Tolerance of Fomes annosus isolates to pine oleoresins and pinosylvins

By J. N. Gibbs

Abstract

Ten Fomes annosus isolates from pine and ten from non-pine-hosts were subjected to the volatile components of pine oleoresin and to various concentrations of pinosylvin (PS) and pinosylvin monomethyl ether (PSM). Both the volatiles and the PS and PSM significantly reduced growth rate compared with controls. However, no detectable difference existed between the two groups of F. annosus isolates in their reactions to the substances tested.

1. Introduction

Fomes annosus (Fr.) Cke causing root and butt rot of trees is a serious problem in the north temperate regions of the world. It has also been recorded from South and South East Asia, Australia, South Africa and Brazil (Commonwealth Mycological Institute Distribution Map No. 271, 2nd Edition 1968). F. annosus is best known as a pathogen of conifers but it can also attack many species of angiosperms. A list of 150 hosts has been compiled by Sinclair (1964) and a further 17 for Great Britain have recently been added by B. J. W. Greig (Personal communication).

This wide habitat and host range has naturally stimulated interest in the possible existence of genetic adaptation in the fungus. Research has been carried out on various growth relationships in culture (Roll-Hansen, 1940; Etheridge, 1955; Cowling and Kelman, 1963); on some aspects of competitive saprophytic ability (Gibbs, 1967); on the capacity of F. annosus to decay wood (Platt, Cowling and Hodges, 1965) and to cause disease (Kuhlman, 1970). From this work there is little evidence with the possible exception of isolates from New Zealand and Australia (Gibbs, 1967; Kuhlman, 1970), that genetically controlled differences exist between F. annosus populations of different origins.

Despite this background, the recent work on the importance of resins and pinosylvins in the resistance of conifers to F. annosus (Shain, 1967; Gibbs, 1968; Cobb, Krstic, Zavarin and Barber, 1968) prompted an investigation into the possible adaptation of F. annosus to these products.

Consequently F. annosus isolates from pine and non-pine hosts were subjected to the volatile components of pine oleoresin and to pinosylvin (PS) and pinosylvin monomethyl ether (PSM).
2. Materials and Methods

2.1. Source of F. annosus isolates

The isolates were collected between February and September 1970. The ten pine isolates came from Scots pine (*Pinus sylvestris* L.) suffering from root rot in Thetford Chase, East Anglia, each isolate being made from a different tree. The ten non-pine isolates came from a variety of species in various parts of Southern England. They were respectively from *Picea sitchensis* (Bong.) Carr in Devon (1, 2) and in Dorset (3, 4), from *Larix decidua* Miller in Gloucestershire (5, 6, 7), from *Abies grandis*, Lindl. in Devon (8, 9) and from *Tsuga heterophylla* (Raf.) Sarg in Gloucestershire (10).

The isolates were made from the root samples on to Russells medium (Russell, 1956) and then maintained under oil until used early in 1971.

2 % Oxoid Malt extract agar was used throughout and all test inoculations were carried out using inoculum discs cut with a No. 2 cork borer from the margin of vigorously growing cultures of the fungus. All experiments were conducted at 22 °C.

2.2. Oleoresins

Oleoresin was collected from Scots and Corsican pine (*Pinus nigra* var. *maritima* (Aiton Melville) in Alice Holt Forest, Hampshire, during the summer of 1970. The technique of collection was that developed to measure resin yields (Gibbs, 1968) and the resin collected after a 24 hour period was stored in sealed tubes at 2 °C until required. A 25-year-old plantation containing a mixture of the two species was the main source of supply. The resin collected from each species was used separately in the experiments.

The technique of assessing the effect of the oleoresin volatiles was based on that used by Cobb et al. (1968). Cultures of the twenty isolates were grown for two days, measured and then held in sealed dessicators containing the resin. Cultures in dessicators without resin acted as controls. After a further three days the cultures were removed and measured.

With the provision of a number of perforated zinc platforms, one replicate of each of the twenty isolates could be contained within a 9 l dessicator. The position of each isolate in the dessicator was randomised although there was no reason to believe that this was necessary. Five ml of resin per dessicator was used and this was poured over glass beads in the base of the dessicator immediately before the introduction of the cultures. The experiment was repeated four times.

2.3. Pinosylvins

The technique followed closely that used by Rennertfelt (1943). Pinosylvin (PS) or Pinosylvin monomethylether (PSM) was dissolved in ethyl alcohol and the solution further diluted with alcohol so that addition to the medium resulted in concentrations of phenol of 12, 25 and 50 ppm, and a standard concentration of alcohol (1 %). The control plates also contained 1 % alcohol.

Two cultures were prepared for each of the twenty isolates. After two days and again after five days two diameters per culture were measured and the growth rate calculated. This experiment was repeated three times.
3. Results and Discussion

The growth rate of the twenty \textit{F. annosus} isolates on 2\% malt agar at 22 °C is shown in Table 1. There was no difference between isolates of pine and non-pine origin. Table 1 also shows the effect of Scots pine and Corsican pine oleoresin on the isolates. With both species the volatiles reduced the growth rate of the fungus but no difference was found between the responses of the two groups of isolates. The average reduction in growth rate of 39\% with Scots pine resin and 24\% with Corsican pine resin is rather less than that found for the oleoresin from \textit{Pinus ponderosa} Dougl. by Cobb et al. (1968).

Table 2 shows the effect of pinosylvin. With increasing concentrations of both PS and PSM a progressive reduction in the growth of the \textit{F. annosus} occurred, but again there was no evidence for a difference between the two groups of isolates. PSM was more inhibitory than PS at equivalent concentrations. The reduction in growth with the pinosylvin was much as would be expected from previous work. Thus Rennerfelt (1943) found 75\% and 36\% growth with 50 ppm of PS and PSM respectively and Shain (1967) showed mean growth of 40\% in two \textit{F. annosus} isolates when grown in a medium containing 25 ppm of pinosylvin (PSM : PS = 3 : 1).

In considering the results it is worth pointing out that the host species from which the non-pine isolates used in this work were made are not native to Britain and that the original source of these isolates may well have been pine. If work on the adapta-
Growth of *F. annosus* isolates on malt agar containing various concentration of pinosylvins as a percentage of growth in the control

<table>
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<th>PS 50 ppm</th>
<th>PSM 12 ppm</th>
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The tolerance of *F. annosus* to oleoresins and to pinosylvins is to be pursued further it would be desirable for the non-pine isolates to be collected from areas where they have been existing in association with non-pine species for a much longer time. Nevertheless the results of these experiments agree with virtually all earlier work on *F. annosus* in failing to find evidence for genetical adaptation in the fungus.

**Acknowledgements**

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**Summary**

Ten *Fomes annosus* isolates from pine and ten from non-pine hosts were subjected to the volatile components of pine oleoresin and to various concentrations of pinosylvin (PS) and pinosylvin monomethyl ether (PSM). The oleoresin volatiles from Scots pine and Corsican pine caused 39% and 24% reduction in growth rate respectively. Concentrations of PS and PSM from 12 to 50 ppm progressively reduced growth rate, PSM being the more effective. No detectable difference existed between the two groups of *F. annosus* isolates in their reactions to the substances tested.

**Résumé**

* Tolérance du Fomes annosus vis-à-vis des oléorésines et des pinosylvines des pins*

Dix souches de *Fomes annosus* isolées de Pin ex dix souches isolées à partir d'autres hôtes ont été soumises aux composés volatils d'oléorésine de pins et à des concentrations différentes de
Tolerance of *Fomes annosus* isolates to pine oleoresins and pinosylvins

Pinosylvine (PS) et de l’ester monométhylé de la pinosylvine (PSM). Les composés volatils de l’oléorésine de *Pinus silvestris* et de *Pinus nigra* var. *corsicana* ont entrainé respectivement 39 % et 24 % de réduction du taux de croissance du champignon. Des concentrations de pinosylvine (PS) et de son ester monométhylé (PSM) variant de 12 à 50 ppm ont réduit progressivement le taux de croissance, l’ester monométhylé de pinosylvine étant le plus efficace. On n’a pu établir de différence nette entre les deux groupes de souches de *Fomes annosus* quant à leurs réactions vis-à-vis des substances testées.

**Zusammenfassung**

Verträglichkeit von *Fomes annosus*-Isolaten gegen Kiefernharz und gegen Pinosylvin


**References**


Kuhlman, E. G., 1970: Seedling Inoculations with *Fomes annosus* show variation in virulence and in host susceptibility. Phytopathology 60, 1743–1746.


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