NEW OR UNUSUAL RECORDS

Pyrenophora japonica occurring on barley in South Africa

J. P. J. LOUW, P. W. CROUS and G. HOLZ

Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa

A *Pyrenophora* teleomorph was recently found on barley stubble in South Africa. Single-ascospore isolates, mated on water agar containing sterile pieces of barley stubble, produced the teleomorph within 12 months of incubation at 15°C in the dark. Based on morphological characteristics and symptom expression on barley plants, the teleomorph is ascribed to *P. japonica* (anamorph *Drechslera tuberosa*). This is the first report describing the occurrence of the teleomorph of *D. tuberosa* in South Africa.

Net blotch is a common disease of barley (*Hordeum vulgare*), caused by *Pyrenophora teres*. The pathogen has a worldwide distribution and occurs wherever the crop is grown in temperate humid regions (Shipton *et al.*, 1973). Two forms of *P. teres* have been reported, *P. teres* f.sp. *teres* and *P. teres* f.sp. *maculata* which differ in the symptoms incited on barley. On matured plants of susceptible cultivars *P. teres* f.sp. *teres* causes a net-type lesion which is characterized by dark brown blotches criss-crossed with a net-like venation and accompanied by chlorosis (Tekauz, 1986). Conversely, *P. teres* f.sp. *maculata* causes spots of various shapes and sizes, encircled by varying widths of chlorosis (Smedegård-Petersen, 1971). Ito & Kuribayashi (1931) described another species, *P. japonica*, which is closely related to *P. teres* and also causes spot-type lesions on barley. In a recent review of *Pyrenophora* Fr. (Sivanesan, 1987), *P. teres* and *P. japonica* were retained as separate taxa.

Leaf-spot diseases on barley are of great importance to the malt-producing industry in South Africa (Smith & Rattray, 1930; Putterill, 1954; Gorter, 1977; Scott, 1988, 1991). Spot-type lesions are common, and South African isolates investigated by Scott (1991) fit the description of *Drechslera tuberosa* (teleomorph *P. japonica*). The present paper reports on the teleomorph of *D. tuberosa*.

During 1991, surveys for net blotch incidence, severity and pathogen distribution were conducted in the main barley producing areas of the Western Cape Province. Black ascomata were regularly observed on barley stubble left in the field from the previous year's harvest. The perithecial ascomata (Fig. 1a) were typical of *P.*

japonica (Sivanesan, 1987), being black, large, scattered, subglobose to globose, sclerotoid when young, and developing short papillate necks frequently bearing conidiophores and setae which also occurred on other portions of the perithecia. Only beaked perithecia were fertile. Immature as well as mature ascospores were frequently observed in the same ascus, a phenomenon also observed by Smedegård-Petersen (1972). The bitunicate, club-shaped or cylindrical asci were usually eight-spored, and measured (255)–267–(277) × (30)–32–(35) µm. Ascospores were yellow-brown, ellipsoidal, with three transverse (rarely four) and up to two longitudinal septa (42)–50–(62) × (15)–18–(22) µm, being slightly constricted at their septa, with the two central cells being somewhat larger than the rounded end cells (Fig. 1b and c).

Pyrenophora teres and *P. japonica* have perithecia that are morphologically very similar (Ito & Kuribayashi, 1931; Smedegård-Petersen, 1971). Different studies were therefore conducted to verify the identity of the teleomorph. Thirty single-ascospore isolates, obtained from a single perithecium, were mated on water agar containing sterile pieces of barley stubble (autoclaved for 15 min at 103 kPa on two consecutive days). Matings were done in all possible combinations, using three replicate plates for each combination. Plates were sealed with Parafilm, and incubated at 15°C in the dark. After 1 month, black protoperithecia developed in most of the matings, but no ascospores were formed at this stage. Beaked perithecia with ascospores in the early stage of development were observed in one mating after 12 months. When placed in moist chambers these perithecia produced mature ascospores within

Fig. 1. Morphological structures of *Pyrenophora japonica*. (a) Vertical section through a beaked perithecium. (b) Mature, dark brown ascospores with rounded, conical end cells and larger central cells. (c) Bitunicate ascus with developing ascospores. Bar = 20 μ m.

2 weeks, thereby indicating the necessity of condensation for ascospore maturation (Smedegård-Petersen, 1972). The general morphology and dimensions of mature asci and

ascospores matched those originally found in the field (PREM 51136). No fertile perithecia developed in any of the matings where isolates were self-mated. However, protoperithecia were fre-

quently observed to develop, proving this species to be heterothallic.

Scott (1991) used symptom expression as well as morphological criteria to distinguish isolates of *P. teres* from those of *P. japonica*. We also inoculated barley seedlings with inoculum derived from single ascospore and conidial isolates, respectively. Wild-type isolates of *D. tuberosa* were obtained from typical spot-type lesions as described by Scott (1991). Inoculum was prepared by growing the different isolates on Purity agar (PA) (30 ml vanilla Purity (Gerber's Purity, Reckitt & Coleman South Africa Pty. Ltd), 15 g Biolab agar in 1 l water) at 25°C in the dark. Preliminary studies showed that *D. tuberosa* sporulated profusely on PA (J.P.J.L., unpublished data). Fully colonized plates were subsequently placed at 15°C under near-ultraviolet light for 21 days for conidiophore formation, and then in darkness for 7 days to induce production of conidia (Onesirosan & Bantari, 1969). The standard inoculation technique as described by Khan & Boyd (1969) was used. Stirling, an intermediately resistant cultivar, and experimental line B87/14 which is susceptible to *D. tuberosa* (Scott, 1992; Scott *et al.*, 1992), were inoculated in the two-leaf stage. Plants were initially sprayed with sterile distilled water containing 0.01% Tween 20 to reduce surface tension, and subsequently sprayed with suspensions containing 1300 conidia/ml (12 ml inoculum per pot; 10 plants per pot; two pots per isolate). Control plants were sprayed with sterile distilled water. The plants were kept in moist chambers for 24 h, and thereafter returned to the greenhouse (maximum temperature 20°C day, minimum 15°C night). Plants were rated for disease 2 weeks after inoculation.

Symptoms induced by the ascospore isolates were similar to spot-type symptoms induced by the conidial isolates of *D. tuberosa*. Leaves with symptoms were dried for 48 h at 25°C, then placed in moist chambers and incubated at 10°C under near-ultraviolet light for 7–21 days until sporulation occurred. The characteristics of the anamorph produced on these leaves by the teleomorph and by the wild-type isolates of *D. tuberosa* were similar: conidiophores were septate, curved, straight, or with a zigzagged upper part, and a swollen base. Conidia were olivaceous brown, with a brown dehiscence scar (2)–3–(4) µm wide, cylindrical, tapering to an acutely rounded base (0)–4–(7), distoseptate, measuring (25)–106–(161) × (10)–14–(18) µm, with secondary conidiophores frequently present. Similar

taxonomic features were recorded for *D. tuberosa* by Scott (1991).

The occurrence of the teleomorph state is important to pathologists, as it may play a role as a source of new virulence (Smedegård-Petersen, 1972; Shipton *et al.*, 1973; Steffenson & Webster, 1992), and also generate variation for fungicide resistance (Peever & Milgroom, 1992) and act as a source of inoculum (Smedegård-Petersen, 1972; Jordan, 1981). Further studies using more objective molecular techniques are required to resolve the question of the various *Pyrenophora* spp. occurring on barley in South Africa.

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