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VERTICICLADIELLA ALACRIS SP.NOV., ASSOCIATED WITH A ROOT DISEASE OF PINES IN SOUTH AFRICA

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A *Verticicladiella* species found associated with a root disease of *Pinus pinaster* Ait. and *P. radiata* D. Don. in South Africa is described as *V. alacris* Wingfield & Marasas sp.nov. A comparison is made between this and *V. procera* Kendrick and *V. wageneri* Kendrick. Some undescribed morphological characteristics observed in authenticated cultures of the last two species are recorded.

Members of the *Leptographium* complex were reclassified by Kendrick (1962) who divided them into three genera: *Verticicladiella* Hughes, *Leptographium* Lagerberg & Melin, and *Phialocephala* Kendrick. Conidiogenesis in *Verticicladiella* is sympodial whereas *Leptographium* and *Phialocephala* have annellidic and phialidic conidiogenesis, respectively (Kendrick, 1962).

Kendrick (1962) described seven species of *Verticicladiella*: *V. antibiotica* Kendrick, *V. abietina* (Peck) Hughes, *V. brachiata* Kendrick, *V. penicillata* (Grosm.) Kendrick, *V. procera* Kendrick, *V. serpens* (Goid.) Kendrick, and *V. wageneri* Kendrick. Subsequently Kendrick & Molnar (1965) described *Ceratocystis dryocoetidis* Kendrick & Molnar, with *V. dryocoetidis* Kendrick & Molnar as the imperfect state. *Ceratocystis europhioides* Wright & Cain and *C. huntii* Robinson also have *Verticicladiella* imperfect states according to Davidson & Robinson-Jeffrey (1965). In addition, three species of *Europhium*, *E. clavigerum* Robinson & Davidson, *E. aureum* Robinson & Davidson and *E. robustum* Robinson & Davidson described by Robinson-Jeffrey & Davidson (1968) are also reported to have *Verticicladiella* imperfect states.

Verticicladiella species usually inhabit conifer wood and are often associated with bark beetles and blue stain (Kendrick, 1962). *Verticicladiella procera* and *V. wageneri* are known causal organisms of root diseases of conifers (Kendrick, 1962; Towers, 1977; Wagener & Mielke, 1961).

This paper describes a new species of *Verticicladiella* found associated with a root disease of *Pinus* spp. in various parts of the Western Cape, South Africa (M. J. Wingfield, unpubl.). A comparison is also made with isolates of two related species, *V. procera* and *V. wageneri*.

MATERIALS AND METHODS

Cultures of the *Verticicladiella* were isolated from roots of dying *Pinus pinaster* Ait. and *P. radiata* D. Don. and grown on half-strength malt extract agar (10 g Difco malt extract, 15 g Difco Bacto agar/l distilled water) in Petri dishes at 24 °C. Cultures of *V. procera* DAOM 62096 (Kendrick, 1962) and *V. wageneri* (isolated from roots of *Pseudotsuga menziesii* (Mirb.) Franco from Prospect, Oregon) were supplied by D. J. Goheen, U.S.D.A. Forest Service, Portland, Oregon and were used for comparative purposes. Comparative growth rates were determined by calculating the average colony diameters of five replicates of each isolate growing on half-strength malt extract agar in Petri dishes. Temperatures at which measurements were made ranged from 10° to 30° at 5° intervals. Scanning electron microscopy (SEM) was used to examine conidiogenous cells. Blocks of cultures on agar were dried for SEM using the critical point technique of Cohen (1970). Specimens were coated with gold paladium and examined in a Jeol JSM-45 scanning electron microscope.

RESULTS

The morphological characteristics of the *Verticicladiella* species isolated from pines in South Africa were found to differ from those described for all the previously known species. Consequently this fungus is described as new.

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Verticicladiella alacris sp.nov.

(*Etym:* Latin alacris = energetic, alive. The epithet refers to the pathogenic abilities of this species and to the rapid growth in culture and production of robust fruiting structures).

Coloniae in agaro 'malt' apud 25° rapide crescentes, diam aetate 4 dierum 7·6 cm, primum hyalinae deinde nigrae, margine undulata. Mycelium aerium perpaucum vel deest. Mycelium immersum ex hyphis septatis, undulatis vel helicoideis, laevis, interdum ramosis, hyalinis ad brunneis, 1·2–9·6 μm diam compositum. Conidiophora macronematosa, mononematosa, cum hyphis rhizoidalibus ad basas. Stipites erecti, 372–878 μm long., ad 14-septati, 15–22 μm lat. ad basas, attenuati ad summas 9–10 μm lat., atrofusci ad basas, spicem versus pallidiores, simplices, glabro-tunicati, parietibus ad 1·9 μm crass. Apparatus sporogenus 36–100 μm long., ex 3–5 seriebus metularum, distaliter circa 675 sympodulas ferentes. Metulae primariae 4–8, plerumque 5–6, fuscae, 9·0–24·0 × 3·6–8·4 μm, metula primaria centralis 13·2–25·2 × 7·2–13·7 μm; metulae secundariae pallidiores, ceterae hyalinae. Cellulae conidiogenae (sympodulae) densae, numerosae, hyalinae, simplices, subuliformes, laeves sed in parte sporifera minute irregulari quia hili parvi adsunt, 7·8–21·0 × 0·9–2·4 μm. Conidia continua, hyalina, laevia, obovoidea vel ellipsoidea vel clavata, ad basas truncata, 2·4–7·2 × 1·2–2·4 μm, aggregantia et capitulum mucosum cremeum formantia, in aetate dilute fuscum.

Colonies fast growing on half strength malt agar at 25°, reaching 7·6 cm diam in 4 days and covering the plates in 5 days. At incubation temperatures below 25° the growth rate is reduced (Table 2) and little growth occurs at 10°. Colonies are initially hyaline but gradually darken, at first to dark brown then black. Little or no aerial mycelium is produced (Fig. 1). The immersed mycelium at the margin of young colonies consists of sparingly branched helicoid hyphae which give the advancing zone an undulate appearance. As the colony matures the immersed hyphal strands become interwoven to form a dense mycelial mat. *Hyphae* vary from hyaline to dark brown, straight or undulate to helicoid, often with peg-like outgrowths somewhat resembling hyphopodia (Fig. 2), smooth-walled but sometimes very thick-walled and roughened with age, sparingly branched, septate with septa spaced 6–120 μm apart, very variable in diameter, 1·2–9·6 μm. *Conidiophores* produced abundantly over the entire colony in cultures incubated in the dark at 25° after 10 days on half strength malt agar. They are macronematous, mononematous, and have short, occasionally septate, darkly pigmented rhizoidal hyphae attached to the base of the stipe (Figs 3–4). *Stipe* erect, 372–878 μm long, with up to 14 septa, 15–22 μm wide at the base, tapering to 9–10 μm wide just below the slightly swollen and rounded apex, dark brown at the base and becoming paler brown towards the apex. The wall of the stipe is usually smooth, up to 1·9 μm thick at the base. The septa are 1·9–3·8 μm thick and septal pores are clearly visible. *Sporogenous apparatus* (Figs 5–6) 36–100 μm long excluding the conidial mass. There is a central primary metula, 13·2–25·2 μm long and 7·2–13·7 μm wide which is larger than the 3–7, usually 5 or 6, surrounding primary metulae, 9·0–2·40 × 3·6–8·4 μm. Above the primary metulae are 2 to 4 further series of metulae. The central primary metula gives rise to up to 7 secondary metulae which bear 2 further series of metulae; each surrounding primary metula usually gives rise to 3 secondary metulae and these in turn bear 3 further metulae each. The primary metulae are concolorous with the upper portion

Figs 1–11. *Verticicladiella alacris* holotype, PREM 45483.

Fig. 1. Culture showing dark, densely interwoven, immersed mycelium.

Fig. 2. Darkly pigmented hyphae showing peg-like outgrowths. × 600.

Fig. 3. Rhizoidal hyphae at base of conidiophore. × 600.

Fig. 4. Erect conidiophore with rhizoids. × 100.

Fig. 5. Conidiogenous apparatus showing secondary and successive series of metulae and conidiogenous cells. × 2000.

Fig. 6. Conidiogenous apparatus with arrow indicating central primary metula. × 960.

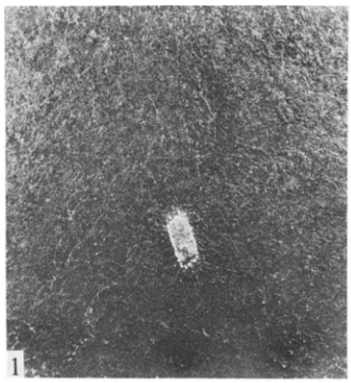
Fig. 7. Sympodial conidiogenesis and irregular walls of conidiogenous cells. × 9400.

Fig. 8. Conidia. × 15000.

Fig. 9. Immature macronematous conidiophore with surrounding micronematous conidiophores and conidia (arrows). × 360.

Fig. 10. Conidiophore with conidiogenous cells developed on the lateral wall of the stipe. × 300.

Fig. 11. Conidiogenous apparatus with proliferation of the secondary metulae to give rise to additional sporogenous apparatus (arrow). × 1000.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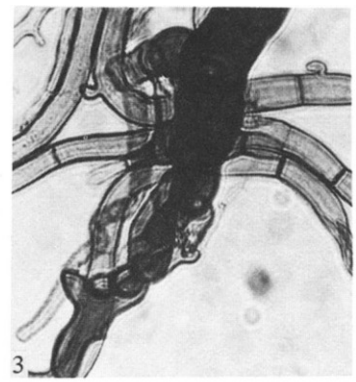
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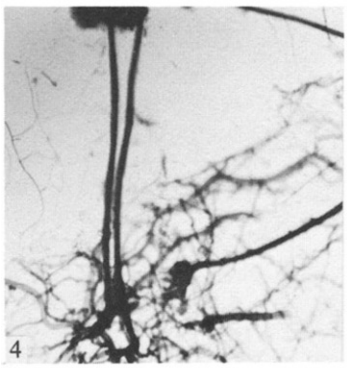
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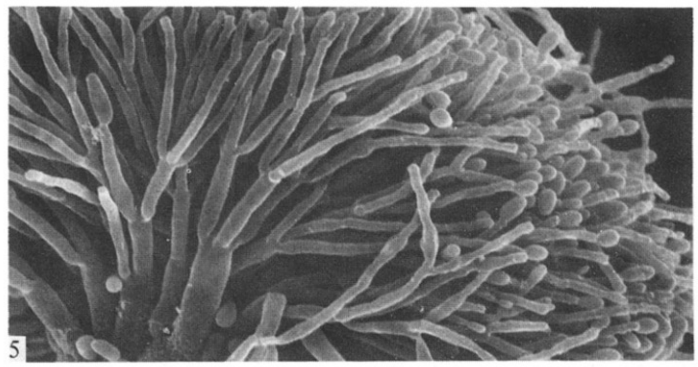
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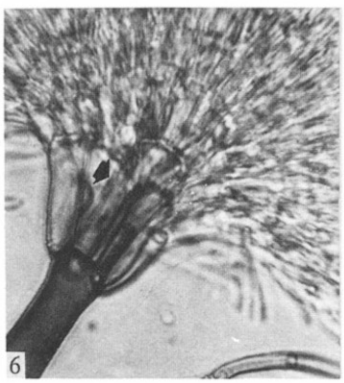
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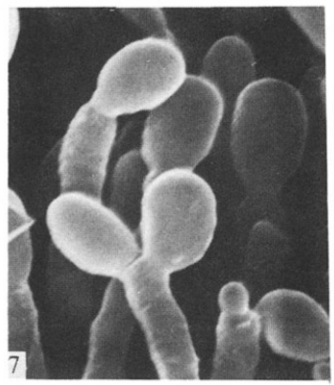
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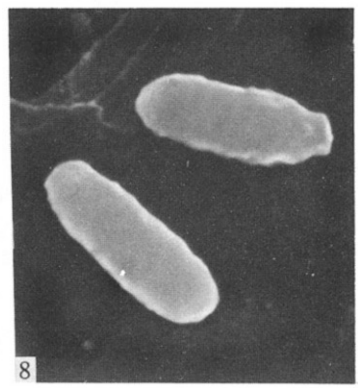
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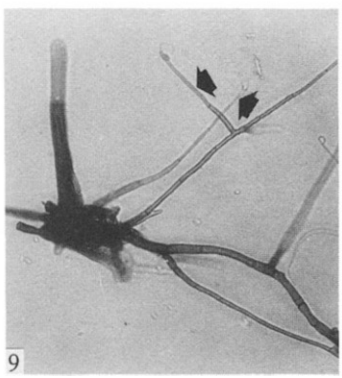
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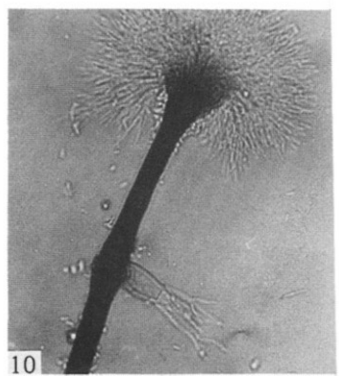
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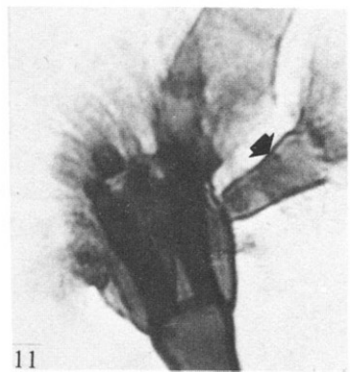
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Table 1. *Comparison of the type culture of* Verticicladiella alacris *with isolates of* V. wageneri *and* V. procera*

	V. alacris†	*V. procera*‡	*V. wageneri*§
Colony colour	Pale brown then black	Chaetura black	Chaetura black
Colony margin	Undulate	Regularly finely fibrillose	Irregular with radial fibrils
Aerial mycelium	Little or none	Present and well developed	Little or none
Hyphal type	Straight, undulate to helicoid (Fig. 12)	Straight, sparingly branched (Fig. 13)	Straight, undulate to helicoid (Fig. 14)
Hyphal colour	Hyaline to dark brown	Hyaline to light brown	Hyaline to dark brown
Hyphal diameter	1·2–9·6 μm	0·6–5·7 μm	1·8–12·0 μm
Rhizoids	Poorly developed (Fig. 15)	Well developed (Fig. 16)	Poorly developed, bulbous (Fig. 17)
Conidiophore distribution	Single	In groups of 2–6 or more (Fig. 13)	Single
Stipe length	372–875 μm	Up to 979 μm	Up to 870 μm
Stipe width at base	15–22 μm	3·0–14·9 μm	6·0–16·4 μm
Stipe septa	Up to 14	Up to 8	Up to 12
Sporogenous apparatus length	36–100 μm	12·6–108 μm	33·8–87·8 μm
Primary metulae: number	4–8	2–3	2–10
Primary metulae: size	9·0–25·2 × 3·6–13·7 μm	1·8–5·4 × 13·2–29·4 μm	3·0–12·0 × 9·0–30·0 μm
Robust central primary metula	Present (Fig. 18)	Absent (Fig. 19)	Present (Fig. 20)
Series of metulae	3–5	3–5	3–5
Sympodulae: size	7·8–21·0 × 0·9–2·4 μm	7·2–15·6 × 1·0–1·8 μm	8·4–24·0 × 1·2–2·4 μm
Conidial shape	Obovoid, ellipsoidal or clavate, usually straight, rarely slightly curved	Obovoid, usually straight, seldom slightly curved	Obovoid, frequently curved
Conidial size	2·4–7·2 × 1·2–2·4 μm	1·5–7·8 × 0·6–2·4 μm	3·0–9·0 × 1·2–2·4 μm
Micronematous conidiophores	Sparse (Fig. 21)	Sparse (Fig. 22)	Abundant, well developed (Fig. 23)
Micronematous conidia	Similar, but smaller than other conidia	Similar, but smaller than other conidia	Larger, distinct from other conidia, 3·6–15·0 × 1·2–5·4 μm (Fig. 23)

* Morphological characteristics of cultures on half strength malt extract agar incubated at 25°.
† *V. alacris* type strain PREM 45483.
‡ *V. procera* strain DAOM 62096.
§ *V. wageneri* isolate supplied by D. J. Goheen.

Figs 12–23. Comparison of morphological characteristics of *Verticicladiella alacris* (holotype, PREM 45483), *V. procera* (DAOM 62096) and *V. wageneri* (isolate supplied by D. J. Goheen).

Fig. 12. *V. alacris*, helicoid and undulate hyphae at margin of culture. × 600.

Fig. 13. *V. procera*, margin of young culture showing groups of conidiophores and straight hyphae. × 200.

Fig. 14. *V. wageneri*, helicoid hyphae. × 180.

Fig. 15. *V. alcaris*, rhizoidal hyphae. × 1000.

Fig. 16. *V. procera*, rhizoidal hyphae. × 700.

Fig. 17. *V. wageneri*, rhizoidal hyphae. × 700.

Fig. 18. *V. alacris*, conidiogenous apparatus. × 600.

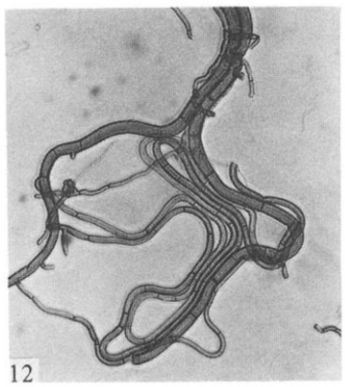
Fig. 19. *V. procera*, conidiogenous apparatus. × 800.

Fig. 20. *V. wageneri*, conidiogenous apparatus. × 760.

Fig. 21. *V. alcaris*, micronematous conidiophores. × 750.

Fig. 22. *V. procera*, micronematous conidiophores. × 720.

Fig. 23. *V. wageneri*, micronematous conidiophores. × 1000.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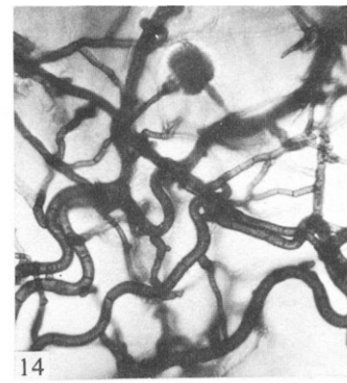
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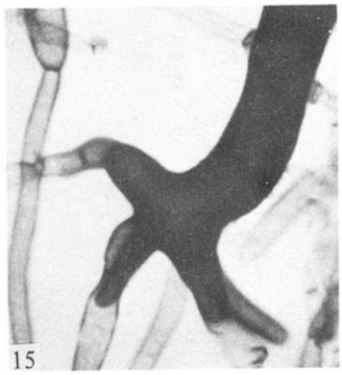
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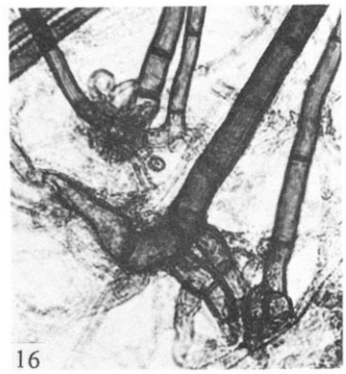
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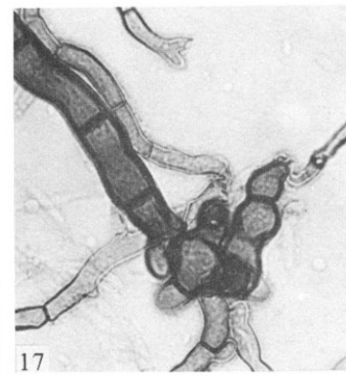
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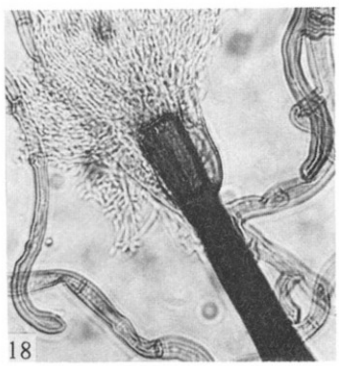
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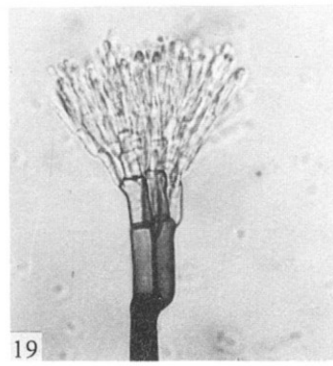
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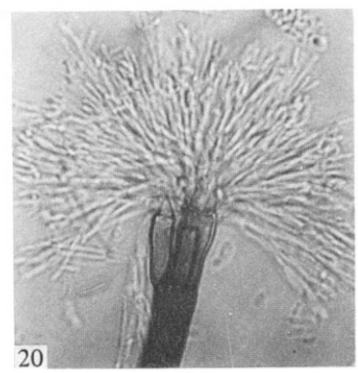
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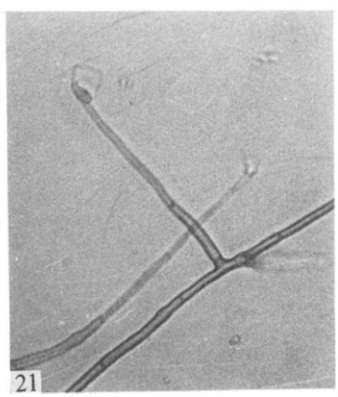
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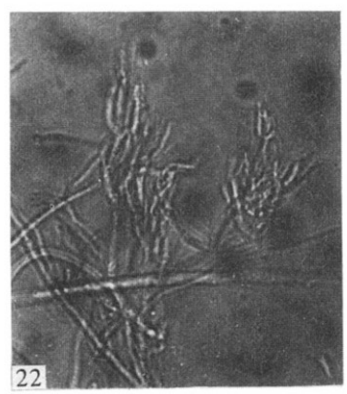
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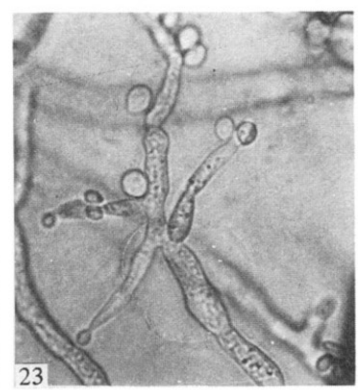
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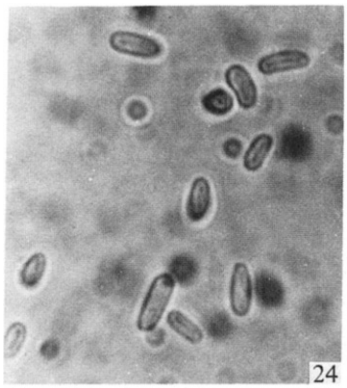

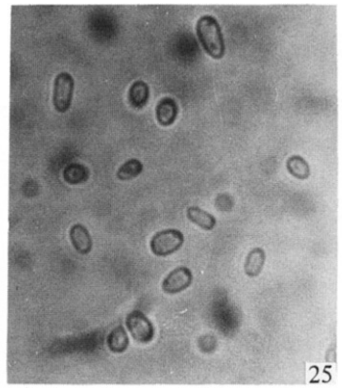

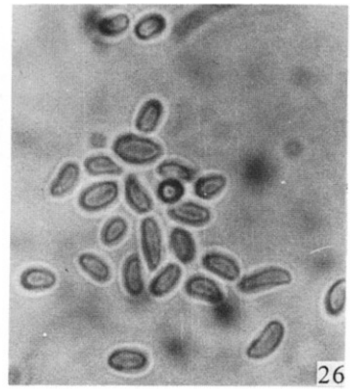

Figs. 24–26. Comparison of conidia of *Verticicladiella alacris* (holotype, PREM 45483), *V. procera* (DAOM 62096) and *V. wageneri* (isolate supplied by D. J. Goheen).

Fig. 24. *V. alacris*, conidia. ×1750.

Fig. 25. *V. procera*, conidia. ×1750.

Fig. 26. *V. wageneri*, conidia. ×1750.

of the stipe, the secondary metulae are paler brown in colour, and the additional series of metulae and conidiogenous cells are hyaline. *Conidiogenous cells* (sympodulae) discrete, densely crowded, up to 675 per stipe, hyaline, simple, elongate, tapering slightly from the base to the fertile apex, smooth-walled except in the apical region where the walls are irregular due to the presence of abscission scars (Fig. 7), polyblastic, sympodial, 7·8–21·0 × 0·9–2·3 μm. *Conidia* one-celled, hyaline, smooth-walled, obovoid, ellipsoidal or clavate, straight or sometimes slightly curved near the truncate base (Figs 7, 8), 2·4–7·2 × 1·2–2·4 μm. The conidia accumulate around the sporogenous apparatus in a mucilaginous mass which is hyaline at first and becomes yellowish-cream to light brown with age. In addition to the macronematous conidiophores, hyaline aerial hyphae at the base of the stipes sometimes function as micronematous conidiophores and give rise to small numbers of conidia at the apex (Fig. 9). A less developed sporogenous apparatus may also form on the lateral walls of the stipe (Fig. 10) and primary metulae sometimes elongate to form a second stipe which gives rise to an additional sporogenous apparatus (Fig. 11).

Specimens examined: Cultures on half strength malt agar, isolated from roots of *Pinus pinaster*, Tokai, Cape Town, South Africa, May 1978, M. J. Wingfield, PREM 45483, holotype; from roots of *Pinus pinaster*, Grabouw, Cape Province, South Africa, Feb. 1978, M. J. Wingfield, PREM 45484; from roots of *Pinus pinaster*, Lebanon State Forest, Cape Province, South Africa, Apr. 1978, M. J. Wingfield, PREM 45485; from roots of *Pinus radiata*, Grabouw, Cape Province, South Africa, Mar. 1979, M. J. Wingfield, PREM 45486.

Table 2. *Comparative growth rates of* Verticicladiella alacris, V. procera *and* V. wageneri *at different incubation temperatures*

Isolate* / Temperature (°C)	Colony diameter (cm)† 10	15	20	25	30
V. alacris	2·8	6·4	6·9	7·6	2·8
V. procera	—	2·6	2·7	4·5	—
V. wageneri	—	3·8	4·2	—	—

* *V. alacris* type strain PREM 45483, *V. procera* strain DAOM 62096, *V. wageneri* isolate supplied by D. J. Goheen.

† Each value represents the mean of five replicates after 4 days' growth on half-strength malt extract agar.

Dried down cultures have been deposited in the Mycological Herbarium of the Plant Protection Research Institute, Private Bag X134, Pretoria, South Africa (PREM). Subcultures of the type strain have also been deposited in the CBS, IMI and DAOM culture collections.

The morphological characteristics of *V. alacris* as determined above most closely resemble those described for *V. procera* and *V. wageneri* as described by Kendrick (1962). All attempts to obtain type cultures of these two species from ATCC, CBS, IMI and DAOM were unsuccessful. However, examination of an authenticated isolate of *V. wageneri* obtained from Dr D. J. Goheen and an isolate of *V. procera* also examined by Kendrick (1962) revealed that both strains differ

in certain respects from Kendrick's (1962) original descriptions. A comparison of the morphological characteristics of *V. alacris* with those of the available isolates of *V. procera* and *V. wageneri* is thus presented (Table 1). The major morphological differences between the three species are illustrated in Figs 12–26. Differences in growth rates are shown in Table 2.

Verticicladiella alacris differs from *V. procera* in having a faster growth rate over a wider temperature range, the absence of aerial hyphae in older cultures, darker pigmented hyphae, less developed rhizoidal hyphae, solitary as compared with groups of up to seven or more conidiophores, and a larger number of primary metulae (Tables 1, 2). Important differences between *V. alacris* and *V. wageneri* include a considerably faster growth rate, a higher optimum temperature for growth, better developed rhizoidal hyphae, a longer sporogenous apparatus, fewer curved conidia and the absence of well-developed micronematous conidiophores (Tables 1, 2).

DISCUSSION

A comparison of *V. alacris* with *V. procera* and *V. wageneri* has resulted in the observation of certain undescribed features of the last two species. Some of the features may represent reported variation within these species (Goheen & Cobb, pers. comm.). Growth rate is an important distinguishing characteristic between the three species of *Verticicladiella* examined. *Verticicladiella alacris* has a considerably faster growth rate than that reported for *V. wageneri* (Kendrick, 1962). As far as is known no growth temperatures have been reported for *V. procera* prior to the present study. It is of interest that *V. alacris* grows optimally at 25° and that the mean annual temperature of areas in which this fungus has been found ranges from 13–18° (Poynton, 1957). However, unlike *V. wageneri*, *V. alacris* is able to grow over a wide temperature range and pathogenicity is unlikely to be affected as in the case of *V. wageneri* (Smith, 1967). The helicoid hyphae observed in *V. wageneri* were not reported by Kendrick (1962). They are, however, less developed than those characteristic of *V. alacris*. Kendrick (1962) reported the presence of rhizoidal hyphae in *V. procera* but not in *V. wageneri*. Although rhizoidal hyphae are present in isolates of all three species examined, these structures are least developed in *V. wageneri*.

The arrangement of the primary metulae appears to be an important distinguishing characteristic in the genus *Verticicladiella*. Although a central primary metula, distinct from the other primary metulae, was not reported in *V. wageneri* by Kendrick (1962), such a distinct primary metula was definitely present in the isolate examined by the present authors. A similar central primary metula was also present in all the isolates of *V. alacris* but was not observed in the isolate of *V. procera* examined. A central primary metula has also been reported in *V. serpens* by Gambogi & Lorenzini (1977).

The arrangement in groups of the macronematous conidiophores in *V. procera* clearly distinguishes this species from *V. alacris* and *V. wageneri*. This characteristic is, however, not mentioned in the original description of the species (Kendrick, 1962). Well-developed micronematous conidiophores are described in *V. wageneri* by Kendrick (1962). These structures are clearly present in the isolate of *V. wageneri* examined but the conidia produced by these structures appear to be different in size and shape from those borne on the macronematous conidiophores. Although present in *V. alacris* and *V. procera*, micronematous conidiophores are poorly developed with no obvious difference in size and shape of the conidia.

No perfect stage has been found associated with *V. alacris*. Presumably the ascigerous state, if it exists, would fall within the genus *Ceratocystis* or a closely related genus as has been found with other *Verticicladiella* species (Davidson & Robinson-Jeffrey, 1965; Goheen & Cobb, 1978; Kendrick, 1962; Robinson-Jeffrey & Davidson, 1968).

As far as the present authors are aware, the description of *V. alacris* represents the first report of this genus from Africa.

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