The morphology and cultural characters of *Pisolithus tinctorius* (Gasteromycetes) in South Africa

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Sporocarp morphology as well as surface ornamentation of the basidiospores of South African collections of *Pisolithus tinctorius* agree in respect of those of specimens from Australia. Cultural characters and micromorphology of isolates made from certain of these collections correspond with those of *P. tinctorius* cultures obtained from sporocarp of strains forming mycorrhizal associations with pines in France and the U.S.A. It is concluded that only strains of the fungus which are unable to form mycorrhizas with pines occur in South Africa.

Sporokarp morfologie en oppervlaksbeeld van basidiospore van Suid-Afrikaanse versamelings van *Pisolithus tinctorius* stem ooreen met die eienskappe van monsters verkry uit Australië. In reinkultuur het die kultureieienskappe en mikromorfologie van isolate wat uit sommige Suid-Afrikaanse versamelings gemaak is, ooreengestem met die van isolate verkry uit sporokarpe van rasse wat met mikorizas van denne in Frankryk en die V.S.A. geassosieer was. Die gevolgtrekking word gemaak dat slegs rasse van die swam wat nie in staat is om mikorizas met denne te vorm nie, in Suid-Afrika voorkom.

**Keywords:** Fungal symbiont, mycorrhiza, *Pisolithus*

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**Introduction**

The Gasteromycete, *Pisolithus tinctorius* (Mich.: Pers.) Coker & Couch (1928) was first reported in South Africa by van der Bijl (1918). He noted that fruit-bodies were common in *Eucalyptus* plantations near Pretoria and, on the basis of careful microscopic observations, concluded that this fungus lived symbiotically on the roots of *Eucalyptus* trees. Subsequently Ashton (1976), Marx & Bryan (1970) and Gibson (1969) among others, reported experimental confirmation of this association. In a survey of the host range and world distribution of *P. tinctorius*, Marx (1977) reported its occurrence in 33 countries of the world. Its ability to form ectomycorrhizas on the roots of 11 species of *Eucalyptus*, 30 of *Pinus*, two of *Quercus* and a number of other coniferous and broad-leaved tree species has been demonstrated experimentally (Marx 1977). It has been reported to form mycorrhizas under natural conditions with nine additional species of *Pinus*, two of *Eucalyptus* and eight of *Quercus* as well as with a number of other tree species (Marx 1977). *P. tinctorius* has been shown to have great potential use in afforestation projects because of its wide host range, adaptability to different sites, and ability to improve tree growth in the nursery and survival in the field. Practical techniques for its artificial introduction into nursery soils are also available (Marx 1976; Marx & Bryan 1976; Marx et al. 1976). However, Marx (1981) later reported significant variation in the ability of *P. tinctorius* isolates from oak or pine hosts south of the Tropic of Cancer, to form mycorrhizas with *Pinus taeda*. Some isolates failed to form mycorrhizas under experimental conditions.

South African *P. tinctorius* is common and widely distributed in association with *Eucalyptus* spp. (Bottomley 1948; Doidge 1950; van der Westhuizen & Eicker 1987). It has also been found in association with wattle, *Acacia mearnsii* De Wild., by the present authors. Its fruit-bodies commonly appear under *Eucalyptus* spp. planted as firebelts around pine plantations but rarely occur under the pines. It is known to occur in association with pines on a site in the vicinity of George, Cape Province, that had previously been planted with *Eucalyptus* sp. (van der Westhuizen & Eicker 1987). It has also been seen in an open stand of *Pinus radiata* in Tokai State Forest (Prof. D.T. Mitchell, University College Dublin, pers. comm.). However, fungi isolated from ectomycorrhizas on roots of *Pinus* spp. seedlings grown in a potting mixture containing basidiospores from fruit-bodies of *P. tinctorius* collected from the pine stand near George, did not include isolates of *P. tinctorius* (Viljoen 1987). Mixing basidiospores of *P. tinctorius* into soil of seedling beds is an accepted practice for promoting mycorrhizal formation by this fungus with pines (Marx et al. 1976). These observations together with the general absence of *P. tinctorius* fruit-bodies from pine stands in South Africa, strongly indicate an absence of mycorrhizal relations with pines. These observations raise doubts about the identity of the fungus known as *P. tinctorius* in South Africa. However, they also tend to support a suggestion by Dring (1973) that both saprophytic and mycorrhizal varieties of *P. tinctorius* may exist.

The sporocarp morphology of *P. tinctorius* has been described from South Africa by van der Bijl (1918) and Bottomley (1948), from Australia by Cunningham (1944), and from North America by Coker & Couch (1928), Grand (1976) and Mims (1980). The two latter authors also described the surface ornamentation and
ultrastructure respectively, of the basidiospores from collections in North America. Bronchart et al. (1975) and Hansen (1986) described and figured basidiospores of Pisolithus arhizus and P. tinctorius respectively from Europe.

The cultural characters and micromorphology in pure culture have been studied by Hile & Hennen (1969) and Miller et al. (1983).

It was decided to investigate the identity of South African specimens designated as P. tinctorius in an effort to find a possible reason for the absence of its association with pines in this country. This paper reports on the results of the investigation.

Materials and Methods
Conventional mycological methods were used to study sporocarps of 22 collections of P. tinctorius, including one from New South Wales, Australia, obtained from the herbarium of the National Collection of Fungi (PREM), Pretoria. Eleven more were collected by the authors from different localities in South Africa during the summer of 1986/87. Fruit-bodies from these collections were deposited in the Fungus Collections (PRUM) of the H.C.W.J. Schweickerdt Herbarium, University of Pretoria. In order to obtain cultures, fresh sporocarps were cut open by means of a sterilized scalpel. From the freshly exposed surfaces, tissue from the lower parts of the gleba was removed by means of sterilized forceps and transferred to plates of Melin-Norkrans agar (MMN) as modified by Marx (1969).

Cultures of eight isolates of P. tinctorius as well as basidiospores from the basidicarps from which six of these cultures had been made, were received from the Department of Forest Science, University of Stellenbosch and were included in the present study. Two of these cultures had been isolated from sporocarps of strains forming mycorrhizas with pines in France and in Georgia, U.S.A.

All cultures were incubated in the dark at 25°C. For comparative studies, five cultures of each isolate were examined after 6 weeks incubation according to the methods described by Miller et al. (1983) and van der Westhuizen (1971).

Basidiospores mounted in lactophenol were examined and photographed by light microscopy. Spore size was determined by measuring 25 spores from each collection. Spore ornamentation was studied by means of scanning electron microscopy (SEM) after mounting spores on adhesive-coated bosses and coating with gold in a sputter coater to about 120 Å. Colour names given in quotation marks are in accordance with the notation by Rayner (1970).

Results
Sporocarps of 33 collections were examined. These varied in size, shape and colouring of the exoperidium but were very similar in anatomical characters (Figure 1).

Basidiospores from 39 sporocarps were studied. They varied somewhat in size, but otherwise appeared very similar under the light microscope (Figures 2 & 3). Basidiospores of six collections, including the one from Australia, were indistinguishable from one another when viewed at 5,000 x magnification by SEM. They were globose to sub-globose, and densely covered with short, thick spines mostly with blunt apices (Figures 4, 5 & 6).

Cultures obtained from seven of the fruit-bodies collected by the authors, as well as eight isolates received from the University of Stellenbosch, grew at different rates and showed slight variation in colour of the mat and colour intensity on the reverse of the plates. However, they were very similar in general appearance, colour, texture of the colonies, and in micromorphology (Figure 7).

Sporocarp morphology
Sporocarps variable, pyriform, broadly ovoid, to pulvinate or sub-globose and with a stout rooting base attached to the substratum by yellowish rhizomorphic strands, 3–20 cm tall and 2–17 cm broad; at first hard spongy, later soft in upper parts but hard, woody towards the base. Peridium smooth, shiny, whitish to 'buff' at first, soon darkening to 'honey' or 'hazel' with 'umber' to 'fuscous black' markings in a snake-skin effect, frequently with 'pure yellow' colours around the base of fresh specimens, thin, brittle, finally cracking into irregular segments from the top downwards, exposing the mature spores. Gleba divided into sub-globose to ovoid or polygonal peridioles, 1.0–5.0× 1.0–3.0 mm, larger in the upper parts, separated by thick, carbonous, persistent tramae plates, the peridioles whitish when young and presenting a mottled appearance when cut, exuding a dark brown, staining fluid, later maturing ochraceous to amber, pulverulent (Figure 1). Spores globose, verruculose to coarsely echinulate, olivaceous brown, 5.3–10.3 μm diam. overall, echinulae 0.3–1.34 μm (Figures 2–6).

Cultural characters
Colonies on MMN slow growing reaching a radius of 20–35 mm after 6 weeks (Figure 7). Margin even, thin, mycelium raised to limit of growth, sparse, forming numerous thin strands, 'pale luteus'; aerial mycelium thin over marginal zone, becoming progressively more dense to woolly and raised over the older parts, finally soft feltly over the oldest parts and incolum plug, darkening to 'honey'. The mat remains soft and easily torn. The reverse is unchanged at first but darkening under the incolum to 'isabelline' then 'greying sepia' to finally 'fuscous black', the darkened area gradually increasing radially.

Microscopic characters
Advancing zone: hyphae hyaline, unbranched at first, later branching, thin-walled, septate, with simple clamps at the septa, often branching behind a septum with the septum and clamp close to the main hypha, or branching from a clamp with a septum and clamp formed close to the origin, 2.0–3.5 μm in diameter (Figure 8).

Aerial mycelium: hyphae as in the advancing zone,
Figures 1-12  *Pisolithus tinctorius*. 1. Mature sporocarp with exoperidium partly removed to show pulverulent gleba in upper part and peridioles. 2. Basidiospores from sporocarp of PRUM 2212, ×1 200. 3. Basidiospores from sporocarp of PREM 46185, ×1 200. 4. Surface view of basidiospores of PREM 46185, SEM, ×4 000. 5. Surface view of basidiospore of PRUM 2241, SEM, ×4 000. 6. Surface view of basidiospore of Stellenbosch no. 3, SEM, ×4 000. 7. Culture of PRUM 2241, on MMN at 4 weeks. 8. Hypha from advancing zone of culture showing branching and clamp connections, ×1 200. 9. Hypha from aerial mycelium of culture showing paarige branching, ×1 200. 10. Hypha from aerial mycelium of culture showing multiple clamps and branches, ×1 200. 11. Hypha with granular deposits on the surface, ×1 200. 12. Branching, tortuous hypha submerged in the agar, ×1 200.
occasionally showing parallel branching (Figure 9) or with multiple branches and clamps at some septa on older hyphae 2.0–5.6 µm diam. (Figure 10) and small granules on some of the older hyphae (Figure 11). Strands of 3–5 hyphae were present in older parts of the mat.

Submerged mycelium: hyphae as in the advancing zone but more tortuous and more frequently branched with short, tortuous branches, 2.0–3.0 µm (Figure 12).

Material examined

*In Herb. PREM:* On ground, Buccleugh Orchards, Natal 1916-11-15, 9792; on ground under *Eucalyptus* sp., Pretoria, Feb. 1919, 11815; on ground Lobatsi, Botswana, 1928-05-02, 18040; on ground under *Eucalyptus*, Pretoria-Johannesburg road, Sept. 1925, 20627; on ground under *Eucalyptus*, Pretoria, Feb. 2929, 23638; on ground under *Eucalyptus* sp., Klampunts, C.P., 1929-06-23, 24842; on ground, Trappe’s Valley, Bathurst, C.P., 1930-09-26, 25495; on ground under *Eucalyptus*, Pretoria, 1939-04-23, 30781; on ground, Newlands, C.P., April 1921, 31358; on ground under *Eucalyptus*, Johannesburg, April 1946, 35532; on ground in *Eucalyptus* plantation, Lions River, Natal, 1948-05-17, 36693; on ground, Marandellas District, Zimbabwe, June 1960, 42083; on ground over termite nest, Pretoria, 1965-01-15, 42981; on ground under pines, Witfontein Forest Station, George, C.P., March 1981, 45893; on soil, in *Eucalyptus* plantation, Stellenbosch, Jan. 1924, 46125; on sandy soil, French’s Forest, N.S.W., Australia, 46185; on soil under *Eucalyptus*, Pretoria Country Club, 1983-11-20, 47303; on ground, Sabie, Tvl., April 1985, 47763; on soil under trees, Bronkhorstspruit, Tvl., 1985-02-15, 47959; on ground under *Eucalyptus saligna*, Umtamvuna Nature Reserve, Natal, April 1985, 48436; on lawn, Pretoria, 1985-03-14, 48446; among grass at side of track, Mhlambanyatsi, Swaziland, 1985-01-06, 48437.

*In Herb. PRUM:* On ground under *Pinus* sp., Witfontein Forest, George, Sept. 1986, 2212; on ground under *Eucalyptus* sp., Boschkop, Pretoria district, March 1987, 2213; on ground under *Eucalyptus* sp., Clewer, Transvaal, March 1987, 2214; on ground under *Acacia mearnsii*, Seralia Wilderness Area, NE Transvaal, April 1987, 2237; on ground under *Eucalyptus* sp., Seralia Wilderness Area, NE Transvaal, April 1987, 2238; on ground under *Eucalyptus* sp., Seralia Wilderness Area, NE Transvaal, April 1987, 2239; on ground under *Eucalyptus* sp., Belfast, Transvaal, April 1987, 2240; on ground under *Eucalyptus* sp., Belfast, Transvaal, April 1987, 2241; under *Acacia mearnsii*, Kraaiibos, near Karatara, C.P., April 1987, 2242; on ground under *Eucalyptus* sp., Kraaiibos, near Karatara, C.P., April 1987, 2243; on ground, Saasveld College of Forestry, George, C.P., April 1987, 2244.

* Cultures from Department of Forest Science, University of Stellenbosch:* No. 3*, from *Eucalyptus* sp., Stellenboschberg, Stellenbosch; No. 22, from *Pinus pinaster*, France, (ex Dr D.H. Marx); No. 36*, *Eucalyptus grandis*, Melmoth, Natal; No. 42*, *Eucalyptus grandis*, Elandshooge, eastern Transvaal; No. 54*, *Eucalyptus* sp., Longmore, Tsitsikamma Forest Region; No. 58, *Pinus taeda*, Georgia, U.S.A.; No. 77*, mixed *Eucalyptus* spp. *Pinus radiata* stand, Wiesenhof, Paarl district; No. 99*, *Eucalyptus diversicolor*, Witte lies bos, Knysna Forest Region.

*also studied in culture.

* spores examined as well.

**Discussion**

The sporocarps of collections examined during the present investigation varied widely in size, shape and general appearance but corresponded with regard to anatomical characters. All correspond with description of sporocarp morphology of *Pisolithus tinctorius* b. Bottomley (1948), Cunningam (1944), Grand (1976) an van der Bijl (1918). Basidiospores from all collection from which they were available, were very similar in morphology when viewed under the light microscope although they were mostly smaller than the sizes given by Grand (1976). Basidiospores from South African sporocarps are larger and more coarsely echinulate that those of the related species *Pisolithus microcarpus* (Cooke & Masseau) G.H. Cunningham (Cunningham 1944). Spore size of the South African collections are within the ranges given by Bottomley (1948), Cunning ham (1944), Mims (1980) and van der Bijl (1918). Unde: the light microscope, basidiospores of the Australasian specimen, PREM 46185, agree in size and morphology with those of the South African collections. Their surface ornamentation also corresponds with that of the South African collections when examined at 5 000 × magnification by SEM. Characters of these spores in turn correspond with the descriptions and electronmicrographs of spores from North American collections (Grand 1976; Mims 1980). They differ however, in surface ornamentation from spores of a collection of *P. tinctorius* reported from Denmark by Hansen (1986) which has sharp-pointed spines. Bronchard et al. (1975) also figured spores with sharp-pointed spines from sporocarps of the European species, *Pisolithus arhizus*, and suggested that this character may make it possible to separate European *Pisolithus* from a similar species frequently occurring in Africa. However Grand (1976) figured scanning electron micrographs of basidiospores of *Pisolithus* which show both sharp-pointed and blunt spines from various localities in the U.S.A. He further reported no differences in spore size and ornamentation related to location, habitat or habit of 167 sporocarps of *P. tinctorius*.

Cultural characters and micromorphology of isolates obtained from South African sporocarps are closely similar to those of isolates from *Pinus pinaster* from France and *Pinus taeda* from Georgia, U.S.A., respectively. All the isolates agree in respect of micromorphological and cultural characters with the descriptions by Hile & Hennen (1969) and Miller et al. (1983). However, inflated hyphae as described and figured by the latter authors were not observed in our cultures although this may be due to the use of different media.

The South African collections thus agree in morphology and cultural characters with specimens and cultures that had been referred to *P. tinctorius* from localities outside of this country. Furthermore the
isolates from *Eucalyptus* were indistinguishable from those obtained from *Pinus* spp. These observations together with descriptions by Grand (1976), Hile & Hennen (1969) and Mims (1980) of *P. tinctorius* associated with *Pinus* spp., reveal no morphological differences between sporocarps of *P. tinctorius* associated with pines and those associated with eucalypts. The absence of *P. tinctorius* sporocarps from pine stands in South Africa can therefore not be ascribed to the occurrence of a different *Pisolithus* species in this country.

Grand (1976) and Marx (1977) reported sporocarps of *P. tinctorius* as being widely distributed in the U.S.A., occurring mostly on poor, acid, sandy or clayey soils and usually in association with various tree species. Occasionally sites without trees such as gardens, lawns, pastures and sand dunes were cited as localities. This led Grand (1977) to support the view put forward by Dring (1973) that mycorrhizal as well as saprophytic forms (varieties) of *P. tinctorius* may exist. Marx (1981) later reported experimental results supporting this view. On the basis of these reports together with observations described in this paper it appears that strains of *P. tinctorius* not generally capable of forming mycorrhizas with pines, generally occur in South Africa.

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