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SOME *VERTICICLADIELLA* SPECIES, INCLUDING *V. TRUNCATA* SP.NOV., ASSOCIATED WITH ROOT DISEASES OF PINE IN NEW ZEALAND AND SOUTH AFRICA

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Verticicladiella procera and two undescribed *Verticicladiella* species were identified from a collection of *Verticicladiella* isolates associated with a *Pinus strobus* L. root disease in New Zealand. One of the undescribed *Verticicladiella* species was also isolated from diseased roots of *Pinus taeda* L. in South Africa and is described as *V. truncata* sp.nov. *V. truncata* was found to be pathogenic and is carried by a scolytid bark beetle.

Verticicladiella species are distinguished from other members of the *Leptographium* complex by their sympodial conidial ontogeny (Kendrick, 1962). They commonly inhabit conifer wood and are often associated with blue-staining and bark-beetle infestations (Davidson & Robinson-Jeffrey, 1965; Goheen & Cobb, 1978; Kendrick, 1962; Wingfield & Marasas, 1980). A number of species have been reported as root pathogens of conifers from various parts of the world (Halambek, 1976; Shaw & Dick, 1980; Wagner & Mielke, 1961; Wingfield & Knox-Davies, 1980*a*). The best known of these are *V. wageneri* Kendrick on various *Pinus* spp. and *Pseudotsuga menziesii* (Mirb.) Franco (Smith & Graham, 1975) and *V. procera* on *Pinus strobus* (Towers, 1977) in the U.S.A.

An unidentified *Verticicladiella* sp. was recently reported causing a root disease of *P. strobus* in New Zealand (Shaw & Dick, 1980). *Verticicladiella* isolates from diseased trees in New Zealand were identified by the present authors as *V. procera* and two unknown *Verticicladiella* spp. One of the unknowns was subsequently found in South Africa associated with diseased roots of *P. taeda*. This paper describes the unknown *Verticicladiella* from South Africa and New Zealand, comments on its pathogenicity and records the presence of *V. procera* in New Zealand.

MATERIALS AND METHODS

Seven *Verticicladiella* isolates from *P. radiata* D. Don and *P. strobus* collected during a previous study in New Zealand (Shaw & Dick, 1980) were supplied by Mrs M. Dick, Forest Research Institute, Rotorua, New Zealand. All isolates were grown in Petri dishes on Difco corn meal agar (CMA) at 20 °C and subjected to 12 h alternating cycles of near-ultraviolet light.

Isolations were made from diseased roots of *P. taeda* from the Wilgeboom seed orchard and Mariti plantation near Sabie, Eastern Transvaal, South Africa. Root samples were surface sterilized in a solution of commercial sodium hypochlorite (1% available chlorine) and plated on CMA. Isolation from bark beetles (Coleoptera: Scolytidae) identified as *Hylastes angustatus* Herbst (National Collection of Insects, Plant Protection Research Institute, Private Bag X134, Pretoria, South Africa) associated with the diseased roots, were made on malt extract agar (MEA) containing Vancomycin (Wingfield & Knox-Davies, 1980*a*).

Verticicladiella species were examined using light and scanning electron microscopy (SEM). Fungi on agar disks were fixed in 2·5% glutaraldehyde and 1·5% osmium tetroxide in a 0·1 M phosphate buffer, dehydrated in a graded acetone series and critical-point dried for SEM. Specimens were coated with gold–palladium and examined in a Jeol JSM-45 scanning electron microscope.

Growth rates of isolates were determined after 4 days on 20 ml of MEA in 90 mm Petri dishes in the dark at temperatures ranging from 12 to 32° at 4° intervals.

Pathogenicity of the new *Verticicladiella* was tested by inoculating roots of ten 11-year-old *P. elliottii* L. trees with agar cultures of the fungus (Wingfield & Knox-Davies, 1980*a*).

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RESULTS

Two of the seven *Verticicladiella* isolates (PREM 45703/Dick 547 and PREM 45704/Dick 725) from New Zealand were identified as *V. procera*, while three isolates (PREM 45701/Dick 778, PREM 45702/Dick 808 and PREM 45705/Dick unnumbered) were of unidentified *Verticicladiella*. The remaining two isolates were of a *Verticicladiella* also found in South Africa and are described as a new species.

Isolations from diseased roots of a small number of dying *P. taeda* trees in the Eastern Transvaal, South Africa and from *H. angustatus* associated with these roots, yielded cultures indistinguishable from one of the *Verticicladiella* species from New Zealand. *Diplodia pinea* Kickx (= *Sphaeropsis sapinea* (Fr.) Dyko & Sutton) and *V. serpens* (Goid.) Kendrick (= *V. alacris* Wingfield & Marasas, 1981) were isolated from the roots of the same trees.

Characteristics of the *Verticicladiella* occurring both in New Zealand and South Africa differ significantly from those of all previously described species. We therefore elect to describe it as a new species.

Verticicladiella truncata Wingfield & Marasas, sp.nov.

Coloniae in agaro 'corn meal' apud 25° rapide crescentes, aetate 3 dierum 52 mm diam, primum hyalinae deinde atro-olivaceae et nigrae, margine aequa. Mycelium aerium deest sed in agaro 'Difco potato dextrose' profusum. Mycelium immersum ex hyphis septatis, rectis, primum laevis et hyalinis, deinde asperis et atro-olivaceis, 1·8–13·7 μm diam compositum. Conidiophora macronematosa, mononematosa, ex hyphis singulariter vel saepe catervatim juxta alia enata, cum hyphis brevis rhizoidalibus et basas. Stipites erecti, hyalines ad atro-olivacei, simplices vel saepe ramosi, 1–11 septati, variabiles in longitudinem, 25·0–447·2 μm long., 3·6–12·7 μm lat. ad basas, 3·6–10·9 μm lat. ad tumidulas summas. Apparatus sporogenus 18·2–103·7 μm long., ex 3–5 seriebus metularum compositus. Metulae primariae 2–4, 10·2–28·0 × 1·7–9·1 μm, pallidiores quam stipites, metulae secundariae et ceterae hyalinae. Cellulae conidiogenae (sympodulae) discretae, attenuatae ad summas sed latissimae in parte media, hyalinae, sympodice elongatae, laeves sed in parte sporifera asperae cum hilis conspicuis, 7·2–50·0 × 1·8–4·1 μm. Conidia continua, hyalina, laevia, pyriformia vel subglobosa vel clavata ad basas late truncata, 2·7–10·9 × 1·8–4·6 μm, conidia secundaria gemmata, aggregantia et capitulum mucosum hyalinum in aetate flavidum formantia.

Holotypus: ex radicibus *Pini taedae*, Wilgeboom, E. Transvaal, South Africa, Dec. 1978, M. J. Wingfield PREM 45698.

Colony diameter on corn meal agar (CMA) at 24°, 52 mm after 4 days. Growth rate similar at 20° and 24° but reduced considerably at lower and higher temperatures (Table 1). *Colonies* hyaline at first, becoming dark olivaceous to black. Aerial mycelium absent on CMA but well developed on Difco potato dextrose agar (PDA). *Hyphae* immersed, septate, straight, smooth-walled, at first hyaline, becoming rougher with deposits and dark olivaceous, 1·8–13·7 μm diam with short branches (Fig. 1) often branching repeatedly (Fig. 2), sometimes appearing knob-like (Fig. 3). *Conidiophores* produced abundantly over the entire colony in cultures incubated at 20° in the dark for 10 days on CMA, macronematous, mononematous arising laterally from the hyphae, single or more usually in groups alongside one another (Fig. 4). One to several celled short rhizoids arise from the basal cells of the stipe and from the hyphal cells giving rise to the conidiophores (Fig. 5). *Stipe* erect, variable in length, 25·0–447·2 μm, with 1–11 septa and hyaline to dark olivaceous in colour. Base of stipe 3·6–12·7 μm wide and apex slightly swollen, 3·6–10·9 μm with attachment points for primary metulae well-developed (Fig. 6), commonly branched (Figs 7, 8) with branches varying from a single primary metula to well-developed side branches bearing a complete sporogenous apparatus. *Sporogenous apparatus* (Fig. 9) 18·2–103·7 μm long, excluding the conidial mass and comprising 2–4 primary metulae 2·7–9·1 μm wide and 10·2–28·0 μm long. Two to four additional series occur above the primary metulae, each giving rise to 1–3 further metulae at the apex or from the side walls (Fig. 10). Primary metulae paler in colour than the stipe, subsequent metulae hyaline. *Conidiogenous* cells (sympodulae) discrete (Fig. 11), hyaline, tapering from base to apex but widest at their centre, 7·3–50·0 μm long, 1·8–4·1 μm wide appearing to elongate considerably in the apical region (Fig. 12), distinctly roughened possibly due to abscission scars which resemble annellations when viewed with the SEM (Fig. 13). *Conidia* hyaline, smooth, one-celled and pyriform to subglobose or clavate with broadly truncate bases (Fig. 14). Under the SEM appearing hat-shaped due to a wide point of attachment at the base (Fig. 15), ranging from 2·7 to 10·9 μm × 1·8–4·6 μm and often budding to produce secondary conidia (Fig. 16). Conidia accumulate around the sporogenous apparatus in a hyaline mucilaginous mass, becoming yellow with age.

Groups of conidiophores may form on aerial hyphae on PDA (Fig. 17) and primary metulae sometimes elongate, giving rise to an additional stipe and conidiogenous apparatus (Fig. 18).

Specimens examined: Holotype: Cultures on CMA, isolated from the roots of *Pinus taeda*, Wilgeboom, near Sabie, Eastern Transvaaal, South Africa, Dec. 1978, M. J. Wingfield, PREM 45698. Paratypes: from roots of

Table 1. *Growth rates of* Verticicladiella *isolates at different temperatures*

Isolate	Temperature (° C)					
	12	16	20	24	28	32
	Colony diam (mm)					
V. truncata (PREM 45698)	26·0	35·0	44·0	52·0	14·0	—
Unidentified *Verticicladiella* sp. (PREM 45702)	31·7	37·5	48·4	22·1	—	—
V. procera (PREM 45703)	17·2	23·8	24·1	33·2	18·0	—

* Mean of five replicates on MEA after 96 h in the dark.

P. taeda, Mariti plantation near Sabie, Eastern Transvaal, South Africa, Feb. 1979, M. J. Wingfield, PREM 45697; from *Hylastes angustatus* in *P. taeda* roots, Wilgeboom, near Sabie, Eastern Transvaal, South Africa, Feb. 1980, M. J. Wingfield, PREM 45696; from roots of *P. radiata*, Hanmer State Forest, North Island, New Zealand, May 1979, M. Dick 903/PREM 45700; from roots of *P. strobus*, Gwavas State Forest, North Island, New Zealand, May 1979, M. Dick unnumbered/PREM 45699.

Dried cultures of *Verticicladiella* spp. examined on CMA have been deposited in the Mycological Herbarium of the Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa (PREM). Subcultures of the type strain of *V. truncata* have also been deposited in the CBS, IMI and DAOM culture collections. Roots inoculated with *V. truncata* developed lesions up to 8 cm on either side of the inoculation point after 5 months. *Verticicladiella truncata* was re-isolated from all lesions.

DISCUSSION

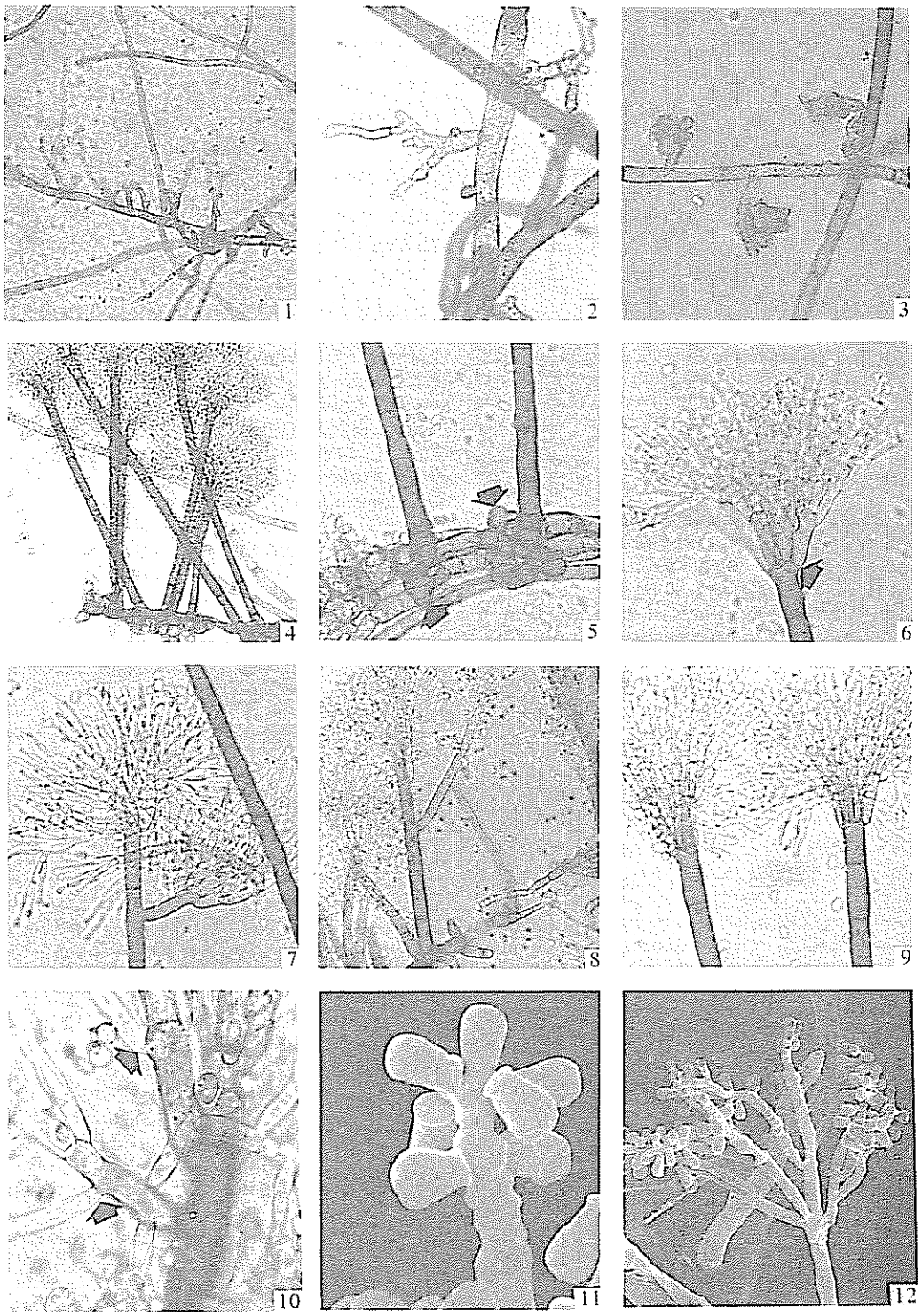
Verticicladiella truncata is distinguished from other *Verticicladiella* species by distinct, broadly truncate conidia and the characteristic arrangement of the conidiophores alongside each other. The 'scars' on the conidiogenous cells, in some cases resembling annellations, are also characteristic of this species. Well-developed rhizoids on the basal cells of the conidiophores and on the mycelium between conidiophores giving rise to these structures differ from rhizoids in other *Verticicladiella* species in which they develop at the base of the individual conidiophores (Kendrick, 1962; Wingfield & Marasas, 1980). Metulae in *Verticicladiella* commonly arise from the apex of the previous metulae in series of threes (Kendrick, 1962), whereas those of *V. truncata* arise from the side walls as well as the apex of the previous metulae.

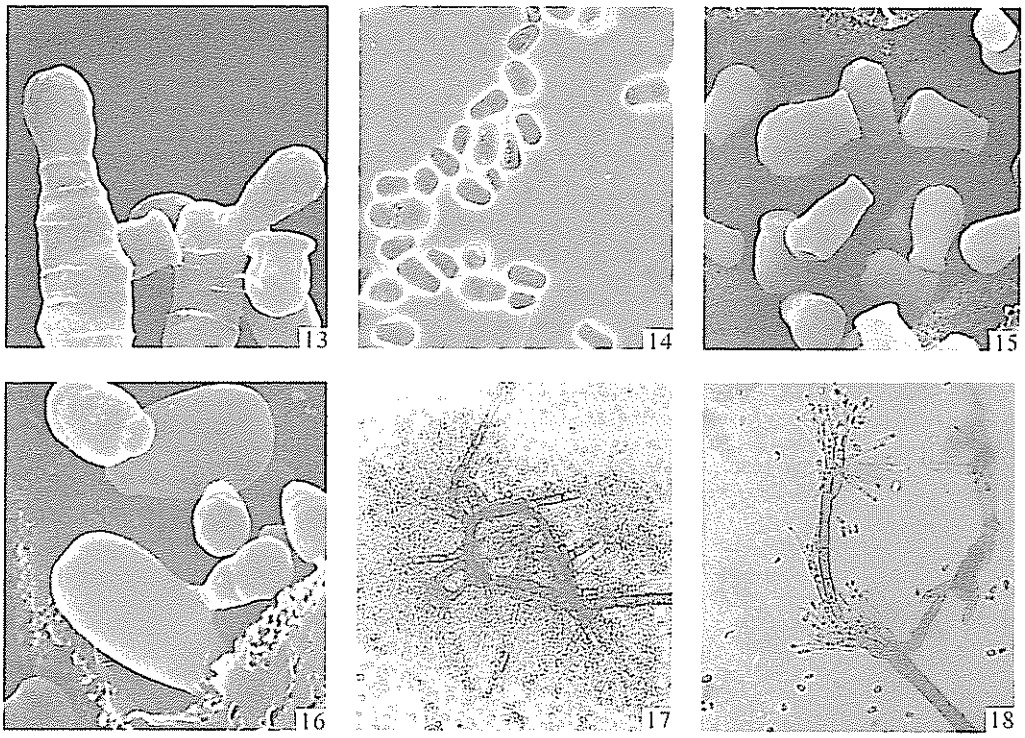
The genera *Verticicladiella*, *Leptographium* and *Phialocephala* are separated by their sympodial, annellidic and phialidic conidial ontogenies respectively (Kendrick, 1962). *Verticicladiella truncata* appears to share characteristics of both sympodial and annellidic ontogeny (R. A. Sampson, pers. comm.). This would lend support to the view (Kendrick, 1980) that the *Leptographium* complex requires taxonomic revision.

Shaw & Dick (1980) suggest that the *Verticicladiella* sp. responsible for the root disease in New Zealand may be similar to *V. procera*, as the symptoms observed were similar to those reported for the *V. procera* root disease of *P. strobus* in the U.S.A. (Dochinger, 1967; Houston, 1969; Towers, 1977). Results of the present study have shown that the isolate (PREM 45703) used in pathogenicity tests in New Zealand (Dick, pers. comm.) is a typical isolate of *V. procera*. The identification of *V. procera* from New Zealand represents a first record of this species from the southern hemisphere.

Shaw & Dick (1980) report different growth rates in two of their *Verticicladiella* isolates. The present study has shown that a number of *Verticicladiella* species are associated with the root disease of *P. strobus* in New Zealand. It is therefore possible that two different *Verticicladiella* species were used in growth comparisons by these authors. The occurrence of more than one *Verticicladiella* associated with tree death in New Zealand supports previous reports (Kulhavy, Chacko & Partridge, 1978; Mielke, 1979) that a number of *Verticicladiella* species may be associated with root disease of a single tree. Similarly the occurrence of *V. truncata* together with other root pathogens such as *V. serpens* (Wingfield & Knox-Davies, 1980*a*) and *D. pinea* (Wingfield & Knox-Davies, 1980*b*) suggests that this fungus is not the only factor involved in the root disease of *P. taeda* in South Africa. Further studies are required to established the significance of the various pathogens associated with tree death in New Zealand and South Africa.

Bark beetles are vectors of *Verticicladiella* species (Davidson & Robinson-Jeffrey, 1965; Goheen & Cobb, 1978; Kendrick, 1962; Robinson-Jeffrey & Griachenko, 1964; Wingfield & Knox-Davies, 1980*a*). The association between *V. truncata* and





Figs 13–18. *Verticicladiella truncata* holotype, PREM 45698. Fig. 13. Abscission scars on conidiogenous cells resembling annellations. × 8600. Fig. 14. Conidia with broadly truncate bases. × 2800. Fig. 15. Scanning electron micrograph showing wide points of attachment of conidia. × 6000. Fig. 16. Conidia budding. × 10000. Fig. 17. Conidiophores on aerial hyphae. × 400. Fig. 18. Primary metula extending to form a second conidiophore. × 700.

H. angustatus provided further evidence for previous suggestions (Goheen & Cobb, 1978; Wingfield & Knox-Davies, 1980*a*) that *Hylastes* spp. may be vectors of pathogenic *Verticicladiella* spp.

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Figs 1–12. *Verticicladiella truncata* holotype, PREM 45698. Fig. 1. Short branches on hyphae. × 360. Fig. 2. Repeated branching of hyphal branches. × 1000. Fig. 3. Hyphal branches appearing knob-like. × 380. Fig. 4. Conidiophores alongside each other on hypha. × 280. Fig. 5. Rhizoids at the base of conidiophores (arrows). × 950. Fig. 6. Sporogenous apparatus with well-developed points of attachment for primary metulae (arrow). × 1000. Fig. 7. Branching conidiophore, × 1000. Fig. 8. Branching conidiophore. × 700. Fig. 9. Typical sporogenous apparatus. × 950. Fig. 10. Progressive series of metulae arising from side walls and apex of previous metulae (arrow). × 2500. Fig. 11. Sympodial proliferation of conidiogenous cell. × 1000. Fig. 12. Large numbers of conidia produced on each conidiogenous cell. × 2400.

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