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HARKNESSIA SPECIES OCCURRING IN SOUTH AFRICA

P. W. Crous

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

M. J. Wingfield

Department of Microbiology and Biochemistry, University of the Orange Free State,
9300 Bloemfontein, South Africa

AND

T. R. Nag Raj

Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

ABSTRACT

Three new species of *Harknessia* are described from leaves of woody hosts in South Africa. *Harknessia eucalyptorum* and its teleomorph, *Wuestneia eucalyptorum*, are described from *Eucalyptus* leaves. In this case, the teleomorph. anamorph connection was proven in culture. *Harknessia fusiformis* is described from *Eucalyptus* leaf litter, while *H. syzygii* is described from *Syzygium cordatum*. Additional collections of *H. uromycoides* and *H. hawaiiensis* are also discussed, and a microconidial state described for the latter species.

Key Words: *Eucalyptus*, foliicolous fungi, *Harknessia*, systematics, *Syzygium cordatum*, *Wuestneia*

Three comprehensive reviews on this genus have been published (Sutton, **1971**, **1980**; Nag Raj and Di Cosmo, **1981**) since *Harknessia* Cooke was first described (Cooke and Harkness, **1881**). Recent studies (Galán et al., **1986**; Sutton and Pascoe, **1989**) have led to the description of three additional species, including the *Cryptosporella* Saccardo teleomorph of *Harknessia karwarrae* Sutton & Pascoe. In a recent study of the genus *Cryptosporella*, Reid and Booth (**1989**) concluded that *Wuestneia* Auerswald is the correct name for fungi previously assigned to *Cryptosporella*.

Several *Harknessia* spp. are known to produce microconidial states. The first record of a microconidial state for *Harknessia* is that described by Sutton (**1971**) for *H. antarctica* Spegazzini. Subsequently, Nag Raj and Di Cosmo (**1981**) described microconidial states for seven species, and Sutton and Pascoe (**1989**) described an additional two.

Harknessia uromycoides (Speg.) Speg. was the first species of the genus reported to occur on *Eucalyptus* leaves in South Africa (Doidge, **1950**). In this paper we describe *Wuestneia eucalyptorum* and its *Harknessia* anamorph from *Eucalyptus* leaves, *H. syzygii* from *Syzygium cordatum* Hochst. and *H. fusiformis* from *Eucalyptus* leaf litter. Additional collections of *H. uromycoides* and *H. hawaiiensis* Stevens & Young are also discussed. A microconidial state is described for the latter species.

MATERIALS AND METHODS

Symptomatic leaves and leaf litter were collected at regular intervals since 1987 at a *Eucalyptus* provenance trial planted on Stellenbosch Mountain in the Western Cape, as well as at different locations in Transvaal, Natal and Orange Free State Provinces. In addition to *Eucalyptus* leaves, leaf litter of *S. cordatum* was collected in Natal and Transvaal Provinces.

Leaves were incubated in moist chambers at 25 C under near-ultraviolet light for 3 days, after which time furfuraceous margins and exuding black spore masses indicated the presence of *Harknessia* conidiomata. To detect the presence of a teleomorph, leaves were incubated in the dark at 4–7 C for 3 days before incubating as explained above. Material was mounted in water, lactophenol cotton blue, erythrosin, 3% KOH as well as Melzer's reagent. Wherever possible, 50 examples of each structure were measured and averages given.

Single conidial and ascospore isolates were obtained using the dilution plating technique on malt extract agar (15 g Difco agar, 20 g Oxoid malt extract, 1 L water) (MEA). To induce sporulation, cultures were placed on MEA, carnation-leaf agar (CLA) (Fisher et al., **1982**; Crous et al., **1992**) or *Eucalyptus* leaf agar (leaf discs sterilized using 1,2-propylene oxide), and subsequently incubated at 20 and 25 C under near-ultraviolet/white light.

The optimum growth temperature was determined for each of the fungi on MEA. One single-conidial isolate was taken as representative of each species, and used in the growth studies. Optimum growth temperature (expressed as colony diameter) was determined after isolates were incubated for 3 days in the dark at eight temperature settings ranging from 5–40 C at 5 C intervals. Each treatment had three replications and the experiment was repeated.

RESULTS AND DISCUSSION

During a study of fungi occurring on *Eucalyptus* leaves in 1988, a *Harknessia* sp. was found on leaves of *E. globulus* Labill., *E. nitens* (Deane & Maid.) Maid. and *E. maidenii* F. Muell. at Stellenbosch in the Western Cape Province. Examination of the conidiomata showed conidia to be 16–22 × 8–14 μm ($\bar{x}$ = 19 × 12 μm), broadly ventricose with apiculate to obtuse apices. The appendages were 2–18 ($\bar{x}$ = 8.5 μm), suggesting that this fungus was *Harknessia eucalypti* Cooke *apud* Cooke & Harkn. (Crous et al., **1989**). Since these initial collections, additional material has been obtained from the same area on leaves of *E. andrewsii* Maid., *E. grandis* Hill: Maid., *E. tereticornis* Sm. and *E. viminalis* Labill. An examination of these collections together with cultural studies has shown that the South African material differs morphologically from *H. eucalypti*. Conidia were found to vary in shape from ventricose to broadly ventricose with apiculate or rounded apices. Conidia were 16–29 × 9–15 μm ($\bar{x}$ = 22 × 12 μm) in size, thus similar to those of *H. eucalypti* (FIG. 1), 19–28 × 11–15 μm, and *H. podocarpi* Lindquist & Sutton *apud* Sutton, 17.5–26 × 11–15 μm (Nag Raj and Di Cosmo, **1981**).

The conidia from these new collections from South Africa could be distinguished from those of *H. eucalypti* by their more obtuse conidial apices and longer appendages. Although conidial dimensions of these collections fit those of *H. podocarpi*, the conidia differ from this species by not being striate and not having persistent mucous sheaths. The fungus previously recorded as *H. eucalypti* in South Africa (Crous et al., 1989) and that noted in the more recent collections are therefore described below as a new species of *Harknessia*.

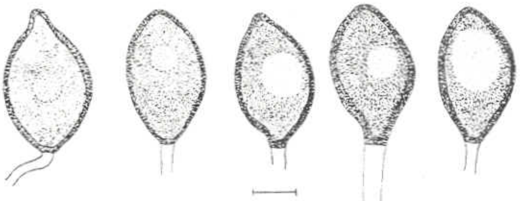


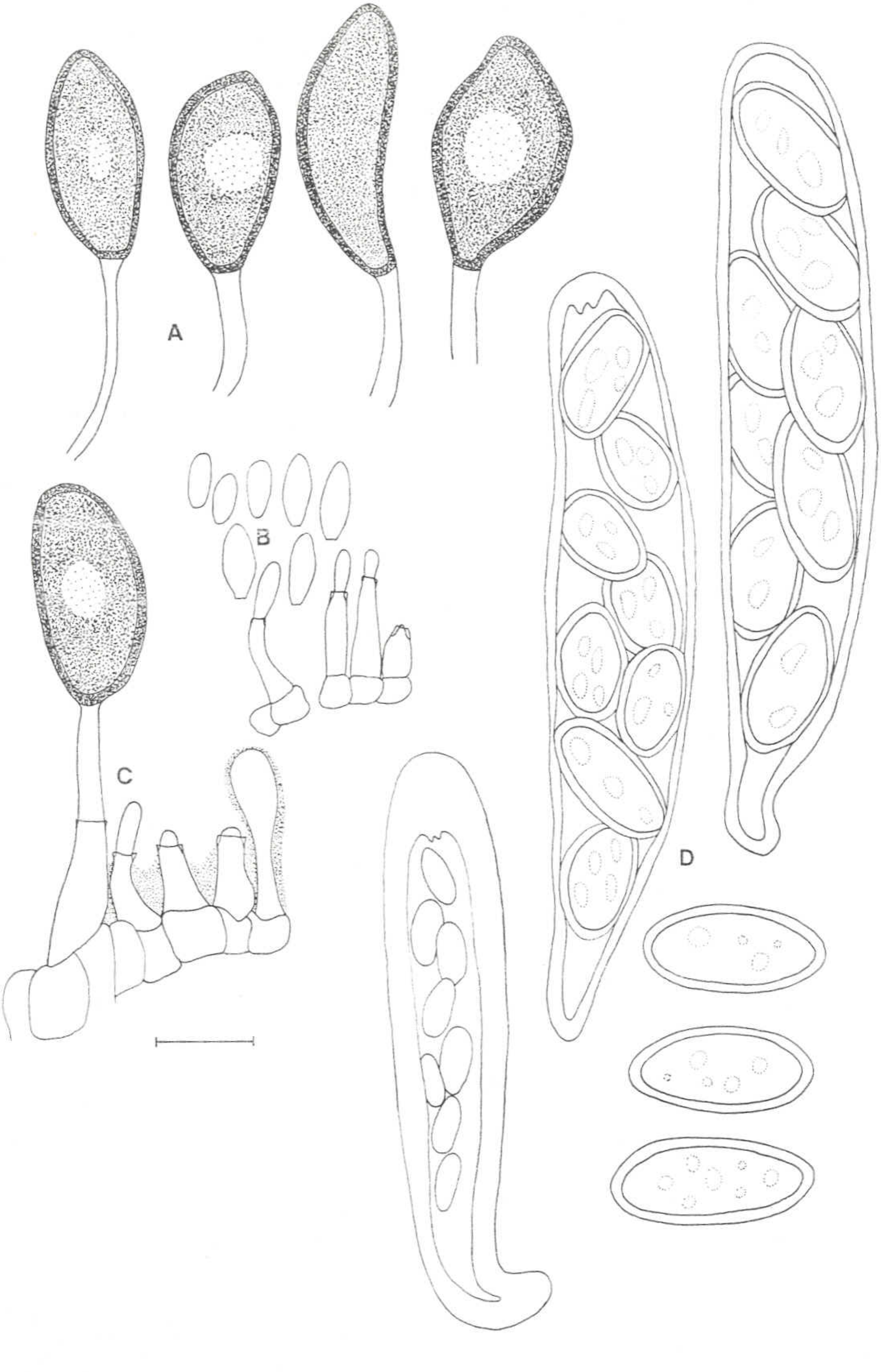
FIG. 1. Conidia of *Harknessia eucalypti* (IMI 146779). Bar = 10 μm.

Harknessia eucalyptorum Crous, Wingfield *et* Nag Raj, *sp. nov.* FIGS. 2, 3, 11

Conidiomata separata, immersa, globosa ad subglobosa, unilocularia, erumpentia et punctiformia, usque ad 350 μm diam, ostiolum margine furfuraceo, pallide brunneo; parietes basales et laterales, 5–7 cellulis crasses, ex textura angulari compositi. Conidiophora ad cellulas conidiogenas deminuta. Cellulae macroconidiogenae discretae, hyalinae, laeves, lageniformes, doliiformes ad cylindricae, 6–20 × 3.5–6.2 μm basi, ex cellulis interioribus parietis conidiomati oriundae, conidium unum efferentes vel proliferatione una enteroblastice. Macroconidia holoblastica, late ventricosa, cum guttula centrali, aseptata, atrobrunnea, apice obtuso ad apiculato, basi truncata, 16–29 × 9–24 μm ($\bar{x}$ = 22 × 11 μm) in foliis, 14.5–24 × 10.5–14 μm ($\bar{x}$ = 19.5 × 12.5 μm) in cultura; appendix hyalina, non ramosa, basalis, 3–16 μm ($\bar{x}$ = 10.5 μm) longa in foliis, usque ad 12 μm longa in cultura. Cellulae microconidiogenae subcylindricae ad lageniformes, hyalinae, laeves, usque ad 15 μm longae, 2.5–4 μm crassae basi. Microconidia holoblastica, apicalia vel lateralia, hyalina, aseptata, laevia, ellipsoidea ad fusiformia, 4.5–9 × 2–3.5 μm.

SPECIMEN TYPICUM in foliis vivis *Eucalypti andrewsii* Maid., Stellenbosch Mountain, Stellenbosch, Western Cape, R.S.A., 20 Dec. 1989, *P.W. Crous*, HOLOTYPUS, PREM 50813 (DAOM 211793, IMI 338270a, ISOTYPI).

Foliicolous and caulicolous. Conidiomata separate, immersed, globose to subglobose, unilocular, erumpent and punctiform, up to 350 μm diam, ostiole with a light brown furfuraceous margin; basal and lateral walls five to seven cells thick, composed of textura angularis. Conidiophores reduced to conidiogenous cells. Macroconidiogenous cells discrete, hyaline, smooth, lageniform, doliiform to cylindrical, 6–20 μm long, 3.5–6.2 μm wide at the base, formed from inner cells of conidiomatal wall, producing a single conidium or proliferating enteroblastically once, periclinal thickening minute, collarette absent. Macroconidia holoblastic, broadly ventricose with central guttule, aseptate, dark brown, astriate, apex obtuse to bluntly apiculate, base truncate, 16–29 × 9–24 μm ($\bar{x}$ = 22 × 11 μm) on


A
B
C
D

leaves, 14.5–24 × 10.5–14 μm ($\bar{x}$ = 19.5 × 12.5 μm) in culture; basal appendage hyaline, unbranched, 3–16 μm ($\bar{x}$ = 10.5 μm) on leaves, up to 12 μm in culture. Conidiogenous cells and appendages sometimes enclosed in a nonpersistent mucilaginous sheath. Microconidiogenous cells in the same or in separate conidiomata, subcylindrical to lageniform, hyaline, smooth walled, with cytoplasmic channel and periclinal thickening but no collarette, up to 15 μm long, and 2.5–4 μm wide at base. Microconidia holoblastic, apical or lateral, hyaline, aseptate, smooth, ellipsoidal to fusiform, 4.5–9 × 2–3.5 μm.

HOSTS: *Eucalyptus andrewsii, E. maidenii, E. globulus, E. grandis, E. nitens, E. tereticornis, E. viminalis.*

SPECIMENS EXAMINED. SOUTH AFRICA. WESTERN CAPE: Stellenbosch, *E. andrewsii*, 20 Dec. 1989, *P.W. Crous* (HOLOTYPE, PREM 50813; ISOTYPES, DAOM 211793, IMI 338270a) *E. andrewsii*, Oct. 1989, *P.W. Crous* (PREM 50814); *E. maidenii*, 20 Dec. 1989, *P.W. Crous* (PREM 50815); *E. maidenii*, Feb. 1988, *P.W. Crous* (PREM 49105); *E. maidenii*, 30 Sept. 1988, *P.W. Crous* (PREM 50816; culture, PPRI 4295); *E. maidenii*, 8 Dec. 1988, *P.W. Crous* (PREM 50817); *E. grandis*, Oct. 1989, *P.W. Crous* (PREM 50818); *E. grandis*, Oct. 1989, *P.W. Crous* (PREM 50819); *E. nitens*, Feb. 1988, *P.W. Crous* (PREM 49104); *E. tereticornis*, 15 Nov. 1988, *P.W. Crous* (PREM 50821); *E. viminalis*, 30 Sept. 1988, *P.W. Crous* (PREM 50822); *E. viminalis*, 15 Nov. 1988, *P.W. Crous* (PREM 50823); *E. viminalis*, 8 Dec. 1988, *P.W. Crous* (PREM 50824); *Eucalyptus* sp., 7 July 1988, *P.W. Crous* (PREM 50826); *Eucalyptus* sp., 29 Sept. 1988, *P.W. Crous* (PREM 50827); *Eucalyptus* sp., 17 Nov. 1988, *P.W. Crous* (PREM 50828); *Eucalyptus* sp., 8 July 1989, *P.W. Crous* (PREM 50829). EASTERN TRANSVAAL: Jessievale, *E. nitens*, 24 Nov. 1988, *P.W. Crous* (PREM 50820).

In this study we observed variation in the symptoms associated with *H. eucalyptorum*. It was usually found associated with a leaf and peduncle necrosis of various *Eucalyptus* spp., and although lesions were always distinct and light brown in color, they were surrounded by a large chlorotic band on *E. tereticornis* but not on other host species. On *E. viminalis*, however, lesions occurred mainly along the leaf margins.

Isolates of *H. eucalyptorum* grew optimally on MEA at 25 C, and sporulated after 2 wk. Colonies were white to pale yellow colored, eventually turning olivaceous green at the center when sporulating. Conidia from cultures derived from different host species were similar in size and appendage length to those occurring on leaves (TABLE I). Conidia from cultures were generally broadly ventricose with obtuse apices, and a central, globose guttule. Although a microconidial state was present on collections made from *E. nitens* and *E. maidenii* leaves, no microconidia were formed in culture.

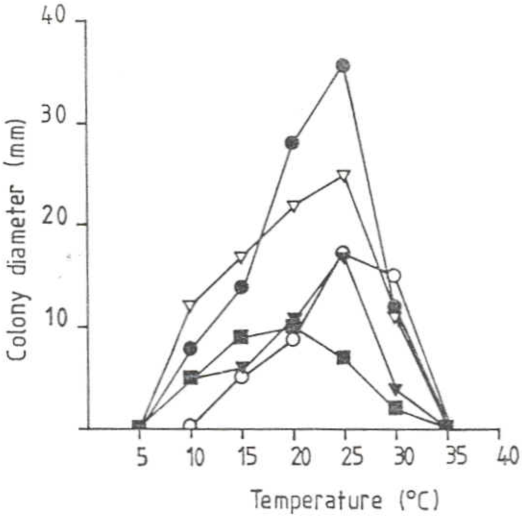


FIG. 3. Colony diameters (mm) of *Harknessia* spp. on MEA after 3 da at various temperatures in the dark. *Harknessia eucalyptorum* (PPRI 4295), ▼; *H. hawaiiensis* (PPRI 4298), ●; *H. uromycoides* (PPRI 4296), ■; *H. fusiformis* (PPRI 4297), ▽; *H. syzygii* (PPRI 4299), ○.

In recent collections of *H. eucalyptorum* [conidia 18–29 × 9–14 μm ($\bar{x}$ = 22 × 11 μm), appendages 3–16 μm ($\bar{x}$ = 10.5 μm)] from leaves of *E. andrewsii* and *E. maidenii*, the conidiomata were associated with the ascocarps of another fungus, and hyphal connections were also observed between the two fructification types. Colonies obtained from single ascospores on MEA were white, flocculent, turning the medium caramel brown in color. Conidiomata with conidia of *H. eucalyptorum* were observed after 3 months on MEA to which sterilized pieces of *Eucalyptus* leaf had been added. We therefore believe that the fungus producing ascomata found on *Eucalyptus* leaves is the teleomorph of *H. eucalyp-*

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FIG. 2. A–D. *Wuestneia eucalyptorum* and its anamorph *H. eucalyptorum*. Bar = 10 μm. A. Macroconidia (PREM 50813). B. Microconidia (PREM 49104). C. Macroconidium and conidiogenous cells. D. Asci and ascospores (PREM 50830).