Relative importance of the barley net blotch pathogens *Pyrenophora teres* f. *teres* (net-type) and *P. teres* f. *maculata* (spot-type) in South Africa

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The distribution of the barley net blotch pathogens *Pyrenophora teres* f. *maculata* (spot-type) and *P. teres* f. *teres* (net-type) was determined during 1991 and 1992 in fields at 26 localities in the Western Cape Province, the major barley-producing region of South Africa. Occurrence of net-type blotch was restricted to three localities. Spot-type lesions were observed on plants at 25 localities, but disease severity rating on the commercial cultivars Clipper and Stirling was generally low. However, the disease was epidemic on the experimental cultivar B87/14 at three localities where it was grown. Dampier, which is field-susceptible to net-type blotch, exhibited only spot-type symptoms at the four localities where it was planted. Barley stubble and seed yielded only *P. teres* f. *maculata*. Spot-type infection was evident on the alternative hosts *Hordeum murinum* and *Bromus diandris*, although only net-type isolates were obtained from the former. These findings indicate a major shift in pathogen populations from the previously prevalent *P. teres* f. *teres* to *P. teres* f. *maculata*, and also suggest that *P. teres* f. *maculata* could be responsible for significant losses in susceptible barley cultivars grown in the Western Cape Province.

Key words: barley, net blotch, *Pyrenophora teres*, survey.

Net blotch caused by *Pyrenophora teres* Dreschler (anamorph *Drechslera teres* (Sacc.) Shoemaker) is an important disease of barley (*Hordeum vulgare* L. emend. Bowden) worldwide (Shipton et al. 1973). Two types of leaf symptoms are associated with net blotch, namely a spot-type and net-type lesion. Originally, *P. japonica* S Ito & Kurib. (anamorph *Drechslera tuberosa* (G F Atk.) Shoemaker) was described as the pathogen causing spot symptoms, whereas *P. teres* was associated with net-type lesions (Ito & Kuribayashi 1931; Shoemaker 1962). McDonald (1967) reported successful mating of spot- and net-type isolates, and referred to the spot isolates as mutant strains of *P. teres*. Using strains from Denmark, Smedegard-Petersen (1971) repeated these matings, and concluded that the strains were two formae of the same biological species, for which he proposed the names *P. teres* f. *teres* (net-type symptoms) and *P. teres* f. *maculata* Smed.-Pet. (spot-type symptoms).

In South Africa, *P. teres* was recorded in 1930 along with two other *Helminthosporium* diseases of barley, namely spot blotch caused by *Cochliobolus sativus* (S Ito & Kurib.) Dreschler ex Dastur, and leaf stripe caused by *Pyrenophora graminea* S Ito & Kurib. (Smith & Rattray 1930). Scott (1991) subsequently reported the anamorph of *P. japonica* from spot-type symptoms of barley, while Louw et al. (1994) reported the teleomorph from barley stubble, and from matings of spot-type isolates in culture. However, in a comparison of *Pyrenophora* species occurring on barley in the Western Cape Province, Louw et al. (1995) found that differences observed in morphological and cultural criteria of South African net-type and spot-type isolates were inconsistent. Using A+T-rich DNA (AT-DNA) polymorphisms, banding patterns of conidial and ascospore strains (spot-type) and conidial strains (net-type) were shown to be similar, supporting the argument that these strains were formae of the same species as proposed by Smedegard-Petersen (1971). In a subsequent study (Crous et al. 1995), South African *Pyrenophora* isolates associated with spot-type and net-type blotch were compared with verified isolates of *P. teres* f. *teres*, *P. teres* f. *maculata* and *P. japonica*. Based on the *F*-values (Hausner et al. 1993) derived from DNA banding patterns of local and overseas spot-type and net-type isolates digested with *Hae*III and *Msp*I, 74–100 % similarity was observed. Furthermore, spot-type and net-type isolates could be mated, and a Danish spot-type × Australian net-type isolate produced viable progeny. Single-ascospore isolates inoculated on differential barley cultivars produced spot-type, net-type or mixed symptoms, suggesting that recombination may have occurred. Based on these findings, *P. japonica* was placed in synonymy with *P. teres* f. *maculata* (Crous et al. 1995).

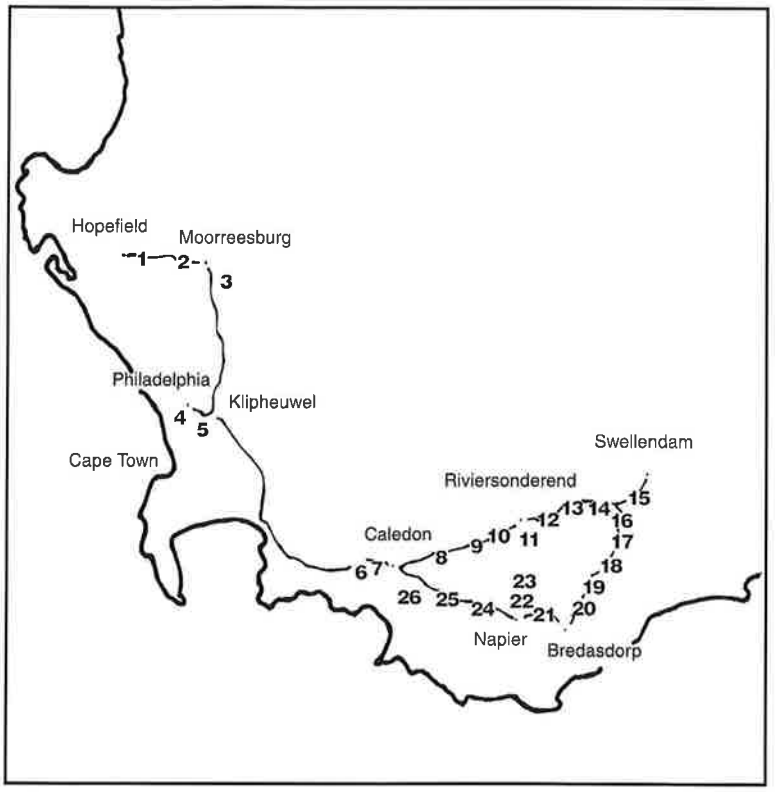

Fig 1. Barley fields in the Western Cape Province of South Africa surveyed for the presence of the net blotch pathogens *Pyrenophora teres* f. *maculata* (spot-type) and *P. teres* f. *teres* (net-type). *P. teres* f. *maculata* was isolated from barley at localities 1–14 and 16–26, and *P. teres* f. *teres* from barley at localities 2, 9 and 17. Spot-type symptoms on *Hordeum murinum* at localities 2, 4, 5, 7, 9, 18, 23 and 25 yielded *P. teres* f. *teres*.

The two forms of *P. teres* are capable of causing economically significant yield losses in barley (Khan 1989; Steffenson et al. 1991). Losses can be reduced by crop rotation, ploughing-under infected crop stubble, applying foliar fungicides and growing resistant cultivars. However, different strategies are required for the chemical control of the two forms of the pathogen (Van den Berg & Rossnagel 1990; Scott et al. 1992), whereas successful resistance breeding programmes depend largely on a thorough understanding of the pathogen population, requiring that it be sampled regularly to monitor changes in distribution and pathotype composition.

Little information is available regarding the incidence and pathotype distribution of the two formae of *P. teres* in South Africa. The two types of *P. teres* cannot be readily distinguished on resistant barley cultivars by field symptoms (Scott 1991). They can also vary considerably in morphological and cultural characteristics (Smedegard-Petersen 1971; Louw et al. 1995), especially after successive subculturing (Kenneth 1962). The primary purpose of this study was to determine the relative importance of the two forms of *P. teres* in local barley fields, 92 % of which are planted to the cultivar Clipper. Both forms of *P. teres* can occur on crop residues (Evans 1969; Jordan 1981; Van den Berg & Rossnagel 1991), in seed (Smith & Rattray 1930; Matthews & Hampton 1977; Hampton 1980; Jordan 1981; Reeves & Ball 1991) and on weeds. For instance, *Hordeum murinum* L. (wild barley) has been described as an alternative host of the spot-type of *P. teres* (Ito & Kuribayashi 1931; Scott 1991), whereas wild barley and *Bromus diandris* Roth (ripgut brome) can harbour the net form of the pathogen (Butler & Jones 1949; Kenneth 1962; Khan & Boyd 1968; Shipton et al. 1973). These sources were therefore also included in the survey.

Materials and methods

Distribution of net-type and spot-type isolates in barley fields

Two surveys, one at early tillering and the second at the booting stage, were conducted in commercial barley fields during the 1991 and 1992 growing seasons. Fields were examined at 15 km intervals (except between localities 3 and 4, and 5 and 6), along the route marked in Fig. 1. During 1991, fields were classified as positive or negative for the occurrence of the two types of the disease, whereas in 1992, disease incidence and severity were recorded at each locality. Disease incidence was determined on the three topmost leaves of 10 main stems removed randomly at three sites per 15 ha field. Severity was determined by the method of Khan (1987). Leaves with characteristic or suspected net- and spot-type lesions were collected and placed in paper bags. The infected leaves were subsequently dried in an oven at 25 °C and stored at room temperature. Previous studies using this method showed that the pathogen remained viable and retained its virulence in infected material for several years (Arabi et al. 1992; Steffenson & Webster 1992).

In a separate experiment, experimental blocks in commercial fields at localities 5, 11 and 22 were planted to the differential cultivars Clipper (field-susceptible to spot-type but resistant to net-type isolates, Khan & Tekauz 1982), Dampier (field-susceptible to net-type and intermediate in reaction to spot-type isolates, Khan & Tekauz 1982) and B87/14 (field-susceptible to spot-type isolates, Louw 1993). At each locality, two replicate blocks, 1 m long with three rows spaced 20 cm apart (one row per cultivar), were planted at a rate of 150 kg seed per hectare on 12 May 1992. Plants were rated for disease, and infected material was sampled as described previously.

Barley stubble

Barley stubble from the previous year was collected during the same period from the fields included in the survey. Stubble was placed in paper bags and stored at room temperature until processed. Black ascomata observed on barley stubble were crushed, mounted in lactophenol and examined microscopically for the presence of ascospores. The presence of other fungi occurring on stubble was also noted.

Alternative hosts

Wild barley and ripgut brome grass growing in barley fields were examined for spot- and net-type symptoms. Infected leaves from two plants were sampled at localities 2, 5, 6–13, 16–21, 23–26, placed in paper bags and dried as described above.

Barley seed

Untreated seed of the commercial cultivars Clipper, Stirling, Schooner, and of the experimental cultivar B87/14, was obtained from experimental field plots planted in the Riviersonderend area. Seeds were surface-sterilised in 50 % ethanol (15 seconds) and 2 % NaOCl (1 minute), rinsed in 50 % ethanol and dried on filter paper in a laminar-flow cabinet. Four hundred seeds of each cultivar were placed on moist filter paper in Petri dishes (20 seeds/dish) and incubated at 15 °C under continuous near-ultraviolet light to allow conidiophore formation by *P. teres*. Dishes were subsequently incubated at 15 °C for 7 days in darkness to induce conidium formation (Onesirosan & Banttari 1969).

In a separate experiment, 1980 untreated seeds of the experimental cultivar B87/14 from the Riviersonderend area were planted in 15 cm pots filled with sterilised river sand (10 seeds/pot). Pots were maintained in a greenhouse (15 °C night and 20 °C day), and seedlings were assessed for infection at the two-leaf stage as described by Jordan (1981). Both experiments were repeated once.

Verification of P. teres *isolates*

Infected leaves from barley and alternative hosts, and pieces of stubble, were cut into 5 cm sections, surface-sterilised in 50 % ethanol (15 seconds) and 2 % NaOCl (30 seconds), and rinsed in 50 % ethanol. The sections were placed on moist, sterile filter paper in Petri dishes and incubated at 15 °C under near-ultraviolet light to allow conidiophore formation, and subsequently in the dark to induce conidiation. Ten single spores per tissue section were transferred aseptically onto Purity agar (PA) (30 ml vanilla Purity, Gerber's Purity, Reckitt & Coleman South Africa (Pty) Ltd, 15 g Biolab agar in 1000 ml distilled water) and incubated at 25 °C in the dark. Fully colonised plates were treated as above to induce conidium formation. Both forms of the pathogen sporulate profusely on PA (Louw et al. 1994). Sporulating conidiophores on naturally-infected material were

Table 1. Disease indices in barley plants infected with *Pyrenophora teres* f. *maculata* at two growth stages in experimental plots at three localities during 1992.

	Tillering				Heading			
	Clipper		B87/14		Clipper		B87/14	
Locality	Incidence[a]	Severity[b]	Incidence	Severity	Incidence	Severity	Incidence	Severity
5	4	1	5	1	25	1	25	5
11	23	1	25	1	65	5	100	10
22	27	1	30	1	72	5	100	25

[a]Incidence = percentage plants with symptoms on the three topmost leaves.
[b]Severity = percentage leaf area affected.

mounted in lactophenol and examined microscopically.

Isolates were verified by inoculating single-conidial strains on the differential barley cultivars Stirling and B87/14 (susceptible to net- and spot-type isolates, respectively). 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 standardised with a haemacytometer to contain 1300 spores ml^{-1}. The cultivars were grown in 15 cm plastic pots filled with river sand (10 plants per pot). Pots were arranged in a greenhouse (15 °C night and 20 °C day) in a randomised complete block design with two replicates per isolate. Plants were inoculated at the two-leaf stage using the inoculation technique described by Khan & Boyd (1969). This involved spraying them with sterile distilled water containing 0.01 % Tween 20 to reduce surface tension, and then with a conidial suspension of the isolate (12 ml per pot). Control plants were treated similarly, but were sprayed with sterile distilled water only. Pots were kept at high humidity for 24 hours, and thereafter returned to the greenhouse. Plants were rated for disease two weeks after inoculation.

Results

Distribution of net-type and spot-type isolates in barley fields

Spot-type blotch symptoms characteristic of *P. teres* f. *maculata* occurred in all fields examined in 1991 and 1992, except at locality 15, where it was absent in 1992. Low spot-type incidences (2–25 %) were recorded at tillering on all commercial cultivars, and disease severity ratings based on the total area affected in the top three leaves were low (1 %). Higher disease indices (incidence 50 %, severity 10 %) were recorded on the experimental cultivar B87/14 grown at Dunghye Park (locality 26). Disease ratings were notably higher during heading (Table 1). Of the commercial cultivars, spot-type blotch was recorded at a relatively high incidence only on cultivar Stirling at locality 9. However, the disease was epidemic on B87/14, grown commercially at localities 3 and 26 (Table 2).

Net-type symptoms characteristic of *P. teres* f. *teres* occurred sporadically, and were observed only at localities 2, 9, and 17. Disease indices were nevertheless low (incidence ≤1 %, severity 1 %), the exception being locality 2 near Moorreesburg, where a disease incidence of 12 % was recorded at heading.

No net-type symptoms were observed in the experimental blocks on Clipper or B87/14 plants, nor on Dampier, which is field-susceptible to net-type blotch. By contrast, spot-type symptoms occurred in all experimental blocks on these cultivars. Disease indices at localities 11 and 22 were similar to those observed at most of the other sampling sites, but were markedly lower at locality 5, where barley had not been planted for nearly a decade.

Infected material collected at the various localities generally produced conidia of *P. teres* when incubated on moist filter paper, the exception being leaves with atypical lesions. All isolates were virulent on at least one of the differential barley cultivars. Spot-type isolates comprised 83.4 % and net-type isolates 16.6 % of the 120 *P. teres* isolates screened.

Barley stubble

Little stubble was available in fields sampled at the various localities. Conidiophores of the target organisms were noted primarily on the internodes of stubble kept in moist chambers. Based on

conidial morphology and symptom expression of isolates on barley plants, only the spot form (*P. teres* f. *maculata*) could be identified. Other fungi commonly isolated from stubble included *Alternaria alternata* (Fr.) Keissl., *C. sativus* and a number of *Fusarium* and *Drechslera* spp.

Pseudothecia of *Pyrenophora* spp. were rarely found in commercial fields, but occurred commonly on stubble collected at locality 26, where B87/14 had been grown. Cultural and inoculation studies showed that these pseudothecia belonged to *P. teres* f. *maculata.*

Alternative hosts

Primarily spot-type symptoms occurred on wild barley growing in all fields at the different localities, but only *P. teres* f. *teres* could be isolated from lesions. Eight of nine randomly selected isolates from wild barley elicited a typical hypersensitive reaction of the net-type in Stirling seedlings (field susceptible to *P. teres* f. *teres*). The remaining isolate was more virulent and produced typical net-type symptoms. It also incited a resistant reaction in B87/14, which is highly resistant to net-type blotch (J P J Louw et al., unpubl. data).

Only spot-type lesions, from which *P. teres* f. *maculata* was consistently isolated, occurred on ripgut brome grass. In inoculation studies, intermediate and susceptible reactions of the spot-type were caused by these isolates on Stirling and B87/14, respectively.

Barley seed

B87/14 yielded the highest percentage infected seed (9.75 %), followed by Clipper (1.5 %) and Schooner (0.25 %). Stirling seed was free of *P. teres.* Based on conidium morphology and symptom expression of isolates on barley plants, only *P. teres* f. *maculata* occurred on barley seed incubated in moist chambers in the laboratory. In the greenhouse, 1.5 % of the B87/14 seedlings showed symptoms indicative of seedborne infection, i.e. blight-like lesions on the central portion of the second leaf (Jordan 1981). *P. teres* f. *maculata* was consistently isolated from these lesions.

Verification of P. teres *isolates*

Conidia assigned to *P. teres* f. *maculata* measured (37–)101(–166) µm, were cylindrical, tapering to an acutely-rounded base, with a brown dehiscence scar, (2–)3(–4) µm wide. Those of *P. teres* f. *teres* measured (18–)70(–100) µm, were cylindrical, tapering to a subtruncate base, with dehiscence scars (3–)4(–6) µm wide, being darker brown and wider than those of the spot-type isolates (Louw et al. 1995). The identity of isolates was further confirmed by inoculations on the barley cultivars Stirling and B87/14.

Table 2. Disease indices at heading of barley plants infected with *Pyrenophora teres* f. *maculata* in commercial fields in 1991/92.

Locality[a]	Cultivar	Incidence[b]	Severity[c]
1	Stirling	15	1
2	Stirling	45	5
3	B87/14	100	75
4	Stirling	18	1
6	Clipper	35	1
7	Clipper	35	1
8	Stirling	30	1
9	Stirling	75	25
10	Stirling	46	5
12	Clipper	36	5
13	Clipper	27	1
14	Clipper	18	1
15	Clipper	0	0
16	Clipper	20	1
17	Clipper	35	5
18	Clipper	35	5
19	Clipper	35	5
20	Clipper	35	5
21	Clipper	38	5
23	Stirling	44	5
24	Clipper	38	5
25	Clipper	38	5
26	B87/14	100	75

[a]Localities are presented in Fig. 1.
[b]Incidence = percentage plants with symptoms on the three topmost leaves.
[c]Severity = percentage leaf area affected.

Discussion

Early reports on *P. teres* described net blotch as one of the commonest barley diseases in South Africa (Smith & Rattray 1930; Putterill 1954). Positive identification of spot-type blotch was not made until 1988, when the spot form, produced by *P. teres,* was reported on barley from Tygerhoek and Dunghye Park (Scott 1988). In the present survey, mainly spot-type symptoms were recorded at all localities and 83.4 % of the 120 *P. teres* isolates collected from infected plant material proved to be *P. teres* f. *maculata.* Net-type symptoms were observed only in a few barley fields and not in the experimental blocks planted with Dampier as

catch crop. Dampier is known for its lack of field-resistance against net-type blotch (Khan 1987). These results thus indicate that *P. teres* f. *maculata* is currently the predominant form of *P. teres* in the Western Cape Province, occurring not only on barley plants, but also in stubble, seed and alternate hosts like ripgut brome grass.

It is not clear when this shift in pathogen population occurred. In earlier years, the cultivars Elsa and Swaneck, which are field-susceptible to *P. teres* f. *teres,* were mostly grown in South Africa. The Australian cultivar Clipper, which is field-susceptible to the spot-type but resistant to the net-type, was introduced to South Africa in 1978. It is now planted in 93 % of commercial fields in the Western Cape Province, the main barley-producing region of South Africa. The predominance of Clipper may have offered selective advantage to the spot-type of *P. teres,* hence its current prevalence. The net-type pathogen nevertheless appears to be maintained in barley fields by wild barley, although mainly as strains low in virulence.

The predominance of *P. teres* f. *maculata* in the Western Cape should be of great concern to the local malt industry, considering the susceptibility of cultivars like Clipper, Schooner and Stirling to leaf scald, the major foliar disease of barley in South Africa (Scott 1988; Scott et al. 1992). An extensive breeding programme aimed at developing a leaf scald-resistant cultivar adapted to local conditions is being conducted by various institutions. The experimental cultivar B87/14, one of several to be released in the near future, is a product of this programme. However, although resistant to leaf scald, it is highly susceptible to net blotch. *P. teres* f. *maculata* is furthermore difficult to control with fungicides on susceptible cultivars (Van den Berg & Rossnagel 1990; Scott et al. 1992). A more intensified fungicide programme on B87/14 may not be practical in the Western Cape due to current economic restraints in the industry. It may also lead to the development of resistance to triazole fungicides in local *P. teres* f. *maculata* populations, as has been reported from other countries (Sheridan & Grbavac 1985; Peever & Milgroom 1992). Indeed, symptoms of seed infection were noticed on B87/14 seedlings originating from seed treated with triadimenol at locality 26. The sexual state of *P. teres* f. *maculata,* which does occur locally (Louw et al. 1994), could also be important in generating variation to triazole resistance, which is then selected for by fungicide application during the growing season (Peever & Milgroom 1992).

Scott et al. (1992) reported that climatic conditions in South Africa are not conducive to spot-type net blotch early in the season. The disease usually develops late and apparently causes little damage to susceptible genotypes. In our study, the incidence and severity of spot-type symptoms on the commercial cultivars Clipper and Stirling was low, thus confirming the greenhouse experiments conducted by Scott (1992), which indicated that these cultivars are resistant to local isolates of *P. teres* f. *maculata.* By contrast, spot-type symptoms occurred on 50 % of the B87/14 seedlings at the early tillering stage in a commercial field (locality 26), and reached epidemic proportions with a high severity index at heading. Similar observations were made at other localities in the production area where this cultivar was planted. This suggests that *P. teres* f. *maculata* is capable of causing substantial losses in susceptible cultivars grown in the Western Cape Province. In Australia, Khan (1989) estimated crop losses incurred by spot-type infection to be as high as 34 % in susceptible barley cultivars.

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