Resistance of wheat genotypes to *Ramulispora herpotrichoides* in South Africa

B Robbertse, P W Crous & G Holz

Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa

Robbertse B, Crous P W & Holz G 1996. Resistance of wheat genotypes to *Ramulispora herpotrichoides* in South Africa. *African Plant Protection* **2**(1): 45–49.

Ramulispora herpotrichoides causes a significant reduction in yield of spring wheat cultivars grown in the Swartland and Koeberg areas of the Western Cape. Based on esterase isozyme banding patterns, a previous study identified 16 groups of *R. herpotrichoides* within the local population. In the present study a representative isolate from each group and one from England were evaluated against the resistant and susceptible winter wheat cultivars VPM-1 and Armada, respectively. The most virulent isolate was subsequently selected to evaluate eyespot resistance in the cultivars Adam Tas, Nantes, Palmiet, SST16 and a newly bred spring wheat line, 92M166, with resistance obtained from *Aegilops ventricosa*. The winter wheat cultivar VPM-1 and 92M166 were significantly more resistant to eyespot than local spring wheat cultivars. Adam Tas, Nantes, Palmiet and SST16 were equally susceptible to eyespot.

Key words: eyespot resistance, *Ramulispora herpotrichoides, Triticum aestivum*.

Eyespot of wheat causes serious yield losses in the Swartland and Koeberg areas of the Western Cape in South Africa (Welgemoed 1987). Lodging can occur as a result of the 'eye'-shaped lesions induced by *Ramulispora herpotrichoides* (Fron) von Arx (syn. *Pseudocercosporella herpotrichoides* (Fron) Deighton) and *Ramulispora acuformis* (Nirenberg) Crous at the base of the wheat plant. *R. herpotrichoides* (W-type) and *R. acuformis* (R-type) were previously regarded as two varieties of *R. herpotrichoides* (Boerema et al. 1992). However, based on differences in pathogenicity, conidial morphology, culture morphology, mating populations, infection processes, isozyme zymograms and DNA polymorphisms (Lange-de la Camp 1966; Scott et al. 1975; Moreau et al. 1989; Julian & Lucas 1990; King 1990; Daniels et al. 1991; Nicholson et al. 1991), these varieties were recognised as distinct species (Robbertse et al. 1995). Only *R. herpotrichoides* has been reported from South Africa (Julian & Lucas 1990; Campbell et al. 1995).

Resistance to eyespot was first observed in the French wheat cultivar Cappelle-Desprez (Batts & Fiddian 1955). The moderate resistance of Cappelle-Desprez protected the crop from severe losses until the more effective resistance identified in wild-goat grass, *Aegilops ventricosa* Tausch. (Sprague 1936), was successfully bred into the hexaploid wheat VPM-1 (Maia 1967). VPM-1 expresses a high level of resistance to eyespot, even at high inoculum levels (Roberts & Allan 1990). The resistance in VPM-1, inherited from *A. ventricosa,* is controlled by a single gene *Pch1* on chromosome 7D (Worland et al. 1988). The eyespot resistance gene in VPM-1 can be easily detected as it is closely linked to the *EP-Dlb* marker gene that encodes for the endopeptidase enzyme. Electrophoresis can therefore be used to detect the presence of the endopeptidase enzyme, and resistant wheat lines selected accordingly (McMillin et al. 1986.). Using this technique, a homozygous resistant spring wheat line (92M166), adapted to South African growing conditions, was developed (92M166 = VPM-1/*3 (5710/Inia 66); 5710 = unknown entry obtained from the International Wheat Rust Screening Nursery in the mid-1970s) (G F Marais, pers. comm.).

The virulence of South African isolates of *R. herpotrichoides* towards resistance of VPM-1 has not been determined. Local isolates of *R. herpotrichoides* and one from England were therefore evaluated for virulence towards the resistance gene in this cultivar and 92M166. This resistance was also compared to resistance of local spring wheat cultivars currently under cultivation in the Swartland area.

Material and methods

Isolation

Stem tissue from wheat plants bearing eyespot lesions was surface-sterilised in 1 % NaOCl for two minutes, rinsed twice in sterile distilled water, dried on sterile filter paper under a laminar flow hood, and placed in Petri dishes with moistened

Table 1. Selected isolates of *Ramulispora herpotrichoides* used in this study.

Isolate[a]	Locality	Host	Collector
GC 66A	Klipheuwel	Barley	G Campbell
GC 66	Klipheuwel	Barley	G Campbell
GC 125	Caledon	Wheat	G Campbell
BR 50	Gouda	Wheat	B Robbertse
GC 68	Klipheuwel	Barley	G Campbell
BR 92	Klipheuwel	Wheat	B Robbertse
BR 98	Klipheuwel	Wheat	B Robbertse
BR 58	England	Wheat	J Lucas
BR 15	Gouda	Wheat	W van Jaarsveld
GC 78	Darling/Malmesbury	Wheat	G Campbell
GC 72	Klipheuwel	Barley	G Campbell
BR 53	Moorreesburg	Wheat	B Robbertse
BR 14	Gouda	Wheat	W van Jaarsveld
BR 140	Moorreesburg/Riebeek-west	Wheat	B Robbertse
GC 21	Klipheuwel	Wheat	G Campbell
BR 91	Klipheuwel	Wheat	B Robbertse
BR 4	Moorreesburg	Wheat	F Bester

[a]All isolates collected from South Africa, except BR 58 from England.

filter paper. Dishes were sealed with Parafilm and incubated for two weeks at 10 °C under near-ultraviolet light to induce sporulation. After sporulation, conidia were suspended in sterile water and dispersed on potato-dextrose agar (PDA) (20 g dextrose, 15 g Biolab agar, and infusion of 200 g potatoes) amended with 0.05 g/l streptomycin sulphate (PDAS). PDAS plates were left open under a laminar flow hood to dry. Single-spore isolates were established from conidia located under a stereo-microscope.

Isolates of *R. herpotrichoides* collected from various localities in the Koeberg and Swartland areas and near Caledon were selected according to criteria indicated in a previous isozyme study (Campbell et al. 1995). Seventeen groups were identified at 40 % similarity on the single linkage CLUSTER procedure of SAS, and an isolate representative of each group was used for the inoculation study (Table 1). All isolates had isozyme profiles similar to that of *R. herpotrichoides* BR 58 from England.

Inoculum preparation

The method described by Bruehl & Nelson (1964) was adapted for inoculum production. Dry oat kernels (125 g) were soaked in water for 12 hours, drained to remove excess fluid, transferred to flasks and autoclaved for 30 minutes on two consecutive days. After cooling, the oat kernels were inoculated with a 10 ml suspension of mycelial fragments obtained from a three-week-old PDA culture of the respective *R. herpotrichoides* isolate. Flasks were shaken after six days to ensure thorough colonisation of the kernels, and then maintained undisturbed for four weeks at 20 °C in the dark before being exposed for two weeks to near-ultraviolet light at 12 °C.

Inoculation of winter wheat seedlings

Winter wheat cultivars Armada (susceptible) and VPM-1 (resistant) were used for assessing the virulence of the various *R. herpotrichoides* isolates. Colonised oat kernels were macerated in a blender, incorporated into river sand, and dispensed into 15 cm diameter pots, with each pot receiving 250 ml oat kernel inoculum. Controls consisted of pots containing sand supplemented with uncolonised autoclaved oat kernels. Ten seeds were sown 1 cm deep in each pot, and covered with a thin layer of sterilised sand to prevent rapid colonisation of the inoculum by other organisms. Pots were arranged randomly in two blocks in a growth chamber, with one pot per isolate per block. Humidity in the growth chamber was enhanced with a humidifier and varied between 65 and 100 %. Temperature ranged from 10–15 °C, and was continuously recorded with a Hanna HI9161 thermo-hygrometer. At second-leaf stage, seedlings were covered with plastic bags

Table 2. Disease ratings of two winter wheat cultivars to 17 single-conidial isolates of *Ramulispora herpotrichoides.*

Isolate	VPM-1		Armada	
	Logit ($LSD_{(0.05)}$ = 1.6831)	Score[a]	Logit ($LSD_{(0.05)}$ = 2.6301)	Score[a]
BR 4	–1.3990	0	–2.0085	3
BR 14	–0.5825	0	0.8025	5
BR 15	0.0295	3	0.5710	5
GC 21	–0.9520	0	0.4470	5
BR 50	0.7770	3	0.3895	5
BR 53	–0.5420	0	1.6510	5
BR 58	0.2055	3	–2.2575	3
GC 66	1.3795	4	0.5785	5
GC 68	0.7600	3	2.2700	5
GC 72	–0.3495	3	0.9610	5
GC 78	–0.1805	3	1.9425	5
BR 91	–0.9520	0	–0.2980	3
BR 92	0.6525	3	1.0835	5
BR 98	0.2840	3	–1.1910	3
GC 125	1.0690	4	–0.3640	3
BR 140	–0.8745	0	–2.4030	3
GC 66A	2.0750	5	1.6510	5

[a]Disease index according to Scott (1971).

for a period of seven days to ensure a relative humidity of 100 %. Disease was assessed on six seedlings per pot two months after inoculation according to the number of leaf sheaths penetrated (Scott 1971).

Comparative resistance of spring wheat genotypes and VPM-1

Resistance of four commercial South African spring wheat cultivars (Palmiet, SST16, Adam Tas, Nantes) and 92M166 was compared with VPM-1 using the most virulent isolate of *R. herpotrichoides* selected from the previous seedling test. Procedures were the same as described above. Fifteen seeds were planted per pot in four replicate blocks each containing three pots per cultivar. Disease was assessed on ten plants per pot.

Data analysis

In both cases data were submitted to logistic analysis of deviance using the SAS computer program to test for interaction. Disease scores were transformed to logit values by the logit function: $y = ln\,[P_i/1\text{-}P_i]$, where $P_i = count_i/total_i$ ($count_i$ = number of ratings per category; $total_i$ = number of plants evaluated per experimental plot where an experimental plot consisted of 10 plants for each isolate per cultivar screened in the first experiment, and 30 plants for each cultivar in the second experiment). A significant cultivar-isolate interaction occurred when the data of VPM-1 and Armada were analysed. A separate analysis of deviance and a Student's *t* LSD value were obtained for each cultivar.

Results and discussion

Results of this study showed that the eyespot resistance originally detected in *A. ventricosa* (Sprague 1936) towards European isolates of *R. herpotrichoides* is also effective against local strains of the fungus (Table 2). This was not unexpected as a separate isozyme and RAPD study found local and overseas isolates to have a very high degree of similarity (Campbell et al. 1995). A host-specific adaptation of various isolates to particular host genera has nevertheless been demonstrated in other countries (Scott et al. 1976; Cunningham 1981). Subsequent research confirmed *R. acuformis* (R-type) and *R. herpotrichoides* (W-type) to be different species (Robbertse et al. 1995). Within *R. herpotrichoides,* however, different host-specific types such as the C- (pathogenic to couch grass) and S-type (pathogenic to wheat and *Aegilops squarrosa*) are acknowledged

Table 3. Analysis of variance of disease ratings on the wheat cultivars Armada and VPM-1 with 17 single-spored isolates of *Ramulispora herpotrichoides.*

Source	*d.f.*[a]	SDev[b]	MSDev[c]	*F*	*P*
Blocks	1	0.113	0.113	0.1649	0.6872
Cultivar (C)	1	53.117	53.117	77.5272	<0.0001
Isolate (I)	17	94.201	5.54124	8.0877	<0.0001
C × I	17	37.169	2.18641	3.1912	0.0018
Error	35	23.980	0.68514		
Total	71	208.580			

[a]Degrees of freedom.
[b]Sum of squares of deviances.
[c]Mean square of deviances.

(Cunningham, 1981). Nicholson et al. (1995) further demonstrated that isolates of *R. herpotrichoides* (W-type) were sexually compatible with those of the C- and S-types.

Only the W-type of *R. herpotrichoides* has been recorded in South Africa (Campbell et al. 1995). Results obtained in the present study comparing the virulence of 17 single-conidial isolates on the wheat cultivars VPM-1 and Armada, showed VPM-1 to be generally more resistant to the local eyespot isolates than Armada (Table 2). Although a significant cultivar-isolate interaction was observed (Table 3), physiological races have not been reported for *R. herpotrichoides.* Scott & Hollins (1977, 1980) observed significant interactions between cultivars and isolates of *R. herpotrichoides* in one experiment, but when tested over two experiments the three-way interaction between cultivars, isolates and experiments was not significant. Neither the differential affinity between cultivars and isolates in each experiment nor the cause of the variation from one experiment to another could be explained (Scott & Hollins 1980).

Isolate GC 66A penetrated 5–6 leaf sheaths of VPM-1 in the first experiment as opposed to only 2–3 in the second (Tables 2, 4). Since leaf sheath penetration by *R. herpotrichoides* increases with increasing temperature from 6 to 18 °C (Scott 1971; Fitt 1985; Higgins & Fitt 1985) this could have been due to the higher mean temperature prevailing during the first experiment (13 °C) as opposed to 10 °C in the second experiment.

Heun et al. (1988) found the method of inoculation used in this study reliable for evaluating cultivar resistance. The mean disease index of local spring wheat cultivars, with the exception of 92M166, was significantly greater than that of the resistant winter wheat cultivar VPM-1 (Table 4). VPM-1 and 92M166 were equally resistant to the eyespot pathogen. This verified the presence of eyespot resistance in the F3 spring wheat line after selection based on the *EP-Dlb* marker gene (McMillin et al. 1986). 92M166 was more resistant to disease than cultivars currently cultivated in the Swartland and Koeberg area, whereas the local cultivars Nantes, Adam Tas, Palmiet and SST 16 were equally susceptible (Table 4). These results confirm that effective cultivar resistance against eyespot is obtainable in local spring wheat cultivars, and that further research focusing on yield and protein content of resistant genotypes should be considered.

Table 4. Mean disease score of different spring wheat genotypes after inoculation with isolate GC 66A of *Ramulispora herpotrichoides.*

Cultivar	Mean logit value ($LSD_{(0.05)}$ = 1.8)	Mean score
VPM-1	–1.8	2 & 3
92M166[a]	–1.8	2 & 3
SST16	0.1	5
Palmiet	0.4	5
Adam Tas	1	5
Nantes	1.7	6

[a]92M166 = new spring wheat line.

Acknowledgements

The authors are grateful to G F Marais (Department of Genetics, University of Stellenbosch) and the Small Grain Institute (Bethlehem) for providing the wheat germplasm evaluated in this study.

References

Batts C C V & Fiddian W E H 1955. Effect of previous cropping on eyespot in four varieties of winter wheat. *Plant Pathology* **4**: 25–28.

Boerema G H, Pieters R & Hamers M E C 1992. Check-list for scientific names of common parasitic fungi. Supplement Series 2b (additions and corrections): fungi on field crops: cereal and grasses. *Netherlands Journal of Plant Pathology* **98**: 1–32.

Bruehl G W & Nelson W L 1964. Technique for mass inoculations of winter wheat in the field with *Cercosporella herpotrichoides. Plant Disease Reporter* **48**: 863–865.

Campbell G F, Janse B J H, Marais G F & Crous P W 1995 (in press). Only one species of *Ramulispora* is associated with eyespot disease of wheat in South Africa. *South African Journal of Science* **91**.

Cunningham P C 1981. Occurrence, role and pathogenic traits of a distinct pathotype of *Pseudocercosporella herpotrichoides. Transactions of the British Mycological Society* **76**: 3–15.

Daniels A, Lucas J A & Peberdy J F 1991. Morphology and ultrastructure of W and R pathotypes of *Pseudocercosporella herpotrichoides* on wheat seedlings. *Mycological Research* **95**: 385–397.

Fitt B D L 1985. Factors affecting the development of eyespot (*Pseudocercosporella herpotrichoides*) lesions in wheat. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **92**: 455–463.

Heun M, Mielke H & Martin R 1988. Analysis of resistance to eyespot in wheat using small seed-samples. *Journal of Phytopathology* **121**: 75–80.

Higgins S & Fitt B D L 1985. Effects of water potential and temperature on the development of eyespot lesions in wheat. *Annals of Applied Biology* **107**: 1–9.

Julian A M & Lucas J A 1990. Isozyme polymorphism in pathotypes of *Pseudocercosporella herpotrichoides* and related species from cereals. *Plant Pathology* **39**: 178–190.

King A C 1990. First record of *Tapesia yallundae* as the teleomorph of *Pseudocercosporella herpotrichoides* var. *acuformis,* and its occurrence in the field in the Federal Republic of Germany. *Plant Pathology* **39**: 44–49.

Lange-de la Camp M 1966. Die Wirkungsweise von *Cercosporella herpotrichoides* Fron dem Erreger der Halmbruchkrankheit des Getreides. II. Aggressivität des Erregers. *Phytopathologische Zeitschrift* **56**: 155–190.

Maia N 1967. Obtention de blés tendres résistants au piétin-verse par croisements interspécifiques blés X *Aegilops. Comptes Rendus des Seances Academie d'Agriculture de France* **53**: 149–154.

McMillin D E, Allan R E & Roberts D E 1986. Association of an isozyme locus and strawbreaker foot rot resistance derived from *Aegilops ventricosa* in wheat. *Theoretical and Applied Genetics* **72**: 743–747.

Moreau J M, Schingen J C V & Maraite H 1989. Detection of *Tapesia yallundae,* the teleomorph of *Pseudocercosporella herpotrichoides* on wheat stubbles in Belgium. *Mededelingen van de Faculteit der Landbouwetenschappen, Rijksuniversiteit Gent* **54**: 555–559.

Nicholson P, Dyer P S, Rezanoor H N, Lucas J A, Walkowiak-Cagara I & Hollins T W 1995. Mating relationships between different pathotypes of *Tapesia yallundae,* the teleomorph of the eyespot pathogen *Pseudocercosporella herpotrichoides. Mycological Research* **99**: 457–465.

Nicholson P, Hollins T W, Rezanoor H N & Anamthawat-Jonsson K 1991. A comparison of cultural, morphological and DNA markers for the classification of *Pseudocercosporella herpotrichoides. Plant Pathology* **40**: 584–594.

Robbertse B, Campbell G F & Crous P W 1995. Revision of *Pseudocercosporella*-like species causing eyespot disease of wheat. *South African Journal of Botany* **61**: 43–48.

Roberts D E & Allan R E 1990. *Cephalosporium* stripe resistance and grain yield potential of wheat lines with strawbreaker resistance derived from *Aegilops ventricosa. Plant Disease* **74**: 852–857.

Scott P R 1971. The effect of temperature on eyespot (*Cercosporella herpotrichoides*) in wheat seedlings. *Annals of Applied Biology* **68**: 169–175.

Scott P R, Defosse L, Vandam J & Doussinault G 1976. Infection of lines of *Triticum, Secale, Aegilops* and *Hordeum* by isolates of *Cercosporella herpotrichoides. Transactions of the British Mycological Society* **86**: 205–210.

Scott P R & Hollins T W 1977. Interactions between cultivars of wheat and isolates of *Cercosporella herpotrichoides. Transactions of the British Mycological Society* **69**: 397–403.

Scott P R & Hollins T W 1980. Pathogenic variation in *Pseudocercosporella herpotrichoides. Annals of Applied Biology* **94**: 297–300.

Scott P R, Hollins T W & Muir P 1975. Pathogenicity of *Cercosporella herpotrichoides* to wheat, barley, oats and rye. *Transactions of the British Mycological Society* **65**: 529–538.

Sprague R 1936. Relative susceptibility of certain species of Gramineae to *Cercosporella herpotrichoides. Journal of Agricultural Research* **53**: 659–670.

Welgemoed Z 1987. Keer omvalsiekte by koring betyds. *Landbouweekblad* **463**: 16–19.

Worland A J, Law C N, Hollins T W, Koebner M D & Guira A 1988. Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. *Plant Breeding* **101**: 43–51.

Accepted 30 November 1995
Associate Editor was F C Wehner