs)

6.1.1 General Characterization

They are produced by several species belonging to *Fusarium* genus, but mainly *F. verticillioides* and *F. proliferatum* which are common contaminant of maize and maize based foods and feeds in many parts of the world. This mycotoxin has been also found in wheat and others agricultural crops (Palacios et al. 2011). At least 28 different FBs have been reported (Rheeder et al. 2002). Three groups of FBs (A–C) have been identified based on structural similarities. Groups A and B are characterized by the presence of an amide and amine group, respectively. Group C is similar to the B-group, except for the absence of the methyl group at the C_1. Of all the FBs identified to date, the fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) are the most important (Fig. 6.1). FB_1 usually constitute about 70 % of the total FBs content found in naturally contaminated foods and feeds.

6.1.2 Biological Effects

The action mechanism of these toxins is related to the fact that interfere with the metabolism of sphingolipids, due to the structural similarity of FBs with sphingoid bases sphinganine (Sa) and sphingosine (So) (Theumer et al. 2008). FB_1 has been

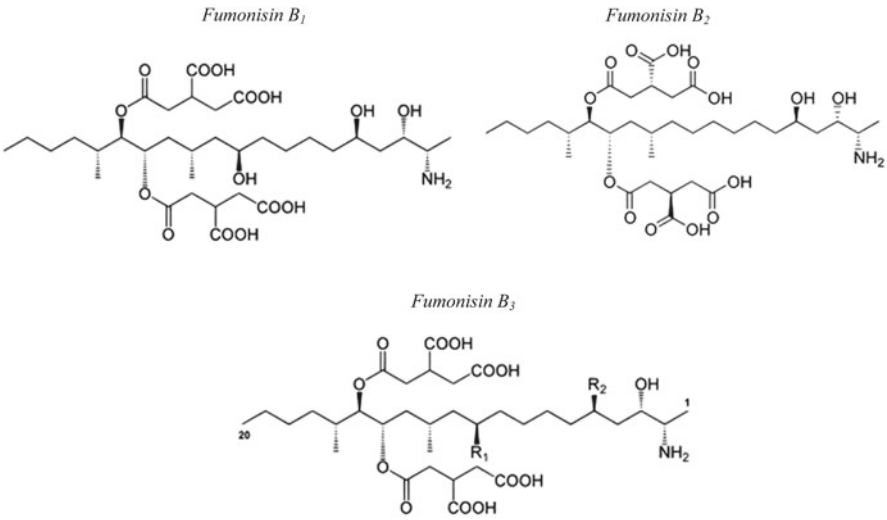

Fig. 6.1 Chemical structure of the main fumonisins

shown to give rise to a wide range of adverse biological effects, include fatal diseases in farm and laboratory animals, such as fatal leukoencephalomalacia in horses and rabbits, pulmonary edema in pigs and nephrotoxicity and liver cancer in rats. In humans, there is a probable link with esophageal cancer.

6.1.3 Detection Procedures

The absence of a UV chromophore in the FB structure needs a chemical modification to make them detectable if using either a UV or fluorescence detector. The presence of four carboxyl and hydroxyl groups in all FBs makes them readily soluble in water and polar solvents such as methanol (MeOH) and acetonitrile. The acid property of FBs and their metabolites requires the use of a strong acid buffer for good reverse-phase high-performance liquid chromatography (HPLC) peak shape (Wilkes and Sutherland 1998).

Mullett et al. (1998), used a immunosensor based on surface plasmon resonance (SPR) to detect FB_1 in spiked samples. That was the first time that detected mycotoxin by this method. The SPR technique is based upon the property that binding of materials to a surface can alter the refractive index near that surface. SPR devices measure the small changes in the angle, or intensity, of internally reflected light that result from the binding event. The magnitude of the response is influenced by the amount of material adhering to the surface. An advantage of SPR is that it does not necessarily require competition, or labelled reagents, for detection. The number of devices that use SPR has increased substantially in recent years and a wide variety of devices are commercially available. The application of SPR to mycotoxins has also been reviewed more recently (Maragos 2004, 2009; Lacy et al. 2008).

HPLC with fluorescence detection (FLD) is the method of choice for the detection and quantification of FBs in food and feeds. In a worldwide survey of fumonisin levels in maize and maize-based products, over 90 % of participating laboratories used pre-column derivatization and HPLC with FLD for quantification (Shephard et al. 1996). Other chromatographic techniques such as gas chromatography (GC) and thin layer chromatography (TLC), as well as immunochemical methods like an enzyme-linked immunosorbent assay (ELISA) are also used for FBs detection and will be described in the following paragraphs. TLC methods developed for the determination of FBs include normal phase (silica) and reverse phase (C_{18}) chromatography (Gelderblom et al. 1988; Rottinghaus et al. 1992). One or two dimensional TLC has been used for the detection and quantification of high levels of FBs in feeds and in fungal cultures. For complex matrix such as feeds, one dimensional TLC was not sufficient to remove interfering substances (Plattner et al. 1990; Ross et al. 1991). A cleaner plate can be obtained by the use of a two-step sequential development process for the silica TLC plate in which the lower section of the plate was removed between stages (Dupuy et al. 1993; Le Bars et al. 1994). Since the FB molecule lacks UV absorption, chemical modification is necessary to make it detectable. Various pre-column derivatization techniques involving reaction of

the primary amine group have been reported. Derivatization is commonly performed with *o*-phthal dialdehyde (OPA) with 2-mercaptoethanol. The reaction takes place at room temperature in a short time (3 min). A worldwide survey of fumonisin levels in maize showed this reagent to be the method of choice in most participating laboratories (Shephard et al. 1996).

Liquid chromatography-mass spectrometry (LC-MS) methods have higher sensitivity than HPLC methods but they are generally reserved for metabolic studies. They were also used to confirm the identity of FBs and related compounds. A method based on LC with electrospray ionisation coupled with mass spectrometry (ESI-MS) was validated for analysis of low level contamination of rodent feeds (Churchwell et al. 1997).

Gas chromatography methods involve the hydrolyzed derivatives of fumonisins (HFBs), followed by esterification of the 1, 2, 3-propane tricarboxylic acid (Sydenham et al. 1990) and/or acylation of the aminopolyol moiety (Plattner et al. 1990).

Several ELISA methods have been developed for determination of FBs in foods and feeds. They are easy, rapid, inexpensive, and adapted for rapid screening purposes but they are less sensitive than LC methods. These methods can also be used for quantification of HFBs (Maragos et al. 1996). Before use, ELISA methods are usually validated by comparison to LC or GC methods. A high correlation ($r > 0.9$) between LC and ELISA has been reported by several authors (Sutikno et al. 1996; Sydenham et al. 1996; Kulisek and Hazebroek 2000). ELISA generally overestimates the concentration of FBs present in samples (Bird et al. 2002). Others methods like immunosensors (biosensors) and capillary zone electrophoresis, were developed for the determination of FBs in food, but they have not found widespread application. In conclusion, the most applied analytical method for FBs quantification in foods and feeds is LC-FLD after derivatization. For food analysis, LC after OPA derivatization has become the standard technique and has been adopted by the AOAC as the method of choice for the determination of FB_1, FB_2 and FB_3.

Electrospray MS is another ideal technique to detect and measure fumonisins (Plattner 1995). Fumonisins tend to be ionic and produce abundant signals in both the positive and negative ion modes.

6.2 Trichothecenes

6.2.1 General Characterization

Trichothecenes are named by the *Thricothecium roseum* fungus, which the tricotecina, the first member of the group, was isolated as antifungal metabolite in 1949 (Desjardins 2006). The trichothecenes constitute a family of more than 60 sesquiterpenoid metabolites produced by a number of fungal genera, including *Fusarium, Myrothecium*, *Phomopsis, Stachybotrys*, *Trichoderma*, *Trichothecium*, and others.

These mycotoxins are tricyclic sesquiterpenes with a double bond between C_9 and C_{10}, and an epoxide ring. Also characterized by oxygenation and esterification at positions C_3, C_4, C_7, C_8 and C_{15}. Trichothecenes are a family of related cyclic sesquiterpenoids, which are divided into four groups (types A–D) according to their characteristic functional groups. This chemical characteristic and the fact that type-A trichothecenes generally have fewer hydroxyl groups makes the type-A trichothecenes less polar, which affects analytical procedures from extraction and clean-up to separation and detection.

The trichothecenes are most commonly found in Europe and are known to be produced on many different grains like wheat, barleys, oats or maize.

6.2.2 Biological Effects

Trichothecenes are responsible for a wide range of toxicity in animals, including feed refusal, weight loss and vomiting. The symptoms produced by various trichothecenes include effects on almost every major system of the vertebrate body; many of these effects are due to secondary processes that are initiated by often poorly understood metabolic mechanisms related to the inhibition of protein synthesis.

6.2.3 Chemical Composition of Main Trichothecenes

6.2.3.1 Trichothecene Type A

The first trichothecene type A by *Fusarium* was isolated in 1961 as a phytotoxic metabolite of *F. scirpi*, for which it's received the name of diacetoxyscirpenol (DAS) (Brian et al. 1961). In 1968, T-2 toxin was isolated by *F. tricinctum* (now *F. sporotrichioides*) from maize in France (Tatsuno et al. 1968). Since then, they have identified more than 40 trichothecenes produced by different *Fusarium* species. Within the group of trichothecenes type A, T-2 and HT-2 toxin are the mycotoxins most important, which differ only in the functional group at C_4. It has detected the presence of these toxins in grains such as wheat, corn, oats, barley, rice, beans and some cereal products. These toxins are produced by *F. sporotrichioides*, *F. poae*, *F. equiseti*, *F. acuminatum* and *F. langhsetiae* (being the first the most important producer).

6.2.3.2 Trichothecene Type B

Within the group of trichothecenes type B, the most important are: deoxynivalenol (DON), nivalenol (NIV) and its acetylated derivatives (3-acetil-DON, 15-acetil-DON). DON is probably the most common *Fusarium* toxin that contaminates many

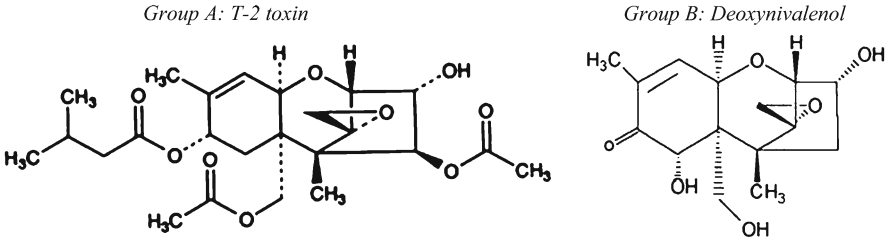

Fig. 6.2 Chemical structure of the main trichothecenes

cereals, especially maize and wheat, both in developed and in developing countries. This mycotoxin is commonly known as "vomitoxin" due to emetic syndromes and acute mycotoxicoses caused in the population of India, China and rural Japan (Bhat et al. 1997). The NIV was first isolated in 1968 from *F. nivale* (now *F. kyushuense*) in Japan (Tatsuno et al. 1968). This chemical substance is very stable thanks to the ring structures that are present, it is slightly toxic: an inhibitor of protein synthesis by binding to ribosomes.

Chemical structures representative of the different classifications of trichothecenes appear in Fig. 6.2.

6.2.3.3 Trichothecene Type C and D

Type C and D trichothecenes, respectively characterized by a second epoxide (C-7, 8 or C-9,10) or an ester-linked macrocycle (C-4, 16), are not associated with Fusarium Head Blight (Sudakin 2003). Type D contains macrocyclic trichothecenes. Type C trichothecenes is represented by crotocin and baccharin while type D trichothecenes by roridin A, verrucarin A, satratoxin H, etc.

6.2.4 Detection Procedures

6.2.4.1 Thin Layer Chromatography (TLC)

TLC has become an extremely powerful, rapid and in most instances, inexpensive separation technique in mycotoxicology, especially in developing countries, for surveillance purposes and control of regulatory limits (Gilbert and Anklam 2002). This technique is still worldwide important for screening purposes prior to use methods that require more specialized staff and more time-consuming. Schaafsma et al. (1998) identified and quantified deoxynivalenol (DON), fumonisin B_1 (FB_1) and zearalenone (ZEA) in grain samples comparing to immunoassay (ELISA) and HPLC methods to determine the reliability of the less expensive

TLC and recommended that researchers choose which analytical method to use based upon individual considerations of cost and precision. Initially this technique had also been selected as a simple and economical method used for routine screening for DON by several authors (Gelderblom et al. 1988; Rottinghaus et al. 1992; Gilbert 1993; Fernandez et al. 1994).

6.2.4.2 High – Performance Liquid Chromatography

The polarity of the trichothecenes lends them readily to reversed phase (RP) LC. HPLC with UV detection is generally not applicable to most trichothecenes exhibiting weak UV absorption, whereas it is routinely used for quantitative analysis of deoxynivalenol and its acetylated derivatives in cereals with good accuracy and precision (Kotal and Radová 2002; Klötzel et al. 2005; MacDonald et al. 2005; Stroka et al. 2006). Among the chromatographic methods, HPLC-UV has the advantage of not requiring a derivatization step before detection. However, given the rather non-specific UV absorption of DON and its derivatives at 200–225 nm, an immunoaffinity column (IAC) clean up is required to obtain chromatograms sufficiently free from interfering peaks.

HPLC-FLD, with either a pre- or post-column derivatization step, uses the light emission of the molecules that have been excited to higher energy levels by absorption of electromagnetic radiation. It is more sensitive than UV or diode array detector (DAD), and is used to increase sensitivity. However, often must perform a derivatization of the mycotoxin in order to make possible the detection or to improve sensitivity. This method provides high sensitivity, selectivity and repeatability of measurements, but it is not applicable to the detection of trichothecenes lacking fluorophore groups in their chemical structure. The possibility of using HPLC-FLD for trichothecenes determination therefore depends on the availability of suitable derivatizing reagents. Pre-column derivatization with coumarin-3-carbonyl chloride as fluorescence label has been reported for HPLC-FLD analysis of T-2, HT-2, neosolaniol (NEO) and diacetoxyscirpenol (DAS) in cereal cultures of *F. sporotrichioides* on maize, rice and wheat (Mateo et al. 2002). Recently, 1-anthroylnitrile (1-AN) was shown to be efficient as a fluorescent-labeling reagent for T-2 and HT-2 under mild conditions (Pascale et al. 2003). This method did not allow determination of HT-2 in oats because of interfering chromatographic peaks eluting at the retention time of the HT-2-(1-AN) derivative (Visconti et al. 2005). A slight modification of the clean up procedure removed interfering matrix compounds and also obtained reliable results with oats (Trebstein et al. 2008). Derivatization via 1-AN was also applied to HPLC-FLD determination of T-2 and HT-2 in eggs (Maragos 2006). To improve the sensitivity of the HPLC-FLD method for T-2 and HT-2 in foodstuffs, including oats, three different commercially-available fluorescent reagents were tested as labeling dyes: 1-naphthoyl chloride (1-NC); 2-naphthoyl chloride (2-NC); and, pyrene-1-carbonyl cyanide (PCC) (Urraca et al. 2004; Lippolis et al. 2008). FLD detection for trichothecene analysis was introduced only recently and is currently used to a lesser extent than

UV or MS detection (Koch 2004). However, given the severe problems with GC analysis, HPLC-FLD detection could represent a reliable alternative tool for the simultaneous determination of type-A and type-B trichothecenes for laboratories not equipped with more sophisticated and expensive LC/MS instrumentation.

HPLC-MS technique is one of the most advanced in the detection of mycotoxins. However, the methods are slow and require expertise. For simultaneous detection of both, type A and B trichothecenes, the use of LC with MS detector is much more promising (Churchwell et al. 1997; Rosenberg et al. 1998; Plattner 1999; Gilbert 2000; Cavaliere et al. 2005; Häubl et al. 2005; Lattanzio et al. 2008; Vishwanath et al. 2009; Zachariasova et al. 2010) Berger et al. (1999) used positive ion atmospheric pressure chemical ionization (APCI) for the determination of nine trichothecenes extracted from wheat and rice while Razzazi-Fazeli et al. (1999) applied negative ion APCI to the LC-MS detection of NIV and DON in wheat.

6.2.4.3 Liquid Chromatography – Tandem Mass Spectrometry

Increasingly, LC–tandem MS (MS-MS) has been applied to mycotoxin analysis despite higher costs and the need for experienced personnel. The main advantages of the technique include its general applicability to a broad range of compounds, high sensitivity and outstanding selectivity. Several methods already have been reported for the simultaneous determination of mycotoxins, which offer significant advantages over conventional techniques. Liquid chromatography-tandem mass spectrometry (LC-MS^2) is becoming the technique of choice for the simultaneous determination of type-A and type-B trichothecenes (Razzazi-Fazeli et al. 2002; Laganá et al. 2003; Berthiller et al. 2005; Biselli and Hummert 2005). Also for quantification of T-2 and HT-2 mycotoxins in cereal (Tölgyesi and Kunsàgi 2012). An LC-ESI-MS^2 method was recently developed for simultaneous determination of major *Fusarium* toxins (i.e. DON, T-2, HT-2, fumonisin B_1, fumonisin B_2 and zearalenone) together with aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) and ochratoxin A in maize based on the use of new multi-toxin immunoaffinity columns (Myco6in1, Vicam, USA) containing antibodies for all these mycotoxins (Lattanzio et al. 2007). More recently, these authors reported an LC-APCI-MS^2 method for simultaneous determination of NIV, DON, T-2 and HT-2 in cereals and cereal based foods (Lattanzio et al. 2008).

6.2.4.4 Gas Chromatography

In the past, GC methods have been used routinely for quantitative determination of trichothecenes because they are non-fluorescent compounds and do not adsorb intensively in the ultravioletvisible (UV-Vis) range. Several researchers applied different derivatization procedures (trimethylsilation or fluoroacylation) to quantify trichothecenes by GC with electron capture detection (ECD) (Scott et al. 1981; Krska 1998; Langseth and Rundberget 1998). Vega and Castillo (2006) determined

deoxynivalenol in wheat by GC-ECD and compared these results with those obtained analyzing the same samples by a HPTLC-FLD high-throughput method. The values found for the spiked samples by both chromatographic systems were very similar without significative difference.

6.2.4.5 Rapid Methods and Emerging Techniques

Although, as discussed above, the instrumental methods such as GC or HPLC-MS, are the reference methods for the determination of mycotoxins, the initial investment, operating expenses and the need for operating personnel as one of the major limitation to the widespread use of them.

Furthermore, these techniques generally require a laborious preparation; clean-up stages of the sample those extend the analysis time and add costs. For this reason alternative methods have been developed that have a greater simplicity of use, require less sample processing, have shorter analysis time (minutes or few hours) and installation and operating costs are much lower. Although comparatively considered these alternative methods have less precision and accuracy, they are valuable tools to estimate the mycotoxin contamination, particularly in screening situations that require a systematic analysis of a large number of samples (Shim et al. 2007) or in low-income countries where the availability of instrumental methods is a limiting factor (Wagacha and Muthomi 2008).

The vast majority of rapid methods are immunochemical assays that use antibodies as recognition elements target specific toxin. Alternatively there are other recognition molecules known as MIP (molecular imprinted polymers) or chemosensory. Other rapid methods include TLC, which has already been discussed and noninvasive methods such as infrared spectroscopy allows estimation of the amount of toxin present in the sample directly without extracting (Hernández-Hierro et al. 2008).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is also widely used for rapid monitoring of DON and T-2 in cereals. This method does not require clean up procedures and analyze mycotoxins directly after sample extraction (Schneider et al. 2004). For the simultaneous determination of NIV and DON, Maragos et al. (2006) proposed a competitive direct and indirect ELISA format by developing a monoclonal antibody exhibiting cross-reactivity for both toxins.

Fluorescence Polarization (FP)

FP immunoassay is a technology that has been used extensively in the area of human clinical diagnostics and after extended to the detection of several mycotoxins such as Maragos and Plattner (2002) who applied a rapid FP immunoassay for

deoxynivalenol in wheat. Unlike most immunoassays, where antibodies or antigens are attached to a solid surface, the FP assay is a solution phase assay. Fluorescence polarization instruments indirectly measure the rate of rotation of a fluorescent molecule in solution. Because the rate of rotation is related to the size of the molecule, larger molecules rotate slower and give a higher FP signal than smaller molecules. If the fluorescent molecule is a mycotoxin conjugate (tracer), the binding of the antibody to the mycotoxin can restrict the rotation of the fluorescent portion of the conjugate, increasing the FP signal. In the presence of toxin (sample) less of the tracer attaches to the antibody, and the FP signal is decreased. FP immunoassay therefore allows the detection of mycotoxins in solution, without the requirement for the washing steps.

Lateral Flow Devices (LFDs)

It involves immunoassays in which the test sample flows by capillary forces along an analytical membrane that contains immobilized immunoreagents (Krska and Molinelli 2009). These tests are mostly based on a visual evaluation of the results and provide a binary yes/no answer. Most commercial strip tests are devoted to T-2 in wheat and oats (Molinelli et al. 2008), DON and T-2 in cereals (Kolosova et al. 2008) Nevertheless, problems with reproducibility and reliability with different matrices, together with lack of extensive method validation, may limit their application.

Competitive Surface-Plasmon Resonance (SPR)

SPR-based immunoassays have been reported for selective and quantitative determination of DON and NIV in wheat with or without clean up (Schnerr et al. 2002; Tudos et al. 2003; Kadota et al. 2010). The results of SPR-based assays were in good agreement with GC-MS or LC-MS^2 measurements when naturally-contaminated wheat samples were analyzed. A fluorescence-based array biosensor (NRL-array) using a competitive immunoassay format and fluorescent-labeled anti-DON antibody has been developed and applied to determination of DON in foods (cornmeal, cornflakes, wheat, barley, and oats) without clean up of the extract (Ngundi et al. 2006). The use of affinity-based biosensor systems, with less demanding sample clean up, might be a useful alternative for screening and cost-effective quantitative determination.

Infrared Spectroscopy (IR)

IR is a rapid analytical technique requiring very little labor and no sample extraction, although it requires calibration. Some IR techniques have been developed as a means of identifying samples infected by fungi and estimating DON content.

Petterson and Aberg (2003) used NIR transmittance for determination of DON in whole wheat-kernel samples at levels >500 μg/kg, and NIR reflectance has been used for detection of scab and estimation of DON and ergosterol in single kernels of highly- infected wheat (Dowell et al. 1999). With Fourier-transform infrared (FT-IR) instruments, measurements can be performed rapidly with excellent signal-to-noise ratio and high resolution. FT-NIR analysis can be suitable for the determination of DON in unprocessed wheat at levels far below the maximum permitted limits for DON set by the European Commission (2006).

6.3 Zearalenone

6.3.1 General Characterization

The zearalenone (ZEA) is a nonsteroidal oestrogenic mycotoxin belongs to a large family of fungal metabolites that are derived from different cyclization precursors and modifications acetylated. These chemical compounds come from a cyclization which is formed resorciclic acid-lactone. The zearalenone differ in the presence and state of reduction of hydroxyl groups and their degree of acetylation. The zearalenones are biosynthesized through a polyketide pathway by *Fusarium graminearum*, *F. culmorum*, *F. equiseti*, *F. semitectum* and *F. crookwellense*. All these species are regular contaminants of cereal crops worldwide such as maize, barley, rice, sorghum and wheat, both before and after harvest (Silva and Vargas 2001). The natural incidence of ZEA in cereals and their co-occurrence with trichothecenes, particularly DON, has been reported in 19 countries by Tanaka et al. (1988). ZEA derivatives, such as α-zearalenol (α-ZEA), β-zearalenol (β-ZEA), α-zearalanol (α-ZOL), β-zearalanol (β-ZOL), zearalanone (ZAN) can be detected in infected corn stalks with *Fusarium* in the field and in rice (Zinedine et al. 2007). ZEA has been detected and β-ZEA in corn and soybean products at low levels (Schollenberger et al. 2006) (Fig. 6.3).

6.3.2 Biological Effects

The zearalenone not produce acute toxicity and have not been associated to date, to fatal mycotoxicosis in animals or humans, but it has been involved in estrogenic syndrome in swine and other animal's species. The acute toxicity of ZEA is relatively low upon oral administration in animals. Nevertheless, this mycotoxin causes alterations in the reproductive tract of both laboratory and domestic animals and, in humans; it has been associated with precocious puberty, hyperplasic and neoplastic endometrium, and human cervical cancer (Ingle and Martin 1986; Kuiper et al. 1998). The oestrogenic properties mainly associated with the toxic effects of ZEA

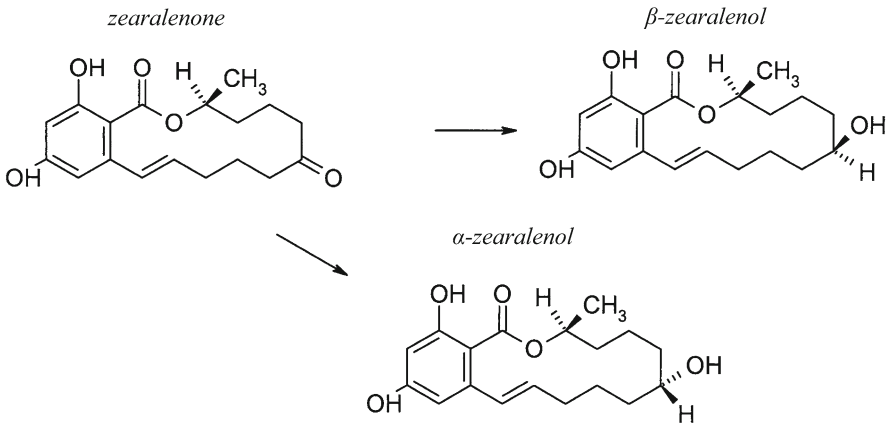

Fig. 6.3 Chemical structure of zearalenone and derivatives

and related compounds have recently been reviewed (Zinedine et al. 2007). The μ-ZEA is 3–4 times more active than ZEA or ZEA β-isomer due to topological resemblance to compete with estradiol and estrogen receptor binding to human cell ERβ, which can cause damage to tissues with high rates of expression of this receptor. Given its properties have been described pathologies as breast: cancer, fetal abnormalities, embryopathy and uterotropic activity.

6.3.3 Detection Procedures

The main analytical techniques used for ZEA analysis in cereals include TLC (Schaafsma et al. 1998; Shumacher and Krska 2001); GC-MS (Tanaka et al. 2000); HPLC using different detection principles such as UV diode array (Briones-Reyes et al. 2007; Zougagh and Ríos 2008), mass spectrometry (Zöllner et al. 1999; Pallanori and Von Holst 2003; Berthiller et al. 2005; Cavaliere et al. 2005; Songsermsakul et al. 2006; Tanaka et al. 2006), fluorescence (Silva and Vargas 2001; Radová et al. 2001; Mateo et al. 2002; De Saeger et al. 2003; Urraca et al. 2004; Reza et al. 2005; MacDonald et al. 2005; Schollenberger et al. 2006) as well as electrochemical detection (Andrés et al. 2008); and capillary electrophoresis (Maragos and Appell 2007). Ostry and Skarkova (2000) have also developed a method to quantify zearalenone (ZEA) in cereals and cereal products combinating the immunoaffinity chromatography and high performance thin layer chromatography (HPTLC) and they increased the selectivity and sensitivity of the method used.

These methods, however, require expensive equipment, complicated cleanup procedures, pre-concentration steps, and skilled operators. Immunoassays, on the other hand, have proven to be an excellent analytical alternative as they are

sensitive, specific, inexpensive, rapid, simple, and high-throughput methods that can be used as an "alarm" to detect different chemical contaminants in a wide variety of food matrices (Zacco et al. 2006).

Immunoanalytical techniques that have been used for ZEA detection include enzyme-linked immunosorbent assay (ELISA) methods (Barna-Vetró et al. 1994; Bennet et al. 1994; Tanaka et al. 1995; Nuryono et al. 2005), fluorescence polarization immunoassay (FPIA) (Maragos and Kim 2004), dipstick immunoassay (Schneider et al. 1995), and an automated flow-through immunosensor (Urraca et al. 2005). More recent techniques include an open sandwich immunoassay in which the antigen induced an enhanced VH/VL interaction (Suzuki et al. 2007), a colloidal gold-based lateral-flow immunoassay (Kolosova et al. 2007), ELISA methods which made use of a new scFv antibody (Wang et al. 2008) and a new specific monoclonal antibody (Thongrussamee et al. 2008) that were developed for detecting ZEA in cereal samples. Additionally, a number of commercial ELISA-based kits have been developed for detecting ZEA in food and feed products. Alternatively, specific molecular imprinted polymers have also been developed with recognition properties for ZEA which are not affected by storage limitations and stability problems usually associated with immunoassays when organic solvents are used. However, lower sensitivity is reached compared to immunoassay (Weiss et al. 2003; Urraca et al. 2006).

Electrochemical immunoassays are receiving special focus because of their inherent simplicity, suitability for mass production, low-cost fabrication, and high sensitivity, as well as their large number of labels, including enzymes and electroactive molecules, and their nanotechnology applications, such as for gold particles and semiconductor crystals. Although direct electroanalytical detection of ZEA has also been studied (Ramírez et al. 2005; Zougahg et al. 2008), the sensitivity reached is not very high. Recently, an electrochemical immunosensor involving magnetic beads and disposable carbon screen-printed electrodes (SPEs) for zearalenone (ZEA) sensing has been developed by Hervás et al. (2009, 2010) from baby food samples. They obtained an excellent accuracy with a high recovery yield (95–108 %) proposing this method as a very powerful and timely screening tool for the food safety scene.

Surprisingly, however, no electrochemical immunoassays for ZEA have been reported to date even though this detection principle is one of the most attractive alternatives to optical detection. In addition, magnetic beads have proven to be a powerful and versatile tool in the development and applications of immunoassays for food contaminants. It has been reported that the use of immunobeads improves the performance of the immunological reaction due to an increase in the surface area, as well as the assay kinetics are achieved more rapidly since the beads are in suspension and the probability that antibody-coated magnetic beads meet the analyte is very high, keeping the solution under stirring. Furthermore, the magnetic beads can easily be manipulated through the use of permanent magnets, and the matrix effect is also minimized due to improved washing and separation steps despite this increased surface area. Therefore, this strategy allows the analysis of samples to be made without any pre-enrichment, purification, or pre-treatment steps as is normally required.

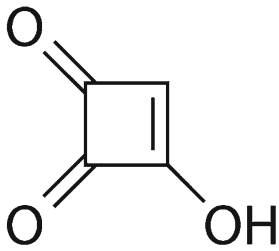

Fig. 6.4 Chemical structure of moniliformin

6.4 Moniliformin

6.4.1 General Characterization

Moniliformin is a quite unusual mycotoxin, a feed contaminant that is quite lethal to fowl. It is formed in many cereals by a number of *Fusarium* species that include *F. avenaceum*, *F. subglutinans* and *F. proliferatum*. It was structurally characterized in 1974 and was identified as the sodium salt of the previously known semisquaric acid (3-hydroxy-3-cyclobutene-1, 2-dione) (Hoffmann et al. 1971; Springer et al. 1974). Therefore, it can be assumed that chemically the relatively small semisquaric anion behaves similarly to inorganic anions (Fig. 6.4).

6.4.2 Biological Effects

This mycotoxin has not yet received much attention because it does not appear to be carcinogenic and relatively high amounts appear to be necessary to cause significant toxicological effects. These have been demonstrated in a limited number of animal species.

Filek and Lindner (1996) determined this mycotoxin in cereals by HPLC-FD. Few years later, Sewram et al. (1999) determined moniliformin in cultures of *F. subglutinans* and in naturally contaminated maize by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Since this mycotoxin has not enough toxicological importance or causes economical losses, there are not many studies related in the last years.

6.5 Simultaneously Detection

In response to the risk of great economic losses in the industry and the threat to human health as a result of exposure to those toxins, various analytical techniques have been developed for the detection of DON and ZEA simultaneously in different

matrices (Krska and Josephs 2001; Krska et al. 2001, 2005; Koch 2004). Commonly used methods include thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and immunochemical methods such as enzyme-linked immunosorbent assays (ELISA). In addition to the ELISA format (Maragos et al. 2006; Suzuki et al. 2007), other instrumental immunochemical techniques, such as fluorescence polarization immunoassay (FPIA) (Maragos et al. 2002; Maragos and Kim 2004; Lippolis et al. 2006), multiplex dipstick immunoassay (Lattanzio et al. 2012) and biosensors (Carter et al. 2000; Schnerr et al. 2002) have been developed in recent years for the analysis of main *Fusarium* toxins (namely zearalenone, T-2 and HT-2 toxins, deoxynivalenol and fumonisins in wheat, oats and maize). The simultaneous identification of several mycotoxins in food and feed samples with one single test is the most attractive approach for practical purposes. It can reduce the time and cost per analysis, allow for simpler assay protocols, and decrease the sample volume required. The development of multi-mycotoxin chromatographic techniques, such as LC–MS/MS, allowing the simultaneous determination of co-occurring mycotoxins, including DON and ZEA, has been described (Berthiller et al. 2005; Sulyok et al. 2006). Several applications of biosensors to multi-mycotoxin testing have also been reported (Van der Gaag et al. 2003; Ngundi et al. 2006). Non-instrumental immunochemical methods proposed for simultaneous mycotoxin detection include membrane-based techniques and tandem columns (Schneider et al. 1995; Goryacheva et al. 2007; Saha et al. 2007).

The use of membrane-based immunoassay tests for rapid on-site screening provides a simple and low-cost alternative to expensive, laborious and time-consuming instrumental methods and to more sophisticated immunoassay formats. Such methods, which use membranes as solid supports, have proven to be very promising formats, and allow parallel assays to be performed in the same sample. They therefore offer certain advantages for multianalyte tests (Gabaldón et al. 2003). The lateral-flow technique or immune chromatography is the most advanced and user-friendly format in terms of simplification and rapid on-site testing. The one-step lateral-flow method with the use of colloidal gold-labeled antibodies, based on the transportation of a reactant to its binding partner immobilized on the membrane surface, offers several advantages, including short assay time, visual interpretation of results, long-term stability and cost-effectiveness. Few studies have been performed on the development of lateral-flow immunoassays for mycotoxin testing (Delmulle et al. 2005; Wang et al. 2006). To our knowledge, this is the first publication reporting the development of a lateral-flow colloidal gold-based technique for the simultaneous detection of mycotoxins (DON and ZEA).

References

Andrés F, Zougagh M, Castaneda G, Ríos A (2008) Determination of zearalenone and its metabolites in urine samples by liquid chromatography with electrochemical detection using a carbon nanotube-modified electrode. J Chromatogr A 1212:54–60

Barna-Vetró I, Gyöngyösi A, Solti L (1994) Monoclonal antibody-based enzyme-linked immunosorbent assay of *Fusarium* T-2 and zearalenone toxins in cereals. Appl Environ Microbiol 60:729–731

Bennet BA, Nelsen TC, Miller BM (1994) Enzyme-linked immunosorbent assay for detection of zearalenone in corn, wheat, and pig feed: collaborative study. J AOAC Int 77:1500–1509

Berger U, Oehme M, Kuhn F (1999) Quantitative determination and structure elucidation of type A- and B-trichothecenes by HPLC/Ion trap multiple mass spectrometry. J Agric Food Chem 47:4240–4245

Berthiller F, Schuhmacher R, Buttinger G, Krska R (2005) Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 1062:209–216

Bhat RV, Shetty HPK, Amruth RP, Sudershan RV (1997) A foodborne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. J Toxicol 35:249–255

Bird SB, Herrick JE, Wander MM, Wright SF (2002) Spatial heterogeneity of aggregate stability and soil carbon in semi-arid rangeland. Environ Pollut 116:445–455

Biselli S, Hummert C (2005) Development of a multicomponent method for *Fusarium* toxins using LC-MS/MS and its application during a survey for the content of T-2 toxin and deoxynivalenol in various feed and food samples. Food Addit Contam 22:752–760

Brian PW, Dawkins AW, Grove JF, Hemming HG, Lowe D, Horries GLF (1961) Phytotoxic compounds produced by *Fusarium equiseti*. J Exp Bot 12:1–21

Briones-Reyes D, Gómez-Martinez L, Cueva-Rolón R (2007) Zearalenone contamination in corn for human consumption in the state of Tlaxcala, Mexico. Food Chem 100:693–698

Carter RM, Blake RC, Mayer HP, Echevarria AA, Nguyen TD, Bostanian LA (2000) A fluorescent biosensor for detection of zearalenone. Anal Lett 33:405–432

Cavaliere C, Foglia P, Pastorini E, Samperi R, Lanana A (2005) Development of a multiresidue method for analysis of major *Fusarium* mycotoxins in corn meal using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 19:2085–2093

Churchwell MI, Cooper WM, Howard PC, Doerge DR (1997) Determination of fumonisins in rodent feed using HPLC with electrospray mass spectrometric detection. J Agric Food Chem 45:2573–2578

De Saeger S, Sibanda L, Ban Peteghem C (2003) Analysis of zearalenone and alpha-zearalenolin animal feed using high-performance liquid chromatography. Anal Chim Acta 487:137–143

Delmulle BS, De Saeger MDG, Sibanda L, Vetero IB, Peteghem CHV (2005) Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B_1 in pig feed. J Agric Food Chem 53:3365–3368

Desjardins AE (2006) *Fusarium* mycotoxins. Chemistry, genetics, and biology. The American Phytopathological Society, St. Paul, 260 p

Dowell FE, Ram MS, Seitz MS (1999) Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem 76:573–576

Dupuy J, Le Bars P, Le Bars J, Boudra H (1993) Determination of fumonisin B_1 in corn by instrumental thin layer chromatography. J Planar Chromatogr 6:476–480

European Commission (2006) European Commission (Recommendation 2006/576/EC of 17 August 2006). The presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Off J Eur Union L 229:7–9

Fernandez C, Stack ME, Musser SM (1994) Determination of deoxnivalenol in 1991 U.S. winter and spring wheat by high performance thin layer chromatography. J AOAC Int 77:628

Filek G, Lindner W (1996) Determination of the mycotoxin moniliformin in cereals by high-performance liquid chromatography and fluorescence detection. J Chromatogr A 732:291–298

Gabaldón S, López S, Carda JB (2003) Legislación y gestión medioambiental en la producción de baldosas cerámicas. Boletín de la Sociedad Española de Cerámica y Vidrio 42:169–179

Gelderblom WC, Jaskiewicz K, Marasas WF, Thiel PG, Horak RM, Vleggaar R, Kriek NP (1988) Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol 54:1806–1811

Gilbert J (1993) Recent advances in analytical methods for mycotoxins. Food Addit Contam 10:37–48
Gilbert J (2000) Overview of mycotoxin methods, present status and future needs. Nat Toxins 7:347–352
Gilbert J, Anklam E (2002) Validation of analytical methods for determining mycotoxins in foodstuffs. Trends Anal Chem 21:468–486
Goryacheva IY, De Saeger S, Delmulle B, Lobeau M, Eremin SA, Barna-Vetró I, Van Peteghem C (2007) Simultaneous non-instrumental detection of aflatoxin B_1 and ochratoxin A using a clean-up tandem immunoassay column. Anal Chim Acta 590:118–138
Häubl G, Berthiller F, Krska R, Schuhmacher R (2005) Suitability of a fully 13C isotope labeled internal standard for the determination of mycotoxin deoxynivalenol by LC–MS/MS without clean-up. Anal Bioanal Chem 384:692–696
Hernández-Hierro JM, García-Villanova RJ, González-Martín I (2008) Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market. Anal Chim Acta 622:189–194
Hervás M, López A, Escarpa A (2009) Electrochemical immunoassay using magnetic beads for the determination of zearalenone in baby food: an anticipated analytical tool for food safety. Anal Chim Acta 653:167–172
Hervás M, López MA, Escarpa A (2010) Simplified calibration and analysis on screen-printed disposable platforms for electrochemical magnetic bead-based inmunosensing of zearalenone in baby food samples. Biosens Bioelectron 25:1755–1760
Hoffmann RW, Bressel U, Gelhaus J, Hauser H (1971) Tetramethoxyethylene, VII. 2 + 2-cycloadditions to tetramethoxyethylene. Chem Ber 104:873–885
Ingle MB, Martin BW (1986) Precocious puberty in Puerto Rico. J Pediatr 109:390–391
Kadota T, Takezawa T, Hirano S, Tajima O, Maragos CM, Nakajima T, Tanaka T, Kamata Y, Sugita-Konishi Y (2010) Rapid detection of nivalenol and deoxynivalenol in wheat using surface Plasmon resonance immunoassay. Anal Chim Acta 673:173–178
Klötzel M, Schmidt S, Lauber U, Thielert G, Humpf HU (2005) Comparison of different clean-up procedures for the analysis of deoxynivalenol in cereal-based food and validation of a reliable HPLC method. Chromatographia 62:41–48
Koch P (2004) State of the art of trichothecenes analysis. Toxicol Lett 153:109–112
Kolosova AY, De Saeger S, Sibanda L, Verheijen R, Van Peteghem C (2007) Development of a colloidal gold-based lateral-flow immunoassay for the rapid simultaneous detection of zearalenone and deoxynivalenol. Anal Bioanal Chem 389:2103–2107
Kolosova AY, Sibanda L, Dumoulin F, Lewis J, Duveiller E, Van Peteghem C, De Saeger S (2008) Lateral-flow colloidal gold-based immunoassay for the rapid detection of deoxynivalenol with two indicator ranges. Anal Chim Acta 616:235–244
Kotal F, Radová Z (2002) A simple method for determination of deoxynivalenol in cereals and flours. Czech J Food Sci 2:63–68
Krska R (1998) Performance of modern sample preparation techniques in the analysis of *Fusarium* mycotoxins in cereals. J Chromatogr A 815:49–57
Krska R, Josephs R (2001) The state-of-the-art in the analysis of estrogenic mycotoxins in cereals. Fresenius J Anal Chem 369:469–476
Krska R, Molinelli A (2009) Rapid test strips for analysis of mycotoxins in food and feed. Anal Bioanal Chem 393:67–71
Krska R, Baumgartner S, Josephs R (2001) The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals. Fresenius J Anal Chem 371:285–299
Krska R, Welzig E, Berthiller F, Molinelli A, Mizaikoff B (2005) Advances in the analysis of mycotoxins and its quality assurance. Food Addit Contam 22:345–353
Kuiper GJ, Lemmen JG, Carlsson B, Corton C, Safe SH, Van der Saag PT (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor b. Endocrinology 139:4252–4263
Kulisek ES, Hazebroek JP (2000) Comparison of extraction buffers for the detection of fumonisin B_1 in corn by immunoassay and high performance liquid chromatography. J Agric Food Chem 48:65–69

Lacy A, Dunne L, Fitzpatrick B, Daly S, Keating G, Baxter A, Hearty S, O'Kennedy R (2008) Rapid analysis of coumarins using surface plasmon resonance. J AOAC Int 89:884–892

Laganà A, Curini R, D'Ascenzo G, De Leva I, Faberi A, Pastorini E (2003) Liquid chromatography/tandem mass spectrometry for the identification and determination of trichothecenes in maize. Rapid Commun Mass Spectrom 17:1037–1043

Langseth W, Rundberget T (1998) Review: instrumental methods for determination of nonmacrocyclic trichothecenes in cereals, foodstuffs and cultures. J Chromatogr A 815:103–121

Lattanzio VM, Solfrizzo M, Powers S, Visconti A (2007) Simultaneous determination of aflatoxins, ochratoxin A and *Fusarium* toxins in maize by liquid chromatography tandem mass spectrometry after multitoxin immunoaffinity clean-up. Rapid Commun Mass Spectrom 21:3253–3261

Lattanzio VMT, Solfrizzo M, Visconti A (2008) Determination of trichothecenes in cereals and cereal-based products by liquid chromatography–tandem mass spectrometry. Food Addit Contam 25:320–330

Lattanzio VMT, Nivarlet N, Lippolis V, Della Gatta S, Huet A, Delahaut P, Granier B, Visconti A (2012) Multiplex dipstick immunoassay for semi-quantitative determination of *Fusarium* mycotoxins in cereals. Anal Chim Acta 718:99–108

Le Bars J, Le Bars P, Dupuy J, Boudra H, Cassini R (1994) Biotic and abiotic factors in fumonisin B_1 production and stability. J AOAC Int 77:517–521

Lippolis V, Pascale M, Visconti A (2006) Optimization of a fluorescence polarization immunoassay for rapid quantification of deoxynivalenol in Durum wheat-based products. J Food Prot 69:2712–2719

Lippolis V, Pascale M, Maragos CM, Visconti A (2008) Improvement of detection sensitivity of T-2 and HT-2 toxins using different fluorescent labeling reagents by high-performance liquid chromatography. Talanta 74:1476–1483

Macdonald SJ, Anderson S, Brereton P (2005) Determination of zearalenone in barley, maize and wheat flour, polenta, and maize-based baby food by immunoaffinity column cleanup with liquid chromatography: interlaboratory study. J AOAC Int 88:1733–1740

Maragos CM (2004) Emerging technologies for mycotoxin detection. Toxin Rev 23:317–344

Maragos CM (2006) Measurement of T-2 and HT-2 toxins in eggs by high-performance liquid chromatography with fluorescence detection. J Food Prot 69:2773–2776

Maragos CM (2009) Biosensors for mycotoxin analysis: recent developments and future prospects. World Mycotoxin J 2:221–238

Maragos CM, Appell M (2007) Capillary electrophoresis of the mycotoxin zearalenone using cyclodextrin-enhanced fluorescence. J Chromatogr A 1143:252–257

Maragos CM, Kim EK (2004) Detection of zearalenone and related metabolites by fluorescence polarization immunoassay. J Food Prot 67:1039–1043

Maragos CM, Plattner RD (2002) Rapid fluorescence polarization immunoassay for the mycotoxin deoxynivalenol in wheat. J Agric Food Chem 50:1827–1832

Maragos JE, Crosby MP, McManus JW (1996) Coral reefs and biodiversity: a critical and threatened relationship. Oceanography 9:83–99

Maragos CM, Jolley ME, Nasir MS (2002) Fluorescence polarization as a tool for the detection of deoxynivalenol in wheat. Food Addit Contam 19:400–407

Maragos C, Busman M, Sugita-Konishi Y (2006) Production and characterization of a monoclonal antibody that cross-reacts with the mycotoxins nivalenol and 4-deoxynivalenol. Food Addit Contam 23:816–825

Mateo JJ, Mateo R, Hinojo MJ, Lorens A, Jimenez M (2002) Liquid chromatographic determination of toxigenic secondary metabolites produced by *Fusarium* strains. J Chromatogr A 955:245–356

Molinelli A, Grossalber K, Führer M, Baumgartner S, Sulyok M, Krska R (2008) Development of qualitative and semiquantitative immunoassay-based rapid strip tests for the detection of T-2 toxin in wheat and oat. J Agric Food Chem 56:2589–2594

Mullett W, Lai EPC, Yeung JM (1998) Immunoassay of fumonisins by a surface plasmon resonance biosensor. Anal Biochem 258:161–167

Ngundi MM, Qadri SA, Wallace EV, Moore MH, Lassman ME, Shriver-Lake LC, Ligler FS, Taitt CR (2006) Detection of deoxynivalenol in foods and indoor air using an array biosensor. Environ Sci Technol 40:2352–2356

Nuryono N, Noviandi CT, Böhm J, Razzazi-Fazeli E (2005) A limited survey of zearalenone in Indonesian maize-based food and feed by ELISA and high performance liquid chromatography. Food Control 16:65–71

Ostry V, Skarkova J (2000) Development of an HPTLC method for the determination of deoxynivalenol in cereal products. J Plan Chromatogr Modern TLC 13:443–446

Palacios SA, Ramirez ML, Cabrera Zalazar M, Farnochi MC, Zappacosta D, Chiacchiera SM, Reynoso MM, Chulze SN, Torres AM (2011) Occurrence of *Fusarium* spp. and fumonisin in durum wheat grains. J Agric Food Chem 59(22):12264–12269

Pallanori L, Von Holst C (2003) Determination of zearalenone from wheat and corn by pressurized liquid extraction and liquid chromatography-electrospray mass spectrometry. J Chromatogr A 993:39–45

Pascale M, Haidukowski M, Visconti A (2003) Determination of T-2 toxin in cereal grains by liquid chromatography with fluorescence detection after immunoaffinity column clean-up and derivatization with 1-anthroylnitrile. J Chromatogr A 989:257–264

Pettersson H, Aberg L (2003) Near infrared spectroscopy for determination of mycotoxins in cereals. Food Control 14:229–232

Plattner RD (1995) Detection of fumonisins produced in *Fusarium moniliforme* cultures by HPLC with electrospray MS and evaporative light scattering detectors. Nat Toxins 3:294–298

Plattner RD (1999) HPLC/MS Analysis of *Fusarium* mycotoxins, fumonisins and deoxynivalenol. Nat Toxins 7:365–370

Plattner RD, Norred WP, Bacon CW, Voss KW, Peterson R, Shackelford DD, Weisleder D (1990) A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia. Mycologia 82:698–702

Radová Z, Hajslová J, Králová J (2001) Analysis of zearalenone in wheat using high-performance liquid chromatography with fluorescence detection and/or enzyme linked immunosorbent assay. Cereal Res Commun 29:435–442

Ramírez EA, Molina PG, Zón MA, Fernández H (2005) Development of an electroanalytical method for the quantification of zearalenone in maize samples. Electroanalysis 17:1635–1640

Razzazi-Fazeli E, Böhm J, Luf W (1999) Determination of nivalenol and deoxynivalenol in wheat using liquid chromatography-mass spectrometry with negative ion atmospheric pressure chemical ionization. J Chromatogr A 854:45–55

Razzazi-Fazeli E, Rabus B, Cecon B, Böhm J (2002) Simultaneous quantification of A-trichothecene mycotoxins in grains using liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry. J Chromatogr A 968:129–142

Reza OM, HJajimahmoodi M, Memariam S (2005) Determination of zearalenone in corn flour and a cheese snack product using high-performance liquid chromatography with fluorescence detection. Food Addit Contam 22:443–448

Rheeder JP, Marasas WFO, Vismer HF (2002) Production of fumonisin analogs by *Fusarium* species. Appl Environ Microbiol 68:2101–2105

Rosenberg E, Krska R, Wissiack R, Kmetov V, Josephs R, Razzazi E, Grasserbauer M (1998) High performance liquid chromatography-atmospheric-pressure chemical ionization mass spectrometry as a new tool for the determination of the mycotoxin zearalenone in food and feed. J Chromatogr A 819:277–288

Ross PF, Rice LG, Plattner RD, Osweiler GD, Wilson TM, Owens DL, Nelson HA, Richard JL (1991) Concentrations of fumonisin B_1 in feeds associated with animal health problems. Mycopathologia 114:129–135

Rottinghaus GE, Coatney CE, Minor HC (1992) A rapid, sensitive thin layer chromatography procedure for the detection of fumonisin B_1 and B_2. J Vet Diagn Invest 4:326–329

Saha D, Acharya D, Roy D, Shrestha D, Dhar TK (2007) Simultaneous enzyme immunoassay for the screening of aflatoxin B_1 and ochratoxin A in chili samples. Anal Chim Acta 584:343–349

Schaafsma AW, Nicol RW, Savard ME, Sinha RC, Reid LM, Rottinghaus G (1998) Analysis of *Fusarium* toxins in maize and wheat using thin layer chromatography. Mycopathologia 142:107–113

Schneider E, Usleber E, Märtlbauer E, Terplan G (1995) Multimycotoxin dipstick enzyme immunoassay applied to wheat. Food Addit Contam 12:387–393

Schneider E, Curtui V, Seidler C, Dietrich R, Usleber E, Märtlbauer E (2004) Rapid methods for deoxynivalenol and other trichothecenes. Toxicol Lett 153:113–121

Schnerr H, Vogel RF, Niessen L (2002) A biosensor-based immunoassay for rapid screening of deoxynivalenol contamination in wheat. Food Agric Immunol 14:313–321

Schollenberger M, Muller HM, Rufle M, Suchy S, Plack S, Drochner W (2006) Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. Mycopathologia 161:43–52

Scott PM, Kanhare RS, Lau PY (1981) Gas chromatography with electron capture and mass spectrometric detection of deoxynivalenol in wheat and other grains. J AOAC Int 64:1364

Sewram V, Nieuwoudt TW, Marasas WFO, Shephard GS, Ritieni A (1999) Determination of the mycotoxin moniliformin in cultures of *Fusarium subglutinans* and in naturally contaminated maize by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Chromatogr A 848:185–191

Shephard GS, Thiel PG, Stockenstrom S, Sydenham EW (1996) Worldwide survey of fumonisin contamination of corn and corn-based products. J AOAC Int 79:671–687

Shim W-B, Yang Z-Y, Kim J-S, Kim J-Y, Kang S-J, Woo G-J, Chung Y-C, Eremin SA, Chung D-H (2007) Development of immunochromatography strip-test using nanocolloidal gold antibody probe for the rapid detection of aflatoxin B_1 in grain and feed samples. J Microbiol Biotechnol 17:1629–1637

Shumacher R, Krska R (2001) International interlaboratory study for the determination of the *Fusarium* mycotoxins zearalenone and deoxinivalenol in agricultural commodities. Food Addit Contam 18:417–430

Silva CMG, Vargas EA (2001) A survey of zearalenone in corn using Romer Mycosep 224 column and high performance liquid chromatography. Food Addit Contam 18:39–45

Songsermsakul P, Sontag G, Cichna-Markl M (2006) Determination of zearalenone and its metabolites in urine, plasma and faeces of horses by HPLC-APCI-MS. J Chromatogr B Analyt Technol Biomed Life Sci 843:252–261

Springer JP, Clardy J, Cole RJ, Kirksey JW, Hill RK, Carlson RM, Isidor JL (1974) Structure and synthesis of moniliformin, a novel cyclobutane microbial toxin. J Am Chem Soc 96:2267–2269

Stroka J, Derbyshire M, Mischke C, Ambrosio M, Kroeger K, Arranz I, Sizoo E, Van Egmond H (2006) Liquid chromatographic determination of deoxynivalenol in baby food and animal feed: interlaboratory study. J AOAC Int 89:1012–1020

Sudakin DL (2003) Trichothecenes in the environment: relevance to human health. Toxicol Lett 143:97–107

Sulyok M, Berthiller F, Krska R, Schuhmacher R (2006) Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. Rapid Commun Mass Spectrom 20:2649–2659

Sutikno A, Abouzied MM, Azcona-Olivera JI, Hart LP, Pestka J (1996) Detection of fumonisins in *Fusarium* cultures, corn and corn products by polyclonal antibody-based ELISA: relation to fumonisin B_1 detection by liquid chromatography. J Food Protect 59:645–651

Suzuki T, Munakata Y, Morita K, Shinoda T, Ueda H (2007) Sensitive detection of estrogenic mycotoxin zearalenone by open sandwich immunoassay. Anal Sci 23:65–70

Sydenham EW, Gelderblom WCA, Thiel PG, Marasas WFO (1990) Evidence for the natural occurrence of fumonisin B_1, a mycotoxin produced by *Fusarium moniliforme*, in corn. J Agric Food Chem 38:285–290

Sydenham EW, Thiel PG, Vleggaar R (1996) Physicochemical data for some selected *Fusarium* toxins. J AOAC Int 79:1365–1379

Tanaka T, Hasegawa A, Yamamoto S, Lee US, Sugiura Y, Ueno Y (1988) Worldwide contamination of cereals by the *Fusarium* mycotoxins, nivalenol, deoxynivalenol, and zearalenone. Survey of 19 countries. J Agric Food Chem 36:979–983

Tanaka T, Teshima R, Ikebuchi H, Sawada J, Ichinoe M (1995) Sensitive enzyme-linked immunosorbent assay for the mycotoxin zearalenone in barley and job's-tears. J Agric Food Chem 43:946–950

Tanaka T, Yoneda A, Inoue S, Dugiura Y, Ueno Y (2000) Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography–mass spectrometry. J Chromatogr A 882:23–28

Tanaka H, Takino M, Sugita-Konishi Y, Tanaka T (2006) Development of a liquid chromatography/time-of-flight mass spectrometric method for the simultaneous determination of trichothecenes, zearalenone and aflatoxins in foodstuffs. Rapid Commun Mass Spectrom 20:1422–1428

Tatsuno T, Saito M, Enomoto M, Tsunoda H (1968) Nivalenol, a toxic principle of *Fusarium nivale*. Chem Phann Bull 16:2519–2520

Theumer MG, López AG, Aoki MP, Cánepa MC, Rubinstein HR (2008) Subchronic mycotoxicoses in rats. Histopathological changes and modulation of the sphinganine to sphingosine (Sa:So) ratio imbalance induced by *Fusarium verticillioides* culture material, due to the coexistence of aflatoxin B_1 in the diet. Food Chem Toxicol 3:967–977

Thongrussamee T, Kuzmina NS, Shim WB, Jiratpong T, Eremin SA, Intrasook J, Chung DH (2008) Monoclonal-based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals. Food Addit Contam 25:997–1006

Tölgyesi A, Kunsági Z (2012) Quantification of T-2 and HT-2 mycotoxins in cereals by liquid chromatography-multimode ionization-tandem mass spectrometry. Microchem J 106:300–306

Trebstein A, Seefelder W, Lauber U, Humpf HU (2008) Determination of T-2 and HT-2 toxins in cereals including oats after immunoaffinity cleanup by liquid chromatography and fluorescence detection. J Agric Food Chem 56:4968–4975

Tudos AJ, Lucas-van den Bos ER, Stigter EC (2003) Rapid surface plasmon resonance-based inhibition assay of deoxynivalenol. J Agric Food Chem 51:5843–5848

Urraca JL, Marazuela MD, Moreno-Bondi MC (2004) Analysis of zearalenone and R-zearalenol in cereal and swine feed using solvent accelerated solvent extraction and liquid chromatography with fluorescence detection. Anal Chim Acta 524:175–183

Urraca JL, Benito-Peña E, Perez-Conde C, Moreno-Bondi MC, Pestka JJ (2005) Analysis of zearalenone in cereal and swine feed samples using an automated flow-through immunosensor. J Agric Food Chem 53:3338–3344

Urraca JL, Marazuela MD, Merino ER, Orellana G, Moreno-Bondi MC (2006) Molecularly imprinted polymers with a streamlined mimic for zearalenone analysis. J Chromatogr A 1116:127–134

Van der Gaag B, Spath S, Dietrich H, Stigter E, Boonzaaijer G, Van Osenbruggen T, Koopal K (2003) Biosensors and multiple mycotoxin analysis. Food Control 14:251–254

Vega M, Castillo D (2006) Determination of deoxynivalenol in wheat by validated GC/ECD method: comparison with HPTLC/FLD. Electron J Food Plants Chem 1:16–20

Visconti A, Lattanzio VMT, Pascale M, Haidukowski M (2005) Analysis of T-2 and HT-2 toxins in cereal grains by immunoaffinity clean-up and liquid chromatography with fluorescence detection. J Chromatogr A 1075:151–158

Vishwanath V, Sulyok M, Labuda R, Bicker W, Krska R (2009) Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/tandem mass spectrometry. Anal Bioanal Chem 395:1355–1372

Wagacha JM, Muthomi JW (2008) Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. Int J Food Microbiol 124:1–12

Wang S, Quan Y, Lee N, Kennedy IR (2006) Rapid determination of fumonisin B_1 in food samples by enzyme-linked immunosorbent assay and colloidal gold immunoassay. J Agric Food Chem 54:2491–2495

Wang S, Du XY, Lin L, Huang Y, Wang Z (2008) Zearalenone detection by a single chain fragment variable (scFv) antibody. World J Microbiol Biotechnol 24:1681–1685

Weiss R, Freudenschuss M, Krska R, Mizaikoff B (2003) Improving the analysis of mycotoxins in beverages. Molecularly imprinted polymers for deoxynivalenol and zearalenone. Food Addit Contam 20:386–395

Wilkes JG, Sutherland JB (1998) Sample preparation and high-resolution separation of mycotoxins possessing carboxyl groups. J Chromatogr B 717:135–156

Zacco E, Pividori MI, Alegret S, Galve R, Marco MP (2006) Electrochemical biosensing of pesticide residues based on affinity biocomposite platforms. Anal Chem 78:1780–1788

Zachariasova M, Lacina O, Malachova A, Kostelanska M, Poustka J, Godula M, Hajslova J (2010) Novel approaches in analysis of *Fusarium* mycotoxins in cereals employing ultra performance liquid chromatography coupled with high resolution mass spectrometry. Anal Chim Acta 662:51–61

Zinedine A, Soriano JM, Moltò JC, Manes J (2007) Review on the toxicity, occurrence, metabolism, detoxification, regulation and intake of zearalenone: an oestrogenic mycotoxin. Food Chem Toxicol 45:1–18

Zöllner P, Leitner A, Jodlbauer J, Mayer BX, Linder W (1999) Improving LC–MS/MS analyses in complex food matrices, part II-Mass spectrometry. J Chromatogr A 858:167–174

Zougagh M, Ríos Á (2008) Supercritical fluid extraction as an on-line clean-up technique for determination of riboflavin vitamins in food samples by capillary electrophoresis with fluorimetric detection. Electrophoresis 29:3213–3219

Zougahg M, Téllez H, Sánchez A, Chicharro M, Ríos A (2008) Validation of a screening method for rapid control of macrocyclic lactone mycotoxins in maize flour samples. Anal Bioanal Chem 391:709–714

Part III
Interaction Plant Pathogen

Chapter 7
Fungal Infection and Disease Progression. *Fusarium* spp. Enzymes Associated with Pathogenesis and Loss of Commercial Value of Wheat Grains

Teresa M. Alconada Magliano and Gisele E. Kikot

Abstract *Fusarium* Head Blight (FHB) one of the most devasting diseases on small-grain cereals has caused severe epidemics worldwide, altering yield and quality parameters of the grains, as their weight, carbohydrate and protein composition and contamination with fungal toxins. The aggressiveness of *Fusarium* spp. utilizes different mechanisms, such as the production and release of extracellular plant-cell-wall-degrading enzymes so crucial in the processes of fungal colonization and disease establishment. A reduced secretion of enzymes might retard both the growth of the fungus on the host surface and the overall infection process, thus giving the host more time for a defensive response. Once infection is established, mycotoxins are released, which interfere with the metabolism of the host. Among the early extracellular enzymes secreted by fungal pathogens during infection, the pectic enzymes are often required for full virulence because the hydrolytic activity softens the cell walls, thus enabling the success of further infection steps and the spread of the mycelium into the inner tissues of the plant. Another group of enzymes relevant are the proteases, which degrade the storage proteins and thus have the greatest influence on quality of the grains. The nature and concentration of the proteins in wheat are some of the main determinants of its commercial value. Different techniques and methodologies are used in laboratories and in industry to analyze the protein content of wheat flours, since the result obtained enables a classification with respect to their quality and a categorization in terms of their end use.

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7.1 Introduction

Cereals are the main nutrient source of human society and because of their nutritional properties, moderate cost and ability to achieve immediate satiation they have always been a basic agricultural product. Cereals have historically been associated with the origins of the cultures and wheat – together with corn and rice is – one of the three grain having the greatest production worldwide and is also the most widely consumed by man throughout western civilization since antiquity. Wheat is used in different forms in a variety of food products; though its main destination is in traditional bread making, both industrial and artisanal processes that include a previous fermentation.

Fusarium Head Blight (FHB) is one of the most devastating diseases of small-grain cereals. Severe epidemics have occurred all over the world, altering the yield and quality of grains, as manifest in their weight, carbohydrate and protein composition and the presence fungal-toxins contaminants such as deoxynivalenol (DON) (Lazzari 2000). The Improvement International Center of Maize and Wheat (CIMMyT) has identified the FHB as the main cause of limitations in wheat production throughout many regions of the world (Bai and Shaner 1994; Xu 2003). The main etiologic agents of the FHB are *Fusarium graminearum, F. culmorum, Microdochium nivales var nivale* y *var majus*, *F. avenaceum* and *F. poae.* The dominant fungal agent associated with this disease on wheat in particular is the first of these, *Fusarium graminearum* (Schwabe) the anamorph of *Gibberella zeae* (Schw.) Petch. (Galich and de Galich 1996; Kikot et al. 2011). *F. graminearum* is prevalent in regions of Asia, North America and South America and Europe, while *F. culmorum* is more common in temperate regions (Parry et al. 1995). The distribution and prevalence of these pathogens is determined largely by weather conditions such as the temperature and humidity. In various occurrences that have been analyzed, the observed extent of damage varies according to the geographical and climatic situation surrounding the *Fusarium* spp. isolate (Walker et al. 2001; Gagkaeva and Yli-Mattila 2004; Láday et al. 2004).

The aggressiveness of *Fusarium* spp. involves different mechanisms or components, with the production and release of extracellular enzymes that degrade the cell wall being crucial in the processes of colonization and establishment of the disease (Martínez et al. 1991; Alconada and Martínez 1994, 1996; Mestherhazy et al. 1999; Kikot et al. 2009, 2010). A reduced secretion of enzymes might retard both the growth of the fungus on the host surface and the infective process, thus giving the host more time to muster a defensive response (Jenczmionka and Schäfer 2005; Kang and Buchenauer 2000a, b).

Once the infection is established, mycotoxins are released and interfere with the metabolism, physiologic processes and structural integrity of the host cell. Mycotoxins may have a more consequential influence on the progress of infections on cereal plants than the pathogenicity factors determining the capability of infection (Proctor et al. 1995; Harris et al. 1999). The major mycotoxin produced by F. *graminearum* is the vomitoxin deoxynivalenol (DON) and its precursors. In some instances a strong association has been found between the severity of the FHB and DON concentration in infected grains. The genetic diversity of *F. graminearum*, as well as the presence of other species of *Fusarium*, is related to the variety of

mycotoxins produced during the infection process, a condition that influences the severity of the disease (Langevin et al. 2004).

An exhaustive and thorough review on the histology and physiology of FHB has been written by Bushnell et al. (2003). Our interest in the current revision is to focus specifically on the enzymatic aspects of the infection process. Only scant studies have been carried out in Latin America investigating infection and disease progression within the context of the particular enzymes related to pathogenesis and wheat-grain damage. The present review thus includes the most recent information on the issue worldwide that will complement and hopefully provide a basis for future regional research.

7.2 Pathogen

7.2.1 Fungal Infection and Disease Progression

FHB is considered a floral and monocyclic disease, with an initial parasitic phase when the pathogen is developed on functional tissue and a final saprophytic one on tissues after death. Although the anthers are clearly the first parts of the floret parts to be infected, the fungus may be able to initiate the infection on the surface of the spikelet (Ireta Moreno and Bekele 1987; McMullen et al. 1997). Reis and Carmona (2002) concluded that perithecia (sexual phase, with ascospores) and conidia (asexual spores) develop on the surface of infected plants or crop residues where the fungus grows saprophytically and thus guarantee the availability of an annual inoculum (Guenther and Trail 2005). Ascospores are released from the perithecia for at least 18 h under favorable conditions of temperature, from 12 to 34 °C (preferably 25 °C) and a relative humidity greater than 80 % (McMullen et al. 1997) and can be dispersed hundreds of kilometers by the wind. Conidia are produced in viscous masses called sporodochia (cushion-shaped hyphal structures). The conidia, however, can be dispersed only a short distance through rain runoff.

If ascospores or macroconidia make contact with spikelets of wheat through the wind or rain flow, the fungus initiates the pathogenic phase. During the short period between the flowering and the beginning of grain development (about 10–20 days), the wheat plants – and especially those with exposed anthers – are highly susceptible to infection by *Fusarium* spp. Anthers, for their part, are reported to be the primary infection site since fungal spores may land there and then grow into the kernels, glumes, or other head parts. The infection can thus occur from the time of anthesis to the soft dough stage of kernel development (McMullen et al. 1997).

The *Fusarium* spp. pathogens have no specialized structures such as appressoria or haustoria for the penetration of the host cell; rather, the fungus either enters the host through natural openings (Pritsch et al. 2000), or penetrates the epidermal-cell walls directly with short infection hyphae (Wanjiru et al. 2002). Jansen et al. (2005) observed that the hyphae of germinating *F. graminearum* spores use different paths of infection on wheat upon analysis of the progress of infection on isolated caryopses. The ability of *F. graminearum* macroconidia to adhere to and germinate on the host

tissue presumably plays a critical role in the localized dissemination of FHB. Once inside the tissue, *F. graminearum* is able to spread systemically. The hyphal growth on the infected spikelet spreads to adjacent spikelets throughout the rachis (Kang et al. 2005). The extension of the hyphae of the pathogen both inter- and intracellularly throughout the parenchyma and vascular tissues of the lemma, glume, ovary and rachis causes severe damage to the host tissues. Subcuticular fungal growth occurs and the tissue eventually becomes abundantly colonized intra- and intercellularly (Kang and Buchenauer 2000a, b; Pritsch et al. 2000; Wanjiru et al. 2002). Rittenour and Harris (2010, 2012) observed that two morphologically distinct hyphal structures – subcuticular and bulbous infection hyphae – are produced by *F. graminearum* during the invasion of detached wheat glumes.

Several research groups studied the progression of infection by focussing on the sequence of histologic changes produced. Ireta Moreno and Bekele (1987), analyzing the *F. graminearum* penetration of wheat in Paraguay, observed that the disease caused alterations in the chlorenchyma and other tissues. In Argentina, the exomorphologic characteristics of Sumai 3 and original native susceptible wheat varieties were observed to be able to either help or hinder the invasion and expansion of the pathogen on the spike (Ribichich and Vegetti 2001). Both cultivars also underwent a horizontal progression of disease, from the anthers to the glumes, and a vertical one, from the anthers to the rachis (Ribichich et al. 2000). When disease development was followed after point inoculations of *F. graminearum* isolates from Argentina on the spikes of field-grown wheat, Malbrán et al. (2012) observed a variation in aggressiveness and analysed the infection efficiency along with both the size of the lesion attained and the movement of the pathogen over the spike.

Stated in brief, the generalized model for the progression of infection involves the entry of the pathogen through an exposed anther followed by the penetration of the ovary and the successive infections of the floral bracts (Pritsch et al. 2000). The hyphae develop on the outer surfaces of the flowers and glumes, allowing the fungus to make growth extensions toward the stomata and other sensitive inflorescence sites (Bushnell et al. 2003). The fungus next grows both inter- and intracellularly in the ovary and bracts and spreads from one spikelet to the other along the rachis. Finally, the growth of fungal hyphae during the later stages of the infection is accompanied by a disintegration of the organelles of the host cell along with a degeneration of the cytoplasm and subsequent collapse of some of the parenchymal cells (Wanjiru et al. 2002). All of these drastic effects results from the action of the enzymes and the mycotoxins, with both playing an essential role in the fungal pathogenicity (Fig. 7.1).

7.2.2 Morphogenesis in Germinating Macroconidia

Spore maturation and germination are essential developmental steps in the fungal life cycle and are critical for plant colonization (Seong et al. 2005). The cyclic adenosine-3′,5′-monophosphate (cAMP) and mitogen-activated protein kinase

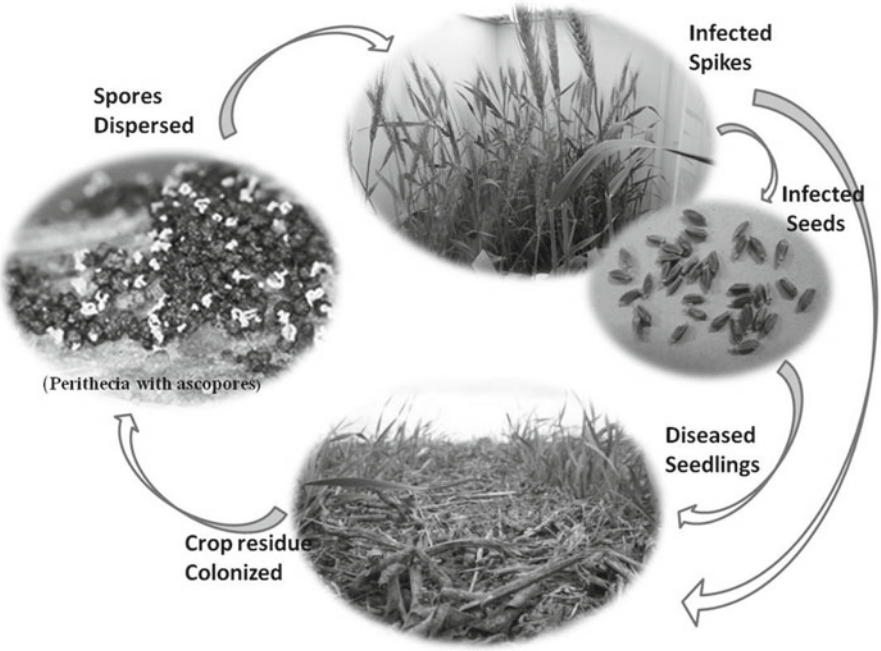

Fig. 7.1 Infection cycle of wheat by *Fusarium graminearum*

(MAP-kinase) signaling pathways have a general role in coordinating multiple events that regulate the morphogenesis during spore germination (as reviewed by Xu 2000). The identification of landmarks associated with spore germination represents a preliminary step toward the development of tools that can be used to characterize early events in the interaction between the pathogen and its hosts. Germination must occur at both the correct time and the appropriate location in order for infection to be successful. The ability of *F. graminearum* macroconidia to adhere to and germinate on host tissue presumably plays a fundamental role in the localized dissemination of FHB.

F. graminearum forms multicellular macroconidia or spores that are essential in the dissemination of the disease. Germ tubes emerge preferentially from the apical cells in a bipolar pattern that appears to be common to filamentous fungi. Chitin deposition occurs at two locations; the spore apices plus the cortical regions of the macroconidial cells that subsequently produce a germ tube. The spatial pattern of morphogenesis involves the participation of the functional microtubules that may be responsible for the transport of key polarity factors to specific target sites. Consistent with this notion, the spatial pattern of morphogenesis in germinating macroconidia was analyzed by Harris (2005) through the use of fluorescent microscopy. Different patterns of chitin deposition were observed that were associated with germination and involved a discrimination between the chitin-rich and chitin-poor regions of the

new germ tube. This pattern suggested that *F. graminearum* possessed a mechanism for determining the sites of germ-tube emergence. In addition, the marker proteins that designate these sites may participate in a fundamental way in the interaction with the host surface. A perturbation of this site-determination mechanism may constitute an effective approach to inhibiting colonization of host-plant surface.

7.2.3 The Structure of Plant Cell Walls

Upon initial association with the hosts plant, a pathogen encounters the epicuticular waxes and cuticle that coat the host epidermal cells. This wax consists in a relatively soluble complex mixture of long-chain fatty acids, aldehydes, alkanes, primary and secondary alcohols, ketones and wax esters; whereas the cuticle is a continuous layer of lipid material that is comprised of an insoluble polymeric material called cutin. This cuticular matrix also decreases the vulnerability of plants to pathogen attacks both by providing a mechanical resistance and by triggering cellular signals for defense responses (Feng et al. 2005; Feng 2007).

Directly under the waxes and cuticle, the plant cell wall – one of the principal barriers for the protection of plants against pathogens – plays a crucial role in the apoplastic diffusion of water and ions in addition to shielding the internal protoplast. Cell walls contain a middle lamella, the outermost part of the wall, shared by adjacent cells. A primary-wall layer is initially laid down inside the middle lamella, but upon the cessation of cell enlargement a secondary layer may form. The cell wall contains predominantly cellulose, matrix materials, and water. Cellulose, a major component of the primary- and secondary-wall layers, is a straight-chain polymer of glucose (β-1,4-glucan). Groups of 100–10,000 cellulose molecules are linked by hydrogen bonding to form microfibrils. The microfibrils contain crystalline areas, termed micelles, while the rest of the structures are paracrystalline. The matrix materials – distributed between the cellulose microfibrils (Carpita and Gibeaut 1993) is composed of pectic substances, hemicelluloses, and structural proteins. These amorphous materials are distributed between the cellulose microfibrils. Pectic substances – with pectin being a family of complex and highly heterogeneous polysaccharides – constitute the main components of the middle lamella and also make up a large portion of the primary cell walls of young plant, where they form an amorphous gel filling the spaces between the cellulose microfibrils (Alkorta et al. 1998). The pectin backbones mainly consist in a α-1.4-linked-D-galacturonic-acid residues that form homogalacturonan chains. The side chains of this backbone structure contain L-rhamnose, arabinose, galactose and xylose (An et al. 2005; Jayani et al. 2005). The carboxyl groups of galacturonic acid are partially or completely neutralized by sodium, potassium or ammonium ions. According to the type of modifications in the backbone chain, the pectic substances are classified into protopectin, pectic acid, pectinic acid and pectin (Kashyap et al. 2001). Hemicelluloses are polymers of diverse groups of sugars such as xylose, glucose, and arabinose, and are abundant in the primary-walls layers. Xylan – the major constituent of hemicellulose, particularly in the primary-cell walls layers of monocotyledoneous plants – forms a complex

structure composed of a D-xylose backbone linked by β-1,4-bridges, more or less substituted by various groups (acetyl, arabinofuranosyl, galactosyl, glucuronyl and methyl glucuronyl) (Hatshc et al. 2006).

Cell walls also contain a variety of protein-containing compounds, such as glycoproteins and extensin. Glycoprotein is a complex of sugars and proteins, where the latter are characterized by abundant hydroxyproline and proline residues within the wall. Extensin – often considered to be the major structural protein (Carpita and Gibeaut 1993) – can bond covalently with other proteins and wrap around other wall constituents (Kieliszewski and Lamport 1994). In addition to the cellulose and matrix materials, water fills approximately 50 % of the space in typical primary-walls layers (Kikot et al. 2009).

7.2.4 Cell-Wall-Degrading Enzymes

FHB is the result of the individual or combined action of different pathogenic mechanisms such as the production and release of extracellular cell-wall-degrading enzymes (CWDEs) that facilitate the colonization of tissues (Martínez et al. 1991; Alconada and Martínez 1994, 1996; Kikot et al. 2009, 2010). Kang and Buchenauer (2000a, b), Kang et al. (2005) and Wanjiru et al. (2002) demonstrated that *F. graminearum* and *F. culmorum* produced CWDEs such as cellulases, xylanases and pectinases in wheat spikes at early stages of infection, and observed alterations in the cell-wall components of the infected host tissue. Those studies suggested that the CWDEs produced by this phytopathogen, after initially enabling tissue penetration, facilitated the consequent establishment of disease and a rapidly ensuing colonization of the wheat spikes. In several instances conclusive evidence has been obtained for the essential participation of CWDEs in the infection process by *Fusarium* spp. (Nightingale et al. 1999; Pekkarinen et al. 2000; Roncero et al. 2003; García Maceira et al. 2001; Feng et al. 2005; Jenczmionka and Schäfer 2005; Feng 2007), and those hydrolases have also been considered as fundamental virulence factors in several studies on *F. graminearum* (Hou et al. 2002; Jenczmionka et al. 2003; Dyer et al. 2005; Seong et al. 2005; Voigt et al. 2005). Phalip et al. (2005) analyzed the diversity of the exoproteome of *F. graminearum* grown on plant cell wall, and identified 24 different enzyme types necessary for the digestion of the complete wall.

Early studies suggested that fungal cutinases exerted an essential action in the penetration of plant surfaces (Feng et al. 2005). Accordingly, cutinases were thought to have been involved in the lysis of the cuticle tissues of wheat kernel upon infection by *F. culmorum* (Kang and Buchenauer 2000b; Kang et al. 2005).

Upon observation of the subcuticular growth of *F. graminearum* after host penetration, Pritsch et al. (2000) suggested that lipases might have participated to a certain extent in the prior degradation of the cuticle. Lipases are hydrolases that cleave substrates such as triglycerides and phospholipids that contain long-chain fatty acids (Lopes et al. 2011). The relevance of lipases to phytopathogenicity has been shown for different fungi, including *F. graminearum* (Feng et al. 2005; Comménil et al. 1998; Berto et al. 1999; Jenczmionka and Schäfer 2005).

Among all the lipase genes identified from plant pathogenic fungi, only the *fgl1* from *F. graminearum* has been demonstrated by direct evidence to be a pathogenicity factor (Voigt et al. 2005).

Moreover, the crucial participation of the pectinases in the establishment of infection by diverse phytopathogenic fungi has likewise been indicated (ten Have et al. 1998; García Maceira et al. 2001; Valette Collet et al. 2003; Roncero et al. 2003). Pectic enzymes produce modifications in the structure of the cell wall and so doing increase the accessibility to other cell-wall components for degradation by additional enzymes, cell lysis and tissue maceration (Panda et al. 2004). The pectic enzymes (in multiple forms) are the first to be induced when fungi are cultured on plant cell walls – and the first to be produced in infected tissue (Martínez et al. 1991; Niture et al. 2006) – followed by cellulases and hemicellulases (Kikot et al. 2010).

The complete hydrolysis of xylans requires the action of several enzymes, probably analogous to the synergistic enzymic action involved in crystalline-cellulose degradation (Kluepfel et al. 1990). Xylanases are assumed to be a likely virulence factor for the pathogens of monocotyledons, as xylan is the most abundant component of the cell wall in this group of plants (Apel et al. 1993). Among these xylan-degrading enzymes, the action of the feruloyl esterases deserves mention: These enzymes hydrolyze phenolic groups involved in the cross-linking of arabinoxylan to other polymeric structures present in the cell wall – particularly within Gramineae family – so as to facilitate the access of depolymerases to the polymers of the wall backbone (Shimokawa et al. 2002; Hermoso et al. 2004; Beliën et al. 2005). The endoxylanases randomly cleave the β-1,4-bonds in the polyxylose backbone, yielding oligosaccharides of varied chain lengths. The degradation by β-xylosidase produces D-xylose both from short-chain oligosaccharides and from xylobiose (Huang et al. 1991). The action of exoxylanases is less frequent (Kluepfel et al. 1990), although whether or not this exoenzyme is a separate entity from the β-xylosidase remain unclear (Hayashida et al. 1988). These same enzymes are required for the total hydrolysis of cellulose to glucose, but there through the synergistic action between exocellulase, endocellulase and β-glucosidase (Wood and García Campayo 1990). A rapid secretion of xylanases and cellulases has, in fact, also been thought to be essential for the infection of various other plant pathogenic fungi (Gómez Gómez et al. 2001; Lev and Horwitz 2003).

Another group of enzymes relevant in the pathogenic process are those catalyzing proteolysis, referred to as proteinases, proteases or peptidases. These hydrolases cleave the peptide bonds of proteins and occur naturally in all living organisms, and constituting from 1 to 5 % of the genomic content. Together with the CWDEs or carbohydrases, the proteases act at an early stage of infection to degrade the structural proteins of the cell walls in order to invade the host. At a later stage these enzymes are responsible for the degradation of the grain's storage proteins, thus altering the quality parameters of the raw material (Nightingale et al. 1999; Barneix 2007; Brzozowski et al. 2008a). The protease activities from *Fusarium* spp. have been studied with respect to their inhibition kinetic, have been purified and characterized, and were judged to be virulence factors by several groups of investigators (Pekkarinen et al. 2002, 2003; Pekkarinen 2003; Pekkarinen and Jones 2003).

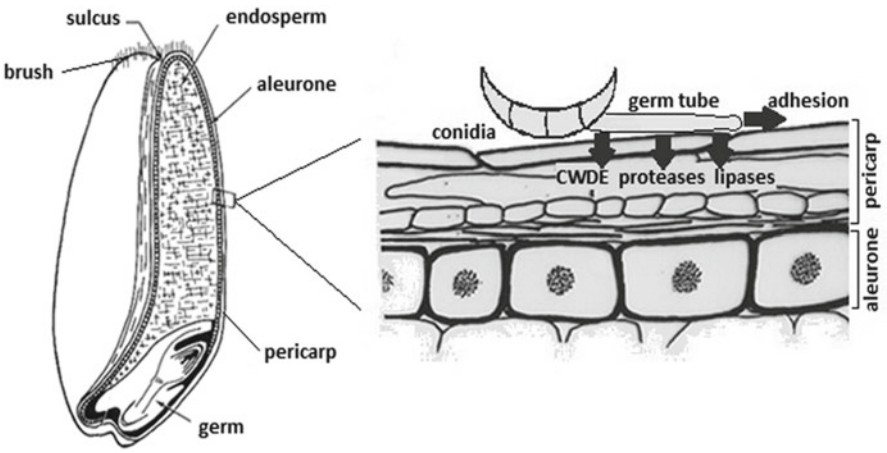

Fig. 7.2 Schema of wheat-grain tissues. Longitudinal section of a grain (*left*) and detail of the external grain layers showing the pericarp and aleurone cells and the principal enzyme activities in relation to the degradation of cell walls and storage substances (*right*)

That the pectinase enzymes have a prevailing role of in the successful establishment of infection explains the classification of this group in the following greater detail.

The pectic enzyme group comprises the pectinesterases, catalyzing the deesterification of the pectin plus the depolymerising enzymes, those cleaving the α-(1,4) glycosidic bonds of the pectin chains. The pectinesterases belong to the group of the carboxyl-ester hydrolases. The depolymerases catalyze the breakdown of α-(1,4) glucosidic linkages of pectic substrates either by hydrolysis (via the D-galacturonase hydrolases) or by a ß-elimination mechanism (via transeliminase enzymes or lyases). These latter two groups have been further divided into four subgroups: first, according to the preferred substrate between pectate (pectic-acid salts) or pectin (esterified linkages); second, in view of the terminal-action pattern in the scission of glycoside bonds – i.e., either end (exo) or random-internal (endo) cleavage. Thus within this classification, the depolymerizing enzymes are divided into eight subgroups, four corresponding to hydrolases and four to lyases or transeliminasas (Jayani et al. 2005; Kikot et al. 2009) (Fig. 7.2).

7.2.5 Microscopy Techniques for the Analysis of Infection

Cytological studies were carried out to elucidate the functioning of the CWDEs during the infection process and the colonization pathway on wheat spikes by *Fusarium* spp. In these investigations, the different forms of visualization – light and epifluorescence microscopy, scanning electron microscopy and transmission

electron microscopy – were relevant for clarifying the mechanism studied (Kang and Buchenauer 2000a, b; Wanjiru et al. 2002). In some instances enzyme-gold and immune-gold-labelling techniques were utilized for microscopial visualization to elucidate the roles of specific CWDEs such as cellulases, xylanases and pectinases (Kang and Buchenauer 2000a; Wanjiru et al. 2002). These results enabled a confirmation that *F. graminearum* penetrated and invaded its hosts with the help of secreted CWDEs. By scanning electron microscopy, Pritsch et al. (2000) observed with inoculated glumes that the fungus penetrated, grew subcuticularly, colonized glume parenchymal cells, and finally sporulated within 2–3 days. Good results were obtained for infection visualization when the infected tissue was stained with calcofluor and observed by epifluorescence microscopy with ultraviolet irradiation. Calcofluor is a fluorochrome of the diaminostilbene-disulfonate type that stains fungi in culture and during plant infection (Pritsch et al. 2000). Scanning electron micrographs revealed that a few hours after inoculation of single spikelets with the macroconidia of *F. graminearum,* the fungus germinated, forming several germ tubes, and then developed a dense hyphal network in the cavity of the spikelets. Next, the fungal hyphae invaded the ovary and inner surface of the lemma and palea. Finally, when the grains were infected, the starch granules of the endosperm became extensively degraded (i.e. pitted; Jackowiak et al. 2002), with the storage-protein matrix that had surrounded the starch granules being no longer present (Nightingale et al. 1999; Pekkarinen et al. 2000). These observations were confirmed by transmission electron microscopy; where the fungi were found to extended inter- and intracellularly within the ovary, lemma and rachis causing considerable damage throughout along with alterations on the host-cell walls.

Through the use of immuno-gold and enzyme-gold labelling followed by transmission electron microscopy, the production of CWDEs such as pectinase, cellulase and xylanase was demonstrated during the infection and colonization of wheat spikes by *Fusarium* ssp. The localization of cellulose, xylan and pectin indicated that the host-cell walls that were in direct contact with the pathogen surface had reduced gold labelling compared to the considerably higher densities in the walls distant from the pathogen-host interface or in those in noncolonized tissues. The reduced gold labelling densities in the infected host-cell walls indicated that these polysaccharide-degrading enzymes were very likely consequential pathogenic factors during the infection of wheat spikes by *F. graminearum* (Kang and Buchenauer 2000a; Wanjiru et al. 2002).

7.2.6 Genomic Analysis in the Study of Enzymes

Significant progress has been made in our understanding of the infection process of wheat by *F. graminearum* (Miller et al. 2004; Skadsen and Hohn 2004). The impact of rapid advances in fungal genomic technologies has enabled the identification of numerous new genes that are potential candidates for involvement in the disease process (Kruger et al. 2002; Trail et al. 2003). The genomic sequence of *F. graminearum*

was published in 2003 by the Broad Institute (http://www.broad.mit.edu) and this information enabled the creation of deletion constructs, knock-out mutations, and a breakage in the secretion pathway of the exoproteome in order to dissect out the various enzymic function (Cuomo et al. 2007; Trail 2009). In addition, recombinant-DNA technology has been employed in the attempt to provide evidence concerning the functioning of CWDEs in plant pathogenesis. When *F. graminearum* knock-out mutants and disrupted genes were studied during wheat infection, a lipase-deficient mutant was strongly reduced in virulence relative to the respective wild-type isolate (Voigt et al. 2005). Beliën et al. (2005) cloned and characterized two endoxylanases from *F. graminearum* and analyzed their inhibition profile against endoxylanase inhibitors from wheat. The MAP kinase regulates the pathogenic stages of many filamentous fungi (Di Pietro et al. 2001; Hou et al. 2002). Jenczmionka and Schäfer (2005) observed that the Gpmk MAP kinase-disruption mutants of *F. graminearum* exhibited a reduced induction of lipolytic activities and determined that the enzyme regulated the early induction of the extracellular endoglucanase plus the xylanolytic and proteolytic activities.

7.3 Wheat

7.3.1 The Structure of the Grain

Cereal grains, are dry fruits with a single seed called caryopses. The grain size varies widely depending on the wheat variety and the placement of the grain within the spike. The average grain length is 8 mm with a weight of 35 mg. The grains are rounded on the dorsal side (the germ); possess a sulcus or furrow, along the ventral side; and have a group of trichomes, or hairs on the apical surface, called the brush. Both the sulcus and the hairs are sites for adhesion and shelter of the microorganisms (Dimitri 1978). The pericarp that surrounds the whole grain is composed of several layers of different cell types, whose characteristics bear a relationship to their ease of removal during the grinding process. The pericap constitutes 5 % of the grain, and its chemical constitution enables a high water-absorption capacity. The seed coat is strongly attached to the pericarp and also consists of three layers with different characteristics: a thick outer cuticle, a middle layer (pigmented in the wheat) and a thin inner cuticle. The endosperm, or grain reserve substance, is rich in carbohydrates and proteins. The endosperm's texture can be hard or soft -referring to the resistance offered by the grain to become separated into flour particles. The outer layer – in general a single cell layer with thick walls, a lack of starch, and numerous proteins granules – is called the aleuron and contains high enzymatic activity. In the milling of wheat this layer is removed along with other exterior layers, which chaff constitutes the bran. The germ or embryo – making up about 2.5–3.5 % the weight of the grains – contains no starch but is relatively rich in protein and certain vitamins in addition to being a source of many enzymes (Mabile et al. 2001; Shewry and Halford 2002) (Fig. 7.2).

7.3.2 Chemical Composition and Enzymes of the Grain

The grain of wheat can be considered as composed fundamentally (by weight) of starch (60–68 %), protein (7–18 %), non-starch-containing polysaccharides or crude fiber (2–2.5 %), lipids (1.5–2.0 %), mineral or ashes (1.5–2.0 %) and vitamins (Matz 1999; Shewry and Halford 2002). The enzyme composition includes both α- and β-amylase – the latter an exoenzyme responsible for degrading the nonreducing ends of the polymer to effect a rapid decrease in the size of the starch molecules and the release of maltose. This enzyme is present in whole, healthy grains, and the concentrations do not change appreciably with germination. By constraint, α-amylase is present at low levels in dried grains, but increases in amount significantly during germination. Thus, the *de-novo* synthesis and secretion of this enzyme is an indicator of the pre- and postharvest status of the grain. Proteases are also found in mature, and healthy grains, but at relatively low levels of activity. Lipases too are found in all cereals at varying concentrations, and are consequential because the free fatty acids generated by lipolysis are more susceptible to autooxidation, and rancidity than in the esterified form in triglycerides. The lipases generated by the microorganisms present in flours also produce the same effect, but can be inactivated by heat treatment in order to prevent deterioration in quality (Ponzio 2010).

7.3.3 Genotype, Environmental Influences, and Quality Traits of Wheat

The choice of which wheat genotype to sow is determined by the end use of the product along with the technological or commercial destination (Turnbull and Rahman 2002). According to the texture of the endosperm, given wheat is classified as either hard or soft. Hard wheats give a generally higher flour yield and a higher percentage of protein than do soft wheats. Durum-wheat flours are destined for baked products that must withstand severe conditions depending on the type of product and the industrial processing involved (De Sá Souza 2009).

The growth curve of a grain of wheat has a typical sigmoid shape and is divided into three phases: The first is characterized by active cell division, when the spaces where starch and proteins will accumulate are created; the second, or grain-filling phase, is the period of synthesis and accumulation of the greatest amount of starch and protein within the grain; and the final physiologic-maturity phase is when the reserve substances in the grain cease from accumulating (Altenbach et al. 2003).

The yield and quality of wheat, depend on both the genotype and the influence of the environmental conditions such as availability of nitrogen and water, the soil type, the temperature and the organisms that interact with the crops (Triboï et al. 2003; Abbate et al. 2010). Any alteration, such as hydric stress or extreme temperature conditions will cause changes in the grain composition. Nitrogen fertilization is an appropriate management strategy to enhance the protein content of wheat, as indicated both by an increased percentage of protein and by the relationship among

the different types of proteins present, whose nature and interaction can improve the quality of the raw material. An essential characteristic for consideration within this context is the ability of a cultivar to fix nitrogen along with the management practices to be used. The use of the most correct cultivar in the best environment with the most appropriate form of management in order to attain the highest possible yield and quality per unit are is the challenge to be met. Extensive and significant research has been conducted in order to evaluate the relative magnitude of the influences of genotype, environment and overall genetic-environmental interactions on the wheat quality of cultivars developed within different agroecological zones in Latin America. To that end, genotypes released in Argentina, Brazil, Chile, Mexico, Paraguay, and Uruguay, were cultivated in different environments. Each environment was characterized according to cultural practices, soil type and climatic conditions, and the grain yield determined along with analyses of the flour product's; protein, ash and gluten contents; Alveograph and Farinograph values; Falling Number; sodium-dodecylsulfate-sedimentation properties; and color (Vázquez et al. 2012).

7.3.4 Wheat Storage-Protein-Degrading Enzymes

Several fungal enzyme activities are responsible for grain damage, with the proteases that degrade the wheat's protein stores being those having the greatest impact on grain quality. Accordingly, the action of the proteases synthesized by *Fusarium* spp. result in quantitative and qualitative changes in the proteins of wheat endosperm (Eggert et al. 2011), while the hydrolysis products released from fungi infected cereal proteins, in addition to diminishing the quality of the wheat products, can cause toxicity and provoke allergic reactions (Brzozowski et al. 2008a, b; Yike 2011).

More than 2,000 different proteases have been found and this number continues to increase. The magnitude and diversity of these enzymes has led to the need for an adequate system of classification and nomenclature. The proteases are associated with a large number of physiologic reactions ranging from the simple digestion of food to complex metabolic pathways, but differ from most other enzymes in that the substrate specificity of a given protease can be extremely difficult to define. Therefore, a classification has been proposed based on the characteristics of catalytic mechanisms of the enzymes so that different groups can be distinguished; with those being, namely, the serine, cysteine, aspartate, and threonine proteases plus the metallopeptidases (Hartley 1960). Whereas the specific chemical residues involved in the different stages of proteolysis differ among the enzymes of these protease groups, the cleavage of the peptide bond – the main process and end objective – is identical in all instances. Although each of the five types of proteases has a distinctive catalytic mechanism, the five modes of action can be grouped into two broad categories: those that form covalent complexes between the enzyme and the substrate (e.g. serine, cysteine and threonine proteases) and those that do not form such covalent complexes (e.g. aspartic proteases and metallopeptidases). The initial contribution of Hartley (1960) to the classification regulation of the

proteolytic enzymes contributed toward the eventual establishment of the two current systems and nomenclature for the proteases: the Enzyme Commission (EC) and Merops system. The EC system recognizes two major groups according to the location of the linkages that are hydrolyzed; the exopeptidases hydrolyzing at the ends of peptide chains and the endopeptidases cleaving internal peptide bonds in the chain. The latter group is, in turn, divided into subclasses based on the catalytic mechanism involved, as Hartley (1960) had originally proposed. In the MEROPS database, the classification of proteases is based on the essential structural features of the enzyme and the evolutionary relationships among them through the use of the available information on their amino-acid sequences. The hierarchical structure is therefore based on the concepts of catalytic type, clan, family, and exo- or endopeptidase activity (MEROPS http:/merops. sanger.ac.uk/) (Fig. 7.2).

FHB produces both a reduction in the yield and an alteration in the quality of the grains. The yield losses result from a decrease in the number, size and weight of the grains (Bai et al. 2001; Snijders 2004), while the diminution in grain quality is brought about when the components are altered by the pathogen (Boyacioglu and Hettiarachchy 1995; Eggert et al. 2011). These alterations occur in the form of an enzymic degradation of the cell walls and storage substances and a contamination with mycotoxins. In this regard, the effect of infection by *Fusarium* spp. on grain-cell structure and composition has been well documented by several authors. Different histochemical studies have demonstrated that grains damaged by *Fusarium* spp. show significant changes in the cellular structure as well as in the composition of carbohydrate, protein, and starch granules of the endosperm reserve (Meyer et al. 1986; Chelkowski et al. 1990; Boyacioglu and Hettiarachchy 1995). These last authors showed that moderate *F. graminearum* infection caused significant compositional changes in the carbohydrates, lipids, and proteins of American hard red spring wheat. In another study cereal grains were found to contain cavities and furrows in the endosperm of the starch granules, thus evidencing damage caused by amylases (Hammond Kosack and Jones 2000; Jackowiak et al. 2002) along with protein degradation by proteases from *Fusarium* spp. (Nightingale et al. 1999). Pekkarinen (2003) characterized the proteases synthesized by *Fusarium* species that degrade grain proteins during infection and examined proteins that could inhibit those enzymes. In that investigation the proteases were characterized and identified as endopeptidases – those having a fundamental role in the degradation processes that reduce the quality of the proteins in flour and consequently the properties conducive to productive baking (Pekkarinen et al. 2007). Bechtel et al. (1985) likewise observed that *F. graminearum* was capable of destroying starch granules, storage proteins, and cell walls during the invasion of wheat grains. Dexter et al. (1996) showed that grain sample from Canadian hard red spring wheat that had been damaged by *Fusarium* exhibited weak dough properties and unsatisfactory baking quality. The *Fusarium* proteases also continue to hydrolyze endosperm proteins during dough mixing and fermentation so as to result in weaker dough and a decreased loaf volume. In other cereals, such as barley *Fusarium* was also observed to reduce the yield and quality of the food products made from infected grain (Schwarz et al. 2001, 2002; Pirgozliev et al. 2003).

Fusarium spp. damage to wheat – both in the field and during the fermentation of the dough mass – as a result of the presence of enzymes in the raw material, effects changes in the color of the flour and the rheological properties of the dough, resulting in a deterioration in the quality of the bread.

7.3.5 Grain Analysis

Early infections can cause grain abortion and a limitation in the total number that the spike will produce; whereas most late infections only go so far as stunting the development of the grains so as to result in a shrivelled, wrinkled appearance and an extremely small size with a consequently low hectolitric weight. The tegument takes on a whitish color with occasional pink areas, and the endosperm has a chalky appearance of intensely white color. In the crops, spikes and spikelets of whitish color are present in contrast to the green of unaffected plants. The fungus develops in grains of moisture content higher than 22–25 % and resides in the tissues of the plant and in the grains before maturity. For this reason, during grain storage fungal development would not be possible because the moisture of the grain is usually lower than that value so that as a consequence no risk is incurred of a production of mycotoxins. Since the toxins become concentrated in the periphery of the grain, the milling removes most of them a notable detail.

The protein content of wheat is one of the main determinants of the commercial value since the industrial qualities of the grain depend on the concentration and type of its proteins with those parameters, in turn, being a function of the variety of wheat, the environmental conditions, and the level of infection present in the grains before and after harvest (Barneix 2007; Zhu and Khan 2001).

One approach used to help understand how wheat-protein composition relates to the functional properties of flour is to survey a large number of individual wheat samples and examine how well different quantitative variations in the protein composition correlate with a range of quality parameters. Although high correlations do not necessarily signify cause-and-effect relationships, this procedure is useful in conjunction with other independent approaches for providing insight (Huebner et al. 1997).

7.3.5.1 Protein Composition

Protein is the major constituent of the wheat flour after carbohydrate and has been traditionally classified into albumins, globulins, gliadins, and glutenins as a reflection of their solubility in different solvents. The storage proteins, gliadin and glutenin, are the main constituents of the gluten, a viscoelastic network necessary to support the rest of the chemical components of the wheat, primarily carbohydrates, and retain the gas produced during fermentation. The functionality of gluten is one of the main parameters that governs the quality of flour and consequently the

breadmaking potential of the wheat. The gliadins were classified as ω, α, β and γ in relation to their electrophoretic mobilities, with the first of these having the highest molecular mass – it in the range between 50 and 65 kD (Hamauzu et al. 1974). These proteins have little or no resistance to extension and appear to be responsible for the coherence and viscosity of the dough. After subsequent studies enabling a more complete understanding of their composition, the glutins were grouped according to their sulfur content (Shewry et al. 1986). Accordingly, the gluten proteins can also be classified as: sulfur-rich or sulfur-poor. In the gluten network, the elasticity is determined by the intermolecular disulfide bonds between glutenin molecules; whereas the viscosity is determined by the monomeric fraction of gliadins, with the latter having only intramolecular disulphide secondary structures. The glutenins are a heterogeneous group of proteins, whose molecular weight ranges from about 100,000 to several million, with an average of about three million (Arfvidsson et al. 2004). The glutenin subunits are formed by high- and low- molecular weight species (HMWG and LMWG respectively) (D'Ovidio and Masci 2004; Belton 2005) linked by disulphide bonds. The gliadin-glutenin molar radio, and the size of glutenin polymers, determine the rheological properties of the dough. HMWG subunits are responsible for the fingerprint of wheat cultivars since the various combinations of these protein species form a unique pattern for each variety. These subunits furthermore have been the most studied in relation to the elasticity of the dough and its baking performance since, along with the LMWG subunits and gliadins, the molecular associations among the larger subunits are responsible for the formation of the viscoelastic network of the gluten occurring in the presence of water and upon mechanical work (Ponzio 2010).

The retention of CO_2 during fermentation and subsequent baking of the bread depends on the quality of the constituent proteins. The proteolytic activity that is present in infected grains leads to the breakdown of protein structure, resulting in breadmaking masses with low cohesive properties and high extensibility, with little retention of the gas liberated during fermentation: the end result is a reduced loaf volume and a poor bread texture (Hariri et al. 2000).

7.3.5.2 Protein Measurement

Itemized below are different methodologies for analyzing the protein composition of wheat flours; all have different levels of sensitivity and/or accuracy. The type of measurement of choice depends on the purpose, the experience of the analyst, and the time available for obtaining the result.

The indirect detection of alterations in wheat protein through the action of proteases is done with an alveograph (measuring the extensibility of the gluten network), a farinograph (evaluating the variation of the consistency of the dough), or an extensograph (detecting a modification in the extensibility) (Cuniberti et al. 2003).

The direct detection of changes in the constituent proteins is carried out by spectrophotometry. A fast method of determining a diminution in wheat breadmaking quality as a result of excessive thermal treatment during drying was proposed by

Tosi et al. (2000), where the extracted proteins are stained with Coomassie Brilliant Blue G 250 and measured at 595 nm.

An analysis by sodium-dodecylsulphate-polyacrylamide-gel electrophoresis (SDS- PAGE) is the technique most widely used worldwide for identifying the subunits of glutenin and gliadin through the differential electrophoretic mobility of the subunits (Payne et al. 1981; Nightingale et al. 1999; Brzozowski et al. 2008a, b; Eggert et al. 2011). In a study of Argentine wheat, Steffolani et al. (2007) concluded that when the protein range of a given sample was narrow, the electrophoretic profile was able to group a cultivar according to its flour quality by means of cluster analysis.

Analysis by high-performance liquid chromatography (HPLC): The application of HPLC to the analysis of cereal proteins (Bietz 1986), facilitated the establishment of a precise relationship between measurements of protein composition and the quality parameters. Changes in the profile of the reserve proteins within a grain product through proteolytic activity were then analyzed efficiently by the size-exclusion form of HPLC in numerous studies (Nightingale et al. 1999; Larroque et al. 2000; Ueno et al. 2002; Wang et al. 2005; Prange et al. 2005; Eggert et al. 2011). Cuniberti et al. (2003), for example, analyzed the protein composition-functionality relationship for a set of Argentine wheats using this methodology. The reliability of size-exclusion HPLC and the small amount of sample required for the determination make this technology an ideal tool for the early detection of promising genetic material in plant-breeding programs (Larroque et al. 2000; Ueno et al. 2002).

The advent of *capillary electrophoresis* allowed the introduction of an either complementary or alternative technique to conventional electrophoresis and HPLC, the two most commonly used protein-analytical methods. Colombo et al. (2008) optimized the conditions for the separation of Argentine wheat gliadins through the implementation of capillary electrophoresis and assessed the ability of this technique to discriminate between different cultivars. As an added advantage, only small amounts of sample are required, and sample preparation is simple. Moreover, this technique involves straightforward protocols for capillary- column cleaning and maintenance. These characteristics enable the analysis of many samples in a relatively short time. In this regard, Brzozowski et al. (2008a, b) analyzed the changes in the storage proteins of wheat – and mainly the gliadins – that had resulted from the activity of the proteases of *Fusarium* spp. by means of capillary electrophoresis.

7.4 Conclusions

Although the quality of the wheat grains depends on the genetic of cultivars, environmental conditions can markedly affect both the yield and quality of the grains. The quality of the flour will furthermore depend not only on the variety of wheat and the state of the environment, but also on the level of infection present in the grain and after the harvest. For this reason, a knowledge of the processes of primary infection and consequent plant-pathogen interaction at different levels in

the commercial development of wheat flour will provide a basis for further research on and interpretation of the components of pathogen resistance in order to establish new strategies for disease management.

References

Abbate PE, Gutheim F, Polidoro O, Milisich HJ, Cunibert M (2010) Fundamentos para la clasificación del trigo argentino por calidad: efectos del cultivar, la localidad, el año y sus interacciones. Agriscientia 27:1–9

Alconada TM, Martínez MJ (1994) Purification and characterization of an extracellular endo-1, 4-ß-xylanase from *Fusarium* oxysporum f. sp. melonis. FEMS Microbiol Lett 118:305–310

Alconada TM, Martínez MJ (1996) Purification and characterization of an ß-glucosidase from *Fusarium* oxysporum f. sp. melonis. Lett Appl Microbiol 22:106–110

Alkorta I, Garbisu C, Llama MJ, Serra JL (1998) Industrial applications of pectic enzymes: a review. Process Biochem 33(1):21–28

Altenbach SB, DuPont FM, Kothari KM, Chan R, Johnson EL, Lieu D (2003) Temperature, water and fertilizer influence the timing of key events during grain development in US Spring Wheat. J Cereal Sci 37:9–20

An HJ, Lurie S, Greve LC, Rosenquist D, Kirmiz C, Labavitch JM, Lebrilla CB (2005) Determination of pathogen-related enzyme action by mass spectrometry analysis of pectin breakdown products of plant cell walls. Anal Biochem 338:71–82

Apel CP, Panaccione DG, Holden FR, Walton JD (1993) Cloning and targeted gene disruption of XYL1, a β-1,4-xylanase gene from the maize pathogen Cochliobolus carbonum. Mol Plant Microbe Interact 6:467–473

Arfvidsson C, Wahlund KG, Eliasson AC (2004) Direct molecular weight determination in the evaluation of dissolution methods for unreduced glutenin. J Cereal Sci 39:1–8

Bai GH, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78:760–766

Bai AE, Desjardins RD, Plattner RD (2001) Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia 153:91–98

Barneix J (2007) Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. J Plant Physiol 164:581–590

Bechtel DB, Kaleidau LA, Gaines RL, Seitz LM (1985) The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem 62:191–197

Beliën T, Van Campenhout S, Van Acker M, Volckaert G (2005) Cloning and characterization of two endoxylanases from the cereal phytopathogen *Fusarium graminearum* and their inhibition profile against endoxylanase inhibitors from wheat. Biochem Biophys Res Commun 327:407–414

Belton PS (2005) Review: new approaches to study the molecular basis of the mechanical properties of gluten. J Cereal Sci 41:203–211

Berto P, Comménil P, Belingheri L, Dehorter B (1999) Occurrence of a lipase in spores of Alternaria brassicicola with a crucial role in the infection of cauliflower leaves. FEMS Microbiol Lett 180:183–189

Bietz JA (1986) High-performance liquid chromatography of cereal proteins. Adv Cereal Sci Technol 8:105–170

Boyacioglu D, Hettiarachchy NS (1995) Changes in some biochemical components of wheat grain that was infected with *Fusarium graminearum*. J Cereal Sci 21:57–62

Brzozowski B, Dawidziuk K, Bednarski W (2008a) Gliadin degradation by proteases of *Fusarium* genus fungi in different in vivo and in vitro conditions. Pol J Nat Sci 23(1):188–206

Brzozowski B, Tatarczuk K, Szymkiewicz A, Bednarski W (2008b) Immunoreactive properties of wheat cv. tonacja storage proteins infected with *Fusarium graminearum* fungi. Pol J Food Nutr Sci 58(1):53–58

Bushnell WR, Hazel B, Pritsch C (2003) Histology and physiology of *Fusarium* head blight. In: Leonard KJ, Bushnell WR (eds) *Fusarium* head blight of wheat and barley. APS Press, St. Paul, pp 44–83

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3:1–30

Chelkowski J, Zawadzki M, Zajkowski P, Logrieco A, Bottalico A (1990) Moniliformin production by *Fusarium* species. Mycotoxin Res 6:41–45

Colombo A, Ribotta PD, León AE (2008) Aplicación de electroforesis capilar para la caracterización de gliadinas de trigos argentinos. Agriscientia 25(2):57–64

Comménil P, Belingheri L, Dehorter B (1998) Antilipase antibodies prevent infection of tomato leaves by Botrytis cinerea. Physiol Mol Plant Pathol 52:1–14

Cuniberti MB, Roth MR, MacRitchie F (2003) Protein composition-functionality relationships for a set of Argentinean wheats. Cereal Chem 80(2):132–134

Cuomo CA, Güldener U, Xu JR, Trail F, Turgeon BG, Di Pietro A, Walton JD, Ma LJ, Baker SE, Rep M, Adam G, Antoniw J, Baldwin T, Calvo S, Chang YL, Decaprio D, Gale LR, Gnerre S, Goswami RS, Hammond- Kosack K, Harris LJ, Hilburn K, Kennell JC, Kroken S, Magnuson JK, Mannhaupt G, Mauceli E, Mewes HW, Mitterbauer R, Muehlbauer G, Münsterkötter M, Nelson D, O'donnell K, Ouellet T, Qi W, Quesneville H, Roncero MI, Seong KY, Tetko IV, Urban M, Waalwijk C, Ward TJ, Yao J, Birren BW, Kistler HC (2007) The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science 317:1400–1402

De Sá Souza E (2009) Tecnología de productos panificados. Procesos de panificación de panes, facturas y repostería, AATA

Dexter JE, Clear RM, Preston KR (1996) *Fusarium* head blight: effect on the milling and baking of some Canadian wheats. Cereal Chem 73:695–701

Dimitri Milán J (1978) Enciclopedia Argentina de Agricultura y Jardinería. 3ro Ed. Tomo I, Familia Gramíneas, pp 145–149

Di Pietro AD, García Maceira FI, Méglecz E, Roncero IG (2001) A MAP kinase of the vascular wilt fungus *Fusarium oxysporum* is essential for root penetration and pathogenesis. Mol Microbiol 39:1140–1152

D'Ovidio R, Masci S (2004) Review: the low-molecular-weight glutenin subunits of wheat gluten. J Cereal Sci 39:321–339

Dyer RB, Plattner RD, Kendra DF, Brown DW (2005) *Fusarium graminearum* TRII4 is required for high virulence and DON production on wheat but not for DON synthesis in vitro. J Agric Food Chem 53:9281–9287

Eggert K, Rawel HM, Pawelzik E (2011) In vitro degradation of wheat gluten fractions by *Fusarium graminearum* proteases. Eur Food Res Technol 233:697–705

Feng J (2007) Molecular characterization of a *Fusarium graminearum* lipase gene and its promoter. Doctoral thesis University of Saskatchewan, Saskatoon, Canada

Feng J, Liu G, Selvaraj G, Hughes GR, Wei Y (2005) A secreted lipase encoded by LIP1 is necessary for efficient use of saturated triglyceride lipids in *Fusarium graminearum*. Microbiology 151:3911–3921

Gagkaeva TY, Yli-Mattila T (2004) Genetic diversity of *Fusarium graminearum* in Europe and Asia. Eur J Plant Pathol 110:551–562

Galich AN, de Galich MTV (1996) Enfermedades de trigo en el área central norte de la región cerealera argentina. Infome Técnico 121. E.E.A INTA Marcos Juárez Córdoba Argentina

García Maceira FI, Di Pietro AD, Huertas González MD, Ruiz Roldán MC, Roncero MI (2001) Molecular characterization of an endopolygalacturonase from *Fusarium* oxysporum expressed during early stages of infection. Appl Environ Microbiol 67:2191–2196

Gómez Gómez E, Roncero MIG, Di Pietro A, Hera C (2001) Molecular characterization of a novel endo-β-1,4-xylanase gene from the vascular wilt fungus *Fusarium* oxysporum. Curr Genet 40:268–275

Guenther JC, Trail F (2005) The development and differentiation of Gibberella zeae (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia 97(1):229–237

Hamauzu Z, Toyomazu T, Yonezawa D (1974) Molecular weight determination of gliadin fractions in gel filtration by ASDS-Page and sedimentation equilibrium. Agric Biol Chem 38:2445–2450

Hammond Kosack KE, Jones JDG (2000) Response to plant pathogens. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. ASPP Press, Rockville

Hariri G, Williams PC, El Haramein FJ (2000) Influence of pentatomid insect on the physical dough properties and two-layered flat bread baking quality of Syrian wheat. J Cereal Sci 31:111–118

Harris SD (2005) Morphogenesis in germinating *Fusarium graminearum* macroconidia. Mycologia 97:880–887

Harris LJ, Desjardins AE, Plattner RD, Nicholson P, Butler G, Young JC, Weston G, Proctor RH, Hohn TM (1999) Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Dis 83:954–960

Hartley BS (1960) Proteolytic enzymes. Annu Rev Biochem 29:45–72

Hatshc DH, Phalip V, Petkovski E, Jeltsch JM (2006) *Fusarium graminearum* on plant cell wall: no fewer than 30 xylanase genes transcribed. Biochem Biophys Res Commun 345:959–966

Hayashida D, Ohta K, Mo K (1988) Xylanase of Talaromyces byssochlamydoides. Methods Enzymol 160:675–678

Hermoso J, Sanz-Aparicio J, Molina R, Juge N, González R, Faulds CB (2004) The crystal structure of feruloyl esterase A from *Aspergillus niger* suggests evolutive functional convergence in feruloyl esterase family. J Mol Biol 338:495–506

Hou Z, Xue C, Peng Y, Katan T, Kistler HC, Xu JR (2002) A mitogen-activated protein kinase gene (MGVI) in *Fusarium graminearum* is required for female fertility, heterokaryon formation and plant infection. Mol Plant Microbe Interact 15(11):1119–1127

Huang L, Hseu TH, Wey TT (1991) Purification and characterization of an endoxylanase from *Trichoderma koningii* G-39. Biochem J 278(2):329–333

Huebner FR, Nelsen TC, Chung OK, Bietz JA (1997) Protein distributions among hard red winter wheat varieties as related to environment and baking quality. Cereal Chem 74:123–128

Ireta Moreno J, Bekele GT (1987) Histopatología de la penetración de *Fusarium graminearum* Schw en trigo. In: Kohli MM (ed) Taller sobre la Fusariosis de la Espiga en America del Sur. Encarnación del Paraguay, Paraguay. CIMMYT, Mexico

Jackowiak H, Packa D, Wiwart M, Perkowski J, Busko M, Borusiewicz A (2002) Scanning electron microscopy of mature wheat kernels infected with *Fusarium* culmorum. J Appl Genet 43(A):167–176

Jansen C, von Wettstein D, Schafer W, Kogel KH, Felk A, Maier FJ (2005) Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. Proc Nat Acad Sci U S A 102(46):16892–16897

Jayani RS, Saxena S, Gupta R (2005) Microbial pectinolytic enzymes: a review. Process Biochem 40:2931–2944

Jenczmionka NJ, Schäfer W (2005) The Gpmk1 MAP kinase of *Fusarium graminearum* regulates the induction of specific secreted enzymes. Curr Genet 47:29–36

Jenczmionka NJ, Maier FJ, Losch AP, Schafer FJ (2003) Mating, conidiation and pathogenicity of *Fusarium graminearum*, the main causal agent of the head-blight disease of wheat, are regulated by the MAP kinase gpmk1. Curr Genet 43:87–95

Kang Z, Buchenauer H (2000a) Ultrastructural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium* culmorum. J Phytopathol 148:263–275

Kang Z, Buchenauer H (2000b) Cytology and ultrastructure of the infection of wheat spikes by *Fusarium* culmorum. Mycol Res 104:1083–1093

Kang Z, Zingen Sell I, Buchenauer H (2005) Infection of wheat spikes by *Fusarium avenaceum* and alterations of cell wall components in the infected tissue. Eur J Plant Pathol 111:19–28

Kashyap DR, Vohra PK, Chopra S, Tewari R (2001) Applications of pectinases in the commercial sector: a review. Biores Technol 77:215–227

Kieliszewski MJ, Lamport DTA (1994) Extensin: repetitive motivs, functional sites, posttranslational codes and phylogeny. Plant J 5:157–172

Kikot GE, Hours RA, Alconada TM (2009) Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. J Basic Microbiol 49:31–241

Kikot GE, Hours RA, Alconada TM (2010) Extracellular enzymes of *Fusarium graminearum* isolates. Braz Arch Biol Technol 53(4):779–783

Kikot GE, Moschini R, Consolo VF, Rojo R, Salerno G, Hours RA, Gasoni L, Arambarri AM, Alconada TM (2011) Occurrence of different species of *Fusarium* from wheat in relation to disease levels predicted by a weather-based model in Argentina pampas region. Mycopathologia 171:139–149

Kluepfel D, Wats Mehta S, Aumont F, Shareck F, Morosoli R (1990) Purification and characterization of a new xylanase (xylanase B) by Streptomyces lividans 66. Biochem J 267:45–50

Kruger WM, Pritsch C, Chao S, Muehlbauer GJ (2002) Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected with *Fusarium graminearum*. Mol Plant Microbe Interact 15(5):445–455

Láday M, Juhász Á, Mulé G, Moretti A, Logrieco A (2004) Mitochondrial DNA diversity and lineage determination of European isolates of *Fusarium graminearum* (Gibberella zeae). Eur J Plant Pathol 110:545–550

Langevin F, Eudes F, Comeau A (2004) The effect of trichothecenes produced by *Fusarium graminearum* during *Fusarium* head blight development in six cereal species. Eur J Plant Pathol 110:735–746

Larroque OR, Gianibelli MC, Gomez Sanchez M, MacRitchie F (2000) Procedure for obtaining stable protein extracts of cereal flour and whole meal for size-exclusion hplc analysis. Cereal Chem 77(4):448–450

Lazzari FA (2000) Control integrado de plagas, manejo de hongos e insectos. Granos y Post-cosecha Latinoamericana VI nº XXIII

Lev S, Horwitz BA (2003) A mitogen-activated protein kinase pathway modulates the expression of two cellulase genes in Cochlobolus heterostroophus during plant infection. Plant Cell 5:835–844

Lopes DB, Fraga LP, Luciana Francisco Fleuri LF, Macedo GA (2011) Lipase and esterase to what extent can this classification be applied accurately? Cienc Tecnol Aliment Campinas 31(3):608–613

Mabile F, Grill J, Abecassis J (2001) Mechanical properties of wheat seed coats. Cereal Chem 78(3):231–235

Malbrán I, Mourelos CA, Girotti JR, Aulicino MB, Balatti PA, Lori GA (2012) Aggressiveness variation of *Fusarium graminearum* isolates from Argentina following point inoculation of field grown wheat spikes. Crop Prot 42:234–243

Martínez MJ, Alconada TM, Guillén F, Vázquez C, Reyes F (1991) Pectic activities from *Fusarium* oxysporum f. sp. melonis. Purification and characterization of an exopolygalacturonase. FEMS Microbiol Lett 81:145–150

Matz S (1999) Bakery technology and engineering, 3rd edn. PanTech International, McAllen

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devasting impact. Plant Dis 81(12):1340–1348

Mestherhazy A, Bartok T, Mirocha CG, Komoroczy R (1999) Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110

Meyer D, Weipert D, Mielke H (1986) Effects of *Fusarium* culmorum infection on wheat quality. Getreide Mehl Brot 40:35–39

Miller SS, Chabot DMP, Ouellet T, Harris LJ, Fedak G (2004) Use of a *Fusarium graminearum* strain transformed with green fluorescent protein to study infection in wheat (*Triticum aestivum*). Can J Plant Pathol 26(4):453–463

Nightingale MJ, Marchylo BA, Clear RM, Dexter JE, Preston KR (1999) *Fusarium* head blight: effect of fungal proteases on wheat storage proteins. Cereal Chem 76:150–158

Niture SK, Kumar AR, Pant A (2006) Role of glucose in production and repression of polygalacturonase and pectate lyase from phytopathogenic fungus *Fusarium moniliforme* NCIM 1276. World J Microbiol Biotechnol 22:893–899

Panda T, Nair SR, Kumar P (2004) Regulation of synthesis of the pectolytic enzymes of Aspergillus niger. Enzyme Microbiol Technol 34:466–473

Parry DW, McLeod L, Jenkinson P (1995) *Fusarium* head blight (scab) in small grain cereal a review. Plant Pathol 4:207–238

Payne PI, Corfield KG, Blackman JA (1981) Correlation between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J Sci Food Agric 32:51–60

Pekkarinen AI (2003). The serine proteinases of *Fusarium* grown on cereal proteins and in barley grain and their inhibition by barley proteins. Academic dissertation, University of Wisconsin Madison USA

Pekkarinen AI, Jones BL (2003) Purification and identification of Barley (*Hordeum vulgare* L.) proteins that inhibit the alkaline serine proteinases of *Fusarium culmorum*. J Agric Food Chem 51:1710–1717

Pekkarinen AI, Mannonen L, Jones BL, Niku-Paavola ML (2000) Production of proteases by *Fusarium* species grown on barley grains and in media containing cereal proteins. J Cereal Sci 31:253–261

Pekkarinen AI, Jones BL, Niku Paavola ML (2002) Purification and properties of an alkaline proteinase of *Fusarium* culmorum. Eur J Biochem 269:798–807

Pekkarinen AI, Tuija H, Sarlin TH, Latila AT, Haikara AI, Jones BL (2003) *Fusarium* species synthesize alkaline proteinases in infested barley. J Cereal Sci 37:349–356

Pekkarinen AI, Longstaff C, Jones BL (2007) Kinetics of the inhibition of *Fusarium* serine proteinases by barley (*Hordeum vulgare* l.) inhibitors. J Agric Food Chem 55:2736–2742

Phalip V, Delande F, Carapito C, Goubet F, Hatsch D, Leize Wagner E, Dupree P, Van Dorsselaer A, Jetsch JM (2005) Diversity of the exoproteome of *Fusarium graminearum* grown on plant cell wall. Curr Genet 48:366–379

Pirgozliev RS, Edwards SG, Hare MC, Jenkinson P (2003) Strategies for the control of *Fusarium* head blight in cereals. Eur J Plant Pathol 109:731–742

Ponzio NR (2010) Calidad panadera de variedades de trigo puras y sus mezclas. Influencia del agregado de aditivos. Tesis de Magister Scientiae. Facultad de Ciencias Agrarias y Forestales. UNLP. Argentina

Prange A, Birzele B, Krämer J, Meier A, Modrow H, Köhler P (2005) *Fusarium*-inoculated wheat: deoxynivalenol contents and baking quality in relation to infection time. Food Control 16:739–745

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP (2000) Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Mol Plant-Microbe Interact 13(2):159–169

Proctor RH, Hohn TM, McCormick SP (1995) Reduced virulence of Gibberella zeae caused by disruption of a trichothecene toxin biosynthetic gene. Mol Plant Microbe Interact 8(4):593–601

Reis EM, Carmona M (2002) Biología, epidemiología y estrategias para su manejo. In: Fusariosis del trigo Buenos Aires, Argentina, 25 pp

Ribichich KF, Vegetti AC (2001) Fusariosis de la espiga de trigo: evaluación de caracteres exomorfológicos asociados a la resistencia. Revista de la Facultad de Agronomía La Plata 104(2):121–127

Ribichich K, Lopez S, Vegetti AC (2000) Histopathological spikelet changes produced by *Fusarium graminearum* in susceptible and resistant wheat cultivars. Plant Dis 84(7):794–801

Rittenour WR, Harris SD (2010) An in vitro method for the analysis of infection-related morphogenesis in *Fusarium graminearum*. Mol Plant Pathol 11(3):361–369

Rittenour WR, Harris SD (2012) In vitro induction of infection-related hyphal structures in plant pathogenic fungi. In: Bolton MD, Thomma BPH (eds) Plant fungal pathogens: methods and protocols, methods in molecular biology. Springer, New York

Roncero MIG, Hera C, Ruiz-Rubio M, García-Maceira FI, Madrid MP, Caracuel Z, Calero F, Delgado Jarana J, Roldán Rodriguez R, Martínez Rocha AL (2003) *Fusarium* as a model for studying virulence in soilborne plant pathogens. Physiol Mol Plant Pathol 62:87–98

Schwarz PB, Schwarz JG, Zhou A, Prom LK, Steffenson BJ (2001) Effect of *Fusarium graminearum* and *F. poae* infection on barley and malt quality. Montsschr Brauwiss 54(3/4):55–63

Schwarz PB, Jones BL, Steffenson BJ (2002) Enzymes associated with *Fusarium* infection of barley. J Am Soc Brew Chem 60(3):130–134

Seong K, Hou Z, Tracy M, Kistler HC, Xu JR (2005) Random insertional mutagenesis identifies genes associated with virulence in the wheat scab fungus *Fusarium graminearum*. Phytopathology 95:744–750

Shewry PR, Halford NG (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. J Exp Bot 53(370):947–958

Shewry PR, Tatham AS, Forde J, Kreis M, Miflin BJ (1986) The classification and nomenclature of wheat gluten proteins: a reassessment. J Cereal Sci 4:97–106

Shimokawa T, Kakegawa K, Ishii T (2002) Feruloyl esterases from suspension-cultured rice cells. Bull FFPRI 1(4):225–230

Skadsen RW, Hohn TM (2004) Use of *Fusarium graminearum* transformed with gfp to follow infection patterns in barley and *Arabidopsis*. Physiol Mol Plant Pathol 64:45–53

Snijders CHA (2004) Resistance in wheat to *Fusarium* infection and trichothecene formation. Toxicol Lett 153(1):37–46

Steffolani ME, Pérez GT, Ribotta PD, León A (2007) Relationship between variety classification and breadmaking quality in Argentine wheats. Int J Agric Res 2(1):33–42

ten Have A, Mulder W, Visser JN, van Kan AL (1998) The endopolygalacturonase gene Bcpg1 is required for full virulence of Botrytis cinerea. Mol Plant Microbe Interact 11:1009–1016

Tosi EA, Re ED, Carbone L, Cuniberti M (2000) Breadmaking quality estimation by fast spectrophotometric method. Cereal Chem 77(6):699–701

Trail F (2009) For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiol 149:103–110

Trail F, Urban M, Gaffoor I, Mott E, Andries C, Hammond Kosack K (2003) Isolation and characterization of *Fusarium graminearum* mutants compromised in mycotoxin production and virulence. Fungal Genet Newsl 50(suppl):127

Triboï E, Martre P, Triboi Blondel AM (2003) Environmentally induced changes in protein composition in developing grains of wheat are related to changes in total protein. J Exp Bot 54(388):1731–1742

Turnbull KM, Rahman S (2002) Endosperm texture in wheat. J Cereal Sci 36:327–337

Ueno T, Stevenson SG, Preston KR, Nightingale MJ, Marchylo BM (2002) Simplified dilute acetic acid based extraction procedure for fractionation and analysis of wheat flour protein by size exclusion HPLC and flow field-flow fractionation. Cereal Chem 79(1):155–161

Valette Collet O, Cimerman A, Reignault P, Levis C, Boccara M (2003) Disruption of Botrytis cinerea pectin methylesterase gene Bcpme1 reduces on several host plans. Mol Plant Microbe Interact 16:360–367

Vázquez D, Berger AG, Cuniberti M, Bainotti C, Zavariz de Miranda M, Scheeren PL, Jobet C, Zúñiga J, Cabrera G, Verges R, Peña RJ (2012) Influence of cultivar and environment on quality of Latin American wheats. J Cereal Sci. doi:10.1016/j.jcs.2012.03.004

Voigt CHA, Schafer W, Salomon S (2005) A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. Plant J 42:364–375

Walker S, Leath S, Hagler WM, Murphy JP (2001) Variation among isolates of *Fusarium graminearum* associated with *Fusarium* Head Blight in North Carolina. Plant Dis 85(4):404–410

Wang J, Wieser H, Pawelzik E, Weinert J, Keutgen A, Wolf G (2005) Impact of the fungal protease produced by *Fusarium culmorum* on the protein quality and breadmaking properties of winter wheat. Eur Food Res Technol 220:552–559

Wanjiru WM, Zhensheng K, Buchenauer H (2002) Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat head. Eur J Plant Pathol 108:803–810

Wood TM, García Campayo V (1990) Enzymology of cellulose degradation. Biodegradation 1:147–161

Xu JR (2000) MAP kinases in fungal pathogens. Fungal Genet Biol 31:137–152
Xu X (2003) Effects of environmental conditions on the development of *Fusarium* ear blight. Eur J Plant Pathol 109:683–689
Yike I (2011) Fungal proteases and their pathophysiological effects. Mycopathologia 171:299–323
Zhu J, Khan K (2001) Effects of genotype and environment on glutenin polymers and bread-making quality. Cereal Chem 78:125–130

Chapter 8
Proteomic Approaches to Analyze Wheat-*Fusarium graminearum* Interaction

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Abstract *Fusarium graminearum*, the causal agent of Head Blight of wheat, was the third filamentous fungus to have a complete genome sequenced, becoming a model for studies of genomics, transcriptomics, proteomics and metabolomics of the plant-pathogen interaction associated with this devastating disease. In modern fungal biology, the major challenge is be able to understand the expression, function and regulation of the entire set of proteins encoded by fungal genomes. Proteomics, in combination with other omics techniques, constitutes a powerful tool for providing important information to understand plant-fungal interactions, pathogenesis and fungal colonization, allowing more solid interpretations on the complex mechanisms involved in the process of infection by *Fusarium* species, as well as mechanisms of host resistance intended to avoid infection. The present chapter summarizes the current worldwide status of proteomics focusing on *F. graminearum*-wheat interaction, provided that, at present, there are no revisions regarding this issue available. Besides, this chapter intends to provide initial research on proteomics of *F. graminearum* pathogenesis in wheat in Latin America, since this area of research is scarcely developed in this region.

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8.1 Introduction

The study of plant-pathogen interactions has been addressed by several and diverse disciplines and approaches including classical genetics, cell biology, and biochemistry, and, more recently, modern, holistic, and high-throughput omic techniques accompanied by proper bioinformatics tools. *Fusarium graminearum*, the predominant causal agent of Head Blight of wheat, was the third filamentous fungus to have a complete genome sequenced, so it has become a model for studies of genomics, transcriptomics, proteomics and metabolomics of the plant-pathogen interaction associated with this devastating disease (Tan et al. 2009). The analysis of *F. graminearum* complete genome had identified distinct regions responsible of encoding several protein categories (Cuomo et al. 2007). The high genetic diversity found in several discrete genomic regions containing genes implicated in plant-pathogen interactions suggests that the fungus has a great ability of adaptation and to induce genetic changes during its interaction with the host species (Cuomo et al. 2007). A defining characteristic of plant pathogenic fungi is the secretion of a large number of degrading enzymes and other proteins, which may play a role in nutrient acquisition, substrate colonization, and ecological interactions. Many of these proteins are of special interest as they may be potential targets for new fungicides (González-Fernández and Jorrin-Novo 2012). In particular, several genes encoding plant cell-wall degrading enzymes, such as xylanases, pectate lyases and cutinases, associated with penetration and maceration of plant tissues as well as acquisition of nutrients from plant polymers, as well as genes encoding effector molecules that trigger host-plant defense responses has been identified in *F. graminearum* genome (Cuomo et al. 2007).

Over the last two decades several studies have been conducted to analyze plant-pathogen interaction through functional post-genomics approaches, including proteomic and transcriptomic research. These studies have contributed to define a large data set of both gene and protein expression profiles related to fungal pathogenicity and plant defense mechanisms. The earliest post-genomic studies of filamentous fungi were carried out in *Trichoderma reesei* and *Aspergillus fumigatus* by Lim et al. (2001) and Bruneau et al. (2001), respectively. The first complete study of the proteomics of fungal secreted proteins was published for the phytopathogenic fungus *Aspergillus flavus* (Medina et al. 2005). Since then, a significant number of post-genomic studies have been published (Trail 2009).

Proteomics is a term coined to encompass a field that intends to understand the expression, function and regulation of the entire set of proteins, or 'proteome', encoded by an organism (Zhu et al. 2003). Two-dimensional gel electrophoresis (2DE) coupled to mass spectrometry (MS) is the method more commonly used to dissect fungal proteomes. However, new emerging high-throughput, gel-free proteomic techniques, based on multidimensional protein identification technology (MudPIT) by liquid chromatography (LC), are rapidly gaining popularity to elucidate fungal proteomes (Mehta et al. 2008; Tan et al. 2009). Hence, proteomics is a modern and functional approach that has emerged as a complementary tool to other omic approaches for the elucidation of complex biological processes.

Early proteomic research on wheat-*F. graminearum* interaction is dated no more than a decade ago (Zhou et al. 2005; Phalip et al. 2005; Wang et al. 2005), but despite the research on this area is still scarce it is increasing. While there are interesting revisions regarding proteomics of plant pathogenic fungi (Mehta et al. 2008; Gonzalez Fernandez et al. 2010, 2012; Bhadauria et al. 2010), none of them described research outcomes regarding *F. graminearum*. The present chapter summarizes the current state of the art on proteomic research on *F. graminearum*-wheat interaction. This field of study has not been explored in Latin America yet, according to the lack of published literature. Therefore, the present revision could provide the basis for future regional research.

8.2 Molecular Plant-Pathogen Interactions

The success or avoidance of the establishment of fungal disease in plants depends on the mutual recognition between the fungus and the plant. Recognition mechanisms involve the expression of virulence and pathogenicity factors in the fungus, while in the plant it relies on the existence of passive, preformed and/or inducible defense compounds. Several fungal proteins have been associated with pathogenicity or virulence, such as cell wall degrading enzymes, inhibitory proteins, and enzymes involved in the toxin synthesis (Walter et al. 2010). The recognition and signaling events that occur in plant cells in response to microorganism challenge need to be extremely rapid, reliable and specific, and are part of the strategy evolved by plants to survive attacks (Metha et al. 2008). The intracellular sensitive perception of pathogens and the recognition of pathogen-associated molecular patterns lead to the activation of a first defense response in the plant that consists of basal, non-specific generic mechanisms that include plant cell wall thickening, papilla deposition, apoplast acidification, signal transduction and transcription of defense genes (Alfano and Collmer 2004; Pritsch et al. 2000, 2001; El Gendy et al. 2001).

As the most commonly observed outcome among plant-fungus interactions are no disease due to incompatible interactions associated with the basal, generic defense mechanisms are the success of the pathogen to colonize plant tissue and to establish disease would depend on the ability to interfere or suppress this basal defense (Mehta et al. 2008).

In addition to the proteins related to basal defense responses, certain plant genotypes expresses a different kind of proteins named R o resistant proteins which trigger a specific, genetically defined hypersensitive response and subsequent programmed cell death. The role of the hypersensitive response is to restrain the pathogen, which involves various biochemical perturbations, known as generic plant responses, including changes in ion fluxes, lipid hyperperoxidation, protein phosphorylation, nitric oxide generation and a burst of reactive oxygen species and antimicrobial compounds (Mohammadi and Kazemi 2002). This rapid incompatibility response effectively puts an end to pathogen invasion and prevents further disease development (Alfano and Collmer 2004).

The capacity of pathogens to overcome plant defense by protecting themselves from the oxidative stress activated by the plant in response to pathogen perception, is critical in the disease outcome. Fungal protein activities, such as catalase and superoxide dismutase, are responsible for the inactivation of H_2O_2 and O_2. The pathogen also produces effector proteins which are expected to assist in the suppression of basal resistance through modification of host-specific proteins. During infection, extracellular enzymes produced by the pathogens are additional critical factors in the establishment of disease (Van Sluys et al. 2002).

8.3 From Structural to Functional Genomic

Since the availability of complete sequenced genomes of several phytopathogenic fungi, a significant challenge has been the identification of genes truly associated with pathogenicity. Several approaches have been used to identify pathogenicity genes including those targeting major signal transduction proteins (Hou et al. 2002; Urban et al. 2003; Yu et al. 2008), screening random insertional mutants for loss of the ability to induce disease (Seong et al. 2008), and identifying orthologous genes to known pathogenicity genes from other fungi (Oide et al. 2007; Shim et al. 2006). Thus, a number of pathogenicity factors have been targeted, and among them, several signaling pathways such as the cyclic adenosine-3′, 5′-monophosphate and mitogen-activated protein kinase (cAMP) and a mitogen activated protein kinase (MAPK) have proved to be crucial to virulence in several phytopathogenic fungi. Notably, the random insertional mutagenesis turns out to be an excellent approach for dissecting complex biological traits, such as pathogenicity, as it does not require any prior information or assumptions on gene function.

F. graminearum genome sequence (36.1 Mb) comprising four chromosomes was publicly released by the Broad Institute in 2003 (www.broad.mit.edu/annotation/genome/ fusarium_group/ MultiHome.html). A set of genes encoding 13,937 predicted proteins (mips.gsf.de/genre/proj/FGDB/) has been identified. Of these genes 2,001 are not similar to those of any other sequenced organism (orphans) while 5,812 have homology to proteins of unknown function. There are fewer repeated sequences and an unusually lower number of paralogous genes in comparison with genome sequences of other filamentous ascomycetes (Cuomo et al. 2007). As an initial premise in the selection of individual genes or groups of genes for functional exploration, regions with high sequence variability should be identified among the strains (Trail 2009).

Genetic manipulation techniques have been well developed for *F. graminearum*, which have enabled enhancing the ability to use the genome for functional analyses. It should be noted that although *F. graminearum* is a homothallic species, it can outcross in laboratory conditions, thus enabling genetic analysis. Random insertion mutagenesis has been one of the most powerful techniques for gene discovery in *Fusarium* species as it provides loss-of-function mutants. In fact, relevant genetic information was obtained from tagged loss-of-function mutants including loss of

virulence (Seong et al. 2008; Kim et al. 2007), loss of mycotoxin production, or arrested perithecium development (Kim et al. 2007; Trail 2009).

With the increase in genomic and postgenomic studies, a large amount of information regarding defense mechanisms in plants, as well as the pathogenicity strategies employed by microbial pathogens has been accumulated. At present, one of the major challenges in postgenomic studies is functional assignment of identified genes and proteins. Other important issue to consider with postgenomic data is the fact that changes detected in transcriptome alone do not necessarily reflect completely the complex regulatory mechanisms occurring in the cell, as post-transcriptional processes which alter the amount of active protein, such as protein synthesis, degradation, processing and post-translational modification, are not taken into account. Thus, complementary approaches, such as proteome-based expression profiling, are needed to obtain a full picture of the regulatory elements. Several studies have revealed that the levels of mRNA do not necessarily predict the levels of the corresponding proteins in the cell. The different stabilities of mRNAs and different efficiencies in translational processes can affect the generation of new proteins. Moreover, proteins themselves can differ significantly in their stability and turnover rate, which should be considered in the accuracy and reliability of the analysis (Mehta et al. 2008; Hallen-Adams et al. 2011).

The large-scale study of the structure and function of the protein complement an organism or proteomics is a new, complex and ambitious discipline. The understanding of the patterns of expression and function of the entire set of proteins encoded by fungal genomes is a major challenge in modern fungal biology. Moreover, this information is undoubtedly invaluable for the understanding plant-fungal interactions, fungal pathogenesis and fungal colonization, as it may be opening up new possibilities for crop disease diagnosis and crop protection.

8.4 Proteomic in Plant-Pathogen Interaction

Proteomic analysis, defined as the global assessment of cellular proteins expressed in a particular biological state, is a powerful tool that can provide a systematic understanding of events at the molecular level. Proteomics allows qualitative and quantitative measurements of large numbers of proteins that directly influence cellular biochemistry, and thus provide accurate analysis of cellular state or system changes during growth, development and response to the environment (Chen and Harmon 2006). Therefore, this approach allows the systematic study of all the proteins expressed by a genome, cell or tissue, focusing particularly on their interactions, modification, localization and functions. Currently, proteomics has proved to be an indispensable technology to interpret the information from genomics and has been successfully applied in protein sequencing, protein quantification, post translational modifications (PTMs) and protein interactions studies (Yang et al. 2011b). Consequently, proteomics provides experimental continuity between genome sequence information and the protein

profile in a specific tissue, cell or cellular compartment during standard growth or different treatment conditions. While genome analysis defines the potential contributions to a set of genes to cellular function, proteome itself represents actual contributions of genetic information. Moreover, by using proteomic approaches, not only differences in the abundance of proteins present at the time of sampling but also different forms of the same protein can be distinguished assessed. Extensive exploration of the proteome of different organisms has been conducted using 2DE coupled with MS; but, new methods are emerging to face limitations in the resolution of proteins. Unequivocal identification of proteins involved in different biological functions can be achieved when proteome data can be complemented with genome sequencing data (Mehta et al. 2008).

Proteomic research has been reported for a number of different plant-pathogen interactions including plant-virus, bacteria, fungi and nematodes interactions. The aims of the research ranged from the detection of plant pathogens; comparison of proteomes and detection of differential protein expression at quantitative and qualitative terms in both plant and pathogen; analysis of post-transcriptional modifications (PTMs) such as phosphorylation, infection-induced protein modifications as well as analysis of protein-protein interaction (Yang 2011a).

At the beginning of the infection process, the observed up-and down-regulation of both secreted and intracellular fungal proteins in fungal cells is likely associated with an improving ability to invade the plant cell. Several of the proteins involved in pathogenicity are secretion proteins. Many of them are cell wall-degrading enzymes or CWDEs such as proteases, cellulases and pectate lyases, which are important, for host plant colonization. In addition to these well-known enzymes, other pathogenesis-related proteins, such as superoxide dismutase and oxidases, have also been reported in the different pathogens, and are associated with conferring protection against the oxidative stress response expressed by the plant upon infection (Van Sluys et al. 2002). A similar scenario was observed with regard to defense-related proteins in plants. The most reported defense-related proteins are PR proteins, including thaumatins, glucanases, peroxidases and chitinases, observed in several pathosystems. The involvement of these proteins in plant defense has been well established, however, their direct role in resistance enhancement still needs to be demonstrated. The general biotic stress response also comprises another kind of regulated proteins, commonly identified in several plant-pathogen proteomic studies, such as glutathione transferase, superoxide dismutase and heat shock proteins (Metha et al. 2008).

8.5 Proteomic Work Flow

A thorough characterization of the changes of plant proteomes occurring in response to biotic stress such as fungal invasion is crucial to understand the molecular mechanisms underlying plant-pathogen interaction and pathogenesis. Over the last years, new technical approaches have arisen, allowing for the identification of large numbers of proteins, as well as detecting for differences in protein repertoire in cells undergoing

various physiological states. Proteomics workflow mainly consists of three steps including protein preparation, separation, and identification by MS. An effectively performed extraction of proteins is a key step for fungal proteomic studies (Yang 2011a) (Fig. 8.1).

8.5.1 Sample

During sample preparation, the extraction methods should minimize protein degradation and eliminate non-protein components such as carbohydrates, lipids, salts, nucleic acids, etc., since they can interfere with the process of separation of proteins. Therefore, for total protein extraction, an ideal protocol would reproducibly capture all the protein species in a proteome with low contamination of other molecules. Different protocols were devoted to overcoming this challenge by providing an effective means of cell lysis for adequate release of intracellular proteins, among which either glass beads, used to liberate cytoplasmic proteins, or chemical or enzymatic extraction methods have been employed. Nevertheless, the most widely used extraction method included grinding the sample in liquid nitrogen using mortar and pestle. Once the cells are lyzed, the protein solution is often purified via precipitation with trichloroacetic acid (TCA) to remove contaminants that can be troublesome during isoelectric focusing analysis. As TCA-treatment hinders subsequent protein solubilization necessary for isoelectric focusing, an additional step of resolubilization is usually included. An effective and frequently used protocol for protein precipitation consists of 10 % trichloroacetic acid (TCA) in acetone with 0.07 % 2-mercaptoethanol or 20 mM DTT. An alternative extraction protocol consisted of extracting proteins from protoplasted cells using a buffer containing 4 % SDS, 2 % DTT, 20 % glycerol, 20 mM PMSF and 100 mM Tris–HCl (pH 7.4) (Shimizu and Wariishi 2005). In fact, the 2DE patterns of protoplast-isolated proteins were better visualized than those obtained by disrupting the fungal cell wall using SDS extraction.

Some strategies have been developed over the years to fractionate protein repertoire into sub-proteomes based on biochemical, biophysical and cellular properties, and to detect the proteome subsets related to membrane proteins and low-abundant proteins. The sub-proteome is defined as the protein species composition of a biological compartment at a certain time and under defined environmental conditions. Fractioning of proteins has greatly improved detection and resolution by reducing the overall sample complexity, such as the detection of low abundant proteins (Bhadauria et al. 2007; Kim et al. 2007). Hence, sub-proteomics describes the proteomic analysis of a defined subset of protein complement of an organism, especially specific organelles. The advantage of a sub-proteomic approach is that it localizes protein expression to a particular organelle, thereby providing additional insight about protein function for cells ongoing a particular physiological state. Whole-organelle as well as sub-organelle proteomics once adequate organelle separation protocols have been achieved. Mitochondria are among the most studied

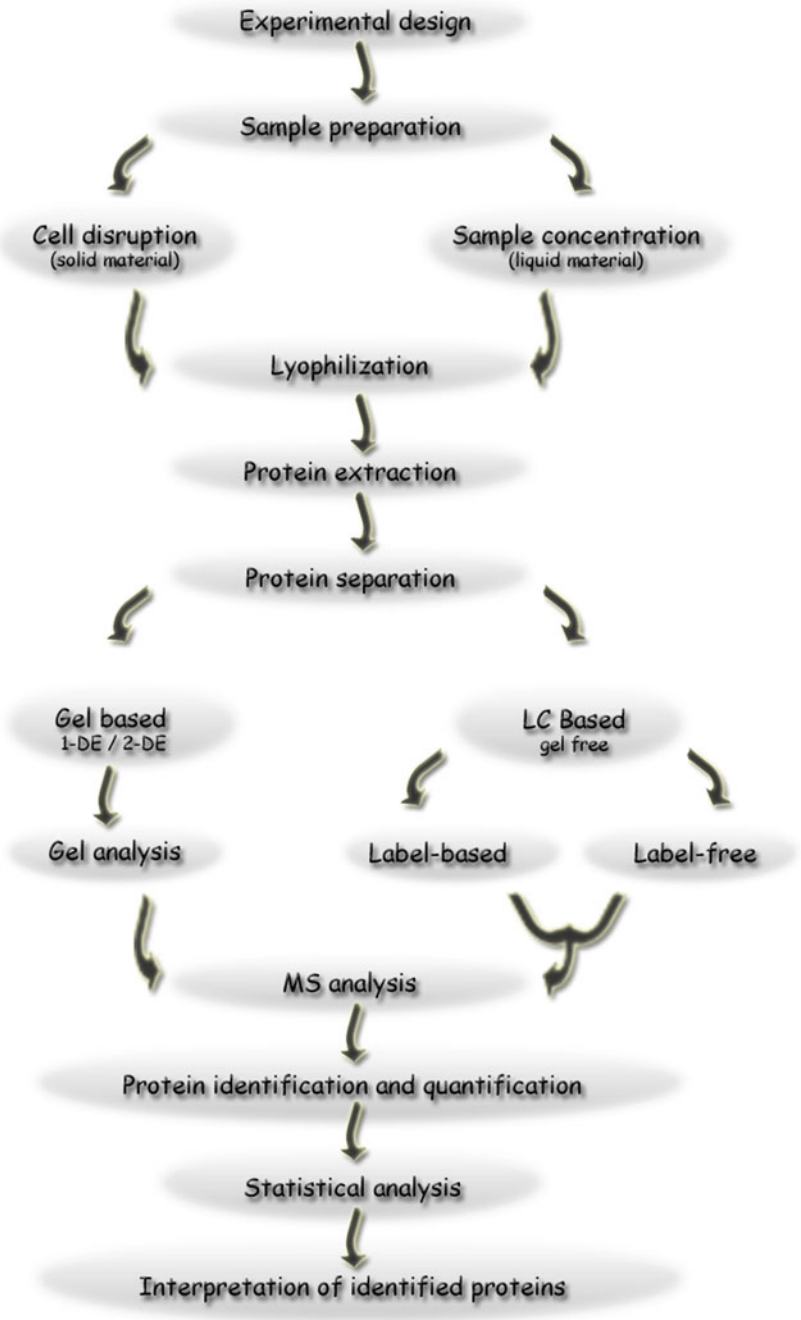

Fig. 8.1 Schematic overview of the work flow in a proteomic study

organelles, being sub-proteome mitochondrial for *Trichoderma harzianum* (Grinyer et al. 2005) the first published study.

Another type of sample to be analyzed is the secretome or extracellular proteins released by the fungus in an appropriate culture media. The secretome has been defined as the combination of native secreted proteins and the cellular machinery involved in their secretion. Secretome-related studies are particularly relevant in understanding filamentous fungi because many fungi secrete a vast number of proteins to accommodate their saprotrophic lifestyle, since they need a large number of extracellular hydrolytic enzymes to break down potential substrates. Many of these proteins are of special interest in the study of pathogens or during production of recombinant proteins in the biotechnology industry, thus a significant number of research has been based on the analysis of fungal secretome. This might also be due to the fact that secretome sample preparation is much faster and simpler than the extraction and preparation of intracellular proteins (Kim et al. 2007).

8.5.2 Protein Identification and Separation

Originally, one-dimensional (1D) sodium-dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) has been one of the most widely used tools for the separation of protein fractions and extracts (Robertson et al. 1997). At present, two-dimensional gel electrophoresis (2DE), two dimensional fluorescence difference gel electrophoresis (2D-DIGE) and multidimensional liquid chromatography protein separation techniques are widely used in fungal proteomics (Bhadauria et al. 2010). 2DE has been the primary tool for obtaining a global picture of the expression profile of a proteome under various conditions. The proteins are first separated in one direction by isoelectric focusing and then in the perpendicular direction by molecular mass. 2D-DIGE allows to perform on a single gel, a direct, high-throughput, differential protein expression analysis, between different samples of complex proteins. The main advantage of 2D-DIGE over 2DE is its unrivaled performance, attributable to a unique experimental design in which each protein spot on the gel is represented by its own internal standard, while on 2DE gels, protein visualization is most often achieved by Coomassie staining because of its simplicity of use and MS compatibility. However, Coomassie staining offers limited dynamic range and sensitivity. The development of the MS-compatible ruthenium-based fluorescent SYPRO Ruby has enhanced detection sensitivity (Lopez et al. 2000) and protein analysis can be multiplexed with a range of other fluorescent dyes that detect post-translational modifications.

Limited power of protein detection using 2DE has been reported for proteins exhibiting extreme isoelectric points, low molecular weight, low abundance or low-solubility.

Protein separation methods coupled with various MS technologies have evolved as the dominant tool in the field of protein identification. A typical MS consists of an ion source, a mass analyzer and a detector. Two "soft ionization" methods, namely matrix-assisted laser desorption ionization (MALDI) and electrospray ionization

(ESI), are designed to volatile and ionize large biomolecules, such as peptides and proteins (Mann et al. 2001). The most currently used mass analyzers are ion trap, time-of-flight (TOF), quadrupole and Fourier transform ion cyclotron. MALDI is usually coupled to TOF analyzers that measure the mass of intact peptides. ESI has mostly been coupled to ion traps and triple quadrupole or hybrid TOF MS and used to generate fragment ion spectra of selected precursor ions. Compared with MALDI, ESI has a significant advantage in the ease of coupling to separation techniques such as liquid chromatography (LC) and high-performance liquid chromatography (HPLC), allowing high throughput and on-line analysis of peptide or protein mixtures. Typically, a mixture of proteins is first separated by LC followed by tandem MS (MS/MS). In this procedure, a mixture of charged peptides is separated in the first MS according to their m/z ratios to create a list of the most intense peptide peaks. In the second MS analysis, the instrument is adjusted so that only a specific m/z species is directed into a collision cell to generate "daughter" ions derived from the "parent" species. LC-MS has been applied to large-scale protein characterization and identification. To counteract this drawback, a new technique has been developed. At present, multidimensional LC coupled with MS (LC-MS) is the most powerful strategy for separation of complex proteins or peptides mixtures. LC separates proteins and peptides according to their affinity for a stationary phase when a mobile phase is forced through a fine capillary. This technology allows detection and identification of low-abundance proteins such as transcription factors and protein kinases which could not identified by other proteome analyses (Bhadauria et al. 2007).

It should be noted that the proteomic studies will continue to be dominated by 2DE in the immediate future. However, as soon as the accessibility to gel-free proteomic technology improves, its adoption is expected to increase (Bhadauria et al. 2007).

8.5.3 Database Search

The protein identification based on peptide mass fingerprints (PMF) is performed by analyzing the sizes of tryptic fragments via the MASCOT (http://www.matrixscience) search engine using the entire NCBI fungal protein database (http://www.ncbi.nlm.nih.gov/)pmc/articles/). The fingerprinting method allows for a maximum of one missed tryptic cleavage per protein. The maximum deviation permitted for matching the peptide mass values is 100 ppm. Scores greater than 65 are considered to be significant ($p = 0.005$) (Bhadauria et al. 2007).

8.5.4 Post-Translational Modifications (PTMs)

The regulation of protein activity is closely linked with covalent modifications of protein structures, which occur either co- or post-translationally. Therefore, the identification of the type of modification and its location often provide essential

information for understanding the function or regulation of a given protein in biological pathways. The most important protein modifications which are related to the protein function are phosphorylation, glycosylation, sulfation, acetylation, myristoylation, palmitoylation, methylation, prenylation and ubiquitylation. As a result, the activity, stability, localization and turnover can be substantially altered. Many of the PTMs are regulatory and reversible, most notably the protein phosphorylation, which controls biological function through several mechanisms. In general, 2DE has sufficient resolution to directly separate different modification states of a protein (Bhadauria et al. 2007).

8.5.5 Protein Localization

To identify the sub-cellular localization of a protein a reporter gene is fused or tagged with an epitope to the gene encoding the protein. Generally, the correct determination of the protein location depends on whether reporter sequences and epitope tags are fused to the N or C termini of target genes. Organelle specific targeting signals such as mitochondrial targeting peptides and nuclear localization signals are often located at the N terminus, while, fusion to the C terminal sequences was decisive for the analysis of the yeast g-tubulin like protein Tub4p (Vogel et al. 2001). Green fluorescent protein (GFP) and its spectral variants efficiently enable protein localization within living cells using epifluorescence microscopy (Tsien 1998).

8.5.6 Protein Interactions

The systematic analyses of protein-protein interaction achieved from isolating individual protein from complexes allow representing the data through the so called cell map proteomics. The importance of such map lies in the fact that, when performing their functions in the organism, the proteins regularly act in cooperation. Cellular functions emerge from the properties of dynamic interconnected protein networks. To understand the complex behavior of networks within cells, individual protein interactions must be analyzed in intact cells with high spatial and temporal resolution. A useful method used for identifying protein-protein interactions is based on the 'fluorescence resonance energy transfer', which describes the energy transfer from an activated donor-fluorophore to an acceptor-fluorophore localized in close vicinity to each other. This methodology can be used if both interaction partners are labeled with suitable fluorophores, as for example, variants of the GFP (Bhadauria et al. 2007). Zhao et al. (2009) presented two possible approaches for predicting protein-protein interactions for *F. graminearum* based on interologs and domain-domain interactions, respectively. The investigation showed the construction of the first interactome map for *F. graminearum,*

which was based on evolutionarily conserved interactions across species and domain-domain interactions. Public access to the *Fusarium graminearum* protein-protein interaction database is available at http://csb.shu.edu.cn/fppi.

8.6 Proteomic Analysis in Wheat-*Fusarium graminearum* Interaction

The first reports of proteomic studies to identify proteins related to pathogenecity in *F. graminearum* on wheat were released by 2005; therein after, the number of studies, involving both *in vitro* as *in planta* approaches has increased in a moderate rate.

To study the extracellular proteins or secretome, various culture media containing either plant cell walls or diverse carbon sources as protein inducers have been used for *F. graminearum* growth (Phalip et al. 2005; Paper et al. 2007). In turn, *in planta* proteomic studies were conducted by several research groups by sampling plant tissues from *Fusarium*-infected wheat (or other cereals) during the progress of the infection (Zhou et al. 2005, 2006; Wang et al. 2005; Geddes et al. 2008; Shin et al. 2011). Proteomic studies have also been carried out in relation to the production of mycotoxins; here Taylor et al. (2008) grew *F. graminearum* on culture media under mycotoxin-inducing conditions in order to identify genes associated with mycotoxin production (Taylor et al. 2008). On the other hand, the differential proteomic analysis between, resistant and susceptible *Fusarium*-infected wheat genotypes under producing and non-producing trichotecene conditions as well as between treated and non-treated plants with mycotoxin DON were studied by Foroud et al. (2008). Regarding analysis of protein associated with regulation pathways, using a mutant of Mat1-2 gene, Lee et al. (2008) identified proteins that were regulated by the mating-type gene and concluded that all protein-encoding genes were down-regulated during the sexual development stage. The use of bioinformatic tools has improved the study of proteomics as demonstrated by comparative analyses performed by Brown et al. (2012), who predicted a *F. graminearum* secretome by combining several bioinformatic approaches. This strategy increased the probability of identifying truly secreted proteins. In this study a secretome of 574 proteins was predicted, from which 99 % was supported by transcriptional evidence. Another interesting analysis was carried out by Kwon et al. (2009). They compared the proteome of virus-free and virus (FgV-DK21) infected *F. graminearum* cultures generated through 2DE compared with LC MS/MS. The viral infection altered fungal aspects related to morphology, pigmentation, sporulations and attenuated its virulence. Numerous proteins showed differences in their abundance; among these proteins, some were up-accumulated and others were down-accumulated. In this study, most of the proteomic results were confirmed at the mRNA level by real time RT-PCR.

The secretome of *F. graminearum* grown on plant cell walls and glucose was studied by Phalip et al. (2005) using a combination of SDS-PAGE 1DE and 2DE followed by LC-MS/MS. It was observed that, when growing on cell wall, 45 %

of the fungal proteins produced, were strictly involved in cell wall degradation and indirectly related to carbon and nitrogen absorption. In contrast, when the fungus was grown in a medium with glucose as the source of carbon, the enzyme patterns notably differed, showing that fungal secretome is regulated according to substrate composition.

High-throughput LC MS/MS was used by Paper et al. (2007) to identify by *F. graminearum*-secreted proteins both *in vitro*, from fungal mycelia growing media with ten different carbon sources and, *in planta*, during infection of wheat heads. Different protein repertoire was observed in both conditions. Most of the proteins from the *in vitro* assay showed predicted signal peptides, whereas *in planta*, only about a half of them had signal peptides. This difference might indicate that significant fungal lysis occurs during pathogenesis. On the other hand, several of the proteins lacking signal peptides that were found *in planta* showed similarities to potent immunogens secreted by several plant pathogenic fungi, and therefore they could be important during the interaction between *F. graminearum* and its host plants.

A different question was addressed by Wang et al. (2005) research. In this case, the proteome of both *Fusarium* and wheat partners was analyzed during infection from protein extracts obtained from *Fusarium*-infected wheat spikes, sampled at different times. Accordingly, differentially-expressed proteins were analyzed by resolving protein extracts by 2DE. Subsequently, for those spots showing change in abundance when compared with control treatments were further characterized by MALDI-TOF MS and searched for matching by querying the mass spectra data in different proteins database or the *Triticeae* EST translation database. These proteins were classified into four categories according to protein patterns. These authors reported many of the proteins identified in the first two categories were related to carbon metabolism and photosynthesis, while most of the proteins identified in the last two categories were related to stress defense of plant (Wang et al. 2005).

Another research has been focused on the analysis of differential protein regulation, determining which proteins were induced or up-regulated in responses to infection by *F. graminearum* in spikes of wheat. The proteins expressed in response to infection were divided into different groups, according to their putative relationship with defense response or metabolism. In these studies the proteins resolved by 2DE were subsequently identified by LC-MS/MS. According to protein identification data obtained by TargetP program, the authors suggested that the chloroplast is the organelle mostly affected by *F. graminearum* infection (Zhou et al. 2005, 2006). Geddes et al. (2008), using 2DE coupled to LC-MS/MS, also studied the differential expression of proteins in response to *Fusarium* infection in six barley genotypes showing varying FHB resistance. Proteins associated with oxidative burst and oxidative stress response showed changes in abundance and absence/presence status. They reported three distinct response patterns from genotypes analyzed.

Shin et al. (2011) isolated and identified proteins from Korean wheat genotype with moderate resistance infected with *F. graminearum* employing 2DE MALDI-TOF/TOF MS. They concluded that differential expressed protein

spots were the result of FHB exposure. The identification of proteins determined that they were associated to carbon metabolism and photosynthesis. Taylor et al. (2008) conducted experiments where *F. graminearum* cells were grown in liquid culture under mycotoxin-inducing conditions. They used different technologies for protein identification. There was good agreement between up accumulated proteins identified by 2DE-MS/MS and isobaric tags for relative and absolute quantification (iTRAQ). RT-PCR and northern hybridization confirmed that genes encoding proteins that were up-regulated based on iTRAQ were also transcriptionally active under mycotoxin conditions. Numerous candidate for pathogenicity proteins were identified using this technique, including many of them contain predicted secreted proteins. Foroud et al. (2008) also analyzed the trichothecene-induced differential transcriptomic and proteomic of wheat by infecting both resistant and susceptible genotypes. They employed 2DE, electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS) as proteomic tools. The results obtained confirmed the differential protein expression in relation to the mycotoxin production observed by Taylor et al. (2008). Yang and collaborators have made substantial contributions to the elucidation of proteomics both *in vitro* and *in planta*. Their work described wheat and barley responses to FHB, on the basis on differential protein profiles using 2DE followed by MALDI-TOF MS, in plants growing under different nitrogen, temperature and drought conditions (Yang et al. 2010b, 2011b). Fungal secretome included enzymes involved in the degradation of cell walls, starch and proteins; of this group, 70 % contained predicted signal peptides and a further 16 %, may be secreted in a non-classical way (Yang et al. 2012). When the early events in susceptible barley cultivar infected by *F. graminearum* were analyzed, an increase in fungal biomass observed 3 days after inoculation coincided with the appearance of discrete *F. graminearum*-induced proteolytic fragments of β-amylase. The protein changes were associated with pathogenesis-related protein synthesis, proteins involved in energy metabolism, secondary metabolism and protein synthesis (Yang et al. 2010a) (Table 8.1).

Table 8.1 Proteomics studies on *Fusarium graminearum*

Host	Proteomic type	Proteomic approach	Remarks	References
Wheat	Intracellular	2DE, LC-MS/MS	Identification of differentially regulated proteins	Zhou et al. (2005)
–	Extracellular	1DE/2DE, LC-MS/MS	Diversity of proteins in liquid culture with plant cell walls or glucose	Phalip et al. (2005)
Wheat	Intracellular	2DE, MALDI-TOF-MS	Differential analysis of proteins during the course of infection	Wang et al. (2005)
Wheat	Intracellular	2DE, LC-MS/MS	Identification of differentially regulated proteins	Zhou et al. (2006)

(continued)

Table 8.1 (continued)

Host	Proteomic type	Proteomic approach	Remarks	References
Wheat	Intracellular/ Extracellular	1DE, LC-MS/ MS	Comparative proteomic *in vitro* and *in vivo*	Paper et al. (2007)
–	Extracellular	2DE, LC-MS/ MS and iTRAQ	Analysis grown under mycotoxin-inducing conditions	Taylor et al. (2008)
Barley	Intracellular	2DE, LC-MS/ MS	Differential expression of proteins on genotypes of varying resistance	Geddes et al. (2008)
Wheat	Intracellular	2D, ESI-qTOF-MS	Analysis of proteins in relation to trichothecene induced and genotypes	Foroud et al. (2008)
–	Extracellular	2DE, MALDI-TOF and ESI-Q-TOF-MS	Analysis comparative of a wild type strain and its self-sterile mat1-2 deleted.	Lee et al. (2008)
–	Intracellular	2DE,LC-MS/MS	Study of effects of virus (FgV.DK21) on development and virulence	Know et al. (2009)
–	–	–	Protein-Protein interactions database and interactome map	Zhao et al. (2009)
Barley	Intracellular	2DE, MALDI-TOF-MS	Protein analysis of early events in the interaction	Yang et al. (2010a)
Barley	Intracellular	2DE, MALDI-TOF-MS/MS	Proteome change in relation to the effect of nitrogen on disease	Yang et al. (2010b)
Wheat	Intracellular	2DE, MALDI-TOF-TOF-MS/MS	Effects of high-temperature and water deficits on protein profiles	Yang et al. (2011b)
Wheat	Intracellular	2DE, MALDI-TOF-TOF-MS	Analysis of defense proteins	Shin et al. (2011)
–	Extracellular	2DE, MALDI-TOF-TOF-MS	Analysis of proteins in liquid culture with barley or wheat flours	Yang et al. (2012)
–	–	–	Prediction of secretome by combining approaches	Brown et al. (2012)

8.7 Conclusions

Substantial progress has been made in the field of fungal proteomics in the last few years. This improvement is the result of upgrade methods for sample preparation, high-resolution protein separation techniques, MS equipment and related software for effective protein identification and characterization, as well as bioinformatics technology. However, there still remain technical challenges to be addressed which

are associated with the dynamic nature of the proteome of diseased cells or tissues. It should be noted that multiple roles found in many proteins impair the progress towards an unambiguous identification of those proteins actually involved in processes of infection. Anyway, proteomic tools are rapidly improving and new methods and equipment are being developed to achieve more precise and accurate results. Besides, proteomic analyses are of extreme importance to validate expression of the genes identified by either genomic or transcriptomic studies. It is expected that future proteomic studies, coupled with functional validation analyses, may provide new insights into disease resistance and pathogenicity, and therefore may contribute to the design of new and efficient strategies for the control of FHB disease.

References

Alfano JR, Collmer A (2004) Type III secretion system effector proteins: double agents in bacterial disease and plant defence. Annu Rev Phytopathol 42:385–414

Bhadauria V, Zhao W-S, Wang L-X, Zhang Y, Liu J-H, Yang J, Kong L-A, Peng Y-L (2007) Advances in fungal proteomics. Microbiol Res 162:193–200

Bhadauria V, Banniza S, Wang L-X, Wei Y-D, Peng Y-L (2010) Proteomic studies of phytopathogenic fungi, oomycetes and their interactions with hosts. Eur J Plant Pathol 126:81–95

Brown NA, Antoniw J, Hammond-Kosack KE (2012) The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: a refined comparative analysis. PLoS One 7(4):e33731. doi:10.1371/journal.pone.0033731

Bruneau JM, Magnin T, Tagat E, Legrand R, Bernard M, Diaquin M, Fudali C, Latgé JP (2001) Proteome analysis of *Aspergillus fumigatus* identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis. Electrophoresis 22:2812–2823

Chen S, Harmon AC (2006) Advances in plant proteomics. Proteomics 6:5504–5516

Cuomo CA, Güldener U, Xu JR, Trail F, Turgeon BG, Di Pietro A, Walton JD, Ma LJ, Baker SE, Rep M, Adam G, Antoniw J, Baldwin T, Calvo S, Chang YL, Decaprio D, Gale LR, Gnerre S, Goswami RS, Hammond- Kosack K, Harris LJ, Hilburn K, Kennell JC, Kroken S, Magnuson JK, Mannhaupt G, Mauceli E, Mewes HW, Mitterbauer R, Muehlbauer G, Münsterkötter M, Nelson D, O'donnell K, Ouellet T, Qi W, Quesneville H, Roncero MI, Seong KY, Tetko IV, Urban M, Waalwijk C, Ward TJ, Yao J, Birren BW, Kistler HC (2007) The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science 317:1400–1402

El-Gendy W, Brownleader MD, Ismail H, Clarke PJ, Gilbert J, El-Bordiny F, Trevan M, Hopkins J, Naldrett M, Jackson P (2001) Rapid deposition of wheat cell wall structural proteins in response to *Fusarium*-derived elicitors. J Exp Bot 54:85–90

Foroud N, Laroche A, Jordan M, Ellis B, Eudes F (2008) *Fusarium graminearum* and trichothecene induced differential transcriptomics and proteomics in resistant and susceptible wheat genotype. B Cereal Res Commun 36:239–243

Geddes J, Eudes F, Laroche A, Selinger BL (2008) Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host *Hordeum vulgare*. Proteomics 8:545–554

González-Fernández R, Jorrin-Novo JV (2012) Contribution of proteomics to the study of plant pathogenic fungi. J Proteome Res 11:3–16

González-Fernández R, Prats E, Jorrín-Novo J (2010) Proteomics of plant pathogenic fungi. J Biomed Biotechnol 2010:1–36

Grinyer J, Hunt S, McKay M, Herbert BR, Nevalainen H (2005) Proteomic response of the biological control fungus *Trichoderma atroviride* to growth on the cell walls of *Rhizoctonia solani*. Curr Genet 47:381–388

Hallen-Adams HE, Cavinder BL, Trail F (2011) *Fusarium graminearum* from expression analysis to functional assays. In: Jin-Rong X, Bluhm H (eds) Fungal genomics: methods and protocols, methods in molecular biology, vol 722. Springer, New York, pp 79–101

Hou ZM, Xue CY, Peng YL, Katan T, Kistler HC, Xu JR (2002) A mitogen activated protein kinase gene (MGVI) in *Fusarium graminearum* is required for female fertility, heterokaryon formation and plant infection. Mol Plant Microbe Interact 15:1119–1127

Kim Y, Nandakumar MP, Marten MR (2007) Proteomics of filamentous fungi. Trends Biotechnol 25:395–400

Kwon SJ, Cho S-Y, Mi Lee K-M, Yu J, Son M, Kim K-H (2009) Proteomic analysis of fungal host factors differentially expressed by *Fusarium graminearum* infected with *Fusarium graminearum* virus-DK21. Virus Res 144:96–106

Lee SH, Kim YK, Yun SH, Lee YW (2008) Identification of differentially expressed proteins in a mat1-2-deleted strain of *Gibberella zeae*, using a comparative proteomics analysis. Curr Genet 53:175–184

Lim D, Hains P, Walsh B, Bergquist P, Nevalainen H (2001) Proteins associated with the cell envelope of *Trichoderma reesei*: a proteomic approach. Proteomics 1:899–909

Lopez MF, Berggren K, Chernokalskaya E, Lazarev A, Robinson M, Patton WF (2000) A comparison of silver stain and SYPRORuby Protein Gel Stain with respect to protein detection in two dimensional gels and identification by peptide mass profiling. Electrophoresis 21: 3673–3683

Mann M, Hendrickson RC, Pandey A (2001) Analysis of proteins and proteomes by mass spectrometry. Annu Rev Biochem 70:437–473

Medina ML, Haynes PA, Breci L, Francisco WA (2005) Analysis of secreted proteins from *Aspergillus flavus*. Proteomics 5:3153–3161

Mehta A, Brasileiro ACM, Souza DSL, Romano E, Campos MA, Grossi-de-Sa MF, Silva MS, Franco OL, Fragoso RR, Bevitori R, Rocha TL (2008) Plant-pathogen interactions: what is proteomics telling us? FEBS J 275:3731–3746

Mohammadi M, Kazemi H (2002) Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Sci 162:491–498

Oide S, Krasnoff S, Gibson D, Turgeon BG (2007) Intracellular siderohores are essential for ascomycete sexual development in heterothallic *Cochliobolus heterostrophus* and homothallic *Gibberella zeae*. Eukaryot Cell 6:1339–1353

Paper JM, Scott-Craig JS, Cuomo CA, Walton JD (2007) Comparative proteomics of extracellular proteins in vitro and *in planta* from the pathogenic fungus *Fusarium graminearum*. Proteomics 7:3171–3183

Phalip V, Delande F, Carapito C, Goubet F, Hatsch D, Leize-Wagner E, Dupree P, Van Dorsselaer A, Jetsch J-M (2005) Diversity of the exoproteome of *Fusarium graminearum* grown on plant cell wall. Curr Genet 48:366–379

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP (2000) Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Mol Plant Microbe Interact 13:159–169

Pritsch C, Vance CP, Bushnell WR, Somers DA, Hohn TM, Muehlbauer GJ (2001) Systemic expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection. Physiol Mol Plant Pathol 58:1–12

Robertson D, Mitchell GP, Gilroy JS, Gerrish C, Bolwell GP, Slabas AR (1997) Differential extraction and protein sequencing reveals major differences in patterns of primary cell wall proteins from plants. J Biol Chem 272:15841–15848

Seong KY, Zhao X, Xu JR, Guldener U, Kistler HC (2008) Conidial germination in the filamentous fungus *Fusarium graminearum*. Fungal Genet Biol 45:389–399

Shim WB, Sagaram WS, Choi YE, So J, Wilkinson HH, Lee YW (2006) FSR1 is essential for virulence and female fertility of *Fusarium verticillioides* and *F. graminearum*. Mol Plant Microbe Interact 19:725–733

Shimizu M, Wariishi H (2005) Development of a sample preparation method for fungal proteomics. FEMS Microbiol Lett 247:17–22

Shin K-H, Mostafa Kama AH, Cho K, Choi J-S, Jin Y, Paek N-C, Lee YW, Lee JK, Park JC, Kim H-T, Heo H-Y, Woo SH (2011) Defense proteins are induced in wheat spikes exposed to *Fusarium graminearum*. POJ 4:270–277

Tan K-C, Ipcho SVS, Trengove RD, Oliver RP, Solomon PS (2009) Assessing the impact of transcriptomics, proteomics and metabolomics on fungal phytopathology. Mol Plant Pathol 10:703–715

Taylor RD, Saparno A, Blackwell B, Anoop V, Gleddie S, Tinker NA, Harris LJ (2008) Proteomic analyses of *Fusarium graminearum* grown under mycotoxin-inducing conditions. Proteomics 8:2256–2265

Trail F (2009) For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiol 149:103–110

Tsien RY (1998) The green fluorescent protein. Annu Rev Biochem 67:2354–2357

Urban M, Mott E, Farley T, Hammond-Kosack K (2003) The *Fusarium graminearum* MAP1 gene is essential for pathogenicity and development of perithecia. Mol Plant Pathol 4:347–359

Van Sluys MA, Monteiro-Vitorello CB, Camargo LEA, Menck CFM, Da Silva ACR, Ferro JA, Oliveira MC, Setubal JC, Kitajima JP, Simpson AJ (2002) Comparative genomic analysis of plant-associated bacteria. Annu Rev Phytol 40:169–189

Vogel J, Drapkin B, Oomen J, Beach D, Bloom K, Snyder M (2001) Phosphorylation of g-tubulin regulates microtubule organization in budding yeast. Dev Cell 1:621–631

Walter S, Nicholson P, Doohan FM (2010) Action and reaction of host and pathogen during Fusarium head blight disease. New Phytol 185:54–66

Wang Y, Yang L, Xu H, Li Q, Ma Z, Chy C (2005) Differential proteomic analysis of proteins in wheat spikes induced by *Fusarium graminearum*. Proteomics 5:4496–4503

Yang F, Jensen JD, Svensson B, Jørgensen HJL, Collinge DB, Finnie C (2010a) Analysis of early events in the interaction between *Fusarium graminearum* and the susceptible barley (*Hordeum vulgare*) cultivar Scarlett. Proteomics 10:3748–3755

Yang F, Jensen JD, Spliid NH, Svensson B, Jacobsen S, Jørgensen LN, Jørgensen HJL, Collinge DB, Finnie C (2010b) Investigation of the effect of nitrogen on severity of Fusarium head blight in barley. J Proteomics 73:743–752

Yang F (2011a) Application of proteomics to investigate barley-*Fusarium graminearum* interaction. Ph.D. thesis, Enzyme and Protein Chemistry Department of Systems Biology, Technical University of Denmark, Lyngby

Yang F, Jørgensen AD, Li H, Søndergaard I, Finnie C, Svensson B, Jiang D, Wollenweber B, Jacobsen S (2011b) Implications of high-temperature events and water deficits on protein profiles in wheat (*Triticum aestivum* L. cv. Vinjett) grain. Proteomics 11:1684–1695

Yang F, Jensen JD, Svensson B, JØrgensen HJL, Collinge DB, Finnee C (2012) Secretomics identifies *Fusarium graminearum* proteins involved in the interaction with barley and wheat. Mol Plant Pathol 13:445–453

Yu HY, Seo JA, Kim JE, Han KH, Shim WB, Yun SH, Lee YW (2008) Functional analyses of heterotrimeric G protein Ga and Gb subunits in *Gibberella zeae*. Microbiology 154:392–401

Zhao X-M, Zhang X-W, Tang W-H, Chen L (2009) FPPI: *Fusarium graminearum* protein-protein interaction database. J Proteome Res 8:4714–4721

Zhou W, Kolb FL, Riechers DE (2005) Identification of proteins induced or upregulated by Fusarium head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). Genome 48:770–780

Zhou W, Eudes F, Laroche A (2006) Identification of differentially regulated proteins in response to a compatible interaction between the pathogen *Fusarium graminearum* and its host, *Triticum aestivum*. Proteomics 6:4599–4609

Zhu H, Bilgin M, Snyder M (2003) Proteomics. Annu Rev Biochem 72:783–812

Part IV
Epidemiology

Chapter 9
Crop Residues and their Management in the Epidemiology of Fusarium Head Blight

Silvia Pereyra and Gladys A. Lori

Abstract Fusarium Head Blight, mainly caused by *Gibberella zeae* (anamorph *Fusarium graminearum*) has emerged as a frequent disease in the Southern Cone of South America. In this region, the area cultivated under no-tillage has considerably increased in the last two decades. No-till generates large quantities of crop residues on the soil surface, which represent the principal reservoir of *F. graminearum* and an ideal site for its sporulation. This chapter will consider current knowledge on the role of infected crop residues and other inoculum sources in the epidemiology of FHB and prospects for its management through cultural practices.

9.1 Introduction

Fusarium Head Blight (FHB or scab) has become one of the most devastating diseases of wheat and barley worldwide (Parry et al. 1995; McMullen et al. 1997; Gilbert and Tekauz 2000; Bai et al. 2003). FHB is of increasing concern in the southern cone of South America (Díaz de Ackermann and Kohli 1997; Galich 1997; Pereyra and Stewart 2001; Del Ponte et al. 2005). Although FHB has been a sporadic disease in the past, its occurrence in Argentina and Uruguay has increased in the past two decades and it now represents a major constraint to wheat and barley production (Moschini and Fortugno 1996; Kikot et al. 2011).

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Wheat grain yield losses caused by FHB in Uruguay during the epidemic years of 1990, 1991, and 1993 have been estimated between 0.5 and 31 % (Díaz de Ackermann and Kohli 1997). In Argentina, the worst outbreaks occurred in 1945–1946, 1978, 1985, 1993 and 2001. Yield losses varied among regions but general estimates ranged 20–30 % (Galich 1989; Galich and Galich 1996; Kohli et al. 1996; Moschini and Fortugno 1996; Kikot et al. 2011). In 1993, grain yield losses up to 50 % were estimated in areas planted under no tillage and corn as a previous crop (Galich 1997). Yield losses for barley have not been thoroughly quantified, but in both crops, FHB is important because of the production of mycotoxins, of which deoxynivalenol (DON) is the most prevalent (Piñeiro 1997). In Argentina and Uruguay, FHB of wheat is incited primarily by *Fusarium graminearum* (Schw.) [perfect stage *Gibberella zeae* (Schwein) Petch] (Boerger 1928; Boasso 1961; Pritsch 1995; Dalcero et al. 1997; Galich 1997; Lori et al. 2003; Pereyra et al. 2006; Umpierrez et al. 2011). Other *Fusarium* species have also been identified in this region including *F. avenaceum* and *F. poae* in wheat and barley grains (Stagno 1980; Lori and Sisterna 2001; Pereyra and Stewart 2001; González et al. 2008; Pereyra and Dill-Macky 2010).

The area cultivated under no-tillage has considerably increased in the last two decades in the southern cone of South America as a need to reduce both soil erosion and costs. This practice generates large quantities of crop residues on the soil surface, which represent the principal reservoir of *F. graminearum* and an ideal site for its sporulation (Sutton 1982; Pereyra et al. 2004; Pereyra and Dill-Macky 2008; Lori et al. 2009). This chapter will consider current knowledge on the role of infected crop residues and other inoculum sources in the epidemiology of FHB and prospects for its management through cultural practices.

9.2 Inoculum Types

Fusarium graminearum may survive as mycelia, ascospores, macroconidia, and chlamydospores (Nyvall 1970; Sutton 1982; Reis 1988; Shaner 2003; Bockus et al. 2010). Inoculation of wheat and barley spikes occurs by aerial dissemination of spores from inoculum sources. Consequently, ascospores and to a lesser extent macroconidia, are the main types of primary inoculum in the epidemiology of FHB (Sutton 1982; Reis 1988; Parry et al. 1995; Shaner 2003; Bockus et al. 2010).

Perithecia are dark blue to black, superficial and gregarious (Booth 1971). Asci (60–85 × 8–11 μm) are formed inside the perithecia and contain eight fusoid ascospores (19–24 × 3–5 μm), usually three- to four-celled. A perithecium may contain as many as 45,000 ascospores (Khonga and Sutton 1988). Perithecia represent a survival structure and provide the primary inoculum to infect wheat or barley the following growing season (Cook 1981; Reis 1988; Shaner 2003; Champeil et al. 2004; Osborne and Stein 2007; Bockus et al. 2010).

Ascospore maturation and ejection appears to be a cyclical process depending upon temperature and moisture levels (Reis 1988). Rainfall may be needed for perithecial and ascospore formation and maturity on crop residues, although rain would not necessarily trigger the actual release of ascospores. Ascospores are generally released from the perithecia during the night, between 2100 and 0600 or 0800 h, and subsequently dispersed by wind (Paulitz 1996). Ascospore discharge appears to be activated by the hydration of the perithecia (Tschanz et al. 1975, 1976; Reis 1988, 1990; Paulitz 1996) and is favored by cool to moderate temperatures (optimum 16 °C) (Tschanz et al.1975, 1976). There appears to be a threshold (>80 %RH) below which ascospore discharge would not occur (Gilbert and Tekauz 2000). Alternative cycles of drying and increases of relative humidity during the day are suggested as the stimulus for the release of ascospores (Paulitz 1996; Fernando et al. 2000; Inch et al. 2005; Trail et al. 2005). Cycles of ascospore ejection continue until the perithecia are empty (Reis 1988). The ejection distance is very small (few millimetres) (Trail et al. 2005). However, long distance dispersal may occur by air movement (Fernando et al. 1997; Maldonado-Ramirez et al. 2005).

Warm conditions are required for perithecia formation, maturation and subsequent ascospore production. Perithecia may form at temperatures from 5 to 35 °C but the optimum temperature is approximately 29 °C (Sutton 1982). Ascospore production has been observed at 13–33 °C, with an optimum around 25–28 °C. Perithecial initiation also requires low intensity ultraviolet light (<390 nM) and the most effective wavelength range is 300–320 nM (Tschanz et al. 1976). Perithecia maturation occurs in 9–10 days under extremely favourable conditions (Tschanz et al. 1975) but generally requires 2–3 weeks in the field (Sutton 1982). Perithecia may form throughout the year in the southern cone of South America as demonstrated by Reis (1990) as temperatures and other environmental conditions permit.

Macroconidia are produced in short lateral phialides and in sporodochia on crop residues (Paulitz 1996; Shaner 2003; Bockus et al. 2010). Because of the sticky and hydrophilic nature of these conidia, splashing or wind driven rain represents their main dispersal mechanism (Sutton 1982).

Temperature requirements for production of macroconidia are similar to those for the formation of perithecia and ascospores. Macroconidia are produced in a temperature range of 16–36 °C, with an optimum of 32 °C (Andersen 1948). Moist conditions are also required for macroconidia formation (Sutton 1982). Production of macroconidia by isolates of *F. graminearum* is maximal at −0.14 MPa, and progressively less as the substrate water potential decreases (Sutton 1982). Cook (1981) reported production of sporodochia under conditions of free moisture on the plant/residue surface, and a shift to perithecial production as the plant tissues dried.

Because of the characteristics mentioned above, macroconidia might be responsible for the short distance dispersal of the disease while ascospores are responsible for the long distance dispersal (Reis 1989; Kohli et al. 1996).

9.3 Crop Residue: Survival and Inoculum Production

9.3.1 Importance of Crop Residues

Most of the *F. graminearum* life cycle in South America occurs under saprophytic growth (300–320 days), while the pathogenic growth is confined to 40–60 days in the year from crop anthesis (September to November) to grain harvest (November to January). Most of the saprophytic growth occurs on the hosts residues. *Fusarium graminearum* may survive in stubble of many agricultural important cereal crops in this region like corn, wheat, barley, oats, rye, among others (Reis 1988; Fernandez 1991; Pereyra and Dill-Macky 2008; Lori et al. 2009; Mourelos et al. 2011b). The fungus may also survive in residues of other gramineous species, either native or exotic, that are components of native or planted pastures or weeds including grasses of the genera *Agropyron, Agrostis, Andropogon*, *Botriochloa*, *Bromus*, *Cenchrus*, *Cortaderia*, *Digitaria*, *Echinochloa*, *Lolium*, *Pannicum*, *Paspalum, Pennisetum, Poa,* and *Setaria* (Costa Neto 1976; Reis 1988; Fernandez 1991; Pereyra and Dill-Macky 2008; Mourelos et al. 2011b). Interestingly, residues of nongramineous species have also been found to host *F. graminearum* under natural conditions such as soybean, sunflower and alfalfa and in inflorescences of various nongramineous weeds (Fernandez 1991; Pereyra and Dill-Macky 2008; Mourelos et al. 2011a, b).

The presence of *Fusarium* species was examined in the residues of wheat, barley, corn, sunflower, pasture, and gramineous weed species common in wheat and barley cropping systems in Uruguay by Pereyra and Dill-Macky (2008). Residues were collected from February 2001 to March 2003 from no tillage and reduced tillage plots established in southwestern Uruguay. *Fusarium graminearum* was recovered from residues of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), corn (*Zea mays*), sunflower (*Helianthus annus*), fescue (*Festuca arundinacea* – a component of pastures) and the gramineous weeds *Digitaria sanguinalis, Setaria spp., Lolium multiflorum,* and *Cynodon dactylon*. *Fusarium graminearum* was not isolated from the other pasture components examined, birds foot trefoil (*Lotus corniculatus*) and white clover (*Trifolium repens*). Recovery of *F. graminearum* declined over time in all residues examined, as residue decomposed. However, survival of the fungus did not necessarily implied inoculum production on all plant residues. Perithecia and ascospore production depended on the plant species. Greater ascospore production was observed on wheat and barley residue than on corn or gramineous weeds species. Sunflower residue did not support ascospore production.

Colonized residue provides an ideal site for sporulation. In the southern hemisphere, the fungus may over summer on residues producing perithecia and sporodochia that would give rise to ascospores and macroconidia, respectively. Perithecia may form since late spring or early summer sometimes immediately before harvesting and ascospores are ejected from perithecia and deposited on wheat heads at the time of flowering (Reis 1988). It has also been observed that *F. graminearum* produced ascospores on infested residues primarily in the first 2 years after the crops were harvested (Pereyra and Dill-Macky 2008).

9.3.2 Fusarium *Species as Residue Colonizers*

Garret (1970) stated that the fungi sharing a given crop residue would relate directly to their competitive saprophytic ability and to their inoculum potential, and inversely to the inoculum potential of their competitors. Competitive saprophytic ability is defined by the summation of physiological characteristics that make for success in a competitive colonization crop residue (Garret 1956). Some characteristics that contribute to competitive saprophytic ability are: rapid germination of fungal propagules and fast growth of young hyphae when stimulated by soluble nutrients that diffuse from a substrate, an adequate enzyme array for decomposition of the more resistant carbon constituents of plant tissues (i.e.: cellulose, lignin), the excretion of fungistatic and bacteriostatic growth products, and the tolerance to fungistatic compounds produced by other microorganisms (Garret 1970). Inoculum potential is defined as the energy of growth of a fungus, available for colonization of a substrate (Garret 1970).

The most significant fitness attributes of pathogenic *Fusarium* species are their relative high saprophytic ability, their long-term survival in the absence of the host, high level of aggressiveness, ability to generate genetic variability (Jamieson 1985) and their ability to colonize a wide range of substrates (Reis 1988). *Fusarium* species readily adapt to new ecological niches (Burgess 1981). There appears to be an inverse relationship between the parasitic ability and saprophytic ability of *Fusarium* species (Jamieson 1985). Changes in the population of the species of *Fusarium* on buried wheat residue are observed accordingly. For instance, *F. graminearum*, *F. avenaceum,* and *F. culmorum*, which have high parasitic ability, would decline following the death of the host plant, whereas other species with low parasitic ability but a greater saprophytic ability, such as *F. equiseti, F. oxysporum, F. poae, F. solani* and *F. sporotrichioides* would increase (Jamieson 1985; Pereyra et al. 2004; Fernandez et al. 2008; Pereyra and Dill-Macky 2008). Ocamb (1991) found that *F. oxysporum*, *F. verticillioides* and *F. proliferatum* were more competent than *F. graminearum* in living maize roots in the field and had a greater competitive saprophytic ability for cellulose, pectin, and xylans.

9.3.3 *Structural and Chemical Composition of Crop Residues*

Wheat residue is composed of stems (nodes and internodes), leaves, floral parts (rachis, glumes, lemmas, paleae), crowns, and kernels that have been lost at harvest. Wheat straw is commonly made up of a variable number of internodes (five to six), separated by nodes, the points at which leaves are attached. The nodes are small areas with meristematic activity in the living plant, but generally include the base of the leaf sheath and small parts of internodes when separated manually from other residues (Harper and Lynch 1981). Nodes and internodes account for 40–80% of the total wheat residue. Wheat straw may also include the rachis, the residual part of the

stem of the flower spike, which is composed of a number of nodes with very short internodes (Theander and Aman 1984). Differences in the biochemical composition of these different residue components may contribute to a differential colonization and *G. zeae/F. graminearum* ascospores production.

It has been observed that the internode walls of wheat straw were composed of 5–7 % epidermis, 25–27 % sclerenchymatous tissue and 65–69 % parenchymatous tissue. The lignified tissues in straw internodes are mainly located in an outer ring, which makes this fraction very resistant to microbial degradation. Therefore, decomposition occurs primarily from the lumen side of the internode or from fractured areas (Harper and Lynch 1981, 1985).

The major chemical components of straw are cellulose and hemicelluloses, which contribute 75–80 % of the dry weight and represent the major substrates for production of acetic acid. Simple water-soluble components make up about 10 % of the straw weight and include 5 % of the total carbon (Harper and Lynch 1985). When the chemical composition of internodes and nodes of wheat straw were compared, internodes contained more cellulose (411 g/kg DM) but similar amounts of hemicellulose (245 g/kg DM) and lignin (216 g/kg DM) (Theander and Aman 1984).

The amount of *Fusarium* species DNA in wheat residues was found to decrease *Fusarium* in nodes and internodes after harvest. However, a different pattern was observed for the population dynamics in stem bases where the amount fluctuated without a clear tendency (Khöl et al. 2007). Particularly, *Fusarium graminearum* appears to survive longer in tissues that resist breakdown: survival being longer in nodal than internodal tissues of wheat stems (Burgess and Griffin 1968; Reis 1988; Pereyra and Dill-Macky 2005). In addition, perithecial formation and ascospore production has been found to be most abundant on *Fusarium* infected kernels than on nodes (Khonga and Sutton 1988; Pereyra and Dill-Macky 2005) and floral bracts (Pereyra and Dill-Macky 2005).

9.4 Residue Decomposition

9.4.1 Factors that Influence Residue Decomposition

Crop residues are decomposed by microorganisms inhabiting the soil. During this process, the natural organic compounds present in residue tissues are attacked by microbial enzymes that degrade them (Parr and Papendick 1978).

A wide range of microorganisms are able to colonize and decompose crop residues, however, fungi are thought to be dominant in the early stages. Residue colonization initially involves microbial growth on the simple soluble components, so cellulolytic fungi capable of growth on these substances will gain initial advantage in having colonized the residue prior to the onset of cellulolysis. The process depends upon the fact that microbial degradation of pectines, hemicelluloses, celluloses, and lignin in the plant cell wall is brought about by enzymes released by the

microorganisms (Garret 1970). Higher lignin content relative to cellulose tends to reduce the decomposition rate (Parr and Papendick 1978; Blevins et al. 1994). The rate at which a fungus can degrade cellulose and other more resistant components of wheat residue tissue may be a consequence of the competitive saprophytic ability for the particular residue. It must be taken into account that the competitive saprophytic ability is relative to the prevalent environmental conditions (Garret 1970).

Studies of residue decay in soil have revealed a succession of colonists, including *Fusarium*, *Mucor*, *Penicillium*, and *Trichoderma* spp. (Harper and Lynch 1985). Burgess and Griffin (1967) showed that the more favourable the conditions for microbial activity (i.e.: 35 °C and 100 % relative humidity), the shorter the life span of *F. graminearum* in the wheat residue.

Consequently, in a situation that most of the crop residues are left on the soil surface, such as is common in no-tillage or minimum tillage systems, the decomposition rate is slow, because the access of the microorganisms is restricted. When crop residues are incorporated into the soil, for instance in a conventional tillage situation, they are decomposed by the microorganisms at a faster rate.

Ubiquitous residue colonizers of the genus *Trichoderma* may directly influence *F. graminearum* growth, parithecial production (Cabrera et al. 2008) and deoxynivalenol production (Naef et al. 2006). At the same time, *Trichoderma* species may indirectly affect *F. graminearum* by outcompeting other soil microorganisms and accelerate residue decomposition by the action of lytic enzymes. Particularly, *T. atroviride and T. koningiopsis* obtained from wheat residue in Uruguay significantly reduced *F. graminearum* perithecial production suggesting that the application of these antagonists might aid in the reduction of primary inoculums on crop residues (Cabrera et al. 2008). Current research in biocontrol of FHB in Uruguay is focusing in the interaction between previous crops or crop sequences and inundative practices with characterized isolates of these two species (Perez and Villar 2011).

Soil fauna may also influence residue decomposition and hence affect *F. graminearum* survival. They not only may feed on mycelia but also fragment residue and organic matter contributing to rapid residue decomposition. Anecic earthworms (*Lumbricus terrestris*) may promote the reduction of *Fusarium* biomass and deoxynivalenol content in wheat straw (Oldenburg et al. 2008; Wolfarth et al. 2012). Also the interaction between nematodes and collembolans may significantly contribute to deoxynivalenol degradation in wheat straw (Wolfarth et al. 2013).

Residue decomposition may also be regulated by several factors of the residue itself, the soil and the climatic conditions. Aspects like the carbon:nitrogen ratio of the residue, size and distribution of the residue particles, soil pH, oxygen content, temperature, and moisture influence the decomposition rate by influencing the microbial population (Cook and Baker 1983). Generally, the rate of residue decomposition decreases as the residue carbon:nitrogen ratio increases (Knapp et al. 1983). Moreover, it has been demonstrated that the decomposition of wheat residue has been strongly dependent on available carbon and nitrogen during initial decomposition (Knapp et al. 1983). Decomposition of wheat residue is quicker if nitrogen in an available form is applied (Garret 1970).

Bigger residue particles as well as those placed on the soil surface would last longer in the field than the smaller and buried ones (Parr and Papendick 1978). When wheat residue was buried at 7.5–20 cm, less than 2 % of the residue remained after 24 months in the field (Pereyra et al. 2004).

Residue placement may also substantially affect microclimatic parameters that control rates of crop residues decomposition (Radke and Honeycutt 1994). Warm temperatures as well as humid conditions accelerate residue decomposition (Blevins et al. 1994; Parr and Papendick 1978). It has been reported that the decomposition rate increases two to three times with a 10 °C increase in temperature (Cook and Baker 1983). On the contrary, low soil temperatures such as those occurring during approximately 6 months in the upper Midwest of United States, cause a slow decomposition rate.

Moisture is a primary factor affecting microbial populations degrading crop residues. Optimal soil water for residue decomposition occurs at approximately −0.03 MPa (Radke and Honeycutt 1994). Soils with low oxygen exchange rates with the atmosphere tend to reduce the rate of residue decomposition (Parr and Papendick 1978).

9.4.2 Residue Decomposition and Tillage Management Practices

The survival of pathogens in crop residues on the soil surface can be influenced by the chemical and physical environment that tillage operations create. Tillage practices influence survival of pathogens mainly through effects in the amount, position and rate of breakdown of crop residues and even more, may modify the microenvironment within subsequent crops (Rees 1987).

Tillage can affect the concentration and distribution of nutrients in the soil. Non-tilled soils usually have a lower concentration of soluble nitrogen and a lower pH (Watkins and Boosalis 1994; Radke and Honeycutt 1994). The physical environment of minimum-tilled or non-tilled soils differs from that of chisel or mouldboard ploughed soils. Non-tilled soils are generally wetter, cooler, and more compacted than tilled soils (Watkins and Boosalis 1994).

Spring or fall mouldboard ploughing used to be the most important common tillage method in temperate climates (Watkins and Boosalis 1994). Ploughing promotes the breakdown of crop residues by burying them and is recommended as an efficient FHB control method as it reduces or eliminates the inoculum source of *F. graminearum* (Khonga and Sutton 1988; Pereyra et al. 2004). Perithecia of *G. zeae* are only formed on crop residues left on the soil surface. When residues are incorporated into the soil, without presence of light, perithecia do not form as light is not available and thus, the available inoculum in the air is reduced (Reis 1988, 1989; Dill-Macky and Jones 2000; Pereyra et al. 2004).

Conventional tillage was widely used after World War II and until the early 1980s. It is defined as a preliminary deep tillage operation, usually by the use of

mouldboard plough, and then followed by some secondary tillage system for seed bed preparation (Sturtz et al. 1997). Mouldboard plough inverts the top 15- to 25-cm layer of the soil profile to bury crop residue. However, the trend in agriculture since the mid-1980s has been the progressive elimination of excessive cultivation in favour of limited or strategic tillage practices (Sturtz et al. 1997). The area cultivated under no-tillage has considerably increased in the last two decades in the southern cone of South America.

Half of the world area cultivated under no tillage corresponds to Latin America. Recent surveys in Argentina show that 27 million hectares of all crops are planted under no tillage, representing 78.5 % of the total arable land in this country (AAPRESID 2012). Particularly, in Uruguay, a plan to soil conservation of each field is mandatory based on a law established in 2009 (MGAP 2011). These plans include adequate crop rotations and residue management as the principal means of reducing soil erosion. Therefore, it is expected that no tillage would continue leading the most important change in residue management practices in this country. Recent surveys report that this system is currently being used on more than 80 % of the cropping area (MGAP 2011).

Although no tillage represents the greatest protection from erosion, it also increases the amount of inoculum of residue-borne pathogens such as *F. graminearum* (Cook et al. 1978; Sutton 1982; Reis 1988, 1989; Bai and Shaner 1994). In the absence of tillage, crop residues and residue borne pathogens are concentrated in the upper 15 cm of soil (Sturtz et al. 1997). The presence of the residue as an energy source for fungal pathogens is critical to the host-pathogen interaction, by influencing fungal survival (Boosalis et al. 1986), germination and infective capability (Sturtz et al. 1997).

Tillage affects residue decomposition rate of wheat residue. Buried wheat residue decomposed after 24 months in the fields under Minnesota (USA) conditions, while 25 % of the dry matter wheat residue on the soil surface was still present (Pereyra et al. 2004).

In a 2-year study on the influence of crop residues and tillage systems on FHB in Uruguay, it has been observed that only in 1 year wheat and barley plants in no-till treatments had significantly higher FHB incidence and severity than plants in reduced tillage plots (Pereyra and Dill-Macky 2008). It is suggested that favourable climate conditions are the main factor determining the infection and development of FHB. In this sense, Lori et al. (2009) found that tillage practices (conventional tillage *vs.* no-till) did not affect FHB development in Argentina.

The relative impact of tillage operations should be evaluated in the context of other sources of inoculum and agronomic practices including the cropping history of a certain field, and of nearby fields, regarding rotation, susceptibility of cereal cultivars/hybrids, and previous occurrence of FHB. All these agronomic factors might determine the risk of disease than the current tillage practice alone as suggested by Miller et al. (1998) and Lori et al. (2009).

Two important agricultural practices, crop rotation and tillage rotation, may attenuate the effect of surface residue on FHB. Crop rotation provides a period for the natural biological destruction of pathogen inoculum in residues and soil to

occur. Planting a crop into its own residue from the previous season, without a fallow period, would more likely aggravate the disease than using crop rotation with non susceptible crops (Watkins and Boosalis 1994). A crop rotation period with non-susceptible crops of at least 2 years is recommended in Uruguay to mitigate the risk of FHB (Diaz de Ackermann and Pereyra 2011).

The colonization and survival of the pathogen on weed species provide an alternative mechanism of survival. As mentioned earlier, *F. graminearum* may be harboured by many gramineous and even non-gramineous species (Costa Neto 1976; Reis 1988, 1989; Fernandez 1991; Pereyra and Dill-Macky 2008; Mourelos et al. 2011a). The ubiquitous nature of *F. graminearum* may negate some of the benefits of crop rotation as a disease control method in conservation tillage systems (Reis 1988, 1990; Bai and Shaner 1994; Watkins and Boosalis 1994).

Alternating tillage systems may also diminish the amount of inoculum, because it can reduce the amount of residue on soil surface compared to no-till. Inclusion of tillage rotation systems with crop rotation is a good alternative for disease management. Watkins and Boosalis (1994) suggested a tillage/crop rotation practice that allows retention of 20–30 % residue cover while also enhancing the weathering of the residue to reduce pathogen inoculum. Additional residue management practices may include.

9.5 Conclusions

Fusarium Head Blight poses a significant threat to wheat and barley yields and grain quality in the southern cone of South America. This disease is highly dependent on the environment, especially during flowering and the early stages of grain development. As climate cannot be controlled, an integrated management of FHB including the use of moderately resistant cultivars, effective fungicide applications deployed with the aid of forecasting models, and cultural practices that reduce primary inoculum may provide in reducing the risk of FHB outbreaks.

Due to the fact that no tillage will remain as the major practice to plant extensive crops in this region of the world, we need to find new alternatives to reduce inoculum originated in soil surface-crop residues. Practices that aid in accelerating residue decomposition such as crop rotation with intervals of at least two years without susceptible crop species, the inclusion of some alternating tillage practice at least after an epidemic year, chopping and even distribution of infested residue, use of green manures that favour soil antagonists such as *Trichoderma* and eventually the use of biocontrol on residues may help in reducing the survival and inoculum potential of *Fusarium* infested residues.

References

AAPRESID (2012) Evolución del área de siembra directa en Argentina. http://www.aapresid.org.ar/images/cms/assets/docs/aapresid.evolucion_superficie_sd_argentina. 1977_a_2011.pdf. Accessed 25 Jan 2013

Andersen AL (1948) The development of *Gibberella zeae* head blight of wheat. Phytopathology 38:595–611
Bai G, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78(8):760–766
Bai G, Chen F, Shaner G (2003) Breeding for resistance to Fusarium head blight in China. In: Leonard KJ, Bushnell WR (eds) Fusarium head blight of wheat and barley. APS Press, St Paul, pp 96–317
Blevins RL, Frye WW, Wagger MG, Tyler DD (1994) Residue management strategies for the southeast. In: Hatfield JL, Stewart BA (eds) Crops residue management – advances in soil science. CRC Press, Boca Raton, pp 63–76
Boasso C (1961) Estado fitosanitario de los cultivos de trigo de la reciente cosecha. Boletín Informativo 854:7
Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW (2010) Compendium of wheat diseases and pests, 3rd edn. APS Press, St Paul, p 171
Boerger A (1928) Observaciones sobre agricultura, quince años de trabajos fitotécnicos en Uruguay. Imprenta Nacional, Montevideo, p 436
Boosalis MG, Doupnik BL Jr, Watkins JE (1986) Effect of surface tillage on plant diseases. In: Sprague MA, Triplett GB (eds) No-tillage and surface-tillage agriculture: the tillage revolution. Wiley, New York, pp 389–408
Booth C (1971) The genus Fusarium. Farnham Royal, Commonwealth Agricultural Bureau. Commonwealth Mycological Institute, Kew, p 271
Burgess LW (1981) General ecology of Fusaria. In: Nelson PE, Tousson TA, Cook RJ (eds) *Fusarium*: diseases, biology, and taxonomy. The Pennsylvania State University Press, University Park, pp 225–235
Burgess LW, Griffin DM (1967) Competitive saprophytic colonization of wheat straw. Ann Appl Biol 60:137–142
Burgess LW, Griffin DM (1968) The recovery of *Giberella zeae* from wheat straws. Aust J Exp Agric Anim Husb 8:364–370
Cabrera M, Pereyra S, Vero S (2008) Biological control of *Gibberella zeae* with *Trichoderma* spp. Phytopathology 98:S29
Champeil A, Fourbet JF, Dore T, Rossignol L (2004) Influence of cropping system on Fusarium head blight and mycotoxin levels in winter wheat. Crop Prot 23:531–537
Cook RJ (1981) *Fusarium* diseases of wheat and other small grains in North America. In: Nelson PE, Tousson TA, Cook RJ (eds) *Fusarium*: diseases, biology, and taxonomy. The Pennsylvania State University Press, University Park, pp 39–55
Cook RJ, Baker KF (1983) The nature and practice of biological control of plant pathogens. APS Press, St Paul, p 539
Cook RJ, Boosalis MG, Doupnik GP (1978) Influence of crop residues on plant diseases. In: Oschwald WR (ed) Crop residue management systems. American Society of Agronomy ASA special publication no 31. American Society of Agronomy, Madison, pp 147–163
da Costa Neto JP (1976) Lista de fungos sobre gramíneas (capins e cereais) no Rio Grande do Sul. Revista da Faculdade de Agronomia UFRGS 1:43–78
Dalcero A, Torres A, Etcheverry M, Chulze S, Varsavsky E (1997) Occurrence of deoxynivalenol and in Argentinian wheat. Food Addit Contam 14:11–14
de Galich MTV (1989) Importancia y difusión de la fusariosis del trigo en Argentina. In: Kohli MM (ed) Taller sobre fisariosis de la espiga en América del Sur. CIMMYT, México, pp 7–26
de Galich MTV (1997) Fusarium head blight of wheat in Argentina. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, Mexico, pp 19–28
de Galich MTV, Galich A (1996) Enfermedades del trigo en el área central-norte de la región cerealera argentina (1982–1994). Inf. Técnico 121 EEA Marcos Juarez INTA Córdoba Argentina
Del Ponte EM, Fernandes JMC, Pavan W (2005) A risk infection simulation model for Fusarium head blight of wheat. Fitopatol Bras 30:634–642
Díaz de Ackermann M, Kohli M (1997) Research on Fusarium head blight in Uruguay. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, Mexico, pp 13–18

Díaz de Ackermann M, Pereyra S (2011) Fusariosis de la espiga de trigo y cebada. In: Pereyra S, Díaz M, Germán S, Cabrera K (eds) Manejo de enfermedades de trigo y cebada. Serie Técnica 189. INIA Uruguay Ed Hemisferio Sur Montevideo, pp 111–128

Dill-Macky R, Jones R (2000) The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis 84:654–660

Fernandez MR (1991) Recovery of *Cochliobolus sativus* and *Fusarium graminearum* from living and dead wheat and non-gramineous winter crops in southern Brazil. Can J Bot 69: 1900–1906

Fernandez MR, Huber D, Basnyat P, Zentner RP (2008) Impact of agronomic practices on populations of *Fusarium* and other fungi in cereal and noncereal crop residue on the Canadian Prairies. Soil Tillage Res 100:60–71

Fernando WGD, Paulitz TC, Seaman WL, Dutilleul P, Miller JD (1997) Head blight gradients caused by *Giberella zeae* from area sources of inoculum in wheat field plots. Phytopathology 87:414–421

Fernando WGD, Miller JD, Seaman WL, Seifert K, Paulitz TC (2000) Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Can J Bot 78:497–505

Garret SD (1956) Biology of root-infecting fungi. Cambridge University Press, London

Garret SD (1970) Pathogenic root-infecting fungi. CambridgeUniversity Press, Cambridge, p 294

Gilbert J, Tekauz A (2000) Review: recent developments in research on fusarium head blight of wheat in Canada. Can J Plant Pathol 22:1–8

González HHL, Moltó GA, Pacin A, Resnik SL, Zelaya MJ, Masana M, Martínez EJ (2008) Trichothecenes and Mycoflora in wheat harvested in nine locations in Buenos Aires Province, Argentina. Mycopathologia 165:105–114

Harper SHT, Lynch JM (1981) The chemical components and decomposition of wheat straw leaves, internodes and nodes. J Sci Food Agric 32:1057–1062

Harper SHT, Lynch JM (1985) Colonization and decomposition of straw by fungi. Trans Br Mycol Soc 85:655–661

Inch S, Fernando WGD, Gilbert J (2005) Seasonal and daily variation in the airborne concentration of *Gibberella zeae* (Schw.) Petch spores in Manitoba. Can J Plant Pathol 27:357–363

Jamieson AR (1985) Aggressiveness and saprophytic ability in species of *Fusarium* associated with wheat. PhD thesis, University of Guelph, Ottawa, p 149

Khonga EB, Sutton JC (1988) Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Can J Plant Pathol 10:232–239

Kikot GE, Moschini RC, Consolo VF, Rojo R, Salerno G, Hours RA, Gasoni L, Arambarri AM, Alconada TM (2011) Occurrence of different species of Fusarium from wheat in relation to disease levels predicted by a weather-based model in Argentina pampas region. Mycopathologia 171:139–149

Knapp EB, Elliot LF, Campbell GS (1983) Carbon, nitrogen and microbial biomass interrelationships during the decomposition of wheat straw: a mechanistic simulation model. Soil Biol Biochem 15:455–461

Köhl J, de Haas BH, Kastelein P, Burgers SLGE, Waalwijk C (2007) Population dynamics of *Fusarium* spp. and *Microdochium nivale* in crops and crop residues of winter wheat. Phytopathology 97:971–978

Kohli MM, Annone JG, Galich MTV (1996) Fusariosis de la espiga y su manejo. In: Kohli MM, Annone JG, García R (eds) Curso de manejo de enfermedades del trigo. Centro Internacional de Capacitación INTA-CIMMYT Pergamino Bs As Argentina1995, pp 164–189

Lori GA, Sisterna MN (2001) Occurrence and distribution of *Fusarium* spp. associated with durum wheat seed from Argentina. J Plant Pathol 83:63–67

Lori GA, Sistema MN, Haidukowski M, Rizzo I (2003) *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area in Argentina. Microbiol Res 158:29–35

Lori GA, Sistema MN, Sarandón SJ, Rizzo I, Chidichimo H (2009) Fusarium head blight in wheat: impact of tillage and other agronomic practices under natural infection. Crop Prot 28: 495–502

Maldonado-Ramirez SL, Schmale DG III, Shields EJ, Bergstrom GC (2005) The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggest the role of long-distance transport in regional epidemics of Fusarium head blight. Agric For Meteorol 132:20–27

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a reemerging disease of devastating impact. Plant Dis 81:1340–1348

MGAP (2011) Anuario Estadísticas Agropecuarias 2011. Ministerio de Ganadería Agricultura y Pesca, Montevideo, p 246

Miller JD, Culley J, Fraser K, Hubbard S, Meloche F, Ouellet T, Seaman WL, Seifert KA, Turkington K, Voldeng H (1998) Effect of tillage practice on fusarium head blight of wheat. Can J Plant Pathol 20:95–103

Moschini R, Fortugno C (1996) Predicting wheat head blight incidence using models based on meteorological factors in Pergamino Argentina. Eur J Plant Pathol 102:211–218

Mourelos CA, Malbrán I, Balatti PA, Ghiringhelli PD, Lori GA (2011a) Fusariosis de la espiga de trigo: monitoreo de malezas como fuente de inóculo y detección de *Fusarium graminearum* In: 2º Congreso Argentino de Fitopatología. Mar del Plata, Argentina. Asociación Argentina de Fitopatólogos (AAF). Libro de Resumenes, p 218

Mourelos CA, Malbrán I, Mengual Gómez DL, Balatti PA, Ghiringhelli PD, Lori GA (2011b) *Fusarium graminearum*: dynamic and survival in plant debris and detection by Polymerase Chain Reaction (PCR). In: Ramírez ML, Barros GG. Chulze S (eds) Strategies to reduce the impact of mycotoxins in Latin America in a global context. International Symposium Mycored (ISM) conference 2011, Mendoza, p 129

Naef A, Senatore M, Défago G (2006) A microsatellite based method for quantification of fungi in decomposing plant material elucidates the role of *Fusarium graminearum* DON production in the saprophytic competition with *Trichoderma atroviride* in maize tissue microcosms. FEMS Microbiol Ecol 55:211–220

Nyvall RF (1970) Chlamydospores of *Fusarium roseum* 'Graminearum' as survival structures. Phytopathology 60:1175–1177

Ocamb CM (1991) Ecology of soilborne *Fusarium* species associated with roots and the rhizosphere of *Zea mays*. PhD thesis, University of Minnesota, Saint Paul, p 94

Oldenburg E, Kramer S, Schrader S, Weinert J (2008) Impact of the earthworm *Lumbricus terrestris* on the degradation of *Fusarium*-infected and deoxynivalenol-contaminated straw. Soil Biol Biochem 40:3049–3053

Osborne LE, Stein JM (2007) Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol 119:103–108

Parr JF, Papendick RI (1978) Factors affecting the decomposition of crop residues by microorganisms. In: Oschwald WR (ed) Crop residue management systems. American Society of Agronomy ASA special publication no 31. American Society of Agronomy, Madison, pp 101–129

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathol 44:207–238

Paulitz TC (1996) Diurnal release of ascospores by *Giberella zeae* in inoculated wheat plots. Plant Dis 80:674–678

Pereyra SA, Dill-Macky R (2005) Colonization and inoculum production of *Gibberella zeae* in components of wheat residue. Cereal Res Commun 33:755–762

Pereyra SA, Dill-Macky R (2008) Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to Fusarium head blight inoculum. Plant Dis 92:800–807

Pereyra SA, Dill-Macky R (2010) *Fusarium* species recovered from wheat and barley grains in Uruguay, pathogenicity and deoxynivalenol content. Agrociencia 14:33–44

Pereyra S, Stewart S (2001) Investigación en fusariosis de la espiga de cebada en Uruguay. In: Minella E (ed) XXI Reuniao Anual de Pesquisa de cevada Anais e ata. EMBRAPA Trigo, Passo Fundo, pp 41–68

Pereyra SA, Dill-Macky R, Sims AL (2004) Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis 88:724–730

Pereyra SA, Vero S, Garmendia G, Cabrera M, Pianzolla MJ (2006) Diversity of fungal populations associated with Fusarium Head Blight in Uruguay. In: Ban T, Lewis JM, Phipps EE (eds)

The global Fusarium initiative for international collaboration: a strategic planning workshop held at CIMMYT El Batán Mexico DF, CIMMYT, pp 35–41

Pérez C, Villar HA (2011) Control biológico en cultivos extensivos: cuando el enfoque condiciona el éxito. In: Pereyra S, Díaz M, Germán S, Cabrera K (eds) Manejo de enfermedades de trigo y cebada. Serie Técnica 189. INIA Uruguay Ed Hemisferio Sur Montevideo, pp 49–62

Piñeiro M (1997) Fusarium toxins in Uruguayan wheat. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, Mexico, pp 125–128

Pritsch C (1995) Variabilidad patogénica en *Fusarium* spp. agente causal del golpe blanco del trigo. FPTA-INIA Informe final, p 79

Radke JK, Honeycutt CW (1994) Residue management strategies in Northeast. In: Hatfield JL, Stewart BA (eds) Crops residue management – advances in soil science. CRC Press, Boca Raton, pp 77–107

Rees RG (1987) Effects of tillage practices on foliar diseases. In: Cornish PS, Pratley PE (eds) Tillage: new directions in Australian agriculture. Inkata Press, Sydney, pp 318–334

Reis EM (1988) Doenças do trigo III. Giberela. Segunda ediçao. Sao Paulo, Brazil

Reis EM (1989) Fusariosis: Biología y epidemiología de *Gibberella zeae* en trigo. In: Kohli MM (ed) Taller sobre fusariosis de la espiga en América del Sur. CIMMYT, México, pp 97–102

Reis EM (1990) Integrated disease management – the changing concepts of controlling head blight and spot blotch. In: Saunders DA (ed) Wheat for the non traditional warm areas. Proceeding of the international conference. CIMMYT, México DF, pp 165–177

Shaner GE (2003) Epidemiology of Fusarium head blight of small grain cereals in North America. In: Leonard KJ, Bushnell WR (eds) Fusarium head blight of wheat and barley. APS Press, St Paul, pp 84–119

Stagno JP (1980) Enfemedades transmitidas por la semilla de trigo. Bol Semagro 3:30–33

Sturtz AV, Carter MR, Johnston HW (1997) A review of plant disease, pathogen interactions and microbial antagonism under conservation tillage in temperate humid agriculture. Soil Tillage Res 41:169–189

Sutton J (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:175–209

Theander O, Aman P (1984) Anatomical and chemical characteristics. In: Sundstol F, Owen E (eds) Straw and other fibrous by-products as feed. Elsevier, Amsterdam, pp 45–78

Trail F, Gaffoor I, Vogel S (2005) Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genet Biol 42:528–533

Tschanz AT, Horst RK, Nelson PE (1975) Ecological aspects of ascospore discharge in *Gibberella zeae*. Phytopathology 65:597–599

Tschanz AT, Horst RK, Nelson PE (1976) The effect of environment on sexual reproduction of *Gibberella zeae*. Mycologia 68:327–340

Umpiérrez M, Garmendia G, Pereyra S, Rodriguez A, Vero S (2011) Las técnicas moleculares en el estudio de los patógenos: ejemplos en patógenos de trigo. In: Pereyra S, Díaz M, Germán S, Cabrera K (eds) Manejo de enfermedades de trigo y cebada. Serie Técnica 189. INIA Uruguay Ed. Hemisferio Sur Montevideo Uruguay, pp 41–47

Watkins JE, Boosalis MG (1994) Plant disease incidence as influenced by conservation tillage systems. In: Unger PW (ed) Managing agricultural residues. CRC Press, Boca Raton, pp 261–283

Wolfarth F, Schrader S, Oldenburg E, Weinert J, Brunotte J (2012) Earthworms promote the reduction of *Fusarium* biomass and deoxynivalenol in wheat straw under field conditions. Soil Biol Biochem 43:1858–1865

Wolfarth F, Schrader S, Oldenburg E, Weinert J (2013) Nematode-collembolan interaction promotes the degradation of *Fusarium* biomass and deoxynivalenol according to soil texture. Soil Biol Biochem 57:903–910

Part V
Management of Fusarium Head Blight

Chapter 10
Integrated Disease Management of Fusarium Head Blight

Erlei M. Reis and Marcelo A. Carmona

Abstract Wheat head blight, caused by the fungus *Gibberella zeae* (anamorph *Fusarium graminearum*), occurs worldwide, but for many years the disease was not considered a major problem. In the last years, however, head blight has increased its frequency and intensity and thus become one of the most damaging diseases of wheat. Fusarium Head Blight (FHB) is considered a floral disease because the infection process begins at the anthers. FHB occurs mainly in wet, warm regions, and its epidemics are associated with long wet periods from anthesis to the soft dough growing stage. The dependence of FHB on weather factors, its nature and epidemiology and its sporadic manifestation have determined that the control measures are not successful. Therefore, FHB is one of the cereal crop diseases most difficult to control. This chapter summarizes the current knowledge of the integrated management of FHB in Argentina and Brazil and includes the success and limitations of control methods such as using crop rotations, cultivars with improved behavior, biological control, chemical control and weather-based warning systems and diversifying the sowing times.

10.1 Introduction

Fusarium Head Blight (FHB), or wheat scab, caused by *Gibberella zeae* Petch, anamorph = *Fusarium graminearum Schwabe,* is a floral infectious disease (Arthur 1891). The occurrence of worldwide epidemics has increased recently. Severe

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epidemics often occur when favorable environmental conditions for fungal infection coincide with vulnerable crop growth stages (Andersen 1948; Dubin et al. 1997). In wheat, initial FHB infection occurs via the anthers (Pugh et al. 1933) due to the presence of high concentrations of the molecule stimulants betaine and choline (Strange et al. 1974). Its occurrence and ability to accumulate mycotoxins in infected grains causes reductions in yield and grain quality in all wheat growing areas (Sutton 1982; McMullen et al. 1997, 2012; Windels 2000).

The dependence of FHB on weather factors, its nature and epidemiology and its sporadic manifestation have determined that the control measures are not successful. Therefore, FHB is one of the cereal crop diseases most difficult to control.

This chapter summarizes the current knowledge of the integrated management of FHB in Argentina and Brazil.

10.2 Damage: Reductions in Grain Yield and Quality

Severe losses have been reported in many wheat-production regions of South America.

In the northeast area of the Pampas region of Argentina, severe epidemics of FHB were recorded in 1978, 1985, 1993 and 2001 (Kikot et al. 2011). Yield losses in 1978, for example, ranged from 10 to 30 % in Marcos Juárez and Oliveros (Córdoba and Santa Fé provinces respectively) (Moschini et al. 2001; 2004), whereas yield losses in 1993 ranged from 24 to 50 % (Galich and De Galich 1996). In the southeast wheat-growing region, severe epidemics occurred in 1963, 1976, 1978 and 1985 (Kikot et al. 2011).

In both Brazil and Argentina, FHB was long considered a disease of secondary importance. However, the increases in frequency and yield losses associated with FHB in southern Brazil have turned this disease into a major problem.

Several works have been carried out to study the problem. In particular, Reis et al. quantified the reduction in grain yield in different wheat cultivars in Rio Grande do Sul state during the 1984 and 2010 growing seasons. All wheat scabbed spikes, from the grain milk stage through ripening, were identified and marked in a 1.0 m^2 area, in three replications per field. The scabby and healthy spikes were harvested, dried, counted and threshed separately. The damages caused by FHB were calculated by the difference between the actual yield and the estimated yield based on the total number of heads, the number of healthy heads and the number of scabby heads (Reis 1986). The average damage caused by wheat scab in 21 growing seasons was 18.6 % (Reis et al. 1996a; Panisson et al. 2003a; Casa et al. 2004, 2010; Telles Neto 2004; Kuhnem Junior et al. 2009) (Table 10.1).

The FHB increase after the 2000 season can be due to the increase in the no till area. Reis (1988) showed that 98 % of the *G. zeae* propagules in the air in southern Brazil were ascospores and that only 2 % were macroconidia. This author also showed that spores were present in the air throughout the year and that they were provided by host debris on the soil. Later, Panisson et al. (2002) determined values similar to those obtained earlier by Reis (1988): 90 % ascospores and 10 %

Table 10.1 Mean wheat grain yield reduction by FHB in different counties of southern Brazil

Season	Yield reduction (%)	County
1984–1994	5.4	Passo Fundo
1999	1.3	Passo Fundo
2000	17.5	Passo Fundo
2001	13.4	Passo Fundo
2002	11.6	Passo Fundo
2003	26.2	Passo Fundo
2004	12.0	Vacaria
2005	22.8	Lages
2007	39.8	Lages
2008	23.2	Vacaria
2009	32.4	Vacaria
2010	17.8	Vacaria
Mean	18.62	

Modified from Reis et al. (1996a), Panisson et al. (2003a), Casa et al. (2004), (2010), Telles Neto (2004), and Kuhnem Junior et al. (2009)

macroconidia. Francl et al. (1999) and Bai and Shaner (1994) pointed out that the density of ascospores in the air is a good indicator of disease potential in the field, when conditions are favorable for infection. In southern Brazil, Uruguay and in the Argentine Pampas, the *G. zeae* inoculum is so abundant that epidemics in small grains do not depend on the debris from a single species such as corn.

In general, FHB intensity has increased as a consequence of the increase in the no-till acreage. The maintenance of crop residues on the soil surface contributes to pathogen survival and high inoculum production between growing seasons, thus, ensuring the presence of abundant inoculum during barley and wheat flowering (Fernandes 1997). Thus, the determining factor for FHB occurrence is the existence of conditions favorable for infection during the susceptible period.

10.3 Strategies for Disease Management

Above-normal rainfall and no-till lead to FHB re-emergence. Since we cannot control weather, we cannot expect a large shift in tillage trends. Thus, we must search for other solutions for FHB management. In order to reduce losses caused by FHB in grain yield, some measures must be taken together. The aim is to interrupt the pathogen-host relationships in their developmental phases, i.e. penetration and colonization.

Periods of warm weather with persistent wetness are key conditions for FHB epidemics. The dependence of FHB on weather factors, its nature and epidemiology and its sporadic manifestation have determined that the control measures are not successful.

In particular, the dependence on weather factors and its sporadic manifestation have determined the need to develop early warning systems based on meteorological conditions. These systems aim to support the development of management strategies and decision making for disease chemical control (Moschini and Fortugno 1996; Moschini et al. 2001; Del Ponte et al. 2005). Nevertheless, control of this sporadic disease continues to challenge researchers worldwide and FHB epidemics continue to depress yields.

Managing FHB includes control methods such as using crop rotations, cultivars with improved behavior, biological control, chemical control and weather-based warning systems and diversifying the sowing times.

10.3.1 Crop Rotation: Does It Allow Reducing the Inoculum?

Mc Mullen et al. (1997) emphasized that crop rotations are the key to reducing the risk of severe scab. Nevertheless, the effectiveness of crop rotations in reducing scab has not been demonstrated in Brazil. Likewise, in Argentina, Lori et al. (2009) suggested that favorable weather conditions are likely to be more important in disease severity than the tillage practice.

Perithecia are formed on infected tissues left in the field after harvest (Parry et al. 1995). The principal sources of inoculum in the southern cone of South America are perithecia saprophytically formed on different small grain residues and on senesced/killed tissues of several gramineae such as weeds, wild plants, and pastures (Reis 1990b). The soybean stubble can also contribute to the inoculum because *F. graminearum* is frequently found in soybean seed and straw (Fernandez and Fernandes 1990).

When residues are incorporated into the soil, perithecia are not formed because of the absence of light, and the inoculum availability in the air is reduced. Moreover, when left on the soil surface, the perithecia survive for a longer period than when buried. On the surface, it is guaranteed that the ascospores will be released into the air.

Perithecia of *Gibberella zeae* are produced on senescent stems of plant species under natural conditions in the following species (Reis 1990b; Carmona et al. 1999; Casa et al. 2010): *Andropogon bicornis* L.; *Avena strigosa*Schrab.; *Botriochloa* sp.; *Brachiaria brizantha*cv. Xaraés; *Brachiaria brizantha* cv. Marandu; *Brachiaria brizantha* cv. Piatã; *Brachiaria decumbens* cv. Basilisk; *Brachiaria plantaginea* (LK.) Hitch; *Brachiaria ruziziensis* Germain & Edvard; *Bromus catharticus* Vahl.; *Cynodon dactylon* (L.) Pers; *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn; *Chenopodium quinoa* Willd ; *Digitaria sanguinalis* (L.) Scop.; *Digitaria ciliaris* (Letz,) Koel.; *Festuca arundinacea* Schreber; *Lolium multiforum* Lam.; *Oryza sativa* L.*; Pannicum maximum* Jacq.; *Pannicum maximum* cv. Aruana; *Pannicum maximum* cv. Massai; *Pannicum maximum* cv. Mombaça; *Pannicum maximum* cv. Tanzânia; *Paspalum dilatatum* Poir.; *Paspalum notatum* Fluegge; *Paspalum urvillei* Steud.; *Pennisetum clandestinum* Chiov.; *P. purpureum* Schumach.; *Sorghum halepense* (L.) Pers.; *S. vulgare* L.

Table 10.2 Interaction between temperature (at 3 °C intervals) and spike wetness duration (*SWD*) to cause different daily values for infection favorability (*DVIF*) of *Gibberella zeae* in wheat spikelets

	DVIF				
	0[a]	1	2	3	4
	Spikelet incidence (%)				
	0	1–15	16–40	41–60	>60
T (°C)			SWD (h)		
10–12	<12	>13	–	–	–
13–15	<8	9–28	>29	–	–
16–18	<6	7–23	24–42	> 43	–
19–21	<6	7–21	22–36	> 37	–
22–24	<5	6–20	21–34	35–45	>46
25–27	<5	6–20	21–33	34-44	>45
28–30	<5	6–20	21–33	34–45	>46
31–33	<6	7–22	23–37	>38	–
34–35	<8	9–29	>30	–	–

[a]0, no infection; 1, 1–15 %; 2, 16–40 %; 3, 41–60 % and 4, >60 % spikelet incidence; (–) Incidence not reached (Zoldan 2008)

Crop rotation allows controlling some plant diseases in several crops by reducing the inoculum during its saprophytic phase. In the case of FHB, the ascospores are present in the air all year round, ensuring the presence of inoculum at flowering, regardless of the crop rotation (Reis 1988). In this case, the inoculum is blown from distant sources (Reis 1990a). Perithecia of *G. zeae* are saprophytically formed and are the most numerous and important source of pathogen inoculum. Even when wheat is rotated with non-susceptible crops, and whether or not infected corn residues are present in the field, FHB can occur in wheat due to the facts explained above. In this way, the nature of the inoculum for FHB is considered ubiquitous and thus FHB occurrence is mainly weather-dependent. Therefore, the scab is not controllable by crop rotation. *Gibberella zeae* produces small light spores produced all year round and blown by the wind to long distances.

10.3.2 Sowing Time – Can Temperature Affect Infection Escape?

Both FHB occurrence and intensity depend on favorable weather conditions (Table 10.2) during the susceptible host period, i.e. from the beginning of anthesis to the grain dough stage (when only partially exerted anthers are present). Thus, early sown plants reach the period of increased susceptibility, when the climatic conditions are less favorable for disease development (temperature below 20 °C). The availability of frost-tolerant cultivars would enable the exploration of this practice to prevent disease.

A measure to prevent scab, however, is the diversification of sowing times. This will allow plants not to bloom at the same time. When long rain periods coincide with flowering, disease occurs. Diversifying the sowing time offers a practical and efficient mechanism to escape infection when the flowering season occurs under climatic conditions unfavorable for disease.

10.3.3 Breeding for Resistance – Is This Control Method Still Far Away?

In 1929, Christensen et al. (1929) stated that "the only effective method to control wheat scab is to grow resistant varieties". Not much has changed since then. Nevertheless new sources of resistance have been found. The alternative is that resistance will be discarded when environmental conditions become unfavorable for scab.

FHB resistance is a priority in wheat breeding programs in the South Cone. The CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) has been working on scab resistance since the mid 1980s (Dubin et al. 1997). The INIA (Instituto Nacional de Investigaciones Agropecuarias) from Uruguay and the INTA (Instituto Nacional de Tecnología Agropecuaria) from Argentina also make significant efforts to improve genotypes. However, most wheat varieties are susceptible or moderately susceptible in the region and no genotype has complete FHB resistance.

Resistance is complex and difficult to achieve. Since polygenic inheritance is greatly influenced by the environment (Schroeder and Christensen 1963; Galich 1997), the results are not consistent and durable.

Two important mechanisms of resistance groups can be considered: those related to the initial infection and those related to penetration and colonization (Wang and Miller 1988; Yan et al. 2011).

10.3.4 Biological Control – Myth or Reality?

The use of beneficial micro-organisms to control plant diseases is considered as an alternative or a supplemental safe option for reducing several diseases. Many microbial (bacterial and fungal isolates) antagonists of *G. zeae* with potential use for FHB biological control have been identified (Luz 2000; Luongo et al. 2005). The main mechanisms involved in the control include competition, antibiosis and mycoparasitism.

In general, the in vitro antagonistic effect is always more effective than that in field conditions. Some causes of the inefficiency under field conditions are related to the survival of biocontrol agents. Regarding this issue, it has been shown that improving the physiological quality of biocontrol agents could optimize survival under field conditions (Palazzini et al. 2009).

Although biological control agents are less effective than the most efficacious fungicides, they can be used as an alternative in organic wheat crops, or in mixture

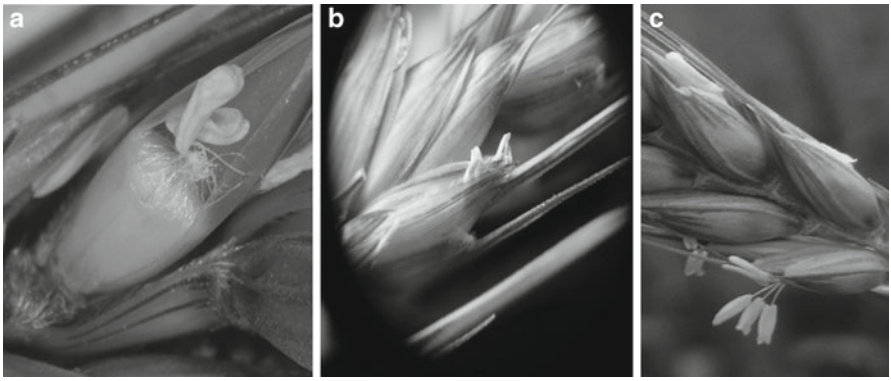

Fig. 10.1 (**a**) Not extruded anthers; (**b**) Partially exserted anthers, and (**c**) Fully exerted (Photo: R. Brustolin)

with a fungicide low rate. So, biological control still shows potential use as part of the integrated disease management program.

10.3.5 Chemical Control

10.3.5.1 Seed Treatment

In good quality seeds, *F. graminearum* incidence is low. The infected seeds are epidemiologically important only if they are left on the ground, forming perithecia on their surface. However, this source of inoculum can be negligible when compared to the inoculum amount saprophytically produced on senesced or frost killed gramineae. Therefore, wheat seed treatment with fungicides specific to *F. graminearum* is not recommended to control FHB, although it is a strategy to manage common root rot.

10.3.5.2 Head Spray with Fungicides – Partially Exerts Anther Protection

Infection Sites – Where Do Fungicides Have to Be Deposited?

FHB is a floral infecting disease (Arthur 1891). During wheat flowering, or anthesis, some anthers remain inside the plant (Fig. 10.1a), whereas others are extruded, but only partially exposed (PEA) (Fig. 10.1b), remaining there until harvest. Finally, others are fully expelled or extruded and easily dehisced from the head (FEA; Fig. 10.1c). Thus, the internal and dehisced anthers should not be important to the infectious process. The internal anthers not exposed to the inoculum and the dehisced

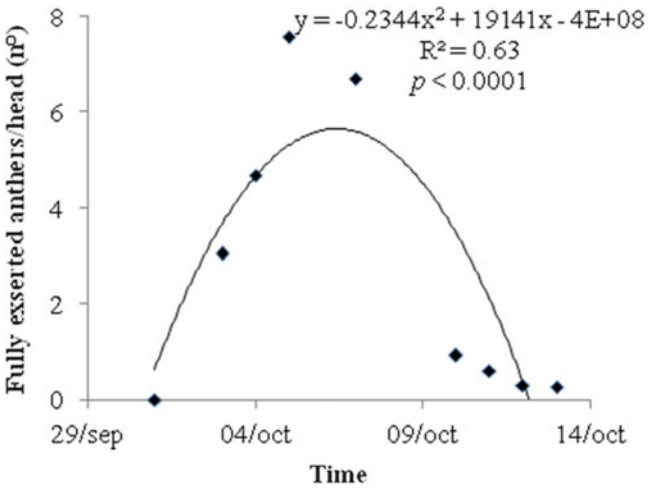

Fig. 10.2 *Curve* of fully exserted anthers in wheat heads, cultivar Marfim (Boaretto, personal communication)

ones remain attached to the spikelets only for a few minutes or hours. Those that remain exposed to the inoculum for a long time are the partially exposed ones (PEA) (Fig. 10.1b). It is likely that PEA should be considered to be protected by the fungicide, in order to prevent infection.

Boaretto (unpublished data) studied the flowering of wheat determining the presence and duration of the different types of anthers. The early flowering (presence of FEA) in the wheat Marfim cultivar took place on October 2nd, 2011, with maximum anther extrusion on October 8th and total dehiscion on October 12th (Fig. 10.2).

Both FEA and PEA were found on the same day, but PEA remained trapped in the ear for a longer time than FEA (Fig. 10.3). It is likely that the most important ones for infection are those that remain exposed to the inoculum for a longer time.

Panisson et al. (2003b) quantified the relative importance of FEA and PEA in the FHB infectious process in wheat. These authors showed that at full flowering stage, the *F. graminearum* incidence was 11.8 % in FEA and 24.3 % in PEA, and explained that the incidence was a function of the time during which anthers were exposed to the inoculum. The PEA remained exposed from the beginning of flowering until the dough stage, and therefore presented the highest fungus incidence. This points out that the wheat predisposition period for *G. zeae* infection should be chemically protected (Fig. 10.4).

In the same research mentioned above (Panisson et al. 2003b), *Fusarium graminearum* was detected in PEA with an incidence up to 75 % on October 5th by using a semi-selective medium (Segalin and Reis 2010). It is thus likely that PEA are the target to be protected by the fungicide. Anthers that did not receive the fungicide would not be protected. In order to protect them, a field spray has to completely cover the head sides, reaching the PEA. The PEA exposed area is very

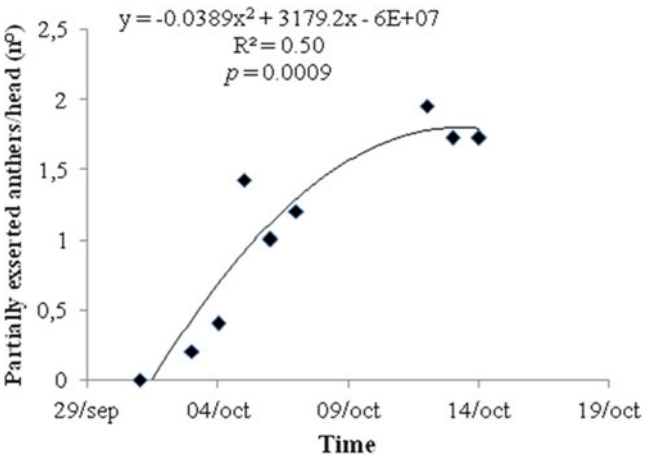

Fig. 10.3 Progress curve of partially exserted anthers in wheat heads, cultivar Marfim (Boaretto, personal communication)

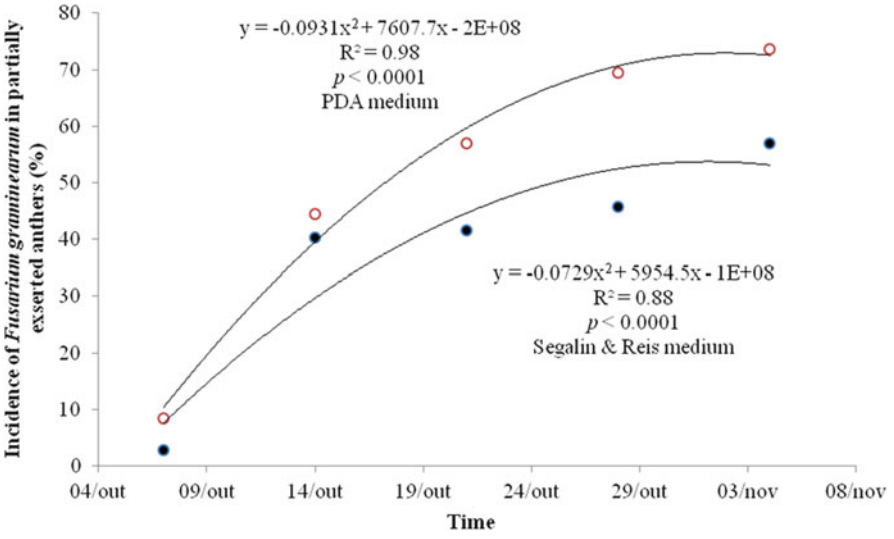

Fig. 10.4 Progress *curves* of *Fusarium graminearum* incidence in partially exserted anthers, wheat cultivar Marfim, isolated in two agar media (Boaretto, personal communication)

small compared to that of other plant organs (i.e. leaves) and its position in the spike and spikelet also hinders the deposition of fungicides. These can be the two main constraints in efficient chemical control of FHB.

10.3.5.3 Fungitoxicity of Fungicides

Fungicide potency should be considered to improve FHB chemical control. Demethylation inhibitor fungicides achieve the lowest mycelial inhibitory concentration (IC_{50}) compared with the strobilurins (Avozani et al. 2011). The IC_{50} ranged from 0.01 (metconazole, prochloraz, prothioconazole) to 0.12 (cyproconazole). For strobilurins the IC_{50} ranged from 0.21 (azoxystrobin) to 1.33 (trifloxystrobin), whereas for methyl benzimidazole carbamates (carbendazim), the IC_{50} was 0.07 mg/L.

According to previous studies (Reis et al. 1996b; Carmona 2003; Formento and de Souza 2008), the fungicides tebuconazole, procloraz and benzimidazole are the most efficient. These were evaluated in both greenhouse and field conditions. During the latter, metconazole also showed high efficiency to control FHB.

10.3.5.4 Technology of Application – How to Reach the Infection Sites?

The effect of fungicide treatment does not usually reach satisfactory levels. One of the important reasons for this low efficacy is the difficulty to reach and cover the anthers.

In general, the sprayers do not provide good ear coverage. The PEA should be the target to be protected by the fungicide. However, under field conditions, the current technology does not allow reaching the target efficiently. Thus, there are differences in the results of fungicide efficacy obtained from glasshouse and field experiments. According to Panisson et al. (2003b), fungicidal sprays to control head blight should use nozzles that deliver medium to fine droplets, at a volume of 200 $L.ha^{-1}$.

10.3.5.5 Fusarium Head Blight Warning Systems

The use of disease prediction models may be useful to predict the disease intensity and generate control recommendations. Nevertheless, a lot of work is necessary to validate them before correct use can be guaranteed. Therefore, model validation and evaluation is necessary before FHB incidence could be predicted in the fields to increase the ability of producers to achieve good disease management through the correct time for spraying chemicals.

In Argentina and Brazil, several models and systems have been developed to analyze and support the chemical control strategy (Moschini and Fortugno 1996; Moschini et al. 1999; 2001, 2004; Del Ponte et al. 2004, 2009; Nicolau and Fernandes 2012).

One of the systems developed as a tool to time fungicide treatment for FHB control used is described below:

The interaction between temperature and head wetness duration and FHB intensity (Table 10.2), used in the warning system, was generated by Zoldan (2008).

Meteorological data were recorded using the Aura® equipment (www.elomed.com.br). Software was used to calculate the interaction between temperature (at 3 °C intervals) and spike wetness duration (SWD), which causes different daily values for infection favorability (DVIF) of *F. graminearum* in wheat spikelets (Table 10.2).

The daily values for DVIF data (Table 10.2) were compared with spike incidence (SI) from field evaluations to identify the sum of daily values for infection favorability (SDVIF) corresponding to the day when infection occurred (Table 10.3).

Figure 10.5 illustrates the data of average values taken from Table 10.3, which indicate the time of FHB occurrence in wheat spikes (Brustolin et al. (2013)).

10.3.5.6 Points to Be Considered for Fusarium Head Blight Chemical Management Strategy – Timing Fungicide

(a) The system considers the wheat predisposition period (beginning of flowering to dough stage) that must be protected by the fungicide.
(b) PEA are considered the infection sites on which the fungicide must be deposited.
(c) Potent fungicides (cited above) should be applied only when, during the predisposition period, environmental conditions will be conducive to infection.
(d) The fungicide must be applied before the occurrence of predicted rainfall during the predisposition period; when it rains, the PEA should already be protected.
(e) When no rain is predicted, fungicide application is not necessary because there will be no infection.
(f) The rain forecast for the next 24, 48 or 72 h is made by the CPTEC/INPE (Centro de Previsão de Tempo e Estudos Climáticos/Instituto Nacional de Pesquisas Espaciais in Brazil, http://www.cptec.inpe.br/) with >95 % accuracy. This is the time for application.
(g) Fungicides must be applied with a spray jet directed to the head sides reaching the PEA.
(h) The occurrence of favorable conditions for infection can be checked using the warning system (cited above).

10.4 Conclusions

FHB is one of the most important diseases on cereal crops in Brazil and Argentina. This sporadic disease continues to challenge researchers worldwide because it is difficult to control. More effective management will require integration of all available strategies and the incorporation of new research specially related to epidemiological aspects and disease forecasting and its relationship with chemical control. Molecular advances will likely allow incorporating effective disease resistance in the near future and generating new ways to reduce the impact of mycotoxins. Studies of biological control should continue to screen and evaluate more potential

Table 10.3 FHB incidence in wheat spikes as a function of temperature and wetness duration and the corresponding DVIF values

Seeding time	Cultivar	Anthesis beginning (date)	Wetting beginning (date)	Wetting end (date)	DVIF	SWD (h)	T (°C)	Symptom onset (date)	SI Assessment (date)	SI (%)
3rd	CEP 0059	06/Oct	06/Oct	09/Oct	3	61.0	16.2	20/Oct	22/Oct	1.7
4th	CEP 0059	14/Oct	14/Oct	18/Oct	2	94.5	14.9	22/Oct	30/Oct	5.1
5th	CEP 0059	20/Oct	21/Oct	23/Oct	3	50.5	18.2	29/Oct	01/Oct	3.2
3rd	BRS 179	10/Oct	10/Oct	11/Oct	2	10.85	17.05	20/Oct	22/Oct	4.7
5th	BRS 179	22/Oct	22/Oct	23/Oct	3	50.5	18.2	29/Oct	01/Oct	3.6
Mean	–	–	–	–	2.6	53.5	16.9	–	–	3.7

DVIF daily values for infection favorability, *SWD* spike wetness duration, *SI* spike incidence

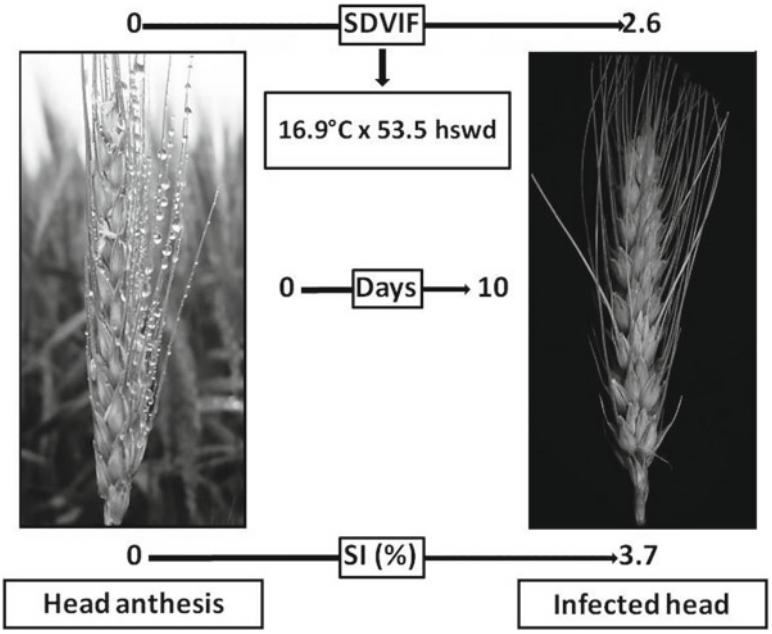

Fig. 10.5 Schematic representation of the warning system for FHB occurrence in wheat. *DVIF* sum of daily values for infection favorability, *C* mean air temperature during the wetting period, *SWD* spike wetness duration (hours), *SI* FHB spike incidence (Taken from Table 4 (Brustolin et al. 2013))

fungal antagonists, especially under field conditions (Luz 2000). Implementation of all such integrated disease management practices should be applied in a coordinated and harmonized manner since multiple management strategies will be surely more successful than a single strategy.

References

Andersen AL (1948) The development of *Gibberella zeae* head blight of wheat. Phytopathology 38:595–611

Arthur JC (1891) Wheat scab. Agric Exp 36:192–232

Avozani A, Tonin RB, Reis EM, Camera J, Ranzi C (2011) Sensibilidade de *Fusarium graminearum* a fungicidas, *in vitro*. In: Reis EM (Organizador) Seminário sobre giberela em cereais de inverno. Gráfica Berthier, Passo Fundo

Bai GH, Shaner GE (1994) FHB of wheat: perspective and control. Plant Dis 78:760–766

Brustolin R, Zoldan SM, Reis EM, Zanatta T, Carmona M (2013) Validation of a weather-based warning system for Fusarium head blight in wheat (in press)

Carmona M (2003) Estado del conocimiento epidemiológico de la fusariosis en el cono Sur. In: INIA (ed) Seminario Internacional Manejo Integrado de la fusariosis de la espiga de trigo. INIA La Estanzuela, Colonia

Carmona M, Pioli RN, Reis EM (1999) Malezas hospedantes de hongos necrotróficos causantes de enfermedades en trigo y cebada cervecera en la región pampeana. Revista Facultad de Agronomía UBA 19:105–110

Casa RT, Reis EM, Blum MMC, Bogo A, Scheer O, Zanata T (2004) Danos causados pela infecção de *Gibberella zeae* em trigo. Fitopatol Bras 29:289–293

Casa RT, Sachs C, Agostinetto L, Ceccon G (2010) Produção de peritécios de *Gibberella zeae* em táxons de gramíneas. Trop Plant Pathol 35:136

Christensen JJ, Stakman EC, Immer FR (1929) Susceptibility of wheat varieties and hybrids to Fusarium head blight in Minnesota. Minn Agric Exp Stn Bull 59:1–24

Del Ponte EM, Fernandes JMC, Pierobom CR, Bergstrom GC (2004) Giberela do trigo – aspectos epidemiológicos e modelos de previsão. Fitopatol Bras 29:587–605

Del Ponte EM, Fernandes JMC, Pierobom CR (2005) Factors affecting density of airborne *Gibberella zeae* inoculum. Fitopatol Bras 30:55–60

Del Ponte EM, Fernandes JMC, Pavan WA, Baethgen WE (2009) A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. J Phytopathol 157:675–681

Dubin HJ, Gilchrist L, Reeves J, McNab A (1997) Fusarium head scab: global status and future prospects. In: CIMMYT (ed) Proceedings of a workshop held at CIMMYT, El Batan, Mexico, p 130

Fernandes JMC (1997) As doenças das plantas e o sistema de plantio direto. Revisão Anual de Patologia de Plantas 5:317–352

Fernandez MR, Fernandes JMC (1990) Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern and central Brazil. Can J Plant Pathol 12:289–294

Formento AN, de Souza J (2008) Control químico de la fusariois de la espiga del trigo (*Fusarium graminearum* y *Fusarium* spp.). Momentos, dosis y eficacia de fungicidas. HM 19 Libro de Resúmenes 1° Congreso Argentino de Fitopatología Córdoba Argentina, p 205

Francl L, Shaner G, Bergstrom G, Gilbert J, Pederson W, Dill-Macky R, Sweet L, Corwin B, Jin Y, Gallenberg D, Wiersma J (1999) Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis 83:662–666

Galich MTV (1997) Fusarium head blight in Argentina. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, Mexico

Galich AN, de Galich MTV (1996) Enfermedades del trigo en el área central norte de la región cerealera Argentina. Informe Técnico EEA INTA, Marcos Juárez, p 121

Kikot GE, Moschini R, Consolo VF, Rojo R, Salerno G, Hours RA, Gasoni L, Arambarri AM, Alconada TM (2011) Occurrence of different species of *Fusarium* from wheat in relation to disease levels predicted by a weather-based model in Argentina Pampas region. Mycopathologia 171:139–149

Kuhnem Junior PR, Casa RT, Kienem RC, Nagata CES, Castro RL, Arns U (2009) Controle químico da giberela pela aplicação de fungicida na floração do trigo. Trop Plant Pathol 34:85

Lori GA, Sisterna MN, Sarandón SJ, Rizzo I, Chidichimo H (2009) FHB in wheat: impact of tillage and other agronomic practices under natural infection. Crop Prot 28:495–502

Luongo L, Galli M, Corazza L, Meeks E, Haas LD, Van Der Plas CL, Kohl J (2005) Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. Biocontrol Sci Tech 15:229–242

Luz WC (2000) Biocontrol of Fusarium head blight in Brazil. In: Erlanger KY (ed) In: Proceedings of the 2000 National Fusarium head blight forum, Erlanger, pp 77–81

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford DA (2012) Unified effort to fight an enemy of wheat and barley: Fusarium Head Blight. Plant Dis 96(12):1712–1728

Moschini R, Fortugno C (1996) Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur J Plant Pathol 102:211–218

Moschini R, Carmona M, Grondona M (1999) Wheat head Blight incidence variations in the Argentinian Pampeana Region associated with the el Niño Southern Oscillation. In: Katan J et al. (eds) XIVth international plant protection congress, Jerusalem, Israel, p 160

Moschini R, Pioli R, Carmona M, Sacchi O (2001) Empirical predictions of wheat head blight in the Northern Argentinean pampas region. Crop Sci 41:1541–1545
Moschini R, Carranza MR, Carmona M (2004) Meteorological-based predictions of wheat head blight epidemic in the southern Argentinean pampas region. Cereal Res Commun 32:45–52
Nicolau MA, Fernandes JMC (2012) Predictive model for daily inoculum levels of *Gibberella zeae* in Passo Fundo, Brazil. Int J Agron (795162):7. doi:10.1155/2012/795162
Palazzini JM, Ramirez ML, Alberione EJ, Torres AM, Chulze SN (2009) Osmotic stress adaptation, compatible solutes accumulation and biocontrol efficacy of two potential biocontrol agents on Fusarium head blight in wheat. Biol Control 51:370–376
Panisson E, Reis EM, Boller W (2002) Quantificação de propágulos de *Gibberella zeae* no ar e infecção de anteras em trigo. Fitopatol Bras 27:489–494
Panisson E, Reis EM, Boller W (2003a) Quantificação de danos causados pela giberela em cereais de inverno, na safra 2000, em Passo Fundo, RS. Fitopatol Bras 28:189–192
Panisson E, Boller W, Reis EM, Hoffmann L (2003b) Fungicidal spray techniques for the control of head blight (*Gibberella zeae*) in wheat. Ciência Rural 33:13–20
Parry DW, Jenkinson P, Mcleod L (1995) *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol 44:207–238
Pugh GW, Johann H, Dickson JG (1933) Factors affecting infection of wheat heads by *Gibberella saubinetii*. J Agric Res 46:771–779
Reis EM (1986) Metodologia para a determinação de perdas causadas em trigo (*Triticum aestivum* L.). Summa Phytopathol 11:951–955
Reis EM (1988) Quantificação de propágulos de *Gibberella zeae* no ar através de armadilhas de esporos. Fitopatol Bras 13:324–327
Reis EM (1990a) Effects of rain and relative humidity on the release of ascospores and on the infection of wheat heads by *Gibberella zeae*. Fitopatol Bras 15:339–343
Reis EM (1990b) Perithecial formation of *Gibberella zeae* on senescent stems of grasses under natural conditions. Fitopatol Bras 15:52–54
Reis EM, Blum MMC, Casa RT, Medeiros CA (1996a) Grain losses caused by infection of wheat heads by *Gibberella zeae* in southern Brazil, from 1984 to 1994. Summa Phytopathol 22:134–137
Reis EM, Blum MMC, Casa RT (1996b) Controle químico de *Gibberella zeae* em trigo, um problema de deposição de fungicidas em anteras. Summa Phytopathol 22:39–42
Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831–838
Segalin M, Reis EM (2010) Semi-selective medium for *Fusarium graminearum* detection in seed samples. Summa phytopathol 36:338–341
Strange RN, Majer JR, Smith H (1974) Isolation and identification of choline and betaine as two major components in anthers and wheat-germ that stimulate *Fusarium graminearum in vitro*. Physiol Plant Pathol 4:277–290
Sutton JC (1982) Epidemiology of wheat head blight and maize era rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:195–209
Telles Neto FXB (2004) Transmissão e controle de *Fusarium graminearum* em sementes e danos causados pela giberela em trigo. Dissertação de Mestrado Universidade de Passo Fundo, Passo Fundo
Wang YZ, Miller JD (1988) Effects of metabolites on wheat tissue in relation to Fusarium head blight resistance. J Phytopathol 122:118–125
Windels CE (2000) Economic and social impacts of fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology 90:17–21
Yan W, Li HB, Cai SB, Ma HX, Rebetzke GJ, Liu CJ (2011) Effects of plant height on type I and type II resistance to fusarium head blight in wheat. Plant Pathol 60:506–512
Zoldan SM (2008) Regiões de risco, caracterização da antese em cereais de inverno e sistema de alerta para giberela, em trigo. *Tese* (Doutorado em agronomia), Universidade de Passo Fundo, Passo Fundo

Chapter 11
Chemical Control of Fusarium Head Blight of Wheat

Martha Díaz de Ackermann and Man Mohan Kohli

Abstract The chemical control of Fusarium Head Blight (FHB) using fungicide has remained an unresolved challenge worldwide. Experiments using fungicides to control the disease have shown large difference of efficiency due to multiple factors (fungicides, moment and method of application, nozzle types, and weather conditions at the time of application, etc.) responsible for disease development. Besides adequate disease control, the fungicides are also expected to act on toxin production. Results have shown that highly susceptible cultivars cannot be fully protected under severe epidemic conditions. For this reason these cultivars need to be withdrawn from commercial production and chemical control be applied as preventive measure based on the FHB developmental models and weather forecasts. Presently one of the most efficient fungicides to control FHB in the Southern Cone region is Metconazole followed by the Tebuconazole. Other fungicides (Prochloraz, Thiabendazole, Carbendazim, etc.) used earlier are less efficient compared to the triazoles mentioned earlier. Under experimental conditions, nozzle types like *Twinjet 60* or Turbo *TeeJet Duo* have shown an increase the disease control efficacy. The earliest time to spray is after complete heading, beginning of flowering or mid flowering depending on the year and weather conditions.

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11.1 Introduction

In general commercial wheat cultivars, except a few, are moderately to highly susceptible to Fusarium Head Blight (FHB) disease. In order to reduce the damage caused by the disease, especially in the epidemic years, it becomes essential to use the fungicides (Mesterhazy 1997). Normally, FHB epidemics occur in crop years dominated by humid and warm weather conditions at the flowering time. In the recent years, however, FHB development has been more frequently associated with changes in weather conditions around flowering stage and the increase of conservation tillage or zero tillage practices recommended for sustainable agricultural systems (Díaz et al. 2002). Under these conditions, the chemical control of the disease using fungicides is complex and its success depends on many factors. Principle aspects are the selection of fungicides and additives, fungicide dose, timing of application, method of application (by ground or aerial), type of nozzles used and their interactions with the environment which determine the success or failure of an application.

The criterion for determining the moment of application in a spike disease, as FHB, is dynamic and must be based on various aspects such as: cultivar reaction, status of the crop (yield potential), phenological stage of the crop (around flowering), weather forecasting and DON Cast, model to predict DON, (Hooker et al. 2002; Schaafsman et al. 2006).

11.1.1 Cultivar Reaction

Commercial wheat cultivars, with very few exceptions, are moderately to highly susceptible to FHB. Whenever cultivars differing in FHB resistance have been used, the efficacy of chemical control was better on the more resistant genotypes (Mesterhazy and Bartok 1996; Wilcoxon et al. 1989). However, in Uruguay susceptible cultivars were used in order to get a good level of FHB infection and to evaluate the efficacy of the fungicides in an extreme situation.

11.1.2 Status of the Crop

The crop development and potential level of productivity are important factors to decide the fungicide control of FHB. Wheat crop planted in the stubble of wheat, barley or corn, is more predisposed to the FHB infection than following another crop. Under these situations, if the weather conditions are favorable to the development of FHB, a severe epidemic will limit the response to the chemical control resulting in lower than expected economic returns.

11.1.3 Growth Stage

The identification of high concentrations of certain stimulatory compounds found in the spike and anthers during the flowering stages suggested that FHB infection is favored at this time (Strange and Smith 1971; Strange et al. 1974). Anthers themselves may offer an initial point of entry for head blight fungi. FHB symptoms appear after flowering, usually following a period of continuous moisture or rains and with temperature between 10 °C to 25–30 °C (Martens et al. 1984).

New studies have showed that FHB infection occurs from anthesis to soft dough stage of the grain (Bushnell et al. 2003). As a result, the period of susceptibility of the wheat crop is limited to 2–3 weeks. The wheat spike is a complex and heterogeneous structure in which all components of the spikes are infected by the fungus *Fusarium graminearum* (Schw.) [teleomorph *Gibberella zeae* (Schwein.) Petch] which is the main specie causing FHB worldwide. Other *Fusarium* species may also incite FHB (Miedaner 2006). Several pathways of infection have been reported including the colonization of external and internal glumes, palea and lemma, anthers and ovaries during the opening of the flower and the progress of invasion of new florets and spikelets through colonization of the rachilla and rachis downward and upward, respectively.

11.1.4 Timing of Application

Given the lack of translocation of contact or systemic fungicides and strobilurins from leaves to head, except in trace amount (Mesterhazy 2003), the timing of fungicide application for FHB control is critical due to the specific period of host susceptibility (Brule-Babel and Fernando 2002). In experiments covering a period of over 30 years (since 1978) at La Estanzuela, the fungicide applications to control FHB have covered the boot stage (Zadoks 45) to milk grain stage (Zadoks 71). The most effective applications were identified around flowering stage Zadoks 61–65, (Diaz de Ackermann 2003; Zadoks et al. 1974).

11.1.5 Selection of Fungicides and Additives

The inflorescence of wheat is a determinate, composite spike with a very complex structure in which the systemic fungicides do not act as in other parts of the plant. Previous studies have shown that the amount of fungicide inside the spike is very low (Parry et al. 1997; Szabolcs et al. 2012b). For this reason the use of fungicides should be preventive to avoid germination and penetration of the fungus in this

complex structure. Once the fungus has penetrated the structure, the amount of fungicides present inside the spike is unable stop the growth of the fungus.

It is important to consider the effect of adjuvants to control this disease. Adjuvants can be surfactants or humectants, adherent, acidifying and penetrating. Conventional surfactants are used in every type of application. However, the use of organo-silicone surfactants, due to their ability to decrease the superficial tension of the droplet and to let its expansion, is recommended when the coverage is a key factor for the success of the application (Jordhal et al. 2001).

In general, the benzimidazole, imidazole and triazole fungicides were tested. The dosage of the fungicide recommended is very important because this disease is not easy to control. In-vitro studies conducted in Uruguay demonstrated high level of sensitivity to Metconazole and Thiabendazole in all isolates. However, different levels of sensitivity were observed among different isolates against Tebuconazole (Pereyra et al. 2006).

11.1.6 Method of Application

Earlier the aerial applications to control FHB were considered unsuitable primarily because of the vertical position the target, the spike, to be protected and low amount of water used in them. In general, the ground applications with the adequate type of nozzles and using more water seemed to be better. However, fungicide applications to control FHB have to be done under wet weather conditions making it difficult to enter the field with ground equipment. Given the advances seen in the spraying technology, the aerial applications could become a possibility if the weather conditions do not allow using the ground applications of the fungicides.

11.1.7 Type of Nozzles

Szabolcs et al. (2012a), studied different types of nozzles used in the application to increase the control efficiency of the disease. They found that *ConeJet* vertically spraying nozzle used for leaf protection was not able to give enough protection to the ears. Hollow cone spray tips, *ConeJet*, and flat spray tips, *TwinJet*, mounted at 0° and 30° angle on the bar used to control the disease at La Estanzuela, Uruguay, demonstrated the superiority of the *TwinJet* nozzle, (Diaz de Ackermann et al. 2002). Newer types of nozzles, Turbo *TeeJet Duo*, Turbo *FloodJet* and *XR TeeJet*, tested recently in Hungary, found significantly higher level of active ingredient in the spike where the fungicide coverage was better. According to these results, Turbo *TeeJet Duo* nozzle was most effective in protecting ear infection (Szabolcs et al. 2012b).

11.1.8 Weather Forecasting

Past studies have shown that FHB infection occurs from anthesis to soft dough stage of the grain (Bushnell et al. 2003), thereby limiting the period of susceptibility of the wheat crop to 2–3 weeks. Weather conditions during this period are one of the most important factors to determine the incidence and severity of the disease (Moschini and Bischoff 2007). The interaction between the temperature and the time of wet spike was studies by Andersen (1948). Temperatures between 10 and 30 °C with an optimal of 25 °C, with at least 48–72 h of wet spikes or rainy days at flowering are considered most favorable to the development of FHB disease. A crop before flowering stage, with the weather reports indicating warm temperature and rain in the immediate future, must be protected before the infection process takes place.

11.1.9 Prediction Models

Several authors have successfully used a modeling approach to explain the development of FHB epidemics at three sites in South America. The models include climate variables and the phenology of the cultivars to predict the impact of the disease (Del Ponte et al. 2009; Fernandez et al. 2004; Moschini 1994, 2011; Moschini and Fortugno 1996; Moschini et al. 2001, 2004, 2006). The change in climate, the phenology of the cultivar and the impact of disease determine the grain yield and the level of toxin contamination. Climate change scenarios are complex and updated regularly.

Berger et al. (2009), created a model to estimate heading stage of the wheat varieties in Uruguay. This tool allows the farmers to work out the heading stage of a crop and take care of FHB disease in a preventive manner.

A Canadian model to predict the presence of DON toxin in the field has been validated for Uruguay and is used to decide if the fungicide applications are necessary or not (Hooker et al. 2002; Schaafsma et al. 2006).

11.2 Materials and Methods

Field experiments to evaluate the timing and application efficacy of the fungicides to control FHB have been conducted at La Estanzuela Experimental Station Alberto Boerger, National Agricultural and Livestock Research Institute, INIA, since 1978. Using different susceptible cultivars each year, the timing of application from boot stage to early milk stage and the fungicidal products viz. benzimidazole, imidazole and triazole were studied in relation to the FHB infection. The results indicated that early applications (boot stage, Zadoks 45) and late applications (milk stage, Zadoks 71) were not effective to control the disease. After these experiences the trials in 2001 and 2002 were modified.

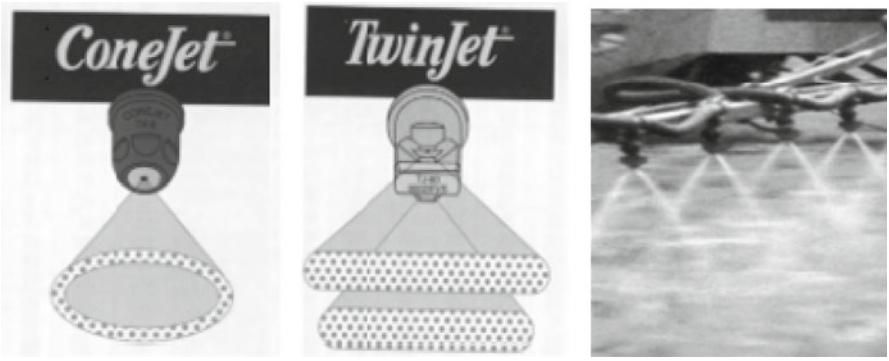

Fig. 11.1 Spray nozzles used: hollow cone spray tips *ConeJet*, and twin even flat spray tips, *TwinJet* (Application coverage shown using twin even flat spray tips)

An experiment using factorial design with complete blocks replicated four times included two or three fungicide treatments (Tebuconazole 450 g. active ingredient/L, rate of 450 cc/ha, Metconazole 90 g. active ingredient/L, rate of 1,000 cc/ha and an experimental product) and three timings of application (beginning of flowering, Zadoks 61, mid flowering, Zadoks 65 and at both stages Zadoks 61 + Zadoks 65). All treatments were applied with a CO_2 backpack sprayer, with hollow cone spray tips; water volume 0.2 L/min. at 3 bars of pressure.

Various agronomic and disease parameters such as grain yield, test weight, thousand kernel weight, visual disease score on a 1–5/1–5 scale, disease incidence (percent of diseased spikes), percent of scabby grains and toxin deoxynivalenol (DON) were evaluated (Fig. 11.1, Tables 11.1 and 11.2). From 2003 on and after confirming the efficiency of the *TwinJet* nozzles to control the FHB, the fungicides were applied with a CO_2 backpack sprayer, with *TwinJet* spray tips; water volume 0.79 L/min. at 3 bars of pressure (Figs. 11.1 and 11.2, Table 11.3).

The interaction among fungicides, types of the nozzles and timing of application were also studied. In 2006, two fungicides (Tebuconazole and Metconazole), three types of spray nozzles in five strategies (hollow cone spray tips, *ConeJet* TXVS-3, twin even flat spray tips, *TwinJet* 60 mounted at 0° and 30° angle on the bar (Figs. 11.1 and 11.2), and *TeeJet* TP 8001 mounted at 30° on the bar), two timings of application (beginning of flowering, Zadoks 61 and mid flowering, Zadoks 65) were tried out. The treatments were analyzed in a factorial design with complete blocks replicated four times. All applications were made with a CO_2 backpack type sprayer. Grain yield, test weight, thousand kernel weight, visual disease score on a 1–5/1–5 scale, disease incidence (percent of diseased spikes) and percent of scabby grains were evaluated (Table 11.4).

In 2009, the treatments: one fungicide (Metconazol), three types of nozzles in four strategies, *Teejet* TP 8001 alone and mounted at 45° on the bar, *ConeJet* TXVS-3 and *TwinJet* 60 alone, and three timing of application were evaluated (Table 11.5).

Table 11.1 Fungicide test against Fusarium Head Blight in wheat, La Estanzuela, Uruguay, 2001

Fungicide	Timing application	% spike with *Fusarium* spp.	Digit 1[a]	Digit 2[b]
Tebuconazole	Z61	40.5[c]	2.8[c]	2.5[c,d]
Tebuconazole	Z65	58.7[b]	3.8[a,b]	3.3[b]
Tebuconazole	Z61 + Z65	43.2[c]	3.0[b,c]	2.5[c,d]
Metconazole	Z61	35.0[c,d]	3.0[b,c]	2.5[c,d]
Metconazole	Z65	53.7[b]	3.8[a,b]	3.0[b,c]
Metconazole	Z61 + Z65	25.0[e]	2.3[c]	1.8[e]
Experimental	Z61	40.2[c]	3.0[b,c]	2.3[d,e]
Experimental	Z65	36.0[c,d]	2.5[c]	1.8[e]
Experimental	Z61 + Z65	30.2[d,e]	2.3[c]	2.0[d,e]
Check		84.8[a]	4.5[a]	4.0[a]
C.V. (%)		8.0	16.9	16.0
Pr > F		0.0001	0.0001	0.0001

Percent spike infection with FHB and its visual score on 1–5/1–5 scale, using three fungicides applied three times at different growth stages of the wheat crop

[a]Digit 1 visual disease note 1–5 (% of spikes affected)

[b]Digit 2 visual disease note 1–5 (% of spikelets in a spike affected)

[c,d,e]Means values within a column followed by the same letter are not significantly different ($p \geq 0.05$) based on LSD test

Table 11.2 Fungicide test against Fusarium Head Blight in wheat, La Estanzuela, Uruguay, 2001

Fungicide	Timing application	Grain Yield (kg/ha)	Test weight (kg/hl)	TKW (g)	DON (ppm)
Tebuconazole	Z61	1,944[a,b,c]	77.5[c,d]	27.8[b,c,d]	9.6[c]
Tebuconazole	Z65	1,542[c,d]	75.3[d]	25.9[d]	10.2[b,c]
Tebuconazole	Z61 + Z65	2,144[a,b]	80.4[a,b]	31.0[a]	5.4[e,f]
Metconazole	Z61	2,165[a]	79.3[a,b,c]	30.0[a,b,c]	5.6[e,f]
Metconazole	Z65	1,710[b,c]	76.1[d]	27.2[c,d]	9.7[c]
Metconazole	Z61 + Z65	2,334[a]	80.9[a]	31.7[a]	3.9[f]
Experimental	Z61	2,225[a]	78.0[b,c,d]	30.8[a]	14.5[a,b]
Experimental	Z65	1,938[a,b,c]	79.2[a,b,c]	30.4[a,b]	8.3[c,d]
Experimental	Z61 + Z65	2,310[a]	80.6[a,b]	31.3[a]	6.0[d,e]
Check		1,231[d]	67.5[e]	20.8[e]	21.0[a]
Mean		1,954	77.5	28.7	
C.V.		15.5	2.4	6.8	
LSD (5 %)		439.97	2.7	2.8	

Data of visual note (infection coefficient), % of grain affected by *Fusarium*, DON (ppm), grain yield (kg/ha), test weight (kg/hl), thousand kernel weight (g) for one cultivar, three fungicides and three times of application

[a,b,c,d,e]Means values within a column followed by the same letter are not significantly different ($p \geq 0.05$) based on LSD test

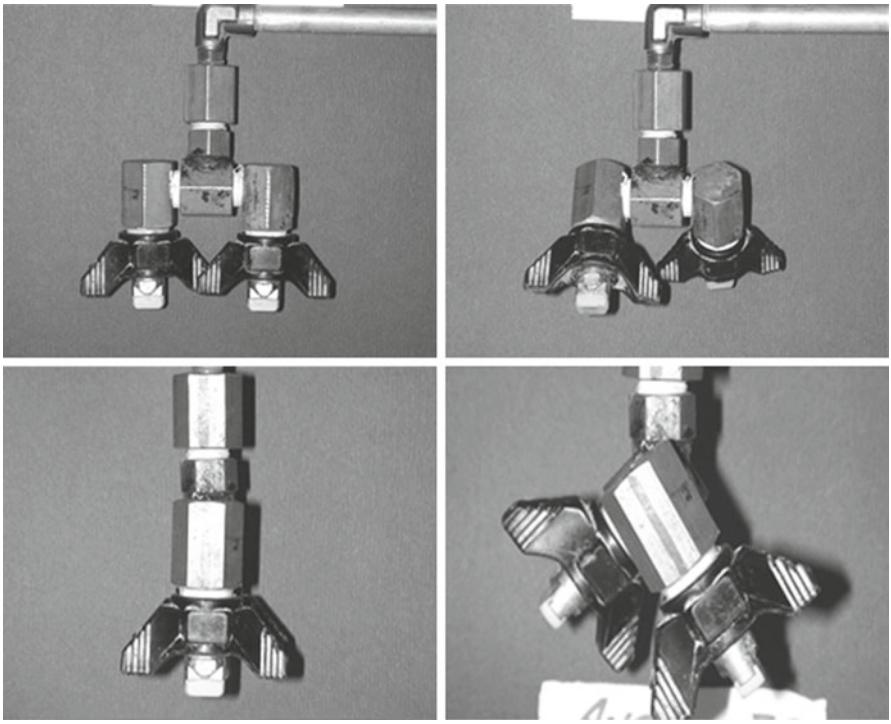

Fig. 11.2 *TwinJet*, mounted at 0° angle on the bar (*left*), *TwinJet*, mounted at 30° angle on the bar (*right*)

11.3 Results

11.3.1 Fungicides and Timing of Application

Earlier trials conducted at INIA La Estanzuela, Uruguay, in 1997, to control the FHB disease in wheat showed the superiority of treatments with Tebuconazole fungicide (43 and 25 %) at the rate of 450 cc/ha and 750 cc/ha respectively, Metiltiofanato (70 %) at the rate of 1,500 cc/ha and Epoxiconazole (12.5 %)+ Carbendazim (12.5 %) at the rate of 1,000 cc/ha (data not shown). The earlier application at growth stage Z61 (beginning of flowering) was better to control the FHB disease infection than the application at Z65 (mid- flowering). Double application at both stages was not included in this trial. The year 2001 was a severe FHB epidemic crop cycle with high disease pressure. Under these conditions, the newly introduced fungicide Metconazole (9 %) at the rate of 1,000 cc/ha showed excellent FHB control compared with Tebuconazole (43 %) at the rate of 450 cc/ha (Tables 11.1 and 11.2). While the double application at Z61 and Z65 was best treatment for disease control, the most economic results were shown by a single early application at Z61.

Table 11.3 Fungicide test against Fusarium Head Blight in wheat for one cultivar, three fungicides and three times of application. La Estanzuela, Uruguay, 2009

Fungicide	**Timing application**	**Visual note Inf. Coeff.**	**% grain with** Fusarium **spp.**	**DON (ppm)**
Metconazole	Z 61	14.0[d,e]	12.1[b,c]	11.0[a,b,c,d]
Metconazole	Z 65	19.5[d,e]	10.3[c,d]	9.0[b,c,d]
Metconazole	Z61 + Z65	4.0[e]	7.6[d]	7.3[d]
Proclorax + Carbendazim	Z 61	27.5[c,d]	12.9[b,c]	11.5[a,b,c]
Proclorax + Carbendazim	Z 65	46.5[b]	13.5[b,c]	11.0[a,b,c,d]
Proclorax + Carbendazim	Z61 + Z65	13.5[d,e]	9.9[c,d]	7.0[d]
Prothioconazole + Tebuconazole	Z 61	42.0[b,c]	14.9[b]	13.5[a]
Prothioconazole + Tebuconazole	Z 65	42.0[bc]	13.3[bc]	14.5[a]
Prothioconazole + Tebuconazole	Z61 + Z65	13.0[d,e]	12.0[b,c]	7.7[c,d]
Check		67.5[a]	19.6[a]	14.5[a]
Fungicides		**Visual note Inf. Coeff.**	**% grain with *Fusarium* spp.**	**DON (ppm)**
Metconazole		12.5[b]	10.0[b]	9.1[b]
Proclorax + Carbendazim		29.2[a]	12.1[a]	9.8[b]
Prothioconazole + Tebuconazole		32.3[a]	13.4[a]	11.9[a]
Check		67.5	19.6	14.5
Timing of application		**Visual note Inf. Coeff.**	**% grain with *Fusarium***	**DON (ppm)**
Z 61		31.4[a]	13.3[a]	12.1[a]
Z 65		33.1[a]	12.3[a]	11.5[a]
Z61 + Z65		13.8[b]	10.5[b]	7.3[b]
Check		67.5	19.6	14.5

(continued)

Table 11.3 (continued)

Fungicide	**Timing application**	**Grain Yield (kg/ha)**	**Test weight (kg/hl)**	**TKW (g)**	**Protein %**
Metconazole	Z 61	4,321^{a,b}	76.6^{a,b,c}	31.5^{b,c,d}	12.6^{a,b}
Metconazole	Z 65	4,341^{a,b}	75.4^{b,c,d}	30.9^{b,c,d,e}	13.0^{a,b}
Metconazole	Z61 + Z65	4,656^{a}	77.8^{a,b}	33.8^{a}	12.8^{a,b}
Proclorax + Carbendazim	Z 61	3,843^{b,c,d}	75.7^{a,b,c,d}	29.4^{e}	12.5^{b}
Proclorax + Carbendazim	Z 65	3,879^{b,c,d}	77.3^{a,b,c}	31.0^{b,c,d,e}	12.7^{a,b}
Proclorax + Carbendazim	Z61 + Z65	4,143^{a,b,c}	77.9^{a,b}	31.8^{b,c}	12.8^{a,b}
Prothioconazole + Tebuconazole	Z 61	3,975^{b,c,d}	75.8^{a,b,c,d}	29.9^{d,e}	12.9^{a,b}
Prothioconazole + Tebuconazole	Z 65	4,103^{b,c}	76.3^{a,b,c,d}	30.6^{c,d,e}	12.9^{a,b}
Prothioconazole + Tebuconazole	Z61 + Z65	4,249^{a,b}	77.7^{a,b}	32.5^{a,b}	12.6^{a,b}
Check		3,486^{d}	73.4^{d}	27.5^{f}	13.2^{a}
Fungicides		**Grain Yield kg/ha**	**Test weight kg/hl**	**TKW (g)**	**Protein %**
Metconazole		4,439^{a}	76.6^{a}	32.1^{a}	12.8^{a}
Proclorax + Carbendazim		3,955^{b}	76.9^{a}	30.8^{b}	12.7^{a}
Prothioconazole + Tebuconazole		4,109^{b}	76.6^{a}	31.0^{b}	12.8^{a}
Check		3,486	73.4	27.5	13.2
Timing of application		**Grain Yield (kg/ha)**	**Test weight (kg/hl)**	**TKW (g)**	**Protein %**
Z 61		3,937^{b}	75.6^{b}	30.1^{b}	12.7^{a}
Z 65		4,068^{b}	76.4^{b}	31.1^{b}	12.9^{a}
Z61 + Z65		4,430^{a}	77.9^{a}	32.5^{a}	12.7^{a}
Check		3,486	73.4	27.5	13.2

Data of visual note (infection coefficient), % of grain affected by *Fusarium*, DON (ppm), grain yield (kg/ha), test weight (kg/hl), thousand kernel weight (g)

^{a,b,c,d,e}Means values within a column followed by the same letter are not significantly different ($p \geq 0.05$) based on LSD test

Table 11.4 Fungicide test against Fusarium Head Blight in wheat for one cultivar, two fungicides, two times of application and five strategies of nozzles. La Estanzuela, Uruguay, 2006

Fungicide	Inf. Coeff.	% spike with *Fusarium* spp.	Yield (Kg/ha)	Test weight (Kg/hl)	TKW (g)
Metconazole	4.3[b]	18.0[b]	5087.4[a]	77.8[a]	32.4[a]
Tebuconazole	10.7[a]	25.3[a]	4868.8[b]	77.0[a]	32.3[a]
Timing of application					
Z61	10.1[a]	22.1[a]	5017.5[a]	77.1[a]	32.2[a]
Z65	4.9[b]	21.2[a]	4938.7[a]	77.7[a]	33.5[a]
Nozzle Type					
ConeJet TXVS-3	11.8[a]	23.4[a]	4778.6[b]	76.9[b]	32.3[a,b]
2 TwinJet 60 (0°)	7.3[b,c]	21.9[a]	4990.5[b]	77.0[a,b]	32.1[b]
2 TwinJet 60 (30°)	8.8[b]	24.5[a]	4895.3[b]	77.4[a,b]	31.6[b]
TwinJet 60	4.8[c]	17.5[b]	5234.2[a]	78.3[a]	33.8[a]
2 TeeJetTP 8001 (30°)	4.8[c]	20.8[a,b]	4991.8[b]	77.3[a,b]	32.0[b]
Check	26.3	37.2	4389.9	76.3	30.7
LSD (5 %)	2.9	3.9	219.1	1.4	1.5

Data of infection coefficient, % of spike infected by *Fusarium*, grain yield (kg/ha), test weight (kg/hl), thousand kernel weight (TKW, g)

[a,b,c,d,e] Means values within a column followed by the same letter are not significantly different ($p \geq 0.05$) based on LSD test

Table 11.5 Fungicide test against Fusarium Head Blight in wheat for one cultivar, one fungicide, three times of application and four strategies of nozzles. La Estanzuela, Uruguay, 2009

Type of nozzle	Timing application	Inf. Coeff.	% Grain of *Fusarium* spp.	Grain Yield (Kg/ha)	Test weight (Kg/hl)	TKW (g)
Teejet TP 8001E	Z 61	35.0[b,c]	16.6[a,b]	4,174[d,e,f]	76.1[b,c,d,e,f]	34.5[e]
ConeJet TXVS 03	Z 61	39.5[a,b]	16.1[a,b]	3,918[e,f]	75.4[d,e,f]	34.9[d,e]
TwinJet TJ 60	Z 61	24.5[c,d]	12.9[b,c,d]	4,501[c,d,e]	74.9[e,f]	35.9[b,c,d,e]
2Teejet TP 8001E 45°	Z 61	35.0[b,c]	15.9[a,b]	4,429[c,d,e]	75.8[c,d,e,f]	36.1[b,c,d,e]
2Teejet TP 8001E 45°	Z 65	14.5[d,e]	16.5[a,b]	4,542[b,c,d,e]	75.3[d,e,f]	35.6[b,c,d,e]
TwinJet TJ 60	Z 65	28.0[c]	14.0[a,b,c]	4,814[a,b,c,d]	76.7[b,c,d,e]	37.6[a,b,c,d]
Teejet TP 8001E	Z 65	16.0[d,e]	10.8[c,d]	4,871[a,b,c,d]	78.2[a,b]	38.4[a,b]
2Teejet TP 8001E 45°	Z61+Z65	5.5[e]	10.9[c,d]	5,014[a,b,c]	77.3[a,b,c,d]	35.4[c,d,e]
ConeJet TXVS 03	Z61+Z65	14.5[d,e]	12.5[b,c,d]	4,527[c,d,e]	77.8[a,b,c]	37.0[b,c,d,e]
TwinJet TJ 60	Z61+Z65	8.5[e]	10.8[c,d]	5,370[a]	79.2[a]	37.9[a,b,c]
Teejet TP 8001E	Z61+Z65	10.0[e]	8.4[d]	5,340[a,b]	79.1[a]	40.0[a]
Check		49.0[a]	18.3[a]	3,560[f]	74.1[f]	34.4[e]
LDS (5 %)		10.78	4.65	798	2.16	2.81

Data of infection coefficient, % of grain infected by *Fusarium*, grain yield (kg/ha), test weight (kg/hl), thousand kernel weight (TKW, g)

[a,b,c,d,e] Means values within a column followed by the same letter are not significantly different ($p \geq 0.05$) based on LSD test

Fig. 11.3 *ConeJet 03* nozzles, water sensitive cards located in the *middle* of the plot, forward support (*left*), back support (*right*)

Recent trials conducted in 2009 confirm the superiority of Metconazole in the FHB control strategy. The new fungicide Prothioconazole + Tebuconazole, used in Europe, did not prove to be good in the formulation tested in Uruguay. The mixture of Proclorax (48 %) and Carbendazim (50 %) at the rate of 1,000 cc/ha of Proclorax and 500 cc/ha of Carbendazim proved to be an alternative treatment to control the disease (Table 11.3). In all the cases, the double application at Z61 and Z65 was better than the single applications.

11.3.2 Fungicides, Timing of Application and Type of the Nozzles

Preliminary results of various FHB control trials conducted during the years show that a good control was achieved by Metconazole fungicide application at Zadoks 61, and using the *TwinJet* nozzles mounted at 0º and 30º angles on a bar rather than the *ConeJet* nozzle (Diaz de Ackermann et al. 2002).

The difference between the coverage of the spike by both types of nozzles can be seen in Figs. 11.3 and 11.4. The *ConeJet* vertically spraying nozzle applied for leaf protection cannot give enough protection to the ears. The *TwinJet* spraying with an angle of 60º covers more the ears forward than backward (Fig. 11.4). Further studies in 2006 confirmed that Metconazole was a better fungicide to control FHB than Tebuconazole. There, was no significant difference between the fungicide applications at Z61 and Z65 and the *TwinJet*60 nozzle was better in terms of percent spikes infected with FHB, Table 11.4.

Recent trials conducted in 2009 confirm the superiority of the double application (Z61 + Z65) to reduce the FHB infection coefficient and that the *TwinJet* 60, *Teejet*

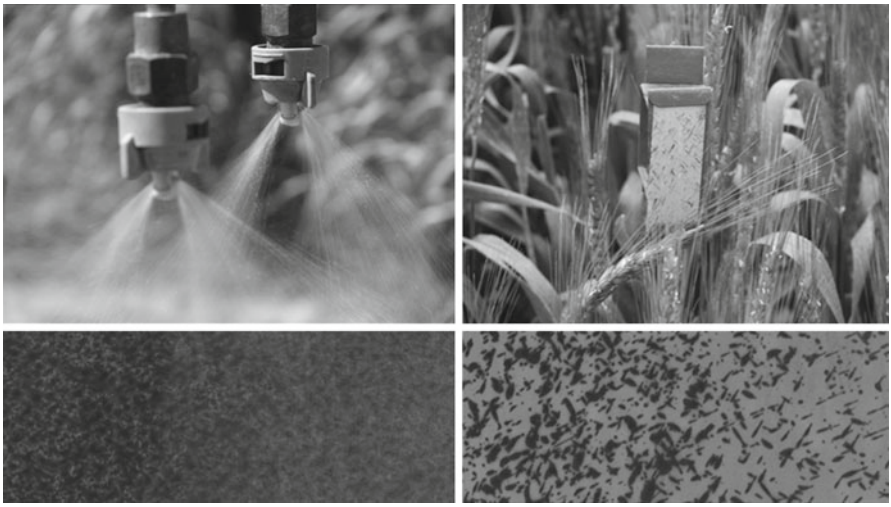

Fig. 11.4 *TwinJet 60* c the plot, forward support (*left*), back support (*right*)

(flat spray only or double mounted) at an angle of 45º on the bar were the most effective nozzles, Table 11.5.

11.4 Conclusions

More than three decades of experience in dealing with the FHB in Uruguay has shown the difficulties one faces to control this disease. For a successful control of the disease the whole production system needs to be organized in a manner that the inoculum from natural sources is minimized while plant nutrition and agronomy are optimized. The weather conditions favorable for the development of the FHB disease are not favorable for the application of fungicidal control. While the chemical control depends a lot on the wheat variety, fungicide and its rate or timing of application, etc., even susceptible cultivars used to generate productivity losses associated with FHB render different results from year to year depending on the climatic conditions favoring the severity of the disease. Although significant differences in the efficacy of fungicides to control FHB has been observed, many years of research shows that the fungicide applications before flowering or past flowering is not effective. The lack of effective acropetal transport of fungicides and only moderate redistribution in the spike underscore the importance of targeting the fungicide directly to spike. Research in this area shows that the best coverage of the ears was obtained with nozzles *TwinJet* 60 or *TeeJet* flat spray only or mounted in a bar at a 45° angle. Depending on the climatic conditions of the year, the most effective timings of application were Z61 or/and Z65. It is quite possible that in spite of poor

economic returns of two applications, they are essential to reduce the damage caused by the disease in a susceptible cultivar or in an epidemic year. The relatively new ranges of fungicides represented by triazoles, benzimidazoles, and imidazole have been more effective to control the disease than their older counterparts. Among the triazoles, the best control was seen with Metconazole followed by Tebuconazole. The formulations of the mixture of Prothiconazole + Tebuconazole, tested in Uruguay, were not more effective than Metconazole or Tebuconazole alone.

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References

Andersen AL (1948) The development of *Gibberella zeae* head blight of wheat. Phytopathology 38:595–611

Berger A, Castro M, Ceretta S (2009) Estimacion del momento de espigazon en trigo. En línea: http://www.inia.org.uy/Servicios y Herramientas

Brule-Babel AL, Fernando WGD (2002) Effect of fungicide treatments on fusarium head blight and leaf disease incidence in winter wheat. In: 2002 National Fusarium Head Blight Forum proceedings, Cincinnati, Erlanger, KY, pp 57–60

Bushnell WR, Hazen B, Pritsch C (2003) Histology and physiology of Fusarium head blight. In: Leonard K, Bushnell W (eds) Fusarium head blight of wheat and barley. APS Press, St Paul, pp 44–83

Del Ponte EM, Fernandes JMC, Pavan W, Baethgen W (2009) A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern. Brazil J Phytopathol 157:675–681

Díaz M, Pereyra S, Stewart S (2002) Antecedentes y perspectivas de control de Fusariosis de la espiga de trigo. In: Jornada Técnica de Cultivos de Invierno, Serie Actividades de Difusión N°282. INIA, Montevideo, pp 1–9

Diaz de Ackermann M (2003) Comportamiento varietal y control químico para fusariosis de la espiga en trigo. pp 23–31. In: Jornada Técnica Cultivos de Invierno, Serie de Actividades de Difusión 312, pp 23–31

Diaz de Ackermann M, Kohli M, Ibanez V (2002) Variations in fungicide application techniques to control fusarium head blight. In: 2002 National Fusarium Head Blight Forum proceedings, Cincinnati, Erlanger, KY, p 62

Fernandes JM, Cunha GR, Del Ponte E, Pavan W, Pires JL, Baethgen W, Gimenez A, Magrin G, Travasso MI (2004) Modeling fusarium head blight in wheat under climate change using linked process-based models. In: Canty SM, Boring T, Wardwell J, Ward RW (eds) Proceedings of the 2nd international symposium on fusarium head blight, 8th European Fusarium seminar, Orlando, FL, pp 441–444

Hooker DC, Schaafsma AW, Tamburic-Ilincic L (2002) Using weather variables pre- and post-heading to predict deoxynivalenol in winter wheat. Plant Dis 86:611–619

Jordhal J, Meyer S, McMuller M (2001) Further studies on the effect of timing application and of adjuvants on fungicide control. In: 2001 National Fusarium Head Blight Forum proceedings, Cincinnati, Erlanger, KY, p 65

Martens JW, Seaman WL, Atkinson TG (1984) Disease of wheat. In: Martens JW, Seaman WL, Atkinson TG (eds) Disease of field crops in Canada. Canadian Phytopathological Society, Harrow, pp 32–47

Mesterhazy A (1997) Fungicide control of Fusarium scab and impact on toxin contamination. In Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, Mexico (1996), pp 120–124

Mesterhazy A (2003) Control of Fusarium head blight of wheat by fungicides. In: Leonard K, Bushnell W (eds) Fusarium head blight of wheat and barley. APS Press, St Paul, pp 363–380

Mesterhazy A, Bartok T (1996) Control of Fusarium head blight of wheat by fungicides and its effect on the toxin contamination of the grain. Pflanzenschutz-Nachrichten Bayer 49:181–197

Miedaner T (2006) Global biodiversity in *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* populations and implications for breeding resistance to Fusarium head blight. In: Ban T, Lewis JM, Phipps EE (eds) The global *Fusarium* initiative for international collaboration: a strategic planning workshop. CIMMYT, El Batán/México, pp 31–34

Moschini R (1994) Modelos predictivos de la incidencia de fusariosis en trigo. In: Actas III Congreso Nacional de trigo. Bahía Blanca, Buenos Aires, Argentina, pp 320–326

Moschini R (2011) Desarrollo y uso de sistemas de pronóstico de epidemias de la Fusariosis de la Espiga de Trigo (*Triticum aestivum L.*) para identificar situaciones sinópticas y predictores meteorológicos en diferentes escalas asociados a la enfermedad en la región pampeana. Tesis Doctoral. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, p 108. En línea: www.digital.bl.fcen.uba.ar

Moschini RC, Bischoff S (2007) Meteorological-based systems for predicting and managing Fusarium head blight epidemics in the wheat growing area of Argentina. In: Proceedings of the 5th Canadian workshop on fusarium head blight, Winnipeg, Canadá

Moschini RC, Fortugno C (1996) Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur J Plant Pathol 102:211–218

Moschini RC, Pioli R, Carmona M, Sacchi O (2001) Empirical predictions of wheat head blight in the northern Argentinean Pampas region. Crop Sci 41:1541–1545

Moschini RC, Carranza MR, Carmona M (2004) Meteorological-based predictions of wheat head blight epidemic in the southern Argentinean Pampas region. Cereal Res Commun 32:45–52

Moschini RC, Sisterna M, Carmona M (2006) Modeling of wheat black point incidence based on meteorological variables in the southern Argentinean Pampas region. Aust J Agric Res 57:1151–1156

Parry MAJ, Andralojc PJ, Parmar S, Keys AJ, Habash DZ, Paul MJ, Alred R, Quick WP, Servaites JC (1997) Regulation of Rubisco by inhibitors in the light. Plant Cell Environ 20:528–534

Pereyra S, Vero S, Garmendia G, Cabrera M, Pianzolla MJ (2006) Diversity of fungal population associated with Fusarium head blight in Uruguay. In: Ban T, Lewis JM, Phipps EE (eds) The global Fusarium initiative for international collaboration: a strategic planning workshop. CIMMYT, El Batán/México, pp 35–39

Schaafsma AW, Hooker DC, Piñeiro M, Díaz de Ackermann M, Pereyra S, Castaño JP (2006) Pre-harvest forecasting of deoxynivalenol for regulatory action in wheat grain in Uruguay using readily available weather inputs. In: Njapeu H, Trujillo S, van Egmond HP, Park DL (eds) Mycotoxins and phycotoxins: advances in determination, toxicology and exposure management. Wageningen Academic Publishers, Wageningen, pp 227–238

Strange RN, Smith H (1971) A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. Physiol Plant Pathol 1:141–144

Strange RN, Majer JR, Smith H (1974) The isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate *Fusarium graminearum* in vitro. Physiol Plant Pathol 4:277–290

Szabolcs L-K, Varga M, Mesterhazy A (2012a) Active ingredient content and ear coverage after spraying wheat with different nozzle types. In: Proceedings of the 4th international symposium on fusarium head blight, Nanjing, China, p 87

Szabolcs L-K, Varga M, Szabo-Hever A, Mesterhazy A (2012b) Translocation and degradation of tebuconazole and prothioconazol in wheat during flowering. In: Proceedings of the 4th international symposium on fusarium head blight, Nanjing, China, p 88

Wilcoxon RD, Ozmon EA, Wiersma J (1989) Effect of tillage, fungicides and wheat cultivars on scab. Biol Cult Test 4:59

Zadoks JC, Chang TT, Konzac CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Chapter 12
Biological Control of Fusarium Head Blight of Wheat: From Selection to Formulation

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Abstract Fusarium Head Blight of wheat is caused mainly by *Fusarium graminearum* (teleomorph = *Gibberella zeae*) and some related species such as *F. culmorum*, *F. poae*, *F. avenaceum*, *Microdochium nivale* var. *nivale* and *M. nivale* var. *majus*. During conducive years, yield lost can achieve up to 70 % in some crop fields with the consequent risk of mycotoxin accumulation on grains, mainly deoxynivalenol (DON). Disease control is mainly achieved by chemicals but ambiguous results have been reported; genetic resistance is also a viable option although transference of resistance genes to commercial lines has not been successfully achieved. Biological control appears as an alternative option to use in an integrated control of the disease. Several techniques are utilized to select potential microorganisms with antagonistic ability under laboratory scale; among them *Index of Dominance* and *Niche Overlap Index*. Further selection is done at greenhouse level where a reduction of the number of candidates is achieved. At this level, disease severity and DON reduction are crucial to select candidates for field experiments. Finally, formulation of the selected candidates is done in order to obtain an easy-handle product to be transported and applied in the field.

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12.1 Introduction

Wheat (*Triticum aestivum* L.) is the most cultivated cereal crop worldwide, occupying 17 % of the total growing areas of the world. The cereal is the main food for the 35 % of the global population and provides more calories and proteins than any other crop (Laegreid et al. 1999). The main wheat producers are European Union, China, India, Russia and US, with a total of 432 million-tons (67.4 % of the global production in 2009/2010 campaign). In Argentina, wheat is produced in 6.24 million hectare, with 12 million-tons produced in 2009/2010 harvest season; ranking Argentina as the thirteenth wheat producer and the fourth exporter in the world.

Fusarium Head Blight (FHB), also known as "Wheat scab" or just "Scab" and was first described in 1884 in UK (Smith 1995). FHB disease has a global distribution, affecting humid and semi-humid regions. Small grain cereals, as oat and barley, are susceptible to this disease but wheat, due to their worldwide distribution, is the most affected. During many years, FHB was considered as a secondary disease, but the increasing epidemics all around the world led to consider FHB as one of the main disease affecting small grain crops during conducive years (Champeil et al. 2004; McMullen et al. 1997). In Argentina, it was first described in 1950 (Galich and Galich 1996) and since then, a total of 17 FHB outbreaks had occurred (1960/1963/1967/1976/1977/1978/1984/1985/1993 and 2001) with yield losses estimated up to 70 % in some fields (Moschini et al. 2002). Since the last outbreak to date, only moderated to low incidence and disease severity was observed in some areas (Moschini, *personal communication*). This increasing in FHB outbreaks in some wheat areas of Argentina has been associated with climate changes but the main reasons are due to tillage practices (direct sowing) and the implementation of maize and soybean crops as alternative cultivars, which help to maintain FHB pathogens´ inoculum in the soil (Galich 1996; Ramirez et al. 2007). Similar cases were observed in US, Canada, Europe, China and Japan (Champeil et al. 2004; Dill-Macky and Jones 2000; Köhl et al. 2007; Luongo et al. 2005; Pirgozliev et al. 2003; Yli-Mattila et al. 2008).

The main pathogen associated to FHB all around the world is *Fusarium graminearum* (teleomorph = *Gibberella zeae*), which causes root rot, seedling blight, crown rot, stem rot and head blight (Champeil et al. 2004). Also, a FHB complex of species has been associated to the disease, including *F. culmorum*, *F. poae*, *F. avenaceum*, *Microdochium nivale* var. *nivale* and *M. nivale* var. *majus* (Osborne and Stein 2007; Xu et al. 2008). In Argentina, several species, among them *F. equiseti, F. semitectum, F. acuminatum, F. verticillioides, F. proliferatum, F. anthophilum, F. oxysporum* and *F. solani*, have been isolated from infected wheat grains, but their participation as pathogens associated to FHB is under discussion (Dalcero et al. 1997; Kikot et al. 2011; Ramirez et al. 2006). *Fusarium graminearum* is grouped into phylogenetic lineages (species complex) according to genealogical concordance phylogenetic species recognition (GCPSR, Taylor et al. 2000) and at least 16 lineages have been described (O'Donnell et al. 2000, 2004; Starkey et al. 2007; Sarver et al. 2011). *Fusarium graminearum* lineage 7 is the principal pathogen

associated with FHB in wheat in Argentina (Kikot et al. 2011; Ramirez et al. 2006, 2007; Reynoso et al. 2011; Sampietro et al. 2012).

Different strategies are used to reduce the impact of FHB including crop rotation, tillage practices, fungicides application, planting less susceptible cultivars. None of these strategies by itself is able to reduce the impact of this disease (Bai et al. 2000, 2001; Dill-Macky and Jones 2000; Mesterházy et al. 2003; McMullen et al. 2012).

Biological control offers an additional strategy and can be used as part of an integrated management of the FHB disease. *In vitro* assays and trials in glasshouse and under field conditions showed that some bacteria included in the genera *Bacillus, Pseudomonas and Lysobacter* were able to reduce *F. graminearum* growth (Schisler et al. 2002; da Luz et al. 2003; Khan et al. 2004; Jochum et al. 2006; Norouzian et al. 2006; Yoshida et al. 2012). Also yeasts, belonging to the genera *Rhodotorula, Sporobolomyces* and *Cryptococcus* were also effective (da Luz 2000a, b; Schisler et al. 2000, 2006; Khan et al. 2001). Among filamentous fungi, *Trichoderma* species were able to reduce the inoculum potential of *F. graminearum* (Fernandez 1992; da Luz et al. 1997; Norouzian et al. 2006; Matarese et al. 2012).

12.2 Strategies Utilized to Select Potential Biocontrol Agents

The selection process for biological control agents (BCA) covers a wide spectrum of techniques utilized to obtain putative microorganisms for pathogen´s biocontrol. These techniques are used in order to diminish the high number of microorganisms isolated from different sources, and this is the first step in the selection process. The *Index of Dominance* (I_d), a technique originally proposed by Magan and Lacey in 1984, was utilized to select potential BCA for *F. graminearum* and allowed us to obtain 22 strains from a total of 354 antagonist isolated from wheat anthers (Palazzini et al. 2007). An additional strategy utilized in the selection step is the utilization of *Niche Overlap Index* (NOI, Wilson and Lindow 1994). This technique evaluates the utilization of Carbon and Nitrogen sources in agar plates by antagonists and pathogens and allows to researchers to deduce the capacity to compete under different environmental conditions. Nesci et al. (2005) isolated several bacteria from maize soils and evaluated them as potential BCA against *Aspergillus* section *Flavi* strains. The utilization of NOI and I_d were successful in comparing the ecological similarity between isolated bacteria and aflatoxin-producers pathogens, suggesting that bacteria can compete against *Aspergillus* section *flavi* strains under natural environmental conditions (Nesci et al. 2005). Khan et al. (2001) isolated a total of 738 microorganisms from wheat anthers as putative BCAs. Therefore, antagonists were subjected to grow under tartaric acid as a selection strategy since the carbon source is a by-product in the processing of grapes and other fruits for juice and wine. A total of 54 strains were selected and evaluated in greenhouse experiments and the authors demonstrated that tartaric acid utilizers´ antagonists were more effective in reducing FHB than non utilizers. In a further study, the same group of microorganisms (738 strains) was evaluated in their capacity to metabolize

choline and betaine (Schisler et al. 2006) since these compounds are found in wheat anthers and others wheat head tissues susceptible to *F. graminearum* (Strange and Smith 1971, 1978). Although it has been shown that *F. graminearum* has specific transport systems for betaine and choline (Robson et al. 1992, 1994, 1995), it was reported that these compounds may stimulate or reduce hyphal growth under *in vitro* conditions (Nkongolo et al. 1993; Strange and Smith 1971, 1978). Schisler et al. (2006) selected 123 from 738 bacterial strains that metabolized choline as the sole carbon and nitrogen source and found that 48 strains were effective in reducing FHB in greenhouse assays. Finally, the authors concluded that choline utilization by antagonists does not ensure effective biocontrol against FHB.

The source from which potential BCA are isolated is also an useful tool and states that microorganisms isolated from specific plant tissue are well adapted to environmental conditions and, under high disease conditions, the antagonists´ activity will be intact for the biocontrol purpose (Schisler and Slininger 1997). In the case of biocontrol of FHB, anthers have been a good source of potential BCA (Palazzini et al. 2007, 2009; Khan et al. 2001). Also, wheat (da Luz et al. 2003), wheat kernels (Norouzian et al. 2006) and grass foliage (Jochum et al. 2006) were good sources of potential BCAs´ isolation.

Köhl et al. (2011) has recently published an extremely interesting research work which groups in different steps the selection strategies that should be followed in order to achieve a biocontrol product. These steps covers several areas of interest, since the potential markets for biological control in targeted crops and diseases at the beginning of the selection process to the formulation of the best candidate and testing at field level in multiple locations during several seasons, considering full integration in existing crop protection strategies. Studies on human toxicology, environmental risks, pilot formulations, mass production, patent protection, and market introduction, between others, are included into these screening steps.

12.3 Narrowing *In Vitro* Antagonist Selections: Greenhouse Assays

Certainly, greenhouse assays are the most utilized to evaluate potential BCAs that have been selected in *in vitro* experiments. The results obtained in this step are the most reliable and conduces to effective biocontrol activity in the most of the cases (da Luz et al. 2003; Jochum et al. 2006; Khan and Doohan 2009).

Using microorganisms as BCA to evaluate reduction in disease severity and deoxynivalenol (DON) production is important since the disease causes yield losses and DON has been reported to be toxic to human and animal health (WHO 2001). At this point, it is crucial to decide what´s better to control: toxin production or disease severity. Maybe both DON and disease are important to reduce. On one hand, scabby grains obtained during a harvest with high disease severity conduces to lowering prices of the grains, diminish the market spectrum and forces to commercialize the grains to a level of animal food. This fact can occur when damaged

Table 12.1 Effect of antagonist bacteria tested against *F. graminearum* RC 276 in greenhouse assays on wheat FHB disease severity and DON production on wheat heads at maturity

Treatment	DS (%)[A]	DON production (μg kg^{-1})[B]
F. graminearum alone	97	1,380[a]
BRC49A	81	130[b,l]
BRC49B	70*	500[b,h]
BRC85	69*	245[b,k]
BRC 87B	69*	0[b,n]
BRC127	63*	880[b,c,d]
BRC 164	78	830[b,d,e]
BRC 166	51*	645[b,f]
BRC 178	89	530[b,g,h]
BRC 184	93	600[b,f,g]
BRC 202	97	330[b,j]
BRC 204	70*	805[b,d,e]
BRC 211	82	500[b,h]
BRC 216	100	585[b,f,g]
BRC 218	45*	590[b,f,g]
BRC 225	100	0[b,n]
BRC 245	87	0[b,n]
BRC 263	29*	925[b,c]
BRC 265	95	300[b,j]
BRC 273	72*	780[b,e]
BRC 314	88	430[b,i]
BRC 325	83	0[b,n]
BRC 335	82	0[b,n]

[A]DS = Disease severity. Within a column, means followed by an asterisk are significantly different from the *F. graminearum* control (Fisher LSD Method. $p \leq 0.05$)

[B]Spikes were harvested after 8 weeks post-inoculation. DON analysis was done using a modified version of that originally reported by Cooney et al. (2001). Within a column, means followed by the same lower-case letter are not significantly different (Fisher´s LSD Method. $p \leq 0.05$)

grains are visible and the presence/absence of DON is unknown. On the other hand, grains that are apparently healthy can be rejected from ports due to elevated DON levels, causing the loss of money and reducing exportation volumes. So, at this step, what is more important to control: severity or DON production?. Palazzini et al. (2007) reported that 20 of 22 bacterial strains were effective in reducing DON in *in situ* experiments over irradiated wheat grains, with values ranging 60–100 % of toxin reduction. Moreover, when these strains were evaluated in greenhouse assays against *F. graminearum*, all the bacterial strains significantly diminished DON content on wheat spikes. However, those strains that controlled DON production were not the best in suppressing FHB disease severity (Table 12.1). Da Luz et al. (2003) suggested the applications of BCAs in combinations, been this fact a possible

answer for total control of FHB with the potential bacterial strains isolated by Palazzini et al. (2007). Schisler et al. (2006) selected 17 strains with the capacity of reducing at least 50 % of the disease severity in greenhouse trials and some strains were identified as *Erwinia* sp., *Pseudomonas* sp., *Pantoea agglomerans* and *Arthrobacter* sp. However, evaluation of DON was not done at this level. Similar results were observed by Jochum et al. (2006) when evaluating the biocontrol agent *Lysobacter enzymogenes* C3 against *F. graminearum* in eight cultivars of hard red spring wheat. The authors observed disease severities <10 % in comparison with the control (>80 %) in five of the eight cultivars evaluated and the utilization of heated broths to inactivate cells was also effective in reducing the disease; suggesting that induced resistance, through an elicitor factor, is a possible mechanism of action of *L. enzymogenes* C3. DON accumulation was not analyzed in this study.

12.4 Field Experiments: The Key Test

Undoubtedly, field experiments under natural environmental conditions are the most reliable and concluding tests to decide which antagonist will be further studied. At this level, effectiveness in suppressing disease severity and DON production is crucial; also, evaluating the tolerance of the candidates to environmental conditions (e.g. UV exposure, desiccation) will allow the selection of the ones that maintains their viability and thus, its biocontrol activity.

Effectiveness of the selected BCA in the field compared with greenhouse assays is, in general terms, lower; but the results obtained in some cases are promising. Schisler et al. (2006) evaluated five antagonists isolated from wheat anthers and selected for field trials after laboratory and greenhouse experiments. All the antagonists evaluated reduced FHB disease severity on the moderately resistant cultivar Freedom at two field locations, but only three antagonists were effective in reducing the disease on the susceptible cultivar Pioneer Brand 2545. The bacteria *Pseudomonas* sp. AS 64.4 was the most effective and consistent through all the field trials. In this study, the fungicide Folicur 3.6 F (based on tebuconazole) was utilized to compare the effectiveness of the antagonists and no statistical differences were found at either both locations and cultivars. The effectiveness of *Lysobacter enzymogenes* C3 was also evaluated on several wheat cultivars, obtaining protection against *F. graminearum* only in some varieties and concluding that biocontrol activity may be cultivar-dependant (Jochum et al. 2006). The antagonist effectiveness in relation to wheat cultivars was also suggested by Khan et al. (2004). The evaluation of two species of *Pseudomonas* was also carried out by Khan and Doohan (2009). In this study, the pathogen *Fusarium culmorum* was challenged with the antagonists on wheat and barley. The authors observed a reduction of the disease (44–55 %) on both crops when the bacteria were applied 24 h before pathogen inoculation. The most striking find was that both antagonists were effective in reducing DON levels on wheat and barley (74–78 %). Also, *Bacillus* species and yeasts have been identified as potential biocontrol agents against FHB (Schisler et al. 2004).

At this stage, effectiveness of several microorganisms has been demonstrated but none of them achieving total disease or DON control, which is obviously an utopian to obtain. So, it is possible that viability and, therefore, biocontrol capacity, may be affected by environmental factors when the antagonists are applied to the field. The strains *Brevibacillus brevis* ZJY-1 and *B. subtilis* ZJY-116 were transformed with a plasmid which expresses a Green Fluorescence Protein (GFP) in order to monitor the bacterial colonization on barley spikes (Zhang et al. 2005). The authors observed that BCA´s population severely decreased after a rain event but the cell number was recovered after several days and biocontrol activity was effective. In addition, although the cells applied on barley spikes were vegetative, the authors suggested that BCAs can survive in the environment as spores, which is also the same conclusion that Bennett et al. (2003) arrived after studying *B. subtilis* MBI600 on different soil treatments. Crane et al. (2010) also evaluated the survival of *B. amyloliquefaciens* on wheat heads up to 14 days after inoculation. Persistence of the BCA on the heads was inconsistent and no effective biocontrol was observed during 2008, 2009 and 2010 field trials, and finally concluded that population levels do not explain the ability of *Bacillus* to control FHB. Under field experiments climatic conditions fluctuate naturally and it is important to analyze how these environmental factors affect BCAs´ survival and biocontrol effectiveness.

12.5 Resistance in the Field: The Importance of Physiological Improvement

Phylosphere is a challenging niche affected by abiotic variations (e.g. temperature, relative humidity, solar radiation, water availability) which directly interacts with biotic factors (plants and microorganisms). These interactions may affect BCAs adaptation on the new niche (host plant tissue) and, therefore, its biocontrol activity. In the nature to deal with these adverse factors, microorganisms had developed several adaptative strategies, among them, the cytoplasmatic accumulation of solutes (Csonka 1989; Oren 1999). Organic osmolites enable organisms to adapt to environmental conditions by protecting cells or molecules without affecting macromolecules or physiological processes and are termed compatible solutes (Wood et al. 2001). Among them, betaine appears to be the most effective osmolyte to be accumulated in *B. subtilis* cells growing under osmotic stress conditions when its precursor, choline, is present. The high level of betaine allows *B. subtilis* cells to grow over a wide range of salinities (Whatmore et al. 1990; Holtman and Bremer 2004). In a previous study, we demonstrated the accumulation of betaine in *B. subtilis* RC218 and *Brevibacillus* sp. RC263 by manipulating growth conditions (water activities) with NaCl and glycerol, respectively (Fig. 12.1) (Palazzini et al. 2009).

Also, we observed that accumulation of betaine did not affect the biocontrol activity of the antagonists in greenhouse trials and, in *B. subtilis* RC218 modified with NaCl $a_w = 0.97$ treatment, the results observed were statistically different from

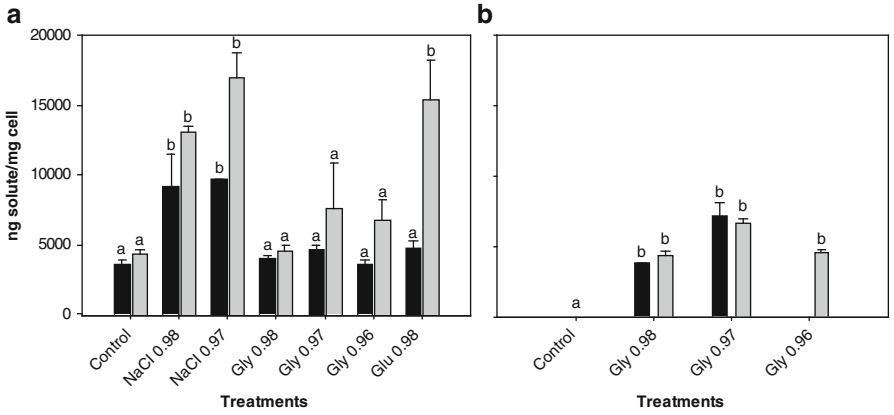

Fig. 12.1 Accumulation of betaine in *Bacillus subtilis* RC 218 (**a**) and *Brevibacillus sp.* RC 263 (**b**) grown in modified media for 24 h (▮) and 48 h (▯) at 28 °C. *Gly* glycerol, *Glu* glucose. Water activities tested on growing liquid media: 0.98; 0.97 and 0.96 a_w. Results are mean of three replicates per treatment. The separation of mean values is based on Dunnet`s test. Columns with different letters indicate significant differences ($p \leq 0.001$) *Nd* Not Detected. Detection limit: 1 μg ml^{-1}

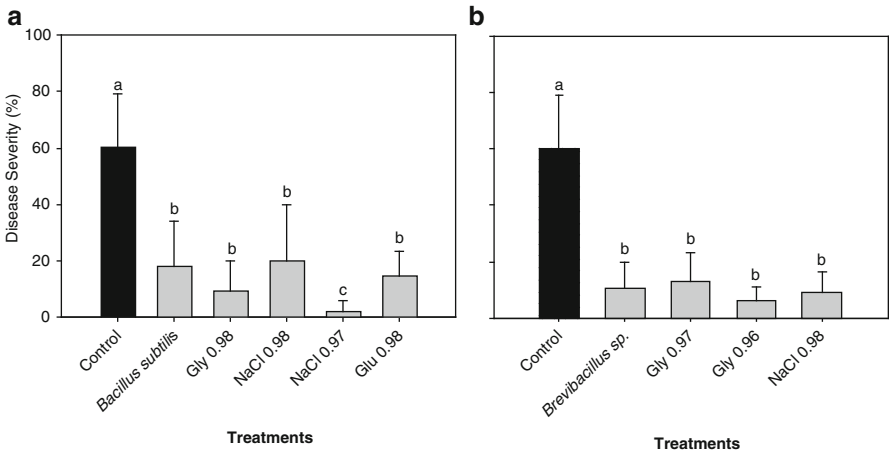

Fig. 12.2 Effect of physiological modified and non modified bacteria on FHB severity in wheat inoculated with *F. graminearum* RC 276 at anthesis. (**a**): *Bacillus subtilis* RC 218; (**b**): *Brevibacillus sp.* RC 263. Control: *F. graminearum* RC 276 inoculated alone; *Gly* glycerol, *Glu* glucose. Water activities tested on growing liquid media: 0.98; 0.97 and 0.96 a_w. The separation of mean values is based on Tukey`s test. On each graph, columns with different letters indicate significant differences ($p \leq 0.001$)

the rest of the treatments suggesting, perhaps, an increased biocontrol activity through the cross protection phenomenon (Fig. 12.2) (Sanders et al. 1999).

The accumulation of betaine and ectoine was also demonstrated by Teixidó et al. (2006) who found that physiological improvement of *Pantoea agglomerans* in NaCl

stressed medium did not affect biocontrol activity against postharvest fungal pathogens in apples and oranges. In addition, solute accumulation in cells exhibited better tolerance to the spray drying process. Schisler et al. (2002) found that *B. subtilis* AS 43.3 cells were less effective in controlling FHB disease severity in the field when compared to greenhouse results. One aspect to consider can be to improve physiologically the biological control agents to allow them to establish more efficiently in the targeted niches to execute its biocontrol activity.

There are several reports indicating that culture medium and cultivation time influences the desiccation tolerance and/or the efficacy of biocontrol agents (Cliquet and Jackson 1999; Engelkes et al. 1997; Montazeri and Greaves 2002a, b; Zhang et al. 2005). In addition, the utilization of physiological improvement has been successful in increasing the desiccation tolerance of BCAs for industrial processes (Cañamas et al. 2007; Cliquet and Jackson 1999; Sartori et al. 2011; Teixidó et al. 2005, 2006). Also, genetic manipulation is an important tool for the improvement of microorganisms for biological control, and is fully reviewed in Klemsdal and Tronsmo (2002).

12.6 Formulation of Biological Products: The Hidden Step

Information about microorganism's formulation is very limited in bibliography mainly due to secrecy issues imposed by commercial companies. Several bacteria and fungi have been isolated and some are being evaluated for commercial development as biopesticides (Khan et al. 2004; Zhang et al. 2005). Nevertheless, one of the main limitations of using biopesticides is the limited tolerance to fluctuating environmental conditions and the difficulties in developing a stable formulated product. Drying a product and maintenance in a dry environment or suspension in oil are common approaches that allow microbial agents to be handled commercially during distribution and storage (Rhodes 1993). Several amendments are used during or after the formulation in order to diminish the impact of storage and/or environmental conditions. Among them, we can find liquid carriers (vegetable oils), mineral carriers (diatomaceous earth), organic carriers (grain flours), protectants (skimmed milk, $MgSO_4$), stabilizers (lactose, sodium benzoate), binders (Arabic gum), surfactants (Tween 80), UV protectants (oxybenzone, lignin) and desiccants (silica gel, anhydrous salt) (Schisler et al. 2004).

Actually, the bacteria *B. subtilis* QST 713 (Serenade, AgraQuest, Inc.) is registered as a biopesticide (U.S. Patent 6.060.051) against a wide range of plant pathogens, including *Fusarium* species. Schisler et al. (2009) had patented three choline metabolizing strains: *Aureobasidium pullulans* AS 55.2, *Arthrobacter* sp. OH 221.3 and *Pseudomonas* sp. AS 64.4 (U.S. Patent 7.601.346) as potential BCA for FHB in wheat and barley. Da Luz (2000a, b) had registered in Brazil three strains *B. megaterium* (Embr 9790), *B. subtilis* (Embr 9786) and *Paenibacillus macerans* (Embr. 9770) as potential biocontrol agents against FHB in wheat. A *B. subtilis* strain (Trigocor 1448) is also been evaluated at field level and resulted in FHB disease and DON reductions (Cornell Research Foundation, Ithaca, NY 14853).

12.7 Conclusions

Testing microorganisms for biological activity against pathogens at lab scale is a laborious but necessary task to reduce the initial number of isolates to be tested at greenhouse. In this "*in vitro*" step, potential strains with good biocontrol activity can be selected. Under greenhouse conditions, the effectiveness of candidates can be measured, but the field tests are the ones that will effectively select the biocontrol strain. Together, research effort on formulation, biomass production and field effectiveness will lead to a commercially viable biocontrol product capable of controlling FHB and deoxynivalenol accumulation.

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References

Bai G, Shaner G, Ohm H (2000) Inheritance of resistance to *Fusarium graminearum* in wheat. Theor Appl Genet 100:1–8

Bai G, Platner R, Desjardins A, Kolb F (2001) Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed 120:1–6

Bennett AJ, Leifert C, Whipps JM (2003) Survival of the biological agents *Coniothyrium minitans* and *Bacillus subtilis* MBI600 introduced into pasteurized, sterilized and non-sterile soils. Soil Biol Biochem 35:1565–1573

Cañamas TP, Viñas I, Usall J, Magan N, Morelló JR, Teixidó N (2007) Relative importance of amino acids, glycine–betaine and ectoine synthesis in the biocontrol agent *Pantoea agglomerans* CPA-2 in response to osmotic, acidic and heat stress. Lett Appl Microbiol 45:6–12

Champeil A, Doré T, Fourbet JF (2004) Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci 166:1389–1415

Cliquet S, Jackson MA (1999) Influence of culture conditions on production and freeze-drying tolerance of *Paecilomyces fumosoroseus* blastospores. J Ind Microbiol Biotechnol 23:97–102

Cooney JM, Lauren DR, di Menna ME (2001) Impact of competitive fungi on trichothecene production by *Fusarium graminearum*. J Agric Food Chem 49:522–526

Crane J, Gibson D, Bergstrom G (2010) Ecology of *Bacillus amyloliquefaciens* on wheat florets in relation to biological control of fhb/don. In: Canty S, Clark A, Anderson-Scully A, Ellis D, Van Sanford D (eds) National FHB proceedings, Milwaukee, KY: University of Kentucky

Csonka L (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol Rev 1:121–147

Da Luz WC (2000a) Bioprotecao da Giberela do trigo. Fitopatol Bras 25:389 (Abstract)

Da Luz WC (2000b) Biocontrol of Fusarium head blight in Brazil. In: Ward R, Canty S, Lewis J, Siler L (eds) National Fusarium Head Blight Forum, Erlanger, KY: University of Kentucky

Da Luz WC, Stockwell CA, Bergstrom GC (1997) Seed microbiolozation for control of *Fusarium* species in cereals. Phytopathology 87:S22 (Abstract)

Da Luz WC, Stockwell CA, Bergstrom GC (2003) Biological control of *Fusarium graminearum*. In: Canty S, Lewis J, Ward R (eds) Fusarium head blight of wheat and barley. APS Press, St Paul

Dalcero A, Torres A, Etcheverry M, Chulze S, Varsavsky E (1997) Occurrence of deoxynivalenol and *Fusarium graminearum* in wheat from Argentina. Food Addit Contam 14:11–14

Dill-Macky R, Jones RK (2000) The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis 84:71–76

Engelkes CA, Nuclo RL, Fravel DR (1997) Effect of carbon, nitrogen, and C:N ratio on growth, sporulation, and biocontrol efficacy of *Talaromyces flavus*. Phytopathology 87:500–505

Fernandez M (1992) The effect of *Trichoderma harzianum* on fungal pathogens infecting wheat and black oat straw. Soil Biol Biochem 24:1031–1034

Galich MT (1996) Fusarium Head blight in Argentina. In: Fusarium head scab: global status and future prospects. Proceedings of a workshop Held at CIMMYT, Mexico

Galich MTV, Galich A (1996) I Jornada de control químico de enfermedades del trigo en sistemas para alta productividad. INTA-CIMMYT Bolsa de cereales de Buenos Aires

Holtman G, Bremer E (2004) Thermoprotection of *Bacillus subtilis* by exogenously provided glycine betaine and structurally related compatible solutes: involvement of Opu transporters. J Bacteriol 186:1683–1693

Jochum CC, Osborne LE, Yuen GY (2006) Fusarium head blight biological control with *Lysobacter enzymogenes* strain C3. Biol Control 39:336–344

Khan MR, Doohan F (2009) Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain. Biol Control 48:42–47

Khan NI, Schisler DA, Boehm MJ, Slininger PJ, Bothast RJ (2001) Selection and evaluation of microorganisms for biocontrol of Fusarium head blight of wheat incited by *Gibberella zeae*. Plant Dis 85:1253–1258

Khan NI, Schisler DA, Boehm MJ, Lipps PE, Slininger PJ (2004) Field testing of antagonist of Fusarium Head Blight incited by *Gibberella zeae*. Biol Control 29:245–255

Kikot GE, Moschini R, Consolo V, Rojo R, Salerno G, Hours RA, Gasoni L, Arambarri A, Alconada T (2011) Occurrence of different species of *Fusarium* from wheat in relation to disease levels predicted by a weather-based model in Argentina Pampas region. Mycopathologia 171:139–149

Klemsdal S, Tronsmo A (2002) Genetic manipulation for improvement of microbial biocontrol agents. In: Albajes R, Lodoviva Gullino M, Van Lenteren J, Elad Y (eds) Integrated pest and disease management in greenhouse crops. Kluwer Academic, New York

Köhl J, de Haas BH, Kastelein P, Burgers SLGE, Waalwijk C (2007) Population dynamics of *Fusarium* spp. and *Microdochium nivale* in crops and crop residues of winter wheat. Phytopathology 97:971–978

Köhl J, Postma J, Nicot P, Ruocco M, Blum B (2011) Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biol Control 57:1–12

Laegreid M, Bockman OC, Kaarstad O (1999) Agriculture and fertilizers. CABI Publishing, New York

Luongo L, Galli M, Corazza L, Meekes E, de Haas L, Lombaers C, Köhl J (2005) Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. Biocontrol Sci Technol 15:229–242

Magan N, Lacey J (1984) Effect of water activity, temperature and substrate on interactions between field and storage fungi. Trans Br Mycol Soc 82:83–93

Matarese F, Sarrocco S, Gruber S, Seidl-Seiboth V, Vannacci G (2012) Biocontrol of *Fusarium* head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*. Microbiology 158:98–106

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D (2012) A unified effort to fight an enemy of wheat and barley: Fusarium Head Blight. Plant Dis 96:1712–1728

Mesterházy Á, Bartók T, Lamper C (2003) Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight. Plant Dis 87:1107–1115

Montazeri M, Greaves MP (2002a) Effects of culture age, washing and storage conditions on desiccation tolerance of *Colletotrichum truncatum* conidia. Biocontrol Sci Technol 12:95–105

Montazeri M, Greaves MP (2002b) Effects of nutrition on desiccation tolerance and virulence of *Colletotrichum truncatum* and *Alternaria alternata* conidia. Biocontrol Sci Technol 12:173–181

Moschini RC, Galich MTV, Annone JG, Polidoro O (2002) Enfoque fundamental-empírico para estimar la evolución del índice de Fusarium en Trigo. Revista RIA 31:39–53

Nesci A, Bluma R, Etcheverry M (2005) *In vitro* selection of maize rhizobacteria to study potential biological control of *Aspergillus* section *Flavi* and aflatoxin production. Eur J Plant Pathol 113:159–171

Nkongolo KK, Dostaler D, Couture L (1993) Effet de la betaine, de la choline et d'extraits d'antheres du ble sur la croissance du *Fusarium graminearum*. Can J Plant Pathol 15:81–84

Nourozian J, Etebarian HR, Khodakaramian G (2006) Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria. Songklanakarin J Sci Technol 28(S1): 29–38

O'Donnell K, Kistler H, Tacke B, Casper H (2000) Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc Natl Acad Sci U S A 97:7905–7910

O'Donnell K, Ward T, Geiser D, Kistler H, Aoki T (2004) Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet Biol 41:600–623

Oren A (1999) Bionergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334–348

Osborne LE, Stein JM (2007) Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol 119:103–108

Palazzini JM, Ramirez ML, Torres AM, Chulze SN (2007) Potential biocontrol agents for *Fusarium* head blight and deoxynivalenol production in wheat. Crop Prot 26:1702–1710

Palazzini JM, Ramirez ML, Alberione E, Torres AM, Chulze SN (2009) Osmotic stress adaptation, compatible solutes accumulation and biocontrol efficacy of two potential biocontrol agents on Fusarium head blight in wheat. Biol Control 51:370–376

Pirgozliev SR, Edwards SG, Hare MC, Jenkinson P (2003) Strategies for the control *Fusarium* head blight in cereals. Eur J Plant Pathol 109:731–742

Ramirez ML, Chulze S, Magan N (2006) Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. Int J Food Microbiol 106:291–296

Ramirez ML, Reynoso MM, Farnochi MC, Torres A, Leslie JF, Chulze S (2007) Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. Food Addit Contam 24:1115–1120

Reynoso MM, Ramirez ML, Torres AM, Chulze SN (2011) Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. Int J Food Microbiol 145:444–448

Rhodes DJ (1993) Formulation of biological control agents. In: Jones D (ed) Exploitation of microorganisms. Chapman & Hall, London

Robson GD, Best LC, Wiebe MG, Trinci APJ (1992) Choline transport in *Fusarium graminearum* A3/5. FEMS Microbiol Lett 92:247–252

Robson GD, Wiebe MG, Trinci APJ (1994) Betaine transport in *Fusarium graminearum*. Mycol Res 98:176–178

Robson G, Wiebe M, Cunliffe B, Trinci A (1995) Choline- and acetylcholine-induced changes in the morphology of *Fusarium graminearum*: evidence for the involvement of the choline transport system and acetylcholinesterase. Microbiology 141:1309–1314

Sampietro D, Aristimuño Ficoseco M, Jimenez C, Vattuone M, Catalán C (2012) Trichothecene genotypes and chemotypes in *Fusarium graminearum* complex strains isolated from maize fields of northwest Argentina. Int J Food Microbiol 153:229–233

Sanders JW, Venema G, Kok J (1999) Environmental stress responses in *Lactococcus lactis*. FEMS Microbiol Rev 23:483–501

Sartori M, Nesci A, Etcheverry M (2011) Production of *Fusarium verticillioides* biocontrol agents, *Bacillus amyloliquefaciens* and *Microbacterium oleovorans*, using different growth media: evaluation of biomass and viability after freeze-drying. Food Addit Contam 29(Part A):287–292

Sarver B, Ward T, Gale L, Broz K, Kistler H, Aoki T, Nicholson P, Carter J, O´Donnell K (2011) Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by mutilocus genealogical concordance. Fungal Genet Biol 48(12):1096–1107

Schisler DA, Slininger PJ (1997) Microbial selection strategies that enhance the likelihood of developing commercial biological control products. J Ind Microbiol Biotechnol 19:172–179

Schisler DA, Khan N, Boehm MJ, Lipps PE (2000) Field tests of antagonists in 2000. In: Canty S, Lewis J, Ward R (eds) National Fusarium Head Blight Forum proceedings, Erlanger. University of Kentucky, KY, pp 105–109

Schisler DA, Khan NI, Boehm MJ, Slininger PJ (2002) Greenhouse and field evaluation of biological control of Fusarium Head Blight on durum wheat. Plant Dis 86:1350–1356

Schisler DA, Slininger PJ, Behle RW, Jackson MA (2004) Formulation of *Bacillus* spp. for biological control of plant diseases. Phytopathology 94:1267–1271

Schisler DA, Khan NI, Boehm MJ, Lipps PE, Zhang S (2006) Selection and evaluation of the potential of choline-metabolizing microbial strains to reduce Fusarium head blight. Biol Control 39:497–506

Schisler DA, Khan NI, Boehm MJ, Slininger PJ (2009) Choline-utilizing microbial strains for biologically controlling Fusarium head blight. United States Patent, US 7.601.346 B1

Smith WG (1995) Diseases of field and garden crops. Cité par Parry. Macmillan, London

Starkey D, Ward T, Aoki T, Gale L, Kistler H, Geiser D, Suga H, Tóth B, Varga J, O'Donnell K (2007) Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. Fungal Gen Biol 44:1191–1204

Strange RN, Smith H (1971) A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. Physiol Plant Pathol 1:141–150

Strange RN, Smith H (1978) Specificity of choline and betaine as stimulants of *Fusarium graminearum*. Trans Br Mycol Soc 70:187–192

Taylor J, Jacobson D, Kroken S, Kasuga T, Geiser D, Hibbett D, Fisher M (2000) Phylogenetic species recognition and species concepts in Fungi. Fungal Genet Biol 31:21–31

Teixidó N, Cañamás T, Usall J, Torres R, Magan N, Viñas I (2005) Accumulation of the compatible solutes, glycine-betaine and ectoine, as an osmotic stress adaptation and heat shock cross-protection in the biocontrol agent *Pantoea agglomerans* CPA-2. Lett Appl Microbiol 41:248–252

Teixidó N, Cañamas T, Abadias M, Usall J, Solsona C, Casals C, Viñas I (2006) Improving low water activity and desiccation tolerance of the biocontrol agent *Pantoea agglomerans* CPA-2 by osmotic treatments. J Appl Microbiol 101:927–937

Whatmore AM, Chudek JA, Reed RH (1990) The effects of osmotic upshock on the intracellular solute pools of *Bacillus subtilis*. J Gen Microbiol 136:2527–2535

Wilson M, Lindow SE (1994) Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. Appl Environ Microbiol 60:4468–4477

Wood J, Bremer E, Csonka L, Kraemer R, Poolman B, Van der Heide T, Smith L (2001) Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comp Biochem Physiol Part A 130:437–460

World Health Organization (2001) Deoxynivalenol. WHO food additives series 47. FAO food and nutrition paper 74

Xu XM, Nicholson P, Thomsett MA, Simpson D, Cooke BM, Doohan FM, Brennan J, Monaghan S, Moretti A, Mule G, Hornok L, Beki E, Tatnell J, Ritieni A, Edwards SG (2008) Relationship between the fungal complex causing Fusarium head blight of wheat and environmental conditions. Phytopathology 98:69–78

Yli-Mattila T, Paavanen-Huhtala S, Jestoi M, Parikka P, Hietaniemi V, Gagkaeva T, Sarlin T, Haikara A, Laaksonen S, Rizzo A (2008) Real-time PCR detection and quantification of *Fusarium poae, F. graminearum, F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. Arch Phytopathol Plant Prot 41:243–260

Yoshida S, Ohba A, Liang Y, Koitabashi M, Tsushima S (2012) Specificity of pseudomonas isolates on healthy and Fusarium head blight-infected spikelets of wheat heads. Microb Ecol 64:214–225

Zhang S, Boehm MJ, Schisler DA, Slininger PJ (2005) Carbon to-nitrogen ratio and carbon loading of production media influence freeze-drying survival and biocontrol efficacy of *Cryptococcus nodaensis* OH 182.9. Phytopathology 95:626–631

Chapter 13
Modeling and Forecasting Systems for Fusarium Head Blight and Deoxynivalenol Content in Wheat in Argentina

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Abstract In Argentina, wheat Fusarium Head Blight (FHB) is predominantly caused by *Fusarium graminearum*, being deoxynivalenol (DON) the main associated mycotoxin. The sporadic weather-induced nature of FHB in the Pampas region led to the development of weather-based disease and DON forecasting systems. Infective events were identified by head wetting resulting from syncronic occurrence of precipitation and high relative humidity, around wheat anthesis. Retrospective model predictions were able to identify synoptic situations and meteorological predictors of increasing space-temporal scale (for developing specific short-range and seasonal weather forecasts), regarding the disease. In the north-eastern quadrant of the Pampas region, greater disease levels were expected with greater August Southern Annular Mode values and dominance of meridional north-northeastern atmospheric circulation in September. In the southern, the Southern Oscillation index and variables associated to blocking action situations in the south (October), strongly helped to explain disease variability. Climate change impact was assessed retrospectively analyzing the trend lines of FHB incidence predictions (1931–2010), which showed light positive slopes, larger towards southern Pampas region. Prospectively, the anomaly map resulting from the difference between disease incidence estimated by future meteorological data (2071–2100, A2 scenario) and baseline climate (1961–1990), presented positive deviations in the southern Pampas region. The spatial distribution of model-based FHB incidence values using only land weather station network data was compared satisfactorily with those using both land and satellite data. Conclusions derived from FHB forecasting systems and specific weather forecasts are being used to assist producers in disease control measures to be employed.

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13.1 Introduction

Fusarium Head Blight (FHB) is a disease caused by several *Fusarium* species. In Argentina, 90 % of the pathogens isolated from blighted heads were *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] (Carranza et al. 2002), which produces exclusively the mycotoxins deoxynivalenol (DON) and zearalenone (Faifer et al. 1992).

Argentinean wheat cropping area is very extensive (4.63 million hectares in 2011–2012 growing season), distributed in five provinces of the Pampas region with different ecological conditions (Buenos Aires, Córdoba, Santa Fe, Entre Ríos and La Pampa). Therefore, no wheat FHB epidemic has ever covered the whole area in a given time. The worst FHB outbreaks occurred in the north-eastern quadrant of the Pampas region, producing wheat yield losses that ranged from 10 to 30 % in 1978. Favorable meteorological conditions once again prevailed in 1985, 1993 and 2001, enhancing FHB infections and causing significant wheat losses. In the south-eastern portion of wheat growing region, the main durum wheat (*Triticum durum* Desf.) area, severe epidemics occurred in 1963, 1976, 1978 and 1985, with crop losses as high as 70 % (Moschini et al. 2004).

The sporadic nature of FHB, strongly related to weather factors, led to development-validation of empirical and fundamental-empirical forecasting systems in the Pampas region. Also, from controlled-environment studies, a forecasting system of DON content in wheat mature grain was developed. Retrospective FHB model predictions were able to make maps showing their regional climate potential and to carry out studies to identify synoptic situations and meteorological predictors of increasing spatial-time scales regarding the pathosystem. A new empirical model for predicting FHB incidence where relative humidity was replaced by thermal amplitude was used to study the effect of climate change retrospectively and by comparing the recent past (1961–1990) and future (2071–2100) climate. All this knowledge is being used for assessing FHB risk and its spatial distribution (supported by land and remote sensing data) in the Pampas region before wheat harvest.

13.2 Development of Weather-Based Fusarium Head Blight/Deoxynivalenol Forecasting Systems

13.2.1 Development of Weather-Based Fusarium Head Blight Forecasting Systems

13.2.1.1 Empirical Approach

Applying linear regression techniques, different models up to a maximum of three independent meteorological variables were fitted to FHB incidence (***I*** %) data in Pergamino (north-eastern of Buenos Aires province) (Moschini and Fortugno 1996). Annual mean percent disease incidence computed from values recorded for wheat

cultivars grouped by their similar heading dates were used to fit the models. Using daily records of maximum (xT) and minimum (nT) temperature, precipitation (Pr) and relative humidity (RH: average of the 0800, 1,400 and 2,000 h observations) obtained from a standard weather station, independent meteorological variables were calculated. Daily average temperature (Td) was calculated as half the sum of the daily minimum and maximum temperatures The closest associations between environmental variables and FHB incidence data were obtained in a time period beginning 8 days prior to heading date (when 50 % of the heads were fully emerged: emergence of first heads) and ending when 530 degree days (***DD***) were accumulated (base temperature: 0 °C). This period was regarded as the susceptible period for infection (**SPI**) lasted 26–32 days in this study.

Variable NP (number of 2-day periods with Pr (≥0.2 mm) and RH >81 % during the first day and RH ≥78 % during the second one) showed the strongest association with disease incidence ($R^2 = 0.81$). Long anther wetness periods (48–60 h) favor the infection of the pathogen. Not having duration measurements of precipitation-induced wetness events, their potential lengths were better considered by combining the occurrence of precipitation with high air relative humidity records in a 2-day window. A low correlation was found between precipitation frequency and disease incidence ($R^2 = 0.17$). The Eq. (13.1) was finally selected for predicting FHB incidence (***PI*** %).

$$\boldsymbol{PI\%} = 20.37 + 8.63\ \boldsymbol{NP} - 0.49\ \mathbf{n}\boldsymbol{DD} \quad R^2 = 0.86 \tag{13.1}$$

in which **n*DD*** represents the daily accumulation of the residuals resulting from subtracting 9 to the nT values (<9 °C) and the exceeding amounts of xT from 26 °C. This equation was adjusted and validated for northern locations than Pergamino (Moschini et al. 2001). For southern areas of the Pampas region (Moschini et al. 2004; Carranza et al. 2007), lower and upper temperature thresholds were changed by 11 and 30 °C and the **SPI** was fit in 450 ***DD***. With the same data, Moschini et al. (2008) developed another FHB incidence predicted model that only requires daily data of maximum and minimum temperature and precipitation to calculate its variables (Eq. 13.2).

$$\boldsymbol{PI\%} = -9.15 + 6.47\ \boldsymbol{ND} + 0.35\ \boldsymbol{pDD} \quad R^2 = 0.81 \tag{13.2}$$

being ND: number of days with simultaneous occurrence of Pr and thermal amplitude (TA = xT − nT) <7 °C; pDD: results of accumulating residuals >9 °C in nT, in those days where xT and nT are <25 °C and ≥9 °C, respectively.

13.2.1.2 Fundamental-Empirical Approach

From 84 FHB incidence (***I*** %: percentage of diseased heads) and severity (***S*** %: percentage of diseased spikelets in the diseased heads) values registered in commercial wheat cultivars (susceptible and moderately susceptible) at Pergamino and M. Juárez (south-eastern Córdoba province) (ten growing seasons), observed

Fusarium index (***FI*** %=***I***%*****S*** %/100) values were calculated and satisfactorily contrasted with predicted ones using a fundamental-empirical approach (Moschini et al. 2002).

Predicted *Fusarium* index (*PFI* %) values were obtained following the next steps:

(a) Daily progress of anthesis (% of wheat heads with exposed anthers): from field observations in a single wheat cultivar, a polynomial function between the logit of the proportion of head with anthers (Anther, values from 0 to 1) and the time in degree days (DD_{12}: daily accumulation of Td ≥ 12 °C) was fit.

$$LogitAnther = -6{,}765052912 + 0{,}136395967\ DD_{12} - 0{,}000694621\ DD_{12}^{2} + 0{,}000001384\ DD_{12}^{3} - 0{,}000000001\ DD_{12}^{4} \quad (13.3)$$

where LogitAnther is the natural logarithm of (Anther/1-Anther); $DD_{12}^{2} = DD_{12} * DD_{12}$; $DD_{12}^{3} = DD_{12} * DD_{12}^{2}$; $DD_{12}^{4} = DD_{12} * DD_{12}^{3}$. Solving [Exp (LogitAnther)/(1 + Exp(LogitAnther))]*100, the daily percentage of heads with anthers were obtained. The ***SPI*** began 4 days prior the observed heading date and finished when 530 ***DD*** were accumulated.

(b) Predicted severity (***PS*** %): in controlled environment, Andersen (1948) established the percentage of infection in wheat heads inoculated with *Fusarium graminearum* conidia, exposed to different wetness periods (***W***: from 18 to 72 h) and temperatures during wetness periods (T_W = 15, 20, 25 and 30 °C). A polynomial function was fit between the logit of the severity (S, values from 0 to 1) and ***W*** (h) and T_W (°C), like individual and interactive effects.

$$LogitS = 38{,}77166158 - 0{,}53815698\ W - 6{,}02985565\ T_W + 0{,}26849793\ T_W^{2} - 0{,}00396097\ T_W^{3} + 0{,}04990941\ I_1 - 0{,}00092343\ I_2 \quad (13.4)$$

where LogitS is the natural logarithm of (S/1 − S); $T_W^{2} = T_W * T_W$; $T_W^{3} = T_W^{2} * T_W$; $I_1 = T_W * W$; $I_2 = T_W^{2} * W$. ***PS*** % values were obtained solving [Exp(LogitS)/(1 + Exp(LogitS))] *100.

In order to use Eq. (13.4), **equivalence rules** defining ***W*** and T_W from daily Pr, xT, nT and RH registered at standard weather stations, were established. Using criteria derived from the empirical approach, it was defined that: 1 day with Pr (≥ 0.2 mm) and RH ≥ 81 % is equal to ***W*** = 24 h; two consecutive days with Pr and RH ≥ 81 % is equal to ***W*** = 48 h; three consecutive days with Pr and RH ≥ 81 % is equal to ***W*** = 72 h. A maximum ***W*** period of 72 h was analyzed. If prior and/or after ***W*** period of 24 and 48 h, Pr and RH ≤ 77 % are registered, 3 h of wet are added. If Pr and RH >77 % and <81 % (prior and/or after) are registered, 6 h of wet are added. Occurrence of RH > 77 % and <81 % after ***W*** periods, 3 h are added. The temperature during the wet period (T_W) resulted of averaging the mean daily temperatures (Td), weighted for the wet duration in each day involved. If T_W is <15 °C, severity values are only calculated for wet periods ≥48 h.

(c) The final ***PF***I % value resulted from adding the partial products between (a) and (b) (divided by 100), calculated for all the wet periods found throughout the wheat ***SPI***. As a consequence of validation studies, just a few small changes of the original methodology were made. The SPI lasted 450 $\boldsymbol{DD}_{10}$ (daily accumulation of Td ≥ 10 °C) in southern Pampas region (Moschini et al. 2004; Carranza et al. 2007) and also an upper daily mean temperature threshold (If Td > 20 °C then Td = 20 °C) during days with precipitations was included (Moschini et al. 2012).

13.2.2 Development of Weather-Based Deoxynivalenol Content Forecasting System

Environment-controlled experiments were conducted in Castelar (northeastern Buenos Aires province) in 2006 and 2007 to study the effect of wetness duration (***W***) and temperature ($\boldsymbol{T}_W$) on three visual estimates of FHB (***I*** %, ***S*** %, ***FI*** %) and grain DON content, and to develop a weather-based forecasting system of the mycotoxin (Martinez et al. 2012). Artificial inoculation with toxicogenic local *Fusarium graminearum* strains was used. Both FI % and S % were significantly related to ***W*** and $\boldsymbol{T}_W$ and strongly associated to DON content ($R^2 = 0.66$ and 0.73, respectively). Relationships between environmental factors and DON content were not significant. The $\boldsymbol{T}_W$ and ***W*** treatments allowed to establish three important thresholds. The first was 9.5 °C, at which there were no FHB symptoms, the second was 15 °C, at which both S % and FI % increased when ***W*** increased and the third was around 20 °C, at which S % and FI % had less response to ***W***. Symptom expressions started around 14 °C for all ***W***, producing different S % levels (5.5–24.3 %). In a similar experiment with *F. graminearum* and environmental-controlled conditions, Andersen (1948) did not find S % levels higher than 1.5 % at 15 °C. Differences in the adaptation of pathogen strains to the place of origin could explain the differences between both studies. Coincidently, Zoldan (2008) observed low disease levels in wheat spikes inoculated with local *F. graminearum* isolates (southern Brazil) at 10 °C, concluding that the pathogen is adapting to lower temperatures and is more aggressive.

From the information generated by the environment-controlled experiments from Martinez et al. (2012), it was possible to modify the earlier explained fundamental-empirical forecasting systems of *Fusarium* index (Moschini et al. 2002) as well as to predict DON content. As first step, the next polynomial equation (Eq. 13.5) was adjusted between the logit of FHB severity (S, from 0 to 1) and the different chamber treatments (***W*** and $\boldsymbol{T}_W$) ($R^2 = 0.96$):

$$\begin{aligned} \boldsymbol{LogitS} = &-52.68809110 + 0.03705345\,\boldsymbol{W} + 8.17557456\,\boldsymbol{T}_W \\ &- 0.45642424\,\boldsymbol{T}_W{}^2 + 0.00856901\,\boldsymbol{T}_W{}^3 \end{aligned} \tag{13.5}$$

being LogitS: natural logarithm of (S/1 − S); $\boldsymbol{T_W}^2 = \boldsymbol{T_W} * \boldsymbol{T_W}$; $\boldsymbol{T_W}^3 = \boldsymbol{T_W}^2 * \boldsymbol{T_W}$. Solving [Exp(LogitS)/(1 + Exp (LogitS))]*100, ***PS*** % values were found. After incorporating Eq. (13.5) into Moschini et al. (2002) forecasting system, 82 predicted *Fusarium* index (***PFI*** %) values were obtained and regressed against field observed ones (Eq. 13.6; $R^2 = 0.54$).

$$\boldsymbol{PFI}_f\% = -4.0089 + 0.4351\,\boldsymbol{PFI}\% \tag{13.6}$$

being PFI_f % the predicted *Fusarium* index values adjusted to field conditions. Finally, a linear regression was fit between DON grain content and observed *Fusarium* index (***FI*** %) values (37 pair values resulting from chamber treatments). Under the assumption that in field conditions low FHB intensity leads to low DON contamination, the regression line was corrected to zero intercept. The determination coefficient (R^2) was 0.66 and the equation was the following:

$$\boldsymbol{DON(mg\,/\,Kg)} = 57.865\,\boldsymbol{FI}\% \tag{13.7}$$

The new forecasting system developed was run for each cultivar with its flowering date and daily meteorological data from Oliveros (Santa Fe province), for the 2007 wheat growing season. Twelve $\boldsymbol{PFI}_f$ % values were obtained and adjusted to field conditions (Eq. 13.6). Using Eq. (13.7), 12 DON values were estimated. The *t*-test determined no significant differences between mean observed ***FI*** % and ***PFI*** % and DON values.

Unlike the results from Martinez et al. (2012), other studies found significant relationships between the environment and grain DON content (Xu et al. 2007; Hooker et al. 2002; Lacey et al. 1999). Varying degrees of association between FHB intensity and DON accumulation in harvested grain, including high positive, low, and negative correlations, as well as correlations close to zero, have been reported (Paul et al. 2005). According to Snijders and Kretching (1992) the mycotoxin can be translocated from floral tissues to the grain, and considering that *F. graminearum* may colonize the floral and grain tissues (Xu et al. 2007), the symptoms on the floral tissues may be relevant for estimating DON content. Using meta-analysis of transformed correlations of published and unpublished studies on the relation between FHB visual intensity measures and DON content, Paul et al. (2005) concluded that the *Fusarium* index had a significantly higher correlation with DON than either incidence or severity. According to Paul et al. (2005), between 27 and 53 % of the variation in DON content is explained by the variation in disease intensity. Other factors may influence DON content and interfere in the precision of the estimation. It is essential to continue collecting samples of wheat grains under field conditions, with the respective disease information to strengthen the reliability of several relationships, for example Eq. (13.7), which relates FI % with DON. The inclusion of field data may increase the variability of values and thus make the proposed system more possible to extrapolate.

13.3 Applications of Fusarium Head Blight Forecasting Systems

13.3.1 Climate Risk of the Pampas Region Regarding Fusarium *Index*

For 37 stations of the Pampas region with daily meteorological data for 1971–2011 time series, levels of *Fusarium* index for each year were estimated by the fundamental-empirical forecasting system (Moschini et al. 2012). The probability of occurrence of severe *Fusarium* index (considered here as PFI % > 10) was calculated for each station. The spatial distribution of these probabilities for the Pampas region can be observed in Fig. 13.1. Northeastern quadrant of the region shows the highest probability of severe attacks (four to six severe epidemics out of 20 years). Towards the southern, the disease gradually decreases, expecting two to three severe outbreaks out of 20 years in the southeastern of the region. The minimum climate risk of the region regarding the disease is observed in the southwestern quadrant.

13.3.2 Development of Specific Fusarium Head Blight Short Range Meteorological Forecasts

Many weather-based forecasting systems in plant pathology estimate epidemic risk in order to make sound disease management decisions, reaching their maximum potential in areas where weather conditions sporadically favor epidemics, unlike those areas in which conducive conditions always prevail. Numerous disease forecasting systems recommend post-infection chemical control after identifying favorable weather events, when contact fungicides are ineffective against already established infections. Past weather patterns are analyzed instead of analyzing present and future weather conditions. Only a few disease predictive systems have incorporated short range meteorological forecasts for truly predicting the infection (Vincelli and Lorbeer 1988; Raposo et al. 1993; Baker and Kirk 2007). Bourke (1970) pointed out the potential value of analyzing the conducive weather factors for infection and identifying the types of recurrent meteorological situations over synoptic weather charts (surface and higher levels; actual and forecast), complementing the role played by simple disease predictive models.

FHB infection events depend strongly on the occurrence of both precipitation-induced long wetness periods (24–72 h) and warm temperatures around wheat flowering. Little time after infection is available for effective triazole fungicide applications (1 or 2 days). Therefore both, FHB weather-based forecasting systems and specific short range meteorological forecasts can help farmers assess

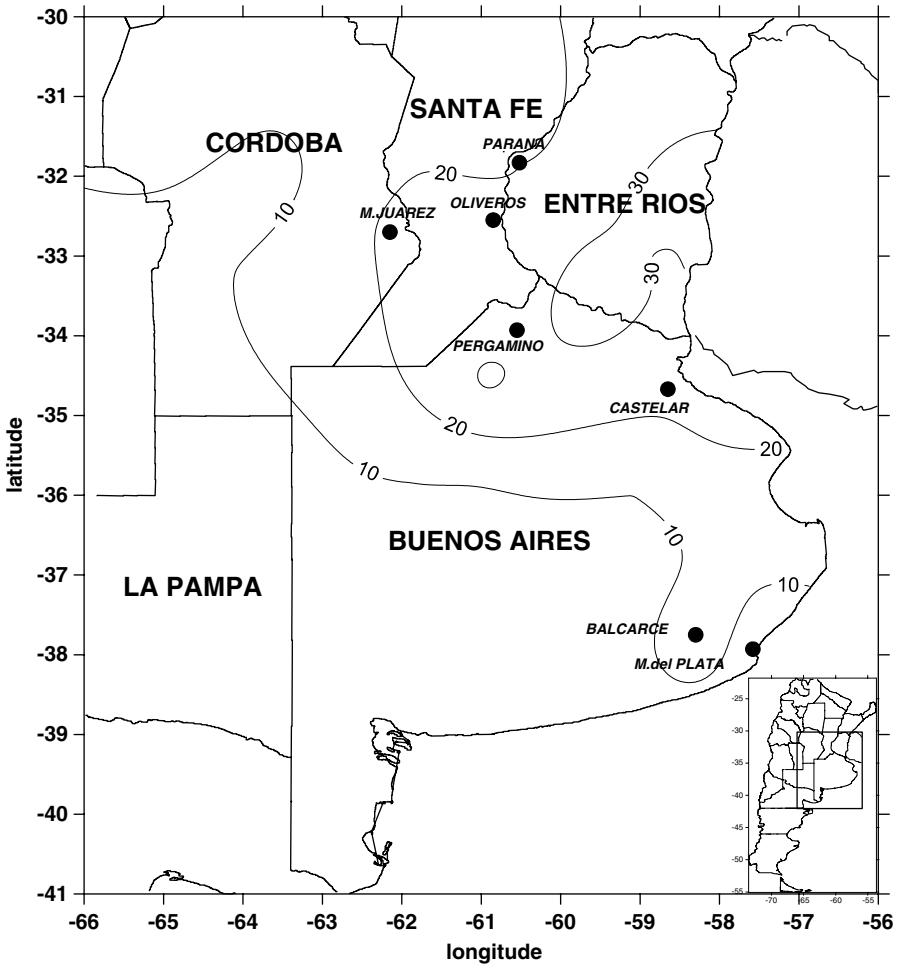

Fig. 13.1 Percentage of years with a severe level of *Fusarium* index (PFI > 10 %) predicted by the fundamental-empirical forecasting system (1971–2011 series; 37 meteorological stations) in the Pampas region (Córdoba, Santa Fé, Entre Ríos, La Pampa and Buenos Aires provinces)

the risk of FHB infection when deciding whether to apply a fungicide. For this goal, studies were carried out for characterizing types of atmospheric circulation at synoptic scale associated to the occurrence of FHB infection events. From Alessandro (2003) studies about the influence of blocking action anticyclones in the south of South America on temperature and precipitation in the Argentinean territory, the effect of this synoptic phenomenon was analyzed in relation to FHB epidemics. Results derived from these researches may be useful for the interpretation of available short range weather forecasts (1–5 days), making them specific for the disease.

13.3.2.1 Synoptic Weather Patterns Related to Fusarium Head Blight Infection Events

Synoptic weather patterns associated to FHB infection events in several sites of the Pampas region were characterized (Moschini 2011). Infection events were identified by head wetting resulting from syncronic occurrence of precipitation and high relative humidity, around wheat anthesis. FHB infection event severities were estimated by Eq. 13.4 (fundamental-empirical approach) in five sites (eastern Pampas region) throughout 45-day periods (in which wheat head with anthers could be available) for the 1971–2006 wheat growing seasons. Predicted FHB severities were categorized into levels: severe ($\boldsymbol{PS}\,\% > 3.7$, percentile 75 %), moderate ($\boldsymbol{PS}\,\% \leq 3.7$ and >1), light ($\boldsymbol{PS}\,\% \leq 1$, percentile 25 %). In Paraná (western Entre Ríos province), 51 and 27 FHB infection events were identified as severe and light respectively, including their initial day. From NCEP/NCAR reanalysis (Kalnay et al. 1996), 3-day sequences (initial day and the two previous) of mean daily 1,000 hPa geopotential height (gph) maps were obtained for South America. Using the technique developed by Lund (1963), each 3-day sequence of mean daily 1,000 hPa gph map was correlated (r: Pearson correlation coefficient) with all the other 3-day sequences analyzed. In Paraná, one 3-day sequence map (initial day infection event: 12 October 1993) had the most $r \geq 0.70$ (33 out of 51). The same process was carried out for 27 light infection events. In this case, one 3-day sequence map (initial day: 16 September 1982) had the most $r \geq 0.70$ (14 out of 27). Figure 13.2 shows both synoptic patterns, resulting of averaging (point by point of the 1,000 hPa gph grid) the 33 (severe infections) and 14 (light infections) 3-day sequence maps. The mean synoptic pattern related to severe FHB infection events (Fig. 13.2, left) presented a low pressure centre located central-north of Argentina, involving ascendant air inducing precipitations (primary source of wetness periods required for FHB infection events). When the 3-day sequence (initial day and the two previous) of gph at 1,000 hPa was analyzed, two strong anticyclones (Pacific and Atlantic) and a low pressure axis over Argentina was characteristic. The strong Atlantic high pressure centre produced advection of warm and humid air during the two previous days to the beginning of FHB infection episodes. The mean synoptic pattern associated to light FHB events (Fig. 13.2, right) showed a latitudinal high pressure axis and two low pressure areas, one strong over central northern Argentina and the other southern. A weakening in the south Atlantic anticyclone activity decreased the incoming of northern air masses. The strong Pacific anticyclone circulation shifted rapidly the continental cyclone to the north of Argentina. This fast displacement of the low pressure area might explain the occurrence of shorter periods of wetness (precipitation-induced) related to light FHB infection events.

13.3.2.2 Influence of Blocking Action Situations in the Southern of South America on Fusarium Head Blight Infection Events

From daily data of maximum and minimum temperature, precipitation and relative humidity, annual model-based *Fusarium* index (fundamental-empirical approach) values were estimated (1971–2006), throughout the susceptible period for infection

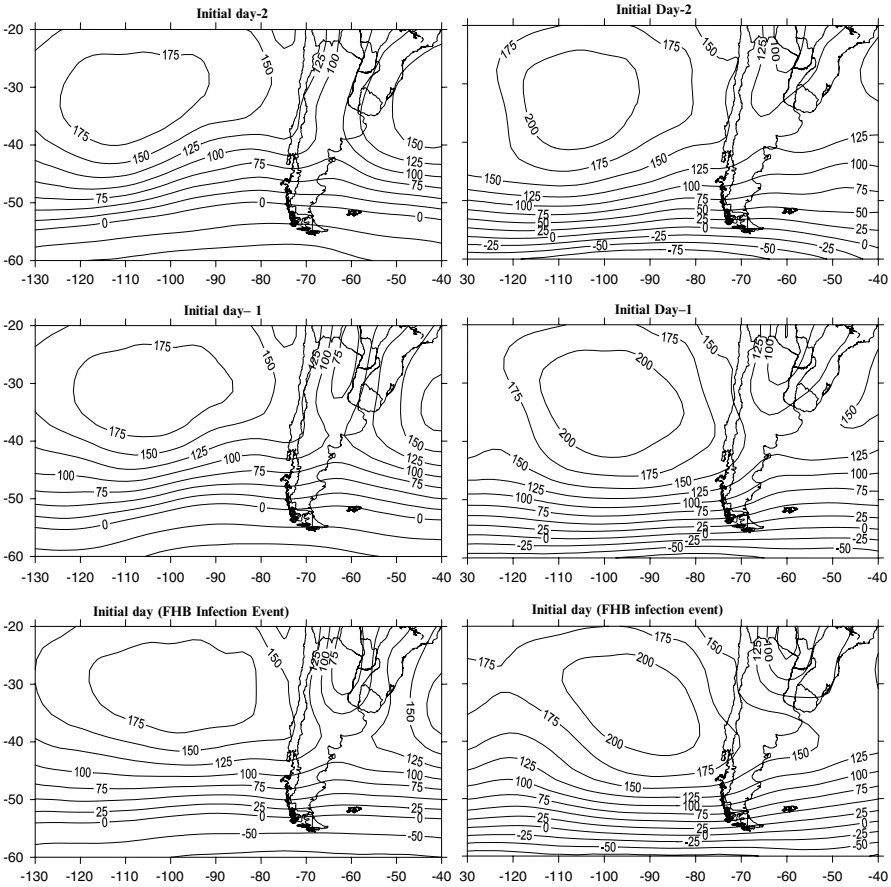

Fig. 13.2 Mean synoptic weather patterns related to severe (*left*) and light (*right*) FHB infection events

(SPI) in Paraná, Pergamino and Balcarce (Fig. 13.1) (Moschini 2011). Combining precipitation occurrence and relative humidity threshold values, wetness periods or infection events (24–72 h) were identified. A zonal index (ZI) was calculated for 100°, 70° y 40°W longitudes, throughout the SPI. ZI = U(30°S) + U(60°S) − 2U(45°S), where U is the wind zonal component (m/s) in 500 hPa. Positive values of ZI indicate weaker westerly Patagonia winds at 45°S latitude. The highest mean percentage of days coincident with FHB infective events (69.7 %) observing ZI > 0, was reached by the zonal index calculated in 70°W. A greater frequency of days with ZI > 0 values was observed accompanying the occurrence of FHB infective events of longer duration (≥ 30 h), especially in Balcarce. More persistent blocking action situations (5 or more days with ZI > 0) were registered more frequently in

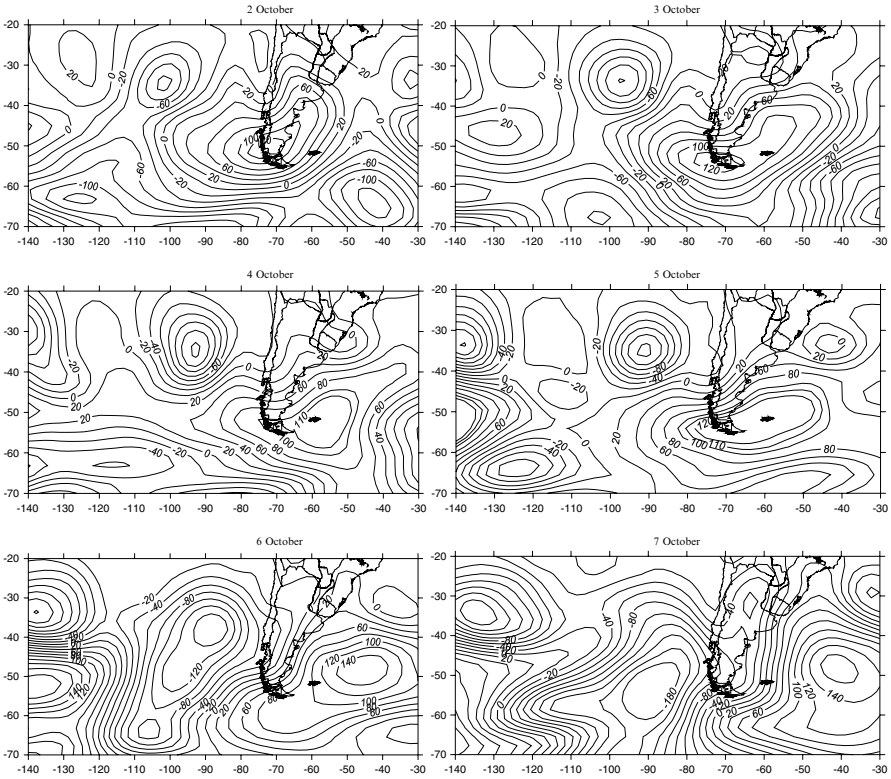

Fig. 13.3 Six day-sequence (2 October to 7 October) of anomaly maps of geopotential height at the level of 1,000 hPa, along the blocking action situation at 70°W observed at the southern South America in 1978 (severe FHB epidemic in the northeastern Pampas region quadrant)

severe annual epidemic cycles, especially in southern Pampas region. For the most severe FHB epidemics (observed and predicted by models) in the Pampas region, 55–100 % of days with occurrence of infective events in the SPI observed positive values of ZI. The lowest values were observed in Pergamino and Paraná in 1978, where 55 and 69 % of days with FHB infection events were accompanied by daily values of ZI>0, respectively. In other severe epidemics, everywhere there were higher percentages, reaching highs ranging from 92 % in Pergamino in 1985, to 93 % in 2001 in Paraná, up to 100 % of the days in 2001 and 2004 in Balcarce. Figure 13.3 show the 6 day-sequence (2 October to 7 October) of anomaly maps of geopotential height at the level of 1,000 hPa, along the blocking action situation at 70°W observed in 1978. Throughout the period under the influence of this blocking action anticyclone (plus 8 October), 96 % of the total cumulative *Fusarium* index was estimated in Paraná for the wheat campaign of 1978.

13.3.3 Development of Specific Fusarium Head Blight Seasonal Forecasts Based on Hemispheric-Scale Meteorological Predictors

The atmospheric conditions present irregular fluctuations on wide range of time scales, from weekly to greater scales causing intraseasonal (26–60 days), interannual and interdecadal variability (Garreaud et al. 2008). El Niño Southern Oscillation (ENSO) constitutes the most important oceanic-atmospheric phenomenon causing interannual climate variability. Walker and Bliss (1932) discovered the existence of an irregular interannual fluctuation called Southern Oscillation (SO), which involves changes in the rainfall and wind over the tropical Pacific and Indian oceans. Bjerknes (1969) associated the SO with fluctuations in the surface temperature of the eastern equatorial Pacific Ocean. ENSO phenomenon recognizes a neutral phase and two extreme phases, El Niño (warm sea surface in the central-eastern equatorial Pacific ocean and pressures greater than average in the Indian Ocean and Australia) and La Niña (processes in the opposite direction than in El Niño years). ENSO affects atmospheric circulation systems located at remote sites on the planet (teleconnections), causing thermal and rainfall anomalies. Zhao and Yao (1989) were able to successfully predict 4 months in advance wheat FHB epidemics in eastern China, measuring the surface temperature in the central Pacific Ocean. This association was explained by the ENSO-induced advance of the summer monsoon on East Asia, increasing rainfall and FHB infection events. In southern Brazil, the increment of spring rains coupled with warm anomalies in the tropical Pacific Ocean, was associated with a higher frequency of FHB outbreaks (Del Ponte et al. 2009). The Southern Annular Mode (SAM) or Antarctic Oscillation constitutes another relevant hemispheric-scale meteorological predictor. SAM is the main mode of variability of the extra-tropical circulation in the Southern Hemisphere and is characterized by zonal-symmetric structures or annular, with geopotential height perturbations of opposite sign in Antarctica and in a surrounding zonal band centered near 45°S latitude. Several studies verified the influence of SAM on precipitation in various regions of the planet (Silvestri and Vera 2003; Reason and Rouault 2005; Gillett et al. 2006; Garreaud et al. 2008). Camilioni et al. (2005) observed an increase in the intensity and frequency of winds from the east, probably associated with the southward shift of the Atlantic Ocean subtropical anticyclone in recent decades. These studies supported the idea of calculating indices that consider the dominant meridional circulation (S-SW or N-NE), in response to the power and relative location of the southern subtropical anticyclones of the Atlantic and Pacific oceans.

The effect of many hemispheric-scale meteorological predictors on the probability of occurrence of wheat FHB incidence levels in the Pampas region was identified and quantified, calculating their Kendall correlation coefficients (r_K) and fitting logistic regression models (Moschini 2011). The Pampas region was divided in homogeneous areas in relation to temporal variation (1971–2006) of FHB incidence

values (PI %) estimated by Eq. (13.1) (empirical approach). The probabilities of occurrence of three PI % levels (severe, moderate and light to nil) were related to the following meteorological predictors (monthly up to 6-month mean values), processed previously to the occurrence of the wheat susceptible period for infection: Oceanic El Niño index (OENI: from NOAA-USA: sea surface temperature anomaly in El Niño 3.4 region); Southern Oscillation index (SOI: from NCC-Australia: differences of pressure anomalies between Tahiti and Darwin according to Troup 1965); Southern Annular Mode or Antarctic oscillation (SAM: from NERC-UK: monthly differences between surface pressure anomalies registered at six meteorological stations around 40°S and at six near 65°S, according to Marshall 2003); Meridional geopotential height index (Agph or Dgph: absolute or differences between values of gph at 1,000 hPa) (NCEP/NCAR; Kalnay et al. 1996) registered at 100 and 45°W or 60°W, around 40°S; Zonal index (ZI) using for identifying blocking action situations in 100°, 70° y 40°W; interactive effects among variables (product).

In the north-eastern quadrant of the Pampas region (Fig. 13.1), greater disease levels were expected with greater August SAM values (direct relationship, positive r_K) and dominance of meridional north-northeastern atmospheric circulation in September (inverse relationship, negative r_K for the Dgph variable: gph difference between Pacific and Atlantic anticyclones). The best logistic model (five variables) developed for the northeastern region (Model A, Table 13.1) classified correctly 72 % of total years, including the most severe FHB epidemics (1978, 1985, 1993 and 2001). For the central-eastern region, the five-variable model B (Table 13.1) reached a prediction accuracy of 83 %. In both areas, SOI only participated interacting with other variables. In accordance with these findings, maps made for the spring season by Reboita et al. (2009) showed a negative anomaly of precipitation related to a negative MAS phase for the whole Pampas region and a positive to neutral relationship for the north-eastern regional quadrant. Similarly, Silvestri and Vera (2003) found in the central-northern of the Pampas region significant positive correlations (0.35–0.45) between MAS index and precipitation anomaly values for September-October.

In the southern Pampas region, the MAS-linked index did not emerge as significant predictor, coinciding with the non-significant correlations found by Silvestri and Vera (2003) between MAS and precipitation anomaly for the bi-monthly period November-December. Predictors associated to the occurrence of blocking action situations ($ZI > 0$) in South America in 100°W and 70°W (October), were remarkable. Average ZI values in 100°W in October peaked Kendall correlation coefficient ($r_K = 0.49$). The best logistic models (Model C and D, Table 13.1) adjusted for the southern Pampas region, classified satisfactorily the most severe FHB epidemics (1976, 1977, 1985 and 2001), reaching prediction accuracies of 89 and 83 % respectively. In the southern, SOI made a significant contribution to explain the variability in disease levels. When ENSO phenomenon reaches its peak (November-December), wheat heads are in full anthesis (November; more susceptible for FHB infections), unlike northern locations (much earlier anthesis

Table 13.1 Logistic models fitted in four homogeneous areas (NE: model A; central-E: model B; S-SW: model C and SE: model D) of the Pampas region for estimating the probabilities of occurrence of severe (*Sv*), moderate (*M*) and light to nil (*L*) epidemics, based on hemispheric-scale meteorological predictors. *PA* prediction accuracy

	Model equations[a]	PA %
A	***LogitPrSv*** = −14.79 + 0.76 ***SAMa*** + 0.09 ***Agph1s*** − 0.104 ***Dgph2s*** − 0.001 ***It1*** − 0.015 ***It2***	72
	LogitPrMc = −12.25 + 0.76 ***MASa*** + 0.09 ***Agph1s*** − 0.104 ***Dgph2s*** − 0.001 ***It1*** −0.015 ***It2***	
B	***LogitPrSv*** = −2.04 + 0.75 ***SAMa*** − 0.08 ***Dgph2s*** − 0.11 ***ZI7015s*** − 0.006 ***It3*** + 0.0013 ***It4***	83
	LogitPrMc = −0.0024 + 0.75 ***SAMa*** − 0.08 ***Dgph2s*** − 0.11 ***ZI7015s*** − 0.006 ***It3*** + 0.0013 ***It4***	
C	***LogitPrSv*** = −4.12 − 0.54 ***SOImjj*** + 0.44 ***SOIjas*** + 0.155 ***ZI10030o***	89
	LogitPrMc = −2.31 − 0.54 ***SOImjj*** + 0.44 ***SOIjas*** + 0.155 ***ZI10030o***	
D	***LogitPrSv*** = −1.96 + 0.15 ***ZI7030o*** + 0.18 **ZI10030o** + 0.015 ***It5***	83
	LogitPrMc = 0.29 + 0.15 ***ZI7030o*** + 0.18 **ZI10030o** + 0.015 ***It5***	

[a]LogitPrSv = ln(PrSv/1-PrSv); LogitPrMc = ln(PrMc/1-PrMc). Solving the expressions Exp (LogitPrSv)/[1 + Exp (LogitPrSv)] and Exp(LogitPrMc)/[1 + Exp (LogitPrMc)], PrSv values (probability of observing a severe epidemic (*Sv*) and PrMc (cumulative probability of observing an epidemic => to moderate (*M*)). Ln: natural logarithm. PrM = PrMc-PrSv. PrL = 1-(PrSv + PrM), being PrL the probability of observing a light to nil epidemic (L). ***SAMa***: average value of the Southern Annular Mode (*SAM*) for August. ***Agph1s***: September (s) value of 1,000 hPa geopotential height (*gph*) in the point 40°S–100°W. ***Dgph2s***: difference between September values of 1,000 hPa gph of the points 40°S–100°W and 40°S–60°W. **It1 = Dgph1a*Dgph2s**, being ***Dgph1a*:** difference between August values of 1,000 hPa gph of the points 40°S–100°W and 40°S–45°W; ***It2 = Dgph1s*SAMja***; being Dgph1s: difference between September values of 1,000 hPa gph of the points 40°S–100°W and 40°S–45°W and ***SAMja***: average value of the SAM for July-August.; ***It3 = Dgph2s*SOIa***, being ***SOIa*** the Southern Oscillation index (*SOI*) for August**;** ***It4 = Dgph2s*ZI7015s***, being ***ZI7015s:*** average of the first September 15-d zonal index values calculated at 70°W by ZI = U30°S + U60°S − 2 U45°S (U: zonal wind at 500 hPa). ***SOImjj*** and ***SOIjas***: average values of the SOI for May–June–July and July–August–September respectively. ***ZI10030o; ZI7030o*:** average of the first October 30-d zonal index values calculated at 100°W and at 70°W by ZI equation, respectively. ***IT5 = SOIas*ZI10030o***, being ***SOIas***: average values of the SOI for August–September

stage). In accordance, the probability of occurrence of dry periods (derived from conditional probabilities of rain: first order Markov chains and seasonal trend) was up to 30 % lower in El Niño years than in those with La Niña episodes, concentrating this difference in late spring (Moschini et al. 1997). In the same sense, Tanco and Berri (1996) reported that in November-December more than 60 % of the Pampas region registered rainfalls below normal in years with La Niña events. Consistent with these results, Grimm et al. (2000) showed that during the late winter (trimester August-October) precipitation coincided with the median in the central-northern Pampas region for years with El Niño phase, unlike southern regional areas with above-median precipitation in the spring trimester (October-December).

13.3.4 Assessing Climate Change Impacts on Fusarium Head Blight

Currently, one of the major challenges is to predict the variations in plant pathosystems in response to anthropogenic greenhouse gas-induced climate change. Assessing the most likely climate change impacts on pathosystems can be made by a retrospective analysis, which identify fingerprints related to climate change in long-term disease observations, or by using mathematical or statistical models. Time series containing standardized disease records are unavailable for the majority of pathogens. In case of having, trends are confounded by changes in management and biological factors over time. When using predictive disease models, major constraints are originated in the uncertainty of the input variables (general circulation climate model-based), the difficulty in estimating biological responses in the presence of nonlinearities and thresholds and the high probability of host-pathogen genetic adaptation to man-induced atmospheric change (Scherm 2004).

Keeping the previous constraints in mind, retrospective and prospective studies were undertaken to analyze climate change effect on wheat FHB in the Pampas region. For both approaches, FHB incidence values were estimated by a simple weather-based model (Eq. 13.2: $\boldsymbol{PI}\ \% = -9.15 + 6.47\ \boldsymbol{ND} + 0.35\ \boldsymbol{pDD}$, being ND: number of days with simultaneous occurrence of Pr and thermal amplitude <7 °C; pDD: results of accumulating residuals >9 °C in nT, in those days where xT and nT are <25 °C and ≥ 9 °C, respectively). Long-term retrospective FHB incidence predictions (1931–2010) and weather variable (***ND*** and ***pDD***) values were analyzed for Paraná (western Entre Rios province), Pergamino (northern-eastern Buenos Aires province) and Mar del Plata (southern Buenos Aires province). According to Fig. 13.4, the trend lines showed a light increase of FHB incidence over time, with positive slopes larger towards southern Pampas region (Paraná: 0.08 %, Pergamino: 0.16 % and Mar del Plata: 0.24 % per year). In accordance, the largest slope of the trend lines fitted to the variable ***ND*** was presented by Mar del Plata (0.031), comparing with Pergamino (0.019) and Paraná (0.006). The trend lines for the thermal variable (***pDD***) showed positive slopes, being Parana the site with the maximum slope. As it was concluded earlier, in the southern Pampas region (Mar del Plata) 67 and 75 % of years with El Niño and La Niña episodes observed predicted FHB incidence values above and below median, respectively. ENSO phenomenon influence was gradually less clear towards the northern. It is worth pointing out the decline of the FHB incidence levels in the last 9 years, which could indicate that the climate system is still in transition to a new equilibrium, driven by anthropogenic changes in radiative forcing.

Climate change impact on FHB in the Pampas region was also assessed following a prospective approach. FHB incidence values were estimated by Eq. (13.2) using meteorological data from the future (2071–2100), predicted by PRECIS (Providing Regional Climates for Impact Studies) under the medium emission SRES IPCC A2 scenario (Marengo et al. 2009), and from the baseline climate (1961–1990). The anomaly map (future – baseline climate) (Fig. 13.5) showed positive values in southern Buenos Aires province, suggesting an increase in the number of years with moderate to severe FHB incidence under the future climate scenario.

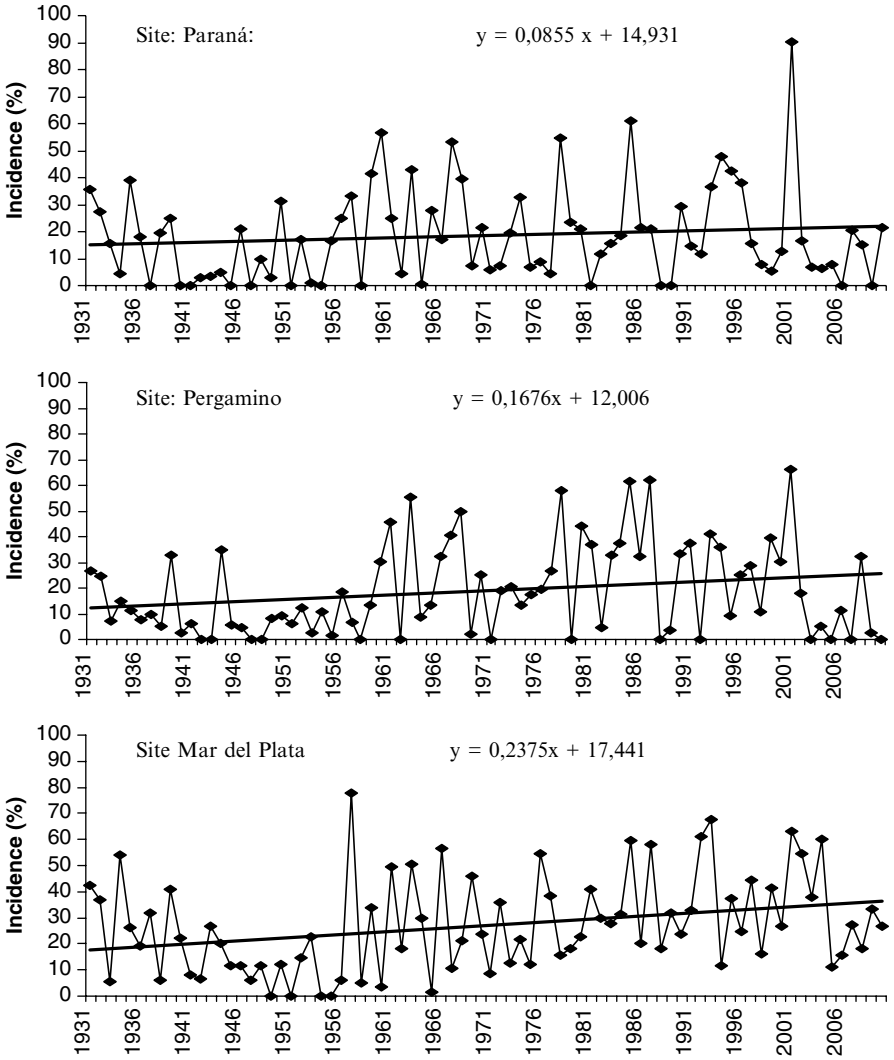

Fig. 13.4 Trend lines of FHB incidence values estimated by Eq. (13.2) (1931–2010) in three sites of the Pampas region

13.3.5 Assessing Fusarium Head Blight Risk in the Pampas Region

Multiple inoculation episodes in areas with moderate and severe outbreaks suggest that multiple infections contribute to cumulative head blight severity. This fact affects prophylactic disease management options (Francl et al. 1999). There is no

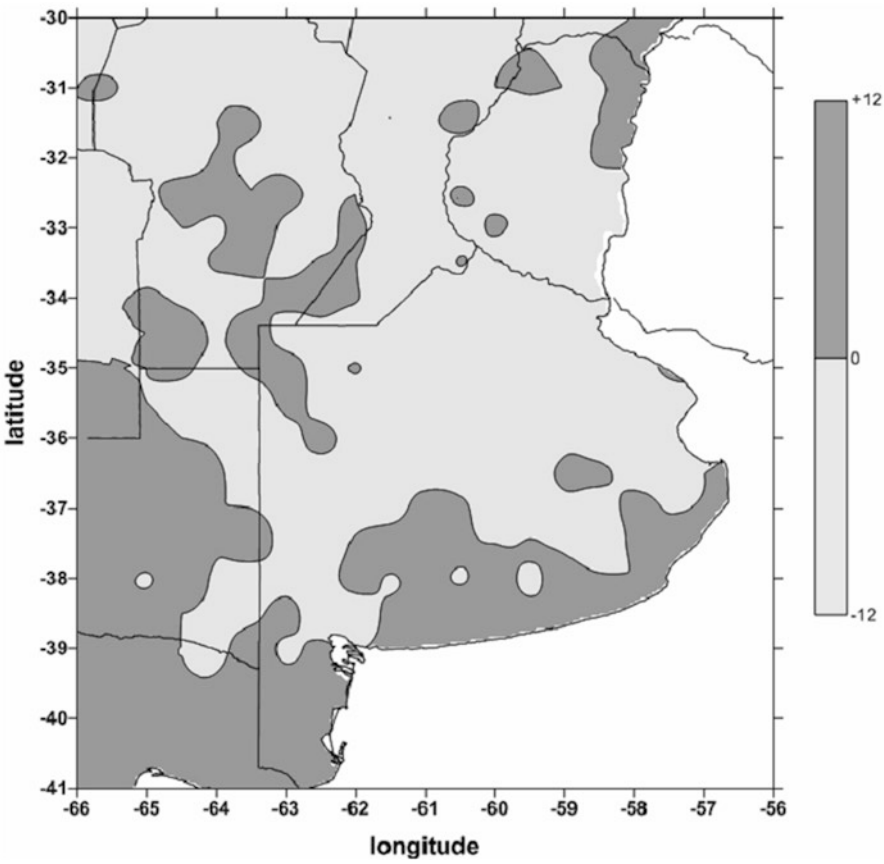

Fig. 13.5 Anomaly map: difference between the number of years with moderate or severe predicted FHB incidence (Eq. 13.2) using future data (2071–2100) and recent past weather data (1961–1990), in the Pampas region

effective fungicide treatment once the wheat head is infected and colonized. Fungicides with *Fusarium* active ingredients, such as the triazoles, are recommended to be applied prior to infection (preventive treatment) or at maximum 1–2 day post-infection (Annone 2003).

The weather-based model (Eq. 13.1) for predicting FHB incidence has limitations in establishing the stepwise evolution of the epidemic and evaluating the intensity of each infection event. Its meteorological variables are calculated after finishing the entire wheat susceptible period for infection, when the impact of having the estimated disease incidence is not useful for making chemical control decisions. However, González Montaner (2004) explained (southern Pampas region) that disease control decisions are taken when at least two FHB infection periods (defined by the variable ***NP*** of Eq. 13.1) are detected, complemented by the occurrence of

average relative humidity above 70 and 80 % in the 10 days prior to anthesis, for durum and bread wheat respectively. Similarly, Mazzilli et al. (2011) concluded in Uruguay that at least two infection periods (variable ***NP***) are needed for FHB epidemics. Following the criterion of no fungicide sprays until two infections events occur, chemical control was avoided in 22 out of 28 flowering dates analyzed (2003–2006), with infection levels similar to those wheat samples sprayed with fungicide at early flowering.

Conclusions derived from the fundamental-empirical FHB forecasting system can be used to assist producers in disease control measures to be employed. From the start of the wheat susceptible period for infection (onset of wheat heading), weather monitoring can detect each FHB infection event and the corresponding predicted FHB index values, in order to make fungicide spray decisions. Since 2005–2006 wheat growing season, a system for assessing FHB risk was implemented for the Pampas region (divided in three sub-regions: northern, central and southern). The system, three times a week (Monday, Wednesday and Friday), updates daily meteorological data from 45 standard weather stations for running predictive FHB models (empirical and fundamental-empirical approaches). Also, a specific short range weather forecast is elaborated. After analyzing all together (weather forecast and disease model outputs), comments and maps showing the spatial distribution of the three possible FHB risk grades (severe: red, moderate: yellow and/or light: green), are presented at the web page: http://climayagua.inta.gob.ar

13.3.6 Estimating Spatial Distribution of Fusarium Head Blight Incidence Using Land and Remote Sensing Data

Disease estimates from weather-based models are usually reduced to punctual data because weather station data are used as inputs. To generate disease maps, these results need to be taken to an extensive area through interpolation methodologies. As a consequence, estimated values in sites where weather station data are not available contain additional uncertainties. This is clearly observed in areas where weather station network is sparse and irregularly distributed (Solis Villagran and Flores Garnica 2003). Hence, the inclusion of remote sensing data into disease models could be a solution. Remote sensing is a multiple purpose tool with many important specific applications such as regional estimation of precipitation amount/occurrence and surface temperature, evaluation of natural resources and land cover classification (Fattorelli et al. 1995).

In order to improve the estimated spatial distribution of FHB incidence in the Pampas region, Sepulcri (2010) and Sepulcri et al. (2010) included satellite data in the calculation of disease incidence by Eq. (13.1) (empirical approach). The spatial distribution of model-based FHB incidence values using only land weather station network data (Fig. 13.6a) was compared with those using land and satellite data. Firstly, precipitation occurrence data from satellite Tropical Rainfall Measurement Mission (TRMM, 3B42 product), previously tested against pluviometer records

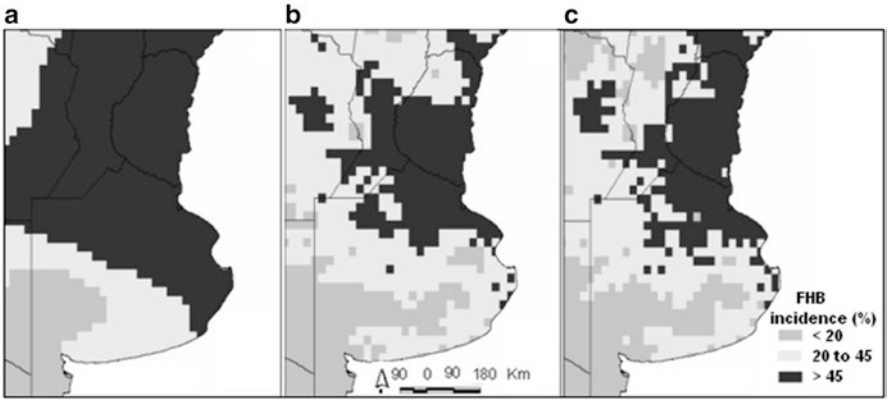

Fig. 13.6 Spatial distribution of FHB incidence values estimated by Eq. (13.1) (classified in percentage categories: >45 %, 20–45 % and <20 %), using only land weather station network data (**a**), land and precipitation satellite data (**b**) and land and precipitation-temperature satellite data (**c**) for 2001 wheat growing season (mean heading date)

(85 % accuracy), combined with interpolated temperature and relative humidity weather station data were used for estimating FHB incidence by Eq. (13.1) (Fig. 13.6b). The product 3B42 provides precipitation estimations every 3 h with a spatial resolution of 0.25º, for all the longitudinal range and from 50ºN to 50ºS latitude (Huffman et al. 2007). Secondly, it was added temperature data obtained from a climatic zoning based on NOAA-AVHRR images, together with satellite (TRMM) precipitation estimates and interpolated relative humidity station data (Fig. 13.6c). Evaluations with field data suggested that when the Eq. (13.1) used satellite data, FHB incidence was represented in a reliable way, mainly in data-sparse areas.

13.4 Conclusions

From simple daily meteorological elements (maximum and minimum temperatures, precipitation and relative humidity), FHB intensity and grain DON content values can be satisfactorily estimated by forecasting systems (empirical and fundamental-empirical approaches) developed/validated in the Pampas region. Retrospective and real time model outputs were crucial to address many applications, previously described.

Nevertheless, due to the inherent variability accompanying the precise definition of each disease pyramid component, it should be noted the lack of mathematical precision in the estimates of the disease forecasting systems. In relation to the pathometry, it was often found that FHB incidence values observed from samples collected from a particular field crop presented high variability, complicating their contrasts with predicted values. Also in already cited controlled environment

studies with artificial inoculation, it was pointed out that local *Fusarium* strains showed greater aggressiveness and adaptation to lower temperatures (Zoldan 2008; Martinez et al. 2012) than the strain used by Andersen (1948). In respect to the host, McMullen et al. (1997) indicated that wheat is susceptible to disease from anthesis until soft dough stage. This susceptible period for infection is highly variable among wheat cultivars, due to head characteristics, and years, in response to prevailing weather conditions. De Souza and Formento (2004) and Reis (1989) described different field crop anthesis progress curves in Paraná (Pampas region) and Passo Fundo (southern Brazil), responding to the wheat cultivar-environment interactive system. From this fact, inaccuracies in model-based predictions (fundamental-empirical approach) may be inferred because a unique model was fitted to the anthesis progress curve observed in a particular wheat cultivar and one growing season. Finally, weather-based forecasting systems were developed under the assumption of non-limiting inoculum and lack of response to crop rotation, due to the marked ubiquity of *Fusarium* species, wide range of hosts and high anemophilous spore dissemination (Reis and Carmona 2002). It was also assumed that the effects of wheat cultivar behavior regarding the disease and cultural practices such as tillage systems do not play a significant role for explaining the observed disease variability (Schaafsma et al. 2001; Lori et al. 2009).

References

Alessandro AP (2003) The influence of blocking events on temperature and precipitation in Argentina during the 1990s. Meteorológica 28(1 y 2):39–52

Andersen AL (1948) The development of *Gibberella* zeae head blight of wheat. Phytopathology 38:599–611

Annone JG (2003) Particularidades del control químico de la FET. Seminario: problemas asociados a la Fusariosis en trigo y estrategias para su prevención. Bolsa de Cereales de Buenos Aires, Argentina

Baker KM, Kirk WW (2007) Comparative analysis of models integrating synoptic forecast data into potato late blight risk estimate systems. Comput Electron Agric 57:23–32

Bjerknes J (1969) Atmospheric teleconnections from the equatorial Pacific. Mon Weather Rev 97:163–172

Bourke PMA (1970) Use of weather information in the prediction of plant disease epiphytotics. Annu Rev Phytopathol 8:345–370

Camilioni I, Barros V, Escobar G, Di Luca A (2005) Tendencias en la posición del Anticiclón del Atlántico Sur y su representación por modelos climáticos globales: impactos sobre el estuario del Río de la Plata y océano adyacente. IXCongreso Argentina de Meteorología Buenos Aires Argentina

Carranza MR, Lori GA, Sisterna MN (2002) Fusaria involved in the head blight complex of wheat in Argentina. Fitopatología 37:164–168

Carranza MR, Moschini RC, Kraan G, Bariffi JH (2007) Examination of meteorology-based predictions of Fusarium head blight of wheat grown at two locations in the southern Pampas region of Argentina. Aust Plant Pathol 36:305–308

De Souza J, Formento N (2004) Estudios de antesis en trigo y su relación con la Fusariosis (*Fusarium graminearum y Fusarium* spp.) VI Congreso Nacional de Trigo. Bahía Blanca Argentina

Del Ponte EM, Fernandes JMC, Pavan WA, Baethgen WE (2009) A model-based assessment of the impacts of climate variability on Fusarium Head Blight seasonal risk in southern Brazil. J Phytopathol 157:675–681

Faifer GC, Zabal O, Godoy HM (1992) Further studies on the hematopoietic damage produced by a single dose of T-2 toxin in mice. Toxicology 75:169–174

Fattorelli S, Casale R, Borga M, Da Ros D (1995) Integrating radar and remote sensing techniques of rainfall estimation in hydrological applications for flood hazard mitigation. The European contribution: perspectives and prospects. Rue de la Loi B-1049, Brussels, Bélgica

Franc L, Shaner G, Bergstrom G, Gilbert J, Pedersen W, Dill-Macky R, Sweets L, Corwin B, Jin Y, Gallenberg D, Wiersma J (1999) Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis 83:622–666

Garreaud RD, Vuille M, Compagnucci R, Marengo J (2008) Present-day South American climate. PALAEO3 Spec Issue (LOTRED South America) 281:180–195. doi:10.1016/j.palaeo.2007.10.032

Gillett NP, Kell TD, Jones PD (2006) Regional climate impacts of the Southern Annular Mode. Geophys Res Lett:L23704. doi:10.1029/2006GL027721

González MJ (2004) Avances en el control de enfermedades en trigo. A Todo Trigo, un Congreso para todos Mar del Plata, Argentina, pp 43–54

Grimm AM, Barros VR, Doyle ME (2000) Climate variability in southern South America associated with El Niño and La Niña events. J Clim 13:35–58

Hooker DC, Shaafsma AW, Tamburic-Ilincic L (2002) Using weather variables pre-and post-heading to predict deoxynivalenol content in winter wheat. Plant Dis 86:611–619

Huffman GJ, Adler RF, Bolvin DT, Gu G, Nelkin EJ, Bowman KP, Hong Y, Stocker EF, Wolff DB (2007) The TRMM Multisatellite precipitation analysis (TMPA): quasi-global, multiyear, combined-sensor precipitation estimates at fine scales. J Hydrometeorol 8:38–55

Kalnay EM, Kanamitsu M, Kistler R, Collins W, Deaven D, Gandia L, Iredell M, Saha S, White G, Woollen J, Zhu Y, Chelliah M, Ebisuzaki W, Higgins W, Janowiak J, Mo KC, Ropelewski C, Wang J, Leetmaa A, Reynolds R, Jenne R, Joseph D (1996) The NCEP/NCAR 40 year reanalysis project. Bull Am Meteorol Soc 77:437–471

Lacey J, Bateman GL, Mirocha CJ (1999) Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. Ann Appl Biol 134:277–283

Lori GA, Sisterna MN, Sarandón SJ, Rizzo I, Chidichimo H (2009) Fusarium head blight in wheat: impact of tillage and other agronomic practices under natural infection. Crop Prot 28:495–502

Lund IA (1963) Map pattern classification by statistical methods. J Appl Meteorol 2:56–65

Marengo JA, Jones R, Alves LM, Muniz L, Valverde M (2009) Future change of temperature and precipitation extremes in South America as derived from PRECIS regional climate modeling system. Int J Climatol. doi:10.1002/joc.1863

Marshall GJ (2003) Trends in the Southern annular mode from observations and reanalyses. J Clim 16:4134–4143

Martínez MI, Moschini RC, Barreto D, Comerio R (2012) Effect of environment on Fusarium head blight intensity and deoxynivalenol content in wheat grains: development of a forecasting system. Cereal Res Commun 40:74–84

Mazzilli S, Pérez C, Ernst O (2011) Una alternativa para optimizar el uso de fungicidas para controlar fusariosis de espiga en trigo. Agrociencia 15:60–68

McMullen M, Jones R, Gallemberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Moschini RC (2011) Desarrollo y uso de sistemas de pronóstico de epidemias de la Fusariosis de la Espiga de Trigo (*Triticum aestivum L.*) para identificar situaciones sinópticas y predictores meteorológicos en diferentes escalas asociados a la enfermedad en la región pampeana. Tesis Doctorado Universidad de Buenos Aires, Argentina

Moschini RC, Fortugno C (1996) Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur J Plant Pathol 102:211–218
Moschini RC, Casagrande G, Vergara G, Conti HA (1997) Efectos del ENSO sobre las probabilidades de períodos secos derivadas de modelos markovianos de primer orden, en La Pampa. Rev Facultad de Agronomía 17:71–76
Moschini RC, Pioli R, Carmona MA, Sacchi O (2001) Empirical predictions of wheat head blight in the northern Argentinean Pampas region. Crop Sci 41:1541–1545
Moschini RC, de Galich MTV, Annone JG, Polidoro O (2002) Enfoque Fundamental-Empírico para estimar la evolución del Indice de *Fusarium* en trigo. RIA 31:39–53
Moschini RC, Carranza MR, Carmona M (2004) Meteorological-based predictions of wheat head blight epidemic in the southern Argentinean pampas region. Cereal Res Commun 32:45–52
Moschini RC, Bischoff S, Martínez MI (2008) Variabilidad climática y enfermedades. Caso de estudio: Fusariosis de la espiga de trigo. Horizonte A. Magazine de las Ciencias Agrarias. Año 5(21):10–15
Moschini RC, Castellarín JM, Martínez MI, Ferraguti F (2012) Análisis de la Fusariosis de la espiga de trigo en Oliveros en la campaña 2011/12. Sistemas de pronóstico basados en variables meteorológicas para estimar su distribución anual y el riesgo climático en la región pampeana. Serie Para Mejorar la Producción. Cultivos Invernales (47) EEA Oliveros, pp 49–54
Paul PA, Lipps PE, Madden LV (2005) Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol content in harvest what grain: a meta-analysis. Phytopathology 95:1225–1236
Raposo R, Wilks DS, Fry WE (1993) Evaluation of potato late blight forecasts modified to include weather forecasts: a simulation analysis. Phytopathology 83:103–108
Reason CJC, Rouault M (2005) Links between the Antarctic oscillation and winter rainfall over western South Africa. Geophys Res Lett 32, L07705. doi:10.1029/2005GL022419
Reboita MS, Ambrizzi T, Da Rocha RP (2009) Relationship between the southern annular mode and southern hemisphere atmospheric systems. Rev Bras Meteorol 24:48–55
Reis EM (1989) Fusariosis: biología y epidemiología de *Gibberella zeae* en trigo. In: Kholi M (ed) Taller sobre Fusariosis de la espiga en América del Sur. CIMMYT, Mexico, pp 97–102
Reis EM, Carmona M (2002) Fusariosis del trigo. Biología, epidemiología y estrategias para su manejo, 1°Edición. Buenos Aires, p 25. ISBN: 98743–3959
Scherm H (2004) Climate change: can we predict the impacts on plant pathology and pest management? Can J Plant Pathol 26:267–273
Sepulcri G (2010) Predicción de la Fusariosis de la espiga de trigo a partir de modelos que incorporan información satelital. Tesis Magister área Producción Vegetal, Universidad de Buenos Aires, Argentina
Sepulcri G, Moschini RC, Di Bella CM (2010) Estimación de Fusariosis incorporando información satelital. Horizonte A. Magazine de las Ciencias Agrarias. Año 6(30):32–33
Shaafsma AW, Tamburic-Ilinic L, Millar JD, Hooker DC (2001) Agronomic consideration for reducing DON in wheat grain. Can J Plant Pathol 23:279–285
Silvestri GE, Vera CS (2003) Antarctic Oscillation signal on precipitation anomalies over southeastern South America. Geophys Res Lett. doi:10.1029/2003GL018277
Snijders CHA, Krechting CF (1992) Inhibition of deoxynivalenol translocation and fungal colonization in Fusarium head blight resistant wheat. Can J Bot 70:1570–1576
Solis VZ, Flores GJG (2003) Análisis comparativo de técnicas de interpolación en la estimación de la variación espacial de factores en una cuenca hidrográfica. XIII Reunión Nacional SELPER Puerto Vallarta México DF
Tanco R, Berri GJ (1996) Acerca del efecto del fenómeno El Niño sobre la precipitación en la Pampa Húmeda Argentina. Actas del VII Congreso Argentino y VII Congreso Latinoamericano e Ibérico de Meteorología Bs As Argentina
Vincelli PC, Lorbeer JW (1988) Relationship of precipitation probability to infection potential of *Botrytis squamosa* on onion. Phytopathology 78:1078–1082
Walker GT, Bliss EW (1932) World weather V Mem. Royal Meteor Soc 4:53–84

Xu XM, Monger W, Ritieni A, Nicholson P (2007) Effect of temperature and duration of wetness during initial infection periods on disease development, fungal biomass and mycotoxin concentrations on wheat inoculated with single, or combinations of *Fusarium* species. Plant Pathol 56:943–956

Zhao S, Yao C (1989) On the sea temperature prediction models of the prevailing level of wheat scab. Acta Phytopathol Sin 19:229–234

Zoldan SM (2008) Regioes de risco, caracterizacao da antese em cereais de inverno e sistema de alerta para a Giberela, em trigo. Tesis Doctor en Agronomía área de Fitopatología, Universidad de PassoFundo, Brazil

Part VI
Resistance

Chapter 14
Genetic Resistance to Fusarium Head Blight in Wheat (*Triticum aestivum* L.). Current Status in Argentina

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Abstract Fusarium Head Blight (FHB) caused by *Fusarium graminearum* is a common and devastating disease of hexaploid wheat (*Triticum aestivum* L.) in all temperate growing regions of the world, including Argentina. Host resistance has been considered a highly economical and efficient way to manage the disease, however, progress of breeding for FHB resistance has been limited because of the lack of effective resistance to FHB and the complex inheritance of the partial resistance currently available in wheat. First part of this chapter deals with the impact of FHB in bread and durum wheat from Argentina, as well as the early efforts to improve for FHB resistance in bread wheat. The second part addresses some examples of successful introgression of the locus*Fhb1* (the best validated gene for FHB resistance from Sumai 3) into local backgrounds by marker assisted selection, and more briefly, the recent progress in the identification of alternative sources of FHB, future projects for QTL mapping, and the development of molecular tools for marker assisted selection.

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14.1 Introduction

Fusarium Head Blight (FHB) caused by *Fusarium graminearum* Schwabe, teleomorph *Gibberella zeae* (Schw.) is a common and devastating disease in bread wheat (*Triticum aestivum* L.) affecting growing regions where flowering and early grain filling phases are coincident with humid and warm-temperate weather conditions. Infected spikelets are killed, and the fungus then may girdle the rachis so that the head dies above that point. A distinct salmon-pink ring of fungus develops at the base of the glumes. Damage from FHB or scab includes shrunken and discoloured (pink or chalky white "tombstone") kernels, reductions in yield and seed quality, and toxin contamination produced by presence of deoxynivalenol, also known as DON or vomitoxin. These factors also reduce test weight, lower market grade (Trigo-Stockli et al. 1998) and threaten animal and human health. Presence of DON has been associated with vomiting and immunosuppression in humans and swine (Pettigrew et al. 2010). Some of the most common effects of prolonged dietary exposure of experimental animals to DON are decreased weight gain, anorexia, decreased nutritional efficiency and altered immune function with species differences (Pestka 2007). It should also be mentioned that several species can cause head blight, although *F. graminearum* is the predominant pathogen in most regions of the world. Good examples are *F. verticillioides* and *F. proliferatum* producing fumonisins. Several studies worldwide have reported fumonisins in different crops susceptible to *Fusarium* spp., including wheat and maize, rice, sorghum, barley and black tea, among others (Sutton 1982; Abbas et al. 1998; Leslie et al. 1990; Goswami and Kistler 2004; Omurtag and Yazicioglu 2004). Presence of fumonisins has been associated in animals with equine leukoencephalomalacia (ELEM) and porcine pulmonary edema (Ross et al. 1990). In the case of humans, studies on the prevalence of esophageal cancer in regions of South Africa and China revealed an association between this disease and the consumption of corn contaminated by *Fusarium* spp. (Rheeder et al. 1992; Chu and Li 1994).

14.2 Fusarium Head Blight Outbreaks in Argentina

The wheat growing area in Argentina is known as Pampas region and includes nearly 5,000,000 ha distributed in five provinces with different agro-ecological conditions: Buenos Aires, Córdoba, Santa Fe, Entre Ríos and La Pampa. Latitude, temperature and susceptible rotational crops (particularly maize) influence the pathogen distribution, and frequency and timing of spring rainfall appear to regulate disease outbreaks (Kikot et al. 2011). Severe FHB outbreaks have been reported in years 1945–1946, 1978, 1985, 1993 and 2001, with yield losses varying among zones, but with an estimated average between 20 and 30 % (Galich 1997; Formento and Firpo 2003). In 1993, the highest estimated losses reached 50 % in areas of no-till over maize stubble. The extent of the damage was magnified by a considerable loss in the trading value of the grain evidenced by a low test weight caused by

presence of scabby grains, combined by the threat for food and feed mycotoxin contamination (Galich 1997). More recently, Ramírez et al. (2006) analyzed *F. graminearum* isolates from bread wheat obtained in three locations mainly at North West of Buenos Aires Province (32–33°S, 60–62°W), and observed that 90 % of tested samples produced the toxin deoxynivalenol.

Severe FHB epidemics also occurred in durum wheat (*Triticum dicoccoides* var. durum) at the South East of Buenos Aires Province -the main durum wheat producing region in Argentina- during 1963, 1976, 1978 and 1985, with crop losses as high as 70 %. Consequently, and due to other contributing factors, the durum wheat planting area was reduced and replaced with bread wheat (which is more resistant to FHB) to production levels lower than the home market demand (Galich 1989). Lori et al. (2003) analyzed samples from 20 adapted durum wheat cultivars and breeding lines obtained in five locations in the Southeast region of Buenos Aires Province (37–39° S, 57–64°W) and detected different levels of mycotoxin contamination (deoxynivalenol, from 0 to 8 ppm) and *F. graminearum* infection (0–42 %). In this study differences in *F. graminearum* infection were more associated with environment than with cultivar. More recently, Palacios et al. (2011) observed that durum wheat samples collected during two consecutive not-FHB epidemic years in Argentina, showed presence of *Fusarium* spp. different from *F. graminearum* (mainly *F. proliferatum*) and natural contamination with fumonisins in grain.

Unfortunately, crop management and chemical measures are only partly effective to control the disease. Therefore, the cultivation of varieties with improved disease resistance plays a key role as strategy to reduce the incidence of FHB in the production of wheat and to prevent mycotoxins contamination in grain.

14.3 Genetic Control of the Disease

Host resistance has been considered a highly economical and efficient way to manage the disease. However, progress of breeding for FHB resistance has been limited because of the lack of effective resistance to FHB and the complex inheritance of the partial resistance currently available in wheat. Currently, most important sources of partial resistance to FHB identified in bread wheat and widely used in breeding programs, come from Asia (Sumai 3 and derivatives), Brazil (Frontana) and winter wheat (Arina). More detailed information about the genetic and molecular basis of main sources of FHB resistance in wheat can be obtained in Buerstmayr et al. (2009).

In Argentina, first efforts for the development of wheat germplasm with improved genetic resistance to FHB combined with good agronomic traits were reported in Galich (1997) and more recently in Galich (2004). In these studies, sources of FHB resistance were identified under controlled conditions through artificial inoculations using local isolates of *F. graminearum.* FHB resistance from superior cultivars and/or breeding lines was incorporated into genotypes with good agronomic traits using a scheme of recurrent selection. Table 14.1 shows foreign and local sources of FHB resistance validated with artificial inoculations.

Table 14.1 Sources of FHB resistance identified through artificial inoculations with local isolates of *F. graminearum*[a]

Introductions with high resistance	
China 7	Pekin 8
Fan 1	Shanghai #5
Ning 8343	Sumai 3
Nobeoka Bozu	Suzhoe #6
Nyubai	YMI #6
Germplasm from CIMMYT and South America	
Catbird	Pampeano
CEP 75203	Pel 73101
Chum/Seri	Pel 74142
Frontana	PF 7815
Kvz/3/Tob/Cfen//Bb/4/Blo/F35.70/Mo/Nac/6/Bow	WRM/Ptm//Coc/Ning 68026
Kvz-K 4500 L.A.4 OC 813	
Old and modern local cultivars	
Biointa 2005	Klein Pegaso
Buck Charrúa	La Paz INTA
Cargill Trigal 706	Las Rosas INTA
Don Mario Cronox	Prointa Federal
Klein Atlas	Prointa Granar
Klein Cacique	Prointa Molinero
Klein Orión	Vilela Sol

[a]Based on Galich (2004) with modifications

This type of evaluation is performed every year in Estación Experimental Agropecuaria (EEA) Marcos Juárez, which belongs to the Instituto Nacional de Tecnología Agropecuaria (INTA) with foreign accessions, local cultivars from private breeding programs and cultivars and advanced breeding lines from the INTA National Wheat Breeding Program, in order to identify, validate and/or select sources of FHB resistance (Fig. 14.1).

14.4 Introgression of Fusarium Head Blight Resistance by Marker Assisted Selection

During the past 15 years, numerous studies have been published on molecular mapping of FHB resistance in wheat. Buerstmayr et al. (2009) summarized in three main ideas the relevant findings from 73 research articles focused on quantitative trait loci (QTL) mapping, marker-assisted selection, and germplasm evaluation: (1) QTL for FHB resistance were found on all wheat chromosomes except chromosome 7D (2 years later Li et al. (2011) discovered a main QTL for FHB resistance in 7D from a Chinese landrace) corroborating the complex inheritance of this trait; (2) some QTL were found in several independent mapping studies indicating that

Fig. 14.1 Heads of F7-BC2 lines obtained from the hybridization Prointa Granar/Sumai 3 artificially infected with FHB during 2012 at Marcos Juárez (Argentina). From *right* to *left*, progressive FHB severity in heads expressed as prematurely whitening of glumes and awns

such QTL are stable and therefore useful in breeding programmes; (3) within this group, the most validated QTL for FHB (and widely used in breeding programs) is *Fhb1* on chromosome 3BS from Sumai 3.

This major QTL was first tagged by RFLPs and referred as*Qfhs.ndsu-3BS* (Waldron et al. 1999). Secondly, Anderson et al. (2001) tagged *Qfhs.ndsu-3BS* using the breeder- friendly markers SSRs*Xgwm533* and *Xgwm493*within a 4.8 cM gap. These markers have been widely used in marker assisted selection (MAS) programs; however, they still define a relatively large chromosome interval for the QTL. *Qfhs.ndsu-3BS* was later re-named as *Fhb1* (Liu et al. 2006), and more recently, Bernardo et al. (2011) saturated the 4.8 cM gap with seven SNPs useful for MAS and future cloning of *Fhb1* resistance gene.

To our best understanding, the first hybridizations in Argentina to introgress *Fhb1* into adapted background by MAS, were made by 2004 at the Instituto de Recursos Biológicos (IRB) INTA Castelar. Local cultivars with high yield potential-Prointa Puntal, Prointa Oasis, Biointa3000-, and with good performance against FHB -Prointa Granar (Table 14.1)-were used as genetic backgrounds and Sumai 3 as *Fhb1* donor. Locus *Fhb1* was traced with SSRs *Xgwm533* and *Xgwm493* using standards procedures of PCR amplification and detection (Silvina Lewis IRB Castelar, personal communication). During 2006 four F2-BC2 (F2 from the second backcross generation) populations segregating for *Fhb1* were planted for identification of *Fhb1* homozygous individual plants using SSRs *Xgwm533* and *Xgwm493* (as before) at the Laboratory of Biotechnology of the INTA EEA Marcos Juárez. *Fhb1* homozygous F3-BC2 (50 plants) and F4-BC2 (200 plants) were advanced as

bulk populations (one per background) at the Experimental Field of INTA EEA Marcos Juárez in 2007–2008. In 2009, F5-BC2 individual plants from Prointa Puntal, Prointa Oasis, Biointa3000 and Prointa Granar backgrounds were inspected and selected on the basis of general agronomic appearance. About 30 F6-BC2 head rows per background were planted in Marcos Juárez in June 2010 as single 1-m rows. In 2011 24–28 F7-BC2 lines per background were advanced to non-replicated observation plot trials at Marcos Juárez (5 m long, 6 rows wide). In 2012, three lines from Prointa Puntal, 27 from Prointa Oasis, four from Biointa 3000 and 19 lines from Prointa Granar backgrounds were advanced to the Preliminary Yield Trials (PYT) at Marcos Juárez on the basis of different traits evaluated during 2011 like grain yield, general agronomic appearance and performance against FHB natural infection occurred at Marcos Juárez. The experimental design of PYT trial used a 10×9 alpha lattice design with two replications and plot sizes as before. It is worth mentioning that months of September and October 2012 showed rainfall and temperature conditions conducive to scab development in Marcos Juárez with outstanding performances of lines derived from Prointa Granar and, in a slightly lower degree, lines derived from Prointa Oasis (Fig. 14.2a, b, respectively). Differences in heading time between tested materials were 3 and 1 day (see legend of Fig. 14.2 for detail).

Additionally, based on excellent yield potential, two lines with Prointa Oasis background were advanced to the Regional Yield Trials (RYT) 2012, grown in the provinces of Buenos Aires (five locations), Córdoba (two locations), Entre Ríos (one location) and Chaco (one location). Advanced breeding lines with prominent yield performance during 3 years of evaluation in the RYT are expected to become commercial cultivars.

Every year, the INTA National Wheat Breeding Program selects 1–2 commercial cultivars among advanced breeding lines on the basis of grain yield (previously evaluated in the RYT for three consecutive years) and additional agronomic attributes like resistance against main pathogens in Argentina (*Puccinia triticina* –leaf rust- and FHB) and bread making quality. This program has already released cultivars combining conventional breeding with MAS. A good example is Biointa 2004 with the leaf rust resistance gene *Lr47* (combined with *Lr34*) introgressed into an adapted background using molecular markers (Bainotti et al. 2009). It is expected that the next step in the program could be the release of a new commercial cultivar carrying *Fhb1*. On the other hand, it is important to mention that lines carrying *Fhb1* with Prointa Oasis background have been part of the Crossing Block of the INTA National Wheat Breeding Program since 2008 as a strategy to increase the basis of genetic resistance against FHB.

14.5 The Case F3-BC2 Prointa Granar

As shown previously, Prointa Granar is a local cultivar with good resistance against FHB, which, according to its pedigree data (MJI//PAK3563/CHAP70/3/DEI) is not related to Sumai 3. Alberione et al. (2008) analyzed FHB severity and scabby

Fig. 14.2 Local cultivars and advanced breeding lines under natural infection of FHB at field in Marcos Juarez (Argentina), year 2012. (**a**), Biointa 1005 (*left*) and line R111171 carrying *Fhb1* with Prointa Granar as background (*right*). (**b**), line R111117 carrying *Fhb1* with Prointa Oasis as background (*arrow* in *gray at left*) and Prointa Oasis without *Fhb1* (*arrow* in *black at right*). Entire head infections or portions of heads infected are visible as prematurely whitened glumes and awns. Heading times of tested materials were: October 9 (Biointa 1005), October 12 (R111171), September 29 (Prointa Oasis) and September 28 (R111117)

kernels in a population of 80 families F3-BC2 derived from the hybridization Prointa Granar/Sumai3, artificially infected with FHB, and arrived at two relevant conclusions: (1) *Fhb1* was confirmed as an effective source of FHB resistance considering local isolates of the pathogen (Table 14.2, Fig. 14.3b);(2) at least 10 families of the 24 not carrying *Fhb1,*exhibited FHB severity values lower than Sumai 3 (Fig. 14.3a).

Table 14.2 Association between presence of *Fhb1* and FHB development[a]

Source of variation	N[b]	FHB severity[c]	LSD[d]	% Scabby kernels[e]	LSD
Families not carrying *Fhb1*[f]	24	40.5	a	32	a
Families heterozygous *Fhb1*	40	33	b	28.5	a
Families homozygous *Fhb1*	16	31	b	22.5	b

[a]Based on Alberione et al. (2008)
[b]Number of F3-BC2 families analyzed from the hybridization Prointa Granar x Sumai 3
[c]Visual determination of the amount of head infected per family (average of 12 spikes) according to Stack and McMullen (1995)
[d]Different letters indicate statistically significant differences ($\alpha < 0.05$)
[e]Visual determination of scabby kernels per spike per family (average of 12 spikes)
[f]Presence of *Fhb1* was determined per F2-BC2 single plant by scoring with SSRs *Xgwm533* and *Xgwm493* (Anderson et al. 2001)

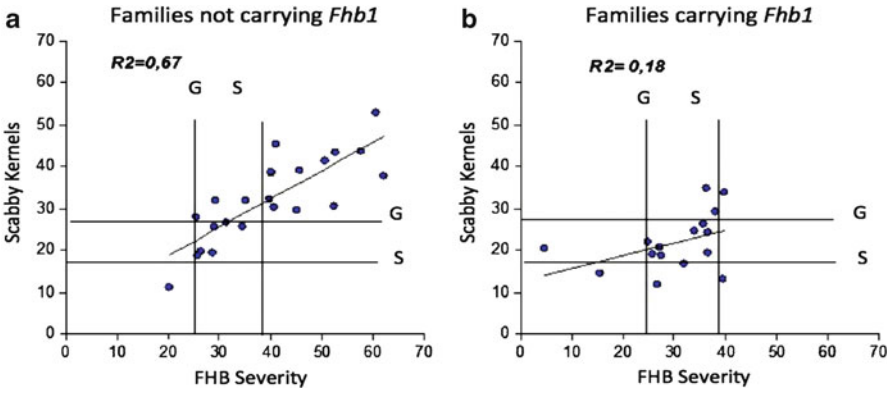

Fig. 14.3 Values of FHB severity and scabby kernels obtained in F3-BC2 families from the hybridization Prointa Granar/Sumai 3 under artificial infection. (**a**) includes F3-BC2 families without *Fhb1* and (**b**) includes F3-BC2 families carrying two copies of *Fhb1*. Severity values were obtained by visual determination of the amount of head infected per family (average of 12 spikes) according to Stack and McMullen (1995). Scabby kernels values were obtained by visual determination per spike per family (average of 12 spikes). Presence of *Fhb1* was determined per F2-BC2 single plant by scoring with SSRs *Xgwm533* and *Xgwm493* (Anderson et al. 2001)

These data would suggest the presence of a second source of FHB resistance in this population probably coming from Prointa Granar, with an additive effect with *Fhb1*. Further QTL mapping studies using a mapping population of Prointa Granar hybridized with a FHB susceptible material (Biointa 1005, Fig. 14.2a) are being carried out to confirm the hypothesis.

It should be mentioned that additional QTL mapping projects are being carried out in Argentina with alternative sources of FHB resistance like Catbird (CIMMYT material, pedigree CHUAN-MAI-18/BAGULA) and Pampeano (cultivar from Brazil, pedigree: ORL-91274/ORL-93807//ORL-95711-S). The final goal is to

develop molecular tools to stack different sources of FHB resistance into adapted backgrounds in order to get superior performance of adapted cultivars against this disease.

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References

Abbas HK, Cartwright RD, Shier WT, Abouzied MM, Bird CB, Rice LG, Ross PF, Sciumbato GL, Meredith FI (1998) Natural occurrence of Fumonisins in rice with Fusarium sheath rot disease. Plant Dis 82:22–25

Alberione E, Helguera M, Bainotti CT, Lewis S, Masiero B, Vanzetti L, Aurelia R (2008) Evaluación del QTL de resistencia a fusariosis de la espiga de trigo de la variedad Sumai3 en una población segregante. In: Actas del VII Congreso Nacional de trigo. Santa Rosa La Pampa: GB15 (in Spanish)

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Fetch J, Mitchell Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Bainotti C, Fraschina J, Salines JH, Nisi JE, Dubcovsky J, Lewis SM, Bullrich L, Vanzetti L, Cuniberti M, Campos P, Formica MB, Masiero B, Alberione E, Helguera M (2009) Registration of "BIOINTA 2004" Wheat. J Plant Regist 3:165–169

Bernardo AN, Ma H, Zhang D, Bai G (2011) Single nucleotide polymorphism in wheat chromosome region harboring Fhb1 for Fusarium head blight resistance. Mol Breed 29: 477–488

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128:1–26

Chu FS, Li GY (1994) Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. Appl Environ Microbiol 60:847–852

Formento AN, Firpo RR (2003) Comportamiento sanitario de cultivares de trigo EEA Paraná. Ciclo agrícola 2002/03. Serie extensión EEA Paraná (24) (in Spanish)

Galich MTV (1989) Importancia y difusión de la Fusariosis de trigo en Argentina. In: Kohli MM (ed) Taller sobre fusariosis de la espiga en América del Sur. CIMMYT, México, pp 7–26 (in Spanish)

Galich MTV (1997) Fusarium head blight in Argentina. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects, El Batan México, pp 19–28

Galich MTV (2004) Fusariosis de la espiga del trigo: Desarrollo de cultivares resistentes. IdiaXXI: 50–57 (in Spanish)

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol 5:515–525

Kikot GE, Moschini R, Consolo VF, Rojo R, Salerno G, Hours RA, Gasoni L, Arambarri AM, Alconada TM (2011) Occurrence of different species of *Fusarium* from wheat in relation to disease levels predicted by a weather-based model in Argentina pampas region. Mycopathologia 171:139–149

Leslie JF, Pearson CAS, Nelson PE, Toussoun TA (1990) Fusarium spp. from corn, sorghum, and soybean fields in the Central and Eastern United States. Phytopathology 80:343

Li T, Bai G, Wu S, Gu S (2011) Quantitative trait loci for resistance to Fusarium head blight in a Chinese wheat landrace Haiyanzhong. Theor Appl Genet 122:1497–1502

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct Integr Genomic 6:83–89

Lori GA, Sisterna MN, Haidukowski M, Rizzo I (2003) *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area of Argentina. Microbiol Res 158:29–35

Omurtag G, Yazicioglu D (2004) Determination of fumonisins B1 and B2 in herbal tea and medicinal plants in Turkey by high-performance liquid chromatography. J Food Prot 67:1782–1786

Palacios SA, Ramirez ML, Cabrera Zalazar M, Farnochi MC, Zappacosta D, Chiacchiera SM, Reynoso MM, Chulze SN, Torres AM (2011) Occurrence of *Fusarium* spp. and fumonisin in durum wheat grains. J Agric Food Chem 59:12264–12269

Pestka JJ (2007) Deoxynivalenol: toxicity, mechanisms and animal health risks. Anim Feed Sci Technol 137:283–298

Pettigrew HD, Selmi CF, Teuber SS, Gershwin ME (2010) Mold and human health: separating the wheat from the chaff. Clin Rev Allergy Immunol 38:148–155

Ramirez ML, Reynoso MM, Farnochi MC, Chulze S (2006) Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) Isolates from wheat in Argentina. Eur J Plant Pathol 115:139–148

Rheeder JP, Marasas WFO, Theil PG, Sydenham EW, Shephard GS, Van Schalkwyk DJ (1992) *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82:353–357

Ross PF, Nelson PE, Richard JL, Osweiler GD, Rice LG, Plattner RD, Wilson TM (1990) Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. Appl Environ Microbiol 56:3225–3226

Stack RW, McMullen MP (1995) A visual scale to estimate severity of Fusarium head blight in wheat. North Dakota State University Extension Service Bulletin PP-1095

Sutton JC (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:195–209

Trigo-Stockli DM, Sanchez-Martinez RI, Cortez-Rocha MO, Pedersen JR (1998) Comparison of the distribution and occurrence of *Fusarium graminearum* and deoxynivalenol in hard red winter wheat for 1993–1996. Cereal Chem 75:841–846

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805–811

Chapter 15
Development and Characterization of International Maize and Wheat Improvement Center (CIMMYT) Germplasm for Fusarium Head Blight Resistance

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Abstract Fusarium Head Blight (FHB) also known as head scab, is an important fungal disease of wheat worldwide. The disease causes yield loss, low test weights, low seed germination and contamination of grains with mycotoxins which makes it unfit for human and animal consumption. Breeding for FHB resistant cultivars is the most effective, economical and environmentally friendly means to combat this grave disease. The FHB research began at International Maize and Wheat Improvement Center (CIMMYT) in early 1980s, since then CIMMYT has initiated various breeding activities for development of FHB resistant germplasm including large scale FHB screening of the promising breeding lines, genetic resources, and crosses have been made between parents with complimentary disease resistance and agronomic traits. At CIMMYT, automated programmable misting system and precision CO_2 spraying for liquid inoculums application allow the systematic, accurate and detailed screening of large sets of germplasm for Type I and Type II resistance. DON contamination is assayed in the laboratory for promising lines. Furthermore, a haplotyping system has also been established to diagnose well known QTL for FHB resistance. Promising lines with good FHB resistance are compiled as Fusarium Head Blight Screening Nursery (FHBSN) regularly and distributed worldwide. Selections for FHBSN are made based on FHB disease scores, phenological traits like days to heading and height, haplotyping, and pedigree information.

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15.1 Introduction-Wheat

Historical evidences indicate that the development of human civilization may be directly connected to the domestication of wheat (*Triticum* spp.). Villages developed when primitive man discovered that he no longer needed to follow game and forage for his food. Civilized humans could grow wheat during summer, store it for food in the winter and use the remaining seed to plant in the spring. Actual wheat cultivation may have started in the Levant region of the Near East and Ethiopian Highlands around 6,000–8,000 B.C. or earlier, but now is cultivated worldwide. In 2010 world production of wheat was 651 million tons (Tables 15.1 and 15.2), making it the third most-produced cereal after maize (844 million tons) and rice (672 million tons). This grain is grown on more land area than any other commercial crop and is the most important staple food for humans. World trade in wheat is greater than for all other crops combined. In the rapidly developing countries of Asia and Africa, westernization of diets associated with increasing prosperity is leading to growth in per capita demand for wheat at the expense of the other food staples.

Wheat grains are highly nutritive as they are rich in energy, carbohydrates, dietary fibre, fat, protein, thiamine, riboflavin, niacin, pantothenic acid, vitamin B_6,

Table 15.1 Leading countries/groups in wheat grain production in recent years (million metric tons)

Country	Group	2008–2009	2009–2010	2010–2011	2011–2012[a]
5	EU (France)	151.1	138.1	135.7	132.4
4	USSR (Russia)	115.5	113.8	81.0	99.5
1	China	112.5	115.1	115.2	118.0
2	India	78.6	80.7	80.8	93.9
3	USA	68.0	60.4	60.1	61.7
6	Canada	28.6	26.9	23.2	27.0
World production		683.7	683.8	648.2	662.4
World use		641.8	652.6	656.6	670.2

[a]Estimated source: USDA (2012)

Table 15.2 Global wheat production, acreage, consumption, international trade and leading exporters in recent years

	2009–2010	2010–2011	2011–2012[a]	2012–2013[b]
Production (mmt)	679	653	696	665
Acreage (mhac)	222	218	221	225
Consumption (mmt)	653	659	691	682
A-Feed	117	120	144	130
B-Industrial	19	19	19	20
C-Food	452	457	461	465
Trade (mmt)	128	126	145	135
Major exporters	78	72	70	54
Carryover stocks (mmt)	199	193	198	182

[a]Estimated

[b]Projected Source: International Grains Council 2012

folate, calcium, iron, magnesium, phosphorus, potassium, zinc and manganese (USDA-National Nutrient Database for Standard Reference, Release 19, 2006). Due to the high nutritive value, wheat grains are eaten in various forms across cultures and continents. The global average contribution of wheat to the human dietary energy (2,794 kcal/capita/day) is estimated at 19 % (529 kcal/capita/day). Average daily per capita consumption of wheat varies greatly in different regions with maximum consumption in Central Asia (1,305 kcal) and least in Middle Africa (124 kcal) (FAO 2010). Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, biscuits, cookies, cakes, breakfast cereal, pasta, noodles, couscous and for fermentation to make beer, other alcoholic beverages, or biofuel. Wheat is planted to a limited extent as a forage crop for livestock, and its straw can be used as a construction material for roofing thatch. The whole grain can be milled to leave just the endosperm for white flour. The by-products of this are bran and germ. The whole grain is a concentrated source of vitamins, minerals, and protein, while the refined grain is mostly starch.

Since green revolution (1960s), wheat production increased globally with almost 300 % beyond 600 MT in 2008 (Table 15.1) on a virtually stable cultivation area of 200 million hectare, hence the progress was largely achieved by increased average yields rather than expansion of arable land (FAO 2010). The global average wheat yield increased from 1 to 3 tons per hectare, with a parallel expansion of consumption from 400 to 530 kcal/capita/day during the last four decades, due to human population growth that doubled since 1961 and is projected to triple to nine billion people in 2050 (FAO 2010). However, the annual growth rate of global wheat production is below 1 %, which eventually cannot meet the global market requirements during the four decades ahead (Fischer and Edmeades 2010). The generation of cultivars with enhanced resistance to biotic and abiotic stress along with optimized management practices is currently considered to be the best strategy to achieve this goal (Fischer and Edmeades 2010). However, due to climate change and agricultural practices, wheat production has brought new challenges for farmers, including the increased incidence of FHB.

In 2011–2012, the leading exporters of wheat were USA (35.08 mmt), Australia (18.66 mmt), Canada (16.58 mmt) and Argentina (9.49 mmt). The other countries exporting wheat include France, Ukraine, Kazakhstan, Russia and Turkey. The major importers of wheat included North-Africa (24.18 mmt) comprising Egypt, Algeria, Morocco, Tunisia and Libya, South-East Asia (15.80 mmt) including Indonesia, Malaysia, Thailand, Vietnam and Philippines, and Middle-East (13.57 mmt) comprising Iraq, Saudi Arabia, Lebanon, Yemen and Israel (USDA, September 2012). Other wheat importing countries include Japan, Italy, Brazil, Spain, Mexico and South Korea.

15.2 Introduction-Fusarium Head Blight

Fusarium Head Blight (FHB) or scab is a devastating disease caused by several species of fungi from the group known as *Fusarium* and *Microdochium nivale*. More than 17 *Fusarium* species are known to cause FHB, while *F. graminearum* (Teleomorph

Gibberella zeae) is the predominant species in many countries. This fungus can produce two important mycotoxins, deoxynivalenol (DON) and zearalenone, on scabby grains that have been shown to be harmful to both human and animals (Bai and Shaner 2004). Wheat is most susceptible at anthesis, but some infection can still occur during kernel development. The primary symptom of the disease is bleaching of some of the florets in the head before maturity. Severe infections can cause premature blight or bleaching of the entire spike or head. *Fusarium* pathogens overwinter in soil, grass and crop residue as well as in the seed. Seedlings may become infected at emergence. Spores are produced first on stem infections at the base of the plant. These spores are spread by rain or wind to infect flower parts, glumes or other portions of the head. Temperatures between 18 and 30 °C and extended periods of moisture in the form of rain or dew favour the reproduction of the fungus on crop residues and also promote infection and disease development. The incidence and severity of FHB have been increasing in the last decades due to an extension of the global maize acreage in rotation with wheat (maize stubble is an excellent inoculum source) and the increasingly popular practice of conservation agriculture wherein reduced tillage with crop residue colonized by the fungus is being maintained on or near the soil surface. In addition, it is predicted that global warming will further exacerbate the problem, as the disease thrives in high humidity and warmer climates (van Ginkel et al. 2004a). The disease often results in 30–40 % yield loss, which may increase up to 70 % for susceptible cultivars under severe epidemics. Yield loss may also be caused when the infected grains are used as seeds, leading to low germination rate, poor seedling vigour, and seedling blight (Parry et al. 1995). Seed from FHB infected spikelets are small, shrivelled, and white or chalky in appearance and are commonly referred to as tombstones. Globally, serious efforts are in progress to manage FHB in order to attain sustainable production of wheat. Breeding for FHB resistant cultivars is the most effective, economical and environment-friendly way to combat this global challenge.

The resistance to FHB is a multi-gene controlled quantitative trait, with various resistance mechanisms. The first two mechanisms, resistance to initial infection (Type I) and resistance to fungal spread within plant tissues (Type II), were defined by Schroeder and Christensen (1963) and were generally accepted. Later, more resistance mechanisms were proposed (Kosová et al. 2009), but only resistance to toxin accumulation (Type III), resistance to kernel infection (Type IV), and resistance to yield loss (Type V) were widely accepted (Mesterhazy et al. 1999). The most well studied mechanisms are Type I and Type II, with the formal evaluated by spray or spawn inoculation and the latter by point inoculation (Buerstmayr et al. 2009). With the growing concern on food safety, Type III resistance gradually drawn more attention, and so did Type IV resistance, with Type V being the least studied (Liu et al. 2009; Mesterhazy 2010). Resistance QTL for Type I to IV, residing on all the 21 wheat chromosomes, have been reported in numerous researches (Liu et al. 2009). Five resistance genes with major effects were nominated, i.e. *Fhb1* on 3BS from Sumai 3 (Cuthbert et al. 2006), *Fhb2* on 6BS from Sumai 3 (Cuthbert et al. 2007), *Fhb3* on T7AL·7Lr#1S from *Leymus racemosus* (Qi et al. 2008), *Fhb4* on 4B from Wangshuibai (Xue et al. 2010), and *Fhb5* on 5A from Wangshuibai (Xue et al. 2011). Efforts on cloning these resistance genes are being made, which will greatly improve the efficiency and preciseness of

marker-assisted selection (MAS) by the application of functional markers that developed from the polymorphic sites within resistance genes (Liu et al. 2012).

15.3 International Maize and Wheat Improvement Center and Fusarium Head Blight

15.3.1 Early Research Activities: Before 1990

The FHB research at International Maize and Wheat Improvement Center (CIMMYT) started in early 1980s (Gilchrist et al. 1997), when local adapted cultivar with good FHB resistance was unavailable, thus the main tasks were to (1) set up a systematic methodology for FHB screening; (2) screen CIMMYT wheat germplasm, as well as distantly related *Triticeae* germplasm, for FHB resistance; and (3) evaluate introduced FHB resistance materials in Mexican environments (CIMMYT 1984, 1985, 1986). The main testing site was at Toluca, a very humid location with an average annual rainfall of 800 mm at an altitude of 2,640 m above sea level (masl), latitude 19°N, in the State of Mexico. FHB occurs naturally on wheat at Toluca, and satisfactory disease is assured when artificial inoculation is applied. In addition, two other FHB hot spots, Patzcuaro in the State of Michoacan and Sierra del Tigre in the State of Jalisco, were also used as in farm testing sites (Ireta and Gilchrist 1994). The cotton and spray inoculation methods were mainly used for inoculation, and the disease was visually scored 40 days after inoculation using a linear scale from 0 to 5 (CIMMYT 1988). Numerous FHB resistant lines were identified in these pilot research activities, making disease resistance breeding possible.

The FHB resistance breeding at CIMMYT started in 1985, using resistance sources mainly from three geographic regions: China and Japan, Brazil, and Eastern Europe (Singh and van Ginkel 1997). Chinese varieties played a very important role in early FHB resistance breeding activities. In the mid 1980s, a shuttle breeding and germplasm exchange program was launched by CIMMYT and China, and a set of Chinese germplasm resistant to FHB were introduced (He et al. 2000). These materials showed better FHB resistance than the local adapted cultivars, but their agronomic characters were poor, e.g. high stature, late maturity, low yields, and generally susceptible to rusts (CIMMYT 1988). In order to transfer the FHB resistance into CIMMYT germplasm, the Chinese materials were extensively used in crossings, notably Sumai 3, Ning 7840, Chuanmai 18 etc. (Singh and van Ginkel 1997).

15.3.2 Re-emphasized Researches in Toluca: 1997–2005

After the large scale germplasm screening and breeding for FHB resistance in 1980s, the research on FHB slowed down. Only sporadic and isolated studies were carried out in early and mid 1990s, e.g. Singh et al. (1995) and van Ginkel et al.

(1996). The research was re-emphasized in 1997, with great efforts devoted to the identification and utilization of all five resistance types, i.e. infection, spread, toxin level, yield loss, and grain appearance (van Ginkel and Gilchrist 2002; van Ginkel et al. 2003). In the past, most FHB resistance-breeding programs aimed at reducing disease in the farmer's field (Types I and/or II); but with the growing awareness of food safety, DON contamination (Type III) has drawn great attention, which made CIMMYT adjusted its research on FHB and modified breeding strategy (van Ginkel and Gilchrist 2002). Another major task was put on diversification of FHB resistance gene pool. Early CIMMYT FHB resistance breeding depended heavily on Chinese germplasm, particularly Sumai 3 and its derived lines (Singh and van Ginkel 1997; van Ginkel et al. 2004b). This narrow base of FHB resistance is unfavourable in terms of both wheat breeding and disease resistance, highlighting the need of new resistance sources. Since late 1990s, the identification and utilization of non-Chinese (especially non-Sumai 3) resistance germplasm has been launched, with studies performed in both common wheat and its relatives, aiming at diversifying FHB resistance gene pool (Gilchrist et al. 1997; van Ginkel et al. 2000, 2004b).

One of the main diversity sources is synthetic hexaploid wheat (AABBDD), derived by crossing durum wheat (AABB) and *Aegilops tauschii* (DD). The large genetic variation in the two relative species provides promising prospects in broadening wheat genetic diversity, and hopefully more new FHB resistance genes could be identified (Gilchrist et al. 1999). To this end, 582 synthetic wheats and derived lines were screened for FHB resistance, and many of them showed both Type I and Type II resistance (Gilchrist et al. 1997). These elite synthetic wheat lines have been intensively used in crossings, while new lines have been synthesized and characterized for FHB resistance (Mujeeb-Kazi et al. 2000). In the tertiary gene pool, resistance genes identified in some *Thinopyrum* and *Leymus* species were introgressed into wheat using cytogenetic transfer protocols associated with *ph* manipulation and molecular diagnostics (Mujeeb-Kazi et al. 2000).

Along with the identification of non-Chinese resistance germplasm, the utilization of Chinese resistance genes was continued through China/CIMMYT shuttle breeding during this period. CIMMYT received around 700 Chinese commercial varieties, and a set of resistant lines with good agronomic performance were developed at CIMMYT through the use of Chinese germplasm (He et al. 2000).

The development of molecular markers linked to major resistance genes provided an opportunity for fast identification and evaluation of FHB resistance germplasm. The SSRs flanking the major QTL on 3BS (*Fhb1*) were used to characterize a set of 500 diverse FHB resistant varieties, and less than 10 % carried the *Fhb1* gene (van Ginkel et al. 2004b), reflecting the progress of utilization of non-Sumai 3 resistance sources.

In addition to common wheat, FHB resistance studies have also been performed in barley, triticale, and durum wheat, leading to the identification of a set of resistant barley and triticale lines but only a few moderately resistant durum wheat lines (Capettini et al. 2006; Gilchrist et al. 2002, 1997; van Ginkel et al. 2003). In order to improve the FHB resistance of durum, tertiary gene pool were exploited by generating AAAABB and AABBBB hexaploids, through the hybridizations of durum

(AABB) with diploid wheat (AA) and *Ae. Speltoides* (BB), respectively, and a few promising lines were identified (Ban et al. 2005; Mujeeb-Kazi et al. 2000). In addition, attempts on transferring resistance genes from *Ae. taushii* (DD) to the A genome of durum through homoeologous exchange were also made (Mujeeb-Kazi et al. 2000).

15.3.3 Researches in El Batán: Since 2006

15.3.3.1 Advantages of the New Screening Nursery

In 2006, the FHB screening program was moved to El Batán, where CIMMYT headquarters is located, and the screening system underwent a series of modification in terms of inoculum, inoculation method, and the misting system (Duveiller et al. 2008). The main reason for this transfer is the heavy natural FHB epidemic in Toluca that complicated the artificial inoculation, making the disease unverifiable and sometimes out of control. Additionally, natural incidence of stripe rust and Septoria tritici blotch in Toluca are also frequent, making plants response to multiple biotic stresses; whereas the natural infection of all the three diseases are much lower in El Batán, greatly reducing the background 'noise' for disease evaluation and thus leading to more reliable results. The proximity of laboratory facilitates the preparation of inoculum and makes it possible for inoculation targeted at each line's flowering time (Duveiller et al. 2008).

15.3.3.2 Novel Screening Tools

CIMMYT uses a high throughput immunochemical assay (ELISA) for myctoxin quantification, which is costly, and new approaches thus were introduced as alternatives to estimate the resistance against DON. Quantitative real-time PCR (qPCR) is one of the new techniques for the assessment of host resistance via determining fungal biomass. Highly positive correlations were found between fungal DNA amount and DON content, as well as fungal DNA and FHB index in a set of 64 entries comprising bread wheat, durum, and barley (Murakami et al. 2008). This result showed the suitability of qPCR in mycotoxin quantification in comparison of the direct detection methods like HPLC and ELISA.

Haplotyping is a new tool to diagnose the presence of known FHB QTL in breeding materials, which is helpful to understand the resistance composition, to identify germplasm with novel resistance genes, and to make crosses toward diversifying resistance. In collaboration with USDA-ARS Small Grain Genotyping Center, Fargo (North Dakota, USA), a haplotyping system has been established to diagnose 10 well known FHB QTL through their closely linked molecular markers (Duveiller et al. 2008; He et al. 2013). The 13th FHBSN was the first to be haplotyped, and the results showed that except for the QTL on 2DL from Wuhan 1 and 2DS from Gamenya,

most QTL are not abundant in this nursery (He et al. 2013). Similarly, the 2DL QTL was also highly frequent in the 14th FHBSN (Table 15.3). The results demonstrated clearly the progress achieved at CIMMYT of using non-Sumai 3 resistance genes in breeding. Except the five entries that have not been haplotyped, all the remaining lines did not have any resistance QTL from Sumai 3. However, the high frequencies of the 2DL QTL may imply low resistance diversity. Although there must be undetected QTL to confer high levels of FHB resistance, heavy dependence on this QTL is not favourable for the diversification of resistance sources. Our goal is that all the resistance QTL present in FHBSN entries, with similar frequencies. Obviously, the utilization of Wuhan 1 and its derivatives should be limited in future FHB resistant breeding programs.

15.3.3.3 Improving FHB Resistance for White Grained Varieties

It is generally accepted that white grained varieties are more likely to be FHB susceptible than red grained varieties (Lewis et al. 2007). Considering its significant acreage globally, CIMMYT has paid great attention to improve the FHB resistance in white grained materials and significant progress has been achieved. According to the field evaluation in 2011, 867 of 1470 white grained entries showed damage rates lower than 2 spikelets per spike (an index for our preliminary screening materials, where Sumai 3 was 0 and Gamenya was 12.4). This demonstrates that white grained varieties with resistance levels similar to red grained materials become available, which is a big progress in bread wheat breeding. It is noteworthy that 3 (18.7%) of the 16 entries of the 13th FHBSN were of white grained lines (He et al. 2013), and this proportion increased to 45 % in the 14th FHBSN (Table 15.3), which is preferred in order to maintain a similar proportion of red vs. white entries in a FHBSN.

15.3.3.4 Screening for New Resistance Sources in Chinese Wheat Varieties Collected in the CIMMYT Germplasm Bank

Most of the Chinese wheat varieties collected in the CIMMYT germplasm bank have not been characterized systematically for FHB resistance in Mexico, or their level of resistance is unknown. Considering the effectiveness of resistance screening for FHB in China and the high prevalence of the disease there, unknown sources of resistance may exist. Therefore a study was performed in 2009 on the Chinese varieties collected in CIMMYT's genebank, to determine their FHB resistance and to find new resistance sources that could be used in CIMMYT's breeding programs (Schlang et al. 2009b).

A total of 491 entries out of 583 genotypes planted were evaluated for FHB reaction in 2009, and 84 genotypes (17.1 %) showed an FHB index lower than that of Sumai 3 (1.5 %, and the FHB index of Gamenya was 76.9 %). Of the 491 lines, 304 genotypes (62 %) showed a FHB index below 10 % and were selected for seed increase in Ciudad Obregón, where the entries were screened for leaf rust resistance

Table 15.3 Entries of CIMMYT's 14th Fusarium Head Blight Screening Nursery (14th FHBSN) and results of field screening and haplotyping

			2009[b]		2010[b]		2011[b]	2012[b]	FHB resistance QTLs[c]										
									Sumai 3			Frontana		Wuhan 1		CJ9306	*T. dicocoides*	
GID	Cross name	Grain colour[a]	FHB	DON	FHB	DON	FHB	FHB	3B	5A	6B	3A	5A	2D	4B	2D	3A	7A
5686798	KS82W418/SPN//WBLL1/3/BERKUT	R	3.2	1.4	9.3	2.2	2.4	1.5						X				
5686808	CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*FCT/5/KAUZ*2/YACO//KAUZ/6/BERKUT	R	2.0	3.1	10.9	2.6	2.2	3.9						X				X
5793395	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	W	5.0	4.1	11.2	2.4	0.6	5.3						X				
5793927	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	W	6.5	3.6	8.2	3.4	1.3	7.5						X				
5794547	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	R	1.8	1.2	9.5	4.9	1.1	4.1										
5794812	WBLL1*2/TUKURU//KRONSTAD F2004	R	0.5	1.1	15.8	4	1.0	4.7						X				
5794829	INQALAB 91*2/KUKUNA//2*KRONSTAD F2004	R	3.7	0.7	12	2.8	2.1	3.9										
5794843	WHEAR/2*KRONSTAD F2004	R	7.2	–	4.3	1.1	2.1	2.0					X		X			
5849348	ATTILA*2/PBW65//KRONSTAD F2004	W	9.6	–	13.9	4.4	0.5	4.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5894933	CO99W329/2*BERKUT	W	1.7	4.5	10.5	1.3	1.0	3.7						X				

(continued)

Table 15.3 (continued)

GID	Cross name	Grain colour[a]	2009[b] FHB	2009[b] DON	2010[b] FHB	2010[b] DON	2011[b] FHB	2012[b] FHB	FHB resistance QTLs[c] Sumai 3 3B	Sumai 3 5A	Sumai 3 6B	Frontana 3A	Frontana 5A	Wuhan 1 2D	Wuhan 1 4B	CJ9306 2D	*T. dicocoides* 3A	*T. dicocoides* 7A
5894946	T.DICOCCON PI94625/AE. SQUARROSA (372)//TUI/ CLMS/3/2*PASTOR/4/ EXCALIBUR	R	2.5	0.9	7.2	3.7	2.3	1.2										
5895215	YAV79//DACK/RABI/3/ SNIPE/4/AE.SQUARROSA (460)/5/2*EXCALIBUR/6/ VEE/LIRA//BOW/3/BCN/4/ KAUZ	R	3.6	1.6	9.5	0.8	0.8	4.5						X				
5895256	CALINGIRI/SOKOLL	R	2.1	1.7	5.9	2.6	0.0	1.6										
5895317	BERKUT/HTG	R	0.3	1.4	5.7	2.2	0.2	6.4						X				
5895423	ASTREB*2/NING MAI 9558	W	0.8	6.4	11.9	3.2	0.2	2.7						X		X		
5895427	ASTREB*2/3/WUH1/VEE#5// CBRD	R	2.9	1.2	10.9	2.3	1.8	6.2						X				
5995411	TUKURU//BAV92/RAYON/6/ NG8201/KAUZ/4/SHA7// PRL/VEE#6/3/FASAN/5/ MILAN/KAUZ	W	3.4	–	12.6	4.9	1.5	5.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5995800	NG8675/CBRD//MILAN/7/ CAL/NH//H567.71/3/ SERI/4/CAL/NH// H567.71/5/2*KAUZ/6/ PASTOR/8/CNDO/R143// ENTE/MEXI_2/3/ AEGILOPS SQUARROSA (TAUS)/4/ WEAVER/5/2*PASTOR	W	5.7	–	9.3	4.5	0.6	6.6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

5996087	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	W	6.1	–	13.1	3	1.4	7.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5996556	PBW343/PASTOR//OTUS/TOBA97/3/PBW343/TONI	W	5.2	–	13.4	6.2	1.0	8.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10004	SUMAI #3 (CHECK)	W	0.2	0.1	1.2	0.1	0.0	0.0	X	X	X			X		X		X
5536	GAMENYA (CHECK)	W	77.8	–	83.6	8.1	80.7	46.1										
4877754	GONDO/CBRD (CHECK)	R	0.5	0.7	0.3	0.3	0.1	–										

[a]W and R stand for white and red grained materials, respectively

[b]Results of field screening in El Batán, Mexico, are present. In 2009 and 2010, both FHB indices and DON concentrations (ppm) are shown, while in 2011 and 2012 only FHB indices are presented

[c]The detected resistant QTL are marked with 'X'; n.a. for 'not analyzed'

as well as agronomic traits (Schlang et al. 2009b). Afterwards, 140 entries that showed high resistance and well adaptability to Mexican environments were selected and evaluated in El Batán in 2012. One hundred and sixteen (83 %) entries showed FHB index lower than 10 %, while 120 (86 %) had DON content lower than 2.0, exhibiting that most of the lines have high levels of resistance. A subset of 102 elite entries was selected for haplotyping, using markers linked to 10 well known FHB QTL (Duveiller et al. 2008). The results indicated that around 50 % of the lines have the 2DL QTL from Wuhan 1 or CJ9306, and 27 % have the 3BS QTL from Sumai 3, while the other 8 QTL are of low frequency. These materials could be used in breeding programs as new resistance sources.

15.3.3.5 Mapping Populations

There have been more than ten mapping populations developed and evaluated for FHB since 2006; but high attention was paid to two DH populations that were developed in 2009. The two populations share a same susceptible parent Ocoroni F86, while their resistant parents are TRAP#1/BOW//TAIGU DERIVATIVE and IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190), respectively, with the resistance source of the former from Chinese germplasm and that of the latter from synthetic wheat. According to haplotyping, no known QTL was detected in the two resistant parents. The two populations have been phenotyped in El Batán for 3 years from 2010 to 2012. Besides FHB traits (FHB index, FDK, and DON), days to heading, plant height, and anther extrusion were also scored. Genotyping are ongoing and QTL mapping will be done to identify resistance genes in the two populations.

15.3.3.6 Exploiting Exotic Gene Pools

Efforts on exploiting second and tertiary gene pools for new FHB resistance genes continued in this period. Mapping populations based on synthetic wheat derived lines were generated for the identification of novel resistance genes (He et al. 2012; Zhu et al. 2012). Translocation and addition lines with alien chromosomal segments from *Leymus* and *Thinopyrum* were produced, but high resistance was not obtained, implying the need of multiple translocations (Kishii et al. 2006). Likewise, the FHB researches on barley and durum kept on going (Capettini et al. 2006; Kishii et al. 2006).

15.3.4 International Cooperation and Fusarium Head Blight Screening Nurseries

International cooperation has been playing a very important role in combating FHB, and it has also contributed greatly to the FHB research at CIMMYT. The China/CIMMYT shuttle breeding program contributed greatly to the incorporation of the

FHB resistance of Chinese germplasm into the high yielding, semi-dwarf and rust resistant CIMMYT varieties, generating numerous elite cultivars released in both CIMMYT and China (He et al. 2000; Singh and van Ginkel 1997). Besides, CIMMYT has developed close bi-lateral and multi-lateral collaborations with the countries where FHB is of economical importance (Duveiller et al. 2008; van Ginkel et al. 2004a). CIMMYT also launched a Global Fusarium Initiative (Ban et al. 2005; Duveiller et al. 2008) and participated in the United States Wheat and Barley Scab Initiative (USWBSI, van Ginkel et al. 2004a), in which CIMMYT played an important role as a communication platform to facilitate information exchange, germplasm enhancement, and breeding methods development.

One of the main breeding objectives of CIMMYT is to develop wide adapted genotypes that could be cultivated in various environments (Rajaram 2001). In light of this, FHB resistant cultivars developed at CIMMYT should be tested in other environments for further evaluation. According to van Ginkel et al. (2004b), significant genotype-by-location interaction was detected when CIMMYT FHB resistant materials were evaluated in different environments, highlighting the importance of multi-location testing. The distribution and evaluation of CIMMYT FHB resistant germplasm worldwide makes the materials exposed to a range of environments as well as *Fusarium* isolates, which will help the identification of globally stable resistant liens. In addition, this is also beneficial to the national breeding programs, in terms of the selection of resistant germplasm adapted to local environments.

Several temporary FHB screening nurseries were compiled and distributed by CIMMYT in the past, e.g. Wheat Scab Resistance Screening Nursery (WWSRSN, van Ginkel et al. 2004a, b), Fusarium International Elite Spring Wheat Nursery (FIESWN, Lewis et al. 2006), and Fusarium International Preliminary Spring Wheat Nursery (FIPSWN, Lewis et al. 2006); but the most important, influential, and long-lasting one is the Fusarium Head Blight Screening Nursery (FHBSN), which was named as Scab Resistance Screening Nursery (SRSN) when the first nursery was released in 1985 (Bekele et al. 1988). Until 2009, 12 SRSNs had been developed and distributed globally. Since 2010, the nursery adopted the current name as FHBSN, but the numbering continued from the former SRSN, and the 13th FHBSN and the 14th FHBSN were made available in 2011 and 2012, respectively. The FHBSNs are provided free of charge to the institutions working on FHB resistance breeding, upon their requests. In 2012, 70 sets of the 14th FHBSN were requested by 62 institutions in 30 countries, and promising results are expected which will contribute to global FHB resistance breeding.

15.4 International Maize and Wheat Improvement Center's Breeding Strategy

FHB is a disease of quantitative inheritance, which is controlled by several major genes, conditioned by numerous minor genes, and influenced by environments (Buerstmayr et al. 2009). Therefore, pyramiding resistance genes from diverse sources

in an adapted recipient cultivar is highly preferred, and this has been the FHB breeding goal at CIMMYT. Three breeding strategies have been adopted at CIMMYT (Singh and van Ginkel 1997), all aiming at the combination of FHB resistance with other favorable traits.

15.4.1 Simultaneous Utilization of Two Resistance Sources

The crossing mode for this strategy is 'resistant donor 1/resistant donor 2//recipient parent'. This allows the utilization of resistance genes from two donor parents and maintains 50 % genetic background of the adapted parent. In practice, a three-way (or top) cross is suggested where F1 is generated by crossing the two resistant parents and is subsequently top-crossed with the adapted recipient cultivar. An example of this strategy is 'Ning 7840/Frontana//Pastor', where Ning 7840 and Frontana are two diverse resistance cultivars from China and Brazil, respectively, and Pastor is a CIMMYT advanced breeding line showing wide adaptation. Mild or no selection for FHB resistance is applied in early segregating generations (F1-top, F2, and F3) to increase homozygosity, while high selection pressure for desirable traits and resistance to other diseases (e.g. rusts) is applied to maintain the adaptivity from the recipient parent. From F4 generation, intense selection pressure for FHB resistance is carried out. F5 plants are harvested in pedigree fashion to achieve pure F6 lines, which may be further tested for FHB resistance and other traits.

15.4.2 Parent Building – Strategy A

This is a four-way (or double) crossing strategy with a mode of 'resistant donor 1/resistant donor 2//resistant donor 3/resistant donor 4/3/recipient parent'. In this strategy, resistance genes from various sources are combined in one parent, which is then crossed to an adapted recipient cultivar. Selection criteria are the same as described above, except for that one backcross could be made with the adapted recipient cultivar, which is called 'single-backcross crossing strategy' and is able to retain most of the desired alleles of the recipient parent during the incorporation of resistance genes (Ortiz et al. 2008).

15.4.3 Parent Building – Strategy B

The crossing mode is 'resistant donor 1/recipient parent//recipient parent/3/resistant donor 2/recipient parent//recipient parent', in which the FHB resistance genes from each of the two (or more) donor parents are transferred into a recipient parent to generate intermediate materials, which are then crossed to obtain improved cultivars.

Notably 'single-backcross' is used here to reinforce the genetic background of the recipient parent; and two (but no more) backcrossings are also applicable in practice. The selection criteria are the same as described earlier. Since the intermediate lines used in the last cross are related by at least 75 %, a smaller population size is needed to identify progenies with combined resistance from two sources. This is an advantage of the strategy over the previous two, while another advantage is the intermediate materials can readily be released as commercial cultivars until the identification of the highly resistant progenies in the final step.

A critical limit factor for the above mentioned breeding strategies is population size (Singh and van Ginkel 1997). Large population size certainly favors the successful identification of desired progenies, which, unfortunately, is resource demanding and sometimes unmanageable. The ways to overcome this problem, as recommended by Singh and van Ginkel (1997), are (1) avoiding highly susceptible recipient cultivar; and (2) applying a mild FHB selection pressure in early generations. However, with the availability of molecular markers linked to resistance QTL, MAS could be effectively and efficiently used to replace the phenotypic selection in early generation, which is helpful to identify and discard lines without resistance genes and thus to maintain a small population size. Nevertheless, MAS cannot completely replace phenotypic evaluation, since there are unidentified resistance genes that unable to be assessed by MAS.

The breeding strategies proposed by Singh and van Ginkel (1997) did not separate different FHB resistance mechanisms; but as shown in the literature, there are five resistance types, with their respective resistance mechanisms (Mesterhazy 1995; Schroeder and Christensen 1963). Based on this theory, van Ginkel and Gilchrist (2002) suggested that crosses should be done between parents that fully complement one another in terms of resistance type, so that progenies exhibiting high levels for all five resistance types could be identified. Considering this strategy, the resistance donors in the above mentioned three strategies should be complimentary not only in terms of genetic diversity, but also in terms of resistance types. This requires the effective incorporation of Type III, IV, and V resistances into the FHB screening and evaluation system, in addition to the traditional Type I and II resistances.

15.5 Fusarium Head Blight Screening at International Maize and Wheat Improvement Center

15.5.1 Plant Materials and the Screening Field

The materials tested in the screening field are mainly bread wheat varieties from different breeding programs of CIMMYT, genebank collections, mapping populations and other genetic resources including introductions. Additionally, synthetic wheat, durum, barley, and triticale are also evaluated.

The experimental field is located at El Batán at an altitude of 2,240 masl, latitude 19°N. It covers approximately 2 ha with a yearly screening capacity of up to 10,000 plots. The materials are planted in 1 m double row plots for routine screening and in 5 m long plots for yield loss trials, and are tested in up to 3 subsequent years, with two or three replications from the second year onwards. To provide suitable references, one to three sets of checks are included depending on population size. Three susceptible lines (Gamenya, Ocoroni F 86 and Falcin/*Ae. squarrosa* (312)/3/THB/CEP7780//SHA4/Lira) and three resistant lines (Sumai 3, Gondo/CBRD and Heilo) are included as checks.

The average annual rainfall in El Batán is 625 mm and natural FHB epidemic is low. A fine misting system is set up in the screening field to provide uniform humidity condition to promote FHB development. The system includes about 1,500 DAN modular microsprinkler (11.7 gph flow rate at 30 psi) spaced in 3 × 4 m dimension, and operated automatically by a programmable timer from 9 a.m. to 8 p.m., with 10 min spraying per hour.

15.5.2 Collection and Characterization of Fusarium graminearum *Isolates*

The inoculum used for artificial inoculation is a mixture of five *F. graminearum* isolates, which have been characterized in terms of chemotype, DON productivity, and aggressiveness (He et al. 2013). To keep the pathogen viable and virulent for the field inoculation, around 70–90 *Fusarium* strains were collected every year in late summer from naturally infected wheat spikes in different places in Mexico. The new strains were characterized firstly through a series of diagnostic PCR assays using species-specific and chemotype-specific molecular markers to select DON-producing *F. graminearum* strains. This is a rapid and accurate method compared with the traditional micro- and macro- morphological characterization approaches. Based on our results, the biodiversity within both *Fusarium* genus and *F. graminearum* species is low in Mexico. For example, 82 *Fusarium* isolates were collected in 2007, of which 70 were identified as *F. graminearum*, and all of them were characterized as 15-acetyl-DON producer using the multiplex PCR assay for DON/NIV specific primers (He et al. 2013).

For those DON-producing *F. graminearum* isolates, a rice medium cultivation assay was employed to determine their DON productivity. The isolates were inoculated on 30 g polished rice and incubated for 2 weeks, and then DON levels were measured for the selection of isolates with high DON production (He et al. 2013).

Around ten strains with high DON production were selected and evaluated in greenhouse for their aggressiveness to two resistant (Sumai 3 and Heilo) and three susceptible cultivars (SERI/CEP80120, BCN//DOY1/*Ae. squarrosa* (447), and Gamenya). Two to four *F. graminearum* strains with known aggressiveness that had been used for field inoculation before were used as controls in greenhouse tests.

The spikes were evaluated at 7, 14, and 21 days post inoculation (dpi) by counting symptomatic spikelets and rachis segments.

Based on DON productivity and aggressiveness, four highly ranked isolates were selected and mixed with a control strain with known aggressiveness to compose the new inoculum for the year's field screening. This yearly renewed system effectively avoids the reduction in pathogen's aggressiveness and vitality during long-term storage, and ensures a consistent disease pressure and epidemic mode that leads to more comparable results across different years.

15.5.2.1 Field Inoculation and Phenotyping Assays

At anthesis, ten spikes of each line (5 per row) are tagged by coloured sticky tape in the morning, and the lines are spray inoculated in the afternoon using precision CO_2 backpack sprayers with flat fan nozzle and a pressure of 40 psi and a rate of 39 ml per meter. The inoculation is repeated 2 days later. Twenty five days after inoculation, FHB symptoms are scored on the ten tagged spikes by counting the numbers of total and infected spikelets of each spike. Subsequently, the FHB index can be calculated by the following formula: FHB index (%) = (severity × incidence)/100 (Stack and McMullen 1994), where *severity* is the averaged percentage of symptomatic spikelets, and *incidence* is the percentage of spikes which show infection. In addition, phenology traits like date of anthesis, plant height and spike morphology characteristics are also recorded. Due to the high cost of DON assay, only a subset of materials that showed low FHB indices, desired agronomic characteristics, and genetic diversity based on pedigree information are tested for DON concentration. After harvest, a sample of 20 g grain is milled, from which a 2 g sub-sample is taken for DON testing using the Ridascreen® Fast DON ELISA kit (R-Biopharm, Darmstadt, Germany).

15.6 Development of International Nurseries

15.6.1 Strategy for Developing the Fusarium Head Blight Screening Nursery

The general workflow for developing a FHBSN is presented below and summarized in Fig. 15.1

1. The candidate lines (approximately 2,000 + entries) are tested in the 1st year screening in the summer cycle (May-September) at El Batán in 1 m double rows. Due to the large number of entries to be evaluated, the screening is done without replications. FHB disease evaluation is performed and only promising material (500 + entries) are selected for future screening. Due consideration is given to pedigree information to maintain genetic diversity in the selected materials.

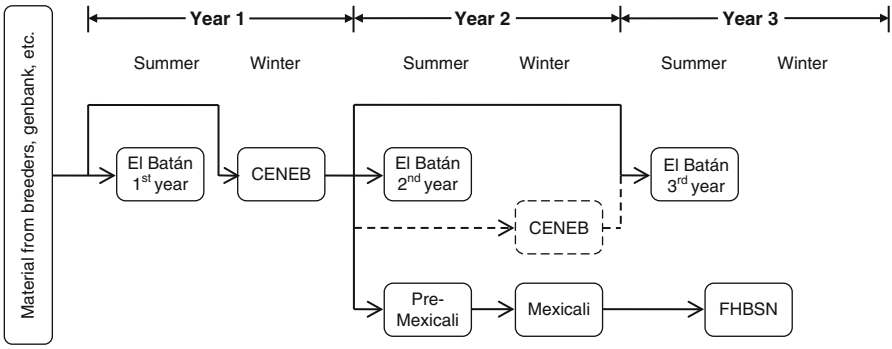

Fig. 15.1 The workflow for the development of a Fusarium head blight screening nursery

2. Since the amount of initial seed is normally limited and the quality of the seed derived from the screening plots is not adequate and have poor germination and vigor, a seed increase for the selected seed from the 1st year screening is done in the winter cycle (November–April) at the Centro Experimental de Norman E. Borlaug (CENEB) station, near Ciudad Obregón at 39 masl, latitude 27°N, with average annual rainfall of 330 mm.
3. The CENEB seed goes back to El Batán for the summer cycle and is planted in two plots:
   (a) Screening plot, in which lines are planted in replications (normally two) and are tested in the 2nd year screening. Promising germplasm (250 + genotypes) is selected for future screening.
   (b) Pre-Mexicali plot, which is fungicide treated to provide clean seed for the seed increase in Mexicali (due to the risk of Karnal bunt infection, seed cannot go directly from CENEB to Mexicali or any other place).
4. After data analysis of the 2nd year screening, selection is done to reduce the number of lines again and only keep promising materials which has proved to be valuable or interesting in 2 years of screening. From now on the selected lines are assembled in a set called "Candidates for the FHBSN" with the according number of the FHBSN (i.e. C13FHBSN). DON contamination is evaluated for the candidate lines (250 + entries). Based on DON contamination information, a further selection of superior genotypes is conducted (200 + entries).
5. Only the candidate lines for the FHBSN are sent from the Pre-Mexicali plot to CIMMYT's field station in Mexicali for seed increase for international distribution.
6. The candidates for the FHBSN are planted in the 3rd year in El Batán in the summer cycle with remnant seed from CENEB. The screening is done in two replications.
7. After data analysis of the 3rd year screening, a selection is made based on FHB disease score and both replicates are tested for DON analysis. Lines showing consistent low DON and disease score are selected and haplotyped. Based on

results from FHB testing involving disease evaluation, DON contamination and haplotyping, diverse genetic materials are assembled for the FHBSN.

8. After the final list of the FHBSN entries is made, the seed from Mexicali is sent to the Seed Inspection and Distribution Unit for seed health check and then distributed worldwide as an international nursery.

15.6.2 The Identification and Characterization of the 14th Fusarium Head Blight Screening Nursery

The 14th FHBSN is presented here as an example to show how a FHBSN is developed.

Based on FHB index and pedigree information, 37 lines with low FHB infection and diverse origin were selected in 2010 from the 468 elite wheat entries of different breeding programs. The lines were compiled as candidates for the 14th FHBSN (C14thFHBSN) and were planted in 2011 with three replications. DON data for the C14th FHBSN were available from 2009 as an additional selection parameter, given the fact that the correlation between FHB index and DON content may not be strong in some experiments (Schlang et al. 2009a) and the growing concern on food safety. There were several candidates that showed high DON content despite their low FHB indices, demonstrating the importance of DON tests. Based on the 2010 data of FHB index and DON content, a further selection was made in 2011, and 20 entries were selected as members of the 14th FHBSN (Table 15.3). In 2012, the 20 entries of the 14th FHBSN were tested again at El Batán and were distributed worldwide, and feedbacks will be available in 2013.

Unlike the 13th FHBSN, which showed significant correlation of FHB index among different years (He et al. 2013), 14th FHBSN did not show this correlation, and the entry ranks differed from year to year (Table 15.3). The reason may be attributed to the similar genetic resistance potential of the entries, which were confounded by environment factors through G×E interaction, leading to different disease levels. There were several lines with disease levels similar to the two resistant controls, Sumai 3 and Gondo/CBRD, in 2009, 2011, and 2012; but not in 2010, when very severe epidemic occurred (Table 15.3). Therefore, the resistant levels of the 14th FHBSN entries were still not as good as the two resistant checks, which showed high and stable resistances across all years. There is still long way to go to increase the FHB resistance of FHBSNs to the level similar to Sumai 3, especially under high disease pressure.

In conclusion, the approach for developing a FHBSN is efficient and effective as shown through the development of the 14th FHBSN, whose members showed satisfactory resistance in terms of both field FHB symptom and DON content in diverse environments. The FHBSNs have been distributed to both developed and developing countries, serving as an important FHB resistance source for national breeding programs to combat the disease through development of resistant varieties with superior agronomic and quality characteristics.

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References

Bai GH, Shaner G (2004) Management and resistance in wheat and barley to *Fusarium* head blight. Annu Rev Phytopathol 42:135–161

Ban T, Kishii M, Ammar K, Murakami J, Lewis J, William M, Peña RJ, Payne T, Singh R, Trethowan R (2005) CIMMYT's challenges for global communication and germplasm enhancement for FHB resistance in durum and bread wheat. In: Proceedings of the National Fusarium Head Blight Forum, pp 6–10

Bekele G, Singh RP, Alcala M (1988) Results of the first international scab resistance screening nursery (SRSN) 1985–86. CIMMYT, Mexico

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. Plant Breed 128:1–26

Capettini F, Grando S, Ban T, Valkoun J (2006) Searching for novel sources of resistance to Fusarium Head Blight in barley. In: Ban T, Lewis JM, Phipps EE (eds) The global *Fusarium* initiative for international collaboration: a strategic planning workshop. CIMMYT/El Batán, Mexico, pp 118–120

CIMMYT (1984) CIMMYT report on wheat improvement 1982. CIMMYT, Mexico

CIMMYT (1985) CIMMYT report on wheat improvement 1983. CIMMYT, Mexico

CIMMYT (1986) CIMMYT report on wheat improvement 1984. CIMMYT, Mexico

CIMMYT (1988) CIMMYT report on wheat improvement 1985–1986. CIMMYT, Mexico

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112:1465–1472

Cuthbert PA, Somers DJ, Brulé-Babel A (2007) Mapping of *Fhb2* on chromosome 6BS: a gene controlling *Fusarium* head blight field resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:429–437

Duveiller E, Mezzalama M, Murakami J, Lewis J, Ban T (2008) Global Fusarium networking. Cereal Res Commun 36:11–19

FAO (2010) http://www.fao.org/news/story/en/item/44570/icode. Accessed July 2013

Fischer R, Edmeades GO (2010) Breeding and cereal yield progress. Crop Sci 50:85–98

Gilchrist L, Rajaram S, Mujeeb-Kazi A, GinkeI M, Vivar H, Pfeiffer W (1997) Fusarium scab screening program at CIMMYT. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) *Fusarium* head scab: global status and future prospects. CIMMYT, Mexico, pp 7–12

Gilchrist L, Velazquez C, Mujeeb-Kazi A (1999) Resistance to Fusarium head blight in synthetic hexaploid wheats (2n=6x=42, AABBDD). In: Proceedings of the National Fusarium Head Blight Forum, pp 162–164

Gilchrist L, Hede A, Gonzalez R, Lopez RM (2002) Sources of combined resistance to Fusarium head blight, stripe rust, and BYD in triticale. In: Proceedings of the National Fusarium Head Blight Forum, pp 242–244

He ZH, van Ginkel M, Gilchrist L, Rajaram S (2000) Progress of China/CIMMYT shuttle breeding and germplasm exchange aimed at combining high yield potential with scab resistance. In: Proceedings of the National Fusarium Head Blight Forum, Cincinnati, USA, pp 264–268

He X, Lillemo M, Singh PK, Shi J, Bjørnstad Å, Duveiller E (2012) Characterization of non-Sumai 3 FHB resistance sources at CIMMYT Mexico. In: Proceedings of the 4th international symposium on Fusarium Head Blight, Nanjing, China, p 28

He X, Singh PK, Duveiller E, Schlang N, Dreisigacker S, Singh RP (2013) Identification and characterization of international *Fusarium* head blight screening nurseries of wheat at CIMMYT Mexico. Eur J Plant Pathol 136:123–134

Ireta J, Gilchrist L (1994) Fusarium head scab of wheat (*Fusarium graminearum* Schwabe), Wheat special report (21b). CIMMYT, Mexico

Kishii M, Delgado R, Rosas V, Cortes A, Cano S, Sanchez J, Mujeeb-Kazi A, Lewis J, Ban T (2006) Utilization of wild genetic resources for the improvement of FHB resistance in wheat breeding. In: Ban T, Lewis JM, Phipps EE (eds) The global *Fusarium* initiative for international collaboration: a strategic planning workshop. CIMMYT/El Batán, Mexico, pp 24–27

Kosová K, Chrpová J, Šíp V (2009) Cereal resistance to *Fusarium* head blight and possibilities of its improvement through breeding. Czech J Genet Plant Breed 45:87–105

Lewis JL, Velazquez C, Murakami J, Capettini F, Ban T, Ward RW (2006) Facilitation of international Fusarium nurseries and improvements of FHB screening system at CIMMYT. In: Proceedings of the National Fusarium Head Blight Forum, p 109

Lewis JM, Trethowan R, Ban T, Duveiller E, Ward R (2007) Preliminary examination of the influence of grain color in FHB resistance. In: Proceedings of the National Fusarium Head Blight Forum, Kansas City Missouri, USA, p 193

Liu SY, Hall MD, Griffey CA, McKendry AL (2009) Meta-analysis of QTL associated with Fusarium Head Blight resistance in wheat. Crop Sci 49:1955–1968

Liu Y, He Z, Appels R, Xia X (2012) Functional markers in wheat: current status and future prospects. Theor Appl Genet 125(1):1–10

Mesterhazy A (1995) Types and components of resistance to *Fusarium* head blight of wheat. Plant Breed 114:377–386

Mesterhazy A (2010) Control of mycotoxin contamination in cereals by breeding. In: Rai M, Varma A (eds) Mycotoxins in food, feed and bioweapons. Springer, Heidelberg, pp 163–177

Mesterhazy A, Bartok T, Mirocha CG, Komoroczy R (1999) Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110

Mujeeb-Kazi A, Delgado R, Cano S, Rosas V, Cortés A (2000) Alien genetic diversity for wheat improvement: focus on scab resistance. In: Proceedings of the National Fusarium Head Blight Forum, pp 220–224

Murakami J, Lewis J, Egusa M, Gargouri S, Kodama M, Duveiller E, Ban T (2008) Evaluation of a real-time quantitative PCR assay for an effective screening of genotypes with FHB resistance and low mycotoxin accumulation in wheat and barley. Cereal Res Commun 36:147–148

Ortiz R, Braun H-J, Crossa J, Crouch JH, Davenport G, Dixon J, Dreisigacker S, Duveiller E, He Z, Huerta J, Joshi AK, Kishii M, Kosina P, Manes Y, Mezzalama M, Morgounov A, Murakami J, Nicol J, Ortiz Ferrara G, Ortiz-Monasterio JI, Payne TS, Peña RJ, Reynolds MP, Sayre KD, Sharma RC, Singh RP, Wang J, Warburton M, Wu H, Iwanaga M (2008) Wheat genetic resources enhancement by the International Maize and Wheat Improvement Center (CIMMYT). Genet Resour Crop Evol 55:1095–1140

Parry DW, Jenkinson P, Mcleod L (1995) *Fusarium* Ear Blight (Scab) in Small-grain cereals – a review. Plant Pathol 44:207–238

Qi LL, Pumphrey MO, Friebe B, Chen PD, Gill BS (2008) Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to *Fusarium* head blight disease of wheat. Theor Appl Genet 117:1155–1166

Rajaram S (2001) Frontier science in the CIMMYT wheat program (2001–05). In: Reeves J, McNab A, Rajaram S (eds) Proceedings of the Warren Kronstad symposium. CIMMYT, Mexico, pp 87–94

Schlang N, Mezzalama M, Chao S, Dreisigacker S, Duveiller E (2009a) Results from the second Fusarium International Spring Wheat Nursery (FIEPSN). In: Canty S, Clark A, Mundell J, Walton E, Ellis D, Van D, Sanford DV (eds) Proceedings of the National Fusarium Head Blight Forum. University of Kentucky, Orlando, p 145

Schlang N, Mezzalama M, Payne T, Duveiller E (2009b) Screening for new sources of *Fusarium* head blight resistance in Chinese wheats from the CIMMYT germplasm bank. In: Canty S, Clark A, Mundell J, Walton E, Ellis D, Van D, Sanford DV (eds) Proceedings of the National Fusarium Head Blight Forum. University of Kentucky, Orlando/Lexington, p 146

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella Zeae*. Phytopathology 53:831–838

Singh RP, van Ginkel M (1997) Breeding strategies for introgressing diverse scab resistances into adapted wheats. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, Mexico, pp 86–92

Singh RP, Ma H, Rajaram S (1995) Genetic-analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis 79:238–240

Stack RW, McMullen MP (1994) A visual scale to estimate severity of Fusarium head blight in wheat. North Dakota State University Extension Service, p 1095

USDA, Agricultural Research Service (2006) USDA National Nutrient Database for Standard Reference, Release 19. http://www.ars.usda.gov/Services/docs.htm?docid=15973

van Ginkel M, Gilchrist L (2002) How to make intelligent crosses to accumulate Fusarium Head Blight resistance genes based on knowledge of the underlying resistance mechanisms. In: Proceedings of the National Fusarium Head Blight Forum, Cincinnati, USA, pp 268–272

van Ginkel M, van der Schaar W, Yang ZP, Rajaram S (1996) Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis 80:863–867

van Ginkel M, Gilchrist L, Velazquez C (2000) New resistances in CIMMYT bread wheat germplasm. In: Proceedings of the National Fusarium Head Blight Forum, pp 297–302

van Ginkel M, Gilchrist L, Capettini F, Kazi M, Pfeiffer W, William M, Ban T, Lillemo M (2003) Breeding for Fusarium head blight resistance: an international approach. In: Proceedings of the National Fusarium Head Blight Forum, Minneapolis, USA, pp 289–293

van Ginkel M, Gilchrist L, Capettini F, Mujeeb-Kazi A, Pfeiffer W, Manilal W, Ban T, Lillemo M (2004a) Breeding for global resistance to FHB. In: Ban T (ed) Collaborative research for *Fusarium* Head Blight resistance in wheat and barley. Japan International Research Center for Agricultural Sciences, Tsukuba, pp 19–24

van Ginkel M, William M, Lillemo M (2004b) CIMMYT's FHB field research approach, evidence for host-by-location interaction and potentially new genetic diversity in resistance in wheat. In: Proceedings of the 2nd international symposium on Fusarium Head Blight, pp 200–202

Xue S, Li GQ, Jia HY, Xu F, Lin F, Tang MZ, Wang Y, An X, Xu HB, Zhang LX, Kong ZX, Ma ZQ (2010) Fine mapping *Fhb4*, a major QTL conditioning resistance to *Fusarium* infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 121:147–156

Xue S, Xu F, Tang M, Zhou Y, Li G, An X, Lin F, Xu H, Jia H, Zhang L, Kong Z, Ma Z (2011) Precise mapping *Fhb5*, a major QTL conditioning resistance to *Fusarium* infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 123:1055–1063

Zhu Z, Bonnett D, Ellis M, Heslot N, Gao C, Yu D (2012) Characterization of putatively novel resistances to Fusarium head blight from a synthetic hexaploid bread wheat. In: Proceedings of the 4th international symposium on Fusarium Head Blight, Nanjing, China, p 54

Chapter 16
Resistance to Fusarium Head Blight in South American Wheat Germplasm

Man Mohan Kohli and Martha Díaz de Ackermann

Abstract Fusarium Head Blight (FHB) identified in the early part of the twentieth century in South America, remained relatively irregular in appearance till the 1980s. However, the early epidemics recorded in Argentina, Brazil and Uruguay must have been severe enough to wipe out production, thereby forcing wheat breeders to look for sources of resistance among the local varieties and/or landraces that survived. It is these old landraces such as Barletta or Lin Calel and Americano selections from La Plata River basin as well as Polyssu and Alfredo Chaves lines from Brazil that formed the initial base of subsequent commercial varieties with moderate resistance to FHB. While the genetic basis of such locally selected resistance has not been researched, it received a further boost from the introduction of two sister lines from Italy, Ardito and Mentana, which led to development of world famous Frontana and other varieties. Both sister lines have 50 % of contribution from a Japanese variety Akagomoughi used as male parent in the cross. Since the 1970s, the Japanese and Chinese germplasm, firstly NobeokaBozu, Nyu Bay and Pekin 8 distributed from Brazil and lately Sumai#3, Catbird and many others distributed by International Wheat and Maize Improvement Center, CIMMYT, in the form of international nurseries have become the backbone of the FHB resistance in the region. Recently, the national wheat breeding programs are exploring the role of synthetic wheats and other alien species to widen the base of FHB resistance and also combine it with additional sources for low mycotoxin generation to safeguard the human and animal health.

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16.1 Introduction

Fusarium Head Blight (FHB) of wheat caused mainly by *Fusarium graminearum* (Schw.) [telemorphic stage *Gibberella zeae* (Schw.) Petch] is an important grain production and quality limiting disease in the Southern Cone region of South America (Kohli 1989, 1999; Reis and Kohli 1993). The disease manifesting itself in severe spike infections, especially in the wet years, is common and predominant in Argentina, Brazil, Paraguay and Uruguay. In Chile, it is more important as a root disease (*Fusarium* crown root) than in the spike. FHB has also been observed in the mid altitude valleys of Bolivian highlands but not in the lowlands where another spike disease (Wheat Blast) caused by *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) (Kato et al. 2000; Couch and Kohn 2002; Tosa et al. 2004) has recently become important.

While *Fusarium graminearum* is the most predominant species isolated from the grains of wheat and barley, other species such as: *F. avenaceum, F. equiseti, F. culmorum, F. poae* have also been isolated from wheat and *F. poae, F. equiseti, F. avenaceum, F. trincictum, F. sporotrichioides* from barley (Survey Reports 2001 and 2002 INIA Uruguay).

The FHB causing fungus has been found to be present and reproducing in the stubble of wheat, barley, maize, sorghum, millet, dried remains of grass weeds [Bermuda grass (*Cynodon dactylon*) and Crab grass (*Digitaria sanguinalis*) etc.] and pasture species such as *Festuca* sp. and *Lolium* sp. in the cattle farming region of the western coastal, litoral, region of Uruguay. However, the stubble of different species contributes differently to the primary inoculum load. For example, wheat stubble contributes more inoculum than barley which, in turn, contributes more than maize and grassy weeds. Similarly, wheat and barley stubbles left on the soil surface after the harvest, contribute inoculum for a longer period (24–30 months) in comparison to the buried stubble doing so for a period of 12–18 months. The superficial maize stubble contributes to inoculum up to a period of 48 months after the harvest.

The first observation of the FHB pathogen was reported in Argentina in 1927, though it took almost two more decades for the first epidemic to be recorded in 1945 (Galich 1989). In Uruguay, on the other shore of the La Plata River, the rainfall pattern and climatic conditions, in general, are a lot more favorable for the FHB epidemics to develop. Thus it seems a little odd that the pathogen was first reported by Dr. Alberto Boerger in 1928 (Boerger 1928) while the first outbreak occurred in 1977 (Tavella et al. 1979). In Brazil the first FHB epidemic was reported in 1939 (Luzzardi and Pierobom 1989) and in Paraguay in 1972 (Viedma 1989).

During the early and mid-twentieth century, the FHB was considered a sporadic disease appearing only in some years. However, its occurrences in the region have increased significantly since the 1970. For instance, the frequency of an FHB epidemic to occur in Uruguay between 1915 and 1977 was one in 16 years. During 1915–1993, the frequency of appearance increased to one in 11 years and to one in 8 years between 1915 and 2001. In general, FHB epidemics occur in the years with

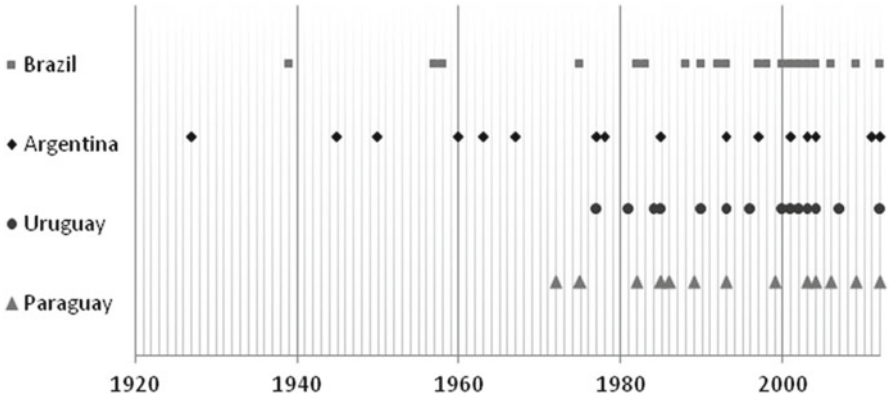

Fig. 16.1 Frequency of reported FHB epidemics in the Southern Cone Region

humid and warm weather conditions at the flowering time. However, over the last two decades, there have been 7 FHB epidemics of different intensities in Argentina, 12 in Brazil, 7 in Paraguay and 10 in Uruguay. This increased frequency of epidemics has been mostly associated with the intensification of agriculture and the seeding of wheat in the maize stubble under zero tillage management, Fig. 16.1.

Given the history of the disease in the region, various wheat breeding programs have identified and selected a number of varieties and landraces which demonstrate a high degree of FHB resistance over the years. Some of these landraces, tested by the Chinese scientists under their conditions, were reported as highly resistant in the greenhouse and moderately resistant under field conditions (Weng et al. 2001, 2003; Zhang and Jin 2002, 2003). More recently, regional and international networks of germplasm have allowed identification of several genetic sources with acceptable level of FHB resistance both under field and greenhouse conditions.

16.2 Earliest Sources of Genetic Resistance to Fusarium Head Blight Identified in the Southern Cone

16.2.1 La Plata River Region

The traditional germplasm of the La Plata River region derives its resistance to FHB from the landraces and local varieties brought in by the European immigrants upon their arrival to this continent. The fact that most of the older germplasm based on the landraces such as Barletta (or its selection Lin Calel MA) and its crosses with Americano 44D (Klein Universal II), led to the development of first Argentine resistant varieties Klein Sin Rival and Klein Vencedor in the 1920s strengthens the importance of the older landlines and germplasm. It is quite

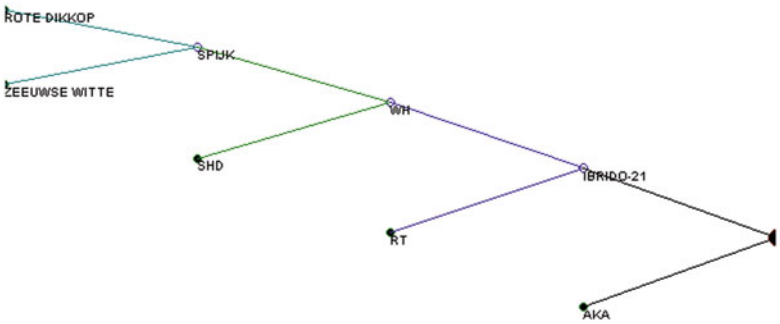

Fig. 16.2 Dendrogram of the Italian variety Ardito used as a source of resistance to FHB in Argentina

possible that the resistance of these varieties was demonstrated in the epidemic of 1927 making them the basis of future improvements. Another older introduction, Ardito, also played an important role in the progress of Argentine germplasm. Developed by famous Italian wheat breeder Nazareno Strampelli, Ardito probably derives its resistance to FHB from Japanese variety Akagomoughi (Fig. 16.2). However, the contribution of other varieties in the background such as Chino (probably Chinese Spring), Bage 1971, Bage 2018, Marquis and others, used in the wheat breeding programs and development of FHB resistant germplasm, is less understood. These varieties were used to develop other moderately resistant FHB lines such as 38MA, Klein Sinmarq, Klein 47, Klein 66, Klein Otto Wulf, Vencelel MA, Magnif Entreriano etc.

All the above mentioned varieties were used in various combinations with other introductions and/or local germplasm to develop commercial varieties of the 1960s. The FHB epidemics of 1960, 1963 and 1967 (Fig. 16.1) helped identify the next set of superior varieties such as, Klein Atlas, Vilela Sol, Oncativo INTA, Pergamino Gaboto and Tezanos Pintos Precoz, which demonstrated a moderate to high level of FHB resistance under field conditions. The dendrograms showing their genealogy going back five or six generations help to confirm the importance of earlier identified land races and advanced lines in the development of FHB resistant varieties (Figs. 16.3, 16.4, 16.5 and 16.6). It is significant to mention that all these varieties, except for Pergamino Gaboto, exhibit spike infections consistent with resistance Types I (penetration) and II (spread) as described by Schroeder and Christensen (1963). The variety Pergamino Gaboto, in spite of being based on Sinvalocho MA (Klein Sin Rival derivative) and two Brazilian lines, seems to show only Type I resistance (V de Galich 1997).

In Uruguay, the selection of the popular variety Pelon 33C (1918) from an unknown local variety is an interesting case as it was used very widely in the wheat breeding programs on both sides of the La Plata River. Although, any possible resistance to FHB in this variety has not been determined, it did participate with

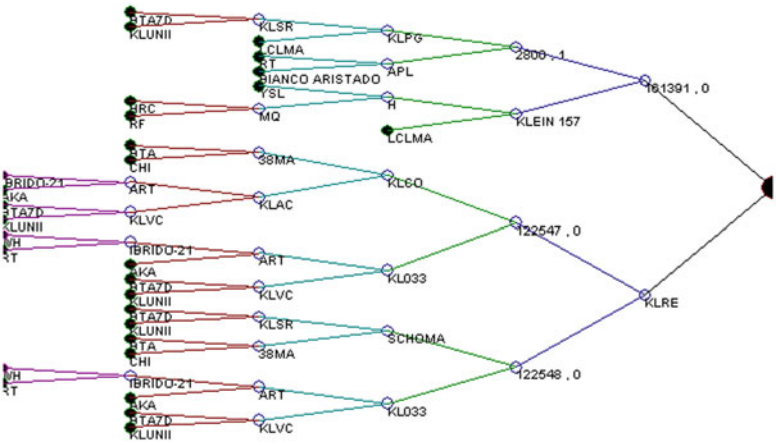

Fig. 16.3 Dendrogram of FHB resistant variety Klein Atlas

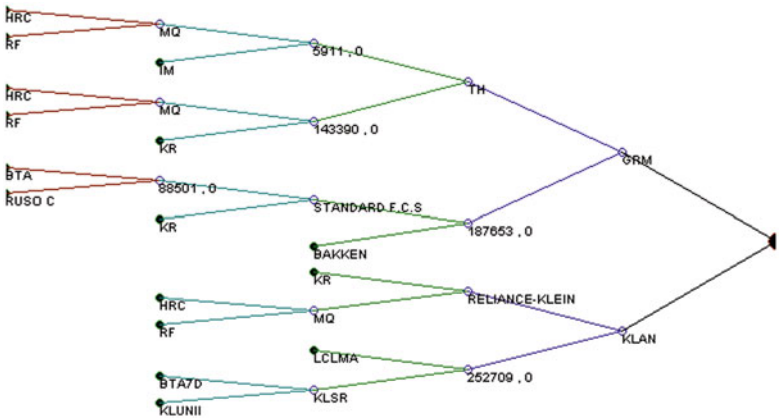

Fig. 16.4 Dendrogram of FHB resistant variety Vilela Sol

Argentine variety 38 MA to develop a set of Litoral varieties in Uruguay during the mid-1930s, a period when the first FHB resistant varieties were being released in Argentina.

Given that famous Argentine wheat breeder Enrique Klein (creator of the Klein group of varieties) had started his initial wheat research in collaboration with Dr. Alberto Boerger at La Estanzuela, Uruguay, both countries shared a similar set of germplasm. The first FHB infection in Uruguay was observed 1 year after its report in Argentina (1927), which allowed Dr. Boerger to take advantage of the germplasm being identified and selected in Argentina. Thus, the variety Petiblanco, a selection

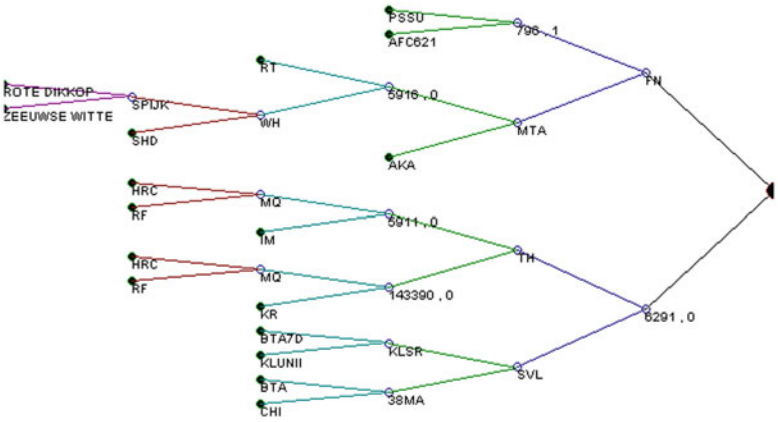

Fig. 16.5 Dendrogram of FHB resistant variety Tezanos Pintos Precoz

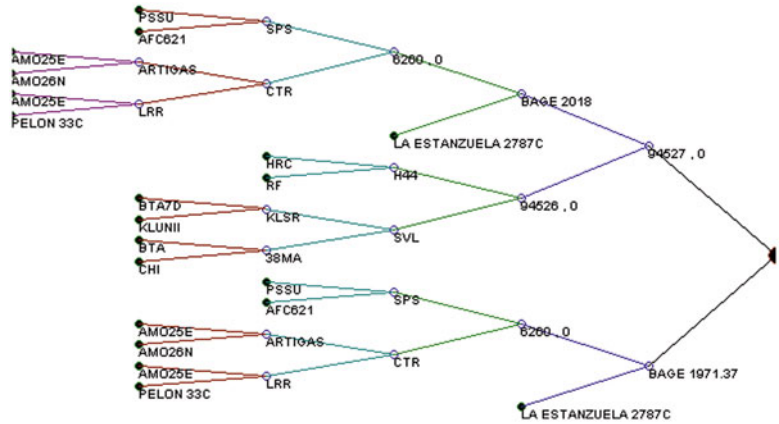

Fig. 16.6 Dendrogram of FHB resistant variety Pergamino Gaboto

from a mixed Petiso line derived from the cross Ardito/Kl Vencedor was recognized as one of the earliest sources of FHB resistance. In Argentina, Dr. Klein had released Klein 033, Kl. Acero and Kl. Palantelen from the same cross a few years earlier (see dendrogram for Klein 033 in Fig. 16.3). Weng et al. (2001) studied several landraces and five Petiblanco derived lines from Sinvalocho/Petiblanco cross. All of them were moderately resistant in the field and very resistant to point-inoculation in the greenhouse.

Considering the relative absence of FHB epidemics in the La Plata River basin for a long time, till the 1960s, it was then that the Uruguayan wheat breeding

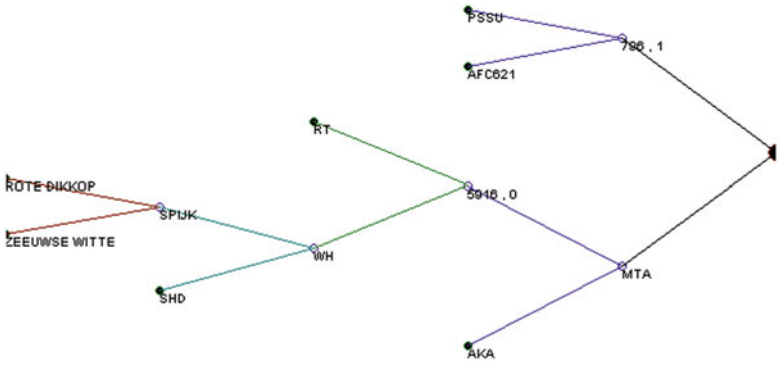

Fig. 16.7 Dendrogram of FHB resistant variety Frontana

program released a new variety EstanzuelaDakuru (Lee/North Dakota 34), with moderate resistance to FHB.

16.2.2 Brazil

Like in the cases of Argentina and Uruguay, it is quite likely that the first FHB infections in Brazil occurred in the 1920s, well before the first reported epidemic in 1939. It was during this period (1920s) that the first varieties such as Polyssu and a set of Alfredo Chavez lines, considered moderately resistant to FHB, were selected from the local wheats. These local wheats or land races were probably brought in by the immigrants in the State of Rio Grande do Sul and selected over generations as the survivors to local conditions and pathogen populations. Dr. Ivar Beckman, at the Experimental Station in Bage, Rio Grande do Sul, who also maintained close contacts with Dr. Boerger in Uruguay, crossed Polyssu and Alfredo Chavez lines, especially AFC 6.21 that led to the development of several varieties (Fronteira, Frondoso, Guarany, Minuano, Missões, Surpresa, etc.) in the 1930s. It was the cross of Fronteira with Mentana (a sister line of Ardito from Italy) that led to the development of world renowned Frontana variety in 1943 (Fig. 16.7). Besides resistance to FHB, Frontana has been widely used as a source for adult plant resistance to leaf rust of wheat and pre-harvest sprouting.

After the 1939 epidemic in the state of Rio Grande do Sul, the next severe epidemic did not occur till 1957–1958. Since then, the infections became more regular and of variable intensities in the southern states of Rio Grande do Sul, Santa Cartarina and Parana. Given this new importance of the disease in the major wheat growing region, research efforts to reduce the losses were also renewed and strengthened. The initial screening results showed that the varieties Frontana and

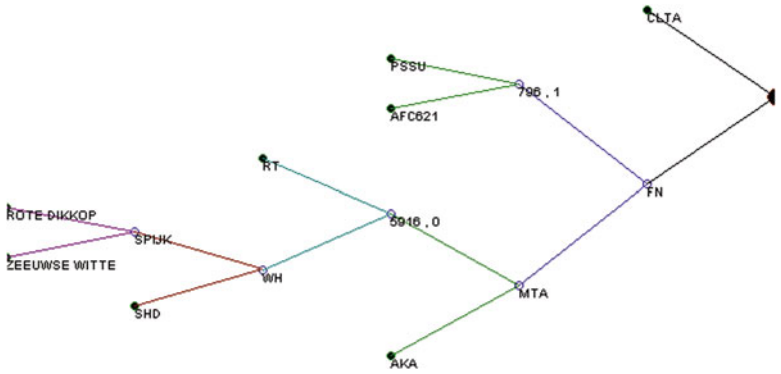

Fig. 16.8 Dendrogram of Frontana derived varieties Colotana, Carazinho and Preludio

three varieties derived from its cross with Colonista variety (Colotana, Carazinho and Preludio) demonstrated high level of resistance to the disease and could be considered good sources of resistance. Three other varieties derived from the same cross (Colonista/Frontana) viz. Fortaleza, Piratininga, Trapeano were not analyzed during the study. The dendrograms of sister varieties Colotana, Carazinho and Preludio and is presented in the Fig. 16.8. However, later screening under field conditions and in epidemic years demonstrated that neither Colotana nor Preludio or Carazinho matched the level of resistance shown by Frontana. In the mid-1960s, another variety Toropi (or S1) developed by Dr. B.O. Paiva combined the FHB resistance of Frontana, Quaderna (Mentana derivative) from Italy and Petiblanco from Uruguay (FN/QUADERNA A//PETIBLANCO 8) and remains till date an excellent source of resistance to FHB disease besides maintaining its leaf rust resistance for almost 50 years.

In order to strengthen the FHB research effort in Brazil in the 1960s, a Japanese scientist, Dr. Motooki Nakagawa was invited to join the Southern Agricultural Research Institute (IPEAS) team. It was Dr. Nakagawa who introduced new sources of resistance (NobeokaBozu, Nyu Bay and Pekin 8) from Japan and China to widen the genetic variability for FHB resistance available in the local lines. Together with these new sources, he also introduced artificial inoculation techniques to screen germplasm and identify better level of resistance. However, the incorporation of these exotic sources into the Brazilian germplasm resulted to be quite difficult due to poor agronomic characters of the Japanese lines. Later with the creation of Brazilian Agricultural Research Corporation (EMBRAPA) in 1973, the National Wheat Research Center (EMBRAPA TRIGO) initiated a backcross program in 1975 to introduce this exotic FHB resistance in a good agronomic background. One of the backcross lines from this program (Londrina*3/Nyu Bay) is a parent of the cultivar EMBRAPA 27 (PF869107) which has been used widely by the wheat breeding programs in the recent times.

16.2.3 Paraguay

Wheat was introduced into Paraguay during the colonial times; however, the crop did not get established here probably due to high temperature regime and disease epidemics favored by high humidity (Kohli et al. 2011). The first local varieties (Jhiá´seVeva, JhiáVeva and JhosáVeva) released in the 1950s were probably selected from the Brazilian germplasm (Frontana derived) and did not suffer the FHB epidemics that spread through Brazil during the 1950s and 1960s. However, their low yield and poor agronomic type did not encourage local farmers to take up wheat production seriously. It was after the creation of the National Wheat Program in 1966 and the release of new semi-dwarf high yielding varieties (P128, P214, P281) that the first FHB epidemic was recorded in 1972 (Viedma 1989). The hot and humid climatic conditions of 1972 were not only responsible for the large scale spread of FHB but also of septoria glume blotch which brought the national average yield to 547 kg/ha. As a result, the wheat area dropped from 51,500 ha in 1971 to 20,300 ha in 1973.

It was under this epidemic that two lines from the International Rust Nursery were identified to have lower FHB infection and released as Itapúa 1 (MGF/2*FCR/4/N/MT//MT/K/3/BAGE/5/FCR) and Itapua 2 (LEE/ND34//ND81/FCR). It is interesting to observe that the resistance of Itapúa 1 was based on a combination of FHB resistant sources from Argentina (Magnif), Brazil (Bage), Japan (Akagomoughi), Italy (Rieti), USA (Froccor) and Canada (Newthatch), Table 16.1. The cultivar Itapúa 2 derives its resistance entirely from the germplasm from North Dakota, USA, which was not as good as that of Itapúa 1. As a result and in spite of its higher yield, Itapúa 2 variety did not succeed commercially.

After another disastrous epidemic in 1975, the program was able to identify another advanced line from International Maize and Wheat Improvement Center, CIMMYT, to be moderately resistant to FHB and released it commercially as Itapúa 25 in 1979. The resistance of Itapúa 25 is based on the Argentine line Tezanos Pintos Precoz (Ardito and Barleta derived) and a Canadian line Knott2 on one side and Frontana on the other which makes it one of the first semi-dwarf varieties to have such a high level of FHB resistance, Fig. 16.9.

16.3 Widening Genetic Variability for Resistance to Fusarium Head Blight from 1970s Onwards

The intensification of agriculture during the 1970s, the introduction of semi-dwarf wheat varieties and the occurrences of Niño events in various parts of the Southern Cone region, favored several foliar disease and FHB epidemics during this decade. Brazil and Paraguay suffered from severe FHB epidemics in 1972 and 1975. In 1977, all the countries of the region suffered from the disease but its impact was more severe in Brazil and Uruguay, when the latter reported its first epidemic.

Table 16.1 Mendelogram of FHB resistant wheat variety Itapúa 1

Variety or line name	Abbreviation	Country	Percent contribution to pedigree
[a]C.O.	C.O.	BRA	17.19
[a]C.R.	C.R.	BRA	17.19
[a]AKAGOMUGHI	AKA	JPN	12.11
[a]POLYSSU	PSSU	BRA	10.55
[a]ALFREDO CHAVES 6.21	AFC621	BRA	10.55
[a]LA ESTANZUELA 2787C	LE2787C	URY	6.25
[a]RIETI	RT	ITA	6.05
[a]SANTA CATALINA	STC	BOL	3.13
[a]KENYA	K	KEN	3.13
[a]SQUAREHEAD	SHD	USA	3.03
[a]AMERICANO 25E	AMO25E	URY	1.56
[a]ROTE DIKKOP			1.51
[a]ZEEUWSE WITTE	ZW	NLD	1.51
[a]HARD RED CALCUTTA	HRC	IND	0.78
[a]RED FIFE	RF	CAN	0.78
[a]AMERICANO 26 N	AMO26N	URY	0.78
[a]PELON 33C	PEL 33C	URY	0.78
[a]IUMILLO	IM	ESP	0.68
[a]KANRED	KR	USA	0.68
[a]BARLETA 7D	BTA7D	ARG	0.39
[a]KLEIN UNIVERSAL II	KLUNII	ARG	0.39
[a]BARLETA	BTA	ARG	0.39
[a]CHINO	CHI	ARG	0.39
[a]YAROSLAV	YSL	USA	0.2

[a]Landrace

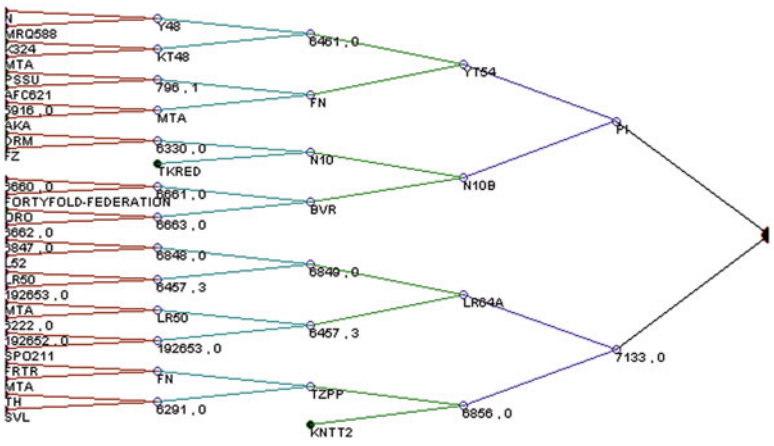

Fig. 16.9 Dendrogram of Itapua 25 variety, moderately resistant to FHB

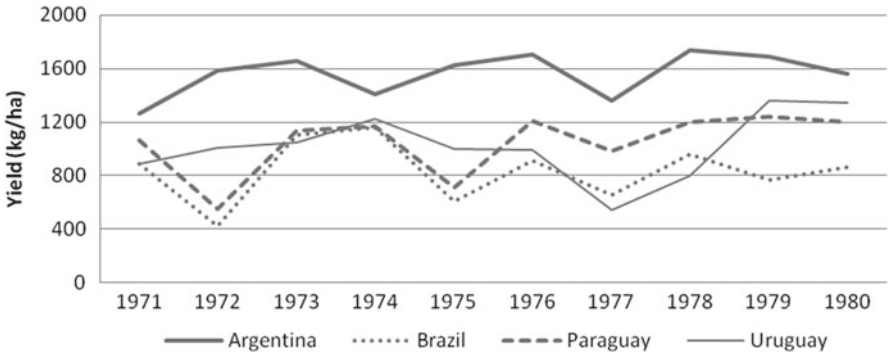

Fig. 16.10 Average yield of wheat in the Southern Cone countries during the 1970s

The average wheat yields during the epidemic years dropped to below 800 kg/ha (Fig. 16.10).

This situation served to demonstrate that the genetic variability for FHB resistance available in the region was probably not enough under severe disease pressure and needed to be strengthened. The distribution of new Japanese sources (NobeokaBozu, Nyu Bay, Abura and Pekin 8) by EMBRAPA to the regional programs started a concerted effort to explore their resistance in spite of known undesirable agronomic type, low production potential and susceptibility to others diseases. Additional sources of resistance from Brazil (Toropi, Encruzilhada, Pel 73007 and Pel 73081) were also used by several wheat breeding programs including Brazil and Uruguay (Sartori 1982).

In the late 1970s International Maize and Wheat improvement Center, CIMMYT, decided to establish a regional office in the Southern Cone. This proved to be a turning point in the availability of newer sources of resistance to FHB from 1980 onwards. Besides introducing large number of CIMMYT advanced lines with multiple disease resistance and high yield potential, the program aimed at strengthening its efforts to widen the genetic variability available for specific characters including FHB resistance.

16.4 Role of International Maize and Wheat Improvement Center and Introduction of Chinese Germplasm into Southern Cone

CIMMYT initiated its collaboration with China in the mid-1970s and soon realized the serious limitations that FHB infections placed on the adaptation of semi-dwarf wheat germplasm there. Although Chinese programs used CIMMYT wheats for their short stature, high yield potential, earliness and excellent rust resistance, yet the progeny of their crosses suffered seriously from the FHB, especially in the

Yangtze River valley basin. As a result, CIMMYT decided to start a concerted FHB research program in the early 1980s in collaboration with Chinese collaborators and using their sources of resistance. The FHB germplasm development effort in Mexico used three hot spot locations (Toluca, Patzcuaro, and Sierra del Tigre) to identify and screen for two types of resistances (Type I and Type II). The major objective of this effort was to develop high yielding germplasm with an adequate level of resistance to sustain good production in scab affected areas.

It was through this collaboration that the Chinese cultivar Sumai#3 was first introduced in the region through international nursery program in 1984. Given the problem of poor agronomic type of Sumai#3, it could not be used directly in a breeding program. Thus it was EMBRAPA, Brazil, that first started a backcross program to improve the FHB resistance of a new semi-dwarf variety BR 14 carrying resistance to aluminum toxicity. In Uruguay, Sumai#3 based resistance was introduced and selected through a Nanjing 7840 derived line (Alondra/Pavon//Nanjing7840), which demonstrated excellent adaptation under Uruguayan conditions.

The initial breeding efforts of CIMMYT with head scab were masked by the predominance of pink snow mold disease caused by *Microdochium nivale* (ex *Fusarium nivale*) in the nurseries at Toluca, Mexico. Its confusion with head scab and/or cross infections made it difficult to select for a high level of FHB resistance in the segregating populations. Once it was identified that *Fusarium graminearum* was the most important scab producing pathogen in these localities, efforts were taken to enhance its inoculum load through artificial inoculations. Further, there was a serious intent to combine the germplasm resources from the Southern Cone, Japan, and China into high yielding genotypes.

As soon as the first lines from these crosses became available, they were distributed worldwide and made their appearance in the Southern Cone region after 1986. Several CIMMYT nurseries viz. Scab Screening Nursery (SCABSN), Advanced Lines from Yangtze (AL Yangtze), Scab Resistant Screening Nursery (SRSN), and Fusarium Head Blight Screen Nursery (FHBSN), have contributed wide variability for FHB resistance to the regional germplasm. Although the exact genetic basis of resistance of the lines in the nurseries was unknown, each nursery provided a few newer sources of resistance that were selected for their adaptation to the region over the years. It is understood that most of the sources selected in the region are based on Sumai#3 and older Chinese germplasm used in the crosses.

In the 1990s, several advanced lines such as Guam92//Pheasant//Bobwhite(6SRSN 22), NG8675/Catbird (7SRSN 05), Milan/Shanghai #7 (7SRSN 07), NG8201//Kauz (7SRSN 26) and Zuo 1330 were observed to carry as good a resistance as Sumai#3(7SRSN 49). A new CIMMYT line, Catbird (Chuan Mai #18//Bagula), deriving its FHB resistance from a genetic source different from Sumai#3, was selected by virtually all the countries of the region (Fig. 16.11). Catbird, a semi dwarf line with excellent agronomic type and high yield potential, has become a parent of choice for FHB resistance in the Southern Cone region. Not all the sister lines of Catbird have the same level of resistance in all the countries of the region. In Uruguay, the line Catbird 1073 was selected after years of screening and remains till date one of the best sources of FHB resistance in the region.

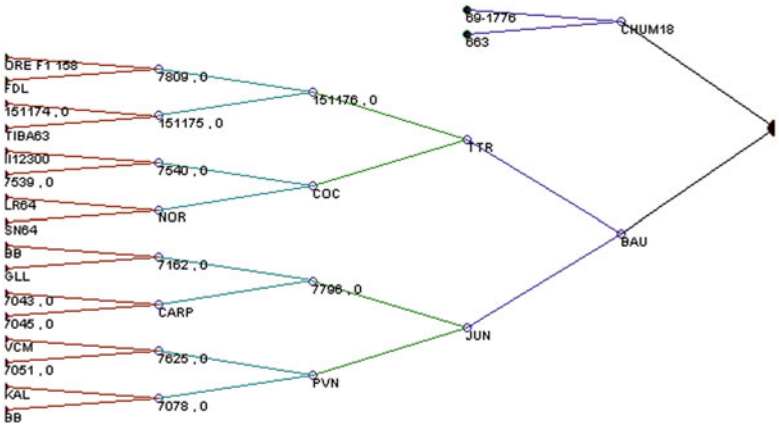

Fig. 16.11 Dendrogram of FHB resistant CIMMYT advanced line Catbird

Despite slow progress in developing high FHB resistance in good agronomic type of plant with superior yield potential, important results have been obtained. Some of the advanced lines developed through this collaborative network, viz. Ningmai# 7 (Shangai#4-3B-0Y), Longmai#19 (Long755778/Ke74-207//Long 82 H522/Alondra), and Chuanmai#25 (1414/Shuanyu5//Genaro81) were released in large areas of China, (Gilchrist et al. 1997). Another group of resistant bread wheat lines with good agronomic type viz. Bagula/Milan, Shanghai #5/Weaver, Nanjing 82149/Kauz, Golden Valley/Azteca//Musala/3/Dodo/4/Bobwhite and Chilero/ChuanMai#18, were selected in the Southern Cone region.

16.5 Fusarium Head Blight Resistance and International Collaboration

16.5.1 Sothern Cone Regional FHB Nurseries

The severe epidemics of FHB in the 1970s affecting grain production and quality served as an alert to strengthen the regional collaboration in developing better germplasm for resistance. Although an informal germplasm exchange among the countries of the Southern Cone started in 1975, formal efforts were enhanced by the opening of a Regional Office by CIMMYT in 1978. In the 1980 the National Research Programs of the region were supported by the Inter-American Institute of Agricultural Cooperation, IICA, and Inter-American Development Bank, IDB to create a Regional Cooperative Program in Agricultural Research, PROCISUR. Emulating the example of CIMMYT's Latin-American Cereals Rust Nursery, ELAR, being distributed from the Andean Region, the Southern Cone Regional Program and

PROCISUR joined hands to create a set of other regional nurseries under the auspices of the PROCISUR. Thus the first regional FHB nursery (the Southern Cone Cooperative Nursery – Scab), was established in 1981 (IICA-Cone Sul/BID (1981)), and coordinated by J.F. Sartori, (National Wheat Center, EMBRAPA, Brazil). This allowed for the exchange of a large number of early FHB resistant germplasm among the countries to incorporate them into national advanced lines.

A few years later, the CIMMYT regional office, then based in Paraguay, took over the coordination of the Southern Cone *Fusarium* Resistant Nursery (FUCOSUR) along with the national wheat program, under the same auspices. With the move of CIMMYT regional program to Uruguay in the 1990s, another regional FHB Resistant Nursery (VIRFET) was organized (2000–2002) and coordinated by CIMMYT, with the help of INIA staff. The organization of VIRFET and other regional FHB research activities were supported by the Inter-American Fund for Agricultural Research, FONTAGRO, and CIMMYT under a special project. Four countries, Argentina: INTA Marcos Juárez and INTA Pergamino; Paraguay: DIA Capitan Miranda; Uruguay: INIA La Estanzuela and México: CIMMYT, provided valuable information on resistance from field and semi controlled shaded house or greenhouse conditions. The data generated in these trials served to identify newer sources of resistance which have maintained their superior performance to the disease till date.

The information generated by the FONTAGRO supported Project: *Development of technologies for the integrated management of the Fusarium Head Blight of wheat* and VIRFET led to the identification of 60 advanced wheat lines with stable resistance to FHB in the Southern Cone region. A new Regional Project started in 2006 and supported by CIMMYT, INIA Spain, INIA Uruguay and PROCISUR was based on the 60 resistant lines mentioned above, 24 differential lines and one susceptible check variety. The objectives of the new project were to reaffirm the FHB resistance of the germplasm and also test it for the production of Deoxynivalenol (DON) toxin in Argentina, Brazil, Paraguay and Uruguay (Diaz de Ackermann 2006). Since 2006, a new FHB nursery coordinated by INIA Uruguay, with identified sources of resistance from the Southern Cone is also being tested for DON production in Argentina, Brazil, Paraguay and Uruguay.

16.5.2 International FHB Nurseries

Other international nurseries, especially those organized and distributed by the International Maize and Wheat Improvement Center, CIMMYT, such as Scab Screening Nursery (SCABSN), Advanced Lines from Yangtze (AL Yangtze), Scab Resistant Screening Nursery (SRSN), and Fusarium Head Blight Screening Nursery (FHBSN) have been tested in the region since the mid-1980s. They have been a continuous source of germplasm variability introduced from different parts of the world as well as their crosses with CIMMYT's high yielding advanced lines selected from multi-location trials.

The international nursery network has been a key to visualize the weakness of the regional germplasm selected for FHB including some of the known sources of resistance. Their interaction with the disease can vary based on location, year or climatic conditions. While most of the superior lines show better performance in general, there are locations and years where the disease pressure or continued favorable climate conditions overcome the resistance. Under such scenario, it is important to look for stability of resistance, especially in the sources to be used in a breeding program.

16.6 Searching Newer Sources Through Artificial Inoculations

The relocation of CIMMYT Southern Cone regional office to Uruguay (1994–2004) permitted a much broader effort on the FHB research activities to be undertaken in collaboration with National Institute for Agriculture Research (INIA) and the region. During this period it became possible to connect Southern Cone with FHB researchers worldwide and also introduce a larger set of resistant germplasm for the regional breeding program. Given the irregular frequency of FHB epidemics at a country level and/or the region (Fig. 16.1), wheat breeding programs were motivated to develop alternative methodologies to screen germplasm each year. Mesterházy (2003) stated that in spite of abundant presence of inoculum in the field; the outbreak of an epidemic depends largely on climatic conditions. In the Southern Cone, the most important aspect of this uncertainty is related with the level of humidity and rainfall during the flowering period.

In general, the FHB germplasm is planted at appropriate dates so that the flowering period coincides with highest probability of favorable climatic conditions (wet season). In order to assure a moderate to high level of infection in the regional nursery VIRFET, all the lines under test were inoculated at pre-flowering, flowering and 3 or 4 days post flowering at INIA, La Estanzuela, Uruguay. Other programs distributed infected grains to serve as initial source of inoculum. But this method needed warm and humid climatic conditions at the flowering time or a supplementary irrigation (spray) system to ensure infection. Some programs used a suspension of conidia as inoculum to spray the spike (Type I resistance) or inject into a central floret of the spike (Type II resistance). Depending on the objective of the screening, single fungal isolates or field mixture were used to evaluate the germplasm. The inoculation technique to evaluate Type I resistance comprised of using a pressurized sprayer at CIMMYT, Mexico, and a hand sprayer in Argentina and Uruguay. The Type II resistance was evaluated using single floret inoculation, with inoculum soaked cotton, in Mexico and by pipette injection in all other locations.

As with the inoculation methodology, the scales to evaluate the disease under field conditions also varied from two double digit scales 0–9/0–9 and 0–5/05 to estimating the percent of infected spikes. In the double digit scale, the first digit represents the percent of infected spikes and the second the percent of infected

Table 16.2 Percent infection in spikes and grains and associated loss in kernel weight in known sources of resistance to FHB

	Spike infection	Infected grains		Thousand kernel weight		Loss in
	Inoculated plot	Inoculated	Fungicide	Inoculated	Fungicide	TKW
Line or cultivar	(%)	(%)	(%)	(g)	(g)	(%)
Nobeoka Bozu	0.00	0.67	0.83	25.75	27.33	5.8
E1-31-	0.00	1.33	0.17	30.72	30.20	–1.7
E4-25	0.00	3.00	0.33	38.38	39.23	2.2
Sumai#3	0.00	3.25	0.83	30.40	30.95	1.8
Catbird 1073	0.00	4.50	1.17	38.37	38.92	1.4
SAGV…	0.00	4.83	4.83	25.38	25.37	–0.1
Alsen	0.00	7.83	2.50	33.22	33.38	0.5
E Pel 90/Suzoe 8	0.05	9.50	2.50	31.65	35.00	9.6
E2-28	0.10	3.83	1.00	31.85	32.35	1.5
Frontana	0.28	0.83	0.83	40.05	39.37	–1.7
ORL99192	0.33	4.33	3.50	32.08	32.78	2.1
Toropi	0.35	6.33	3.50	37.00	38.62	4.2
Cbrd/IMir	3.30	7.75	5.50	39.70	41.43	4.2
I Boyero (SW Check)	11.70	13.50	4.83	32.02	33.83	5.4
LE 2299 (FW check)	2.00	35.75	13.25	25.28	29.75	15.0
I Cabure (FW check)	24.30	19.83	1.67	30.35	32.53	6.7

spikelets, but in terms of 0–9 or 0–5. Under semi-controlled or controlled conditions, the Type I resistance is measured on the 0/5 scale or the percentage of infected spikelets and Type II as percent of spikelets infected in a spike from point of inoculation or the 0/5 scale (Diaz de Ackermann 2006).

Inoculations conducted on the major sources of resistance being used by the breeding programs not only confirmed that many combined Type I and Type II resistance, but also demonstrated very little loss in the kernel weight caused by the infection, Table 16.2. The infection in the susceptible check INIA Cabure affected almost 25 % of the spikes. While in most cases, the spike infection was represented in high or low grain infection, there were cases such as LE2299 where fewer spikes were infected but high percent of grains were infected in each spike resulting in a significant loss in grain weight. A high grain weight loss in two of the parents being used in the crossing program in Uruguay viz. NobeokaBozu and E.Pelon 90/Suzhoe 8 needs to be reconfirmed (Table 16.2).

Large scale screening of lines and varieties under field and shaded house conditions allowed the identification of additional sources with low infection to be used by the breeding program, Table 16.3. However, no effort was made to study the genetic basis of these lines, assuming that a majority of them derived their resistance from Sumai#3 or other Chinese sources. The average grain infection caused by the disease served to identify those lines that may also have low levels of toxin. While the relationship between low FHB infection under semi-controlled conditions and lower percent of infected grains holds good for sources with high degree of resistance, it may not be a true indicator for lines with moderate level of resistance.

Table 16.3 Percent average grain infection in selected lines with different levels of FHB infection, Uruguay

Field 2003	Line or variety	FHB 01	FHB 02	Average infected grains (%)
28776	MIANYANG81-5//PC B084.985/JIANZIMAI	R	R	1
28781	SHANGAI	R	R	1
28770	CATBIRD	R	R	3.5
28763	SHA3/CBRD	R	R	4.5
28787	SODAT/SUM3//NING820/3/NING8626	R	MR	6
28825	TURDA 2317-90	MR	R	1.5
28778	PC B084.985/JIANZIMAI//8744	MR	R	3
28766	NG8675/CBRD	MR	R	4
28798	BCN/3/68112/WARD//AE.SQ(369)	MR	R	4.5
28772	CATBIRD	MR	R	6
28784	GOV/AZ//MUS/3/DODO/4/BOW	MR	MR	6
28803	CROC1/AE.SQ(205)/5/BR12*3/4/IAS55*4/CI14123/3/IAS55*4/EG.AUS//IAS55	MR	MS	7
28809	CS/LE.RA//CS/3/PVN	MR	MR	7.5
28785	RECURRENT SELECTION 1	MR	MR	7.5
28783	GOV/AZ//MUS/3/DODO/4/BOW	MR	MR	8
28773	CHUM 18//JUP/BJY	MR	MR	8.5
28799	CHIR3/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	MR	MR	11
28774	CHUM 18//JUP/BJY	MR	R	12
28769	MILAN/SHA7	MS	R	4.5
28790	BCN//DOY1/AE.SQ(447)	MS	MS	11
28797	MAYOOR/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	MS	MS	11.5
28802	MAYOOR	S	MSS	10

Over the years more than 500 advanced lines and varieties originating from the region (especially Argentina and Brazil), Austria, China, Japan, Hungary, Mexico and USA, selected for moderate to high levels of FHB resistance, were added to the INIA's Germplasm Bank held at La Estanzuela, Uruguay. This collection also contains 18 ancestral sources or resistant lines from Japan viz. Soba Komugi IB, Soba Komugi IC, Su Mai#3, AburaKomugi, AsoZairai II, AzoZairai (YuubouKappu), Chile, Itou Komugi, Kagoshima, Kikuchi, Nyubai, Qiaomai (Xiaomai), ShiroNankin, ShouKomugi II, Sotome, Sotome A, ZairaiYuubou, NobeokaBozu, Komugi provided by Dr. Tomohiro Ban, JIRCAS, Tsukuba. It will be worthwhile to study the genetic diversity of their resistance in order to combine them with other sources to develop a higher level of resistance for this disease.

For the facultative and winter wheats, new sources of resistance from Hungary (Courtesy Dr. AkosMesterhazy), and China (Courtesy Dr. Zhonghu He) provide ample genetic variability to be utilized by the wheat breeders (Kohli et al. 2002). Some of the newer sources of resistance from China were inoculated at three stages and their field reactions to FHB are presented in Table 16.4.

Table 16.4 Percent spike infection of FHB at three stages of inoculation and associated field reaction of new Chinese germplasm, La Estanzuela, Uruguay

	Percent infected spikelets				
Line or variety	Pre-flower	Flower	Post-flower	Average	FHB field
NEIXIANG 184	8.2	9.5	28.3	15.3	0
PH 82.2.2	19.7	17.5	12.8	16.9	0
LUMAI-14	22.6	23.1	25.0	23.7	0
E AIL	29.8	20.7	20.7	27.8	T
NING9415	29.4	17.0	13.0	20.0	T/2
SHAAN 229	18.9	27.6	44.4	30.4	T/4
MIAN YANG 20	16.7	17.9	25.0	18.8	T/5
I. BOYERO(susceptible)	70.8	56.7	70.1	65.8	5/5

Table 16.5 Selected local crosses with lower FHB grain infection after 14 and 21 days post-inoculation

		% grain infection	
Line or variety	Selection history	14 days	21 days
NING8331/I.CAB	CGF9706-2E-0E-1E-2ESP-0E	0.00	0.00
Sumai-3-81,60/Kicsó//JAGGER	UR309-0LE-9LE-3LE-0LE	0.00	0.83
CHUAN MAI#18/BAU"S"/3/I.BOY// CLEO/INIA66	CGF9702-2E-0E-21E-0E-0E	0.00	1.04
NING8331/I.CAB	CGF9706-2E-0E-1E-1ESP-0E	1.25	1.25
CHUAN MAI#18/BAU"S"/3/I.BOY// CLEO/INIA66	CGF9702-2E-0E-1E-0E-0E	0.00	2.29
I.MIRLO//CBRD (DIF 1073)	CGF9701-1E-0E-44E-0E-0E	0.66	2.83
NG8675/CBRD (R Check)	CMSS92Y00639S-4-1SCM-0CHN-015Y-3SCM-0F US-0LE	1.32	6.53
NOBEOKA BOZU/JAGGER	URO92-0LE-8LE-2LE-0LE	6.53	7.37
I.CAB//LI 107/YMI#6	CGF9704-5E-1E-12ESP-0E	2.50	8.07
LE2221/RingoSztar-MM/NB	UR97130-5LE-3LE-0LE	–	10.09
NOBEOKA BOZU/JAGGER	UR092-0LE-6LE-1LE-0LE	7.90	12.34
MILAN/SHA7 (MRMS check)	CM97550-0M-2Y-030H-3Y-3Y-0Y-3M-010Y-0LE	5.43	21.73
LE 2278 (S check)		24.52	30.48

The strategy to develop local pre-breeding program to incorporate the genetic variability in regionally adapted materials made ample use of sources such as Sumai#3 (China), NobeokaBozu (Japan), Catbird (México), Frontana (Brazil) and Alsen (USA). In spite of the selection of many F_6 advanced lines with high level of resistance from these local crosses, very few of them combined high yield component. Percent grain infection (14 and 21 days post inoculation) in a set of lines representing different groups is presented in (Table 16.5). Most of these lines varied between 67 and 102 % of the high yielding check variety INIA Torcaza (Table 16.6). Several sister lines of the cross Shanghai 4/Chilero//U1275.1.4.2/WGRC16

Table 16.6 Selected local crosses combining moderate level of FHB resistance with high yield potential

Cross or variety	Selection history	Heading date	FHB reaction[a]		Yield kg/ha	% yield	
			2002	2003		INIA Torcaza	INIA Tijereta
Sha4/Chil/U1275-1-4-2/WGRC16	UR96-152-0LE-2le-0LE-5LE-0LE-0LE	19-Oct	MR	MR	7,181	102	145
Sha4/Chil/U1275-1-4-2/WGRC16	UR96-152-0LE-1le-0LE-5LE-0LE-0LE	18-Oct	MR	MR	7,092	101	143
Sha4/Chil/U1275-1-4-2/WGRC16	UR96-152-0LE-10le-0LE-9LE-0LE-0LE	19-Oct	MS	MR	6,732	96	136
Catbird/MN92151	UR95185-9LE-0LE-0LE-6LE-0LE-0LE	24-Oct	MS	MR	6,152	88	124
Shanghai4/Chilero//Milan	UR96165-0LE-3LE-0LE-2LE-0LE-0LE	28-Oct	MR	M	6,152	88	124
Mason/Catbird (G90) (R Check)	97-1040-10-5	21-Oct	R	MRR	5,588	80	113
ND2928 (MR Check)		2-Oct	MR	R	4,690	67	95
LE2221//Ringo Sztár-MM/NB	UR97130-0le-7LE-0LE-1BG-0LE	20-Oct	MR	R	4,640	95	94
Sumai3-81.60/Kincsó//I.Tijereta	UR97316-0le-2LE-0LE-1BG-0LE	24-Oct	MR	R	4,490	92	91
NobeokaBozu/I.Tijereta	UR97112-0le-6LE-0LE-1BG-0LE	21-Oct	MR	MR	4,468	91	90
LE2221//Ringo Sztár-MM/NB	UR97130-0le-14LE-0LE-1BG-0LE	20-Oct	MR	R	4,169	85	84
Ringo Sztár-MM/NB/I.Tijereta	UR97315-0le-11LE-0LE-18LE-0LE	23-Oct	MR	MR	3,901	80	79
INIA Torcaza (MR Check)		20-Oct	MR	MR	7,040		
INIA Tijereta (MRMS Check)		17-Oct	MRMS	MRMS	4,952		

[a]*R* resistant, *MR* moderately resistant, *M* = *MRMS* moderately resistant to moderately susceptible, *MS* moderately susceptible

demonstrated high degree of FHB resistance combined with the high yield potential. However, the progress in attaining a higher degree of resistance from the local crosses has been slow and of small magnitude (Diaz de Ackermann and Pereyra 2011).

Early in the 2000s, the Uruguayan program received an access to several double haploid populations developed around the world to identify molecular markers associated with the resistance to FHB. These populations were CM82036/Remus from Austria, Milan/Catbird from England, Mayoor//TK SN1081/Ae.squarrosa (222)/3/Flycather, NobeokaBouzu/Sumai#3, Sumai#3/Gamenya, Fukuhokomugi/Oligoculm 380 and Frontana/Inia from Mexico and Sagvari-N.Bozu/MM-Sumai#3 from Hungary. Although, phenotyping these populations at La Estanzuela, Uruguay, in a critical FHB cycle of 2001–02 produced several lines with high level of resistance in a better agronomic type, initial molecular work conducted by Dr. HiroSuenaga in CIMMYT, Mexico, did not identify any significant markers associated with the resistance. Considering the advances in molecular genetics over the past decade, it should be worthwhile to reanalyze these populations to identify newer sources of FHB resistance which have probably not been detected so far.

16.7 Identification of Stable Sources of Fusarium Head Blight Resistance

In order to determine stable sources of FHB resistance in the regional and introduced germplasm, used by various breeding programs, a new collection of elite FHB lines were put together in a Regional Fusarium Head Blight Nursery (VIRFET). Cooperators seeding VIRFET germplasm used different methodologies to make certain high levels of infection. While all collaborators took disease notes on the percentage of spikes affected with FHB in the field, participants from Uruguay also evaluated the percentage of tombstone kernel affected after harvest. (Diaz de Ackermann 2006). The stability of the lines was determined statistically based on their disease score over the locations.

A total of 118 lines and varieties (Argentina = 21, Brazil = 21, CIMMYT, Mexico = 44, Paraguay = 12 and Uruguay = 20), including resistant and susceptible checks, were classified into Type I and Type II resistance based on at least 2 years of infection data from the field, shaded covers or controlled glasshouse conditions (Tables 16.7 and 16.8).

Only three of the tested lines (Frontana, Shangahi#5/Weaver and Shanghai#3/Catbird) showed both types (Type I and Type II) of resistance. It was interesting to observe the Type I resistance in a new high yielding cultivar, PROINTA Granar (Marcos Juarez INTA//PAK3563/Chenab 70/3/Diamante INTA) from Argentina, which has shown field resistance to FHB over the years. Although high yielding cultivars viz. Klein Cacique, Buck Charrúa (Argentina), and CEP 24 (Brazil) were found to be good for Type II resistance, they are later in maturity class which may be responsible for their low infection scores. These varieties need to be re-inoculated under controlled conditions to verify the resistance they demonstrate under field conditions. An additional group of 38 lines of well-known sources of resistance to

Table 16.7 Stable sources of Type I resistance

Line or variety	Field reaction[a]
Shanghai5/Weaver	R
CM95103-25Y-0 M-0Y-2 M-0RES-5PZ-0Y-10PZ-0Y-2SCM	
Nanjing 8331	MR
BC-207	
Shanghai3/Catbird	MR
CMSS92Y00595S-1SCM-0CHN-015Y-3SCM	
Frontana (CHECK MR)	MR
–0BRA	
ProintaGranar	MR
MJI//PAK3563/CHAP70/3/DEI	
Bobwhite´S´/´Nobeoka Bozu//CEP75203/Veery´S´	MR
LFJ-I-7	
Klat/Pel74142//Lri/Nyubay/3/Klat/CEP75203//LAJ1409/PF7815	MR
LFJ-IV-59	

[a]*R* resistant (0 -<1 on 0–5 scale), *MR* moderately resistant (1–2 on 0–5 scale)

Table 16.8 Stable sources of Type II resistance

Line or variety	Field reaction[a]
Shanghai 5/Weaver	R
CM95103-25Y-0M-0Y-2M-0RES-5PZ-0Y-10PZ-0Y-2SCM	
Vilela Sol/NobeokaBozu//Pel73101/Las RosasInta	R
LFJ-III-38	
CEP24	R
BR 3/CEP 7887//CEP 7775/CEP 11	
EPelon90/Suzhoe F3#8	R
31B-0Y (PLANTA 2)	
BuckCharrua	R
RAF/K.PET//K.REN/3/K.IMP//RAF/K.PET/4/LOV/5/ RAF/K.PET//K.REN/3/K.IMP	
Remus/CM 82036	R
E1	
Frontana/Remus	R
E4	
Neixiang 184	R
Klein Atlas (Moderately resistant check)	MR
K.LUCERO/K.157//K.RENDIDOR	
Corre Caminos/Piamontes	MR
LAJ2231	
CEP24/PF87107//Pavon/Ani´S´	MR
B36293-B-0A-1A-2A-0A-0 V	
CooperacionCabildo	MR
BATL/4/KLAN/BLACKHULL/PENTAD/3/MQ/ G.ROCA/5/VMAR//VSOL/JAR“S”	
Klein Cacique	MR
BCIM/25348//VEERY“S”	
LI 107/Yang Mai#6	MR
BC-208	
Shanghai 3/Catbird	MR
CMSS92Y00595S-1SCM-0CHN-015Y-3SCM	

[a]*R* resistant (0 -<1 on 0–5 scale), *MR* moderately resistant (1–2 on 0–5 scale)

FHB in the region was selected by several collaborators, but they are not stable (IICA-BID ATN/SF 6486 RG 2003 – Project).

The organization of this regional nursery and its evaluation in different locations is considered an important step forward because it allowed to bring together all germplasm selected in the region for FHB resistance over the past several decades. The identification of a reduced number of lines with specific characteristics and different types of resistance, such as Frontana (used world-wide), Shanghai#5/Weaver (CM95103-25Y-0 M-0Y-2 M-0RES-5PZ-0Y-10PZ-0Y-2SCM) and Shanghai#3/Catbird (CMSS92Y00595S-1SCM-0CHN-015Y-3SCM) is of great value for the regional breeding programs and adds to the stable sources of resistance in the world.

16.8 Sources of Resistance to Fusarium Head Blight from Alien Species

Given the limited variability for scab resistance found and used in the wheat germplasm worldwide, which could lead the crop to disease vulnerability, Chen et al. (1997), suggested an exhaustive exploration of new genetic resources in wheat and related alien species.

Earlier efforts by Kazi et al. (1983), had reported a high level of FHB resistance in *Elymus giganteus* (=*Leymus racemosus*) and produced hybrid progenies with wheat for head scab resistance. Wang et al. (1986, 1991) confirmed the scab resistance of *E. giganteus.* Furthermore, *Roegneria kamoji* and *R. ciliaris,* growing in the humid and warm areas of southern China, were also identified as sources of high level of resistance to head scab disease, Liu et al. (1989).

In order to expand the genetic variability for FHB resistance, CIMMYT started a large collection of the *Aegilops squarrosa* in the 1990s and also initiated a concerted effort to create a large number of synthetic bread wheats (SH:*Triticum turgidum*/*Aegilops squarrosa*; 2n = 6x = 42, AABBDD). The first group of 582 synthetic wheats and derived lines (BW/SH) developed by the Wheat Wide Crosses group at CIMMYT were tested under field conditions in Mexico to identify available diversity for FHB resistance. The initial results indicated that both type I and II resistance were present in the synthetic wheats (Table 16.9). One of the synthetic crosses Ganso/*Ae. squarrosa* (427) demonstrated both Type I and Type II resistance while another cross Ganso/*Ae.squarrosa* (408) showed only Type II resistance. Other crosses showing good level of FHB resistance were Rabi//Ganso/Crane/3/*Ae. squarrosa*(190) and 68.111/RGB-U//Ward Resel/3/Stifftail/4/*Ae. squarrosa*(633), (Gilchrist et al. 1997).

While CIMMYT was combining resistances from numerous sources into well-adapted backgrounds, (Gilchrist et al. 1997), a Canadian group led by George Fedak identified other resistant accessions of *Triticum monococcum*, *Triticum miguschovae* and *Aegilops speltoides* to be highly resistant to head scab disease and also lower in level of mycotoxin Deoxynivalenol (DON) production, Fedak et al. (2006). The backcross lines derived from these accessions with the cultivar Superb demonstrated a very high level of genetic diversity both for FHB resistance and

Table 16.9 Synthetic hexaploid (*SH*) lines resistant to *Fusarium graminearum* evaluated at Atizapan Station, Toluca, Mexico

	FHB severity (%)		
	Type I	Type II	
Synthetic hexaploids, wheat cultivars and check lines	1996	1995	1996
Rabi//Ganso/Crane/3/*Ae. squarrosa (190)*	10.7[a]	16.9[b]	17.15[c]
Ganso/Ae. *squarrosa (437)*	6.9	10.0	11.46
Ganso/Ae. *squarrosa (408)*	32.9	14.3	21.09
Rabi//Ganso/Crane/3/*Ae. squarrosa (891)*	8.9	5.9	27.82
6973/Ward.7463//74110/*3/Ae. squarrosa (665)*	–	5.5	20.96
Decoy1/*Ae. squarrosa (446)*	8.5	6.2	21.69
Decoy1/*Ae. squarrosa (511)*	12.6	16.0	20.07
68.111/Rgb-U//Ward resel/*3*/Stifftail/*4/Ae. squarrosa (783)*	11.2	10.8	17.98
Snipe/Yavaro79/Dackiye/Teal/3/*Ae. squarrosa (633)*	25.2	15.7	21.92
Chinese line (Bread wheat resistant check)	6.3	16.0	6.92
Frontana(Bread wheat resistant check)	3.2	6.3	15.13
Mayoor (Bread wheat moderately resistant check)	12.3	30.0	–
Flycatcher (Bread wheat susceptible check)	35.2	79.0	42.36
Altar 84 (Durum wheat susceptible check)	100.0	100.0	100.0

Data: Drs. Gilchrist L, Kazi M CIMMYT, Mexico
[a]Average of 21 spikes
[b]Average of 10 spikes
[c]Average of 25 spikes

Table 16.10 FHB symptoms and DON content of progenies from interspecific crosses with bread wheat

Source of resistance	Generation	Number of lines	FHB index	DON (ppm)
Triticum monococcum	BC2F6	22	0.3–3.0	0.1–1.7
Aegilops speltoides	BC3F6	70	0.3–7.5	0.3–2.0
Checks				
Superb			24.0	9.2
Roblin			76.5	9.1
Fukuho			1.5	1.9
Sumai#3			1.3	2.9

Source: Fedak et al. (2006) (field data 2005)

DON production (Table 16.10). Fedak believes this strategy to be a pragmatic approach considering that it should be possible to pyramid resistance genes from numerous unique sources over a period of time.

After the 2001 epidemic in Uruguay, 171 synthetic derived double haploid lines from Mayoor//TK SN1081/*Aegilops squarrosa* (222)/3/Flycatcher cross, selected for FHB resistance by CIMMYT were introduced to the region. While there was ample genetic diversity for FHB resistance in the cross, all the lines were extremely susceptible to leaf rust. A set of double haploid populations selected as resistant over a 2 year period 2002 and 2003 are presented in the Table 16.11.

Table 16.11 FHB reaction of a selected set of doublé haploid population derived from a synthetic wheat line, 2002/03, Uruguay

Field id. 2003	Line or variety	History of selection	Heading date	FHB reaction[a]
32030	I. Mirlo (Bread wheat susceptible Check)		16-Oct	MS
32032	I. Boyero (Bread wheat susceptible Check)		29-Oct	AS
32033	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-1DH-0DH	24-Oct	R
32062	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-30DH-0DH	24-Oct	R
32034	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-2DH-0DH	29-Oct	R
32038	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-6DH-0DH	29-Oct	R
32049	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-17DH-0DH	29-Oct	R
32060	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-28DH-0DH	29-Oct	R
32164	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-132DH-0DH	29-Oct	R
32202	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-170DH-0DH	29-Oct	R
32083	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-51DH-0DH	24-Oct	RMR
32045	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-13DH-0DH	29-Oct	RMR
32059	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-27DH-0DH	29-Oct	RMR
32096	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-64DH-0DH	29-Oct	RMR
32097	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-65DH-0DH	29-Oct	RMR
32098	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-66DH-0DH	29-Oct	RMR
32147	Mayoor//TK SN1081/*Ae.squarrosa*(222)/3/Flycatcher	CAWS98B00012S-115DH-0DH	29-Oct	RMR

[a]*MS* moderately susceptible, *AS* highly susceptible, *R* resistant, *MR* moderately resistant

16.9 Genetic Resistance to Fusarium Head Blight and Mycotoxin Production

Ever since the severe epidemics of 1996–1997 in the region, the search for better sources of FHB resistance has added another dimension. It is the production of toxins belonging to the Trichothecene family associated with the susceptibility to the disease. In a study conducted on the grains collected from experimental and commercial fields, the Uruguayan Food Technology Laboratory (LATU) identified mycotoxins such as DON (Deoxynivalenol), 15-Ac DON (15 Acetyldeoxynivalenol), and T2 (Piñeiro et al. 1996). DON was the most predominant mycotoxin isolated from all the samples analyzed. Although direct relationship between the disease severity based on the *Fusarium* field infection and toxin concentration (DON content) is not always clear; in general, high level of DON is found only in the heavily infected plants, (Table 16.12). The lines with low disease infection, especially those of Chinese origin, showed low mycotoxin level but this relation was less than perfect. Lower presence of toxin can represent a high degree of resistance in the germplasm as well as a lack of disease incidence in the field. From a group of 68 sources of resistance from China and the region, it was possible to identify lines with low FHB susceptibility and low DON concentration in the epidemic year of 1996 (Table 16.13).

Soon after the identification of stable sources of resistance through VIRFET in the project "Development of Technologies for the Integrated Management of the Fusarium Head Blight of Wheat", 2000–2003, the search for associated lower toxin germplasm became a priority. It was carried out in the second phase through a "Cooperative Regional Project on Genetic Resources of Wheat for Sustainable

Table 16.12 Relation between field evaluation of the FHB infection and the presence of toxins in the grain

Advanced line	FHB field reaction[a]	DON µg/kg	15-Ac DON µg/kg	T2 µg/kg	Toxin resistance[b]
Parula/Veery 6	35	167	800.0	>1,000	S
Catbird 1073	TT	119	800.0		R/LT
Luan	TT	320			R/LT
Catbird"S"	24	1,600			S
Shanghai# 8/Genaro	44	n.d.			LT
CAR853/Cocoraque72	44	2,235			S
Buck Guarani (susceptible check)	55	640			S

Source: Piñeiro et al. (1996)

n.d.: Toxin level below detection (DON <80 µg/kg)

[a]FHB infection on 00/55 scale (Kohli 1989). Lines with FHB score less than T2 are considered resistant under field conditions

[b]Resistance to the toxin level; *R* resistant, *S* susceptible, *LT* low toxin

Table 16.13 Percent of spikelet infection caused by the FHB and respective DON concentrations

Line or variety	% Infected spikelets	DON µg/kg[a]
Shanghai3/Seri//Nanjing8331/Lira	11	107.7
Yangmai87-158/3/Suzhoe10//Alondra/Pavon	10	107.7
Shanghai3/Seri//Yangmai87-142	11	236.9
Shanghai3/Seri//G.C.W.1/Seri	11	108
Shanghai4/3/2*Chuan Mai18//Jupateco/Bluejay	11	344.6
Catbird (Bread wheat resistant Check)	11	43
Milan (Bread wheat susceptible Check)	60	4,307.6
I.Mirlo (Bread wheat susceptible Check)	59	957.2

[a]Detection level 40 ppb (1 µg/kg = 1 ppb)

Production Systems in the Southern Cone, 2005–2008". However, this period coincided with the lack of natural epidemics in the region, thereby creating a serious limitation on the generation of data.

The new nursery, planted under natural infection conditions in Mexico, Paraguay and two locations in Uruguay (La Estanzuela and Young) aimed to reconfirm the resistance stability of the identified lines and cultivars and evaluate the toxin (DON) content associated with the level of infection. Lack of FHB infection in Argentina and quarantine problems with the seed in Brazil impeded these countries from providing the data.

Lines showing low level of infection in the field (% of spikes affected by *Fusarium*), low percent of grain affected with *Fusarium* and low DON content in El Batan, Mexico and La Estanzuela, Uruguay were: E1-31 (45), Shanghai (52), Shanghai (53), Nanjing 8331 (3), Shanghai3/Catbird (48), ProintaGranar (41), Shanghai (51), Nanjing8675/Catbird (37), Sodat/Sumai3//Nanjing820/3/Nanjing 8626 (55), Shanghai 3/Catbird (47), Recurrent Selection 1 (42), Shanghai 3/Catbird (49), E1-97 (46), LI 107/Yangmai # 6 (34), Catbird (14), VillelaSol/NobeokaBozu// Pel3101//Las Rosas INTA (57), RingoSztar – Mini Mano (MM)//NobeokaBozu (146) (58), Sumai # 3 (1), Catbird (10), Brs Tarumá (78), IniaCaburé (70), Cep24 (17), Buck Charrúa (8), Bagula´S´/Cep87103//Cep14 (6), Suzhoe/E. Pelon90 F3 # 8 (27), Catbird (15), Catbird (12), Tables 16.14 and 16.15 IICA/PROCISUR (2010).

16.10 The Present Status of Fusarium Head Blight Germplasm in the Southern Cone and Future Directions

After years of germplasm development, the present Southern Cone germplasm with resistance to FHB has its origins not only in the earlier local landraces and varieties but also in the germplasm introduced through the international nurseries and

Table 16.14 Selected entries with lower field infection, grain infection and DON content

	Paraguay				Young			La Estanzuela				Mexico			
	Spikes infected	Grain infected	EB DON	LE DON	Grain infected	EB DON	LE DON		Grain infected	EB DON	LE DON	Incidence	Grain infected	EB DON	LE DON
Entry	%	%	Ppm	ppm	%	ppm	ppm	FHB severity	%	ppm	ppm	%	%	ppm	ppm
45	–	0	0.60	0.50	0	0.50	0.50	0.0	0	0.50	0.50	0	2	0.50	0.50
52	10	2	1.00	0.88	0	0.50	0.50	0.0	0	0.50	0.54	12	0.5	0.90	1.10
53	5	1	0.50	0.59	0	0.50	0.83	0.0	0	0.50	0.59	4	0	0.68	0.50
3	–	6	0.50	0.54	1	0.50	0.50	0.0	0	0.50	0.62	18	1.5	0.50	0.50
48	–	1	1.80	0.97	0	0.50	0.50	0.5	0.5	0.50	0.69	14	1	0.50	0.50
41	10	0	1.49	0.50	0	0.50	0.50	0.5	0	0.50	0.71	12	0.5	0.50	0.50
51	10	0	1.10	0.68	0.5	0.50	0.50	0.0	0	0.50	0.72	0	0	0.50	0.50
37	15	0	1.15	1.10	1.5	0.60	0.50	0.0	0	0.50	0.79	10	0.5	0.56	0.50
55	20	6	1.70	1.90	0.5	0.50	0.50	0.0	0.5	0.50	0.79	16	1	0.93	0.50
47	15	1	1.20	0.50	1.5	0.50	0.50	0.0	0	0.50	0.96	0	2	0.50	0.50
42	10	1	1.47	1.10	1	0.50	0.50	0.5	2	0.50	1.00	2	1	0.50	0.50
49	15	1	0.70	1.40	1	0.50	0.77	0.5	1.5	0.50	1.40	4	0	0.50	0.57
46	–	0	0.50	0.50	0.5	0.50	0.50	0.0	0	0.50	0.50	0	0.5	0.50	0.50
34	5	4	1.14	0.50	1	0.50	0.50	0.0	0.5	0.50	0.50	2	7	0.50	0.50
14	15	5	1.40	0.61	0.5	0.50	0.50	0.0	0.5	0.50	0.50	8	0.5	0.50	0.50
57	10	1	1.40	1.10	1.5	0.50	0.77	0.0	1	0.50	0.50	0	2.5	0.65	0.71
58	–	2	1.57	–	0	0.50	0.50	0.0	0	0.50	0.50	0	2	0.50	0.50
1	–	0	–	–	0	0.74	0.50	0.0	0	0.50	0.50	0	1.5	0.50	0.50
10	–	2	0.50	0.94	0	0.50	0.50	1.0	0	0.59	0.70	10	0.5	0.50	0.50
78	20	–	–	–	1	0.50	0.50	0.5	0	0.59	0.34	–	2.5	–	0.91
70	–	3	0.83	–	1	0.50	0.73	3.0	0	0.68	0.69	4	1.5	0.50	0.50

(continued)

Table 16.14 (continued)

	Paraguay				Young			La Estanzuela				Mexico			
	Spikes infected	Grain infected	EB DON	LE DON	Grain infected	EB DON	LE DON		Grain infected	EB DON	LE DON	Incidence	Grain infected	EB DON	LE DON
Entry	%	%	Ppm	ppm	%	ppm	ppm	FHB severity	%	ppm	ppm	%	%	ppm	ppm
17	5	0	0.50	0.50	1	0.50	0.50	0.0	0	0.69	0.50	0	2.5	0.50	0.50
8	–	2	0.50	–	0	0.50	0.50	0.0	0	0.79	0.50	–	0	0.50	0.63
6	10	8	1.19	0.50	0.5	0.63	0.50	1.0	2	0.89	0.52	6	1.5	0.50	0.50
27	–	1	1.63	1.80	1	0.50	0.50	1.5	0	1.04	0.50	2	1	0.50	0.62
15	10	0	0.50	0.59	0.5	0.50	0.50	0.0	0	1.20	0.51	2	0	0.50	0.50
12	5	2	1.22	1.80	0.5	0.50	0.50	0.5	2	1.52	0.87	6	0.5	0.50	0.50

Information from Paraguay, Uruguay (Young and La Estanzuela, LE) and Mexico (El Batan, EB)

Table 16.15 Cross and pedigree identification of the selected lines or cultivars with high degree of FHB resistance and lower DON content identified over the region

Entry	Cultivar/cross	Pedigree
45	E1-31	REMUS/CM82036
52	SHANGAI	–0SHG-19GH-0FGR-0FGR
53	SHANGAI	–0SGH-8GH-0FGR-0FGR
3	NANJING 8331 (Resistant check)	NING7840/YANGMAI#4
48	SHANGHAI 3/CATBIRD	–0SHG-1GH-0FGR-0FGR
41	PROINTA GRANAR	(MARCOS JUAREZ INTA//PAK3563/ CHENAB 70/3/DIAMANTE INTA)
51	SHANGAI	–0SHG-13GH-0FGR-0FGR
37	NG8675/CATBIRD	CMSS92Y00639S-5-5SCM
55	SODAT/SUMAI 3/NANJING820/ NANJING8626	2SCM-0CHN-015Y-2SCM
47	SHANGHAI 3/CATBIRD	–0SHG-2GH-0FGR-0FGR
42	RECURRENT SELECTION 1	2SCM
49	SHANGHAI 3/CATBIRD	–0SHG-1GH-0FGR-0FGR-0SCM-0Y-020SCM
46	E1-97	REMUS/CM82036
34	LI 107/YANG MAI#6	BC-208
14	CATBIRD	CM91045-9Y-0M-5M-0Y-5PZ-0Y-4PZ-010Y-0E
57	V. SOL/N BOZU//PEL 73101/ LAS ROSAS INTA	–0FGR-0FGR-0FGR
58	RINGO SZTAR-MINI MANO (MM)/ NB WW (146)	
1	SUMAI#3 (Resistant check)	FUNO/TAIWAN XIAOMAI
10	CATBIRD	CM91045-9Y-0M-0Y-5M-0Y-5M-1M-0Y-2M-0Y-1SCM
78	BRS TARUMA	Century/BR35
70	INIA CABURE	EFEDERAL/F5-83-7792-15
17	CEP 24	
8	BUCK CHARRUA	(RAF/K.PET//K.REN/3/K.IMP//RAF/K.PET/4/ LOV/5/RAF/K PET//K.REN/3/K.IMP)
6	BAGULA'S'/CEP87103//CEP14	B34410-BY-3A-0A-0A-10A-1V-3A-0V
27	EPELON 90/SUZHOE F3#8	
15	CATBIRD	CM91045-5Y-0M-0Y-4M-2Y-0YZ-010M-0Y-1SJ-0Y-0
12	CATBIRD	CM91045-5Y-0M-4M-7Y-0B-0FC-0FGR-0FGR-0FGR0F

collaborative programs. The most important sources of new genetic variability came from China and Japan and/or lines derived from them via CIMMYT in Mexico, USA, Canada or Europe. This makes the germplasm base of FHB resistance very wide as mentioned by Souza Rosa and Souza Filho (2003). They believe that the new Brazilian germplasm derives its FHB resistance genes from Japan, China, Mexico, USA, Argentina, Uruguay and many other countries. The variation in the sources of FHB resistance is examplified in the set of parents used by the Uruguayan program over a 20 year period (Table 16.16).

Table 16.16 Sources of FHB resistance used as progenitors in wheat breeding, INIA, Uruguay

Year	Sources of resistance
1981	E. Young, Encruzilhada, Toropi, NobeokaBozu, NyuBay, Pel 74142
1982–1984	Abura, NyuBay
1990	LE 2120, Shangai#5, Shangai#7, PF 85513, PF 85516
1991	Nanjing 7840, Wuhan#3, Ning 82149, Shangai#7, Suzhoe#2, Catbird, Ald/Pvn//Ning 7840
1995	LAJ 1409 (Nad//Bb/Inia), Wuhan#3/Star, Catbird, Sha#8/Gen, Ning 82149/Kauz
1996	Sha#7/Vee#5, Ning8201/Kauz, E.Pelon90/Suzhoe#8, Ning 8675/Catbird
1997	Shangai#3/Catbird, LI107/Yang Mai#6
1998	E.Pelon90/Suzhoe#8, LI107/Yang Mai#6
1999–2000	Chuan Mai#18/Bagula (Catbird), Ning 8331 (Ning 7840/Yang Mai#4), E.Pelon90/Suzhoe

Some of the newer lines and commercial cultivars showing superior performance against FHB over the last two decades are as follows:

16.10.1 Argentina

Trigal 706, CooperaciónCabildo, La Paz INTA, Nadadores//Bluebird/Inia (LAJ1409), CorreCaminos/Piamontes (LAJ2231) and Buck Namuncura/Cruza natural// PatoRojo/Calidad/3/7Cerros//Bluebird/Ciano67 (LAJ 2955) and many other lines derived from Japanese and Chinese sources viz. Nuy Bay, NobeokaBozu, Pekin#8, Ning 8343, Ning 82109, and Sumai #3.

Among the commercial cultivars with moderate to high level of Type II resistance are: Buck Charrua, Buck Guapo, Buck Poncho, Klein Cacique, Klein Granador, Klein Don Enrique, KeinPegaso, Klein Volcan, Klein Chaja, Klein Escorpion, Klein Sagitario, Klein Capricornio, Klein Gaviota, Klein Guerrero, Klein Nutria, Prointa Granar, Prointa Molinero, Prointa Quintal, Baguette 19, Biointa 2004 and Biointa 1006.

16.10.2 Brazil

Cep 24, Embrapa 27, Orl 99192, Brs 177, Brs 179, Brs Camboim, Brs Guamirim, Brs Louro, Brs Tarumã, Brs Timbaúva and Brs Umbu. Many other advanced lines derive their resistance from the older regional germplasm as well as the new sources of resistance from China, Japan, Mexico, USA and others Souza Rosa and Souza Filho (2003).

Newly released cultivars such as Brs 296, Brs 327, Fundacep Campo Real, Pampeano, Topázio, Turqueza are all considered moderately resistant to FHB infections under field conditions (EMBRAPA 2011).

16.10.3 Paraguay

Itapúa 25, Itapúa 35, Itapúa 65 and IAN 15. Newer advanced lines with Type II resistance to FHB are primarily based on the crosses with Ning 8331, Catbird and Shangai#4.

All the commercial cultivars being grown at present viz. Itapúa 40, Itapúa 45, Itapúa 65, Itapúa 70, Itapúa 75, IAN 10, Canindé 1, Canindé 2, Canindé 3, Canindé 11, Canindé 12 and Canindé 13 are moderately susceptible to susceptible (Kohli et al. 2010).

16.10.4 Uruguay

Estanzuela Young, Estanzuela Lusitano, Estanzuela Hornero, E. Pelon90/Suzhoe8, Prointa Superior/Catbird. Advanced lines with higher level of resistance are based on the regional germplasm, Chinese and Japanese sources viz. Frontana, Sumai #3, Shangai #3, Catbird, YMI#6, Suzhoe and Sagvari-NB/MM-Sumai #3. Newer advanced lines with low level of infection derive their resistance from a Brazilian line ORL 99192 e.g. Génesis 2375 (LE2302/3/ORL99192), LE 2382 (LE2265/3/ORL99192), LE 2387 (ORL99192/3/Baguette10) and LE 2394 (ORL99192/Inia Gavilán).

Commercial cultivars INIA Torcaza, I. Churrinche, I. Tijereta, I. Gorrión and I. Caburé demonstrate moderate Type I and II resistance (Diaz de Ackermann 2006). Other cultivars with low level of infection of FHB include: Buck Charrua and Klein Capricornio. Several cultivars with low to intermediate FHB infection are: Klein Gaviota, INIA Gorrion, Genesis 2358, Genesis 2366 and Klein Guerrero among the facultative wheats and Baguette 19, Biointa 2004, LE 2357, LE 2375 (Genesis 2375) and Klein Nutria among the spring wheats.

While wide genetic base of resistance in these cultivars provides a safety-net for reduced FHB losses, there are large number of other susceptible to highly susceptible cultivars which add to the inoculum load in an epidemic year. The knowledge that chemical control of the disease is only partially effective, the national programs need to make a determined effort to eliminate or not release susceptible or highly susceptible varieties in the future. This will become more important as the countries enforce their limits on the presence of DON or other mycotoxins in the grain and flour for commerce. Since grain yield remains an important factor in the selection of varieties, the national programs will have to make FHB resistance as a priority to combine the above varieties and others to develop new high yielding cultivars. However, it is the successful selection of segregating populations through successive generations, especially in non-epidemic years, that will lead to the development of highly resistant germplasm.

Given the complex nature of FHB resistance and its genetics, a renewed effort needs to be undertaken to identify molecular markers and quantitative trait loci (QTL) associated with resistance in the regional germplasm and exotic sources.

Pyramiding of multiple resistance factors and low DON germplasm through marker assisted selection (MAS) is the only way to guarantee an accumulation of resistance in the newer varieties and lines. The second critical aspect is the mycotoxin analysis of FHB advanced lines, especially in the epidemic years, to accumulate not only resistance but also low DON level in the future germplasm.

16.11 Conclusions

Almost 90 years of Fusarium Head Blight epidemics recorded in the Southern Cone region have been important to help identify and select large numbers of local land races and older cultivars with moderate to high levels of resistance. Many of these earlier sources derived their resistance from unknown European wheats brought into the region by the European immigrants in the late nineteenth century or even earlier. Their exposure to local climatic and disease conditions must have led to the harvest of most successfully adapted types which continued from generation to generation to help solidify wheat production in the region and eventually become land races. It was these land races such as Barleta selections from Argentina and Federico Chaves lines and Polyssu from Brazil, Americano selections and Pelon 33C from Uruguay that first gave rise to FHB resistant local cultivars viz. Klein Universal II, Klein Sin Rival, Fronteira, etc. These varieties or cultivars have remained the backbone of the FHB resistance in the regional germplasm till date.

Early FHB epidemics in the region (1920s) indicating the moderate resistance of these lines may have served the need to broaden the local genetic base through introduction of exotic sources. The most important contribution was made by two sister lines Ardito and Mentana, both developed by famous Italian wheat breeder Nazareno Strampelli in the 1930s. The crosses of regional germplasm with these varieties led to the development of world famous FHB sources of resistance such as Klein 33, Frontana and Petiblanco. It may have also been the first time that a resistance source from Japan, Akagomoughi (parent line of Ardito and Mentana) was introduced in the region. Later in the 1960s, Brazil led a more organized introduction of Japanese sources of resistance (Abura, Nyu Bay and NobeokaBozu) and Pekin 8, probably from China.

The strengthening of CIMMYT activities in the Southern Cone region in the 1970s and establishment of a base here opened doors to a major influx of international germplasm, introduced directly from the countries or developed by CIMMYT in Mexico. It was this effort that led to large scale introduction of Chinese germplasm, especially Sumai#3 and its derivatives, from 1980s onwards. The introduction and identification of a CIMMYT cross Chuan Mai#18/Bagula (Catbird), in the 1990s, is probably the peak of high FHB resistance in high yielding and good agronomic type germplasm. These lines and many others since then have become the basis of all new resistant germplasm being developed in the region.

In spite of this broad genetic base, virtually all cultivars and advanced lines selected for FHB resistance demonstrate low to moderate level of infection in

epidemic years such as 2012. While this level of resistance combined with good agronomic practices and adequate chemical control of the disease is manageable, it is not sufficient to eliminate or reduce the level of mycotoxin production (DON) or contamination in the harvested grain. In spite of a close relationship between low infection and low DON content, it does not hold true in all varieties. So all newer effort to enhance the level of FHB resistance in the germplasm must take low mycotoxin production into account. With the advances in biotechnology, identification of molecular markers and QTLs as well as genetic transformations, these techniques can well become a key to any future progress we make with this disease. Meantime, simple elimination of very susceptible or susceptible varieties from the market can serve as a first step to reduce the losses caused by this disease.

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References

Boerger A (1928) Observaciones sobre agricultura, quince años de trabajo fitotécnico en Uruguay. [Agricultural notes: Fifteen years of crop breeding in Uruguay] Montevideo, p 436

Chen P, Liu D, Sun W (1997) New countermeasure of breeding wheat for scab resistance. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT, 1996, Mexico DF, pp 59–65

Couch BC, Kohn LM (2002) A multilocus gene geneology concordant with host preference indicates segregation of a new species, *Magnaporhte oryzae*, from *M. grisea*. Mycologia 94:683–693

de Ottoni Sousa Rosa, de Ottoni Sousa Rosa Filho (2003) Estrategia para combinar alto rendimiento e resistencia a giberelaem trigo. In: Estrategias y metodologías utilizadas en el mejoramiento de trigo. Seminario Internacional La Estanzuela Uruguay CIMMYT-INIA 2001, pp 129–136

de Viedma L (1989) Importancia y distribución de la fusariosis del trigo en el Paraguay. In: Kohli MM (ed) Taller sobre la fusariosis de la espiga en América del Sur. CIMMYT Encarnación Paraguay1987, Mexico DF, pp 39–48

Diaz de Ackermann M (2006) Germplasm exchange in the Southern Cone of Latin America. In: Ban T, Lewis JM, Phipps EE (eds) The global Fusarium initiative for international collaboration: a strategic planning workshop. CIMMYT El Batán, México, pp 103–108

Diaz de Ackermann M, Pereyra S (2011) Fusariosis de la espiga de trigo y cebada. In: Pereyra S, Diaz de Ackermann M, GermanS, Cabrera K (eds) Manejo de enfermedades en trigo y cebada. Serie Técnica INIA 189. INIA, Montevideo, Uruguay, pp 111–128. www.inia.org.uy

EMBRAPA (2011) Informacões técnicas para trigo e triticales- safra 2012. Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale. 5:2011: Dourados MS, p 204

Fedak G, Cao W, Xue A, Savard M, Gilbert J, Clarke J, Somers D (2006) Fusarium Head Blight resistance from wide crosses in bread wheat and durum. In: Ban T, Lewis JM, Phipps EE (eds)

The global Fusarium initiative for international collaboration: a strategic planning workshop. CIMMYT El Batán, México, pp 20–23
Galich MT (1989) Importancia y difusión de la fusariosis del trigo en Argentina. In: Kohli MM (ed) Taller sobre la fusariosis de la espiga en América del Sur. CIMMYT Encarnación Paraguay1987, México DF, pp 7–26
Galich MTV (1997) Fusarium Head Blight in Argentina. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium Head Scab: global status and future prospects. CIMMYT 1996, Mexico DF, pp 19–28
Gilchrist L, Rajaram S, Mujeeb-Kazi A, van Ginkel M, Vivar H, Pfeiffer W (1997) Fusarium Scab Screening Program at CIMMYT. In: Dubin HJ, Gilchrist L, Reeves J, McNab A (eds) Fusarium head scab: global status and future prospects. CIMMYT 1996, Mexico DF, pp 7–12
IICA-BID ATN/SF 6486 RG (2003) Informetécnico final. Proyecto: development of technologies for the integrated control of FHB in wheat, Agreement IICA-BID ATN/SF 6486 RG, FONTAGRO/PROCISUR. Montevideo, Uruguay, p 82
IICA-Cone Sul/BID (1981) Southern Cone Meeting on *Septoria* and *Gibberella*, IICA-Cone Sul/BID. IICA/PROCISUR, Montevideo, Uruguay, p 133
Instituto Interamericano de Cooperación para la Agricultura (IICA) (2010) Fusarium. In: Diaz R (ed) Proyecto Regional Trigo: principales logros y avances, IICA-PROCISUR. Montevideo
Kato H, Yamamoto M, Yamaguchi-Ozaki T, Kadouchi H, Iwamoto Y, Nakayashiki H, Tosa Y, Mayama S, Mori N (2000) Pathogenicity, mating ability and DNA restriction fragment length polymorphism of Pyricularia populations isolated from Gramineae, Bambusideae and Zingiberaceae plants. J Gen Plant Pathol 66:30–47
Kohli MM (ed) (1989) Análisis de la Fusariosis del Trigo en el Cono Sur. Taller sobre la Fusariosis de la Espiga en America del Sur. 7–11 September 1987. CIMMYT, Asuncion
Kohli MM (1999) Breeding wheats for South America: collaboration with CIMMYT. In: Proceedings of the 3th Assembly Wheat Breeding Society of Australia. Vision 2020. University of Southern Queensland, Toowoomba
Kohli MM, Quincke M, de Ackermann MD (2002) Screening winter and facultative wheats for Fusarium Head Blight infection. National Fusarium Head Blight Forum, Cincinnati
Kohli MM, de Viedma L, Cubilla LE (2010) Manual del Agricultor. In: Kohli MM, de Viedma L, Cubilla LE (eds) Guía para la producción de trigo. MAG/DIA/CRIA/CARECO, Asuncion, Paraguay, p 40
Kohli MM, Pedretti R, de Viedma L (2011) Chapter 19: History of wheat breeding in Paraguay. In: Bonjean AP, Angus WJ, van Ginkel M (eds) The world wheat book, vol 2: History of wheat breeding. Lavoisier. Paris, France, pp 467–500. ISBN 2743011025, 9782743011024
Liu DJ, Weng YQ, Chen PD, Wang YN (1989) Gene transfer of scab resistance from *Roegneria kamoji* and *Elymus giganteus* to common wheat. Jiangsu Agric Sci 1(suppl):97–100
Luzzardi G, Pierobom C (1989) Importancia y distribución de la fusariosis del trigo en Brazil. In: Kohli MM (ed) Taller sobre la fusariosis de la espiga en América del Sur. CIMMYT Encarnación Paraguay1987, Mexico DF, pp 33–37
Mesterházy A (2003) Breeding wheat for Fusarium Head Blight resistance in Europe. In: Leonard K, Bushnell W (eds) Fusarium Head Blight of wheat and barley. APS Press, St Paul, Minnesota, USA, pp 211–240. ISBN 978-0-89054-302-3
Mujeeb-Kazi A, Bernard M, Bekele G, Miranda J (1983) Incorporation of alien genetic information *from Elymusgiganteus* into *Triticum aestivum*. In Sakamoto S (ed) Proceeding 6th international wheat genetic symposium, Kyoto, pp 223–231
Piñeiro MS, Kohli MM, Silva GE (1996) Mycotoxin presence and field infection in selected wheat lines varying in resistance to *Fusarium*. In: Miraglia M, Brera C y Onori R (eds) IX international IUPAC symposium on mycotoxins and phycotoxins, Rome 1996
Reis E, Kohli MM (1993) Wheat diseases in South America and strategies for their control. In: Tanner DG (ed) Developing sustainable wheat production systems. The eighth regional wheat workshop for Eastern, Central and Southern Africa. CIMMYT 1994, Addis Ababa
Sartori JF (1982) Giberella. In: Trigo no Brasil. Fundaçao Cargill, Campinas, Brasil (1):537–541

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831–838

Tavella CM, Gonnet M, Díaz M (1979) El golpe blanco del trigo. Revista de la Asociación de Ingenieros Agrónomos del Uruguay 13:3–6

Tosa Y, Hirata K, Tamba H, Nakagawa S, Chuma I, Isobe C, Osue J, Urashima AS, Don LD, Kusaba M, Nakayashiki H, Tanaka A, Tani T, Mori N, Mayama S (2004) Genetic constitution and pathogenicity of Lolium isolates of Magnaporthe oryzae in comparison with host species-specific pathotypes of the blast fungus. Phytopathology 94:454–462

Wang Y, Chen P, Liu D (1986) Studies on transfer of *E. giganteus* germplasm into wheat. I. Production of wheat x *E. giganteus* hybrids. J Nanjing Agric Univ 1:10–14

Wang Y, Chen P, Wang Z, Liu D (1991) Studies on transfer of *E. giganteus* germplasm into wheat. II. Cytogenetics and scab resistance of backcross derivatives. J Nanjing Agric Univ 14(2):1–5

Weng Y, Zhang X, Yen Y, Jin Y (2001) Characterization of Fusarium head blight resistant germplasm with SSR markers linked to FHB resistance in Sumai#3. In: 2001 National Fusarium Head Blight Forum proceedings, Holiday Inn Cincinnati-Airport, Erlanger, pp 212–215

Weng Y, Yen Y, Jin Y (2003) Results of SSR fingerprinting of 94 newly identified Fusarium head blight resistance sources. In: 2003 National Fusarium Head Blight Forum proceedings, Holiday Inn Select, Bloomington, p 236

Zhang X, Jin Y (2002) Putative sources of Fusarium head blight resistance in spring wheat identified from the USDA small grains collection. In: 2002 National Fusarium Head Blight forum proceedings, Holiday Inn Cincinnati-Airport, Erlanger, pp 220–222

Zhang X, Jin Y (2003) Evaluation of spring wheat germplasm for Fusarium head blight resistance. In: 2003 National Fusarium Head Blight forum proceedings, Holiday Inn Select, Bloomington, pp 238–241

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