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# Genetic structure of *Fusarium verticillioides* populations isolated from maize in Argentina

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**Abstract** *Fusarium verticillioides* (sexual stage *Gibberella moniliformis*) is a common fungal pathogen of maize worldwide that also produces fumonisin mycotoxins. Populations of this fungus can be diverse with respect to neutral and selectable genetic markers. We used vegetative compatibility groups (VCGs) and amplified fragment length polymorphisms (AFLPs) to evaluate the genetic structure of three *F. verticillioides* populations from commercial maize fields in Argentina. Based on work with similar populations from outside South America, we expected individuals within the populations to be genetically diverse, that genotypic variation would be distributed in a manner consistent with random mating, and that populations from different locations would be genetically indistinguishable from one another. We analysed 62 AFLP loci for 133 fungal isolates. All three populations were genotypically diverse but genetically similar and potentially part of a larger, randomly mating population, with significant genetic exchange occurring between the three subpopulations. There was no evidence for linkage disequilibrium at $P=0.05$. The low values of $G_{ST}$, the lack of frequent private alleles, and the lack of a systemic pattern of linkage disequilibrium all suggest that sexual reproduction is sufficiently common in *F. verticillioides* and that the dispersal of strains is sufficiently efficient for the population of *F. verticillioides* in the main maize growing region to be a single randomly mating population with no detectable genetic subdivision. Thus differences in disease and/or toxin production observed in this region are best attributed to differences other than the genetic composition of the population.

**Keywords** AFLP · Ear rot · *Gibberella fujikuroi* mating population A · Vegetative compatibility · Stalk rot · *Zea mays*

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## Introduction

In Argentina, *Fusarium verticillioides* (= *Fusarium moniliforme*, teleomorph *Gibberella fujikuroi* mating population A = *Gibberella moniliformis*) is the most common *Fusarium* species recovered from maize, although *Fusarium proliferatum* (teleomorph *G. fuji-*

*kuroi* mating population D = *G. intermedia*) and *Fusarium subglutinans* (teleomorph *G. fujikuroi* mating population E = *Gibberella subglutinans*) also may occur at significant frequencies (Chulze et al. 2000, 1996; Gonzalez et al. 1995; Ramírez et al. 1996; Reynoso et al. 2006; Sydenham et al. 1993). Similar results are known for *Fusarium* populations recovered from other locations (Bottalico 1998; Desjardins et al. 1994). Both *F. verticillioides* and *F. proliferatum* may produce troublesome levels of the fumonisin mycotoxins. Strains from Argentina produce between 40 and 20,000 μg $g^{-1}$ of fumonisins under laboratory conditions, but there is no known correlation between the location at which a strain was recovered and its ability to synthesise fumonisins (Reynoso et al. 2004).

Both biological characters, e.g., vegetative compatibility groups (VCGs; Leslie 1993) and mating properties such as mating type and male/female fertility (Leslie and Klein 1996), and anonymous molecular markers, e.g., Amplified Fragment Length Polymorphisms (AFLPs; Vos et al. 1995) have been used to characterise *Fusarium* populations (e.g., Zeller et al. 2004). These characters can be used to determine the genetic relatedness of individuals and populations and potential and historic levels of genetic exchange. Collectively, these data can be used to determine the speed at which a population is evolving and to determine the relative ease of generation of new multi-locus genotypes by recombination in response to selection or otherwise.

In this study we used VCGs and AFLPs to evaluate *F. verticillioides* populations collected from maize grown in Argentina. Strains in the same VCG often are inferred to be clonal in origin, although this inference need not always hold, e.g. (Chulze et al. 2000), as the critical question is whether two strains carry the same alleles at all of the *vic* loci. While VCGs are used primarily to distinguish 'self' from 'non-self', AFLPs provide a much more detailed evaluation of genetic variation as the number of possible multi-locus. AFLP genotypes is limited only by the number of primer pairs used to generate the AFLPs and often is large enough to be effectively infinite. AFLP data also can be used to estimate genetic relatedness of fungal strains and populations (e.g. González et al. 1998; Majer et al. 1996; Zeller et al. 2000, 2004), to gauge whether sexual reproduction has been occurring and to estimate the relative amount of genetic exchange occurring between non-contiguous populations.

The objective of this study was to characterise the genetic structure of *F. verticillioides* populations present in the major maize growing areas of Argentina, and to determine if their structure was similar to that observed in other parts of the world. We tested the hypotheses that individuals within the populations were genetically diverse, that the distribution of genotypic variation was consistent with that expected if the population was randomly mating, and that populations from different locations were not genetically distinguishable from one another. This report is the first for *F. verticillioides* from multiple locations in Argentina and provides important base line data for comparing the fungi from this region with those present in other parts of the world.

## Materials and methods

### Sampling

*Fusarium* species were isolated from maize kernels of a single hybrid (Pioneer 32K61) planted in 11 commercial fields cultivated by conventional tillage during the 1996/1997 cropping season in three maize growing regions in Argentina: Santa Fe (SF; three fields), Cordoba (CO; four fields) and Buenos Aires (BA; four fields). The fields sampled within a province were located within a 300 km-diam region. In the fields, maize was planted in rows 100 m length with 70 cm between rows and five plants $m^{-1}$ within a row. Maize ears were taken arbitrarily from 10 rows with a sampling intensity of 0.7%, or 42 ears per sample. Each ear was shelled by hand and the grain pooled; from each sample a 200 g sub-sample was taken.

### *Fusarium* isolation and identification

The grain in each sub-sample was surface-disinfected by soaking in 1% aqueous NaOCl for 1 min, and then rinsed three times with distilled water. One hundred kernels were plated (10 kernels per Petri dish) on a peptone + PCNB medium (Leslie and Summerell 2006). These plates were incubated at 24°C for 7 days with 12/12 h photoperiod under cool white and black light fluorescent lamps (Leslie and Summerell 2006).

*Fusarium* isolates (one or more per kernel) were subcultured as single conidia and then transferred to

carnation leaf agar (CLA) and potato dextrose agar (PDA) and incubated with a 12/12 h photoperiod under cold under cool white and black light fluorescent lamps (Leslie and Summerell 2006). Of the 420 *Fusarium* isolates recovered, 377 belonged to Section *Liseola*. Based on sexual crosses with *MAT-1* and *MAT-2* testers for *G. moniliformis*, *G. intermedia* and *G. subglutinans* (Leslie and Summerell 2006) there were 203 isolates of *F. verticillioides*, 78 isolates of *F. proliferatum* and 96 isolates of *F. subglutinans* (Reynoso et al. 2006). We arbitrarily selected 133 of the *F. verticillioides* isolates for further study. Female fertility of these isolates was determined by using the field isolate as the female parent and the complementary *G. moniliformis MAT* tester as the male parent.

The isolates were maintained as spore suspensions in 15% glycerol and frozen at −80°C. Duplicate subcultures of each isolate are held in the culture collection at the Department of Microbiology and Immunology, Universidad Nacional de Río Cuarto, Córdoba, Argentina, and at Kansas State University, Manhattan, USA.

## Vegetative compatibility tests

Sixty arbitrarily selected isolates of *F. verticillioides*, 20 strains from each region, were evaluated for vegetative compatibility (Leslie 1993); *nit* mutants were generated and assigned phenotypes as described by Leslie and Summerell (2006). At least two different *nit* mutants were obtained from each strain and complementary pairings of *nit1*, *nit3*, and NitM mutants were made to establish heterokaryon self-compatibility (Correll et al. 1989). Wherever possible, VCG assignments were based on complementation reactions between NitM and *nit1* mutants. Complementation tests were made in 24-well hybridoma plates as previously described (Klittich and Leslie 1988).

## DNA isolation

A suspension of spores (1 ml, aproximately $10^6$ spores $ml^{-1}$) from CLA was used to inoculate complete medium (CM; Correll et al. 1987) and incubated on an orbital shaker (150 rpm) for 3 days at 25±1°C. Fungal DNA was extracted from mycelia harvested by filtration with a cetyltrimethylammonium bromide (CTAB) method (Leslie and Summerell 2006).

## AFLP reactions

AFLPs were performed as described by Vos et al. (1995), as modified by Leslie and Summerell (2006) in a PTC-2000 Thermal Cycler (MJ Research Inc., Watertown, MA). We used all buffers and DNA modifying enzymes following either the manufacturer's instructions or standard protocols (Sambrook et al. 1989). Genomic DNA was digested to completion with *Eco*RI and *Mse*I and ligated to AFLP adapters in a single overnight reaction at room temperature (21–24°C), and then diluted in nine volumes of Tris–EDTA buffer prior to preamplification. We used two primer pair combinations (*Eco*RI+TT/*Mse*I+CA and *Eco*RI+GG/*Mse*I+CA) to prime the PCR-AFLP reactions. The *Eco*RI primer was labelled with $\gamma^{33}$P for detection of bands by autoradiography.

We manually scored the presence or absence of polymorphic AFLP bands ranging from 200 to 400 bp in length, and recorded the data in a binary, present/absent format. All bands in this size range were scored, including those that were monomorphic. Bands migrating at the same position were assumed to be homologous and to represent the same allele and locus. Bands of differing mobility were treated as independent loci with two alleles (present or absent). Unresolvable bands and missing data were treated as missing data. Multiple runs of DNA from the same strain were at least 98% similar, so a cut-off of 98% similarity was used to identify clones.

## Genetic distance

To estimate the genetic distance between individuals, similarity coefficients ($S$) were calculated using the formula: $S = 2N_{xy}/(N_x + N_y)$, where $N_x$ and $N_y$ are the number of fragments amplified in isolates $x$ and $y$, respectively, and $N_{xy}$ is the number of fragments shared by the two isolates (Nei and Li 1979). Genetic distance ($D$) was derived from similarity coefficients as $D = 1 - S$. Genetic distance matrices were constructed for isolates from the compiled AFLP data. Dendrograms were prepared by using the UPGMA (unweighted pair-group method using arithmetic averages) algorithm in NTSYSpc 2.0 (Numerical Taxonomy System) software package (Rohlf 1990). The AFLP data were subjected to bootstrap analysis with 1000 replications with PAUP* version 4.0 (Swofford 1999) to determine if there was significant

genetic substructure or clustering among isolates as they were resolved by the AFLPs.

### Genotype diversity assessments and population genetic analyses

We identified indistinguishable *F. verticillioides* isolates, i.e., clones, as those isolates with AFLP haplotypes that shared ≥98% of the scored AFLP bands. Populations were clone-censored, i.e., only one strain of each clone was retained for analyses of genetic diversity and linkage disequilibrium. We estimated genotypic diversity $(\widehat{G})$ for each population as described by Milgroom (1996) and the index for each population was normalised by dividing each estimated $\widehat{G}$ by the number of genotypes identified in that population.

We estimated (1) allele frequencies of polymorphic loci and gene diversity within and between populations as described by Nei (1973); (2) $G_{ST}$ (fixation index) as described by Nei (1973); (3) *Nm* (effective migration rate) as described by McDermott and McDonald (1993), and (4) genetic identity among populations as described by Nei (1978) with the shareware programme POPGENE version 1.32 (available at http://www.ualberta.ca/~fyeh, Yeh et al. 1997). All data analysed by this programme were treated as haploid with dominant markers. $G_{ST}$ and *Nm* were estimated with both the complete data set and with a subset of data that included only those loci for which both alleles were present at ≥5% frequency in at least one of the three populations. The results of these analyses were compared to determine if the inclusion of loci with rare alleles in these analyses altered the estimates of genetic differentiation of the populations. We also used POPGENE to estimate linkage disequilibrium for AFLP loci if both alleles at both loci were present in the population at a frequency of ≥5%. We calculated two-locus gametic disequilibria between all pairs of these loci, and conducted $\chi^2$ tests for significance as described by Weir (1979).

## Results

### *nit* mutants isolation and phenotype identification

The frequency of chlorate-resistant sectors varied among the isolates and upon the amount of chlorate in the medium MM + 2% $KClO_3$ was the most efficient for inducing *nit* mutants among the tested *F. verticillioides* isolates. Of the 654 chlorate-resistant sectors recovered, 381 (58%) were *nit* mutants and 273 (42%) were *crn* (chlorate-resistant nitrate utilising). Amongst the 381 *nit* mutants, 186 (49%) were *nit1*, 140 (37%) were *nit3* and 55 (14%) were NitM. NitM mutants were obtained from only 39 of the 60 isolates tested.

All 60 strains were heterokaryon self-compatible and produced robust wild-type growth when complementary *nit* mutants were derived from the same parent paired on MM. Forty-three VCGs were identified amongst these 60 isolates, of which 35 were each represented by a single isolate and were vegetatively incompatible with all tested strains and represent 35 unique VCGs. The remaining 25 isolates were compatible with at least one other isolate and could be grouped into eight VCGs containing two to five isolates each. Two of the eight multi-member VCGs contained isolates originating from more than a single location.

### Genetic distance

Sixty-two AFLP bands were identified in the 200–400 bp range for the 133 analysed isolates (Table 1). Of these 62 AFLP loci, 37 were polymorphic (60%, overall). Primer pair *Eco*RI+TT/*Mse*I+CA produced 35 bands of which 21 were polymorphic, while *Eco*RI+GG/*Mse*I+CA produced 27 bands of which 16 were polymorphic. The number of polymorphic bands for the *Eco*RI+TT/*Mse*I+CA primer pair drops from 21 to 15 and from 16 to 12 for the *Eco*RI+GG/*Mse*I+CA primer pair if only loci at which both alleles are present at between 5% and 95% of the entire population are included in the analysis. The number of clones repeatedly sampled or recovered as indicated by these AFLPs within the population was small (11/133). When genetic relationships amongst the strains analysed are displayed as a dendrogram (data not shown) there was no clustering of isolates from different populations. Within all locations, the bootstrap values of non-terminal branches generally were <50%, showing a lack of any clear geographic differentiation of the strains at a regional level. There were seven clones recovered more than once from Buenos Aires, and four from Cordoba.

**Table 1** Estimates of genotypic diversity ($\widehat{G}$) in Santa Fe, Buenos Aires and Cordoba clone-censored populations of *Fusarium verticillioides* based on 37 polymorphic loci

| Population | SF | BA | CO |
|---|---|---|---|
| Sample size | 34 | 47 | 52 |
| Percent polymorphic loci | 52 | 48 | 48 |
| Number of *F. verticillioides* haplotypes | 34 | 42 | 50 |
| $\widehat{G}$[a] | 34 | 44 | 49 |
| $\widehat{G}/n$[b] | 1.00 | 0.95 | 0.94 |

[a] Calculated as described in Milgroom (1996) from comparisons of AFLP allelic data at 62 AFLP loci. $\widehat{G} = 1/\sum p_i^2$ where $p_i$=the observed frequency of the $i$th multilocus genotype in a population.

[b] Calculated by dividing $\widehat{G}$ by the number of AFLP haplotypes observed in each population.

The genetic distance ($D$) was calculated for all pairs of isolates in the combined population. Across all populations the genetic similarity between any pair of isolates averaged 91% and ranged from 81% to 100%. Within populations the genetic similarity between any two isolates ranged from 82% to 98% for the Santa Fe population, 82% to 100% for the Cordoba population, and 85% to 100% for the Buenos Aires population. If only the 27 loci in which both bands were present in at least 5% of the population are evaluated, then the overall genetic similarity ranges from 56% to 100%, and the corresponding ranges for the Santa Fe, Cordoba and Buenos Aires populations are 60% to 95%, 57% to 100% and 63% to 100%, respectively.

## Genotypic diversity and recombination

Normalised genotypic diversity ($\widehat{G}$) was high (≥97% of the count) in all three populations, with the highest number of clonal isolates recovered from BA population. The highest genotypic diversity (100%) occurred in the Santa Fe population, in which all 34 isolates had unique AFLP haplotypes.

The percentage of locus pairs whose genotypes were present at frequencies statistically distinguishable from linkage equilibrium expectations ($\chi^2$ test, $P<0.05$) was 3.2%. If only loci in which both bands were present in at least 5% of the population are evaluated, then the percentage of locus pairs not in linkage equilibrium drops by one half. These percentage values are all less than the expected 5% cut-off due to error with Weir's methods (Weir 1979).

## Population differentiation

Allele frequencies were generally very similar between these three populations (data not shown). There were three loci with private alleles (both allelic forms present in one population but not the others) in the Santa Fe population, three in the Cordoba population and one in the Buenos Aires population (Table 2). In each of Santa Fe, Buenos Aires and Cordoba there was one private allele with a frequency of >5%.

For the full set of 62 loci, the average gene diversity was 0.128 among all isolates, with no obvious relationship between gene diversity and the size of the population. Removing the 25 loci for which no polymorphism was observed increased the mean gene diversity estimates from 0.128 to 0.215, and removing the 35 loci for which one allele was found in >95% of the isolates further increased the mean gene diversity estimates to 0.278 (Table 2).

For the full set of 62 loci, $G_{ST}$ for individual loci ranged from a 0 to 0.167 (Table 3). The mean $G_{ST}$

**Table 2** Summary of population statistical information comparing *Fusarium verticillioides* populations isolated from three maize growing sites in Argentina (Santa Fe, Buenos Aires and Cordoba)

| Population | Santa Fe | Buenos Aires | Cordoba |
|---|---|---|---|
| Sample size | 34 | 47 | 52 |
| Percent polymorphic loci | 52 | 48 | 48 |
| Number of private alleles | 3 | 1 | 3 |
| Mean frequency of private alleles[a] | 0.039 | 0.071 | 0.067 |
| Range | 0.029–0.059 | – | 0.020–0.160 |
| Mean gene diversity[a,b] | | | |
| 62 loci (all loci) | 0.138 | 0.118 | 0.128 |
| 37 loci (polymorphic loci) | 0.231 | 0.199 | 0.215 |
| 27 loci (highly polymorphic loci[c]) | 0.289 | 0.262 | 0.283 |

[a] 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.

[b] Calculated as in Nei (1973).

[c] Both alleles present at a frequency of >5%.

**Table 3** Statistics on population genetic differentiation between Santa Fe, Buenos Aires and Cordoba clone-censored populations of *Fusarium verticillioides* calculated from all 62 scored loci, the 37 polymorphic loci, and for the 27 loci at which the frequency of both alleles (presence and absence) was >5%

| Statistic | 62 loci | 37 loci | 27 loci |
|---|---|---|---|
| Mean gene diversity[a] | 0.128 | 0.215 | 0.278 |
| Range | 0.119–0.138 | 0.013–0.494 | 0.090–0.494 |
| Fixation index ($G_{ST}$)[a] | 0.0312 | 0.0312 | 0.0309 |
| Range | 0–0.167 | 0.0001–0.167 | 0.0001–0.167 |
| Effective migration rate ($Nm$)[b] | 15.5 | 15.5 | 15.7 |
| Range | 2.5–2,000 | 2.5–2,000 | 2.48–2,000 |
| Genetic identity[c] | 0.995 | 0.990 | 0.986 |

[a] Calculated as in Nei (1973)

[b] Calculated as in McDermott and McDonald (1993)

[c] Calculated as in Nei (1978)

(fixation index or differentiation among populations due to population subdivision) across all 62 loci was 0.0312, indicating that allele frequencies are similar in all three populations (Table 3). Analyses of the subset of 37 polymorphic loci or the subset of 27 loci for which the frequency of the rare allele was >5% gave results similar to those for the full set of 62 loci (Table 3). These results are consistent with the hypothesis that the populations from all three regions are subpopulations of a larger randomly mating population and regularly exchange significant genetic information ($Nm$>15 across all 62 loci).

## Discussion

We evaluated three populations from maize in Argentina for genetic variation as assessed with VCGs and AFLPs. For VCGs, the genotypic diversity was high, 72%, which could be maintained if sexual recombination is occurring within these populations. The presence of some strains in the same VCG might mean that some clonal reproduction is occurring; however, the AFLP genotypes for strains in the same VCG were identical in only one of the multiple member VCGs. Thus, at least in this population, VCG phenotype and AFLP genotype are not closely correlated. This result is similar to that of Chulze et al. (2000) who also found that strains of *F. verticillioides* that were in the same VCG were not necessarily clones of one another. Such results occur because isolates in the same VCG must share only the alleles at the *vic* loci and need not be genetically identical beyond this rather limited set of loci. The 43 VCGs detected require at least six polymorphic *vic* loci with two alleles each to account for the variation observed, as five such polymorphic loci could generate only 32 different VCGs (Leslie 1993). In other ascomycete fungi, the number of independent *vic* loci usually is postulated to be between 6 and 10 (Leslie 1993) which would be consistent with the results obtained here.

VCGs studies are useful to say that most of the isolates within a population are different but cannot be used to determine the degree of genetic relatedness among the isolates. We used AFLPs to make this assessment. The average genetic similarity of the *F. verticillioides* isolates we examined was 91%, ranging from a minimum of 81%, to a few clones with 100% similarity. If AFLP loci in which one allele is rare are excluded from this assessment then the average genetic similarity was 78% with a range from 56% to 100%. These similarity values are consistent with the hypothesis that all of the isolates belong to the same species and are similar to values previously reported for intraspecific variation for this and other *Fusarium* species (Leslie et al. 2004a, b, 2005).

All 133 isolates also were cross-fertile with one of the two mating type tester strains representing *G. moniliformis*, indicating that they all belong to the same biological species. Based on female fertility and mating type frequency the effective population sizes ($N_{e(mt)}$ and $N_{e(f)}$) of these populations are 93% and 90% of the count, respectively (Leslie and Klein 1996; Leslie and Summerell 2006). Thus, genetic drift in these populations is affected more by population size (which is large) than it is by limits on sexual reproduction.

Isolates with the same AFLP genotype, i.e., clones, were rare, with only 11 of 133 strains having a non-unique AFLP genotype. Correspondingly, the normalised genotypic diversity ($\widehat{G}$) was uniformly high (≥97% of the count) in all three populations (Table 2). High genotypic diversity strongly suggests that recombina-

tion has played a significant role in developing the population structure (Milgroom 1996) and has been observed in this and other *Fusarium*/*Gibberella* species with identified sexual reproductive stages (e.g., Chulze et al. 2000; Zeller et al. 2004).

Analyses of linkage disequilibrium also can be used to test if regular recombination has not been occurring in a population, as a population in which recombination occurs regularly should have relatively little linkage disequilibrium, while one in which recombination is rare should have many more loci in linkage disequilibrium. Estimates of gametic phase disequilibrium among locus pairs were uniformly low (24 to 31 locus pairs in each subpopulation) across the 37 polymorphic loci. Estimates from all three populations were each well below the statistical cut-off of 5% (data not shown) of all locus pairs that deviate significantly from expectations, although the sample sizes are somewhat smaller than the 100 recommended for the unambiguous detection of gametic phase equilibrium (Brown 1975). The lack of gametic phase disequilibrium, the high level of VCG diversity, the relatively few clonal strains as defined by AFLPs, the high $N_e$ values, and the high genotypic diversity estimates collectively suggest that regular recombination has occurred in these populations and that the sexual stage plays a significant role in the population biology of this fungus.

Gene flow can have a significant impact on the genetic structure of a population, since in its absence, isolated populations can become differentiated in terms of alleles present and allele frequencies due to genetic drift. The genetic identities of the subpopulations we evaluated were very nearly one even though they were separated by at least 200 km (Table 3). The low $G_{ST}$ values also indicate minimal geographic subdivision among the three subpopulations. If the high degree of similarity is due exclusively to gene flow and the populations are at a stable equilibrium, then $G_{ST}$ can be used to estimate $N_m$, the average number of migrants exchanged per generation to give the present lack of subdivision. $N_m$ averaged 15–16 across all loci and populations, suggesting that the level of gene flow was ~16 times greater than needed to prevent populations from diverging by genetic drift. Low levels of $G_{ST}$ also have been reported for populations of other plant-pathogenic fungi, e.g. *Rhizoctonia solani* (Rosewich et al. 1999), *Cronartium* (Et-touil et al. 1999), *Rhynchosporium secalis* (Salamati et al. 2000), *Mycosphaerella graminicola* (Linde et al. 2002), *Phaeosphaeria nodorum* (Keller et al. 1997) and *Gibberella zeae* (Zeller et al. 2003, 2004), and in each of these cases, the authors also concluded that these fungi exist as larger, well-mixed populations.

In conclusion, our data are consistent with the hypothesis that regular, homogenising gene flow occurs across maize-growing regions of Argentina and that the populations we evaluated are part of a much larger, interconnected pathogen population that includes all of the major maize-growing regions of Argentina. Consequently we think that for host resistance to this pathogen to be an effective control strategy, the resistance must be developed against the species as a whole and not against a single genotype. On the other hand, challenges of breeding material with a relatively few strains should be representative of most of the species, and differences in resistance reactions between locations, at least within Argentina, are more likely to be due to differences specific to the location, e.g. rainfall, tillage, etc., than they are due to differences in the composition of the pathogen population.

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