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Lesion size as a criterion for screening oat genotypes for resistance to leaf spot

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Abstract In Brazil, increased leaf spot disease (*Pyrenophora chaetomioides* Speg.) frequency and intensity on cultivated spring oat (*Avena sativa*) requires that plant pathologists and breeders rapidly identify oat genotypes with greater resistance. Criteria are needed to compare and evaluate oat genotypes to screen large numbers of lines, quantification of resistance components under controlled conditions allowing year-long screening and the rejection of susceptible lines before lengthy field trials. There is a need to determine which resistance components are associated with leaf spot intensity in the field, as estimated from the area under the disease progress curve (AUDPC). We assessed various oat *P. chaetomioides* resistance components under controlled conditions in seedlings of 26 oat genotypes chosen from recommended varieties and elite breeding lines to determine the association of resistance components with the AUDPC, obtained by evaluating each genotype in the field. The resistance components estimated were: initial lesion size (ILS) and final lesion size (FLS); rate of lesion expansion (r); and area under the normalized and corrected lesion expansion curve (AULECc). All correlations were positive and significant at $p=0.01$ and were distributed into moderate ($0.5<r<0.8$) and strong ($0.8\leq r<1$) correlation classes. The strongest average correlations occurred with the AULECc (0.827), ILS (0.801) and FLS (0.801) components. These results indicate which components may be useful in resistance screening, with FLS possibly being the most useful criterion because it is less laborious to obtain and speeds up the selection process for leaf spot resistance.

Keywords *Avena sativa* · *Pyrenophora chaetomioides* · Resistance components · Lesion expansion rate

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Introduction

In southern Brazil, spring, or white, oats (*Avena sativa* L.) are an excellent option for cultivation in the winter-spring season, between major summer crops, such as

soybeans and maize (Rosa et al. 2003). In 2010, white oats occupied an area of 126,400 ha in Brazil, mainly in the southern states of Rio Grande do Sul and Paraná, resulting in a production of 244,100 metric tonnes (CONAB 2009). Since the mid-1970s, oat breeding programs have released nearly 60 varieties of this cereal in Brazil, all adapted to the climate and soil of the region.

Leaf spot, caused by the hemibiotrophic fungus *Pyrenophora chaetomioides* Speg. (syn. *Pyrenophora avenae* Ito & Kurib.; Anamorph = *Drechslera avenae* (Eidam) Sharif), is a disease of oats that has recently increased in importance in Brazil (Martinelli et al. 2003; Bocchese et al. 2006). Depending on the susceptibility of the cultivar, *P. chaetomioides* causes the destruction of much of the leaf tissue of infected plants, interfering with the accumulation of photosynthates and resulting in the production of lightweight, shrivelled and darkened grains of inadequate quality for milling processing (Bocchese et al. 2006).

Studies have shown that direct sowing and monoculture promotes survival and proliferation of hemibiotrophic pathogens, resulting in increased incidence and severity of disease (Prestes et al. 2002; Reis et al. 1997). However, Prestes et al. (2002) found that rotation of wheat crops by at least one winter is sufficient to significantly reduce the intensity of leaf spot. Similarly, Blum (1997) reported that, in the Brazilian state of Rio Grande do Sul, an average of 17 months was needed for the total decomposition of oat straw and that this period also drastically reduced the inoculum of *P. chaetomioides* remaining in the soil, thus confirming the importance of crop rotation in no-tillage systems.

High resistance to leaf spot has not yet been reported in any oat genotype, with, at most, genotypes having been classified as moderately resistant to highly susceptible (Bocchese et al. 2003; Bocchese et al. 2006; Mehta 2001; Rosa et al. 2003). The search for genotypes possessing higher levels of resistance is of extreme importance, with quantitative or partial resistance possibly being an effective tool for the management of this disease.

The primary effect of quantitative resistance is that in resistant or partially resistant plants, leaf spot progresses slower than in fully susceptible plants. Slower development of leaf spot is due to changes occurring in various processes that begin after contact between the pathogen and host, including lower infection efficiency, longer incubation period, reduced rate of lesion expansion, lower spore production and a reduction in the size and number of lesions. Each of these processes is a component of resistance and their combined effects cause large differences in the final disease severity in the field. Resistance components are usually estimated using monocyclic infections carried out in greenhouses or growth chambers (Deadman 2006; Matiello et al. 1997; Parlevliet 1997; Thomé et al. 1999), allowing one or more resistance components to be evaluated in tests throughout the year and hence the elimination of susceptible genotypes as soon as possible in the breeding program. This procedure could speed up the selection of genotypes with resistance to leaf spot in oat breeding programs. However, the criteria used for selection must be associated with the development of the disease in the field, as represented by the area under the disease progress curve (AUDPC). Examples of studies associating epidemiological components with resistance have frequently been reported in the literature for several pathosystems. For example, lesion expansion was the criterion used by Berger et al. (1997) to model and validate epidemics of *Cercospora medicaginis* on alfalfa and *Exserohilum turcicum* on maize and by Menegon et al. (2005) to determine the timing for chemical intervention to control barley leaf spot caused by the fungal pathogens *Bipolaris sorokiniana* and *Pyrenophora teres*. Lesion size has also been a commonly used criterion in various pathosystems, such as sunflower/*Alternaria helianthi* (Kong et al. 1997) and wheat/*Pyrenophora tritici-repentis* (Liu et al. 2004). Other studies have used both the above criteria, with Tredway et al. (2003) using them to evaluate the resistance of *Festuca arundinaceae* to the fungus *Magnaporthe grisea* and Dallagnol et al. (2009) employing them to investigate the active absorption of silicon by rice plants during the control of brown spot caused by the fungus *Bipolaris oryzae*.

However, studies regarding the possible use of resistance components to pre-select oat lines resistant to leaf spot caused by *Pyrenophora chaetomioides* have yet to be developed. The aims of the study reported in this paper were to determine various resistance components to *P. chaetomioides* in spring oat seedlings under controlled conditions, to verify their association with the AUDPC and to indicate which components could be used in a breeding program to select oat genotypes with greater resistance.

Materials and methods

Genetic material

Twenty-six spring oat genotypes were evaluated, five varieties and twenty-one elite lines, developed in the Oat Breeding Program of the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul (RS) state, Brazil (Table 1). In 2007 and 2008 the oat genotypes were tested under field conditions and as seedlings under controlled environmental conditions. The resistant control was UFRGS 19, a variety extensively tested by Brazilian researchers and one of the genotypes most resistant to leaf spot, displaying low incidence and severity of lesions on leaves and a low incidence of spotted kernels (Rosa et al. 2003; Bocchese et al. 2006). The susceptible control was the elite line UFRGS 9912002-1, fully susceptible to leaf and kernel spot.

Field trials

Trials were conducted at the Agronomic Experimental Station of UFRGS in Eldorado do Sul, RS, Brazil (30° 05′S 51°40′W, elevation ≈ 46 m). The local climate is classified as Cfa, a humid subtropical climate with hot summers and the soil is a typical Dystrophic Red with sandy texture at the surface (Streck et al. 2008). Seeds were sown under a no-till planting system using a direct

Table 1 Oat genotypes (*Avena sativa* L.) developed at the Agronomy Faculty, Federal University of Rio Grande do Sul (UFRGS), Brazil and used in field and controlled environment experiments to assess lesion size as a criterion for screening oat genotypes for resistance to leaf spot caused by the fungus *Pyrenophora chaetomioides*

Genotype accession number	Type	Genealogy
UFRGS 16	Variety	CP16CRcpx/C7512//SRcpx/74C8014
UFRGS 19[a]	Variety	UFRGS 841110 × UFRGS 884021-1
UFRGS 039017-3	Elite line	COCKER492/STARTER-1F3 × UFRGS 8
UFRGS 039083-1	Elite line	COCKER492/STARTER-1F3 × UFRGS 10
UFRGS 015050-1	Elite line	UPF 16 × UFRGS 950155
UFRGS 017150-4	Elite line	UFRGS 881971//PC68/5*STARTER F4
UFRGS 017121-2	Elite line	PC68/5*STARTER F4 × UFRGS 10
UFRGS 01B7121-2-4	Elite line	PC68/5*STARTER F4 × UFRGS 10
UFRGS 046048-1	Elite line	UFRGS970216-2 × UFRGS970461
UFRGS 046050-4	Elite line	UFRGS970216-2 × UFRGS970461
UFRGS 046052-4	Elite line	UFRGS970216-2 × UFRGS970461
UFRGS 046053-4	Elite line	UFRGS970216-2 × UFRGS970461
UFRGS 046054-2	Elite line	UFRGS970216-2 × UFRGS970461
UFRGS 046071-5	Elite line	UFRGS984021-1 × UFRGS 19
UFRGS 047062-2	Elite line	UFRGS 91905-17/UFRGS8
UFRGS 15	Variety	COCKER 81C42//CORONADO2/CORTEZ3/PENDEK/ME1563
UFRGS 17	Variety	CORONADO2/CORTEZ3/PENDEK/ME1563//76-29/76-23/75-28/CI833
URS 21	Variety	UFRGS 10 × CTC 84B993
UFRGS 038005-3	Elite line	CTC 89B210 sel 1/X6661-3
UFRGS 038009-1	Elite line	UFRGS-11 sel 1/Belle
UFRGS 046070-1	Elite line	UFRGS984021-1 × UFRGS 19
UFRGS 046103-2	Elite line	UFRGS987016-1 × UFRGS 19
UFRGS 046107-2	Elite line	UFRGS987016-1 × UFRGS 19
UFRGS 047024-1	Elite line	UFRGS 940556 × Unknown
UFRGS 046054-5	Elite line	UFRGS970216-2 × UFRGS970461
UFRGS 9912002–1[b]	Elite line	UFRGS 86A1194-2/UFRGS 8

[a] Resistant Control

[b] Susceptible Control

seed driller on 3 July 2007 and 26 June 2008. Nitrogen (22.5 kg ha^{-1}) was added as urea at the four-leaf and seven-leaf stages in 2007 and at the four-leaf, five-leaf and seven-leaf stages in 2008. A randomised block experimental design was used in each of the two years, in 2007 with three replicate plots, each consisting of two 2-m rows 0.2 m apart, and in 2008 with four replicate plots, each consisting of five 3-m rows 0.2 m apart.

Each genotype was assessed regularly for the severity of leaf spot in each plot, beginning when the plants had five or six open leaves. Five evaluations were performed in 2007 and seven in 2008. For each genotype tested, we constructed disease progress curves and used trapezoidal integration to calculate the areas under the curves based on the equation proposed by Shaner and Finney (1977):

$$\mathrm{AUDPC} = \sum\left[\left(y_i + y_{i+1}\right)/2\right] * \left(t_{i+1} - t_i\right),$$

where:

y_i % leaf area affected by leaf spot (severity at the i^{th} observation)

t_i time in days after first evaluation at the i^{th} observation

In both assessment years, the final severity (FS) of the disease was recorded as the final leaf spot severity reading and was used to group the genotypes into susceptibility classes as proposed by Mehta (2001): where S is susceptible (>11 % severity), MS is moderately susceptible (between 6 and 10 %) and MR is moderately resistant (<6 %).

Data were subjected to analysis of variance (ANOVA) and genotype means were compared by Duncan's test at $p=0.05$ using the SAS statistical package (SAS Institute Inc., Cary, NC). To check for significant interactions between the 2 years of field evaluations, the means for each genotype were compared between years using Student's t-test at $p=0.01$ and $p=0.05$.

Controlled environment seedling evaluation

Seeds of the 26 oat genotypes (Table 1) were disinfected by immersion for 3 min in aqueous sodium hypochlorite (1 %v/v available chlorine) and rinsed three times in sterilised distilled water (SDW). The disinfected seeds were sown in polystyrene trays consisting of 3 cm× 3 cm×5 cm cells arranged in blocks of ten cells per section, each cell containing sieved and autoclaved substrate consisting of 70 % clay, 25 % sand and 5 % organic matter, pH 6.5. Immediately before sowing, we mixed 5-20-20 nitrogen-phosphorus-potassium (NPK) fertilizer with the substrate at the equivalent rate of 500 kg ha^{-1}. Three seeds were planted per cell and five cells were planted per genotype (two genotypes per tray section), for a total of 15 seeds per genotype in each experiment. The polystyrene sections were placed in plastic trays containing about 1 cm of tap water and maintained at 20°C±2°C, illumination for a 14-h photoperiod being provided by three 40 W daylight fluorescent lamps and one 40 W Grolux lamp (model T-12, Sylvania, Danvers, USA) placed 42 cm above the trays. The study was carried out at the Small Grain Cereals Plant Health Laboratory, Phytosanitary Department, Agronomy Faculty, UFRGS, Porto Alegre, RS, Brazil.

The monosporic fungal isolate used to inoculate the oat seedlings was obtained from one plant of the resistant control UFRGS 19 showing typical symptoms of *P. chaetomioides* leaf spot, the isolate being cultivated in petri dishes containing one-quarter strength potato dextrose agar (¼PDA, containing: potatoes, 50 g; dextrose, 5 g; agar, 10 g; distilled water 1000 ml). The inoculum was prepared by washing 15 plates with SDW containing 100 µl l^{-1} of polyoxyethylene-20-sorbitan monolaurate (SDW/Tween 20) and gently rubbing the surface of the fungus colony with a brush to remove the conidia. The conidial suspension was filtered through a double layer of gauze to remove fragments of agar and mycelium and the conidia counted in a Neubauer chamber, the average number of conidia being adjusted to 1.0×10^4 ml^{-1} with SDW/Tween 20.

Oat seedlings with two fully expanded leaves (stage 12: Zadoks et al. 1974) were inoculated with conidial suspension using a Venturi atomizer connected to a constant flow compressor, the jet of the atomizer being directed toward the median adaxial region of the second leaf of each seedling. After inoculation, the seedlings were placed in moist chambers (relative humidity (RH)=100 %) for 24 h and then transferred to a heated room (25°C±3°C, RH=80 %) under a 14-h photoperiod, using the same illumination as described above. Maximum and minimum temperatures were recorded daily. The experimental design was fully randomised. Two replicate experiments were carried out, designated 'replicate experiments 1 and 2'.

Two days after inoculation, lesions that appeared at the median adaxial region of the second leaf and that were not very close to each other were selected and numbered with permanent black ink using a pen with a 1 mm tip. A digital calliper, accurate to 0.1 mm, was used to measure the length and width of each lesion and the dark lesion area was estimated using the modified formula for an ellipse: $S = \pi(L \times W)/4$, where S is the surface area of the lesion, L is the lesion length and is W the lesion width. Measurements of the lesions on all 26 genotypes began on the third day after inoculation and were repeated every three days until it was not possible to take accurate measurements due to lesion coalescence or leaf death. Each plant with at least three lesions on the second leaf was considered a replicate. The number of replicates varied between both genotypes and experiments. Measurements were not destructive, so that each lesion was measured again on each day of evaluation. From these measurements over time, it was possible to quantify the following resistance components: initial lesion size (ILS), defined as the average lesion size (mm^2) on each genotype on the first day of measurement on the third day after inoculation; final lesion size (FLS), defined as the average lesion size (mm^2) on each genotype on the twelfth day after inoculation. For some genotypes, measurements continued until the eighteenth day after inoculation; rate of lesion expansion (r, in $mm^2\ day^{-1}$), with daily r values being obtained by linear regression using the SAS package and the lesion expansion data obtained during the time period evaluated; and the area under the normalized and corrected lesion expansion curve (AULECc).

The AULECc for each lesion on each plant was computed by trapezoidal integration of the lesion expansion curve over time using a similar equation to that shown above to calculate the AUDPC:

$$\text{AULECc} = \{\{\sum[(y_i + y_{i+1})/2]*(t_{i+1} - t_i)\}/n\}*c$$

where:

- y_i area of the lesion at the i^{th} observation
- t_i time in days after initial evaluation of the i^{th} observation (Tredway et al. 2003)
- n period in days between the first and last measurement of the lesion
- c maximum period in days during which the lesions could be measured (Graichen et al. 2010).

The AULEC represents the cumulative size of lesions that were measured every three days. This criterion has been used to evaluate the resistance of cultivars of the forage grass *Festuca arundinacea* to the fungus *Magnaporthe grisea* (Tredway et al. 2003), while Dallagnol et al. (2009) used the AULEC as a component of assessment of rice resistance to brown spot caused by *Bipolaris oryzae*. The normalized and corrected AULECc value has been used by Graichen et al. (2010, 2011) to investigate oat resistance to crown rust caused by *Puccinia coronata* because the maximum period in days during which the lesions could be measured is one of its parameters, allowing direct comparison between the area of leaf lesions occurring at different time periods.

Because the genotype-trial interaction was significant ($p \leq 0.05$), the experiments were not pooled but were analysed separately. The data were subjected to ANOVA and genotype means were compared by the Duncan test ($p \leq 0.05$) using the SAS package (SAS Institute Inc., Cary, NC).

Correlations between the results obtained in a controlled environment and those obtained in the field (AUDPC) were also established. Genotype means were paired and the degree of association was assessed by Pearson's and Spearman correlation coefficients. A correlation matrix was generated using the SAS statistical package (SAS Institute Inc., Cary, NC).

Results

Field trials

The field evaluations showed no qualitative resistance, with leaf spots appearing on the leaves of all 26 genotypes. However, the severity of the disease varied, there being significant differences ($P>F=0.0001$) between the genotypes tested regarding AUDPC and final severity of the disease in both years of the experiment. The resistant control (UFRGS 19) and the susceptible control (UFRGS 9912002-1) showed, as expected, contrasting results and differed significantly in the amount of the disease (Table 2). Visual observations indicated that lesions occurred in greater quantity and size in the susceptible control and were clearly distinct from lesions in the resistant control. In 2007, the final severity in the resistant control was 2 % and ranged from 1.7 % for genotypes UFRGS 17, UFRGS 15 and UFRGS 017150-4 to 16 % for the susceptible control, with a mean of 3.69 %. In this year, most genotypes were statistically different from the susceptible control, the

Table 2 Final severity and area under the *Pyrenophora chaetomioides* leaf spot disease progress curve (AUDPC) for oat genotypes under field conditions. Three replicate plants were assessed in 2007 and four in 2008

Genotype accession number	Final Severity (%)					AUDPC		
	2007		2008		Pr > *t*	2007	2008	Pr > *t*
UFRGS 9912002-1[b]	16.0 a	S	20.00 a	S	<.0001**	335.33 a	400.63 a	<.0001**
UFRGS 047062-2	12.3 b	S	11.33 c	S	0.2857	248.67 b	244.30 b	0.7653
UFRGS 01-B-7121-2-4	6.0 c	MS	14.00 b	S	<.0001**	122.67 c	223.26 b	<.0001**
UFRGS 16	5.3 cd	MR	4.33 def	MR	0.1658	109.83 c	71.34 d	0.0094**
UFRGS 046048-1	5.0 cd	MR	3.00 efghi	MR	0.0559*	106.17 cd	49.19 defg	0.0002**
UFRGS 038009-1	4.3 cde	MR	3.33 efgh	MR	0.4543	75.83 def	50.58 def	0.0861
UFRGS 017121-2	3.7 def	MR	4.66 de	MR	0.1658	95.67 cde	69.85 de	0.0794
UFRGS0 47024-1	3.3 def	MR	19.33 a	S	<.0001**	68.83 ef	379.41 a	<.0001**
UFRGS 015050-1	2.6 ef	MR	2.00 ghijk	MR	0.3927	64.17 f	32.11 fg	0.0300*
UFRGS 038005-3	2.6 ef	MR	3.33 efgh	MR	0.4543	59.50 f	37.60 efg	0.1361
UFRGS 046054-2	2.6 ef	MR	3.00 efghi	MR	0.6687	63.00 ef	41.64 defg	0.1459
UFRGS 046107-2	2.6 ef	MR	3.00 efghi	MR	0.4543	53.67 f	37.44 efg	0.2684
UFRGS 046054-5	2.7 ef	MR	6.00 d	MS	<.0001**	57.17 f	112.35 c	0.0002**
UFRGS 046103-2	2.3 ef	MR	2.00 ghijk	MR	0.8306	54.83 f	36.84 fg	0.2200
UFRGS 046050-4	2.3 ef	MR	3.00 efghi	MR	0.2405	66.50 ef	45.55 defg	0.1538
UFRGS 046053-4	2.3 ef	MR	4.66 efg	MR	0.0334*	54.83 f	50.20 defg	0.7515
UFRGS 046052-4	2.3 ef	MR	2.33 ghijk	MR	0.5928	51.33 f	44.51 defg	0.6411
UFRGS 039017-3	2.3 ef	MR	2.66 gfhij	MR	0.8306	46.10 f	28.86 fg	0.2399
UFRGS 046071-5	2.0 f	MR	0.83 jk	MR	0.1503	45.50 f	17.91 fg	0.0611
UFRGS 19[a]	2.0 f	MR	1.16 jki	MR	0.2624	45.50 f	21.04 fg	0.0963
UFRGS 039083-1	2.0 f	MR	1.50 hijk	MR	0.4228	42.00 f	18.55 fg	0.1107
URS 21	2.0 f	MR	2.33 ghijk	MR	0.5212	54.83 f	36.28 fg	0.2060
UFRGS 046070-1	2.0 f	MR	1.00 jk	MR	0.3364	45.50 f	19.89 fg	0.0818
UFRGS 15	1.6 f	MR	1.00 jk	MR	0.3927	41.43 f	16.46 g	0.0897
UFRGS 17	1.6 f	MR	0.66 k	MR	0.2405	39.67 f	18.26 fg	0.1451
UFRGS 017150-4	1.6 f	MR	2.33 ghijk	MR	0.2857	49.00 f	38.94 defg	0.4919
Mean	3.69		4.68		80.67		82.42	
CV (%)	29.90		20.77		22.65		23.84	

[a] Resistant control; [b] Susceptible control; Pr > *t* = probability of *t*; MR = moderately resistant (<6 % severity), MS = moderately susceptible (6 % to 10 % severity), S = susceptible, (>11 % severity), from Mehta (2001); Means with different letters within columns differ by Duncan's test at $p=0.05$; * = significant at $t \leq 0.05$; ** = significant at $t \leq 0.01$; CV = Coefficient of variation

exceptions being UFRGS 046103-2, which did not differ statistically from the resistant control. Genotype UFRGS 047062-2 showed an intermediate final severity. In 2008, the final severity in the resistant control was 1.3 % and ranged from 0.75 % for UFRGS 17 to 20.5 % in the susceptible control, with a mean of 4.89 %. In both years, the majority of genotypes showed similar responses, although six genotypes showed greater final severity in 2008. Comparing 2007 and 2008, in 2007 UFRGS 047024-1 and UFRGS 046054-5 were classified as moderately resistant but in 2008 UFRGS 047024-1 was classified as susceptible and UFRGS 046054-5 as moderately susceptible. In all, we found that 21 genotypes were classified as moderately resistant (Table 2) according to the criteria proposed by Mehta (2001). The average area under the disease progress curve (AUDPC) was very similar between years (Table 2). In 2007, all genotypes differed significantly from the susceptible control (AUDPC=value 335) while for the resistant control AUDPC=45. Twenty

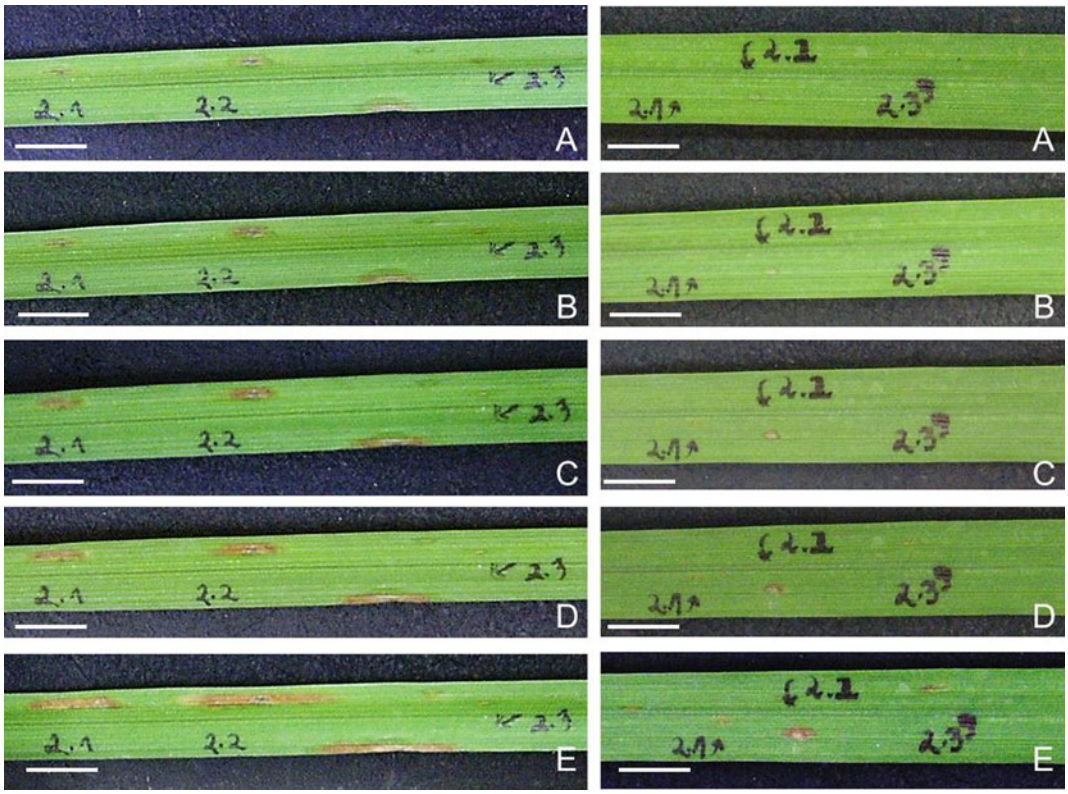

Fig. 1 Expansion of *Pyrenophora chaetomioides* lesions on the second leaves of the susceptible oat genotype UFRGS9912002-1 (left) and the resistant oat genotype UFRGS19 (right) maintained under controlled conditions (25±3°C, 14 h photoperiod). Three days (*A*), five days (*B*), seven days (*C*), nine days (*D*) and 15 days (*E*) after inoculation. Bars=0.5 cm. Lesions were identified with a 1-mm fine-point permanent black marker, with, for example, 2.1 referring to the second replicate (2), first lesion (1)

genotypes had AUDPC values similar to that of the resistant control in 2007 and nineteen in 2008. As with final severity, some genotypes showed very different values between the two years, with the susceptible control and genotypes UFRGS 01B7121-2-4, UFRGS 047024-1 and UFRGS 046054-5 having greater AUDPC values in 2008 than in 2007, while genotypes UFRGS 16 and UFRGS 046048-1 showed lower AUDPC values in 2008 than 2007 (Table 2).

Controlled environment tests on seedlings

The combined analysis of variance showed significant differences between genotypes for all resistance components studied. Lower means were found in the replicate experiment 1, therefore each replicate experiment was analysed separately and a significant interaction between genotype and each replicate experiment was observed. As observed in the field trials, during the controlled environment experiments the susceptible and resistant controls differed in all resistance components studied, the difference in lesion development on these two genotypes being shown in Fig. 1.

For replicate experiment 1, the mean initial lesion size (Table 3) was 0.356 mm^2 and ranged from 0.191 mm^2 for UFRGS 046070-1 to 0.674 mm^2 for the susceptible control, while the lesion size of 12 of the genotypes did not differ statistically from that of the resistant control (0.207 mm^2). The lesion size of UFRGS 047062-2 (0.584 mm^2) did not differ from the susceptible control, with 11 genotypes showing intermediate values. The mean final lesion size was 1.33 mm^2 but ranged from 0.57 mm^2 for UFRGS 046071-5 to 2.935 mm^2 for UFRGS 047062-2, this latter genotype being statistically equal to the final mean lesion size for the susceptible control (2.467 mm^2). The mean final lesion size of 17 genotypes did not differ statistically from the resistant control (0.999 mm^2).

Table 3 Initial and final *Pyrenophora chaetomioides* lesion size on the second leaves of 26 oat genotypes assessed in two replicate experiments carried out under controlled conditions (25±3°C, 14 h photoperiod)

Genotype accession	Initial lesion size (mm^2)							Final lesion size (mm^2)						
	Replicate 1			Replicate 2				Replicate 1			Replicate 2			
	Lesion size	nº leaves	nº lesions	Lesion size	nº leaves	nº lesions	Pr > *t*	Lesion size	nº leaves	nº lesions	Lesion size	nº leaves	nº lesions	Pr > *t*
UFRGS 9912002-1[b]	0.674 a	8	29	0.707 a	9	43	0.696	2.64 ab	8	29	3.94 a	9	43	0.0002**
UFRGS 047062-2	0.584 ab	11	58	0.686 a	9	33	0.129	2.93 a	11	58	3.57 a	9	33	0.0438*
UFRGS 047024-1	0.527 bc	10	38	0.564 ab	5	19	0.566	2.42 bc	10	38	2.23 b	5	19	0.515
UFRGS 017121-2	0.491 bcd	8	34	0.423 bcd	10	40	0.244	2.02 cd	8	34	1.40 cd	10	40	0.0235*
URS 21	0.460 cde	12	55	0.508 bc	12	55	0.406	1.96 cd	12	55	2.35 b	12	55	0.119
UFRGS 01B7121-2-4	0.458 cde	9	45	0.360 cdef	8	27	0.154	1.51 def	9	45	1.20 cd	8	27	0.23
UFRGS 038005-3	0.403 def	12	60	0.569 ab	10	43	0.010**	–			–			–
UFRGS 046053-4	0.383 defg	10	44	0.349 cdef	11	42	0.455	1.03 fghi	10	44	1.06 cd	11	42	0.906
UFRGS 16	0.381 defg	8	36	0.364 cdef	9	28	0.728	1.37 efg	8	36	1.59 bcd	9	28	0.622
UFRGS 046052-4	0.375 defg	10	38	0.334 cdef	6	26	0.621	1.17 fgh	10	38	1.18 cd	6	26	0.925
UFRGS 038009-1	0.348 efg	11	54	0.359 cdef	9	36	0.943	1.80 de	11	54	1.85 bc	9	36	0.82
UFRGS 17	0.345 efg	9	40	0.280 def	8	28	0.351	1.07 fghi	9	40	1.14 cd	8	28	0.926
UFRGS 046054-5	0.342 efg	13	52	0.286 def	8	24	0.38	0.94 fghi	13	52	1.15 cd	8	24	0.59
UFRGS 046048-1	0.329 fgh	10	49	0.340 cdef	6	16	0.822	1.00 fghi	10	49	1.02 cd	6	16	0.941
UFRGS 046107-2	0.315 fghi	11	39	0.319 def	11	36	0.905	0.86 ghi	11	39	1.22 cd	11	36	0.255
UFRGS 15	0.303 fghi	10	48	0.231 ef	8	24	0.287	0.99 fghi	10	48	0.84 d	8	24	0.522
UFRGS 039017-3	0.298 fghi	12	58	0.260 def	7	23	0.603	1.23 fgh	12	58	0.85 d	7	23	0.122
UFRGS 046050-4	0.290 fghi	8	29	0.330 cdef	11	40	0.655	0.73 hi	8	29	1.01 cd	11	40	0.438
UFRGS 039083-1	0.278 fghi	11	54	0.430 bcd	5	15	0.0353*	1.02 fghi	11	54	1.34 cd	5	15	0.379
UFRGS 017150-4	0.275 fghi	7	29	0.414 bcde	10	30	0.065	1.25 fgh	7	29	1.75 bc	10	30	0.165
UFRGS 046054-2	0.273 fghi	11	62	0.292 def	8	19	0.748	0.72 hi	11	62	1.09 cd	8	19	0.346
UFRGS 015050-1	0.266 ghi	9	43	0.349 cdef	9	28	0.247	1.30 efgh	9	43	1.66 bcd	9	28	0.332
UFRGS 046103-2	0.253 ghi	9	33	0.208 f	6	19	0.589	0.95 fghi	9	33	0.81 d	6	19	0.564
UFRGS 19[a]	0.207 hi	12	57	0.290 def	5	23	0.242	1.00 fghi	12	57	1.36 cd	5	23	0.34
UFRGS 046071-5	0.203 hi	10	53	0.439 bcd	4	12	0.0022**	0.57 i	10	53	1.31 cd	4	12	0.0500*
UFRGS 046070-1	0.191 i	13	46	0.357 cdef	8	25	0.0074**	0.76 hi	13	46	1.16 cd	8	25	0.2197
Mean	0.356	10.2	45.5	0.387	8.2	29.0		1.33	10.2	45.5	1.52	8.2	29.0	

Table 3 (continued)

Genotype accession	Initial lesion size (mm^2)							Final lesion size (mm^2)						
	Replicate 1			Replicate 2				Replicate 1			Replicate 2			
	Lesion size	nº leaves	nº lesions	Lesion size	nº leaves	nº lesions	Pr > *t*	Lesion size	nº leaves	nº lesions	Lesion size	nº leaves	nº lesions	Pr > *t*
CV (%)	34.37			38.2				40.2			43.45			

[a] Resistant control; [b] Susceptible control; nº leaves = number of replicate leaves measured; nº lesions = number of replicate lesions measured; Lesion measurements were made until the twelfth (†) or, in some cases, eighteenth (‡) day after inoculation, a dash (–) indicating that data is unavailable because measurements were made only until the ninth day after inoculation; values with different letters within columns differ by Duncan's test at $p \leq 0.05$; Pr > t = probability of Student's *t test* of experiments means are different, where \ast = significant at $p \leq 0.05$ and ** = significant at $p \leq 0.01$; CV = Coefficient of variation

For replicate experiment 2 the mean initial lesion size was 0.387 mm^2 and ranged from 0.208 mm^2 for UFRGS 046103-2 to 0.707 mm^2 for the susceptible control, while 20 genotypes did not differ from the resistant control (0.290 mm^2) and three (UFRGS 047024-1, UFRGS 038005-3 and UFRGS 047062-2) did not differ from the susceptible control, with only URS 21 showing an intermediate lesion size (Table 3). The mean final lesion size was 1.523 mm^2 in the second experiment and ranged from 0.806 mm^2 for UFRGS 046103-2 to 3.945 mm^2 for the susceptible control, with the final lesion size of 21 genotypes not differing statistically from the final lesion size (1.357 mm^2) of the resistant control (Table 3). In the second experiment, as compared to the first experiment, final lesion size was significantly larger for three genotypes (UFRGS 9912002-1, UFRGS 047062-2 and UFRGS 046071-5) and significantly smaller for genotype UFRGS 017121-2 (Table 3).

For replicate experiments 1 and 2, both the average lesion expansion rate and the area under the normalized and corrected lesion expansion curve (AULECc) of the genotypes were relatively consistent, with few genotypes showing significant differences between the two experiments (Table 4). In the first experiment, the mean lesion expansion rate of the susceptible control was 0.223 $mm^2\ day^{-1}$ (range 0.141 $mm^2\ day^{-1}$ to 0.358 $mm^2\ day^{-1}$), while the corresponding value for the resistant control was 64.57 % lower (mean 0.079 $mm^2\ day^{-1}$, range 0.042 $mm^2\ day^{-1}$ to 0.165 $mm^2\ day^{-1}$). In the second experiment, the mean lesion expansion rate of the susceptible control was 0.347 $mm^2\ day^{-1}$ (range 0.222 $mm^2\ day^{-1}$ to 0.486 $mm^2\ day^{-1}$), with the corresponding value for the resistant control being 70 % lower (mean 0.104 $mm^2\ day^{-1}$, range between 0.057 $mm^2\ day^{-1}$ to 0.144 $mm^2\ day^{-1}$). In both replicate experiments, the mean lesion expansion rate of most genotypes did not differ statistically from the resistant control (Table 4). In the first trial, the mean AULECc value was 10.713 in the resistant control, ranging from 7.091 for UFRGS 046071-5 to 30.49 for the susceptible control. In the second trial, the AULECc values ranged from 8.913 for UFRGS 046103-2 to 41.362 for the UFRGS 9912002-1, with a value of 14.844 in the resistant control UFRGS 19 (Table 4).

Pearson's and Spearman's correlation coefficients for the final disease severity and the AUDPC values versus the initial lesion size (ILS), final lesion size

Table 4 The *Pyrenophora chaetomioides* lesion expansion rate (*r*) and area under the normalized and corrected lesion expansion curve (AULECc) estimated from the second leaves of 26 oat genotypes assessed in replicate experiments 1 and 2 carried out under controlled conditions (25±3°C, 14 h photoperiod)

Genotype accession	r (mm2 day-1)[c]							AULECc[c]						
	Replicate 1			Replicate 2				Replicate 1			Replicate 2			
	r	nº leaves	nº lesions	*r*	nº leaves	nº lesions	Pr > *t*	AULECc	nº leaves	nº lesions	AULECc	nº leaves	nº lesions	Pr>*t*
UFRGS 047062-2	0.254 a	11	58	0.284 b	9	33	0.24	27.778 a	11	58	38.383 a	9	33	0.0002**
UFRGS 9912002-1[b]	0.223 ab	8	29	0.347 a	9	43	0.00001**	30.490 a	8	29	41.362 a	9	43	0.0005**
UFRGS 047024-1	0.205 bc	10	38	0.182 cd	5	19	0.376	27.812 a	10	38	28.520 b	5	19	0.6512
UFRGS 038005-3	0.183 cd	12	60	0.272 b	10	43	0.00001**	15.543 bcde	12	60	23.890 bc	10	43	0.0029**
URS 21	0.182 cd	12	55	0.199 c	12	55	0.389	20.383 b	12	55	22.008 bcd	12	55	0.5287
UFRGS 017121-2	0.162 de	8	34	0.119 defg	10	40	0.039*	18.418 bc	8	34	15.117 def	10	40	0.1993
UFRGS 038009-1	0.147 def	11	54	0.158 cde	9	36	0.733	15.765 bcde	11	54	16.834 cdef	9	36	0.7085
UFRGS 01B7121-2-4	0.128 efg	9	45	0.100 efg	8	27	0.222	18.153 bcd	9	45	15.967 cdef	8	27	0.5364
UFRGS 16	0.116 fgh	8	36	0.132 defg	9	28	0.604	19.718 b	8	36	17.550 cde	9	28	0.4373
UFRGS 017150-4	0.108 fghi	7	29	0.147 cdef	10	30	0.181	14.009 cdef	7	29	17.597 cde	10	30	0.3518
UFRGS 015050-1	0.105 ghi	9	43	0.131 defg	9	28	0.356	12.510 efghi	9	43	15.356 def	9	28	0.3591
UFRGS 046052-4	0.103 ghij	10	38	0.099 efg	6	26	0.831	13.692 cdefg	10	38	14.468 def	6	26	0.6445
UFRGS 039017-3	0.103 ghij	12	58	0.071 g	7	23	0.137	12.952 cdefgh	12	58	10.394 ef	7	23	0.4701
UFRGS 17	0.097 ghij	9	40	0.088 fg	8	28	0.663	12.814 defghi	9	40	14.004 def	8	28	0.6253
UFRGS 039083-1	0.093 ghij	11	54	0.112 efg	5	15	0.531	10.125 efghi	11	54	15.512 def	5	15	0.0625
UFRGS 046053-4	0.092 ghij	10	44	0.093 efg	11	42	0.906	12.571 efghi	10	44	13.250 ef	11	42	0.9158
UFRGS 046048-1	0.083 hijk	10	49	0.087 fg	6	16	0.891	10.430 efghi	10	49	13.250 ef	6	16	0.2748
UFRGS 15	0.082 hijk	10	48	0.069 g	8	24	0.539	11.470 efghi	10	48	9.797 ef	8	24	0.6558
UFRGS 046103-2	0.081 hijk	9	33	0.064 g	6	19	0.493	10.402 efghi	9	33	8.913 f	6	19	0.8042
UFRGS 046054-5	0.081 hijk	13	52	0.093 efg	8	24	0.675	10.182 efghi	13	52	12.469 ef	8	24	0.3329
UFRGS 19[a]	0.079 hijk	12	57	0.104 efg	5	23	0.411	10.713 efghi	12	57	14.844 def	5	23	0.1336
UFRGS 046054-2	0.073 hijk	11	62	0.090 efg	8	19	0.496	8.515 fghi	11	62	12.252 ef	8	19	0.1549
UFRGS 046107-2	0.071 ijk	11	39	0.096 efg	11	36	0.313	10.755 efghi	11	39	14.604 def	11	36	0.1853
UFRGS 046050-4	0.064 ijk	8	29	0.087 fg	11	40	0.428	7.924 ghi	8	29	11.020 ef	11	40	0.3788
UFRGS 046070-1	0.060 jk	13	46	0.101 efg	8	25	0.086	7.815 hi	13	46	12.882 ef	8	25	0.0503*
UFRGS 046071-5	0.048 k	10	53	0.107 efg	4	12	0.0402*	7.091 i	10	53	17.216 cdef	4	12	0.0026**

Table 4 (continued)

Genotype accession	r (mm2 day-1)[c]						AULECc[c]					
	Replicate 1			Replicate 2			Replicate 1			Replicate 2		
	r	nº leaves	nº lesions	*r*	nº leaves	nº lesions	Pr > *t*	AULECc	nº leaves	nº lesions	AULECc	nº leaves	nº lesions	Pr>*t*
Mean	0.1157	10.2	45.5	0.1369	8.2	29.0		14.33	10.2	45.5	17.44	8.2	29.0	
CV (%)	35.63			40.33				37.64			38.91			

[a] Resistant control; [b] Susceptible control; nº leaves = number of replicate leaves measured; nº lesions = number of replicate lesions measured; [c] Mean values for each replicate experiment conducted under controlled conditions (25°C±3°C, 14 h photoperiod); values with different letters within columns differ by Duncan's test at $p \leq 0.05$; Pr>t = probability of Student's *t test* of experiments means are different, where \ast = significant at $p \leq 0.05$ and ** = significant at $p \leq 0.01$; CV = Coefficient of variation

(FLS), lesion expansion rate (*r*) and AULECc resistance components were mostly positive and significant at the 1 % probability level, the exception being for Spearman's correlation between FS and FLS, and fell into moderate ($0.5 < r < 0.8$) and strong ($0.8 \leq r < 1$) correlation classes (Table 5). The AULECc, ILS and FLS resistance components showed the strongest associations with FS (AULECc=0.807, ILS=0.784 and FLS=0.774) and AUDPC (AULECc=0.827, ILS=0.801 and FLS=0.801) at $p \leq 0.01$. Lesion expansion rate was the component that showed the weakest correlation with both FS (r=0.694) and AUDPC (0.717) at $p \leq 0.01$, although these values still fell within the moderate correlation class. This weaker correlation may have been related to the initial lesion size occurring in some genotypes, where lesions had already reached a considerable size three days after inoculation.

Discussion

The final disease severities recorded in the field in 2007 were, in general, slightly lower than in 2008 as for the AUDPC values, which maintained the same tendency (Table 2). Differences in the final disease severity occurring in the same genotype in 2007 and 2008 may have been due to variations in temperature and relative humidity between years. Formation of *P. chaetomioides* conidia depends on the simultaneous occurrence of several environmental factors, especially temperatures of about 21°C and relative humidity above 80 % (Rosa et al. 2003). In our experiments, high temperature and humidity combined with existing lesions may have stimulated sporulation and the dissemination of *P. chaetomioides* conidia to the upper leaves of the test plants may have resulted in increased disease severity in the susceptible control UFRGS 9912002-1 and the genotype UFRGS 047062-2, while for the other genotypes only a small increase in disease severity was recorded. The results of our field experiments indicate that although leaf spot is relatively new in the Southern Brazilian environment a good level of field resistance exists in well-adapted genotypes, with most genotypes having a good level of leaf spot resistance and slow disease progress during the two years of the field experiments. However, it should be emphasized that no genotypes showed complete resistance to leaf spot.

Under controlled conditions there was a range of values but all the resistance components discriminated

Table 5 Pearson's and Spearman's mean correlation coefficients for *Pyrenophora chaetomioides* oat leaf spot resistance components assessed under different conditions

Resistance components for controlled condition experiments[a]	Correlation coefficients			
	Resistance components for field experiments[a]			
	Pearson's		Spearman's	
	FS	AUDPC	FS	AUDPC
ILS (mm^2)	0.784**	0.801**	0.711**	0.681**
FLS (mm^2)	0.774**	0.801**	0.499 ^{ns}	0.512**
r (mm^2 day^{-1})	0.694**	0.717**	0.537**	0.512**
AULECc	0.807**	0.827**	0.562**	0.534**

[a] Pooled means for the 2007 and 2008 field experiments: FS = final field severity; AUDPC = area under the disease progress curve

[b] Pooled means for replicate experiments 1 and 2 conducted under controlled conditions (25±3°C, 14 h photoperiod): ILS = initial lesion size; FLS = final lesion size; r = lesion expansion rate; AULECc = area under the normalized and corrected lesion expansion curve

between susceptible and resistant genotypes. Compared to the susceptible control, most genotypes showed low rates of lesion growth, small initial and final lesion sizes and low AULEC values (Tables 3 and 4). Most of the data did not differ significantly between experiments 1 and 2, which gives further support to the identification of resistance in the genotypes. These genotypes are thus potential sources of quantitative resistance to oat leaf spot.

All the parameters measured under controlled conditions showed good correlations with the AUDPC values observed in the field. The initial lesion size (ILS) is not normally used to detect resistance, but in our study ILS data are presented because it was possible to see differences in this variable among the 26 genotypes tested from the first day of measurement. Furthermore, there were high Pearson's and Spearman's correlations (Table 5) between ILS and FS (r= 0.784 and 0.711, respectively) and AUDPC (r=0.801 and 0.681, respectively). In this pathosystem the FLS also showed a high correlation (r=0.801) with the AUDPC (Table 5). The Spearman's correlation coefficient was lower than the Pearson's coefficient due to changes in the ranking of genotypes with intermediate values for all the traits measured.

All resistance components measured in this study show moderate to strong correlations with the development of leaf spot in the field. The area under the lesion expansion curve (AULEC) has been a component used by some authors to screen genotypes for resistance (e.g. Nociti et al. 2006). In our study, as compared to the other components, the AULEC values presented the highest Pearson's correlation (0.827) with the field AUDPC values (Table 5). Since it is necessary to perform at least three measurements of lesion size over time to estimate AULECc and r values these components are more laborious and difficult to obtain than ILS or FLS values, which can thus be used as criteria to easily, rapidly and reliably select white oat lines possessing higher levels of resistance to leaf spot.

Of the 26 genotypes tested, two were released as commercial cultivars in 2009. The line UFRGS 046054-2 became the cultivar URS Taura and the line UFRGS 046103-2 became the cultivar URS Tarimba. In these genotypes, we observed a final severity of leaf spot and AUDPC values similar to those of the resistant control (UFRGS19). These two lines also showed low AULECc values and reduced final lesion sizes in young plants when evaluated in a controlled environment. There is no complete resistance to leaf spot, the response of the genotypes being a continuum from low levels of resistance to high levels. Hence, crosses should be done between the best resistance genotypes when breeding for higher resistance and further genetic studies are presently under way in an effort to understand how resistance to leaf spot is inherited in the *Pyrenophora chaetomioides*/white oat pathosystem and to obtain plants with higher levels of resistance to leaf spot.

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