The morphology of *Dothistroma septospora* on *Pinus canariensis* from South Africa

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The morphology of *Dothistroma septospora* in pure culture and on the host, *Pinus canariensis* needles, collected from Isidenge State Forest, Stutterheim, South Africa, is described. The variation of dimensions on the conidia excludes assignment of these isolates to any specific variety.

S. Afr. J. Bot. 1984, 3: 397–401

Die morfologie van *Dothistroma septospora* in reinkultuur en op die gasheer, *Pinus canariensis*-naalde, versamel te Isidenge Staatsbos, Stutterheim, Suid-Afrika, word beskryf. Die variasie in groottes van konidiums sluit die aanduiding van spesifieke variëteite uit.

S-Afr. Tydskr. Plantk. 1984, 3: 397–401

Keywords: *Dothistroma* needle blight, *Pinus canariensis*

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Accepted 1 August 1984

Introduction

Dothistroma septospora (Dorog.) Morelet (syn. *D. pini* Hulbary; Sutton 1980) was first described from Russia (Doroguin 1912) as *Cytosporina septospora.* Later it was described as *Dothistroma pini,* the cause of needle blight of Austrian pines in Illinois (Hulbary 1941). Since then it has been reported from various countries, notably eastern Africa (Gibson 1962), Britain (Murray & Batko 1962), Rhodesia (Whiteside 1966), South Africa (Ivory 1967), Chile (Dubin & Walper 1967), Swaziland (Browne 1968) and Australia (Edwards & Walker 1978).

The taxonomy of *D. septospora* has been studied by various workers. Hulbary (1941) described the fungus as *Dothistroma pini.* Morelet (1968) regarded *D. pini* as identical with *Cytosporina septospora* Dorog. and made a new combination *D. septospora* (Dorog.) Morelet. The combination was accepted by Sutton (1980) who cites *Septoriella septospora* (Dorog.) Sacc. apud Trotter as an additional synonym.

Thyr & Shaw (1964) described two varieties, viz. *D. pini* var. *pini* and *D. pini* var. *linearis,* on the basis of conidial length. Ivory (1967) added a third variety, *D. pini* var. *keniensis* from Kenya, and related these varieties to the geographical distribution of the fungus. This division of the species has been questioned by Funk & Parker (1966) and Sutton (1980).

The teleomorph was described as *Scirrhia pini* on various species of pines from British Columbia by Funk & Parker (1966). According to Ivory (1967) the corresponding anamorph is *D. pini* var. *linearis* whereas Sutton (1980) relates *S. pini* to *D. septospora* var. *septospora* (syn. *D. pini* var. *pini*) which constitutes an inconsistency.

Hulbary (1941) did not mention red bands in association with conidiomata on the needles. Shain & Franich (1981) reported that the red colour in the lesions was due to accumulation of dothistromin, a difuranoanthraquinone, during the infection process. This red colouration was positively correlated with light intensity and *D. pini* infection (Gadgil & Holden 1976). Shain & Franich (1981) reported a relationship between dothistromin concentration and the length of the lesions. Gibson (1965) reported blight symptoms on naturally infected needles in Kenya, which disappeared when the seedlings were moved into the shade. Development of natural (Gadgil & Holden 1976) and artificially induced lesions (Shain & Franich 1981) were favoured by high light intensity.

Dothistroma septospora was found on *Pinus canariensis*

needles collected from the Isidenge State Forest, Stutterheim, in July 1982.

The purpose of this paper is to report on the morphology of this fungus on the host and in culture to confirm its occurrence in South Africa.

Materials and Methods

Pine needles severely affected by dieback were collected from Isidenge State Forest, Stutterheim, eastern Cape Province, South Africa. This material as well as two pure cultures made by Dr John E. Lundquist, Forest Pathologist, Plant Protection Research Institute, then stationed at Stellenbosch, S.A., were studied. An additional isolation was made by plating out conidia dissected from a stroma on one of the needles on 1,5% malt extract agar (MEA).

Cultures were grown on 1,5%, 2% and 3% MEA (Ainsworth 1971) and 2% MEA pH 5,5 (Dubin & Walper 1967) at 24°C under intermittent illumination 12 h/d, by mixed near ultra violet (Philips TL 40W/08 RS) and day light fluorescent tubes suspended 500 mm above the plates. Material for microscopic examination was mounted in lactophenol (Ainsworth 1971) and alkaline erythrosin (Sutton 1980).

Specimens examined

In herb. PREM: 47248 on *Pinus canariensis*, Isidenge State Forest, Stutterheim C.P. July 1982; dried cultures grown on MEA PREM 47249 *Lundquist 74*, PREM 47250 *Lundquist 75*, PREM 47251 *Roux, Louise.*

Live cultures of these isolates are deposited in the Culture Collection, National Collection of Fungi, Private Bag X134, Pretoria 0001.

Results

Stromata of *Dothistroma septospora* were present as elongated black structures between the rows of stomata on the surface of moribund sections of the needles. They were subepidermal at first, later erumpent and breaking the epidermis longitudinally. Prominent red bands as wide as the conidiomata were associated with some stromata, while brown bands, darker in colour than the surrounding tissue, were associated with others. The black appearance of the stromata was due to dust contamination.

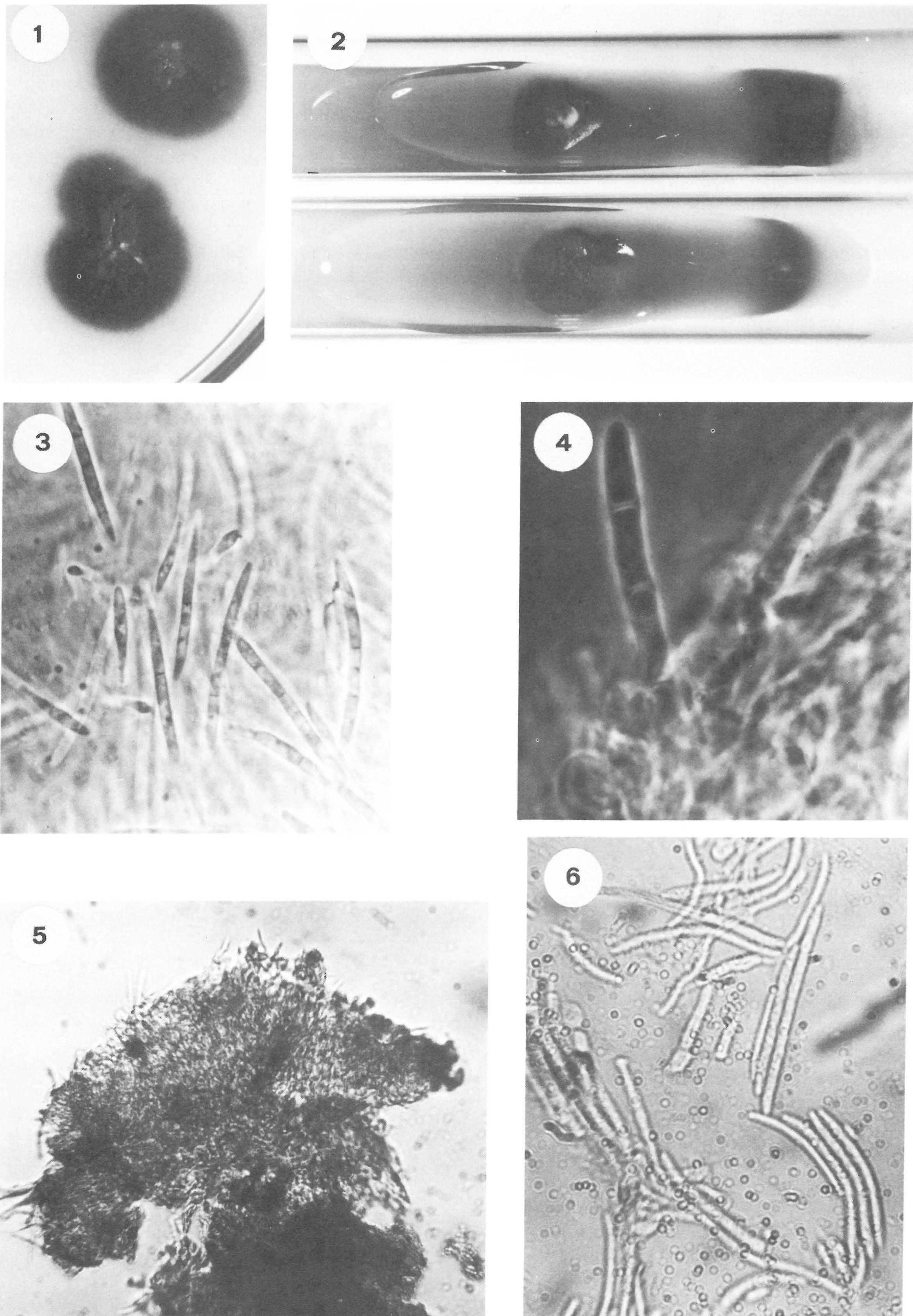
Three isolates were grown on MEA of three concentrations. They grew exceedingly slowly on 1,5% MEA attaining a maximum colony diameter of 10 mm in 30 days (Figure 1). The growth rate could be doubled when the cultures were grown in sealed petri dishes. The colony diameter was directly related to the size of the inoculum used, for single conidia grew much more slowly than greater numbers of conidia inoculated together. The ability of the cultures to sporulate upon sub-culturing declined while the conidia increased in length. This was demonstrated when the descriptions were repeated six months after the initial descriptions were done (Table 1).

Colonies on 3% MEA were dark vinaceus brown (Plate xxxix 1k, Ridgway 1912), the reverse blackish brown (Pl. xxxix 1m). The colonies were covered with conidia which

Table 1 The micromorphology and effect of different concentrations of MEA on three isolates of *Dothistroma septospora* in culture over a period of time, and on needles of *Pinus canariensis*

Isolate and medium	Conidial length (μm) minimum mode maximum	Conidial breadth (μm) minimum mode maximum	Septation least mode most	Conidiogenous cells min. max. length × breadth
At initial isolations				
1,5% MEA				
PREM 47249	21,1–25,6–58,5	1,2–2,3–2,3	1–3–3	*
PREM 47250	21,1–37,4–57,3	0,6–1,2–1,8	1–3–3	*
PREM 47251	26,9–35,1–46,8	2,3–2,3–2,3	1–2–3	*
2% MEA pH 5,5				
PREM 47250	15,2–29,3–37,4	1,8–1,8–2,3	1–3–3	3–5,9 × 5,9–9,4
3% MEA				
PREM 47249	17,6–25,7–30,4	1,2–2,3–2,3	1–3–3	5–5,9 × 3,5–5,9
PREM 47251	23,4–35,1–58,5	2,3–2,3–3,5	1–3–3	2,3–3 × 3,5–5,9
After six months				
1,5% MEA				
PREM 47249	19,9–30,4–46,8	1,8–1,8–3,5	2–3–6	*
PREM 47250	21,1–35,1–56,2	1,2–1,8–2,3	2–4–6	*
PREM 47251	25,7–41,0–50,5	1,8–2,3–2,3	2–4–5	*
2% MEA				
PREM 47249	18,7–30,4–52,7	1,2–1,8–2,3	1–4–6	*
PREM 47250	30,4–37,4–49,1	1,8–2,3–2,3	2–4–6	*
PREM 47251	28,1–37,4–46,8	1,8–1,8–2,3	2–3–5	*
3% MEA				
PREM 47249	23,4–35,4–52,7	1,2–1,8–2,3	1–3–6	*
PREM 47250	21,1–41,0–53,8	1,8–1,8–2,3	1–3–6	*
PREM 47251	23,4–41,0–53,0	1,8–1,8–2,3	2–3–6	*
Pinus canariensis needles				
PREM 47248	16.3–25,7–31,6	1,2–2,3–2,3	1–3–3	2,9–5,9 × 3,9–5,9

* not measured



Figures 1–6 Figure 1 Colonies grown on 1,5% malt extract agar for 30 days, seven colonies per 90 mm petri dish. **Figure 2** Colonies of two different isolates grown on 1,5% malt extract agar slants in test tubes producing an exudate in the medium. **Figure 3** Conidia showing septa from a culture of *D. septospora* on 1,5% malt extract agar × 1 800. **Figure 4** Ampulliform conidiogenous cell and immature septate conidium still attached to conidiogenous cell × 4 500. **Figure 5** Eustromatic conidioma with definite stipe dissected from pine needle × 450. **Figure 6** Conidia produced on host tissue × 1 800.

gave sporulating cultures a pinkish buff (Pl. xiii 3m) appearance but which turned darker with age and merged with total colony colour also giving a sodden texture to the colony. A vinaceus brown pigment (Pl. xxx lx) diffused into the medium resulting in a diamine brown (Pl. xxlx f3) reverse colour (Figure 2). Isolate PREM 47250 was grown on 2% MEA at pH 5,5 (Dubin & Walper 1967) to stimulate the formation of exudate, but without success. This exudate, dothistromin (Shain & Franich 1981) was produced mostly by isolate PREM 47251 on 1,5% MEA during the second set of descriptions.

The conidiomata in culture were unilocular, eustromatic, closely spaced and separated in some cultures by yellowish setose-like hyphae. The stromata covered most of the upper surface of the colony.

The conidia produced in culture were mostly straight, filiform, hyaline, 1–3 septate, continuous, thin-walled, smooth. The mode length varied from 25,7 to 35,1 μm for the first set and 30,4 to 41,0 μm for the second set of descriptions. Initially 3-septate conidia were most commonly found (Figure 3), whereas a tendency to 4-septate conidia was recorded in the second set. The conidiogenous cells were ampulliform to lageniform, hyaline, producing holoblastic conidia, mostly 5,9 $\times$ 5,9 μm (Figure 4).

The conidiomata on the host were sub-epidermal becoming erumpent and acervular. The stromata consisted of pale brown, thin-walled textura angularis, were eustromatic and of darker brown thick-walled tissue. A stipe consisting of a thick hyphal mass anchored the stroma to the hypodermal layers of the pine needle (Figure 5). Masses of slimy hyaline, filiform, straight and curved, smooth, continuous, thin-walled, 1–3 euseptate conidia were produced holoblastically (Figure 6). The conidiogenous cells were ampulliform, hyaline, smooth, non-proliferating and formed by the upper cells of the stroma. Conidiophores were not observed.

Discussion

Red and brown bands on pine needles can be caused by a variety of organisms, e.g. as the result of insect bites and infection by other fungi such as *Scirrhia acicola* (brown spot disease) which is a serious disease, especially of long leaf pine in the U.S.A. (Peterson 1981). Red band disease usually defoliates the trees (Gibson 1972) but the trees studied here were not severely attacked. The needle beyond the point of infection was killed and dropped off eventually. This meant that the photosensitization ability of the affected needle was diminished and the timber yield was less because the trees grew more slowly.

The absence of red bands in some conidiomata of the specimen studied here might be due to a lack of high light intensity and confirms earlier observations of their erratic presence. The presence of red bands, therefore, appears to be an unreliable symptom in the recognition of *D. septospora* infection in pines. The cultures grown on 2% MEA pH (Dubin & Walper 1967) did not consistently produce a red colour in the medium. However, PREM 47251, taken from a conidioma within a red band did produce a red colour irrespective of the medium on which it was grown. This might point to an inherent ability of the particular isolate to produce dothistromin (the red exudate).

The conidial dimensions observed in the cultures grown on various concentrations of MEA showed that conidial size of a particular isolate was definitely affected; higher concentrations giving larger conidia. When the descriptions were repeated after six months in storage, it was found that the conidia measured were significantly larger with more septa. This could be attributed to the selection of a viable culture which turned out to be phenotypically at the top of the range previously studied. Great difficulty was experienced in reviving some of the isolates, especially those which were lyophilized. Lyophilization was not a satisfactory method of storage for these isolates. Conidia produced in culture by some isolates were larger than those produced on the needles. This confirms observations by Thyr & Shaw (1964), Dubin & Walper (1967) and Ivory (1967). The differences in conidial size observed in these isolates which were obtained from different conidiomata from the same collection of needles, indicate that different varieties of *D. septospora* might be present in the needles. The occurrence of more than one variety in one locality, has, however, never been reported before.

The size of the conidia reported here have just as great a range of conidial size within one sample as those reported by Ivory (1967), whereas the mode falls between those given for the types of *D. pini* var. *linearis* [syn. *D. septospora* var. *linearis* (Sutton 1980)] and *D. pini* var. *keniensis* [syn. *D. septospora* var. *keniensis* (Sutton 1980)] (Ivory 1967). Ivory's material was not available for examination because the present locality is unknown. The isolates studied here can, therefore, not be assigned to any variety of *D. septospora* with any certainty. They agree, however, with Sutton's (1980) description of this species.

Acknowledgements

I am grateful to Dr J.E. Lundquist for collecting and submitting the material studied, Dr G.C.A. van der Westhuizen for reading the manuscript and the technical staff of the Mycology unit for their assistance, especially Mrs Louise Schutte.

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