

Short Communication / Kort Mededeling

Amylostereum areolatum (Aphylophorales: Stereaceae) in South Africa

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Amylostereum areolatum (Fr.: Fr.) Boidin has been found in South Africa for the first time. So far, its presence has been confirmed on *Pinus radiata* D. Don in the Tokai Plantation, Western Cape. Its distribution area is expected to follow that of the wood-boring wasp, *Sirex noctilio* Fabricius, with which it has a symbiotic association.

Amylostereum areolatum (Fr.: Fr.) Boidin is vir die eerste keer in Suid-Afrika aangetref. Tot dusver is die teenwoordigheid daarvan op *Pinus radiata* D. Don in die Tokai Plantasie, Westelike Kaap, bevestig. Na verwagting sal die verspreidingsgebied van die fungus met dié van die hout-borende wespe *Sirex noctilio* Fabricius, waarmee dit 'n simbiotiese assosiasie vorm, ooreenstem.

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Amylostereum areolatum (Fr.: Fr.) Boidin was recently found in South Africa for the first time, in the Western Cape. A weak pathogen of *Pinus* L. species (Talbot 1964; Coutts 1969a, 1969b), this fungus is best known for its symbiotic association with the potentially destructive wasp, *Sirex noctilio* Fabricius. Talbot (1977) as well as Neumann and co-workers (1987) have reviewed the literature on the complex biology of the *Sirex*-*Amylostereum*-*Pinus* association and the interaction of biocontrol organisms.

Carrying the fungal symbiont in two internal mycangia, the female wasp injects eggs together with phytotoxic mucus and fungal arthrospores into the sapwood (Neumann *et al.* 1987). The mucus, containing enzymes including phenoloxidase (Wong & Crowden 1976), rapidly induces physiological changes with concomitant foliage symptoms (Coutts 1969b) and increases the susceptibility of trees to the fungus (Coutts 1969a, 1969b). Phenoloxidases are also produced by *A. areolatum* (Stalpers 1978), which causes a white wood rot (King 1966; Chamuris 1988) and grows relatively slowly in standing trees (Vaartaja & King 1964; Coutts 1969a). Neither the fungus nor mucus alone is usually capable of killing trees, but the combination is lethal (Coutts 1969a). In culture, *A. areolatum* optimally grows at 25°C (Madden 1981) and poorly competes with later fungal colonizers of wood infested by *S. noctilio* (King 1966). Immature stages of the wasp are mycophagous (Cartwright 1929) and depend on actively growing *A. areolatum* for their development and survival (Madden 1981). In *S. cyaneus* Fabricius, larvae evidently ingest wood as well as the mycelium of their symbiont, *A. chailletii* (Pers.: Fr.) Boidin, acquiring essential digestive enzymes from the fungus (Kukor & Martín 1983).

In the temperate Northern Hemisphere, where *A. areolatum* occurs mainly on *Picea* (Jülich & Stalpers 1980; Chamuris 1988), *Amylostereum* species are saprotrophs (Jülich & Stalpers 1980). Contrastingly, in the Southern Hemisphere *A. areolatum*

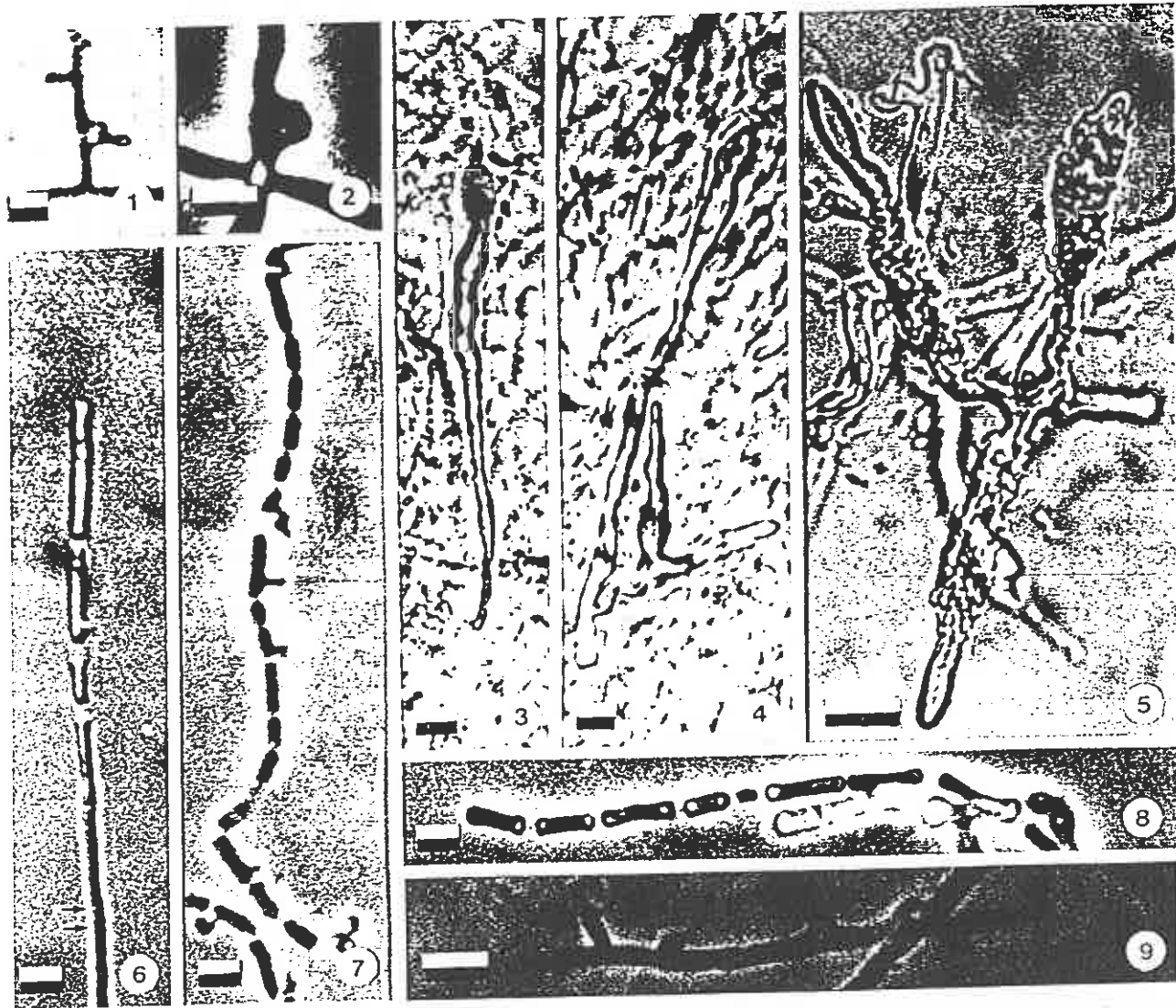
occurs on *Pinus*, which it damages or kills in association with *Sirex* wasps (Talbot 1977). Similarly, the Eurasian *Sirex noctilio*, reportedly also established in Canada (Morgan 1968), is not considered a primary pest in Europe (Neumann *et al.* 1987), whereas it is a major pest of *Pinus* in the Southern Hemisphere, particularly in New Zealand and Australia (Talbot 1977). In South Africa it is expected to become a major pest in the Cape forestry areas due to the Mediterranean climate and abundance of suitable host trees (Tribe 1995).

Although the wasp was reported in South Africa as long ago as 1962 (Morgan 1968), such earlier records were limited to harbour interceptions (C.D. Eardley, pers. commun.). The first South African isolate of the fungus was positively identified in September 1994 from tunnelled *P. radiata* D. Don wood. Its wasp symbiont appears to have been present in the Cape Peninsula for at least two years prior to its discovery in April 1994 (Tribe 1995). *Sirex* host trees recorded in South Africa thus far are *P. radiata*, *P. pinaster* Aiton and *P. taeda* L., and the present distribution area appears to fall within a 90-km arc around Cape Town (Tribe 1995).

Wood samples were initially taken from a larvae-infested *P. radiata* log in Tokai Plantation by forest entomologists of the Plant Protection Research Institute. Later, standing trees with suspected *Sirex* infestation, which was subsequently confirmed, were core sampled. Pieces of wood were placed on 1.5% malt extract agar (MEA) and incubated at 24°C. Cultures of *Amylostereum* were obtained from both sets of samples as well as from female reproductive organs, floated overnight in sterile water and then incubated on MEA. Spot tests for laccase were done with 1-naphthol as well as syringaldazine, as described by Marr (1979) after Käärik (1965) and Harkin *et al.* (1974) respectively.

Cultures laccase positive. Growth rate 55-65 mm after two weeks at 25°C, about 40 mm at 20°C. Colony mat with margins appressed; thin, cottony to somewhat woolly, white to pale cream, becoming subfelty to pelliculose with patches of brown, later almost entirely light yellowish-brown with patches of darker brown; reverse darker. Aerial hyphae mostly 2.0-4.5 µm diameter, thin-walled, sometimes yellow and with oily-appearing contents. Clamp connections (Figures 1 & 2) present at all septa, sometimes sprouting; prominent, ratio hyphal diameter:clamp diameter ≥ 1. Skeletal hyphae present as pointed skeletocystidia (Figures 3-5), originating from generative hyphae and subtended by a clamp connection, mostly yellow to yellow-brown, often ventricose, usually densely encrusted with crystals over at least the apical third of their length; length very variable, 30-150 × 4.5-7.5 µm; walls up to 2.5 µm thick. Basidia formed within four to six weeks, extremely sparse; subclavate, about 14-15 × 4.5 µm, with a basal clamp and four robust sterigmata. Basidiospores hyaline, smooth, amyloid, predominantly ellipsoid. Arthrospores (Figures 6-8) abundant, cylindrical, mostly oblong to bacilliform with rounded ends, sometimes barrel-shaped; 7.0-18.0 × 2.5-4.5 (mostly 11.5 × 3.5) µm. Basidiomata not seen: Talbot (1977) noted that investigators such as King (1966) had not found fruit-bodies of *A. areolatum* in Australia or New Zealand, although isolates from those areas were able to form them in culture, Gilbertson (1984) postulated that dissemination in *Amylostereum* species in some areas had become dependent on the insect symbiont. This would sidestep the uncertainty of propagation by wind-dispersed basidiospores, which are exposed to desiccation and the likelihood of landing on unsuitable sites (Gilbertson 1984).

Specimens examined from *Pinus radiata* D. Don in Tokai Plantation (34°03'S 18°25'E), south-western Cape Province, South Africa: PREM 51842 (culture PPRI 5610), coll. G.D. Tribe, August 1994; PREM 51843 (culture PPRI 5642), coll. J.J. Cillié, November 1994; PREM 51877 (culture PPRI 5858) from



Figures 1-9 Morphological features of *Amylostereum areolatum* (Fr.: Fr.) Boidin: 1-8 from cultures on 1.5% malt extract agar, 9 from female reproductive organs of *Sirex noctilio* Fabricius wasp. Scale bars: 10 μ m. 1-2. A clamp connection is present at each septum in the young generative hyphae; clamps commonly sprout (1) and are mostly very prominent (2). 3-5. Skeletocystidia originate as modified ends of skeletal hyphae. They are usually pointed and apically encrusted, with a hyphoid pedicel (3), or ventricose, sometimes unencrusted (4); some are clearly the ends of branched hyphae (5). 6-8. Arthrospores are formed in long chains, with simple septa (arrowed) demarcating the developing spores (6); clamped septa are often still apparent (7) as well as the continuous outer wall layer that holds the spores together in young chains (8). 9. Hyphae with clamped septa were visible on female wasp reproductive organs suspended overnight in sterile water.

reproductive organs of female *S. noctilio* from the above locality, January 1995. Voucher specimens of the wasp are deposited in the National Collection of Insects, Plant Protection Research Institute, Pretoria.

Cultures on MEA closely corresponded with the characters reported by Stalpers (1978); morphological features matched those described by Talbot (1964). Clamped hyphae (Figure 9) were observed on female reproductive organs that had been suspended in water overnight. All the isolates reported above produced the brownish, acute, encrusted cystidia characteristic of the genus (Chamuris 1988), as well as arthrospores, which are formed in cultures of *A. areolatum* but not in those of *A. chailletii* (Siepmann & Zycha 1968; Stalpers 1978; Chamuris 1988). Although multinucleate, the arthrospores are homokaryotic (Gaut 1969). By means of interfertility tests and starch-gel electrophoresis, Gaut (1969) confirmed the identity of the fungus associated with *S. noctilio* in Australia as *A. areolatum*.

Talbot (1964), acknowledging the help of J. Boidin, was the first to describe the fungal symbiont of *S. noctilio* as a species

of *Amylostereum*. Boidin (1958) had segregated this genus from *Stereum* Hill ex Gray *sensu stricto*, including *A. chailletii*, the type, as well as *A. areolatum* and *A. laevigatum* (Fr.: Fr.) Boidin. Of the four species matching the genus concept of *Amylostereum* accepted by Chamuris (1988), two are symbionts of siricid wasps (Talbot 1977): *A. areolatum* and *A. chailletii*. In addition to *S. noctilio*, *A. areolatum* is associated with *S. juvenicus* (Linnaeus) and *S. nitobei* (author citation not traced); *A. chailletii* is associated with *S. cyaneus*, *S. areolatus* (Cresson) and *S. californicus* (Ashmead) as well as certain *Urocerus* species (Gilbertson 1984).

The nature of the cystidia in *Amylostereum* has been subject to various interpretations. Boidin (1958) was of the opinion that the distinction between cystidia, pseudocystidia and skeletal hyphae was largely based on the position and orientation of the structure in question. Price (1973) interpreted the cystidia in *Amylostereum* as the ends of skeletal hyphae, terming them skeletocystidia. Donk (1964) had used this term to designate the modified tips of skeletal hyphae that usually terminate in the

hymenium. According to Jülich & Stalpers (1980), cystidia in *Amylostereum* originate from skeletal hyphae, rarely from generatives. In the cultures examined in the present study, the cystidium-form elements clearly originated from thin-walled, clamped, generative hyphae. Their length was very variable: the longer ones, in particular, could be interpreted as the modified, sometimes branched ends of skeletal hyphae.

The source of the present *Sirex*-*Amylostereum* infestation is, as yet, unknown. An attempt was made in 1970 by I.P.C. Gaut (Talbot 1977) to trace the geographical origins of Australasian isolates of *A. areolatum* by comparing their protein and enzyme patterns with those of isolates from Belgium and Switzerland. Modern techniques such as restriction fragment length polymorphisms (RFLPs) and multicopy DNA probes have been used with success for this purpose in various fungi (Liew *et al.* 1991; Whisson *et al.* 1992). Comparison of *Amylostereum* isolates from South Africa with material from other regions could provide valuable information on this aspect.

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