# SOME ASPECTS OF THE BIOLOGY OF THE FUNGAL SYMBIONT OF *SIREX NOCTILIO*

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*Summary*

Studies of the cultural characteristics on artificial media of the fungal symbiont of *Sirex noctilio* F. indicate that in Australia only one fungus is involved in the association. A medium containing 20 p.p.m. *o*-phenylphenol was found suitable for the isolation of the Sirex fungus. Histological studies confirmed that the Sirex fungus develops in wood only as vegetative hyphae. Ray parenchyma cells were killed in advance of the growing hyphae, which suggested that a toxin was produced by the fungus. Comparisons of growth in agar culture, of fructifications produced on wood blocks, and of starch gel electrophoresis protein patterns indicated that the Sirex fungus is probably a strain or variety of *Amylostereum chailletii* (Fr.) Boidin.

## I. INTRODUCTION

In Australia, adults of *Sirex noctilio* emerge from attacked trees during the period from late November to early May, with peak emergence occurring in February and March. The symbiotic fungus, which is carried in the form of arthrospores and blastospores by female wasps in the paired intersegmental sacs at the base of the ovipositor (Francke-Grosmann 1939), is injected into the wood during the process of oviposition. The ovipositor is inserted about 0·75 in. into the wood, but eggs are not laid at each insertion. The eggs hatch in 2–6 weeks, depending on the temperature and moisture content of the wood. For about 4 months larvae stay near the annual ring in which they are laid, then turn inward and often enter the heartwood. Larvae pupate from October to March in both the first and second seasons following oviposition, in pupal chambers constructed about 1 in. below the bark, and pupation is completed in about a month, after which the adult bores out of the wood.

The close association of the fungus with developing wood wasp larvae, the transfer of the fungus between successive female larval instars, and its eventual development as arthrospores in the intersegmental sacs of the adult female wasp have been examined and discussed by a number of workers (Buchner 1930; Cartwright 1938; Francke-Grosmann 1939, 1957; Parkin 1942), but there are many points which still need to be investigated.

The first sign of infestation by wood wasps is the exudation of resin from oviposition punctures in the bark. Within a month of oviposition, a strip of bark extending above and below each puncture is killed, probably owing to the activity of the fungus. Wood wasp attack may lead to the killing of trees within 2 months of oviposition, or trees may die over a longer period depending on the time and severity of attack. For example, trees attacked in late autumn usually die in late winter or spring while some are only partly killed. Some trees, however, may recover and show

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few external symptoms of attack. There is much evidence to suggest that siricids attack the least vigorous or weakened trees. The fungal symbiont of *S. noctilio* causes extensive dry white rot of the wood of *Pinus radiata*, both during wood wasp development and after the death of the trees.

## II. Cultural Studies

Isolates of the fungal symbiont of *S. noctilio* (hereafter called the Sirex fungus) were obtained: (*a*) from infested pine wood from Tasmania, Victoria, and New Zealand; (*b*) from the intersegmental sacs of female wasps; (*c*) from the hypopleural organs of female larvae. These isolates were studied in pure culture on artificial media and their cultural characteristics have been described by Talbot (1964).* All isolates grew well on a wide range of media. At 25°C, the growth rates on corn meal agar and neutral Dox + yeast agar (Warcup 1950), the two media used most extensively in the present study, were 1·6–1·8 and 2·8–3·1 mm/day respectively. All isolates were very similar in cultural characteristics and growth rate, and all were apparently of the same fungal species. On the above two media growth occurred at temperatures between 7 and 30°C, the optimal temperature being between 20 and 25°. No growth occurred during incubation for a 3-week period at 3 and at 35°, but the fungus resumed growth when plates were later incubated at 25°. Mycelium on agar slants in test-tubes was killed within 24 hr at 42°.

The effect of hydrogen ion concentration on one isolate of the Sirex fungus was studied in neutral Dox + yeast liquid medium with a phosphate buffer. No growth occurred at or below pH 3·4 nor at or above pH 7·6, although good growth was recorded between pH 4·8 and 6·0.

The effect of the growth of some wood-inhabiting fungi (most of which are commonly found in previously *Sirex*-infested timber) on the growth of the Sirex fungus was studied in paired cultures on agar media. In general, there was a retardation in growth of both fungi as the hyphal fronts approached each other, with the formation of a narrow zone of inhibition. This was usually followed by a partial or complete overgrowth of the Sirex fungus by the other fungus. *Trichoderma* sp. and *Diplodia pinea* (Desm.) Kickx. were found to be strongly antagonistic to the Sirex fungus, causing death of most hyphae. The evidence from agar cultures suggests that the Sirex fungus is a poor competitor with other fungi which are later colonists of wood infested by *Sirex noctilio*.

During the course of cultural work a selective medium was found for the isolation of the Sirex fungus. The selectivity of agar media containing antibiotic and other chemicals at various concentrations was tested on artificially mixed inocula of the Sirex fungus and 12 fungi which commonly inhabit pine wood. The selective medium giving most consistent results was a one-sixth dilution of neutral Dox + yeast agar (Warcup 1950) containing 20 p.p.m. *o*-phenylphenol (Russell 1956). The Sirex fungus produces its characteristic arthrospores and cystidia on this medium which can be used to separate it from other fungi.

* In Talbot's paper, the term "hypopleural sacs" was used in error for "intersegmental sacs". The structures referred to in all cases were the intersegmental sacs of adult female wasps and not the hypopleural organs of female larvae.

## III. Growth in Wood

The growth rate of the Sirex fungus was examined in freshly cut branches of *Pinus radiata* and other softwoods. The pieces used were more than 1·5 cm in diameter and 20–35 cm long and of internodal origin. A hole 3 mm in diameter was drilled into the wood to a depth of about 1 cm towards one end of the test piece, and this was filled with inoculum which consisted of an actively growing culture of the Sirex fungus on a sawdust–maize meal medium. The inoculation hole and the branch ends were sealed with paraffin wax to prevent water loss. The presence of viable mycelium was tested after 3 weeks by taking pieces of bark and wood at 1 cm intervals away from the inoculation point along the length of a branch and plating these on the selective medium described above. The maximum growth rate at 25°C in *Pinus radiata* D. Don was 14 mm/day but the fungus also grew well, though more slowly, in other pine species (*P. canariensis* C. Sm., *P. pinaster* Ait., and *P. halepensis* Mill.). With other softwoods tested, the maximum growth rates were 8 mm/day in *Cedrus deodara* (Roxb.) Loud., 5 mm/day in *Araucaria excelsa* R. Br. (Norfolk Island pine), and 2 mm/day in *A. cunninghamii* Sweet (hoop pine). No growth was obtained in *Cupressus macrocarpa* Hartw., although the inoculum remained viable for the period of the test. It is interesting to note here that in the Pittwater plantation, Tasmania, *C. macrocarpa* windbreaks have not been affected during the severe infestations by *Sirex noctilio* in the surrounding plantations of *P. radiata*.

## IV. Histology

The development of the Sirex fungus in pine wood soon after oviposition was studied in fixed and stained microtome sections. Female wasps were placed on freshly cut lengths of pine trunk, 3–4 in. in diameter and of suitable moisture content, on which all ovipositions were carefully marked. After 2, 4, 7, 14, and 28 days the wood surrounding several oviposition holes was cut into small blocks not more than 2·5 cm long, 0·5 cm wide, and 1·5 cm deep, this being a size suitable for microtome sectioning. Sections 10–20 $\mu$ thick were stained with 0·1% trypan blue in 6% acetic acid (hyphae and parenchymatous ray cells stained blue) and counter-stained with a 1% aqueous solution of safranin (tracheid walls stained red).

Two days after oviposition, the wood surrounding oviposition holes appeared white 2 to 3 cm above and below the hole and 0·1 to 0·2 cm to either side. The white discoloration was much in advance of hyphal penetration, which at this stage could only be traced approximately 4 cm up or down from the oviposition site and 0·2 to 0·3 mm to either side. No arthrospores were seen in oviposition holes, but hyphal development from arthrospores, which obviously occurred very soon after oviposition, would make their identification difficult. No arthrospores were seen in the wood at a later stage. Most hyphae were 1·3 $\mu$ in diameter, with numerous simple clamp connexions, and were observed to pass from one tracheid to the next through simple or bordered pits (Plate 1, Fig. 1). Ray cells about 2 mm in advance of the hyphae appeared normal but those within the area of mycelial development were actively colonized by the fungus. The white discoloration of the wood extended more rapidly than the spread of the fungus, and both processes were largely confined to extension in a longitudinal direction.

After 14 days the white discoloration had reached 10–15 cm up and down the stem from the oviposition holes but only 0·5 cm to either side, and a brown-stained area had developed in the bark around the oviposition puncture; this was similar in shape to the whitened area of wood but with about half its linear dimensions. The Sirex fungus was isolated from the stained bark, from the wood immediately below it, and from wood and bark just beyond the stained area. The contents of the parenchymatous ray cells in the wood beyond the region penetrated by the fungus were darkly discoloured and apparently dead. These affected ray cells were in the wood, which macroscopically appeared white and from which no fungus was isolated. The death of ray cells before invasion by hyphae suggests the production of a toxin by the fungus.

After 28 days the wood surrounding the holes was thoroughly permeated with the fungus. The diameter of the hyphae varied from 1 to 6 $\mu$. As well as passing through pits, hyphae were observed to pass directly through tracheid walls (Plate 1, Fig. 2). Wood near larval tunnels about 4 months old, within the white discoloured area, was also examined. Abundant hyphae were present in the ray cells and in the tracheids near the tunnels, but about 1 cm away they were not plentiful.

None of the sections showed evidence of blockage of tracheids by hyphae or tannin deposits, and no tyloses were observed. At no stage were arthrospores or blastospores observed in infected wood; fungal growth is apparently confined to the production of unmodified vegetative hyphae.

## V. Comparison of the Sirex Fungus with Amylostereum chailletii

Stillwell (1963) reported that the fungus isolated from arthrospores from intersegmental sacs of adult females of *Sirex juvencus* L. and *Urocerus* spp. in Canada is *Amylostereum chailletii* (Fr.) Boidin. Since the Sirex fungus in Australia has been shown by Talbot (1964) to be a species of *Amylostereum*, its cultural characteristics were compared with those of Canadian isolates of *A. chailletii*. To facilitate this, five polysporous isolates originating from natural fructifications of *A. chailletii* were obtained from Dr. M. K. Nobles (Canadian Department of Agriculture, Ottawa). Growth rates and cultural characteristics were compared on neutral Dox + yeast agar at 25°C. The growth rates of the five isolates of *A. chailletii* varied from 3·0 to 4·3 mm/day while that of the Sirex fungus varied from 2·8 to 3·1 mm/day. Some morphological characteristics, such as the production and form of the simple clamp connexions and encrusted cystidia, were very similar in *A. chailletii* and the Sirex fungus, but *A. chailletii* did not form arthrospores or basidia on the agar medium. Although there were some general differences between the growth form of isolates of *A. chailletii* and those of the Sirex fungus on agar, there was also considerable variation in the cultural characteristics of the individual isolates of *A. chailletii*.

Fructifications of *A. chailletii* and of the Sirex fungus were produced on wood blocks by the technique described by Tamblyn and Da Costa (1958). There was considerable similarity in the general appearance of all the fruiting bodies obtained, but because of the well-known difficulties of comparing microscopic characters of stereoid fructifications at different stages of growth no detailed comparative examination was made of these fructifications. Such examination was postponed pending

further more closely controlled fructification studies. Naturally produced fructifications of the Sirex fungus have not been observed in infected forests in New Zealand and Australia.

It is considered that interfertility tests with monosporous cultures would be of importance in determining the relationship between *A. chailletii* and the Sirex fungus. However, although a proportion of basidiospores of the Sirex fungus (from basidia on agar media and from fructifications on wood blocks) germinated on agar, growth ceased at an early stage. With the isolates of *A. chailletii* examined, a high proportion of basidiospores germinated after being shed on agar, and continued growing to form a monocaryotic mycelium devoid of clamp connexions.

Starch gel electrophoresis of mycelial proteins can be used as an aid to identification of fungi (Clare 1963). Extracts of mycelium of *A. chailletii* and the Sirex fungus, obtained from liquid cultures 10 and 18 days old, were compared by starch gel electrophoresis, amido black B10 being used as stain. The methods for preparing hyphal extracts and starch gels described by Clare (1963) were followed. There was general similarity between the protein patterns produced by individual isolates of the Sirex fungus and between those of individual isolates of *A. chailletii*. Although there were minor differences between the protein patterns of *A. chailletii* and the Sirex fungus isolates (Plate 1, Fig. 3), the general similarity is considered as a strong indication that the Sirex fungus is a strain or variety of *A. chailletii*. This opinion has been corroborated by Mr. B. G. Clare.

From the results derived in the comparative studies of growth on agar, from the experimental production of fructifications and from starch gel electrophoresis, there is considerable evidence that *A. chailletii* and the Sirex fungus are monospecific.

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## EXPLANATION OF PLATE 1

Fig. 1.—Tangential longitudinal section of pine wood infected with the Sirex fungus, showing passage of hyphae through bordered pits.

Fig. 2.—Tangential longitudinal section of pine wood infected with the Sirex fungus, showing ray cells filled with fungus and a hypha passing directly through tracheid walls.

Fig. 3.—Starch gel electrophoresis pattern, showing protein bands of three isolates of the Sirex fungus (*S*) alternating with those of two isolates of *Amylostereum chailletii* (*A*).

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