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Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina

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Abstract

Gibberella zeae (anamorph *Fusarium graminearum*) is the main pathogen causing *Fusarium* head blight of wheat in Argentina. The objective of this study was to determine the vegetative compatibility groups (VCGs) and mycotoxin production (deoxynivalenol, nivalenol and 3-acetyl deoxynivalenol) by *F. graminearum* populations isolated from wheat in Argentina. VCGs were determined among 70 strains of *F. graminearum* isolated from three localities in Argentina, using nitrate non-utilizing (*nit*) mutants. Out of 367 *nit* mutants generated, 41% utilized both nitrite and hypoxanthine (*nit1*), 45% utilized hypoxanthine but not nitrite (*nit3*), 9% utilized nitrite but not hypoxanthine (NitM) and 5% utilized all the nitrogen sources (*crn*). The complementations were done by pairing the mutants on nitrate medium. Fifty-five different VCGs were identified and the overall VCG diversity (number of VCGs/number of isolates) averaged over the three locations was 0.78. Forty-eight strains were incompatible with all others, thus each of these strains constituted a unique VCG. Twenty-two strains were compatible with other isolates and were grouped in seven multimembers VCGs. Considering each population separately, the VCG diversity was 0.84, 0.81 and 1.0 for San Antonio de Areco, Alberti and Marcos Juarez, respectively. Toxin analysis revealed that of the 70 strains of *F. graminearum* tested, only 90% produced deoxynivalenol, 10% were able to produce deoxynivalenol and very low amounts of 3-acetyldeoxynivalenol. No isolate produced nivalenol. The results indicate a high degree of VCG diversity in the *F. graminearum* populations from wheat in Argentina. This diversity should be considered when screening wheat germplasm for *Fusarium* head blight resistance.

Introduction

Wheat production in Argentina covers about 6.24 millions hectares. It reached 16 million tons during the 2004 harvest season, ranking Argentina as fourth in the world for export (Garcia, 2004). The main pathogen associated with *Fusarium* Head Blight (FHB) in wheat in Argentina is *Gibberella zeae* (anamorph *Fusarium graminearum*) (Galich, 1997). In Argentina during the last 50 years, 16 FHB epidemics of varying degrees of severity occurred in the north central area. In 1993, during a severe FHB outbreak 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 grain trading value resulting from low grain weight, the presence of scabby grains, and mycotoxin contamination. In the most affected areas, nearly all production was traded below established standards. During this severe epidemic, deoxynivalenol (DON) was the only toxin reported (Dalcero et al., 1997).

The occurrence of DON in cereal grains is of great concern for human and animal health, since this toxin is known to cause food refusal, vomiting and depressed immune functions (WHO, 2001). Although progress is being made in disease reduction through chemical control, cultural control, and the development of resistant cultivars, satisfactory levels of control have yet to be attained (Bai and Shaner, 1994; Leonard and Bushnell, 2003). *Fusarium graminearum* can produce a range of trichothecenes, including 4-acetylnivalenol (4-ANIV), nivalenol (NIV), DON, and other acetylated derivates. The loss of trichothecene production does not affect the ability of an isolate to infect wheat or maize, but it does affect the spread of infection (Desjardins et al., 1996). Chemotypes, based on the dominant trichothecenes produced, have been described in *F. graminearum* (Ward et al., 2002), but the relationship between chemotype and pathogenicity has not been established with respect to barley, wheat, triticale, rye or maize (Logrieco et al., 1990; Perkowsky et al., 1997). On wheat, Cumagun et al. (2004) found a correlation between chemotype (DON or NIV) and aggressiveness, but chemotypes were not useful to distinguish the population at the genetic level (O'Donnell et al., 2000). The lack of a relationship between chemotype and pathogenicity may mean that both the type and the amount of toxin produced affect pathogenicity (Ward et al., 2002). Chemotype variation is, however, important in determining the suitability of methods used to analyse the toxin content of human and animal food.

In many filamentous fungi, physiologically distinct individuals can fuse asexually to form a stable heterokaryon. A heterokaryon is a multinucleate cell containing genetically distinct nuclei in a common cytoplasm. Heterokaryosis provides an opportunity for genetic recombination via the parasexual cycle in sterile, homothallic and imperfect fungi. The individuals that fuse and form a stable heterokaryon are said to be vegetatively compatible and therefore belong to the same vegetative compatibility group (VCG). Genetic control of vegetative compatibility was found to be conditioned by numerous loci in those species where it has been investigated. In *F. graminearum*, heterokaryon formation is governed by a series of at least 5 *vic* loci and it is likely to be much higher (Bowden and Leslie, 1999). These loci control heterokaryon formation in a homogenic manner, i.e. two fungal isolates are able to form a heterokaryon only if the alleles at each of their corresponding *vic* loci are identical. VCGs have been used in a number of fungal species to assess the level of pathogen variability and obtain additional insights into their population structure (Leslie, 1993). In some cases, they have been correlated with pathogenicity to a specific host. In addition to providing a useful genetic marker, VCGs also directly affect the potential asexual exchange (parasexuality) and may play an important role in preserving mycelial individualism during exploitation of substrates. Knowledge about the genotypic variation of *F. graminearum* isolates that infect wheat would help in designing effective screening strategies for the development of FHB-resistant wheat.

In *F. graminearum* VCGs can be determined using two basic techniques: (1) direct assessment of heterokaryon formation using auxotrophic mutants such as nitrate non-utilizing (*nit*) mutants (Bowden and Leslie, 1992) and (2) direct assessment of the inability to form heterokaryons through barrage formation (McCallum et al., 2004).

Bowden and Leslie (1997) stated that the population structure of *F. graminearum* is poorly understood, and a better understanding of spatial and temporal patterns of genotypic diversity, potential sexual and asexual reproduction, and selection pressures, could lead to better control strategies for FHB.

Although there are reports of genotypic variability on *F. graminearum* populations assessed by VCGs from the USA (Bowden and Leslie, 1994; Zeller et al., 2003), Canada (Gilbert et al., 2001; McCallum et al., 2001), Europe (Gagkaeva et al., 2001), Korea (Moon et al., 1999) and China (Gagkaeva et al., 2001), there is no available information about this topic in Argentina or South America.

The objective of this study was to determine the VCGs and mycotoxin production (DON, NIV and 3-ADON) by *F. graminearum* (*G. zeae*) populations isolated from wheat in Argentina.

Materials and methods

Sampling

Wheat samples were collected from three commercial wheat fields, two located in Buenos Aires province, San Antonio de Areco (SAA) and Alberti (ALB), and one in Cordoba province, Marcos Juarez (MJ), Argentina. The samples were collected during the 2002 harvest season. The three localities belong to Region II within the major wheat production area of Argentina (Figure 1). This region has a temperate-humid climate. The two localities ALB and SAA (Buenos Aires province) are 130 km apart, and 300 km from MJ (Cordoba province). The wheat variety planted in the three fields was 'Pro Inta Granar' (highly susceptible), and the disease incidence was estimated as >70% in the three localities. In each location a $5.0 \times 5.0\ m^2$ plot area was chosen for sampling. Fifty spikes with symptoms of FHB were randomly collected in each field, air-dried, and stored at −20 °C in paper envelopes.

For the present study, all isolates sampled from a naturally contaminated field were regarded as a population, namely a group of isolates originating from a limited geographical area in the 2002 harvest season.

Strain isolation and morphological identification

Wheat seeds with symptoms of FHB were transferred on to Nash–Snyder modified pentachloronitrobenzene medium (PCNB). The PCNB plates were incubated at 25 °C for 7 days with a 12/12 photoperiod under cool-white and black-light fluorescent lamps (Nash and Snyder, 1962). Monoconidial cultures were obtained using a stereoscopic microscope. Identification of *Fusarium* species was done on carnation leaf agar (CLA) and potato dextrose agar (PDA) incubated at 25 °C for 15 days with a 12/12 photoperiod under cool-white and black-light fluorescent lamps according to Nelson et al., (1983). The isolates identified as *F. graminearum* were kept as spore suspensions in 15% glycerol and frozen at −80 °C. The strains are deposited in the culture collection at the Department of Microbiology and Immunology, Universidad Nacional de Río Cuarto, Córdoba, Argentina.

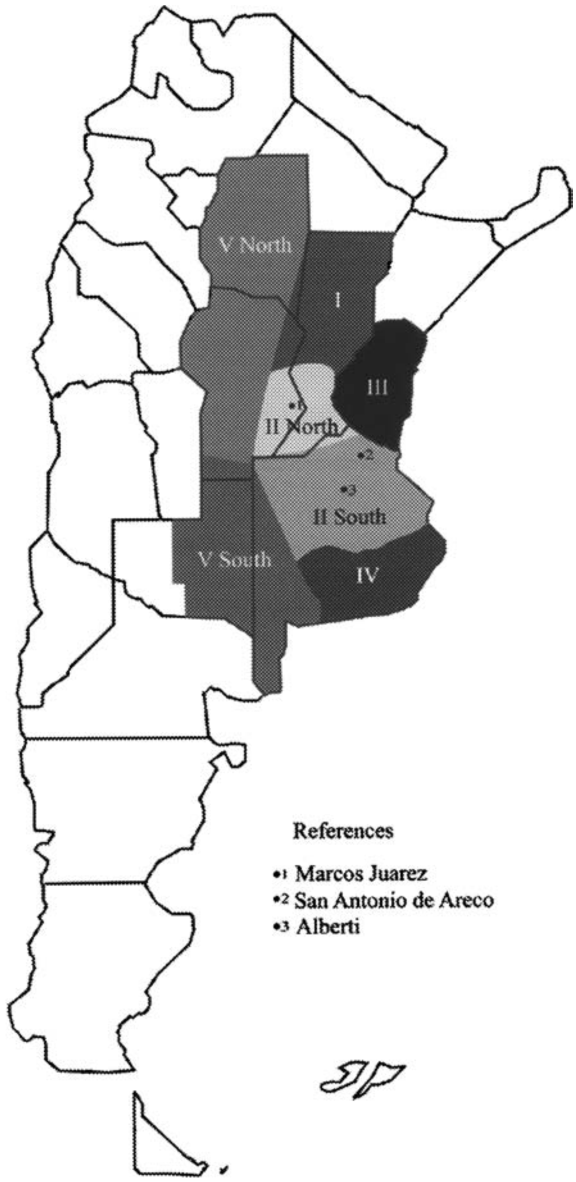

Figure 1. Map indicating the main agroecological regions for wheat production in Argentina, with the localities where *Fusarium graminearum* strains were isolated. Localities: 1, Marcos Juarez; 2, San Antonio de Areco; and 3, Alberti.

Perithecium production

Ability to produce homothallic perithecia was tested for all the putative *F. graminearum* isolates identified by morphology on carrot agar (Bowden and Leslie, 1999; Klittich and Leslie, 1988) and/or CLA (Fisher et al., 1982) at 25 °C under a mixture of cool-white and black-light fluorescent lights with a 12 h photoperiod.

Production and characterization of nitrate non-utilizing mutants

Nitrate non-utilizing mutants were generated by placing isolates onto 1.5% chlorate medium made by the addition of 3.0 g $NaNO_3$, 1.6 g L-asparagine, and 15.0 g $KClO_3$ per litre of basal medium (30.0 g sucrose, 1.0 g KH_2PO_4, 1.0 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 20.0 g agar, and 0.2 ml trace element solution in 1 l of distilled water) (Brooker et al., 1991). In order to find the optimum conditions for *nit* mutant generation the concentration $KClO_3$ in the media was raised up to 2.5%. Wild-type isolates were inhibited by the chlorate and initially grew slowly; fast-growing chlorate-resistant sectors began to appear after approximately 7 days, Sectors were subcultured on nitrate medium slants (basal medium plus 3.0 g l^{-1} $NaNO_3$). Strains that were unable to metabolise nitrate and grew sparsely with little or no aerial mycelium on nitrate medium were designated as *nit* mutants and were saved for later analysis. Strains with heavy growth that resembled wild-type strains were designated as chlorate-resistant, nitrate-utilizing (*crn*) mutants and were discarded because they could not be used to force heterokaryon formation on nitrate medium; *nit* mutants were further tested for their ability to utilize nitrite (basal medium plus 3.0 g l^{-1} $NaNO_2$), hypoxanthine (basal medium plus 0.2 g l^{-1} hypoxanthine) and ammonium (basal medium plus 1 g l^{-1} ammonium tartrate) as sole nitrogen sources. Mutants able to utilize both nitrite and hypoxanthine were classified as *nit1*, those able to utilize nitrite but not hypoxanthine were classified as NitM, and those that could utilize hypoxanthine but not nitrite were classified as *nit3* (Klittich and Leslie, 1988).

Vegetative compatibility test

At least two different *nit* mutants were obtained from each strain and complementary pairings of *nit1*, *nit3*, and NitM were initially made to establish self-compatibility. Wherever possible, VCG assignments were based on complementation reactions between NitM and *nit1* mutants. Otherwise, complementation reactions between *nit3* and *nit1* were used. Complementation tests were conducted by placing pairs of complementary mutants approx 2 cm apart on 6 cm Petri dishes containing nitrate medium (basal medium plus 3.0 g l^{-1} $NaNO_3$) and incubated at 25 °C for 14 days. If the two mutants were complementary, a line of dense heterokaryotic (prototrophic) growth formed at the contact point of the two colonies. Lack of complementation may have meant that the mutants were not complementary and that the mutant strains were in different VCGs. VCGs were established by pairing mutant in all possible pairwise combinations (4900).

Trichothecene production

Wheat grain (14.5% moisture content, 1 kg batches) were gamma-irradiated (10–12 k gray using a Cobalt radiation source and stored aseptically at 4 °C. The irradiated grain contained no microbial infection or mycotoxin contamination and retained germinative capacity of about 75%, although respiration was reduced by about 30% and shoot length by 65–70% (Hamer, 1994). The initial water activity (a_w) of the grain was 0.766; 400 g of irradiated wheat was weighed into sterile beakers and rehydrated to the required a_w (0.995) by addition of sterile distilled water using a moisture absorption curve. Flasks were subsequently refrigerated at 4 °C for 48 h with periodic shaking to allow absorption and equilibration. Finally, the a_w values were confirmed by using an Aqualab Series 3 (Decagon Devices, Inc., WA, USA).

Rehydrated wheat was placed in sterile 9-cm Petri dishes to form a thin layer of grains (20 g); a 3 mm diam agar disk was then taken from the margin of a 7 day-old growing colony of each isolate on synthetic nutrient agar (Gerlach and Nirenberg, 1982) at 25 °C and transferred to the centre of each plate. Plates containing grains were then placed in sealed plastic containers together with beakers of glycerol–water solution at 0.995 a_w in order to maintain a correct equilibrium of relativity humidity inside the boxes. Containers were incubated at 25 °C. After 28 days of incubation each sample was dried at 50 °C for 24 h and stored at −20 °C until toxin analyses were carried out.

Deoxynivalenol, nivalenol and 3-acetyldeoxynivalenol analyses

The toxin analyses were done using a modified version of that originally reported by Cooney et al. (2001). Each sample was finely ground and mixed well. A sub-sample (15 g) was extracted by mixing with acetonitrile/methanol (14:1, v/v; 40 ml), shaken for 2 h and then filtered through filter paper (Whatman N°1). A syringe was plugged with glass wool and dry-packed with alumina/carbon (20:1, w/w; 500 mg) to form a mini-cleanup column. A 2 ml aliquot of extract was applied to the column and allowed to drain under gravity and the eluant collected. The column was washed with acetonitrile/methanol/water (80:5:15, v/v; 500 µl), and the combined eluant was evaporated to dryness (N_2, 50 °C). The cleaned-up residue was dissolved in methanol/water (5:95, v/v; 500 µl).

The HPLC system consisted of a Hewlett Packard model 1100 pump (Palo Alto, CA) connected to a Hewlett Packard 1100 Series variable wavelength detector and a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a Luna™ C18 reversed-phase column (100×4.6 mm^2, 5 µm particle size) connected to a guard column SecurityGuard™ (4×3.0 mm^2) filled with the same phase. The mobile phase consisted of methanol/water (12:88, v/v), at a flow rate of 1.5 ml min^{-1}. The detector was set at 220 nm with an attenuation of 0.01 AUFS. Injection volume was 50 µl and the retention time of NIV, DON, and 3-ADON were 400, 900, and 1300 s respectively. Quantification was relative to external standards of 1–4 µg ml^{-1} in methanol/water (5:95). The quantification limit was 5 ng g^{-1} for each toxin.

Results

We were able to isolate and identify 134 *F. graminearum* strains, 52, 56 and 26 from SAA, ALB and MJ, respectively. From these we arbitrarily selected 70 strains to perform VCG analysis. All isolates produced fertile homothallic perithecia on CLA and carrot agar and were identified as *G. zeae*.

Seven hundred and fifteen chlorate-resistant sectors were produced on chlorate medium from 70 single-spore isolates of *F. graminearum* with a mean of 5.2 sectors per isolate. The chlorate-resistant sectors that grew as thin expansive colonies with no aerial mycelium indicated an inability to utilize nitrate as the sole source of nitrogen. These were designated as *nit* mutants and comprised 51% of the total chlorate-resistant sectors. All *nit* mutants produced wild-type growth on complete medium. Three hundred and sixty-seven sectors were *nit* mutants, of which 41%, 45%, 9% and 5% were *nit1*, *nit3*, NitM and *crn*, respectively.

When *nit1* mutants were paired with either *nit3* or NitM mutants on nitrate medium, all 70 strains were self-compatible. When mutants from different isolates were paired on nitrate medium with each other 55 different VCGs were identified. The overall VCG diversity (number of VCGs/number of isolates) averaged over the three locations was 0.78. Forty-eight strains were incompatible with all others; thus each of these strains constituted a unique VCG. Meanwhile, 22 strains were compatible with other isolates and were grouped in seven multimember VCGs. Among these multimember VCGs we found three that grouped strains belonging to SAA and ALB and two VCGs that grouped strains belonging to SAA and MJ, while others included strains from only one population. Considering each population separately, the VCG diversity was 0.84, 0.81 and 1.0 for SAA, ALB and MJ, respectively. In the SAA population, we were able to identify 27 VCGs. Seven strains were compatible with other isolates and were grouped into two multimember VCGs; 25 strains were incompatible with all others, and thus each of these strains constituted a unique VCG. In the ALB population we identified 22 VCGs, only three were multimember groups, and the remainder (19) were groups with only one member. However, in the MJ population we were unable to identify multimember groups. All the strains (11) were incompatible with all others, and thus each strain constituted a unique VCG (Figure 2).

Toxin production on irradiated wheat revealed that among the 70 strains of *F. graminearum* tested, 90% produced only DON and 10% were able to produce DON and very low amounts of 3-ADON which was occasionally detected as a minor co-metabolite. DON production varied greatly among the *F. graminearum* isolates, ranging from 1.0 to 7.6 µg g^{-1}. No isolate produced NIV.

Discussion

The present study is the first report on VCG diversity of *F. graminearum* populations isolated

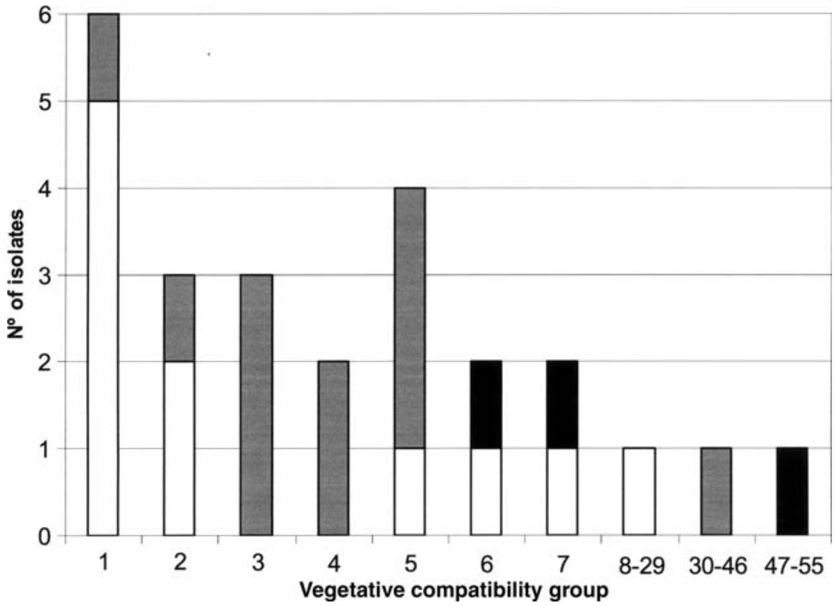

Figure 2. Vegetative compatibility group distribution by number of isolates and geographic origin of the strains (□ San Antonio de Areco; ▒ Alberti; ■ Marcos Juarez).

from different agroecological regions in Argentina. We found a high genotypic diversity, 0.78, considering together the isolates from the three populations under study. It was noticeable that we found multimember VCGs that grouped strains from SAA and MJ and SAA and ALB, although there were no multimember VCGs between MJ and ALB. These results appear to be related to the geographical position of the three localities since SAA is located between ALB and MJ. There may be some movement of similar genotypes between SAA and MJ, and between SAA and ALB since they are relatively close, but not between ALB and MJ since they are separated by a larger distance. Vegetative compatibility is a good indicator of clonal ancestry and predictor of genotypic similarity among isolates. However, this qualitative trait does not reveal how closely related isolates are belonging to the same VCG or how distantly related are isolates of different VCGs. In our study, we cannot confirm that members of the same VCGs are clones; further studies using molecular markers (i.e. AFLP) will be useful to determine the degree of genotypic variation in this population, providing complementary quantitative estimates of similarity or variability at various grouping levels. In a previous study it was shown that members of the same VCG in *F. verticillioides* have different AFLP haplotypes (Chulze et al., 2000). The fact that we found strains belonging to the same VCG in different locations also indicates that it might be reasonable to treat all the strains as members of the same population.

Similar results have been obtained in *F. graminearum* populations around the world using vegetative compatibility tests as a marker. Bowden and Leslie (1992) determined extensive levels of genetic diversity among 24 isolates of *F. graminearum* collected from 23 locations in Kansas. While all the isolates were self-compatible, no isolate was found to be compatible with another isolate. Bowden and Leslie (1994) later confirmed this high level of diversity for loci that determine vegetative compatibility for isolates collected on a much smaller spatial scale. They sampled 50 infected wheat heads from a small area (0.25 m^2) within a commercial wheat field in Kansas and up to three fungal isolates were obtained per wheat head. Though some isolates from different plants were members of the same VCG, indicating some degree of clonality, incompatibility was the rule. For example, 19 VCGs were identified among 26 isolates originating from 19 wheat heads. Even isolates from a single head sometimes belonged to different VCGs. The authors concluded that heads can be infected repeatedly by isolates from unrelated sources, and that mixed infection would provide an opportunity for out-crossing in the primarily homothallic species *F. graminearum* (Bowden and Leslie 1994, 1997; Zeller et al., 2003).

Similar variability has been reported for *F. graminearum* in Korea, Canada, Europe and Asia. Among a collection of 53 Korean isolates, no vegetative compatible pairs were identified (Moon et al., 1999). Likewise, VCG analysis of 15 *F. graminearum* isolates from three provinces in Canada resulted in the description of 14 VCGs. The two compatible isolates were originally isolated from the same research farm in Ontario (Gilbert et al., 2001). The *F. graminearum* population from barley also showed a high VCG diversity in Canada. Twenty-one VCGs were found among 22 *F. graminearum* isolates from different barley fields in Manitoba and 16 VCGs among 21 isolates from four barley fields that were sampled more repeatedly at a local level. The highest diversity was found among isolates from different fields where the average ratio of VCGs by isolates was 0.95, but even within the same barley spike he genetic diversity of the isolates was high, 0.90 (McCallum et al., 2001). VCG studies of isolates of *F graminearum* from Europe and Asia revealed a high degree of genetic diversity (Gagkaeva et al., 2001). In all 29 VCGs were found among 32 isolates. Only one complex VCG was found and it included isolates originating from distant localities, two isolates from Asia (China and the far east of Russia) and one from Europe (Germany). The high genotypic variation revealed in all these studies is somewhat surprising, as many of the ascospores were probably formed in a homothallic manner in which no recombination or genetic exchange occurred.

The high genotypic variability found among *F. graminearum* (*G. zeae*) populations in this study could be explained by out-crossing, mutation, gene flow or balancing selection. The relative importance of these evolutionary forces in *F. graminearum* populations have to be investigated in the future. Due to the lack of data, only general comments can be made here. Mutation can putatively play an important role given the large amounts of propagules produced during an epidemic on a small spatial scale. Evidence from various studies, that included observation and knowledge of fungal morphology, indicates that wind disperses the ascospores of *G. zeae*, whereas splashing rain disperses the macroconidia (Sutton, 1982). However, few studies have carefully documented and quantified spore dispersal. Ooka and Kommendahl (1977) observed that propagules of *Fusarium* species were transported on wind-blown soil particles to distances of 300–400 km. There is the potential for long-distance dispersal of propagules of *F. graminearum* and gene flow may play an important role in enhancing genotypic variation. However, the pathogen's life cycle alternates between saprophytic growth in the soil and a parasitic cycle when a susceptible host is available. This may result in a balancing selection for saprophytic versus parasitic fitness components and thus maintain or generate a high degree of polymorphism.

Although *F. graminearum* isolates have the ability to reproduce both sexually and asexually, and both macroconidia and ascospores can infect cereal heads (Sutton, 1982), the relative proportion of sexual and asexual reproduction is not known. Perithecia can be observed frequently in nature on the residue of maize or small grains (Francis and Burgess, 1977; Sutton, 1982). *Fusarium graminearum* is homothallic (Nelson et al., 1983) and therefore, individual ascocarps can yield either heterozygous progenies from out-crossing or homozygous progenies from selfing. The latter will not result in segregating progenies in haploid organisms. Although heterozygous perithecia and sexual recombination in the field have not yet been found, Bowden and Leslie (1999) reported an out-crossing rate of 35% under laboratory conditions by crossing strains with different nitrate non-utilizing mutants. If this rate is also realized in nature a high genotypic diversity may arise in small field areas, given the large number of propagules produced during an epidemic. Out-crossing appears to be common in *F. graminearum* populations from the USA and China because they display low levels of gametic disequilibrium (Gale et al., 2002; Zeller et al., 2004). Also, the relative frequency of selfing and out-crossing in nature has not been rigorously investigated. If sexual recombination does occur in the field, it could facilitate the assembly of selective advantageous multilocus genotypes through recombination of favourable alleles from diverse strains. This might improve the ability of *F. graminearum* populations to adapt to disease control measures such as resistant varieties, biocontrol organisms, or fungicides.

The likelihood of asexual genetic recombination (parasexuality) appears to be low in this fungus. Firstly, the probability of vegetative compatibility between two random isolates is low. Secondly, when vegetatively compatible strains anastomose, the resulting heterokaryon appears to be limited in stability. The apparent lack of propagation of the heterokaryon would greatly reduce the probability of asexual recombination (Bowden and Leslie, 1992).

The number of vegetative incompatibility, (*vic*) loci segregating in populations of *F. graminearum* is not known, although in Kansas it was concluded that at least five *vic* loci and probably more would be present in the *F. graminearum* population (Bowden and Leslie, 1999). In other ascomycetous, fungi from 6 to 10 independent *vic* loci have been identified (Leslie, 1993). In the present study we found 55 VCGs among 70 isolates; assuming that only two *vic* alleles are found at each locus, at least six recombining loci (2^6) would be present to produce this number of unique VCGs. The *vic* loci and alleles that define VCGs are presumed to be selectively neutral with respect to traits such as pathogenicity and vegetative viability *per se*. If selection acts to maintain a large number of VCGs with in a population, perhaps due to the value of individualism such those described by Rayner (1991), or to reduce the spread of infectious agents, then the frequency-dependent selection may be reflected in the frequency of individual *vic* genes and VCGs, and the number of VCGs that are maintained in the population (Leslie, 1993).

From the toxicological point of view, the toxigenic isolates of *F. graminearum* have been classified into different chemotypes: chemotype I produced DON and/or its acetylated derivatives, while chemotype II produced NIV and/or fusarenone X (Yoshizawa, 1997). Furthermore, DON chemotype strains of *F. graminearum* were subclassified into two types: DON-chemotype Ia producing DON and 3-ADON, essentially from warmer regions (mostly including the European strains) and DON-chemotype Ib, producing DON and 15-ADON from slightly cooler regions (mostly including the American strains) (Miller et al., 1991; Ward et al., 2002). It now appears that DON and NIV chemotypes of *F. graminearum* are not distributed evenly around the climatic areas of the world, and such ecological differences in chemotype distribution may contribute to establish regional grain contamination. In the present study we have demonstrated that chemotype I was prevalent among the *F. graminearum* strains isolated from wheat in Argentina. A small percentage of the strains, belonging to the chemotype Ia were also able to produce 3-ADON. None of the isolates produced both DON and NIV. The levels and the type of toxin found in the present work agree with previous studies carried out in our country (Faifer et al., 1990; Molto et al., 1997). Faifer et al., (1990) tested 24 *F. graminearum* strains isolated from wheat for mycotoxin production, and found that only 33% of the evaluated strains were able to produce DON, 15-ADON and 3-ADON. Molto et al. (1997) showed that 27 strains of *F. graminearum* isolated from maize in Argentina were able to produce DON (0.3–5.7 $\mu g\ g^{-1}$) and 7/27 produced 3-ADON (0.3–1.8 $\mu g\ g^{-1}$). No isolate produced NIV. However, our results partially disagree with a previous report by Lori et al. (1992) who found among 76 *F. graminearum* isolates from wheat, that 7 did not produce any mycotoxin (DON, NIV). Among the toxin producers, 38 produced DON, 17 produced NIV and 15 produced both toxins.

The present work demonstrates the high level of genotypic diversity, assessed by VCG analysis, among *F. graminearum* (*G. zeae*) populations isolated from wheat in Argentina. This high degree of genotypic variation should be considered when devising strategies for screening germplasm to develop FHB-resistant wheat. Cultivar×isolate interaction did not have a dramatic effect on FHB in wheat (Dusabenyagasani et al., 1997; Miedaner, 1997) but isolates differed significantly in aggressiveness (Miedaner et al., 2001). The disease is also influenced by environment (Miedaner, 1997). Inoculation with an isolate mixture of *F. graminearum* should be considered in wheat breeding programmes screening for FHB-resistant germplasm.

Recently, O'Donnell et al. (2000) and Ward et al. (2002) used six gene genealogies to show that the *F. graminearum* clade is composed of eight worldwide lineages. Furthermore, these lineages were biogeographically structured, suggesting limited gene flow among these populations. The *F. graminearum* clade lineages have been formally described as phylogenetically distinct species (O'Donnell et al., 2004). Work is currently in progress in our laboratory in order to provide information about the *F. graminearum* clade lineages present on wheat in Argentina using AFLPs.

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