Euphytica (2007) 153:329–337
DOI 10.1007/s10681-006-9171-y

Fusarium ear rot and how to screen for resistance in open pollinated maize in the Andean regions

E. Silva · E. A. Mora · A. Medina · J. Vasquez · D. Valdez · D. L. Danial · J. E. Parlevliet

Received: 22 April 2005 / Accepted: 6 April 2006 / Published online: 13 October 2006

Abstract Ears infected with ear rot were collected from five provinces in Ecuador. Of the 44 samples analysed 26 carried *Fusarium verticillioides,* 11 *F. subglutinans,* two *F. graminearum* and five carried fungi different from *Fusarium*. The pathogenicity of ten isolates, seven of *F. verticillioides* and three of *F. subglutinans,* were tested. Per isolate 30 ears of the susceptible cultivar Mishca were inoculated by pricking a steel pin, dipped into a spore suspension, through the husks in the central part of the ear 14 days after mid-silk. Ears inoculated with sterile water and ears without any treatment, natural infection, served as controls. The disease severity (DS) of the ears ranged from 14 to 58% ear rot, the range being similar for both species. The DS of the water control, 19%, was much higher than that of the natural control of 2%. Five strains gave a DS of over 40%, significantly higher than the water control. The DS of the others were similar to the water control.

In a series of experiments the effect of various methods of applying Fusarium spores through the husks into young ears were compared. All tested methods resulted in DSs significantly higher than those of the two controls. Inoculation with tooth picks and steel pins dipped in a spore suspension gave similar ear rot percentages. Inoculations at 7 to 14 days after mid-silk produced the highest DS's. There was no significant effect of spore concentration on the DS. Cultivars differed considerably, the range being from around 20% to over 50%. Surprisingly, only wounding the husks, the sterile water control, resulted in a fairly high DS, much higher than that of the natural control. As the ranking order of the cultivars after wounding only and after inoculation did not seem to be different from the ranking order of the natural control it is suggested to use in areas with high inoculum pressures like the Andes only wounding by means of a steel pin for screening for resistance to maize ear rot.

Keywords Ear rot · *Fusarium* · Inoculation methods · Isolate · Maize · Pathogenicity · Resistance · Strain

E. Silva · E. A. Mora · J. Vasquez
E.E. Santa Catalina, INIAP, Km 1, Panamericana Sur, Quito, Ecuador

A. Medina
E.E. Banos del Inca, INIA, A. Postal 169, Cajamarca, Peru

D. Valdez
Programma Maiz, Las Barrancas Km2, Casilla 1158, Tarija, Bolivia

D. L. Danial · J. E. Parlevliet
Lab. Plant Breeding, Dept. Plant Science, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

Introduction

Ear rot in maize occurs world wide and is caused by several *Fusarium* species. *F. verticillioides,* formely *F. moniliforme* (Summerell et al., 2003), teleomoroph

Gibberella fujikuroi and its very close relative *F. subglutinans* (*G. fujikuroi* var. *subglutinans*) are the most frequently occurring species. Some other species, such as *F. proliferatum* and *F. graminearum* (*G. zeae*) are much less frequent, although the latter occurs frequently in NW Europe and N. America. *Diplodia zeae* and a few other fungi are incidentally involved as well (Calvert et al., 1985; Abbas et al., 1988; Logrieco, et al., 1993; Arino & Bullerman, 1994; Bullerman & Tsai, 1994; Szecsi, 1994; Gonzalez et al., 1995; Rava et al., 1996). All these pathogens have a wide host range; they are generalists (Amoah et al., 1995).

The mycotoxins, fumonisins, produced in the infected kernels by the *Fusarium* species, are toxic to man and its domestic animals. They may cause diseases such as leucoencephalomalacia in horses, pulmonary oedema in swine and oesophageal cancer in humans (Marasas et al., 1984; Nelson et al., 1993). Strains within *F. verticillioides* and *F. subglutinans* vary considerably in the rate of fumonisins they produce. Fumonisins production and pathogenicity seem positively associated (Atlin et al., 1983; Nelson et al., 1993).

The Fusarium ear rot is transmitted especially by wind borne spores that enter through the silk and wounds, although seed transmission through symptomless infected seed also occurs (Munkvold et al., 1997; Munkvold & Carlton. 1997).

In the Andean regions of Latin America, where maize is a major food crop for millions of people Fusarium ear rot is a serious problem. It is a health threat in those regions, especially as the local cultivars are quite susceptible to ear rot. A reduction in the amount of mycotoxins in the maize kernels would increase the health standard of the local people and increase the productivity of the domestic animals fed with maize. The best approach to this goal is the use of resistant cultivars.

Resistance is available in maize material elsewhere and at low levels in the highly variable local cultivars. Resistance to Fusarium ear rot is reported to be quantitative (Mesterhazy, 1989; Teich, 1989; Reid et al. (1992). Nankam and Pataky (1996) concluded that the ear rot resistance to *F. moniliforme* in the sweet corn hybrid IL 125b was based on the cumulative effect of at least three minor genes. The ranking order in resistance is not greatly affected by strain or even species of Fusarium (Mesterhazy, 1989; Teich, 1989).

Resistance tests generally use artificial inoculation. Two main methods are in use: (i) Introduction of inoculum into the ears through the husks, or (ii) into the silk channel. Ullstrup (1970) compared the infection levels of inbred lines and hybrids using four infection methods. (1) Spore suspension sprayed onto tip of ears. (2) Toothpicks inserted into the ears with fungal mycelium or (3) with spores. (4) Spore suspensions injected through the husks. The disease incidence was very high with mycelium or spores introduced by wounding (2, 3 and 4) with only moderate differences between the inbred lines and hybrids tested. The first method gave less severe infection but larger genotype differences. Also Koehler (1959) found introduction of inoculum through wounding much more effective than spraying inoculum over the silks. Reid and Hamilton (1996), studying *F. graminearum*, observed that the disease seve rity at harvest was the highest when the inoculation was done through the husks with stainless steel pins, dipped in a macroconidial suspension of at least 100.000 conidia/ml in the centre of the ear 15 days after silk-emergence. Nearly all inoculation experiments were carried out on genetically uniform material such as inbred lines and hybrids.

In the Andean regions nearly all maize cultivars are open pollinated and far from uniform. Artificial inoculation methods may have to be adapted to this different situation. A few experiments were done to investigate which Fusarium species are important and whether strains vary in pathogenicity. Several other experiments were carried out to establish a suitable inoculation procedure.

Materials and methods

Fusarium strains

Experiment 1 was carried out to get an impression about which *Fusarium* species were causing ear rot in maize. Ears with initial symptoms of ear rot were collected from important maize growing areas at altitudes between 2300 and 2900 m in six provinces (Bolivar, Carchi, Chimborazo, Cotopaxi, Imbabura and Pichincha) of Ecuador. Diseased kernel tissue samples were disinfected with sodium hypochlorite 5.25% during three minutes after which they were washed twice in sterile water. Next the samples were plated on selective medium as described by Nash and Snyder (1962) at 20 °C. From the fungal colonies grown from the diseased kernel tissue, 5 mm sections of mycelium were isolated and plated on water-agar (AA) medium at room

temperature. From these, monospore cultures were established and transferred to carnation-leaf agar (CLA) culture medium and potato-glucose-agar (PGA) culture medium at 20 °C as described by Nelson et al. (1983). For identification purposes the cultures on the PGA medium were used for gross morphological appearances and coloration, while the cultures on the CLA medium were used to evaluate microscopic features. For the identification of the *Fusarium* species the key of Nelson et al. (1983) was used.

Experiment 2 was aimed to determine the variation of pathogenicity within the important Fusarium species. Ten single spore based isolates, seven of *F. verticillioides* and three of *F. subglutinans* were compared. The susceptible open pollinated cultivar Mishca was planted in plots consisting of 10 rows of 5.5 m long with a row distance of 0.8 m. Per plant one ear was inoculated 14 days after 50% of the plants in the experimental plots had produced visible silk. The inoculation was performed by pricking a steel pin dipped in a spore suspension with 50,000 conidia per ml through the husks in the central part of the ear. There were two controls, one consisting of ears inoculated with sterile water only, another consisting of non-treated ears, natural infection. A randomised complete plot design with the 12 treatments in 3 replicates was used. Per replicate and per treatment ten plants were inoculated and assessed. At harvest the disease severity (DS) of each treated ear was assessed using the 1–6 CIMMYT scale, where scale 1 = 0%, 2 = 1–10%, 3 = 11–25%, 4 = 26–50%, 5 = 51–75% and 6 = 76–100% affected by ear rot. The mean scale values per plot were retransformed into percentage DS by the equation $DS = (N1 \times 0 + N2 \times 5.5 + N3 \times 18 + N4 \times 38 + N5 \times 63 + N6 \times 88)/\Sigma N$, where N1 to N6 are the number of ears with scale values 1 to 6 which are multiplied with the mean percentage of the scale. The statistical analysis was done on the retransformed DS values. For comparing the means in Table 3 Tukey's w Procedure as well as Duncan's New Multiple-Range Test were used. The latter is less conservative than the former (Steel & Torrie, 1980).

Inoculation experiments

Five experiments were carried out, one in South Bolivia at two sites near Tarija at altitudes of 2060 and 1870 m respectively (Experiment 3), two in Ecuador at the Santa Catalina Experiment Station south of Quito (Exp. 4 and 6) and two in Peru at the Banos del Inca Experiment Station near Cajamarca (Exp. 5 and 7), all at altitudes of about 2700 m. The various treatments were compared through the DS assessed when the seeds were physiologically mature. The *Fusarium* sources from which the inoculum was produced were obtained from infected maize kernels and grown in vitro as described for experiment 1. The DS of each treated ear was assessed using the 1–6 CIMMYT scale. The statistical analyses were carried out on the basis of DS's expressed in those scale values. For comparing means, Tukey's w Procedure was used (Steel & Torrie, 1980). The mean scale values per plot were retransformed into percentage DS as described in exp. 2. Plots consisted of a variable number of rows 0.8 m apart, but the row length was always 5, 5 m with 11 planting holes into which 2 seeds were planted. Per plant the first ear was used. The five experiments together aimed to determine in different Andean environments the effect of cultivar, inoculum dose, time of introducing the inoculum and the method of introducing the inoculum on DS. Experiment 3 was also used to test the suitability of the CIMMYT scale to assess the DS.

Experiment 3. Plants of six cultivars were inoculated with a spore suspension of 50,000 spores per ml of a *F. verticillioides* isolate just below the middle of well developed ears 7, 14 or 21 days after mid-silk, which is when the silks of 50% of the plants are visible. Three methods of inoculation were tested, steel pin and toothpick dipped in the inoculum suspension or injecting the suspension into the ear. The experimental design was a split plot one with cultivars on main plots, inoculation methods on sub-plots and different dates of inoculation on sub-sub-plots in three replicates and at two sites. The sub-sub-plot consisted of four rows. All well developed first ears were used. The DS of each inoculated ear was assessed in two ways; by applying the 1–6 CIMMYT scale and by estimating the percentage area affected by ear rot directly. There were no proper controls.

Experiment 4. Plants of the fairly susceptible cultivar Mishca were inoculated with a spore suspension of 50,000 spores per ml of the mixture of the ten *Fusarium* isolates described in experiment 1. Two inoculation methods and five dates of inoculation were compared in three replicates in a randomised complete block design. Each plot measured 10 rows. Per plot 50 plants with a similar date of female flowering (silk

just emerging) were selected. Those plants were inoculated by pricking a toothpick dipped in inoculum or sterile water or a steel pin either dipped in inoculum or sterile water through the husks in the central part of the ear 13, 15, 19, 21 or 23 days after the start of female flowering while at least 20 non-inoculated plants of a similar flowering date formed a second control (natural infection). To hinder insects entering the ear through the silk opening 3–4 drops of a vegetative oil (palm oil) were applied on the top of each ear 10–15 days after silk emergence and again two to three weeks later.

Experiment 5. Well developed ears of plants of the susceptible cultivar Choclero 201 were inoculated with a suspension of 500,000 spores per ml. of a *F. verticillioides* isolate 7 or 14 days after mid-silk. The plants were inoculated by pricking a steel pin or toothpick, dipped in the inoculum suspension, through the husks in the central part of the ear. Plants inoculated with steel pin or toothpick dipped in sterile water and non-inoculated plants of a similar female flowering date (natural infection) formed the two controls. The experiment was a randomised complete block design with three replicates. Plots consisted of four rows. The main ear of each plant was treated.

Experiment 6. Plants of the fairly susceptible cultivars Mishca and INIAP-122 were inoculated with a suspension of 50,000, 500,000 or 1,000,000 spores per ml of a *F. verticillioides* isolate 18 days after mid-silk. The plants were inoculated by pricking a steel pin dipped in the inoculum suspension through the husks in the central part of the ear. Non-inoculated plants of a similar female flowering date formed the control (natural infection). The experimental design was a split plot one with the cultivars on main plots and the inoculum concentrations on sub-plots in three replicates. Of each sub-plot of eight rows 50–54 ears were inoculated while at least 20 non-inoculated ears formed the control.

Experiment 7. Four cultivars were inoculated with a steel pin dipped in a suspension of 50,000, 500,000, or 1,000,000 spores per ml of a mixture of *F. verticillioides* isolates or not inoculated (natural infection). The inoculation was carried out 14 days after mid-silk. The experiment was a randomised complete block design with three replicates. Plots consisted of ten rows. Of each plot the main ears of 50–55 plants were inoculated and another 50–55 main ears formed the natural control.

Results

Experiment 1. Of the 44 samples analysed, 26 contained *Fusarium verticillioides* (59%), 11 carried *F. subglutinans* (25%) and 2 appeared to have *F. graminearum* (4.5%) as shown in Table 1. From one sample from the province of Pichincha a Diplodia species was isolated. From four samples, three from Bolivar and one from Imbabura unidentified non-*Fusarium* fungal species were obtained.

Experiment 2. The isolates of both *Fusarium* species showed a similar range in pathogenicity, from similar to the sterile water control, about 20% ear rot, to well over 50% ear rot (Table 2). There are several statistical methods available to compare treatments means as obtained here. Two tests were chosen, a rather severe one, Tukey's *w* procedure, and a considerable less severe one, Duncan's New Multiple-Range Test (Steel & Torrie, 1980). Both tests show that the isolates 1 to 5 differed significantly from the controls, while the other five did not differ significantly from the sterile water control. In both tests the sterile water control resulted in a significantly higher DS than the natural control. The isolates differed considerably in pathogenicity.

Experiment 3. The 12 treatment means of the six cultivars (over three inoculation dates, three inoculation

Table 1 Frequencies of *Fusarium* species present in ear rot affected maize samples in five provinces in Ecuador in 1997–1998

Province	No of samples	*Fusarium verticillioides*	*Fusarium subglutinans*	*Fusarium graminearum*
Bolivar	6	3	–	–
Carchi	4	4	–	–
Chimboroza	12	8	4	–
Imbabura	12	7	2	2
Pichincha	10	4	5	–
Total	44	26	11	2

Table 2 Disease severities (DS) in percentage ear rot on cv Mishca of 10 *Fusarium* isolates belonging to two species and originating from six provinces in Ecuador, and of two controls

No.	Species/Isolate	Province	DS in	%
1	*F. subglutinans*	Imbabura	57.8 a*	a**
2	*F. verticillioides*	Pichincha	56.7 a	a
3	*F. verticillioides*	Bolivar	55.8 ab	a
4	*F. verticillioides*	Imbabura	43.0 ab	bm
5	*F. subglutinans*	Chimborazo	40.2 bc	b
6	*F. verticillioides*	Cotopaxi	25.5 cd	c
7	*F. verticillioides*	Chimborazo	20.6 d	cd
	Control; Sterile water		19.2 d	cd
8	*F. verticillioides*	Carchi	18.3 d	cd
9	*F. subglutinans*	Pichincha	15.9 d	cd
10	*F. verticillioides*	Imbabura	14.4 d	d
	Control; Natural infection		1.6 e	e

*Significantly different according to Tukey's w procedure at $P = 0.05$ if letters are different

**Significantly different according to Duncan's new multiple range test at $P = 0.05$ if letters are different

Table 3 Ear rot disease severity evaluated using the 1–6 CIMMYT scale (C-Sc) and the percentage ear rot (%E) assessed directly of six maize cultivars, after three moments of inoculation and after three methods of inoculation at two sites in Bolivia

Treatment	Site 1 C-Sc	%E	Site 2 C-Sc	%E	Mean C-Sc	%E
Cultivar						
Chaparrita	4.08 c*	39.0	5.08 e*	61.1	4.58 c*	50.0
IBTA Erquis 1	4.29 c	43.0	4.26 d	44.2	4.28 c	43.6
IBTA Erquis 5	3.14 b	23.7	3.77 c	35.3	3.46 b	29.5
Pisankalla	3.31 b	24.0	3.41 b	31.3	3.36 b	27.7
IBTA Erquis 2	2.77 a	21.2	3.23 b	27.5	3.00 a	24.4
IBTA Erquis 4	2.71 a	18.1	2.90 a	24.3	2.81 a	21.2
Inoculation after mid-silk						
7 days	3.77 c	35.2	3.89 b	39.2	3.83 c	37.2
14 days	3.42 b	28.0	3.83 b	37.6	3.63 b	32.8
21 days	2.97 a	21.3	3.66 a	35.0	3.32 a	28.2
Inoculation method						
Toothpicks	3.34 b	28.5	3.89 b	40.5	3.62 b	34.5
Steel pins	3.76 c	34.0	3.87 b	37.5	3.82 b	35.7
Hypodermic needle	3.05 a	21.0	3.62 a	33.9	3.34 a	27.5

*Significantly different according to Tukey's w Procedure at 5% probability if letters are different

methods and three replicates) based on the CIMMYT scale assessment values ranged at site 1 from 2.7 to 4.3 and at site 2 from 2.9 to 5.1. The treatment means based on the direct evaluation of the percentage disease severity ranged at site 1 from 18.1% to 43.0% and at site 2 from 24.3% to 61.1% (Table 3). The correlation coefficient, *r*, between these two variables was 0.98 at site 1 and 0.99 at site 2.

The cultivars differed considerably and significantly in DS. There are indications of small cultivar × site interaction effects. 'Chaparrita' was significantly more diseased than 'IBTA Erquis 1' at site 2 but not at site 1. 'IBTA Erquis 5' and 'Pisankalla' showed a similar interaction (Table 3). The cultivar mean effects over the two sites have therefore been tested against the combined error and cultivar × site interaction variance.

The moment of inoculation after mid-silk had a significant effect on the DS, being highest at the earliest inoculation date, the effect being smaller than the cultivar effect. Among the inoculation methods the inoculation with the hypodermic needle gave a significantly lower DS than the other two methods. Inoculation by means of tooth picks or by steel pins did not give a consistent effect.

The CIMMYT scale appeared to assess the DS reliably and can be recommended for screening purposes. Cultivars differed clearly in DS, early inoculation produced the highest DS and introducing the

Table 4 Ear rot disease severity in percentage ear affected (DS %) of two inoculation methods and of five dates of introducing Fusarium spores of a mixture of 10 isolates into maize ears of the cultivar Mishca in Ecuador

Treatment	DS %
Inoculation method	
Toothpick with Fusarium	45.8 a*
Steel pin with Fusarium	44.7 a
Days after mid-silk	
13 days	45.9 b*
15 days	45.7 b
19 days	40.6 b
21 days	32.2 a
23 days	31.8 a
Inoculum	
Fusarium suspension	45.2 c*
Sterile water	33.2 b
Natural infection	1.8 a

*Significantly different according to Tukey's *w* Procedure at 5% probability if letters are different

inoculum using a hypodermic needle produced a lower DS than introduction with a tooth pick or a steel pin.

Experiment 4. According to the statistical analysis only the main effects, pricking methods, dates of inoculation and the differences between the two controls with each other and with the treatments were highly significant. All interactions were non-significant. For this reason only the main effects are shown in Table 4. A highly interesting aspect is the observation that inoculation with sterile water gave a DS much higher than the DS by natural infection.

The earliest inoculation date resulted in the highest DS. Introducing the inoculum with a tooth pick or a steel pin was equally effective. The DS produced by the sterile water control was much higher than that of the natural control, as in experiment 2.

Experiment 5. The analysis of variance indicated highly significant differences between inoculation with Fusarium spores, inoculation with sterile water and natural infection. There were no significant differences between the steel pin (53.7%) and toothpick (51.6%) methods of introducing inoculum, nor between the two dates (53.5% and 51.8% respectively) of inoculation (Table 5). However, the DS of the sterile water treatment using the steel pin was significantly higher (48.4%) than the one using the toothpick (39.8%).

Table 5 Ear rot disease severity in percentage after inoculating maize ears of the cultivar Choclero 201 with Fusarium spores or sterile water by means of steel pins or toothpicks seven or 14 days after mid-silk in Peru

	7 Days		14 Days		
Treatment	Steel	Tooth	Steel	Tooth	Mean
Inoculum	52.3	54.7	55.2	48.4	52.7 c
Sterile water	46.6	36.1	50.2	43.5	44.1 b
Natural infection	11.9	2.1	9.2	12.6	11.5 a

*Significantly different according to Tukey's w Procedure at 5% probability if letters are different

Table 6 Ear rot disease severity in percentage of two maize cultivars inoculated with three concentrations of Fusarium spores, 18 days after mid-silk in Ecuador

	Cultivar		
Concentration	Mishca	INIAP-122	Mean
50,000	30.3	28.4	29.4
500,000	35.4	32.0	33.7
1,000,000	32.9	24.4	28.7
Mean	32.9	28.3	30.6
Natural infection	2.9	2.7	2.8

The only main effect that was significant and large resulted from the difference between the sterile water and the natural control.

Experiment 6. According to the analysis of variance neither the effects of cultivars nor those of spore concentrations were significant (Table 6).

Experiment 7. The cultivar effect was very significant, 'Choclero 201' being the most affected and 'Morocho' the least. Inoculation had a strong effect, the difference with natural infection being highly significant. However, beyond 50.000 conidia there was no significant increase in DS (Table 7).

The period between inoculation and disease assessment, and therefore the period of fungal development, varied greatly between the cultivars. The disease severity measured may therefore be the result of the combined effects of resistance and escape due to earliness. If this effect plays a role, the early cultivar INIA Negro might in fact be more susceptible than its DS suggests.

As in experiment 6, spore concentrations above 50,000 per ml produced no increased DS's. The cultivars differed considerably in DS but these differences may not be due to resistance only.

Table 7 Ear rot disease severity in percentage of four maize cultivars inoculated with three concentrations of Fusarium spores, disease severity from natural infection, days to silk emergence, days to physiological maturity and days from inoculation to disease assessment of four maize cultivars in Peru

	Cultivar				
Inoculum	Choclero 201	INIA Negro	Canchero 401	Morocho 601	Mean
50,000	62.4	32.4	32.1	18.5	36.3 y*
500,000	47.5	31.3	27.7	15.2	30.4 y
1000,000	56.0	25.1	28.7	22.1	33.0 y
Mean	55.3 c*	29.6 b	29.5 b	18.6 a	
Natural infection	19.6 b*	2.0 a	3.8 a	2.0 a	6.9 z
Days to silk emergence	110	94	100	109	
Days to phys. maturity	210	170	210	215	
Days from inoc. to asses.	86	62	96	92	

*Significantly different according to Tukey's w Procedure at 5% probability if letters are different

Discussion

The results of Table 1 agree very well with the observations from other areas in the world (Calvert et al., 1985; Abbas et al., 1988; Logrieco et al., 1993; Arino & Bullerman, 1994; Bullerman & Tsai, 1994; Szecsi, 1994; Gonzalez et al., 1995; Ravaet al., 1996). As in many other areas, *F. verticillioides* is the most dominant species with *F. subglutinans* second in importance. Here only three *Fusarium* species were identified. In other inventories, where more samples were analysed, more species were reported. If the present inventory would have been done on a wider scale more *Fusarium* species might have been observed as well.

Fusarium species usually are highly variable for various traits, including pathogenicity (Nelson et al., 1983; Nelson et al., 1993). Screening for resistance to maize ear rot asks for the right inoculum to use in terms of species and strains. Therefore a number of isolates of the two important *Fusarium* species, *F. verticillioides* and *F. subglutinans,* were tested for their pathogenicity. Two controls were used as the natural infection lacks the damage done to the ear when inoculum is introduced through the husks. In this way a wound is created through which other fungi, present on the outside of the ear, may enter either at the moment of wounding or later. Indeed the sterile water control showed a much higher ear rot DS than the natural control. The isolates of the two most important *Fusarium* species had a similar range in pathogenicity and half the isolates had apparently no or a low level of pathogenicity as they did not differ from the sterile water control.

The Fusarium species causing ear rot in maize belong to the so called generalists, pathogens with a wide host range (Bruehl, 1983; Amoah et al., 1995; Parlevliet, 2002). Resistance to generalists is usually of a quantitative nature and of a durable type (Parlevliet, 2002). It tends to be effective to related pathogens as well (Bruehl, 1983). Resistance to Fusarium is of the quantitative type and the ranking order in resistance is not greatly affected by strain or even species of Fusarium (Gendloff et al., 1986; Mesterhazy, 1982, 1989; Teich, 1989; Chungu et al., 1996; Nankam & Pataky, 1996). However, small cultivar × site (Hunter et al., 1986) and even cultivar × Fusarium isolate interactions (Atlin et al., 1983; Gendloff et al., 1986) have been reported. These interactions may be caused by the fact that fumosin production is also dependent on environmental conditions (Shelby et al., 1994). The observations reported here confirm the quantitative character of ear rot resistance in maize as well as the occurrence of small genotype × site interactions. For screening purposes one is therefore advised to use a suitable strain of established pathogenicity or even better a mixture of pathogenic strains of *F. verticillioides* or *F. subglutinans.*

To assess the DS the CIMMYT scale has been used as this facilitates the assessment considerably. Mean percentages ear rot agreed very well with the mean CIMMYT scale values (Table 3), the correlation coefficient *r* for the two sites being 0.98 and 0.99 respectively. Retransformation from the CIMMYT scale values to percentage ear rot as was done in several experiments here, is therefore allowed.

There were hardly any differences in DS between the methods used to introduce the inoculum through tooth picks or steel pins as shown in Tables 3–5, but injecting through a hypodermis needle produced a lower DS in Bolivia (Table 3). Also there were no significant differences between the concentrations of the introduced inoculum (Tables 6 and 7). The optimal time to introduce the inoculum or to produce an entrance through the husks is about 14 days after mid-silk (Tables 4 and 5), This confirms the observations of Reid and Hamilton (1996). The data from Bolivia deviate somewhat as the DS was highest after 7 days (Table 3). The ranking order of the cultivars after inoculation or wounding (water control) seems at least fairly similar to the ranking order after natural infection, but differences become much larger (Tables 4 and 7). The DS after the sterile water treatment, although significantly lower than the inoculated treatments, was still much higher than the DS of the natural controls (Tables 2, 4 and 5). This indicates that introducing a wound through the husk is more important than the introduction of the inoculum. In the Andean regions maize and ear rot are almost ubiquitous. The wind borne spores therefore can be expected to be ubiquitous as well. The spores are either introduced at the wounding or enter shortly after the wounding. A somewhat higher DS after the sterile water treatment compared with the natural control was to be expected, but its much higher DS was a surprise, indicative for a very high level of natural inoculum.

The DS measured at physiological maturity is taken as a measure for the susceptibility. However, the DS is likely the result of several variables such as the chance to become infected either through wounds or through the silk opening, the rate of disease development after infection and the time between infection and maturity. Inoculation by wounding might especially measure the rate of disease development. The effect of the latter variable may be visible in Table 7 where 'INIA Negro' may seem fairly resistant because of its earliness.

When screening for resistance to ear rot in open pollinated maize populations two problems have to be considered. The populations differ in time of mean silk emergence and within populations the individual plants vary in the time of silk emergence. As the time of inoculation after silk emergence is of importance, populations and plants within populations cannot be inoculated all at the same time if they have to be inoculated a given number of days after the silk emergence. Using a spore suspension to inoculate the ears may introduce an error as it will be very difficult to inoculate all entries and all plants within entries with inoculum of exactly the same quality at different moments. This problem does not exist when only wounding using a steel pin is practised. This is not the only advantage. Wounding only does not require to produce inoculum of a certain concentration and quality and is therefore much easier to apply. The DSs produced, although somewhat lower compared with truly inoculated ears, are much higher than the natural DS level. The discriminating power is probably not less than after true inoculation and certainly much better than relying on the natural situation.

In the Andean regions entries can be screened for Fusarium ear rot resistance very well by wounding and assessing a fair number of ears of each entry. This is done by pricking a steel pin through the husks just below the middle of the ears about 14 days after silk emergence.

References

Abbas HK, Mirocha CJ, Meronuck RA, Pokorny JD, Gould SL, Kommedahl T (1988) Mycotoxins and *Fusarium* spp. associated with infected ears of corn in Minnesota. Appl Environ Microbiol 54:1930–1933

Amoah BK, Rezanoor HN, Nicholson P, MacDonald MV (1995) Variation in the *Fusarium* section Liseola: pathogenicity and genetic studies of isolates of *Fusarium moniliforme* Sheldon from different hosts in Ghana. Plant Pathol 44:563–572

Arino AA, Bullerman LB (1994) Fungal colonization of corn grown in Nebraska in relation to year, genotype and growing conditions. J Food Prot 57:1084–1087

Atlin GN, Enerson PM, Mcgirr LG, Hunter P (1983) Gibberella ear rot development and zearalenone and vomitoxin production as affected by maize genotype and *Gibberella zeae* strain. Can J Plant Sci 63:847–853

Bruehl GW (1983) Nonspecific genetic resistance to soilborne fungi. Phytopathology 73:948–951

Bullerman LB, Tsai WYJ (1994) Incidence and levels of *Fusarium moniliforme*, *Fusarium proliferatum* and fumonisins in corn and corn-based foods and feeds. J Food Prot 57:541–546

Calvert OH, Foudin AS, Minor HC, Krause GF (1985) *Fusarium moniliforme* colonization of corn ears in Missouri. Plant Dis 69:988–990

Chungu C, Mather DE, Reid LM, Hamilton RI (1996) Inheritance of kernel resistance to *Fusarium graminearum* in maize. J Heredity 87:382–385

Gendloff EH, Rossman EC, Casale WL, Isleib TG, Hart LP (1986) Components of resistance to fusarium ear rot in field corn. Phytopathology 76:684–688

Gonzalez HHL, Resnik SL, Boca RT, Marasas WFO (1995) Mycoflora of Argentinian corn harvested in the main production area in 1990. Mycopathologia 130:29–36

Hunter RB, Atlin GN, Muldoon JF (1986) Genotype × environment interactions for ear mold resistance and its subcomponents in maize hybrids. Can J Plant Sci 66:291–297

Koehler B (1959) Corn ear rots in Illinois. III. Agric Exp Sta Bull 639:87

Logrieco A, Moretti A, Altomare C, Bottalico A, Torres EC (1993) Occurrence and toxicity of *Fusarium subglutinans* from Peruvian maize. Mycopathologia 122:185–190

Marasas WFO, Kriek NPJ, Fincham JE, van Rensburg SJ (1984) Primery liver cancer and oesophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme*. Int J Cancer 34:383–387

Mesterhazy A (1982) Resistance of corn to Fusarium ear rot and its relation to seedling resistance. Phytopath Z 103:218–231

Mesterhazy A (1989) Progress in breeding of wheat and corn genotypes not susceptible to infection by Fusaria. In: Chelkowski J (ed) *Fusarium*; Mycotoxins, taxonomy and pathogenicity. Elsevier, Amsterdam pp. 357–386

Munkvold GP, Carlton WM (1997) Influence of inoculation method on systemic *Fusarium moniliforme* infection of maize plants grown from infected seeds. Plant Dis 81:211–216

Munkvold GP, McGee DC, Carlton WM (1997) Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology 77:207– 217

Nankam C, Pataky JK (1996) Resistance to kernel infection by *Fusarium moniliforme* in the sweet corn inbred IL 125b. Plant Dis 80:593–598

Nash S, Snyder WC (1962) Quantitative estimations by plate counts of propagules of bean rot Fusarium in field soils. Phytopathology 52:567–572

Nelson PE, Toussoun TA, Marasas WFO (1983) Fusarium species. An illustrated manual for identification. The Pennsylvania State University Press, University Park & London

Nelson PE, Desjardins AE, Plattner RD (1993) Fumonisins, mycotoxins produced by *Fusarium* species, biology, chemistry and significance. Ann Rev Phytopathol 31:233–252

Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124:147–156

Rava E, Viljoen JH, Kallmeyer H, de Jager A (1996) Fungi and mycotoxins in South African maize of the 1993 crop. Mycotoxin Res 12:15–24

Reid LM, Mather DE, Hamilton RI, Bolton AT (1992) Diallel analysis of resistance in maize to *F. graminearum* infection via the silk. Can J Plant Sci 72:915–923

Reid LM, Hamilton RI (1996) Effects of inoculation position, timing, macroconidial concentration, and irrigation on resistance of maize to *Fusarium graminearum* infection through kernels. Can J Plant Pathol 18:279–285

Shelby RA, White DG, Bauske EM (1994) Differential fumonisin production in maize hybrids. Plant Disease 78:582–584

Steel RGD, Torrie JH (1980) Principles and procedures of statistics, 2nd edn. McGraw-Hill Book Company, New York etc.

Summerell BA, Baharuddin Salleh, Leslie JF (2003) A utilitarian approach to *Fusarium* identification. Plant Dis 82:117–129

Szecsi A (1994) Occurrence of *Fusarium* species in the section Liseola isolated from Hungarial maize samples in 1991 and 1992. Novenyvedelem 30:313–318

Teich A (1989) Epidemiology of corn (*Zea mays* L.) ear rot caused by *Fusarium* spp. In: Chelkowski J (ed) *Fusarium*; Mycotoxins, taxonomy and pathogenicity. Elsevier, Amsterdam pp. 319–328

Ullstrup AJ (1970) Methods of inoculating corn ears with *Gibberella zeae* and *Diplodia maydis*. Plant Dis Rep 54:58–662