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Characterization of *Pyrenophora* Isolates Associated with Spot and Net Type Lesions on Barley in South Africa

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With 3 figures

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Abstract

Single-spored isolates of *Pyrenophora* associated with spot and net type net blotch of barley were compared using total DNA banding patterns, morphological and cultural characteristics, symptomatology and mating studies. Isolates of spot and net type net blotch were found to vary regarding conidium length and cultural growth rate. Mating studies among and between ascospore, spot and net type isolates proved unsuccessful under the conditions studied. Total DNA polymorphisms of the net, spot and ascospore isolates digested with the restriction enzymes *Hpa*II and *Hae*III showed that the isolates have similar banding patterns. Random amplified polymorphic DNA (RAPD) showed that the banding patterns of the spot and net type isolates were again similar, but were distinct from outgroups such as *P. semeniperda* and *P. tritici-repentis*. The homology in DNA banding patterns of local isolates indicated that the difference in conidium length is insufficient to separate them as two species. It is concluded that spot and net type isolates occurring in South Africa belong to *P. teres*. Therefore, spot type lesions are caused by *P. teres* f. sp. *maculata*, and not by *P. japonica* as reported previously.

Zusammenfassung

Charakterisierung von *Pyrenophora*-Isolaten, die Spot- und Net-Typ-Läsionen bei Gerste in Südafrika hervorrufen

Einsporisolate von *Pyrenophora*, die den Spot-Typ und den Net-Typ der Netzfleckenkrankheit bei Gerste verursachen, wurden mit Hilfe der Bandenmuster der gesamten DNA sowie anhand von morphologischen Kriterien, Kulturmerkmalen, Symptomen und Kreuzungsversuchen verglichen. Spot- und Net-Typ-Isolate variierten hinsichtlich Konidienlänge und Wachstumsgeschwindigkeit in Kultur. Kreuzungsversuche innerhalb und zwischen Ascosporen-, Spot-Typ- und Net-Typ-Isolaten waren unter den gegebenen Versuchsbedingungen nicht erfolgreich. Die gesamte DNA der Net-Typ-, Spot-Typ- und Ascosporen-Isolate wurde mit den Restriktionsenzymen *Hpa*II und *Hae*II geschnitten. Die vorhandenen Polymorphismen zeigten, daß die Isolate gleiche Bandenmuster aufweisen. Das Random Amplified Polymorphic DNA (RAPD)-Verfahren ergab, daß die Bandenmuster der Spot-Typ- und der Net-Typ-Isolate gleich waren, sich aber von den Mustern anderer *Pyrenophora*-Arten wie *P. semeniperda* und *P. tritici-repentis* unterschieden. Die Homologie der DNA-Bandenmuster bei lokalen Isolaten zeigte, daß die unterschiedliche Konidienlänge nicht ausreicht, um die Typen als zwei Arten aufzufassen. Aus den Ergebnissen wird gefolgert, daß die in Südafrika vorkommenden Spot- und Net-Typ-Isolate zu *P. teres* gehören. Spot-Typ-Läsionen werden demnach von *P. teres* f. sp. *maculata* und nicht, wie früher angegeben wurde, von *P. japonica* verursacht.

Introduction

Net blotch caused by *Pyrenophora teres* Drechs. (anamorph *Drechslera teres* [Sacc.] Shoem.), is an important disease of barley (*Hordeum vulgare* L. *emend.* Bowden) (Smith and Rattray, 1930; Putterill, 1954; Shipton et al., 1973; Jordan, 1981; Scott, 1991). Two forms of *P. teres* have been reported, *P. teres* f. sp. *teres* and *P. teres* f. sp. *maculata* Smedegard-Pietersen, which differ in the symptoms induced on barley. *P. teres* f. sp. *teres* causes a net type lesion which is characterized by dark brown blotches crisscrossed with a netlike venation and accompanied by chlorosis. *P. teres* f. sp. *maculata*, on the other hand, causes spots of various shapes and sizes, encircled by varying widths of chlorosis (Smedegard-Petersen, 1971).

Prior to the description of the spot and net forms of *P. teres* (Smedegard-Petersen, 1971), this fungus was reported to be exclusively associated with net symptoms, whereas *P. japonica* Ito & Kuribyashi (anamorph *D. tuberosa* (Atk.) Shoem.) was associated with spot symptoms (Ito and Kuribayashi, 1931). According to Ito and Kuribyashi (1931), *P. teres* differs from *P. japonica* in width and colour of conidiophores and conidia, ascospore size and the presence of thick hyphal strands in cultures. However, most of the criteria used by Ito and Kuribyashi (1931) to separate the net and spot forms, were also found to occur in *P. teres* isolates studied in Israel and Denmark by Kenneth (1962) and Smedegard-Petersen (1971) respectively. Kenneth (1962) concluded, therefore, that *P. japonica* could well be a sibling species of *P. teres*. Smedegard-Petersen (1971) was of the opinion that the spot type fungus found in Denmark should be considered a type of *P. teres* since it is identical with the latter in all morphological and cultural characteristics and readily crosses with compatible isolates of *P. teres*. Similar matings were reported by McDonald (1967), who referred to the spot isolates as mutant strains of *P. teres*.

In a review of *Pyrenophora* by Sivanesan (1987), *P. japonica* and *P. teres* were retained as separate species, while the two formae of the latter were also mentioned. Scott (1991) based his comparative morphological study of *Pyrenophora* isolates causing net type and spot type lesions on barley in South Africa on the descriptions given by Sivanesan (1987), and concluded that they represents two species, *P. teres* and *P. japonica*, respectively. Based on this study, the teleomorph state of the spot

type was recently reported for the first time in South Africa as *P. japonica* (Louw et al., 1994). However, Wallwork et al. (1992) stated that Scott (1991) regarded *P. teres* f. sp. *maculata* to be synonymous with *P. japonica*. These studies (Kenneth 1962; Smedegard-Petersen, 1971; Sivanesan, 1987; Scott, 1991; Wallwork et al., 1992; Louw et al., 1994) suggest that the status of the *Pyrenophora* spp. associated with spot type net blotch is still uncertain.

The use of DNA restriction fragment length polymorphisms (RFLP), total DNA banding patterns and randomly amplified polymorphic DNA (RAPD) to study relationships among plant-pathogenic fungi, is well documented (Goodwin and Annis, 1991; Nicholson et al., 1991; Henson, 1992). Several workers (Förster et al., 1988, 1989, 1990; Hwang et al., 1991; De Cock et al., 1992) have used RFLP analysis of mitochondrial DNA in association with general morphological and cultural criteria to compare inter- and intra-specific variation in different isolates of *Phytophthora* de Barry. In *Leptosphaeria maculans* (Desm.) Ces et de Not, different pathotypes could be distinguished on the basis of their RAPD profiles (Goodwin and Annis, 1991), whereas Bulat and Mironenko (1990) could demonstrate that *P. teres* and *P. graminea* Ito & Kuribayashi are intraspecific forms of the same species. Differences among isolates of the formae of *P. teres* could also be shown using this technique (Reeves and Ball, 1991).

The aim of the present study was to characterize local *Pyrenophora* isolates associated with spot and net type lesions using total DNA banding patterns, a technique which has proven satisfactory in solving similar questions in other filamentous fungi (Crous et al., 1993). These data were compared with morphological and cultural characteristics, symptomatology and data from mating studies.

Materials and Methods

Sampling of isolates

Leaves showing characteristic spot and net type symptoms were collected in barley fields in the south-western Cape Province, the major barley producing area in South Africa. The infected leaves were subsequently dried in an oven at 25°C and stored at room temperature. Other studies using this method showed that the pathogen remained viable and retained its pathogenicity in infected material for several years (Arabi et al., 1992; Steffenson and Webster, 1992).

Isolate designation and verification of symptom expression

Leaves were cut into sections, surface sterilized for 15 s in 50% (v/v) ethanol, and 1 min in 2% (v/v) sodium hyphochloride. Leaf sections were subsequently washed in sterile water and incubated on wet filter paper in petri dishes at 15°C under continuous near-ultraviolet light to induce sporulation (Onesirosan and Banttari, 1969). Single spores from sporulating lesions, and single ascospores obtained from *Pyrenophora* perithecia on barley stubble (Louw et al., 1994), were transferred aseptically onto Purity agar (PA) (30 ml vanilla Purity, 15 g Biolab agar in 1000 ml H_2O) (Gerber's Purity, Reckitt and Coleman, South Africa Pty., Ltd.). Previous studies showed that *Pyrenophora* isolates sporulate profusely on PA (Louw et al., 1994).

Symptom expression of 50 isolates obtained from lesions and of 30 single ascospores were verified on barley plants in a glasshouse. Sporulating cultures on PA were flooded with sterile distilled water and the petri dishes sealed with Parafilm. Dishes were shaken to dislodge conidia and spore suspensions were standardized with a haemacytometer to contain 1300 spores/ml. Inoculations were done using the technique of Khan and Boyd (1969). Barley cultivars Stirling and B87/14 (susceptible to net and spot type isolates, respectively) were inoculated in the two leaf stage. Plants were initially sprayed with a wetting agent (Tween 20, 1 drop per 100 ml H_2O), and subsequently atomized with the spore suspension (1300 spores/ml) at a volume of 12 ml per 10 seedlings. Plants were then placed in moist chambers for 24 h after which they were returned to the glasshouse (max 20°C day, min 15°C night). Plants were examined for symptom expression 2 weeks after inoculation. Leaves were harvested from plants showing the most characteristic symptoms of either spot type or net type net blotch, and dried as described above. Harvested leaves were subsequently used for further characterization of the 27 isolates designated in Table 1.

Morphology

Dried leaves were placed in moist chambers and incubated as described above to induce sporulation. Conidiophores and conidia were mounted in lactophenol cotton blue and examined under the 100 × (oil) objective. The formation of secondary conidiophores and microcyclic conidiation was also noted.

Cultural studies

To avoid the use of atypical cultures, single spores were obtained from barley leaves as described above and used in cultural studies. Growth studies were conducted on malt-extract agar (MEA) (20 g malt extract, 15 g Biolab agar in 1000 ml H_2O) and PA. Agar plugs (3 mm diam.) of representative ascospore, spot, and net isolates (Table 2) were transferred to MEA and PA. In preliminary cultural studies, plates were incubated for 7 days in the dark at temperatures ranging from 5 to 35°C in 5°C intervals. Three replicate plates of each medium (MEA and PA) were used for each isolate at each temperature. Based on this work, subsequent studies were conducted at 25°C. Cultural characteristics such as pigmentation of the medium, presence of tufts of aerial mycelium and hyphal strands were rated after 7 days at 25°C in the dark. The PA plates of each isolate were also used to rate sporulation. Five ml sterile H_2O was added to each plate, and the spores dislodged by scraping with a sterile spatula. Spore concentrations were determined by means of a haemacytometer. Isolates were subsequently classed according to the amount of spores produced; $<10^4$ spores/ml = moderate; $>10^4$ spores/ml = abundant.

Mating studies

Net, spot and ascospore isolates listed in Table 1 were mated in all possible combinations. Matings were conducted on Sach's agar (SA) modified according to Hebert (1971). Twenty sterile sorghum seeds were placed on each SA plate. Agar plugs (3 mm diam.) of the respective isolates (two isolates per plate) were mated by placing them on either side of the seeds. Three replicate plates were used for each possible combination. Inoculated plates were sealed with Parafilm and incubated at 15°C in the dark to induce perithecial formation (McDonald, 1963).

DNA isolation

The method described here is a compilation of several techniques (Murray and Thompson, 1980; Fang et al., 1992; Crous et al., 1993), and yields large quantities of high molecular weight DNA that can readily be digested by restriction enzymes.

Single-conidial and -ascospore isolates of the spot type, and conidial isolates of the net type (Table 1), as well as outgroups such as *Pyrenophora semeniperda* (Brittlebank & Adams) Shoem. (Mor 10) (causing leaf spots and seedling blight of grasses and cereal crops) and *Pyrenophora tritici-repentis* (Died.) Drechsler (IMI 190925) (causing tan spot of wheat) were grown on PA. Plugs of 7-day-old cultures were transferred into 200 ml Glucose-yeast extract broth (GYEB) (Zumpetta, 1976) and incubated for 14 days at 26°C in the dark. Mycelia were harvested by filtration (Whatman No. 12 filter paper), frozen at −80°C for 60 min and lyophilized overnight. Lyophilized mycelia were subsequently ground to a powder in the presence of glass beads (0.4–0.6 mm diam, Sigma Chemical Co., St. Louis, MO, USA). Twenty ml lysing buffer (50 mM Tris-HCl [pH 7.2]; 50 mM Na_2EDTA; 3% [w/v] sodium dodecyl sulphate and 1% [v/v] 2-mercaptoethanol) was added and the ground mycelia incubated for 60 min at 65°C. Cetyltrimethylammonium bromide (CTAB) (Sigma) and NaCl were added to a final concentration of 2% (w/v) and 0.9 M, respectively, and

Table 1
Pyrenophora isolates associated with spot and net type lesions of barley used in mating and taxonomic studies

Isolates	Isolate no.	Collector date	Host[a]	Region[b]
Spot type isolates (ascospore)	Cal 13	12 Aug. 1991	B87/14	Caledon
	Cal 14	12 Aug. 1991	B87/14	Caledon
	Cal 15	12 Aug. 1991	B87/14	Caledon
	Cal 16	12 Aug. 1991	B87/14	Caledon
	Cal 17	12 Aug. 1991	B87/14	Caledon
	Cal 18	12 Aug. 1991	B87/14	Caledon
	Cal 19	12 Aug. 1991	B87/14	Caledon
	Cal 20	12 Aug. 1991	B87/14	Caledon
	Cal 21	12 Aug. 1991	B87/14	Caledon
	Cal 22	12 Aug. 1991	B87/14	Caledon
Spot type isolates (conidial)	Cal 5	18 Jul. 1991	B87/14	Caledon
	Cal 7	1 Nov. 1991	B87/14	Caledon
	Cal 8	22 Jul. 1991	B87/14	Caledon
	Cal 9	10 Sept. 1991	Clipper	Caledon
	Cal 10	1 Nov. 1991	Clipper	Caledon
	Nap 4	6 Aug. 1991	Clipper	Napier
	Mor 2	12 Sept. 1991	Stirling	Moorreesburgh
	Hop 1	16 Sept. 1991	Stirling	Hopefield
Net type isolates (conidial)	Cal 12	10 Sept. 1991	Stirling	Caledon
	Nap 5	6 Aug. 1991	Stirling	Napier
	Nap 6	17 Jul. 1991	Clipper	Napier
	Nap 7	6 Aug. 1991	Stirling	Napier
	Nap 9	6 Aug. 1991	Clipper	Napier
	Mor 5	16 Sept. 1991	Stirling	Moorreesburgh
	Mor 6	16 Sept. 1991	Stirling	Moorreesburgh
	Riv 6	10 Sept. 1991	Clipper	Riviersonderend
	Riv 8	10 Sept. 1991	Stirling	Riviersonderend

[a] Different barley cultivars.
[b] Regions in the south-western Cape Province.

incubated overnight at 65°C. Proteins were subsequently denaturated by a phenol/chloroform/isoamylalcohol (25:24:1) (PCI) extraction, followed by two chloroform/isoamylalcohol extractions (Sambrook et al., 1989). Nucleic acids were precipitated by adding two volumes 100% ethanol and subsequently harvested by centrifugation at 12 100 × *g* for 5 min at room temperature. The pellets were washed twice with 70% (v/v) ethanol and dried. The nucleic acids were resuspended overnight at 4°C in 1 ml TE-Buffer (pH 8.0). RNase A was added to a final concentration of 0.2 mg/ml, and the nucleic acids incubated at 37°C for 60 min, after which the DNA was subjected to PCI extraction, precipitated with two volumes 100% ethanol, washed, dried and redissolved overnight at 4°C in 400 µl TE Buffer (pH 8.0). Sodium chloride (4 M) was added to a final concentration of 2.5 M and the samples precipitated with two volumes 100% ethanol, washed, dried and redissolved in 400 µl TE Buffer (pH 8.0). Total DNA was subjected to digestion with the restriction enzymes *Hae*III and *Hpa*II for 4 h according to the recommendations of the suppliers (Boehringer Mannheim, Johannesburg, South Africa). Gel electrophoresis was conducted as previously described (Crous et al., 1993). Phage lambda digested with the restriction enzymes *Eco*RI and *Hin*dIII was used as molecular weight marker during electrophoresis.

RAPD analysis

Oligonucleotides, 10 bases in length, were used as random primers and were purchased from Operon Technologies (Kit K, Operon Technologies Inc., Alameda, CA, USA). The primers from kit K were used. Taq-polymerase, together with its 10 × concentrated PCR buffer (100 mM Tris HCl [pH 8.3] [25°C], 500 mM KCl, 15 mM $MgCl_2$), was supplied by AB-Technologies (Advanced Biotechnology [Ltd.], Hilltop road, London, UK). Amplifications were conducted in a Hybaid Omnigene (Hybaid Ltd., Waldegrave Rd., Middlesex, UK) thermal cycler. The conditions used were those of Devos and Gale (1992), except that 100 nM primer and 200 µm of each dNTP was used. One mM $MgCl_2$ was also added. Twenty µl of the samples were separated on a 2% agarose gel using TAE-buffer (Sambrook et al., 1989).

Results

Morphology

The morphology of conidiophores were extremely variable with both spot and net type isolates. Conidiophores varied from being curved, straight or zigzagged in their upper parts, hampering accurate measurements of these structures. However, conidiophores of spot type isolates were usually longer than those of net type isolates. Conidia of spot isolates were 25–160 × 10–18 µm, (0—)4(—7)-septate, and those of net isolates 18–120 × 10–18 µm, (0—)3(—5)-septate. Furthermore, conidia of spot type isolates were frequently olivaceous brown, cylindric, tapering to an acutely rounded base, with a brown hilum, (2)–3–(4) µm wide. Cultures of net type conidial isolates usually produced cylindrical, olivaceous brown conidia, tapering to a subtruncate base. Conidial hila were usually (3)–4–(6) µm wide, being darker brown and wider than those of the spot type isolates. Secondary conidiophores were present in both the spot and the net type isolates, but microcyclic conidiation was more common in net type isolates.

Cultural studies

No clear differences in cultural characteristics (pigmentation and presence of tufts of aerial mycelium) between spot and

Table 2
Colony diameters of spot and net type isolates of *Pyrenophora* on malt extract agar (MEA) and 3% Purity agar (PA)

Isolates[b]	Colony diameter (mm)[a]	
	MEA[c]	PA[c]
Spot type isolates (ascospore)		
Mp 7	(20)–23–(29)	(54)–56–(59)
Mp 2	(20)–25–(30)	(61)–61–(62)
Mp 4	(18)–21–(29)	(63)–67–(71)
Mp 1	(18)–21–(26)	(60)–63–(66)
Mp 6	(22)–26–(33)	(68)–70–(72)
Spot type isolates (conidial)		
Cal 8	(32)–34–(40)	(54)–56–(59)
Nap 4	(30)–33–(37)	(49)–52–(54)
Mor 2	(19)–22–(24)	(56)–59–(65)
Cal 5	(21)–24–(30)	(50)–55–(61)
Hop 1	(27)–31–(35)	(61)–62–(64)
Net type isolates (conidial)		
Nap 5	(43)–50–(60)	(65)–67–(69)
Riv 8	(64)–68–(73)	(68)–72–(76)
Mor 5	(41)–46–(54)	(70)–73–(75)
Nap 7	(38)–45–(49)	(65)–67–(71)
Cal 12	(47)–60–(69)	(72)–77–(80)

[a] Mean growth of six replicate plates/isolate after 7 d at 25°C in the dark.
[b] Symptoms produced by spot and net type isolates are shown in Fig. 1.
[c] Mean growth of spot and net type isolates differ significantly on MEA (Significance Level, SL = 0.0022) and PA (SL = 0.0021).

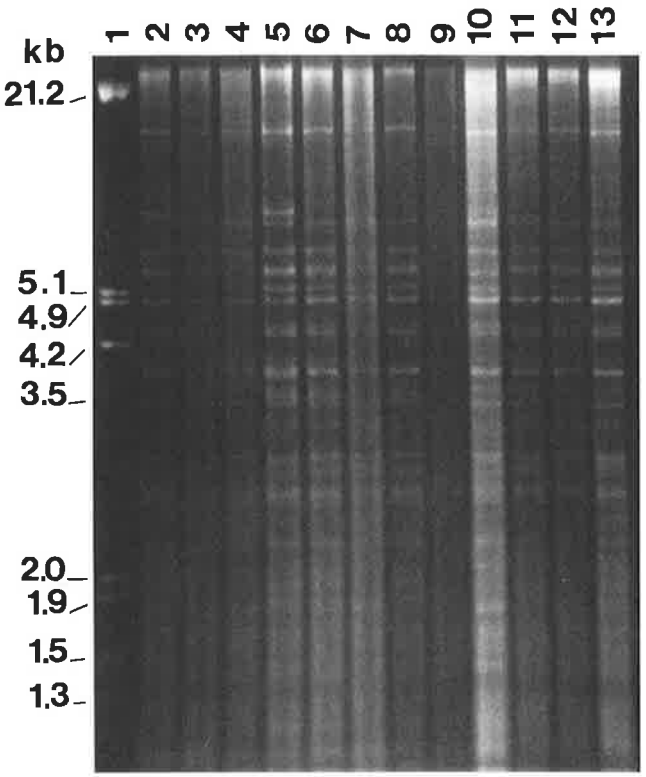

Fig. 1 Total DNA cleaved by the restriction enzyme *Hpa*II and separated on a 0.7% agarose gel. Lane 1: molecular weight marker; lanes 2–6: *P. teres* f. sp. *maculata* strains Cal 5, Cal 8, Hop 1, Nap 4 and Mor 2; Lanes 7–10: *P teres* f. sp. *teres* strains Riv 8, Nap 5, Nap 7 and Mor 5; Lanes 11, 12, 13: ascospore strains MP 4, MP 6, MP 7

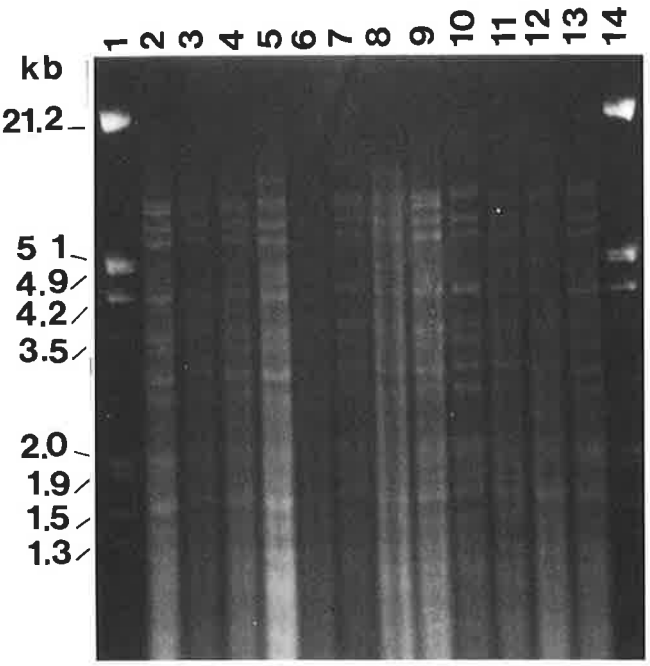

Fig. 2 Total DNA cleaved by the restriction enzymes *Hae*III and separated on a 0.7% agarose gel. Lanes 1 and 14 are the molecular weight markers. Details as in Fig. 1

net type isolates were detected. Net type isolates produced an olivaceous buff to greenish olivaceous pigmentation (Rayner, 1970) in PA after 7 days in darkness. Spot type (ascospore and conidial) isolates however, usually produced a darker pigmentation (greenish olivaceous) in the agar with woolly or tufted aerial mycelium. These criteria were, however, too variable on MEA to distinguish between groups. Although net type isolates sporulated more abundantly ($>10^4$ spores/ml) than spot type isolates ($<10^4$ spores/ml), variation also occurred within the two groups, with the absence of sporulation usually being associated with cultures having a whitish growth or orange cultural pigmentation. On MEA, ascospore and spot type conidial isolates were found to be slow growers in comparison to the net type conidial isolates (Table 2). This was not the case on PA.

Mating studies

Nine months after mating, protoperithecia were observed where isolates were mated with themselves. In matings between isolates of the spot and net types, beaked perithecia indicating fertility developed. However, no ascospores were observed after 12 months under the conditions provided.

DNA polymorphisms

Total DNA polymorphisms of the net, spot and ascospore isolates digested with the restriction enzymes *Hpa*II and *Hae*III respectively, are shown in Figs 1 and 2. Differences between individuals appear as the presence/absence of individual bands but which do not affect the overall pattern of similarity between isolates. Figure 3 shows the PAPD-profiles of a selected spot and net isolate and two outgroup species, *P. semeniperda* and *P. tritici-repentis*. RAPD-profiles of spot and net type isolates were similar, but differed markedly from the profiles of the outgroups.

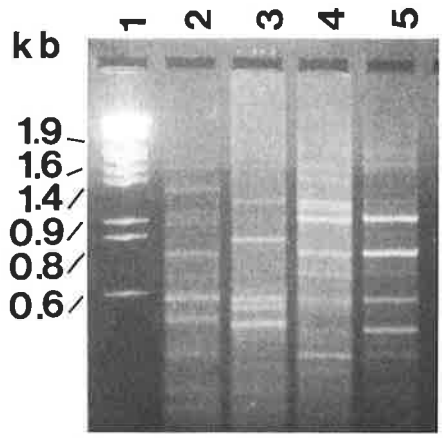

Fig. 3 Agarose gel electrophoresis of DNA fragments amplified from *Pyrenophora* DNA using primer 1 of the operon kit K. Lane 1: molecular weight marker; lane 2: *P. tritici-repentis* (IMI 190925); lane 3: *P. semeniperda* (Mor 10); lane 4: *P. teres* f. sp. *maculata* (Nap 4); lane 5: *P. teres* f. sp. *teres* (Nap 5)

Discussion

Using total DNA polymorphisms, it was previously shown (Crous et al., 1993) that members of the same species isolated from the same host and geographic location show high levels of homology. In this study with *Pyrenophora*, only individual band differences (presence, absence or degree of intensity) occurred between spot (ascospore and conidial) and net type (conidial) isolates using total DNA polymorphisms. The differences that did occur could not be confined to one group, and spot and net isolates could not be typified by an individual specific banding pattern. To further support the validity of our results we subjected selected isolates to RAPD-analysis. This technique has previously been shown to identify close phylogenetic relatedness (Goodwin and Annis, 1991, Nicholson et al., 1991; Henson, 1992). We also included isolates from two morphologically distinct species of *Pyrenophora*, viz. *P. tritici-repentis* and *P. semeniperda*. As with total DNA profiles, very similar banding patterns were obtained when comparing the RAPD-profiles of spot and net type isolates, whereas distinct banding patterns were obtained for both outgroups. The similar profiles of South African isolates agree with those of Wu et al. (1993), who in using RFLP's, also found identical profiles for the two South African *P. teres* f. *teres* isolates used in their study.

Contrary to McDonald (1967) and Smedegard-Petersen (1971), we were unsuccessful in mating spot and net type isolates. However, as only one mating (from numerous crosses) was obtained that produced a viable progeny (Louw et al. 1994), together with the inability of spot × spot and net × net to form viable progeny in this study, and the fact that the teleomorph occurs in nature, we conclude that the conditions as set out by McDonald (1967) and as used in this study are not optimal for mating South African isolates, thereby explaining this anomaly.

Our observation that morphologically only a few inconspicous differences occur between *Pyrenophora* isolates causing spot and net type lesions is in accordance with that reported by other workers (Ito and Kuribayashi, 1931; Kenneth, 1962; McDonald, 1967; Smedegard-Petersen, 1971). Although conidiophores of spot isolates tend to be longer than that of the net form, we found conidiphore dimension and morphology an extremely variable character in local *Pyrenophora* isolates. Secondary conidiophores were regularly formed by isolates obtained from both lesion types, and by ascospore isolates. Conidial catenulation as described by Scott (1991) to occur only with net type isolates was not observed, but microcyclic conidiation (a single conidium borne at the tips of unbranched germ tubes) as described by Boosalis (1962) for *Helminthosporium sativum* Pammel, King & Bakke (=4=*Bipolaris sorokiniana* (Sacc.) Shoem.), was found in both spot and net type isolates, but occurred more frequently in the latter. The teleomorph morphology of the spot form, which we recently found in the south-western Cape Province (Louw et al., 1994), is also very similar to that of *P. teres* (Ito and Kuribayashi, 1931; Smedegard-Petersen 1971). The only constant difference observed between the isolates was in conidium length; conidia of spot type isolates were generally longer than those of the net type. However, the dark colour of conidial hila in spot isolates were not prominant as was described for *P. japonica* (Schoemaker, 1962; Scott 1991).

In conclusion, the homology in DNA banding patterns and morphological relatedness of South African *Pyrenophora* isolates causing spot type and net type lesions on barley indicated that the difference in conidium morphology is insufficient to separate them as two species. These findings indicated that two types of *P. teres* occur locally, as was reported in other countries (McDonald 1967; Smedegard-Petersen, 1971). Spot type net blotch in the south-western Cape Province is therefore caused by *P. teres* f. *maculata*, and not by *P. japonica* as was previously reported (Scott, 1991). The teleomorph of the spot form recorded from South Africa (Louw et al. 1994) should therefore be re-allocated to *P. teres*.

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