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Different types and concentrations of oat grain inoculum to quantify *Septoria tritici* resistance in wheat

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Abstract. Two *Septoria* Monitoring Nursery sets were tested for resistance in the field during three consecutive years. Different concentrations of oat grains covered with mycelia of *Septoria tritici* were applied as inoculum. The position of the disease on the plants and the severity of the *Septoria* leaf blotch infection were recorded at two growth stages. A comparison between leaf pulverisation and grain application as a source of conidia was made in the last year. The percentages of necrotic lesions and pycnidial coverage were recorded on the upper three leaves of the plants at the same growth stages as for previous years. With grain inoculation, the infection reached the 7th leaf of the plants with the maximum concentration applied at tillering stage. The best concentration to obtain the highest discrimination among

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Introduction

Leaf blotch, caused by *Septoria tritici* (teleomorph: *Mycosphaerella graminicola*), is a worldwide disease that causes appreciable economic losses to wheat crops (Eyal *et al*. 1985; Simón *et al*. 2005). Its incidence depends on the inoculum concentration, crop management and the environmental conditions; cool temperatures, high humidity and frequent rain are favourable for its development (Eyal *et al*. 1985). Sources for resistance derived from the international collection of germplasm, the *Septoria* Monitoring Nursery (SMN), and from screening nurseries, confer their resistance through different genes. They can be used by breeding programs in their attempts to increase stable resistance over time. The SMN set was created to measure the virulence spectrum over a geographic area. Inoculum in the field can be applied by different methods. Oat grains colonised by mycelia of *Pyricularia oryzae* or *Drechslera tritici repentis* were described as an inoculation source by Cordo de Balonga *et al*. (1980) and Perelló *et al*. (2003) as an alternative to the traditional pulverisation technique. The aims of this work were to (1) determine the best concentrations of oat grain inoculated with fungal mycelium to be placed on the soil and the efficacy of this technique as compared with leaf pulverisation, and (2) to quantify the resistance of the SMN accessions using both techniques.

Methods

For the field experiment, the inoculum for grain application was prepared in sterilised 500-mL flasks with 100 g of oat grains and 50 mL of a liquid extract malt medium (Perelló *et al*. 1987). The grains were soaked with 10 mL of an inoculum suspension (10^7 conidia/mL) of *Septoria tritici* isolate FALP96088 and incubated for 15–21 days at 23 ± 2°C in darkness and shaken daily to promote good fungal growth. Each flask yielded ~280 g of wet infected grains. After the incubation, the grains colonised by a stromatic mycelium were spread and dried on trays under laboratory conditions, then weighed and stored in nylon bags at 5°C before their use 24 h later in the field.

Two concentrations of oat grain inoculum were compared in 1998 and 1999. Eleven differential spring wheat cultivars and eight lines at a similar vegetative maturation stage belonging to the 6th (1998) and 7th (1999) SMN sets (Gilchrist 1994; Gilchrist *et al*. 1999) were inoculated and evaluated. The best sources of resistance identified by CIMMYT were assayed at the Julio Hirschhorn Experimental Station, Los Hornos, Buenos Aires Province. The accessions (1-BOBWHITE S; 2-TIA.2/4/CS/TH.CU//GLEN/3/ALD/PVN; 3-CHIRYA.1; 4-CHIRYA 4; 5-CS7TH.CU//GLEN/3/ALD/PVN/4/NANJING; 6-EG-A/H567.71//4#EG-A/3/2#CMH79.243; 7-MH86.540-A-1Y-3B-2Y-1B-1B-1B-1Y-1M-1Y; 8-ALD/PVN//YMI#6; 9-SHA5/BOW; 10-ENCOY 1582–1B; 11-BOBWHITE S as the other derivative

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line; 12-DON ERNESTO INTA; 13-SERI M82; 14-BETLEHEM; 15-LAKHISH; 16-KAUZ; 17-PENJAMO; 18-ETIT 38; 19-GLENNSON M81) were sown in a factorial design experiment. The covered grains were spread onto the soil next to the plants during the tillering growth stage (GS23, Zadoks *et al*. 1974). Plants within the plots were assessed for *S. tritici* infection at anthesis (GS60) and at the medium milk stage (GS75).

The pulverisation inoculum was produced using the same isolate as in the previous year following the method of Perelló *et al*. (1987). The conidial concentration of the suspension was adjusted to 1×10^7 conidia/mL.

A comparison between the pulverisation method and the grain application method was made in the field in 2000. The 8th SMN set was sown into plots in a randomised complete block design with two factors, and replicated twice. The inoculum suspension was sprayed onto the leaves at the tillering stage (GS23), until run-off. In total, 200 g/m^2 of inoculated oat grain were spread onto the soil surface below each row of the plots and next to the plants. After inoculation, plants were kept moist by sprinkling water several times a day over 3 days. The severity of the infection was registered on the flag leaf at the beginning of flowering (GS60) and medium milk (GS75) stages using a modified double digit Saari–Prescott scale (Saari and Prescott 1975). The cut for resistant behaviour was estimated as 5.3 (Gilchrist *et al*. 1999). Weather variables (daily temperature, relative humidity and rainfall) were recorded from the date of inoculation to anthesis. Plant height was evaluated. The relationship between the total plant height and the height of *S. tritici* blotch was evaluated at both GS60 and GS75. Digits 1 and 2 of the scale were analysed separately by ANOVA without transformation.

To compare the inoculation techniques, both the necrotic coverage percentage (NCP) and pycnidial coverage percentage (PCP) were scored on the upper three leaves of 15 plants, 21 days after inoculation. The cut-off point between resistant and susceptible response classes was 16.8% NCP following Eyal *et al*. (1985). The variable 'days to heading' was not evaluated because all cultivars had a similar heading date. NCP and PCP were analysed by ANOVA using plant height as a covariant. The data were previously transformed according to the formula $\sqrt{x+1}$.

Results

The intensity of the infection for Digit 1 of the Saari–Prescott scale reached the 5th leaf to emerge as the lowest level of the infection; in other accessions, it ascended to the 6th leaf, while the highest level of the disease was reached on the 7th leaf on the plant. In relation to Digit 2, the infection severity on the leaf varied from a low (8.7%) to a high (35%) PCP, independent of the height reached by the disease on the plant. Combining the results of both digits, Chirya1 was the most resistant and Kauz the most susceptible of the accessions. According to the l.s.d. test ($P < 0.01$), the effect of the inoculum concentration and the environment of the different years, under grain application, are shown in Table 1.

The plants treated with pulverisation compared with grain application showed that the method of inoculation has a significant effect ($P < 0.001$) on NCP and PCP for the two types of inoculum and two growth stages (Table 2). The mean severity on the three upper leaves varied between 26.6% and 64.5% for NCP and between 18.5% and 59.3% for PCP. The mean general values for NCP and PCP were higher ($P < 0.001$) for the pulverisation than for the grain application method. NCP and PCP increased with the appearance of later growth stages, being higher at GS75 than at GS60. In only one case (No.18), both

Table 1. ***Septoria tritici*** **infection average (Digit 1 and 2) for different concentrations of inoculum and different years**
Means followed by the same letter are not statistically different ($P < 0.01$). C1, 120 g/m^2; C2, 280 g/m^2

Accession	Inoculum concentration				Years			
	Digit 1[A]		Digit 2[A]		Digit 1[A]		Digit 2[A]	
	C1	C2	C1	C2	1998	1999	1998	1999
1	6.00b	6.50b	2.50b	1.00a	6.50b	6.00b	2.50b	1.00a
2	5.50b	7.25d	2.25a	0.75a	6.75b	6.00b	2.50b	0.50a
3	2.50a	7.50e	2.75c	1.75a	5.50a	4.50a	3.00d	1.50a
4	7.25c	7.25c	0.50a	1.50a	8.00f	6.50b	1.75a	0.25a
5	6.75b	7.25d	2.00a	2.25a	7.00c	6.00b	2.75c	1.50a
6	6.25b	6.75b	1.25a	1.75a	6.75b	6.25b	1.75a	1.25a
7	6.50b	8.00g	1.00a	0.75a	7.75e	6.75b	1.00a	0.75a
8	6.50b	6.75b	2.75c	0.50a	7.00c	6.25b	2.75c	0.50a
9	6.50b	5.75b	1.50a	2.00a	6.25b	6.00b	2.50b	1.00a
10	6.00b	7.75f	1.00a	1.75a	7.25d	6.50b	1.75a	1.00a
11	6.50b	7.00c	0.75a	2.00a	7.75e	5.75a	1.00a	1.75a
12	6.25b	7.00c	1.00a	5.00g	7.25d	6.00b	3.25d	2.75c
13	6.75b	7.25d	0.75a	1.50a	7.75e	6.25b	0.50a	1.75a
14	7.00c	7.25c	1.25a	2.25a	8.00f	6.25b	2.75c	0.75a
15	6.25b	6.50b	4.00f	3.00d	6.75b	6.00b	3.25d	3.75e
16	7.00c	7.25c	3.00d	3.50e	8.00f	6.25b	2.50b	4.00e
17	6.75b	7.75f	2.25a	2.25a	8.00f	6.50b	3.25d	1.25a
18	6.50b	7.75f	2.00a	0.50a	7.750e	6.50b	1.25a	1.25a
19	6.50b	7.75f	2.61c	1.50a	7.50d	6.75b	3.25d	0.86a

[A] As assessed by a modified double-digit Saari–Prescott scale (Saari and Prescott 1975).

Table 2. Mean necrotic and pycnidial coverage percentages (NCP and PCP, respectively) caused by *Septoria tritici* for different types of inoculum and growth stages

Values are means of three upper leaves. Values followed by different letters are significantly different ($P < 0.01$)

Accession	Inoculum type				Growth stage			
	Pulverisation		Grain application		GS60		GS75	
	NCP	PCP	NCP	PCP	NCP	PCP	NCP	PCP
1	41.60abc	40.94e	28.91abcd	28.02def	3.21a	18.31cde	51.28cde	50.66cd
2	42.59bc	42.68de	37.33defg	37.43i	12.89b	12.88bc	67.03ijk	67.24f
3	62.45fg	61.91g	32.26bcde	28.17ef	30.08bcd	30.08fg	64.63hij	60.00e
4	27.96a	11.67a	25.32ab	25.32cd	1.59a	0.93a	54.88efgh	37.93a
5	31.92a	26.98b	40.91efg	37.14hi	20.12b	12.74bc	52.71def	51.37d
6	46.52bcd	39.60d	18.99a	18.76ab	21.84b	21.70de	43.67abc	36.66a
7	38.89ab	33.34c	26.14abc	17.97ab	22.48bc	13.16bc	42.55abc	38.15a
8	42.73bcd	37.68cd	36.93def	30.61fg	17.67b	16.47bcd	61.99ghij	51.83d
9	70.62gh	67.48g	47.88g	48.56j	29.93bcd	30.55fg	88.58m	85.49h
10	51.60de	47.35ef	35.07cde	34.66ghi	10.83b	11.18b	75.84l	70.82fg
11	48.73cd	41.15de	45.73fg	40.59ij	49.17e	36.89gh	45.29bcd	44.86bc
12	46.77bcd	47.16ef	25.63ab	21.81bc	11.48b	42.07h	60.93fghi	56.90de
13	50.64cde	39.80d	25.32ab	20.23abc	32.39cd	16.93bcd	43.58abc	43.10b
14	58.97ef	51.89f	34.75cde	31.52fgh	24.49bc	17.64cd	69.22jkl	75.26g
15	76.43hi	63.16g	44.35fg	37.24hi	33.84d	17.70cd	86.95m	82.70h
16	83.23i	83.55h	45.79g	35.10ghi	55.20e	45.17h	73.82kl	73.48g
17	62.21f	61.88f	40.54efg	38.19i	27.77bcd	24.81ef	74.97l	65.77f
18	66.24fg	65.99g	19.39a	14.87a	46.40e	41.56e	39.23ab	39.30a
19	33.79a	38.58cd	20.97a	25.48cdf	22.18bc	23.02de	32.57a	41.03ab
Mean	51.78	47.42	33.27	30.09	25.60	21.15	59.46	56.45

parameters of severity had a higher value for GS60 than GS75 (Table 2).

Plant height (to flag leaf) was not associated with resistance within this germplasm according to the analysis of covariance.

Discussion

This paper describes an inoculation technique using oat grains covered with the stromatic mycelia of *S. tritici* to check the resistance of the SMN set. When two types of applications were compared (pulverisation and grain application), except for the variety Bobwhite 'S' CM 33203-K-10M-7Y-3M-2Y-1M-OM and the line Tia.2/4/CSTH.CU//GLEN/3/ALD/PVN CIGM88.734–1B-3PR-0PR-1M that reacted similarly to the observations of Gilchrist *et al*. (1999), all genotypes were more susceptible under Argentine conditions. The higher level of virulence of the Argentine isolates and their frequency of variation could explain this behaviour (Eyal *et al*. 1985; Gilchrist *et al*. 1999; Cordo *et al*. 2006).

The PCPNCP and PCP results in this study are in agreement with previous research (Eyal *et al*. 1985; Gilchrist *et al*. 1999). The advanced resistant lines coming from the crosses with a group of resistant Chinese lines did not show a high level of resistance (Ald/Pvn/YM#6, Milan/Sha#7, Catbird, Talhuen INIA, Sha3/Seri/PSV/Bow and the cultivar with Kavkaz/K4500sources).

The resistant check Bethlehem was not resistant at CIMMYT nor in our conditions. The bread wheat checks SeriM82 and Glennson M81 (with Veery 'S' germplasm) and Lakhish were susceptible, as was expected (Gilchrist *et al*. 1999). The durum wheat Etit 38 and the resistant check Bethlehem had the same level of susceptibility as the bread wheat checks (SeriM82, Lakhish), as they were scored by Kohli (1995). The disease resistance introduced from Brazilian germplasm was detected on a short, early-maturing resistant line derived from IAS 20 spring wheat and a more susceptible reaction on lines derived from IAS 58.

Bobwhite 'S' germplasm and its derivative lines (in Argentina represented by Don Ernesto INTA), showed variable levels of resistance caused by its background with more than one genetic source and the presence of a low number of major genes (Cordo *et al*. 1994). Plant height was not associated with the resistant reaction. The negative associations were present when weather conditions were less conducive to the development of the diseases. Non-conducive conditions and the further distance between leaves in tall cultivars could have reduced the rain-splash dispersal of pycnidiospores, thus causing this negative association (Simón *et al*. 2005).

Two factors were found to influence the expression of the disease on the leaves: (1) the concentration of the grain inoculum (120 g/m^2) is optimum for differentiation between susceptible and resistant accessions, and (2) the wet environment. For the grain application treatment, the density of the plants was as important as rainfall in producing infection. If the rain regimen was not frequent and intensive, the pycnidiospores could not reach the higher leaves, making it difficult for the inoculum to ascend.

In the pulverisation treatment, the surface covered by the inoculum included more than one leaf stratum. A simultaneous proliferation of the pathogen was obtained at all foliage levels, which, in addition to the beneficial structure of the canopy, produced the highest values of severity.

The most susceptible varieties at the GS75 stage were those that had a longer period of green leaf during the growth cycle; however, something different occurred with ETIT 38, which did

not show any difference on NCP and PCP for both growth stages. This could be explained by the quick senescence of the leaves that practically stopped the development of the fungus at the end of GS60.

The higher values of the disease in 1998 compared with those of 1999 were caused by the influence of the climatic conditions. Temperature was not an important factor because there was no statistical differences detected in 3 years of experiments. In 1998, high humidity (30% higher than the following year) and increased rainfall (425.3 mm more than the following year) were responsible for the rapid increase of the disease compared with results of 1999 (data not shown).

Both inoculation techniques were appropriate to monitor the behaviour of the accessions of the SMN set. If the experimental area has good rainfall from tillering to flowering, grain application is recommended. In dry irrigated area, the pulverisation with extra irrigation as a source of humidity is suggested.

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