Tapesia yallundae collected from wheat stubble in South Africa

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Abstract. *Tapesia yallundae* var. *yallundae* is newly recorded from wheat stubble collected near Moorreesburg in the western Cape Province of South Africa. Apothecia were observed on wheat stubble incubated for 8 months at 10 °C under near-ultraviolet light. Single ascospore isolates produced colonies typical of the Wheat (W)-type in culture. Apothecia were also induced after single-conidial isolates were mated on wheat stubble and incubated at 10–15 °C for 6 months. All single-conidial isolates were of the W-pathotype (*Ramulispora herpotrichoides* var. *herpotrichoides*).

Key words: Eyespot, Heterothallism, *Pseudocercospora herpotrichoides*, *Tapesia yallundae*, Teleomorph, *Ramulispora herpotrichoides*, Wheat

Introduction

Eyespot, caused by *Ramulispora herpotrichoides* (Fron) von Arx var. *herpotrichoides* (syn. *Pseudocercospora herpotrichoides* (Fron) Deighton) is a serious disease of spring-wheat in the Swartland area of the western Cape Province of South Africa [1]. The disease is characterized by typical 'eye'-shaped lesions that develop on leaf sheaths at or near the soil surface. Eyespot weakens or kills individual tillers, reduces kernel size and number, causes culms to lodge, and renders plants difficult to harvest [2].

Two main pathotypes of *R. herpotrichoides* can be distinguished on the basis of seedling pathogenicity tests, cultural characteristics, mating populations, isozyme zymograms and DNA polymorphisms [3–6, 14, 15]. Wheat-type (W-type) isolates, which are generally more pathogenic to wheat than to barley and much less pathogenic to rye, produce colonies which are fast-growing and even-edged. Rye-type (R-type) isolates, which are about equally pathogenic to wheat, bar-

ley and rye, produce colonies which are slow-growing and feathery-edged [7]. Results obtained by King & Griffen [8] and Nicholson et al. [6] suggest that the W- and R-type might correspond to the varieties *R. herpotrichoides* var. *herpotrichoides* and var. *acuformis* (Nirenberg) Boerema, Pieters & Hamers respectively. *R. herpotrichoides* var. *herpotrichoides* (W-type) produces curved and straight conidia, whereas *R. herpotrichoides* var. *acuformis* has straight conidia only [9]. In South-Africa, only the variety *R. herpotrichoides* var. *herpotrichoides* has thus far been found [5], and it is not known if other varieties also occur [10].

The teleomorph *Tapesia yallundae* Wallwork & Spooner, has previously been reported on hosts such as *Triticum aestivum* L. cv. Warigal, *Bromus diandrus* Roth. and *Hordeum leporinum* Link [11], occurring in countries such as South Australia [11], New Zealand [12], England [13], Belgium [14] and Germany [15]. In South Australia, New Zealand and England, single-ascospore isolates produced typical W-type colonies,

Table 1. Conidium morphology of isolates of *Ramulispora herpotrichoides*

Species	Isolate no.	Conidium		
		Dimensions ^a (length × width)	Septation	Shape ^b
<i>R. herpotrichoides</i> var. <i>herpotrichoides</i>	BR 1	23–50 × 1–2	1–4	C
	BR 2	20–51 × 1.5–2	1–4	S, C
	BR 3	23–87 × 1–2	1–11	S, C
	BR 4	16–35 × 1.5–2	1–3	S, C
	BR 5A	40–70 × 1–1.5	1–7	C
	BR 6A	50–81 × 1.5	2–6	S, C
	BR 7A	22–85 × 1–2	1–7	C
	BR 8A	27–74 × 1–1.5	3–6	C
	BR 9A	21–85 × 1–2	1–6	C
	BR 10A	38–58 × 1–1.5	1–3	C
	BR 11A	28–100 × 1–2	3–7	C
	BR 13A	37–100 × 1–1.5	3–6	C
	BR 14A	31–82 × 1–1.5	1–7	C
	BR 15A	29–70 × 1	0–6	C
	BR 16A	42–76 × 1–2	1–9	C
	BR 20	25–72 × 1–1.5	3–8	C, S
	BR 21	28–74 × 1–1.5	2–8	C, S
	BR 24A	20–87 × 1–2	1–9	C
	BR 25A	31–74 × 1–2	0–5	C
	BR 26A	24–81 × 1–1.5	0–6	C, S
	BR 27A	33–71 × 1–1.5	1–4	C
	BR 42A	23–100 × 1–1.5	0–10	C
	BR 48A	33–90 × 1–1.5	2–6	C, S
	BR 49A	28–85 × 1–1.5	2–8	C
	BR 50A	sterile	–	–
	BR 51A	26–70 × 1–1.5	1–5	C
<i>R. herpotrichoides</i> var. <i>acuformis</i>	BR 19	28–60 × 1–1.5	1–6	S
	BR 30	30–66 × 1–2	3–9	S
	BR 31	42–83 × 1–1.5	3–10	S

^a Measurements (µm) on SNA (Nirenberg, 1981) after 14 days at 10 °C under near-ultraviolet light.

^b S = straight; C = curved.

when incubated at 10 °C [11–13]. In Germany, however, single-ascospore isolates produced typical R-type colonies in culture, and pathogenicity tests to wheat and rye confirmed them to be representative of the R-type [15]. Boerema, Pieters & Hamers [16] subsequently distinguished two varieties of *T. yallundae* based on the anamorph, and described *T. yallundae* var. *acuformis* Boerema, Pieters & Hamers for isolates of *R. herpotrichoides* var. *acuformis*.

King [15] stated that the teleomorph *T. yallundae* represents a stronghold for overcoming new selection pressures by means of gene recombination and segregation within the fungus. The

present study reports the occurrence of apothecia of *T. yallundae* var. *yallundae* for the first time in South Africa, and the in vitro mating of single-conidial field isolates.

Materials and methods

Isolates studied. Straws with typical eyespot lesions were collected at Moorreesburg and Gouda in the western Cape Province. Straw pieces (5 cm in length) containing eyespot symptoms were surface sterilized for 1 min in 1% NaOCl, and placed in moist chambers at 10 °C under near-ultraviolet

Figs 1–4. Apothecia, asci, ascospores and conidia of *Tapesia yellundae* var. *yellundae*. Fig. 1. Hyaline, curved and straight conidia of the anamorph *Ramulispora herpotrichoides* var. *herpotrichoides* (bar = 10 μ m). Fig. 2. Mature and immature apothecia on stubble after mating an isolate from Gouda and Moorreesburg (bar = 0.7 mm). Fig. 3. Hyaline asci with ascospores (bar = 10 μ m). Fig. 4. Fusiform ascospores (bar = 10 μ m).

light for 2 weeks to induce sporulation. Conidia were suspended in water and dispersed on potato dextrose agar (PDA) (200 g potatoes, 20 g dextrose [BDH], 15 g agar [Biolab]) amended with streptomycin sulphate (PDAS) (0.05 g streptomycin sulphate/L). PDAS plates were left under a laminar flow hood to dry. Conidia were located under a stereo-microscope and transferred to fresh PDAS plates. Single-conidial isolates were subsequently incubated at 25 °C in the dark for 2 weeks. During this period the single-conidial strains were subcultured to provide enough inoculum for the mating experiment.

Cultures studied. UK: *Triticum aestivum*, G.L. Bateman, 1987, BR 19 (87.2H11a), (R-type); T.W. Hollins, 1989, BR 30 (C89/737), (R-type); T.W. Hollins, 1991, BR 31 (C91/760), (R-type); G.L. Bateman, 1987, BR 20 (87.291a), (W-type); G.L. Bateman, 1991, BR 21 (GP6), (W-type). South Africa: Moorreesburg, *Triticum aestivum*, F. Bester, 1991, BR 1–4; Gouda, A.B. van Jaarsveld, 1991, BR 5A, 6A, BR 8A–11A, BR 13A–16A; Gouda, B. Robbertse, 1991, BR 24A–27A, BR 42A, BR 50A, 51A; Moorreesburg, B. Robbertse, 1991, BR 48, 49A. All cultures examined in this study are maintained in the culture

Table 2. Cultural characteristics of single-conidial isolates of *Ramulispora herpotrichoides*

Species	Isolate no.	Colony		
		Colour ^a	Diameter ^b	Margin ^c
<i>R. herpotrichoides</i> var. <i>herpotrichoides</i>	BR 1	Sayal Brown	24	S
	BR 2	Tawny-olive	20	S
	BR 3	Snuff Brown	24.5	S
	BR 4	Olivaceous buff	22.5	S
	BR 5A	Olivaceous buff	20	S
	BR 6A	Sayal Brown	23	S
	BR 7A	Tawny-olive	23	S
	BR 8A	Kronberg's green	25	S
	BR 9A	Kronberg's green	21	S
	BR 10A	Olivaceous buff	23	S
	BR 11A	Kronberg's green	22	S
	BR 13A	Olivaceous buff	22.5	S
	BR 14A	Olivaceous buff	22.5	S
	BR 15A	Olivaceous buff	28	S
	BR 16A	Kronberg's green	23	S
	BR 20	Olivaceous buff	22.5	S
	BR 21	Dark-ivy-green	23	S
	BR 24A	Kronberg's green	23	S
	BR 25A	Dark-ivy-green	25	S
	BR 26A	Olivaceous buff	23	S
	BR 27A	Dark-ivy-green	19	S
	BR 42A	Kronberg's green	20.5	S
	BR 48A	Kronberg's green	24	S
	BR 49A	Olivaceous buff	20.5	S
	BR 50A	Twany-olive	24.5	S
	BR 51A	Olivaceous buff	25	S
<i>R. herpotrichoides</i> var. <i>acuformis</i>	BR 19	Chamois	14.5	F
	BR 30	Tawny-olive	12	F
	BR 31	Pale-smoke-gray	14.5	F

^a Colour designations according to Rayner (1970) on PDA after 14 days at 25 °C in the dark.

^b Colony diameters (mm) measured on PDA after 10 days at 20 °C in the dark.

^c Colony margin determined on PDA after 10 days at 20 °C in the dark; S = smooth; F = feathery.

collection of the Department of Plant Pathology at the University of Stellenbosch, South Africa.

Isolate identity. Isolates used in the mating study were characterized on PDA. Linear growth was determined on three replicate Petri dishes after 10 days at 20 °C in the dark, and the experiment was repeated once. An agar disc (3 mm diam.) obtained from the periphery of an actively growing colony was placed in the centre of each plate. Colony diameters were determined by taking two perpendicular readings of each colony, and determining the averages (mm). Colony colour (reverse) was rated after 14 days at 25 °C in the dark, and designated according to Rayner's mycological

colour chart [17]. Isolates were also characterized according to their conidial morphology. Single-conidial isolates were grown on synthetic nutrient agar (SNA) for 14 days at 10 °C under near-ultra-violet light [9]. Conidia were measured under $\times 1000$ magnification.

Mating single-conidial isolates in vitro. Straw segments (5 cm in length) were sterilized by autoclaving twice on two consecutive days at 121 °C for 15 min. Segments were placed on PDA plates (ten per plate), and inoculated with colonized agar plugs from the respective single-conidial isolates. Inoculated straws were incubated at 20 °C for four weeks in the dark. Isolates were mated

BR	1	2	3	4	5A	6A	7A	8A	9A	10A	11A	13A	14A	15A	16A	24A	25A	26A	27A	42A	48A	49A	50A	51A
1	-																							
2	-	-																						
3	-	-	-																					
4	-	+	-	-																				
5A	-	-	-	-	-																			
6A	-	-	-	-	-	-																		
7A	-	-	+	-	-	-	-																	
8A	-	-	+	+	-	-	-	-																
9A	-	-	+	-	-	-	-	-	-															
10A	-	-	+	-	-	-	-	-	-	-														
11A	-	-	+	-	-	-	-	-	-	-	-													
13A	-	-	+	-	-	-	-	-	-	-	-	-												
14A	+	-	+	+	-	-	-	-	-	-	-	-	-											
15A	-	-	+	-	-	-	-	-	-	-	-	-	-	-										
16A	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-									
24A	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-								
25A	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-							
26A	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-						
27A	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-					
42A	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
48A	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-			
49A	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
50A	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
51A	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 5. Schematic representation of single-conidial isolates of *Ramulispora herpotrichoides* var. *herpotrichoides* mated in all possible combinations. A designated (-) indicates that no apothecia formed, while (+) represents apothecium formation with a viable progeny.

in all possible combinations by placing colonized straws touching along their length in Petri dishes containing 1.5% wateragar (Biolab). Mated isolates were incubated under near-ultraviolet light at 10–15 °C. Two replicates were used for each combination.

Results and discussion

The conidial morphology of local isolates closely matched those of the verified W-type isolates (Table 1, Fig. 1). Local isolates produced colonies which were fast-growing, even-edged and varying

from sayal brown to dark-ivy-green in colour on PDA (Table 2), confirming all local isolates to be representative of the W-pathotype.

Apothecia with viable progeny readily developed on straw in matings between isolates from Gouda and Moorreesburg (Fig. 2). From the possible 300 combinations, 29 produced the teleomorph *T. yallundae* var. *yallundae*. The infrequent occurrence of the teleomorph in the mating study concurs with that observed in the field, suggesting that the teleomorph is not very common. Slide preparations made after 6 months found most asci to have already released their ascospores (Fig. 3). Ascospores were hyaline, fus-

iform with rounded ends, $6-10 \times 1.5 \mu\text{m}$, closely fitting the description of the type specimen as described by Wallwork & Spooner [12] (Fig. 4). No apothecia formed in pairings where single-conidial isolates were paired with themselves, confirming strains to be heterothallic. Isolate BR 3 proved to be a good tester strain, forming apothecia with 14 other isolates, accounting for almost 50% of the overall successful combinations. Isolate BR 6A, however, failed to form apothecia in any of its pairings. The ability to produce apothecia in matings differs considerably between BR 3 and BR 6A, which in part demonstrates the variation present in the isolates. Not all the isolates designated as either MAT1-1 or MAT1-2 could be mated successfully with all those of the opposite mating type, indicating that although the incompatibility is homogenic, it may be more complex (Fig. 5). Further research using a wider range of isolates is therefore required to suitably resolve this matter.

The teleomorph is responsible for genetic recombination [18] and would influence fungicide sensitivity. However, with the infrequent production of apothecia, the development of fungicide resistance would be slower.

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