THE EFFECT OF RADIATA PINE RESIN AND RESIN COMPONENTS ON THE GROWTH OF THE SIREX SYMBIONT AMYLOSTEREUM AREOLATUM

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SUMMARY

The effects of *Pinus radiata* resin (oleoresin) and some individual resin components on the growth of *Amylostereum areolatum*, the fungal symbiont of *Sirex noctilio*, were examined *in vitro*. Among the volatile turpentine compounds tested l-limonene and myrcene were the most inhibitory, while d- α - and l- β - pinene caused the same degree of growth inhibition as volatiles from whole resin. Resin and non-volatile resin acids incorporated in the growth medium at concentrations below 1% w/v also inhibited fungal growth. The results suggest that in radiata pine trees attacked by *Sirex* the inhibitory or toxic effects of resin on *Amylostereum areolatum* are due, at least in part, to chemical factors.

O.D.C. 453: 145.7 x 21.02: 174.7 *Pinus radiata*

INTRODUCTION

Resin is involved in the resistance of conifers to a number of fungal pathogens and insect pests. Gibbs (1968) correlated the resistance of pines to *Fomes annosus* with their ability to mobilise resin, and Shrimpton and Whitney (1968) demonstrated that the resistance of lodgepole pine to blue stain fungi was effected by the initial oleoresin flow and the gradual impregnation of the sapwood with resinous material. Smith (1961), Rudinsky (1966) and van Buijtenen and Santamour (1972) have related insect resistance to both the volatile components and the physical properties of resins.

The inhibitory effects of resins on wood-inhabiting fungi have been associated with the volatile resin turpentine components, and Cobb *et al*.(1968) and de Groot (1972) recorded growth inhibition and other effects on wood rot and blue stain fungi by terpenoids and alcohols present in the oleoresin of a number of pine species. Recently Shain (1971) found that abietic acid, a component of the resin of both spruce and radiata pine, inhibited the growth of *Fomes annosus*.

Resin flow is one of the first observable host responses to attack by *Sirex noctilio* F. on *Pinus radiata* D. Don and precedes the synthesis of

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polyphenols in areas of affected sapwood. Coutts and Dolezal (1966) found that resin accumulation limited the spread of the *Sirex* symbiont, *Amylostereum areolatum* (Fr.) Boiden, in the sapwood of *Sirex* - attacked trees. The effect of resin could be due to its physical properties and ability to act as a mechanical barrier or to a chemical effect on the fungus. The present study was undertaken to determine if *P. radiata* resin and some of its components affected the fungus in a chemical manner.

MATERIALS AND METHODS

Colonies of *Amylostereum areolatum* were cultured under different conditions of exposure to resin and resin components of *P. radiata* in 25 x 25 x 5 cm aluminium dishes containing 500 ml of saturated sodium chloride. Each colony was formed by placing a 10 mm plug, taken from the edge of an 8-day-old culture, in the centre of a 9 cm petri dish containing 2% malt agar. Four petri dishes were placed in each culture chamber on a glass plate supported by aluminium walls so that it was above the salt solution. Another glass plate over the chamber sealed the whole system, and at the temperature of 25°C at which all the experiments were conducted the saturated salt solution maintained a relative humidity of 75%. Two chambers containing a total of eight cultures were used for each treatment and control, and the diameters of the cultures were measured every one or two days during the two weeks after inoculation.

Whole resin was collected from dominant and co-dominant radiata pine trees in Pittwater plantation and thoroughly mixed. In the first experiment a vessel containing either whole resin or one of a number of volatile components of resin (d- α - pinene, 1 - β - pinene, myrcene and 1 - limonene) was placed in each culture chamber along with the fungal colonies.

In the second experiment resin dissolved in ethanol was incorporated in the 2% malt agar used in the *Amylostereum* cultures at concentrations of 0.25%, 0.50% and 1.0% w/v; the final ethanol concentration in all treatments and in the control was 1%. The volatile effects of whole resin were also re-tested at the same time.

For the third experiment the resin acids, dehydroabietic and abietic, were dissolved in ethanol and incorporated in the agar growth medium at concentrations of 0.25%, 0.50% and 1.0%; again the ethanol concentration in all cases was 1%. Other *P. radiata* resin acids were not available for testing.

The final difference in diameter growth between the untreated control cultures and each treatment was regarded as an indication of the inhibitory effect of the treatment, and was expressed as a percentage of the growth made by the controls. The significance of differences between treatment means was tested using Duncan's Multiple Range Test.

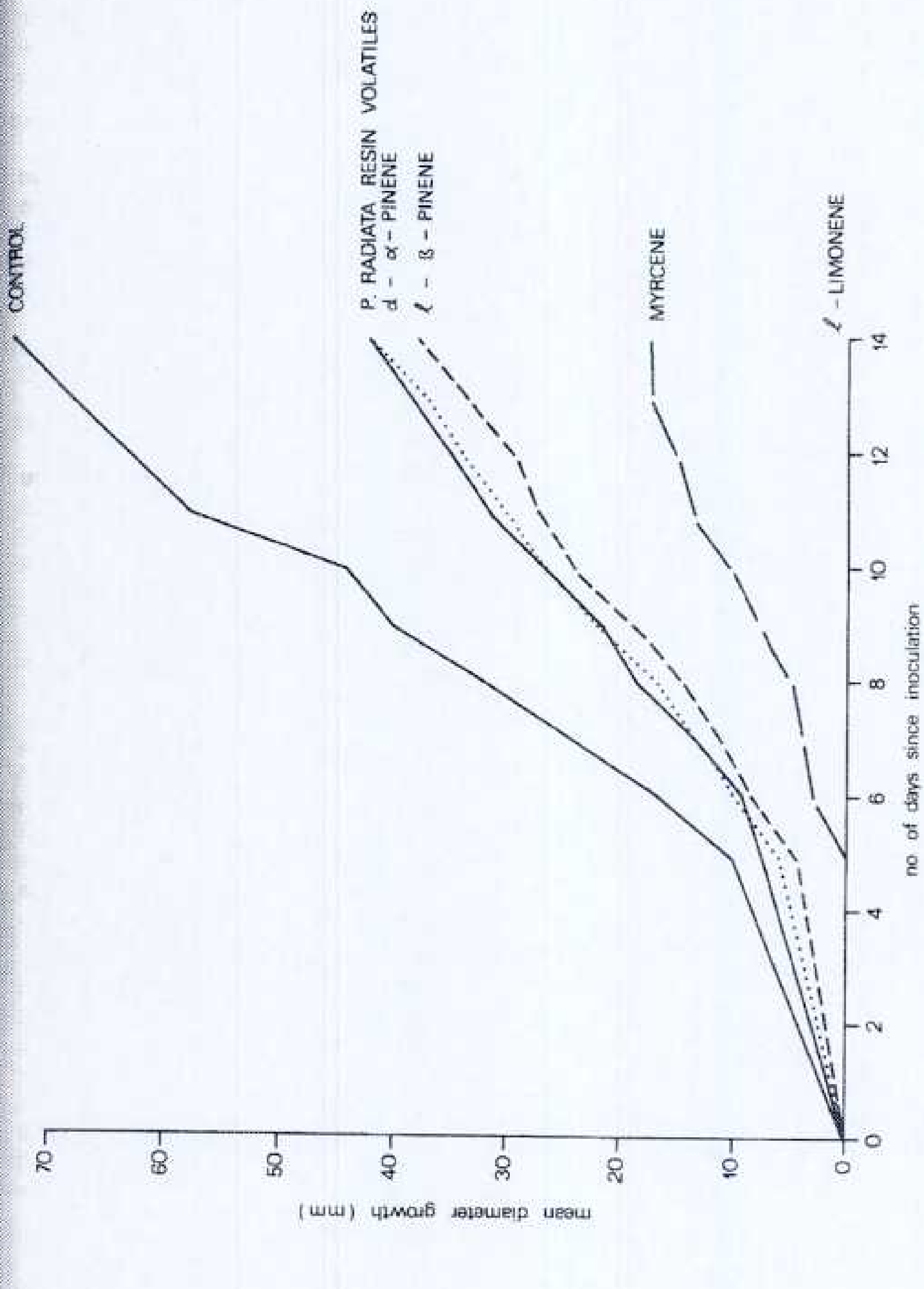

Figure 1. Effects of resin volatiles on growth of *Amylostereum areolatum*.

RESULTS

The growth of A. areolatum was significantly reduced ($P < .01$) when the fungus was exposed to atmospheres containing (1) volatiles from whole resin and (2) a number of volatile monoterpenoid components of P. radiata resin singly (Figure 1). The percentage inhibition caused by volatiles from whole resin was normally in the range of 40-50%, and, when material from individual trees was tested for activity, resin from dominant and suppressed trees inhibited fungal growth to the same extent. L-limonene completely inhibited growth while myrcene reduced growth by 76%. d- α -pinene and 1- β -pinene caused a growth inhibition of 43 and 49% respectively. The reduction in growth caused by d- α -pinene and 1- β -pinene was of the same order as that caused by volatiles from whole resin, and the differences between these three treatments were not significant.

Resin incorporated into the medium inhibited the growth of A. areolatum, but to a lesser extent than the presence of resin volatiles in the atmosphere of the culture chamber (Table 1). Attempts to test growth with resin concentrations higher than 1% were not successful due to the difficulty of incorporating resin uniformly in the medium - the resin tended to aggregate into large drops leading to irregular fungal growth patterns.

TABLE 1 - Effect of resin incorporated into 2% malt agar on the mean diameter growth after 14 days of cultures of A. areolatum

Treatment	Growth (mm)*	Percent Inhibition
Control	63.9	
Resin in medium		
0.25% w/v	53.3	16.6
0.50% w/v	45.3	29.1
1.00 w/v	45.1	29.4
Resin as volatile	28.9	54.8

* Means bracketed by the same line not significantly different at the 0.05 probability level

Resin acids (abietic and dehydroabietic) incorporated in the medium were inhibitory to A. areolatum ($P < .01$)(Figure 2 - page 32). Inhibition increased with increasing concentration of both acids; abietic acid was approximately twice as inhibitory (54-77%) as dehydroabietic acid, which showed inhibitory effects (18-36%) very similar to that produced by the incorporation of whole resin in the growth medium.

Exposure of A. areolatum to atmospheres containing resin volatiles did not affect fungal culture characteristics. However, incorporation of resin or resin acids in the medium promoted an unusual growth of aerial mycelium, and the fungal colonies had a denser and fluffier appearance than normal. As this effect occurred in all treatments including the controls, it was concluded that it was due to the ethanol in the medium.

DISCUSSION

Both volatile and non-volatile components of P. radiata oleoresin inhibited growth of A. areolatum. This indicates that the inhibitory effects of resin on A. areolatum in the tree have a chemical basis, although it does not exclude the possibility that the inhibitory effects of resin are also related to its physical properties.

α - and β - pinene are probably the most important volatile compounds inhibiting A. areolatum in vivo. These compounds comprise 98% of the resin turpentine fraction of P. radiata oleoresin (Bannister et al., 1962), and, while some other volatile compounds are more inhibitory than the pinenes, it is probable they are present in insufficient amounts to be effective inhibitors of A. areolatum. The volatility of the resin turpentine components means that the physical presence of resin in the immediate vicinity of the fungus is probably the only requirement for these materials to be inhibitory, as a saturated atmosphere of volatiles could occur in the lumen of tracheids in Sirex-attacked sapwood

The effects produced by the incorporation of resin and resin acids in the culture medium suggest that the non-volatile fraction of resin also inhibits fungal growth in vivo. As loss of volatiles is likely to have occurred during the process of plate preparation and incubation in an unsaturated atmosphere, the inhibitory effect of resin in the medium was probably dependent on the resin acids. However, the inhibitory effects of resin and resin acids incorporated in the growth medium cannot be attributed with complete certainty to their chemical properties, as the addition of such material may change the physical properties of the medium to the extent of influencing fungal growth.

The difference between the degree of growth inhibition produced by abietic acid and resin when incorporated in the medium probably arises from the fact

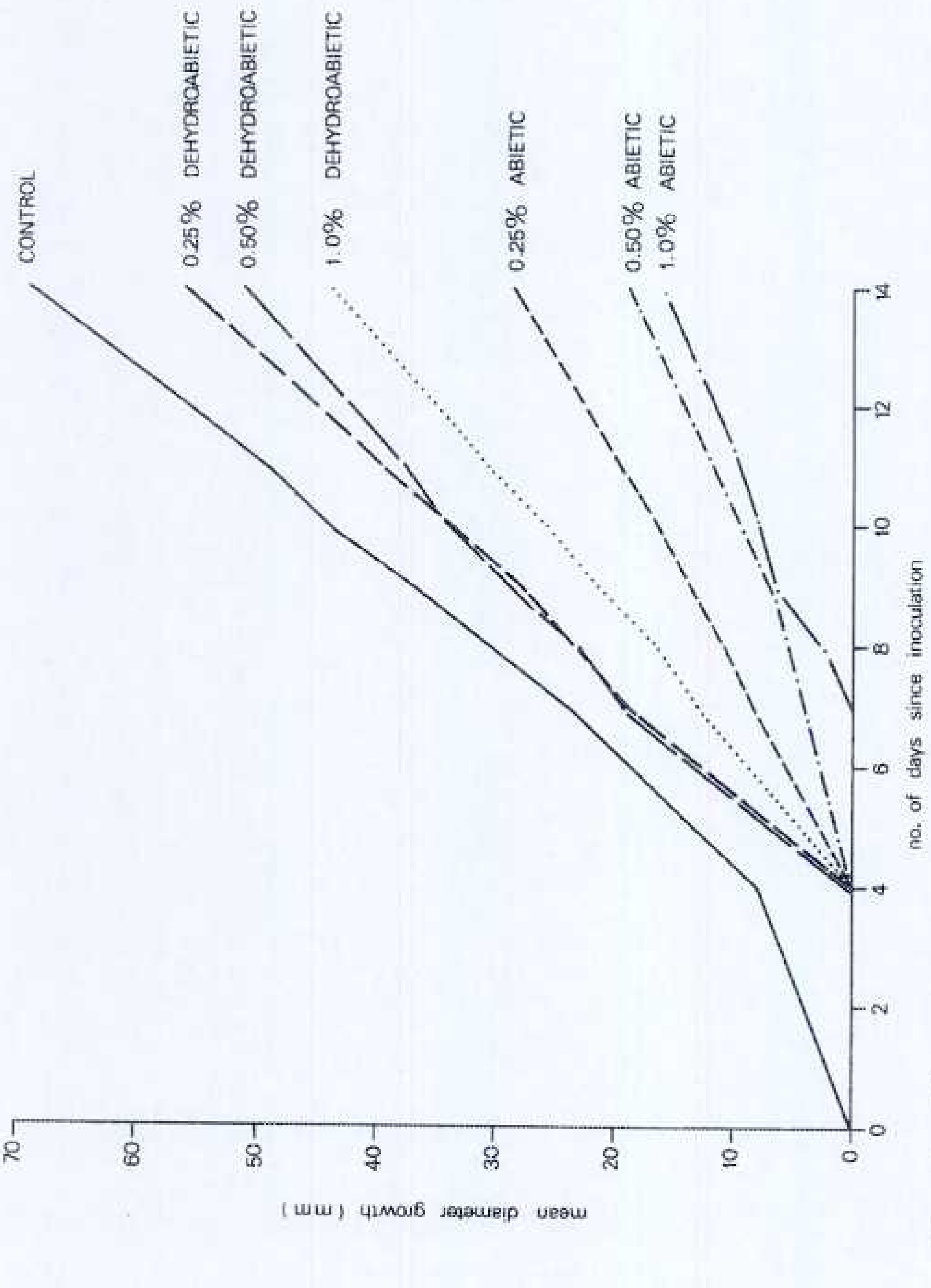

Figure 2. Effects of abietic and dehydroabietic acids on the growth of Amylostereum areolatum

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Figure 2. Effects of abietic and dehydroabietic acids on the growth of Amylostereum areolatum

that abietic acid is not a major component of P. radiata resin in vivo, although its proportion increases with ageing of resin in vitro (Porter, 1969). It may be that the major resin acids (palustric, levopimaric and neoabietic) are less inhibitory than abietic acid or that the concentration of resin in the culture medium was too low to reproduce the effects of abietic acid tested singly. This latter suggestion is supported by the greater inhibition observed where the resin was concentrated in the medium.

ACKNOWLEDGEMENTS

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SUMMARY

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