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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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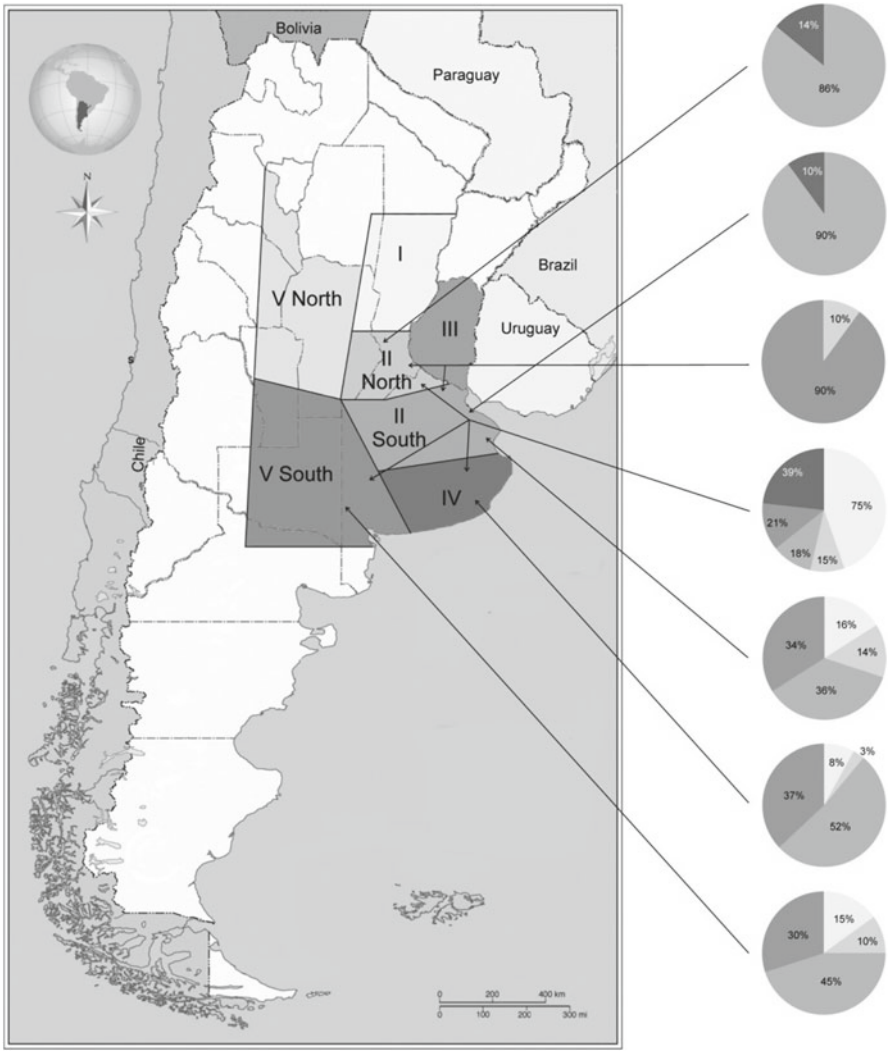

Fig. 1.1 Wheat cultivation regions in Argentina showing the proportions of *Fusarium graminearum* species complex genotypes (Reynoso et al. 2011) and chemotypes (Ramirez et al. 2006; Fernandez Pinto et al. 2008; Alvarez et al. 2011)

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