Euphytica (2006) 147: 403–407
DOI: 10.1007/s10681-005-9037-8
© Springer 2006

Stability of maize resistance to the ear rots caused by *Fusarium graminearum and F. verticillioides* in Argentinian and Canadian environments

Daniel A. Presello[1,*], Juliana Iglesias[1], Grisela Botta[1], Lana M. Reid[2], Gladys A. Lori[3] & Guillermo H. Eyhérabide[1]

[1]*Instituto Nacional de Tecnología Agropecuaria (INTA), CC 31, B2700KXC Pergamino, Argentina;* [2]*Agriculture and Agri-Food Canada (AAFC), Eastern Cereal and Oilseed Research Centre, Central Experimental Farm, Bldg. # 99, Ottawa ON, K1A 0C6, Canada;* [3]*Centro de Investigaciones de Fitopatología (CIDEFI-CIC), Facultad Cs. Agr. For. Universidad Nacional de La Plata, CC 31, (1900) La Plata, Argentina*
*(*author for correspondence: e-mail: dpresello@pergamino.inta.gov.ar)*

Received 27 January 2005; accepted 13 October 2005

Key words: Fusarium verticillioides, *Fusarium graminearum*, maize, disease resistance

Summary

Sources of resistance to *Fusarium* spp. are needed to develop maize hybrids resistant to the accumulation of fungal mycotoxins in the grain. In a search for resistant germplasm in 1999 and 2000, a set of Argentinian maize populations was evaluated in Ottawa, Canada, for resistance to ear rots after inoculation with local isolates of *Fusarium verticillioides* and *F. graminearum*. Sixteen of these populations, varying in observed resistance levels, were re-evaluated in 2003 and 2004 in Pergamino, Argentina, using local isolates of the same fungi. Conidial suspensions of each fungal species were inoculated into the silk channel of primary ears. Disease severity was assessed after physiological maturity using a scale based on the percentage of visibly infected kernels. Genotype effect was more important than genotype-by-fungal species or genotype-by-fungal species-by-environment interaction effects. In addition, disease severity levels associated with each fungal species were positively correlated ($P < 0.05$) ($r = 0.90, r = 0.81, r = 0.87$ and $r = 0.53$, in Ottawa 1999 and 2000, and Pergamino 2003 and 2004, respectively). Populations ARZM 01107, ARZM 07138, ARZM 10041, ARZM 13031, ARZM 16002 and Pora INTA exhibited the highest and most stable resistance to both species. Considering that disease resistance exhibited low specificity to the environment and to the fungal species in evaluations conducted in a wide range of environments and with fungal isolates collected from different hemispheres, the most resistant populations are potential sources of genes for stable resistance to these *Fusarium* spp.

Introduction

Fusarium graminearum (Schwabe) [teleomorph *Gibberella zeae* (Schwein. Petch] and *F. verticillioides* (Saccardo) Nirenberg [=*F. moniliforme* (Sheldon)] {teleomorph *G. fujikuroi* (Sawada) Ito in Ito & Kimura} are two of the most prevalent maize (*Zea mays* L.) ear rotting pathogens around the world. *Fusarium* spp. enter maize ears mainly through kernel wounds and the silks (Hesseltime & Bothast, 1977). These fungi cause loss in grain yield and affect grain quality due to contamination of infected grains with mycotoxins (Marasas et al., 1988; Prelusky et al., 1994; Vigier et al., 2001). Although mycotoxins have been detected in grain samples from ears exhibiting low levels of symptomatic kernels, particularly with infections of *F. verticillioides,* there is a positive association between visible symptoms and mycotoxin concentration for *F. verticillioides* (Desjardins et al., 1998; Clements et al., 2003) and for *F. graminearum* (Reid et al., 1996c). Therefore, it should be possible to reduce grain mycotoxin contamination by selecting for hybrids less susceptible to ear rots.

In the maize growing regions of Argentina and Canada, *F. verticillioides* and *F. graminearum* regularly cause outbreaks, but the prevalence of each species within a given year depends on the environmental conditions during the growing season. Thus, developing resistance to both pathogens is important to prevent yield loss and mycotoxin contamination. In addition, since the response of maize hybrids to *Fusarium* infection is affected by genotype–by-environment interactions (Vigier et al., 2001), the identification of stable sources of resistance is a prerequisite to the development of hybrids resistant to *Fusarium* infection across variable environmental conditions.

Resistance to ear rots caused by these *Fusarium* spp. is available and depends on several genes exhibiting mostly additive effects for *F. verticillioides* (Gendloff et al., 1986) and for *F. graminearum* (Chungu et al., 1996). An evaluation of disease resistance after inoculation of several isolates of the prevalent *Fusarium* species, in variable environmental conditions, may provide indications of resistance stability.

The objective of this work was to assess resistance and stability of resistance to ear rots, after inoculation with local isolates of *F. verticillioides* and *F. graminearum*, in a set of Argentinian maize populations across four environments (two years each in a major maize growing region of both Argentina and Canada).

Materials and methods

In a search for resistant germplasm in 1999 and 2000, fifty-three maize populations, representing a wide range of the earliest germplasm from Argentina, and two local check hybrids were evaluated for ear rot resistance after inoculation with local isolates of *F. verticillioides* and *F. graminearum* in Ottawa, Ontario, Canada (Presello et al., 2004). In 2003 and 2004, 16 of these populations, including fourteen landraces and two breeding populations (Table 1), which varied in disease resistance when evaluated in Canada, were chosen to be re-evaluated in Pergamino, Province of Buenos Aires, Argentina, for resistance to local isolates of the same fungal species. Information on race, geographical origin, and kernel type of each genotype were provided by Presello et al. (2004).

A split-plot design, with the genotype randomized to the main plot units and the fungal species randomized to the sub-plot units, was used in the four environments. Since the search of broad resistance to *F. graminearum* and *F. verticillioides* was considered as a main objective, fungal species was randomized to the sub-plot units to have the highest precision level in comparisons of means of disease severity after the inoculation with either fungus within genotype. However, disease severity means among genotypes were compared with a lower precision level than that for fungal species. The number of replicates was three in Ottawa and two in Pergamino. Each subunit consisted of two 3.5-m long rows separated by 0.7 m and sown at a rate of fifteen plants per row.

In Canada, the inoculum for *F. verticillioides* was produced from the isolate DAOM 195167 and the inoculum for *F. graminearum* was produced from a mixture of three isolates (DAOM 180378, DAOM 194276 and DAOM 212678). All of these isolates were obtained from naturally infected maize in Ontario production fields. In Argentina, the isolate P364, obtained

Table 1. Severity of ear rot symptoms after inoculation with conidial suspensions of *Fusarium verticillioides* and *F. graminearum* into the silk channel of fourteen landraces and two breeding populations of maize evaluated in two years (2003 and 2004) in Pergamino, Argentina

		Symptom severity[a]			
		Fusarium verticillioides		*Fusarium graminearum*	
Genotype	Code	2003	2004	2003	2004
ARZM 01069	G1	0.38	0.29	0.40	0.35
ARZM 01107	G2	0.26	0.23	0.37	0.34
ARZM 01122	G3	0.71	0.33	0.81	0.59
ARZM 01123	G4	0.31	0.23	0.49	0.42
ARZM 01127	G5	0.41	0.29	0.50	0.42
ARZM 07138	G6	0.18	0.20	0.32	0.36
ARZM 09144	G7	0.48	0.34	0.84	0.27
ARZM 10041	G8	0.22	0.26	0.28	0.31
ARZM 12154	G9	0.48	0.40	0.59	0.67
ARZM 13031	G10	0.16	0.23	0.31	0.32
ARZM 16002	G11	0.29	0.24	0.25	0.35
ARZM 16015	G12	0.39	0.37	0.47	0.5
ARZM 16040	G13	0.38	0.40	0.52	0.34
ARZM 19001	G14	0.59	0.55	0.93	0.50
CSDP[b]	G15	0.38	0.30	0.39	0.40
Pora INTA[b]	G16	0.16	0.19	0.24	0.21
Mean		0.36	0.30	0.48	0.40
L.S.D.[c]			0.18		

[a]Arcsin (percentage of the ear affected by the fungus)$^{1/2}$.
[b]Breeding populations.
[c]Least significant difference to compare the means of two genotypes at a probability level of 0.05.

from maize grown in Pergamino, was used to produce the inoculum of *F. verticillioides* and the isolate VI-II-3, obtained from wheat at the Universidad Nacional de La Plata and tested for aggressiveness in maize, was used to produce the inoculum of *F. graminearum*.

Isolates were grown separately in a liquid medium following Reid et al. (1996a). After two weeks, the cultures were filtered through cheesecloth to remove mycelium and conidial concentrations were adjusted to 2.5×10^5 conidia ml^{-1} with sterile water. Suspensions were stored at 4 °C for a maximum of three days prior to inoculation. For inoculation, two ml of conidial suspension were injected into the silk channel 4 to 6 days after silking (Reid et al., 1996a).

Ears were manually harvested at maturity and disease severity was assessed by determining the percentage of each ear covered by fungus using a seven-category rating scale where: 1 = no symptoms; 2 = 1–3%; 3 = 4–10%; 4 = 11–25%; 5 = 26–50%; 6 = 51–75%; and, 7 = 76–100% of the ear covered by the fungus (Reid et al., 1996a). This scale was tested for accuracy of disease severity assessment by Chungu et al. (1997). To perform the analysis of variance and statistical tests on the basis of a linear scale, non-linear ear rot severity scores were converted to percentages of the ear exhibiting symptoms by replacing each score with the mid-point of the interval the score represents on the percentage scale (e.g. 1 = 0%, 2 = 2%, 3 = 7%, etc.), as suggested by Campbell and Madden (1990). The percentages of disease severity were then transformed to arcsin (percentage of the ear affected by the fungus)$^{1/2}$. Transformed percentages were tested for goodness-of-fit to a normal distribution by the Kolmogorov-Smirnov test and for additivity among blocks by the Tukey's test for non-additivity (Reza Hoshmand, 1994), and subjected to analysis of variance. Environment and replicate within environment were considered as random effects. Genotype and fungal species were considered as fixed effects. Environment, genotype and genotype-by-environment sources of variation within pathogen were analyzed following additive main effects and multiplicative interaction (AMMI) models (Zobel et al., 1988). Data analyses were performed with SAS® (SAS INSTITUTE INC., 1999) following Vargas Hernández & Crossa (2000). The same sources of variation were used to estimate genotype-by-environment interaction effects accounted for each genotype. Pearson's correlation coefficients were calculated between means of disease severity after the inoculation of *F. graminearum* and *F. verticillioides* in each environment. All statistical tests were based on transformed means of disease severity, arcsin (percentage of the ear visibly affected by the fungus), at a significance level of $P = 0.05$.

Results and discussion

All genotypes developed symptoms after inoculation with either fungal species. In Ottawa, inoculation with *F. graminearum* caused more severe symptoms to most genotypes compared to those caused by inoculation with *F. verticillioides* [Ottawa data was previously published in Presello et al. (2004) as a subset of a much larger data set]. A similar trend was observed in Pergamino, but differences in disease severity between fungal species were not significant for most genotypes (Table 1). These results are consistent with previous reports suggesting that environmental conditions in Canadian maize growing regions favor the growth of *F. graminearum* (Reid et al., 1999) and environmental conditions in the Argentinian maize growing region favor growth of *F. verticillioides* (Saubois et al., 1996). ARZM16002 and Pora INTA, two of the most resistant genotypes, exhibited no differences between severity of symptoms caused by *F. graminearum* and *F. verticillioides* across the four environments (Presello et al., 2004, Table 1).

Genotype mean square was substantially higher than those of genotype-by-environment and genotype-by-fungal species-by-environment (Table 2), which indicates that there was a general trend of disease severity

Table 2. Analysis of variance of transformed disease severity [arcsin (percentage of the ear affected by the fungus)$^{1/2}$] from a split-plot experiment in which sixteen maize populations (genotype) were evaluated for resistance to ear rot after inoculation with two *Fusarium* spp (fungal species) in four environments

Source of variation	Degrees of freedom	Mean square
Environment	3	0.0890
Replicate/Environment	6	0.0176
Genotype	15	0.3292*
Genotype × Environment	45	0.0290*
Error A	90	0.0127
Fungal Species	1	2.5389*
Fungal Species × Environment	3	0.1950*
Genotype × Fungal Species	15	0.0326*
Genotype × Fungal Species × Environment	45	0.0151*
Error B	96	0.0089

*Significant at a probability level of 0.05.

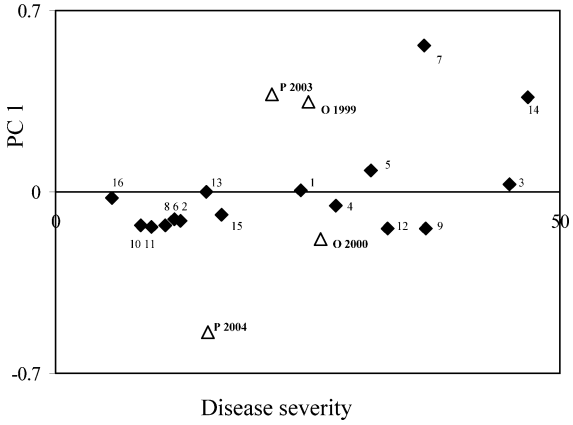

Figure 1. Biplot of disease severity (percentage of the ear affected by the fungus) after the inoculation of *Fusarium graminearum* and the first principal component from an analysis of additive main effects and multiplicative interactions of sixteen maize genotypes (see Table 1 for codes) in four environments: Ottawa 1999 (O1999) and 2000 (O2000), and Pergamino 2003 (2003) and 2004 (P2004).

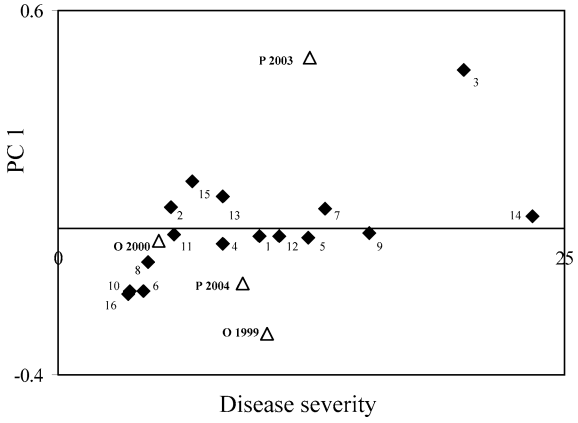

Figure 2. Biplot of disease severity (percentage of the ear affected by the fungus) after the inoculation of after the inoculation of *Fusarium verticillioides* and the first principal component from an analysis of additive main effects and multiplicative interactions of sixteen maize genotypes (see Table 1 for codes) in four environments: Ottawa 1999 (O1999) and 2000 (O2000), and Pergamino 2003 (2003) and 2004 (P2004).

of the genotypes across environments and across fungal species. Analyses of genotype-by-environment interaction with AMMI models within fungal species indicated significant differences for PC1 in the two fungal species [mean squares (%) = 57.0 and 52.3, for *F. graminearum* for *F. verticillioides*, respectively]. For PC1, Pergamino 2004 and Pergamino 2003 exhibited the highest contribution to genotype-by-environment interaction effects for *F. graminearum* and for *F. verticillioides*, respectively (Figures 1 and 2). The most unstable genotypes (largest PC1 scores in absolute values) were ARZM09144 (G7) and ARZM19001 (G14) for *F. graminearum* and ARZM01122 (G3) for *F. verticillioides* (Figures 1 and 2). Most of the other genotypes exhibited PC1 scores close to zero, which indicates that reaction to inoculation tended to be stable across environments for both fungi, and this tendency was observed regardless of the mean disease severity of each genotype.

Populations ARZM 01107, ARZM 07138, ARZM 10041, ARZM 13031, ARZM16002 and Pora INTA exhibited, in most environments, less disease severity compared to that exhibited by the most susceptible genotypes (ARZM 01127, ARZM 01122, ARZM 09144 and ARZM 19001) (Presello et al., 2004, Table 1). In addition, genotype-by-environment effects within fungal species were not significant for all five resistant populations (results not shown) and PC1 scores from the AMMI analyses tended to be close to zero (Figures 1 and 2), which indicates that resistance of these genotypes to either fungi was stable across the four environments. The other genotypes evaluated here exhibited significant genotype-by-environment effects for at least one of the fungal species, even though mean squares of the genotype-by-environment interaction were minor compared with those of genotype or environment.

Within each environment, mean of disease severity observed after inoculation with *F. verticillioides* was positively correlated with that observed after inoculation with *F. graminearum* ($r = 0.90$, $r = 0.81$, $r = 0.87$ and $r = 0.53$, in Ottawa 1999 and 2000, and Pergamino 2003 and 2004, respectively). Similar associations were observed by Al-Heeti (1987) for the ear rots caused by *F. graminearum, F. sporotrichioides* Sherb. and *F. poae* (Peck) Wollenw. The positive correlations that we observed suggest common mechanisms of disease resistance to the two pathogens. If so, then selection for resistance to one pathogenic species would most likely result in an indirect selection for resistance to the other species. Thus, the inoculum for selection could be developed either from the prevalent pathogenic species in a given region or from the species that maximizes the genetic progress. *Fusarium verticillioides* causes a mix of non-symptomatic and symptomatic infections (Munkvold et al., 1997), while *F. graminearum* causes a generalized ear rot (Reid et al., 1996b). Thus, disease severity can be assessed more accurately after the inoculation of *F. graminearum* and consequently, assuming the same genotypic variability

for resistance to each fungal species, genetic progress should be easier to attain using *F. graminearum* as the source of inoculum.

Some of the most resistant maize populations evaluated here exhibited stable resistance across environments. Considering that these evaluations of disease resistance were conducted after the inoculation of fungal species belonging to two different Sections of the genus *Fusarium*, in very heterogeneous environmental conditions in North and South America and using isolates that had been collected from different regions, the low specificity of disease resistance to the environment and the pathogen indicates that resistance of these populations might be quite durable. This is consistent with the fact that several genes affect resistance to both fungal species in maize (Gendloff et al., 1986; Chungu et al., 1996).

Most of the resistant populations identified in this study are ancient landraces with poor agronomic performance. However, since these landraces have rarely been used in breeding programs, they could be potential sources of novel genes for broad-based resistance to two of the most prevalent *Fusarium* spp. in the world. Since several genes affect resistance to *Fusarium* in maize, undesirable linkages may hinder the utilization of these landraces in traditional breeding programs and further research on molecular markers may be required.

Acknowledgments

The authors extend thanks to Catalina Améndola and Juan Annone, from INTA, for assistance with the statistical analysis and reviewing the manuscript. Financial support for this research was provided by the Instituto Nacional de Tecnologia Agropecuaria and the Secretaría de Ciencia y Técnica (PN INTA 520203, PICTO 08-12913).

References

Al-Heeti, A.A., 1987. Pathological, toxicological and biological evaluations of *Fusarium* species associated with ear rot of maize. PhD. thesis, University of Wisconsin-Madison. Univ. Microfilms Int. Diss. Inf. Serv. 8727220.

Campbell, C.L. & L.V. Madden, 1990. Introduction to plant epidemiology. Wiley-Interscience. New York. 532 pp.

Chungu, C., D.E. Mather, L.M. Reid & R. I. Hamilton, 1996. Inheritance of kernel resistance to *Fusarium graminearum* in maize. J Hered 87: 382–385.

Chungu, C., D.E. Mather, L.M. Reid & R.I. Hamilton, 1997. Assessment of ear rot symptoms development in maize hybrids inoculated with *Fusarium graminearum*. Can J Plant Pathol 19: 390–396.

Clements, M.J., C.E. Kleinschmidt, C.E. Maragos, J.K. Pataky & D.G. White, 2003. Evaluation of inoculation techniques for Fusarium ear rot and fumonisin contamination of corn. Plant Dis 87: 147–153.

Desjardins, A.E., R.D. Plattner, M. Lu & L.E. Claflin, 1998. Distribution of fumonisins in maize ears infected with strains of *Fusarium moniliforme* that differ in fumonisin production. Plant Dis 82: 953–958.

Gendloff, E.H., E.C. Rossman, W.L. Casale, T.G. Isleib & L.P. Hart, 1986. Components of resistance to fusarium ear rot in field corn. Phytopathology 76: 684–688.

Hesseltine, C.W. & Bothast, R.J. 1977. Mold development in ears of corn from tasseling to harvest. Mycologia 69: 328–340.

Marasas, W.F.O., T.S. Kellerman, W.C.A. Gelderblom, J.A.W. Coetzer, P.G. Thiel, & J.J. Van der Lugt, 1988. Leukoencephalomalacia in a horse induced by fumonisin B1, isolated from *Fusarium moniliforme*. Onderstepoort J Vet Res 55: 197–203.

Munkvold, G.P., Hellmich, R.L., Showers & W.B. 1997. Reduced fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology 87: 1071–1077.

Prelusky, D.B., B.A. Rotter & R.G. Rotter, 1994. Toxicology of mycotoxins. In: J.D. Miller & H.L. Trenholm (Eds.), Mycotoxins in Grains. Compounds Other than Aflatoxin, pp. 359–403. Eagan Press, St. Paul, MN, USA.

Presello, D.A., L.M. Reid & D.E. Mather, 2004. Resistance of Argentine maize germplasm to gibberella and fusarium ear rots. Maydica 49: 83–91.

Reid, L. M., R.I. Hamilton & D.E. Mather, 1996a. Screening maize for resistance to gibberella ear rot. Agriculture and Agri-Food Canada, Ottawa, ON. Tech Bull Publ 1996-5E.

Reid, L.M., R.I. Hamilton, D.E. Mather & A.T. Bolton, 1996b. Distribution of deoxynivalenol in *Fusarium graminearum*-infected maize ears. Phytopathology 86: 110–114.

Reid, L.M., R.W. Nicol, T. Ouellet, M.E. Savard, J.D. Miller, J.C. Young, J.C., D.W. Stewart & A.W. Schaafsma, 1999. Interaction of *Fusarium graminearum* and *Fusarium moniliforme* in maize ears: disease progress, fungal biomass and mycotoxin accumulation. Phytopathol 89: 1028–1037.

Reid, L.M., D.W. Stewart & R.I. Hamilton, 1996c. A 4-year study of the association between gibberella ear rot severity and deoxynivalenol concentration. J Phytopathol 144: 431–436.

Reza Hoshmand, A., 1994. Experimental research design and analysis. A practical approach for agricultural and natural sciences. CRC Press Inc. Boca Raton. pp. 15–41.

SAS Institute Inc., 1999. SAS/STAT Usersś Guide, Version 8, Vol. 1, Cary, NC: Sas Institute Inc.

Saubois, A., M.C. Nepote & E. Piontelli, 1996. Regional distribution of *Fusarium* strains in corn from the Province of Santa Fe, Argentina. Boletín Micológico 11: 75–80.

Vargas Hernández, M. & J. Crossa, 2000. The AMMI analysis and graphing the biplot. CIMMYT, INT. México D.F. México.

Vigier, B., L.M. Reid, L.M. Dwyer, D.W. Stewart, R.C. Sinha, J.T. Arnason & G. Butler, 2001. Maize resistance to gibberella ear rot: Symptoms, deoxynivalenol and yield. Can J Plant Pathol 123: 99–105.

Zobel, R.W., M.J. Wright & H.G. Gauch Jr., 1988. Statistical analysis of a yield trial. Agron. J. 80: 388–393.