Only one species of *Ramulispora* is associated with eyespot disease of wheat in South Africa

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Eighty-nine Ramulispora *isolates were obtained from eyespot lesions on graminicolous hosts from different regions in the Western Cape of South Africa. Using verified isolates from England and Australia, and based on esterase isozyme electromorphs, all South African isolates were identified as* R. herpotrichoides *(W type). Single linkage cluster analysis of esterase banding patterns indicated that* R. herpotrichoides *isolates showed 23% similarity to the* R. acuformis *isolates. Intraspecific variation was observed within the* R. herpotrichoides *population and at 40% similarity 17 distinct groups could be identified. Restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs) conducted on representative isolates of the 17 different groups confirmed their identity as* R. herpotrichoides *isolates. Using three different restriction enzymes, RFLPs revealed a high degree of homogeneity within the* R. herpotrichoides *population. Combined DNA fingerprints derived from banding patterns of four 10-mer primers could distinguish 13 of the 17 representative isolates. Similarity coefficients (F-values) using 25 RAPD bands were also determined. The average similarity coefficient obtained for pairwise comparisons of the 17* R. herpotrichoides *isolates was 79% whereas the average value for pairwise comparisons of each* R. herpotrichoides *isolate with an* R. acuformis *isolate was 31%. These results suggest that* R. acuformis *is presently not associated with eyespot disease in South Africa, and probably does not occur here. Strict quarantine measures are required, therefore, to ensure that this fungus does not enter the country.*

Knowledge of pathogen population structure is important for breeding programmes directed at producing pathogen-resistant cultivars and for developing strategies to deploy pathogen resistance.[1] Correct identification of the causative agent,[2] therefore, as well as a knowledge of its genetic variability,[3] are important.

Four cercosporoid species have been associated with eyespot disease of wheat, a widespread disease of cereals in temperate regions throughout the world. Losses in crop yield are attributed mainly to severe eyespot lesions which girdle and soften the stem-base, eventually resulting in lodging.[4] Other structural defects of the wheat plant due to eyespot infection may also result in a reduction in yield.[5] Serious yield losses have been reported from chemically untreated fields in England as well as South Africa.[4,6]

Field populations of the eyespot pathogen have been reported to comprise two main pathotypes, namely, the W (*R. herpotrichoides* [Fron] Arx var. *herpotrichoides* = *Pseudocercosporella herpotrichoides* [Fron] Deighton) and R type (*R. herpotrichoides* var. *acuformis* [Nirenberg] Boerema, Pieters and Hamers = *P. herpotrichoides* var. *acuformis* Nirenberg).[7] The low percentage RAPD similarities (50%) between these varieties, however, as well as differences in spore and colony morphology, infection process, and distinct mating populations,[8–10] suggest that these taxa should be treated as separate species.[11] To ensure uniformity, therefore, these pathogens will be referred to as *R. herpotrichoides* and *R. acuformis* (Nirenberg) Crous for the remainder of this paper.

Isolates of *R. herpotrichoides* are more virulent to wheat than to rye, whereas *R. acuformis* isolates are equally virulent to both hosts.[12,13] Pathogenicity testing is time consuming and laborious, however, and requires several replications under controlled conditions to identify species or pathotypes of *R. herpotrichoides* clearly.

Molecular markers have been used to distinguish *R. herpotrichoides* and *R. acuformis*, as well as pathotypes of the former. Isozymes,[14] restriction fragment length polymorphisms (RFLPs),[15–18] and RAPDs[19] have all been used successfully to distinguish isolates of *R. herpotrichoides* and *R. acuformis*. These two species differ in their mode of infection and sensitivity to fungicides. It is therefore essential to know the distribution and species composition of field populations, to facilitate effective disease control and prediction.

Until now, no survey involving a significant number of isolates has been conducted on eyespot populations in South Africa. Therefore, the purpose of this study was to assess the eyespot population in the Western Cape of South Africa and to identify and quantify the genetic variation within the predominant species. This was achieved using isozymes, RFLPs and RAPDs.

Materials and methods

Isolates. Plants with eyespot lesions at their stem bases were randomly sampled from various locations in the Swartland and Overberg regions of the Western Cape during the 1991–92 growing season, and single-spored isolates obtained on potato dextrose agar (PDA).[11] Isolates were obtained from wheat (*Triticum aestivum* L.) (GC1–64, 76–85, 91, 120–125; BR1–36, 38–53, 88–146), barley (*Hordeum vulgare* L.) (GC65–72), canary grass (*Phalaris minor* L.) (GC90, 90A, 105, 105A) and rye grass (*Lolium multiflorum* Lam.) (GC103, 103A, 104, 104A).

Isolates BR58 and BR59 (verified *R. herpotrichoides* [W type] isolates), and BR60 and BR61 (verified *R. acuformis* [R type] isolates) were obtained from Dr J. Lucas (University of Bristol, UK). Isolate BR37 (verified *R. herpotrichoides* [W type] isolate) was obtained from Dr H. Wallwork (Waite Agricultural Research Institute, Glen Osmond, Australia). All isolates cited are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch.

Isozyme analysis. Culture conditions used to cultivate the pathogens were as described by Julian and Lucas.[14] Isolation of total protein extracts were as described by Crous *et al.*,[20] with the following modifications. Subsequent to homogenization of the mycelia, and incubation at 4°C for 12–16 h, the crude extracts were centrifuged at 17 000 × *g* for 1 h. Crude extracts were subjected to dialysis[21] (pore size of dialysis tubing: 12 500 daltons),

and lyophilized. Subsequent procedures were as described by Crous *et al.*[20]

DNA isolation. Colonized agar plugs from 14-day-old colonies grown on PDA were inoculated into 200-ml liquid cultures (8 g l^{-1} yeast extract and 5 g l^{-1} glucose). Cultures were incubated at 25°C for 10 days on an orbital shaker and the mycelia harvested by filtration (Whatman no.1 filter paper). Isolation of total DNA was as described by Crous *et al.*[20]

RFLP analysis and gel electrophoresis. Total DNA was digested with *Hin*dIII, *Hae*III and *Msp*I as recommended by the supplier (Boehringer Mannheim, Johannesburg). DNA fragments were resolved in 1% (w/v) agarose gels using 0.5 × TBE buffer.[21]

RAPD analysis. Taq DNA polymerase together with its 10× concentrated buffer (100 mM Tris-HCl [pH 8.3]; 15 mM $MgCl_2$; 500 mM KCl) was supplied by Boehringer Mannheim. Polymerase chain reaction mixtures (50 µl final volumes) contained 25 ng genomic DNA, dATP, dCTP, dGTP and dTTP each at a final concentration of 200 µM, 5 pmol oligonucleotide primer, 1 × *Taq* polymerase buffer and 1 unit of *Taq* polymerase. One mM $MgCl_2$ was also added, except for reactions with the OPE-3 primer. Each reaction was overlaid with 100 µl of sterile mineral oil to prevent evaporation. The primers used were OPE-2 5'GGTGCGGGAA, OPE-3 5'CCAGATGCAC, OPE-14 5'TGCGGCTGAG and OPE-15 5'ACGCACAACC (Kit E, Operon Technologies Inc., Alameda, USA). Amplifications were conducted in a Biometra TRIO-Thermoblock TB1 (Göttingen, Germany). Samples were subjected to 45 repeats of the following cycle: 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. After the last cycle, a final extension step of 72°C for 5 min was included followed by cooling to 4°C until recovery of the samples. Electrophoresis of aliquots (15 µl) of amplification products was through 3.5% (w/v) polyacrylamide gels using 1 × TAE buffer,[21] at a constant voltage of 15 V cm^{-1}. After electrophoresis, gels were stained with ethidium bromide and visualized by illumination under UV light.

Data analysis. Isozyme and RAPD electromorphs were digitized using a tabletop Genius tablet (Model GT1212A). Matching of the bands was carried out at 97% tolerance. The digitized data were subsequently treated as binary factors, the presence of a band being indicated by a 1 and the absence of a band by a 0 (data available upon request). These data matrixes were then analysed using the CLUSTER procedure of SAS[22] to obtain a single linkage cluster analysis for each set of data. Distances between clusters were indicated using R^2 values. Dendrograms were constructed directly from the single linkage cluster analyses. Electrophoretic data obtained from the RAPD analysis were also analysed using the similarity coefficient (*F*-value) of Nei and Li.[23]

Results

Isozyme analysis. Isozyme gels stained with α-esterase revealed the presence of four to eight bands for each isolate (Fig. 1). When compared to verified *R. herpotrichoides* isolates from England and Australia, all South African isolates were shown to have similar α-esterase electromorphs. Furthermore, South African and verified *R. herpotrichoides* isolates could be clearly distinguished from the verified *R. acuformis* isolates from England (Figs 1 and 2). Seventeen different groups were detected at 40% similarity on the single linkage cluster analysis amongst the 92 isolates of the *R. herpotrichoides* studied. Representatives from each of the 17 groups, including British *R. herpotrichoides* and *R. acuformis* isolates, were examined further by RFLP and RAPD analyses.

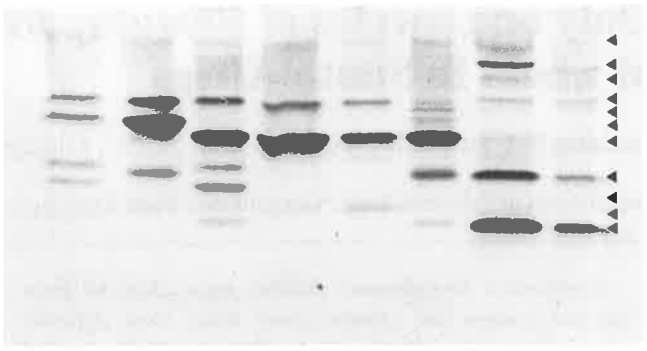

Fig. 1. Alpha-esterase stained polyacrylamide gel indicating scoring of isozyme bands. Accession numbers are representative of isolates of *R. herpotrichoides* and *R. acuformis* (BR60, 61).

RFLP analysis. Total DNA isolated from representatives of each of the 17 groups was subjected to digestion in separate experiments with *Hin*dIII, *Hae*III and *Msp*I. Using these enzymes, the South African *R. herpotrichoides* isolates were clearly distinguished from the verified *R. acuformis* isolate (BR60) (Fig. 3, arrowed), but not from the verified *R. herpotrichoides* isolate (BR58).

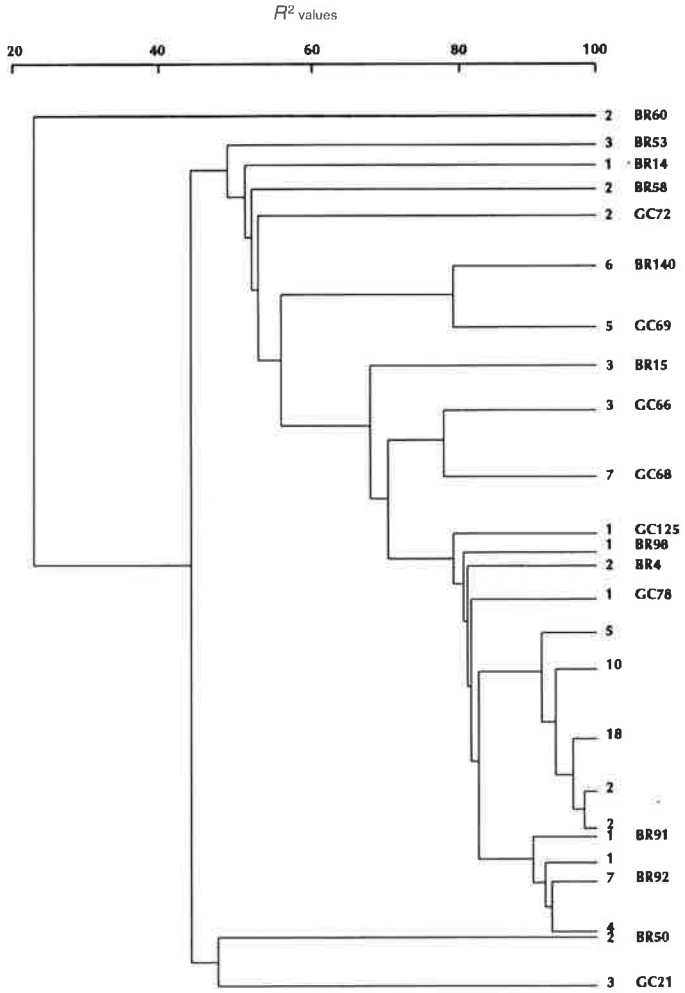

Fig. 2. Dendrogram based on isozyme data illustrating the clustering amongst the 94 isolates studied. This dendrogram was generated using the single linkage cluster analysis of the CLUSTER procedure of SAS. Numbers on the *y*-axis indicate the number of isolates present at that cluster. Accession numbers shown are selected representative isolates of the 17 groups of *R. herpotrichoides* and *R. acuformis* (BR60) which were obtained at 40% similarity on the single linkage cluster analysis.

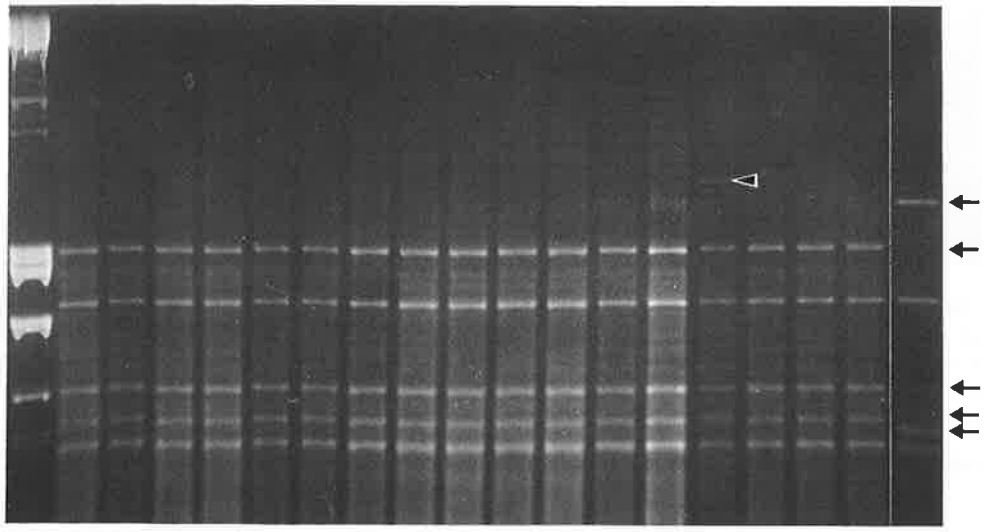

Fig. 3. *Msp*I digests of total DNA of isolates of *R. herpotrichoides*. Illustrated are 17 representative *R. herpotrichoides* isolates of the 17 groups and a *R. acuformis* (BR60) isolate. Lambda DNA has been digested with *Eco*RI and *Hin*dIII. Bands distinguishing the two species are indicated by arrows.

RAPD analysis. A total of 40 primers (Kits E and K) were screened for their ability to produce distinct RAPD profiles. Four were selected for subsequent experiments. The four primers that were selected clearly distinguished the *R. herpotrichoides* isolates from the *R. acuformis* isolate (Figs 4 and 5, arrowed). RAPDs also revealed genetic variability within the South African population. When the fingerprints of each primer were combined and analysed using the single cluster analysis, 13 groups were distinguished (Fig. 6). *F*-values obtained from combined RAPD electrophoretic fingerprints (Table 1) indicated that the average *F*-value (similarity coefficient) for the 17 *R. herpotrichoides* isolates was 79%. The average *F*-value that was obtained after pairwise comparisons involving the *R. acuformis* isolate with each of the *R. herpotrichoides* isolates was 31%.

Discussion

The purpose of the present study was, first, to determine what cercosporoid species are associated with eyespot disease of graminicolous hosts in South Africa, and furthermore to establish the genetic diversity within the South African population. Earlier studies have suggested that some morphological criteria used to distinguish the two species such as colony margins and colour production on PDA could be unreliable.[16,17,24] In contrast, molecular markers have been shown to be useful in taxonomic classification and genetic characterization of *R. herpotrichoides* and *R. acuformis* in European populations.[14–18,25] However, South African eyespot populations of *Ramulispora* Miura have not previously been characterized using molecular markers.

Esterase isozyme studies have previously been used to differentiate between species of the same genus, e.g. *Botrytis* P. Micheli ex Pers.[26] and *Colletotrichum* Corda,[27] to identify subspecific taxa, e.g. *formae speciales* of *Fusarium oxysporum* Schltdl.: Fr.,[28] and to differentiate between aggressive and non-aggressive isolates of *Ceratocystis ulmi* (Buisman) C. Moreau.[29] Using isozyme, RFLP and RAPD electromorphs of verified *R. herpotrichoides* and *R. acuformis* isolates, we were able to identify all the South African isolates as belonging to *R. herpotrichoides*. Using α-esterases, 17 groups could be identified at 40% similarity on the single linkage cluster analysis, each group being represented by a specific electromorph. The single linkage cluster analysis also showed that of the 92 *R. herpotrichoides* isolates examined, 79 (constituting 86% of the *R. herpotrichoides* isolates studied) had R^2 values[22] of 80% or higher. Furthermore, the single linkage cluster analysis also revealed that the two *R. acuformis* isolates showed 23% similarity (Fig. 2) to the 92 *R. herpotrichoides* isolates examined. As five genetically different isolates (each isolate had a distinct esterase electromorph) were isolated in an area 30 × 30 m and two of these isolates were obtained from the same eyespot lesion, this variation may indicate the presence of the teleomorph in that specific field. Burdon and Roelfs[30] previously attributed high levels of heterogeneity in *Puccinia graminis* Pers.: Pers. to the presence of its teleomorph. However, a high degree of heterogeneity exhibited by staining for α-esterase activity may also be attributed to α-esterase being a non-regulatory enzyme.[14] Non-regulatory enzymes often show a disproportionately high level of intraspecific variation. Representatives of each of the 17 distinct electromorphs were chosen in an attempt to determine whether α-esterase grouping represents true genetic heterogeneity. Isolates that were obtained from barley, canary grass and rye grass exhibited isozyme electro-

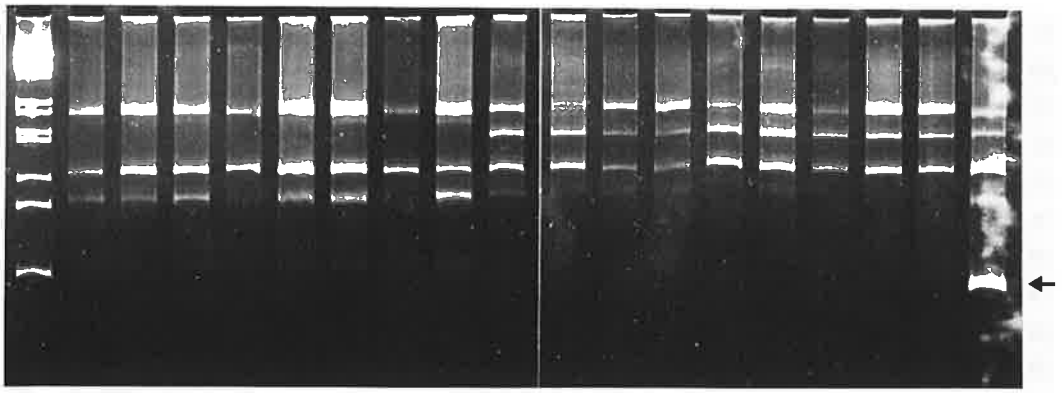

Fig. 4. Random amplified polymorphic DNAs of isolates of *R. herpotrichoides* with primer OPE-15. Illustrated are 17 representative *R. herpotrichoides* isolates and an *R. acuformis* (BR60) isolate. Lambda DNA has been digested with *Eco*RI and *Hin*dIII. The band distinguishing the two species is indicated by an arrow.

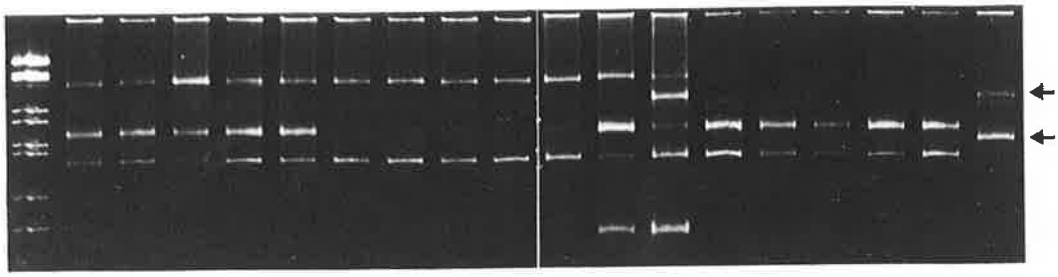

Fig. 5. Random amplified polymorphic DNAs of isolates of *R. herpotrichoides* with primer OPE-14. Illustrated are 17 representative *R. herpotrichoides* isolates and an *R. acuformis* (BR60) isolate. Lambda DNA has been digested with *Eco*RI and *Hin*dIII. The band characteristic of the isolates from England is indicated by a white arrow, while the band distinguishing the two species is indicated by a black arrow.

morphs which fell within one of the 17 groups, that is, no specific band could be assigned to distinguish these isolates from those isolated from wheat or barley.

Nicholson *et al.*[16] distinguished *R. herpotrichoides* and *R. acuformis* using RFLPs. In their study it was reported that no polymorphisms were observed within the individual pathotypes when *Eco*RI, *Bam*HI, *Hin*dIII and *Bgl*II were used. In the present study, a high degree of homogeneity within the *R. herpotrichoides* population was also observed using *Hin*dIII (data not shown). It has been reported that total DNA of fungi digested with GC-specific base-cutting enzymes will show distinct bands of repetitive DNA against a continuous background smear of heterogenous DNA fragments when observed on agarose gels.[20,31,32] In the present study, *Hae*III and *Msp*I were used in an attempt to detect polymorphisms between isolates within repetitive sequences. Despite using four base-pair cutting enzymes like *Hae*III and *Msp*I, only one polymorphism was detected (Fig. 3, arrowed) within the *R. herpotrichoides* population in South Africa.

The RAPD analysis revealed a high level of intraspecific variation which was in agreement with the isozyme results. While 17 groups were distinguished using isozymes, 13 could be distinguished using RAPDs. Intraspecific variation was best observed when using composite fingerprints of all four primers. All bands that were observed were used for data analysis. Although 13 different fingerprints were observed amongst the 17 representative isolates, most of the fingerprints differed from one another by only one or two bands. This accounts for the high *F*-values (similarity coefficients) that were obtained (average *F*-value for the local isolates was 81%) (Table 1). Some groups were differentiated by minor bands. Isolates BR14 and BR15, isolates GC66 and GC69, and isolates BR53 and BR91, which had been differentiated using isozyme analysis, had the same RAPD fingerprints. When comparing the two respective dendrograms (Figs 2 and 6), no correlation is seen with regard to the actual clustering patterns of isolates. However, only 4–8 isozyme bands were used per isolate to construct the dendrogram, whereas the RAPD data were based on a composite of up to 25 bands per isolate. Two shared bands observed amongst the verified *R. herpotrichoides* and *R. acuformis* isolates from England with primer OPE-14 (Fig. 5) were not observed in any of the local isolates. In addition, one isolate obtained from the Overberg region (GC125) in South Africa had a band which was not observed in any of the other isolates which had been isolated from the Swartland region (Fig. 5). However, this band was found to be present in isolate

Table 1. Combined *F*-values (% similarity) following RAPD analysis of 17 representative isolates of *R. herpotrichoides* and an *R. acuformis* isolate (BR60).

	BR4	BR14	BR15	GC21	BR50	BR53	BR58	GC66	GC68	GC69	GC72	GC78	BR91	BR92	BR98	GC125	BR140	BR60
BR4	–																	
BR14	78	–																
BR15	78	100	–															
GC21	82	96	96	–														
BR50	90	70	70	73	–													
BR53	64	88	88	92	64	–												
BR58	67	67	67	67	56	76	–											
GC66	89	67	67	80	89	70	59	–										
GC68	95	83	83	87	86	78	74	84	–									
GC69	89	67	67	80	89	70	59	100	84	–								
GC72	91	80	80	73	82	75	70	80	96	80	–							
GC78	61	85	85	88	61	96	73	67	75	67	72	–						
BR91	73	88	88	92	64	100	76	70	78	70	75	96	–					
BR92	95	73	73	76	95	67	59	94	90	94	85	64	67	–				
BR98	82	80	80	78	73	83	80	80	87	80	83	88	83	76	–			
GC125	76	67	67	78	67	78	90	74	82	74	78	75	78	70	78	–		
BR140	78	92	92	88	61	96	76	67	83	67	80	92	96	73	88	75	–	
BR60	38	25	25	35	29	26	40	32	36	32	35	25	26	30	26	36	25	–

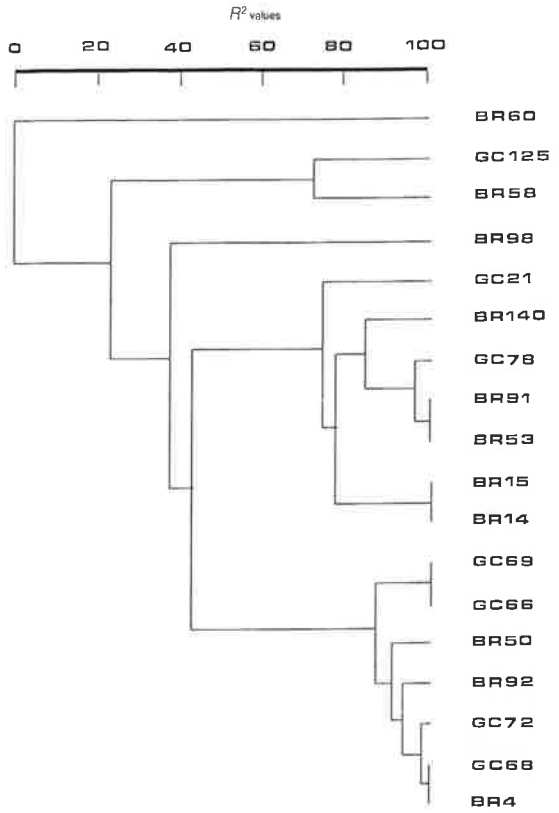

Fig. 6. Dendrogram based on RAPD data illustrating the clustering amongst the 17 groups of *R. herpotrichoides* and an *R. acuformis* (BR60) isolate. This dendrogram was generated using the single linkage cluster analysis of the CLUSTER procedure of SAS.

BR58 obtained from England. To confirm whether these bands can be used to indicate geographic origin, more isolates from England and the Overberg region would have to be screened. Using RAPDs, however, Nicholson and Rezanoor[19] were unable to identify bands correlating with an isolate's geographic origin.

Results obtained in the present study suggested that although there was considerable genetic variation in the local population of *R. herpotrichoides*, the South African population still shared a high degree of similarity with overseas isolates (Figs 3–5). This entails that genetic sources used for breeding resistant wheat cultivars in such countries can also be effectively employed in South Africa.

Robbertse *et al.*[11] showed that the W (*R. herpotrichoides*) and R types (*R. acuformis*) were not two varieties of the same species, but two distinct biological species. Results of the present study have shown that only one species of *Ramulispora*, *R. herpotrichoides,* is presently associated with eyespot disease of wheat in South Africa. As far as we could establish, *R. acuformis* does not yet occur here. Given the complexity of disease control in countries where both species are present, extreme care must be exercised in screening any grain produce being imported into South Africa in future.

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1. Leung H., Nelson R.J. and Leach J.E. (1993). Population structure of plant pathogenic fungi and bacteria. *Adv. Pl. Path.* **10**, 157–205.
2. Maclean D.J., Braithwaite K.S., Manners J.M. and Irwin J.A.G. (1993). How do we identify and classify fungal plant pathogens in the era of DNA analysis? *Adv. Pl. Path.* **10**, 207–244.
3. McDonald B.A. and Martinez J.P. (1990). DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. *Phytopathology* **80**, 1368–1373.
4. Scott P.R. and Hollins T.W. (1974). Effects of eyespot on the yield of winter wheat. *Ann. appl. Biol.* **78**, 269–279.
5. Clarkson J.D.S. (1981). Relationship between eyespot severity and yield loss in winter wheat. *Pl. Path.* **30**, 125–131.
6. Welgemoed Z. (1987). Keer omvalsiekte by koring betyds. *Landbouweekblad* **463**, 16–19.
7. Nirenberg H.I. (1981). Differenzierung der Erreger der Halmbruchkrankheit I. Morphologie. *Z. Pflkrankh. Pflschutz.* **88**, 241–248.
8. Daniels A., Lucas J.A. and Peberdy J.F. (1991). Morphology and ultrastructure of W and R pathotypes of *Pseudocercosporella herpotrichoides* on wheat seedlings. *Mycol. Res.* **95**, 385–397.
9. Dyer P.S., Papaikonomou M., Lucas J.A. and Peberdy J.F. (1994). Isolation of R-type progeny of *Tapesia yallundae* from apothecia on wheat stubble in England. *Pl. Path.* **43**, 1029–1044.
10. Robbertse B., Crous P.W. and Holz G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathologia* **125**, 23–28.
11. Robbertse B., Campbell G.F. and Crous P.W. (1995). Revision of *Pseudocercosporella*-like species causing eyespot disease of wheat. *S. Afr. J. Bot.* **61**, 43–48.
12. Lange-De la Camp M. (1966). Die Wirkungsweise von *Cercosporella herpotrichoides* Fron, dem erreger der Halmbruchkrankheit des Getreides. II. Agressivitat des Erregers. *Phytopath. Z.* **56**, 155–190.
13. Scott P.R. and Hollins T.W. (1980). Pathogenic variation in *Pseudocercosporella herpotrichoides*. *Ann. appl. Biol.* **94**, 297–300.
14. Julian A.M. and Lucas J.A. (1990). Isozyme polymorphism in pathotypes of *Pseudocercosporella herpotrichoides* and related species from cereals. *Pl. Path.* **39**, 178–190.
15. Frei U. and Wenzel G. (1993). Differentiation and diagnosis of *Pseudocercosporella herpotrichoides* (Fron) Deighton with genomic DNA probes. *J. Phytopath.* **139**, 229–237.
16. Nicholson P., Hollins T.W., Rezanoor H.N. and Anamthawat-Jonsson (1991). A comparison of cultural, morphological and DNA markers for the classification of *Pseudocercosporella herpotrichoides*. *Pl. Path.* **40**, 584–594.
17. Nicholson P., Rezanoor H.N. and Hollins T.W. (1993). Classification of a world-wide collection of isolates of *Pseudocercosporella herpotrichoides* by RFLP analysis of mitochondrial and ribosomal DNA and host range. *Pl. Path.* **42**, 58–66.
18. Thomas D.T., Maraite H. and Boutry M. (1992). Identification of rye- and wheat-types of *Pseudocercosporella herpotrichoides* with DNA probes. *J. gen. Microbiol.* **138**, 2305–2309.
19. Nicholson P. and Rezanoor H.N. (1994). The use of random amplified polymorphic DNA to identify pathotype and detect variation in *Pseudocercosporella herpotrichoides*. *Mycol. Res.* **98**, 13–21.
20. Crous P.W., Janse B.J.H., Victor D., Marais G.F. and Alfenas A.C. (1993). Characterization of some *Cylindrocladium* species with three-septate conidia using morphology, isozyme banding patterns and DNA polymorphisms. *Syst. appl. Microbiol.* **16**, 266–273.
21. Sambrook J., Fritsch E.F. and Maniatis T. (1989). *Molecular cloning: a laboratory manual.* Cold Spring Harbor Laboratory Press, NY.
22. Luginbuhl R., Kaufman M. and Crum L. (1985). *SAS User's Guide: Statistics, Version 5 edition,* ed. S.P. Joyner, pp. 255–316. SAS Institute Inc., Cary, North Carolina.
23. Nei M. and Li W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5269–5273.
24. Mauler A. and Fehrmann H. (1987). Erfassung der Anfalligkeit von Weizen gegenüber *Pseudocercosporella herpotrichoides*. I. Untersuchungen zur pathogenitat verschiedener Formen des erregers. *Z. Pflkrankh. Pflschutz.* **94**, 630–636.
25. Priestley R.A., Dewey F.M., Nicholson P. and Rezanoor H.N. (1992). Comparison of isoenzyme and DNA markers for differentiating W, R and C pathotypes of *Pseudocercosporella herpotrichoides*. *Pl. Path.* **41**, 591–599.
26. Backhouse D., Willets H.J. and Adam P. (1984). Electrophoretic studies of *Botrytis* species. *Trans. Br. mycol. Soc.* **82**, 625–630.
27. Bonde M.R., Peterson G.L. and Maas J.L. (1991). Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. *Phytopathology* **81**, 1523–1528.
28. Bosland P.W. and Williams P.H. (1986). An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatability, and geographic origin. *Can. J. Bot.* **65**, 2067–2073.
29. Bernier L., Jeng R.S. and Hubbes M. (1983). Differentiation of aggressive and non-aggressive isolates of *Ceratocystis ulmi* by gel electrophoresis of intramycelial enzymes. *Mycotaxon* **17**, 456–472.
30. Burdon J.J. and Roelfs A.P. (1985). The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis*. *Phytopathology* **75**, 1068–1073.
31. Förster H. and Coffey M.D. (1992). Molecular characterization of *Phytophthora* isolates with non-papillate sporangia causing root rot of raspberry using mtDNA restriction fragment length polymorphisms. *Mycol. Res.* **96**, 571–577.
32. Förster H. and Coffey M.D. (1993). Molecular taxonomy of *Phytophthora megasperma* based on mitochondrial and nuclear DNA polymorphisms. *Mycol. Res.* **98**, 1101–1112.