INOCULATION EXPERIMENTS WITH AMYLOSTEREUM SP. FROM A WOOD WASP

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Abstract

Amylostereum sp., associated with Sirex noctilio F. in Tasmania, killed young seedlings of Pinus radiata in aseptic experiments in test tubes. It did not kill saplings in the greenhouse or larger trees in the field, when inoculated in one place on the trunk and incubated for 3 to 6 months. It survived, however, and spread slowly (0 to 50 mm in 4 months) in the surface wood. The trees showed varying degrees of resistance. We suggest that in natural epidemics this resistance is lowered by drought or overwhelmed by mass-infection from a large number of inoculations by the wasp.

INTRODUCTION

In their native habitats, for example, in Europe, wood wasps (Siricidae) do not occur in a manner considered economically serious (3). In New Zealand and Tasmania one species from Europe (Sirex noctilio F.) has become destructive on Pinus radiata (3). Extensive areas of valuable forests have died. Spread of this wasp into the monocultures on the Australian mainland would be economically serious. This wasp species lives in a close but not well known association with fungi (2) growing in wood, but their role in killing trees has not yet been studied. Solutions to the most urgent problems involved would be facilitated by inoculating trees with the fungi under various conditions and with different techniques in the absence of the wasp. This paper reports a few inoculation experiments with a fungus commonly associated with S. noctilio in P. radiata in Tasmania.

MATERIALS AND METHODS

Isolations were made from intersegmental sacs of female wasps and from wood around the larval galleries in infested trees near Hobart airport, Tasmania. A basidiomycete was isolated consistently and is considered to be Amylostereum sp. (5). Its cultures could be identified on the basis of (a) rather straight hyphae with numerous single clamps and of unusually even thickness (mainly 2.5-4.5μ); (b) abundant transformation of aerial hyphae into arthrospores (oidia) of varying lengths with some clamps remaining distinct (Fig. 1); (c) numerous single or sometimes branched cystidia largely covered by brown crust (Fig. 2); (d) white, later creamy or brownish, faintly sweet smelling colony on malt agar.

Isolates from the wasp and its galleries were utilized in four types of inoculations on P. radiata: (a) In test tubes: seedlings were grown aseptically on cornmeal agar inoculated with the fungus; (b) In the greenhouse: sawdust-maize meal culture of the fungus was placed in a small flat hole in the wood of each of nine saplings 30 to 40 cm high; (c) In the field with sawdust-maize meal culture: placed in a 30-mm deep, 7-mm wide hole, in each of nine trees 2 to 4 inches in diameter, at Hobart; (d) In the field with culture suspension: macerated liquid culture was injected with a hypodermic needle into a hole 1-mm wide in each of three trees; other-

FIGURE 1. Arthrospore chain of Amylostereum sp. (Approx. 525 X)

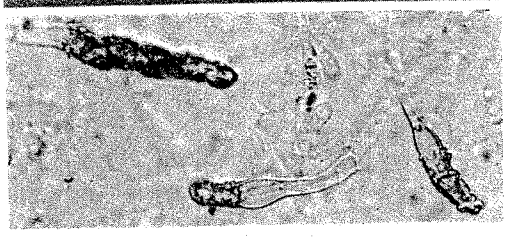

FIGURE 2. Cystidia of Amylostereum sp. (Approx. 525 X)

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wise as in (c). In (b) to (d), the bark was first cut loose with a sterilized knife, replaced after inoculation, and then firmly bound. The hole for (b) was made with a knife, for (c) with an increment hammer and for (d) with a drill. For (c) a part of the removed cylindrical wood core was reinserted in the hole to protect the inoculum underneath.

The greenhouse saplings were grown in cans under three watering treatments, but as these had no influence on the results, they are not described here. The field inoculations were made into healthy trees in December, February and May. After incubation for 3 to 5 months small pieces of surface wood were taken at different distances from the inoculum, plated on agar and, after a few days, the fungal growth was studied under the microscope. Re-isolation of Amylostereum sp. was aided by incorporation of the following selective fungicides, alone or at various combinations, in some of the culture media: orthophenyl phenol (up to 20 ppm), Mycostatin (8 ppm), pentachloronitrobenzene (10 ppm), and streptomycin (100 ppm). Amylostereum sp. tolerated these better than did various other wood-inhabiting fungi and bacteria.

In experiment (a) the aseptic seedlings were obtained as follows: P. radiata seeds were treated with 0.25% aqueous solution of mercuric chloride and germinated on malt agar; contaminated seeds were removed with some surrounding agar. When a radicle reached 1 to 3 mm from the seed, it was aseptically dropped on inoculated agar in large test tubes, 2 to 4 seeds per tube. The tubes were incubated under illumination of about 400 ft-c at room temperature.

RESULTS

In test tubes, Amylostereum sp. killed all 15 seedlings in 2 to 4 months, while 19 of 20 ntrol seedlings remained alive (one failed to root and dried up). The 15 seedlings with Amylostereum developed very poor roots and no or only a few primary needles. All the surviving control seedlings developed vigorous large roots and many needles. Amylostereum sp. was re-isolated from the seedlings in inoculated tubes. No other fungi were present.

In the greenhouse, after 5 months' incubation, none of the nine inoculated saplings appeared essentially different from the nine control saplings. Amylostereum sp. was re-isolated from eight seedlings. The distance the fungus had spread was only 5 to 10 mm, however. Other fungi were also present around the inoculum. Most of these could be identified as Aureobasidium pullulans (DeBary) Arnaud or Hormodendron sp. and a few as Penicillium sp.

In the field, after 4 to 6 months' incubation, none of the 12 inoculated trees appeared essentially different from the surrounding healthy trees. They certainly did not exhibit the chlorosis or browning of needles usually, though not always, seen in 4 to 9 months after heavy natural inoculations by S. noctilio in similar trees on the same site. Amylostereum sp. was re-isolated from nine of the trees, including the three injected with the culture suspension. However, the fungus had usually spread only 5 to 20 mm, mainly up or down from the inoculum. In one tree the spread was 50 mm during four summer months. Various other fungi were also isolated: commonly A. pullulans and occasionally Leptographium sp., Penicillium sp., Trichoderma sp., Diplodia sp. and Candida sp.

In most greenhouse and field inoculations the wood around showed a slight reddish brown stain. This appeared to result from slight to abundant release of resin. In most inoculations resin was much less abundant than in the few natural infections where Amylostereum was found to be apparently encapsulated by excessive resin. No resin stain was detected in one of the inoculations with a hypodermic needle, while it was obvious in the two others. In two trees there was also some blue stain, probably caused by other fungi, such as Diplodia sp. and Leptographium sp.

DISCUSSION

The ability of Amylostereum sp. to kill young seedlings in the test tubes should of course be taken only as an indication of its potential pathogenicity. In forests this fungus perhaps never infects such young seedlings.

Although field and greenhouse inoculations failed to produce the disease and the spread of Amylostereum sp. in wood was slow, the common reisolations from living wood after long incubation are interesting. Apparently, the inoculum had some pathogenic potential but this was almost balanced by the resistance of the host. This is a contrast to the experiment (a) in test tubes and to results with severed pine branches, in which Amylostereum sp. spread rapidly, usually over 10 cm, in 3 weeks (unpublished data).

The resistance factors could conceivably be resin exudation into tracheids or other antifungal substances in living wood cells. Resin appeared to play some part in the resistance of

a tree, but obviously was not the only factor. Bier and Bloomberg (1) have recently established a correlation between water content of poplar cuttings and their resistance to various fungi. Furthermore, Rawlings and others, as summarized by Scott (4), have given circumstantial evidence for the hypothesis that drought predisposed pines to attack by S. noctilio and to the associated fungus when the epidemics started in New Zealand. It is urgent to compare inoculations similar to ours (d) with inoculations in trees under moisture stress. This was in fact attempted with some of our experimental trees by severing part of the stem with a saw. Such treatments, however, were not sufficient to produce drought effects during the moist cool season and should be repeated during a dry summer. The spread of Amylostereum sp. in the greenhouse and field experiments varied greatly, from 0 to 50 mm per 4 months, indicating the relative character of the resistance factors. It is perhaps not mere coincidence that the greatest spread occurred in a tree in the field during a hot summer period.

Because the resistance does not appear absolute but only relative, it is reasonable to postulate that the large number of natural inoculations in Sirex epidemics overwhelms such resistance. Antagonism by other fungi present in wood may be a third factor affecting the infection by Amylostereum sp. This is the subject of another study. Our unpublished data suggest that this factor was not essentially different in our experiments from that in a natural epidemic.

Literature Cited

1. BLOOMBERG, W. J. 1962. Cytospora canker of poplars; factors influencing the development of the disease; the moisture relations and anatomy of the host. Can. J. Botany 40: 1271-1292.
2. FRANCKE-GROSMANN, HELENE. 1939. Uber das Zusammenleben von Holzwespen (Siricinae) mit Pilzen. Zeits. Angew. Entom. 25: 647-680.
3. FRANCKE-GROSMANN, HELENE. 1963. Some new aspects in forest entomology. Ann. Rev. Ent. 8: 415-438.
4. SCOTT, C. W. 1960. Pinus radiata. F.A.O. Forest and For. Prod. Stud. 14: 1-328.
5. TALBOT, P. H. B. 1964. Taxonomy of the fungus associated with Sirex noctilio. (In press).

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