# ACTA FORESTALIA FENNICA

Vol. 117, 1971

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Tauno Kallio

SUOMEN METSÄTIETEELLINEN SEURA

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# PROTECTION OF SPRUCE STUMPS AGAINST *FOMES ANNOSUS* (FR.) COOKE BY SOME WOOD-INHABITING FUNGI

*KUUSEN KANTOJEN MAANNOUSEMASIENI-INFEKTION ESTÄMINEN MUUTAMIA PUUSSA KASVAVIA SIENIÄ KÄYTTÄEN*

TAUNO KALLIO

HELSINKI 1971

Suomalaisen Kirjallisuuden Kirjapaino Oy Helsinki 1971

## PREFACE

In 1959—1964 I studied the chemical control of *Fomes annosus* (Fr.) Cooke, and these studies awakened my interest in the biological control of the fungus. An opportunity of carrying out the relevant studies was afforded in connection with my investigation of the airborne distribution of *Fomes annosus* in 1967—1969.

The study would not have been possible without much assistance. Dr. AINO KÄÄRIK of the Royal College of Forestry, Stockholm, Sweden, taught me the identification of decay fungi from cultures and Mr ARVI SALONEN of the Department of Plant Pathology, University of Helsinki, that of the *Fungi imperfecti*. Dr. VEIKKO HINTIKKA read the manuscript and suggested valuable amendments. Mrs. ANNA-MAIJA HALLAKSELA carried out the bulk of the laboratory work, conscientiously and precisely. The personnel of the Department of Plant Pathology, University of Helsinki, assisted me in various ways towards the completion of the study. The study was carried out in a forest owned by the City of Helsinki.

I was granted financial support by the University of Helsinki and the Foundation for Research of Natural Resources in Finland.

I wish to express my very best thanks to all those mentioned above.

Helsinki, February 1971

*Tauno Kallio*

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## INTRODUCTION

Biological control in plant pathology can be defined as protection against disease by means of one or several living organisms. According to GARRETT (1965), this implies any living organism except the host plant and man. The borderline between biological and other forms of control is open to various interpretations. In the following, the possibilities of controlling *Fomes annosus* with the aid of other wood-decaying fungi are considered. This might also be termed microbiological control.

A large number of microbes are known to be antagonistic to *F. annosus*. They include e.g. the *Actinomyces* spp. (NISSEN 1956 a and 1956 b), *Aspergillus* spp. (ENEBO 1949), *Beauveria bassiana* (Bals.) Vuill. (LAINE and NUORTEVA 1970), *Boletus bovinus* L. and *Boletus variegatus* (Sw.) Fr. (HYPPEL 1968), *Botrytis cinerea* Pers. (GUNDERSEN 1967), *Coryne sarcoides* (Jacq.) Tul. (RICARD 1970), *Hypholoma fasciculare* (RISHBETH 1948), *Hypholoma* sp. (RISHBETH 1951 a), *Lenzites sepiaria* (Wulf.) Fr. (BOYCE 1966), *Penicillium claviforme* Bainier (RENNERFELT 1949), *Penicillium* spp. (RISHBETH 1948, BJÖRKMAN 1949), *Peniophora gigantea* (Fr.) Massee (RISHBETH 1948, 1950, 1952), *Polyporus abietinus* (Dicks.) Fr. (BOYCE 1966), *Polyporus adustus* (Willd.) Fr. (RISHBETH 1970), *Scytalidium* spp. (KLINGSTRÖM and BAYER 1965, RICARD 1970), *Stereum sanguinolentum* (A. & S.) Fr. (RISHBETH 1970), *Streptomyces griseus* (Krainsky) Waksman et Henr. (GUNDERSEN 1962 a), *Trichoderma alba* Preuss (RICARD 1970), and *Trichoderma viride* Pers. (RISHBETH 1948, RENNERFELT 1949). On the other hand, *F. annosus* has also been reported to be antagonistic to other microbes (WILKINS 1946, RENNERFELT and PARIS 1953).

*F. annosus* is aerially distributed in South Finland during the snowless seasons via stump surfaces after fellings (KALLIO 1965). As a result of year-round fellings, fresh stump surfaces are available also in those seasons of the year when the diaspore settling of *F. annosus* is at its maximum (KALLIO 1970). The purpose of the present study was to find out whether the spreading of *F. annosus* via spruce (*Picea abies* (L.) Karst.) stumps can be reduced by treating the stump surfaces, immediately after felling, with laboratory-grown mycelial suspension of a few common spruce-decaying fungi. The following fungi were used: *Fomes pinicola* (Sw.) Gillet., *Lenzites sepiaria* (Wulf.) Fr., *Peniophora gigantea* (Fr.) Massee, *Polyporus abietinus* (Dicks.) Fr., and *Trichoderma viride* (Pers.).

## MATERIAL AND METHODS

### Outline of the study

The main part of the investigation was carried out in a forest about 14 km east of the centre of Helsinki. It was a mixed forest of spruce, pine, birch and aspen, 50—100 years old, and was being thinned. The forest site was mostly Myrtillus Type (MT). Judging from the cut surfaces, an average of 38 per cent of the felled trees were decayed. In the course of thinning operations, twenty to thirty spruces were felled every month for one year in the usual way, but then the surfaces of the spruce stumps, which to the naked eye seemed to be free from decay, were inoculated with the mycelial suspension of the fungi to be tested, using an ordinary paint brush. At the time of felling, a disc about 4 cm thick was sawn from the butt end of every tree included in the study. The disc was kept in the laboratory for 10 days at + 20° C, wrapped up in plastic. After this the surface of the disc was studied by stereomicroscope to identify *F. annosus* conidiophores. If co-

nidiophores were found the relevant stump was excluded from the study.

The mycelial suspensions with which the stump surfaces were inoculated were prepared by culturing the fungi on malt agar in a Petri dish for 4—6 weeks. The diameter of the dishes was 8 cm, and each contained 10 ml agar. The growths of four dishes were crushed and mixed with 240 ml sterile distilled water. This made the suspension with which 5 stump surfaces were inoculated.

About a year after the inoculation, specimens were taken from the stumps. The profuse logging waste resulting from the thinning made it impossible to find all the stumps that had originally been included in the study. A disc about 4—5 cm thick was first removed from the stump surface by power saw as aseptically as possible. Two more discs were then sawn from the stump, each about 3—4 cm thick, the saw blade being immersed in alcohol between the sawings. An arrow pointing towards the north was drawn on these discs. The top surface of the undermost disc was marked out on tracing paper in the laboratory, with the arrow pointing northwards. This disc was kept in the laboratory for 10 days, wrapped up in plastic. On its surface, the areas growing *F. annosus* conidiophores were localized and their borders outlined with pencil. These areas were then copied onto the tracing paper.

Identification of the fungi discolouring the wood was attempted from the top disc. Strips 4 cm wide, running north-south and east-west through the centre of the disc, were drawn on the surface of each disc, and along these strips small specimens were taken from all discoloured areas of wood. The fungi of these specimens were cultured on malt agar for identification. This method had been previously used by many authors (e.g. KÄÄRIK 1967 and von PECHMAN et al. 1968).

After the samples had been taken, a few stumps were excavated with their roots. The roots were cut into lengths starting from the distal ends, and fungi from all discoloured spots visible to the naked eye were cultured on malt agar for identification on the basis of their mycelia.

### Fungi used for inoculations

The fungal mycelia isolated from spruce discs had grown after airborne invasion of the discs in the summer of 1967 in Helsinki. Earlier information on the antagonism of other fungi towards *Fomes annosus* could already be found in the literature, except for *Fomes pinicola*. It was included in the investigation since it was very common in Finland, and also because the author had never found sporophores of *F. annosus* and *F. pinicola* on the same spruce stump.

## RESULTS

### Laboratory tests

Laboratory tests were carried out with the listed fungi in order to verify the properties that were antagonistic to *F. annosus*. On malt agar substrate, *F. annosus* was inoculated into one side of the Petri dish and one of the studied fungi into the other. The growth of the fungi was then observed in the laboratory. A similar method has been used in *F. annosus* studies e.g. by RENNERFELT (1949), GUNDERSEN (1962 a, 1962 b), and FEDOROV and STAICHENKO (1968). In the present study, *Lenzites sepiaria* and *Fomes pinicola* were able to hold their own on malt agar against *F. annosus*. *L. sepiaria* and *Polyporus abietinus*, however, covered almost all *F. annosus* growth in about 10 weeks (typical growths in Figs. 1 and 2). Nevertheless, *F. annosus* conidiophores could still be seen on the substrate after 10 weeks of growth together with the two fungi mentioned. With *Trichoderma viride* on a growth substrate, *F. annosus* held its own relatively well right from the beginning, and in some culture tests was even able to suppress the *T. viride* growth (typical growths

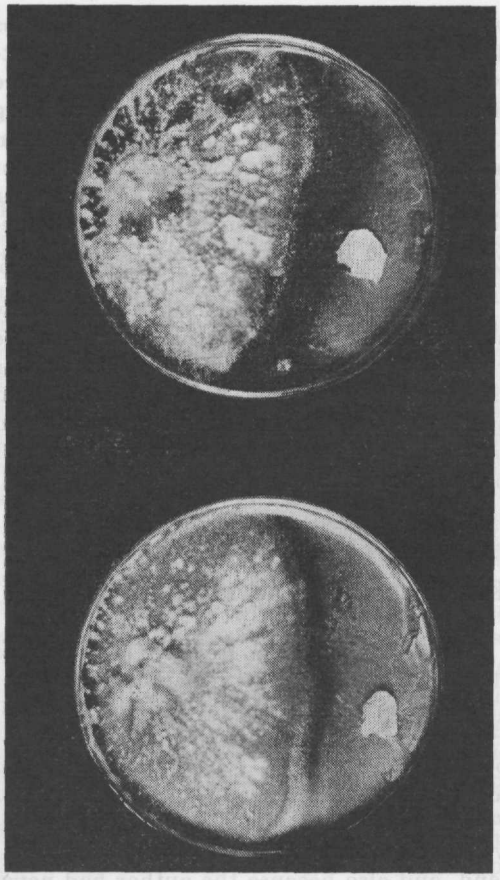

Fig. 1. *Lenzites sepiaria* (left) and *Fomes annosus* (right) growing on their separate areas of malt agar substrate about 10 weeks after inoculation.

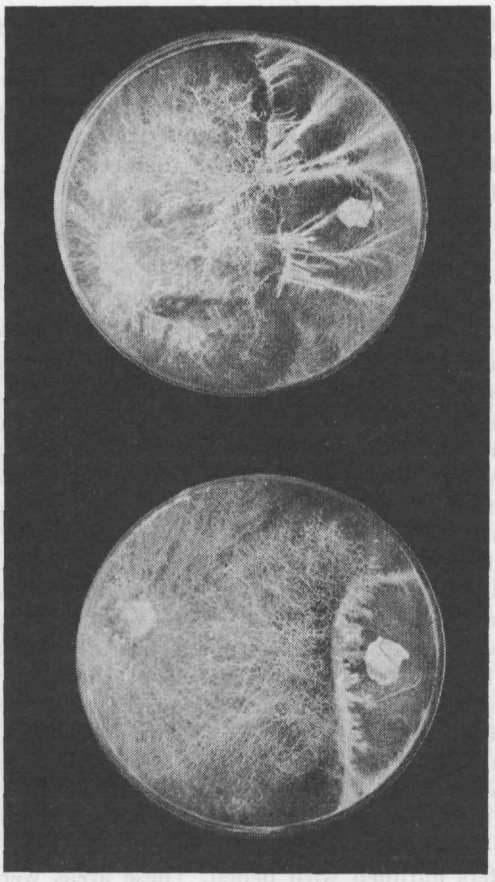

Fig. 2. *Polyporus abietinus* (left) has almost totally covered the *Fomes annosus* (right) growth about 10 weeks after inoculation.

in Fig. 3). *Peniophora gigantea* totally covered the conidiophores of *F. annosus* in 5 weeks (Fig. 4).

Of all the fungi used for the tests, in cultures on malt agar, the *P. gigantea* strain was the most superior to *F. annosus*.

## *Fomes annosus* on cut stump surfaces

The diaspore fall of the root rot fungus in South Finland is most profuse from May to October (KALLIO 1970). Infection of the cut stump surfaces, however, does not depend on the *F. annosus* diaspore fall alone, for e.g. microbes antagonistic to *F. annosus* on the stump surfaces may interfere with infection by the root rot fungus (DRIVER and GINNS 1969). In the present study the root rot fungus invariably began to grow on one or a few cut surfaces of the control stumps after each felling made between May and August 1968. All inoculations with *F. annosus* were successful. According to Fig. 5, *Peniophora gigantea* was the only one of the fungi used to control *F. annosus*, which completely inhibited it. The present result is not in full agreement with that reported by RISHBETH (1970) on the infection of spruce stumps in England. According to him, *P. gigantea* failed to provide complete protection against the invasion of the cut surfaces of spruce stumps by airborne root rot fungus; on the other hand, a number of the stumps he examined were already decayed when the study began.

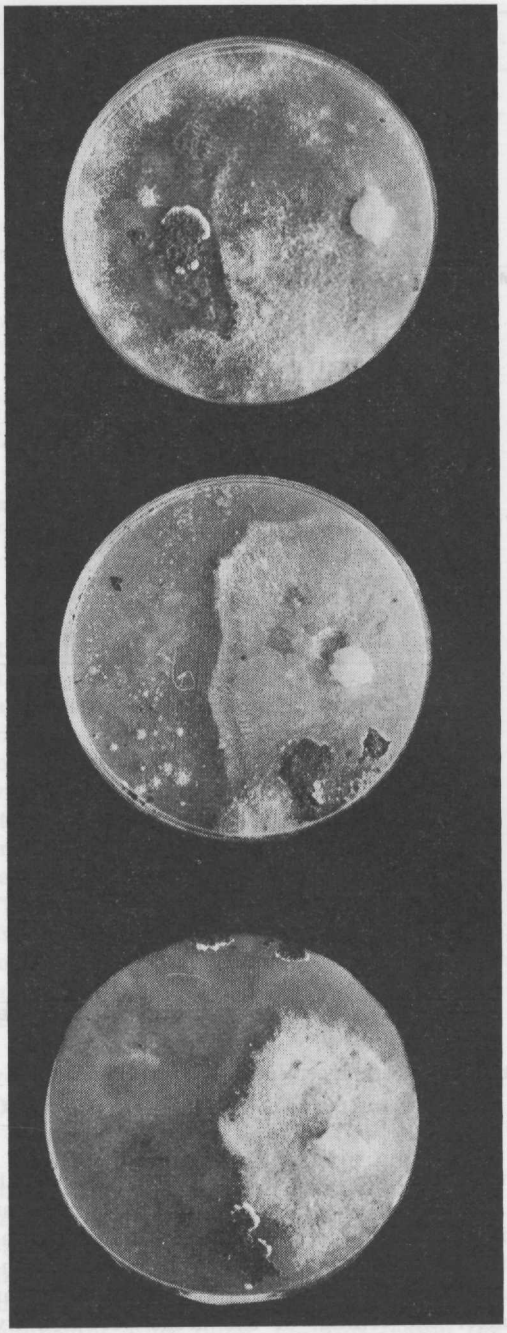

Fig. 3. *Trichoderma viride* (left) and *Fomes annosus* (right) about 8 weeks after inoculation. In the top picture the substrate is predominated by *F. annosus*. In the two lower pictures each fungus is growing in its separate area.

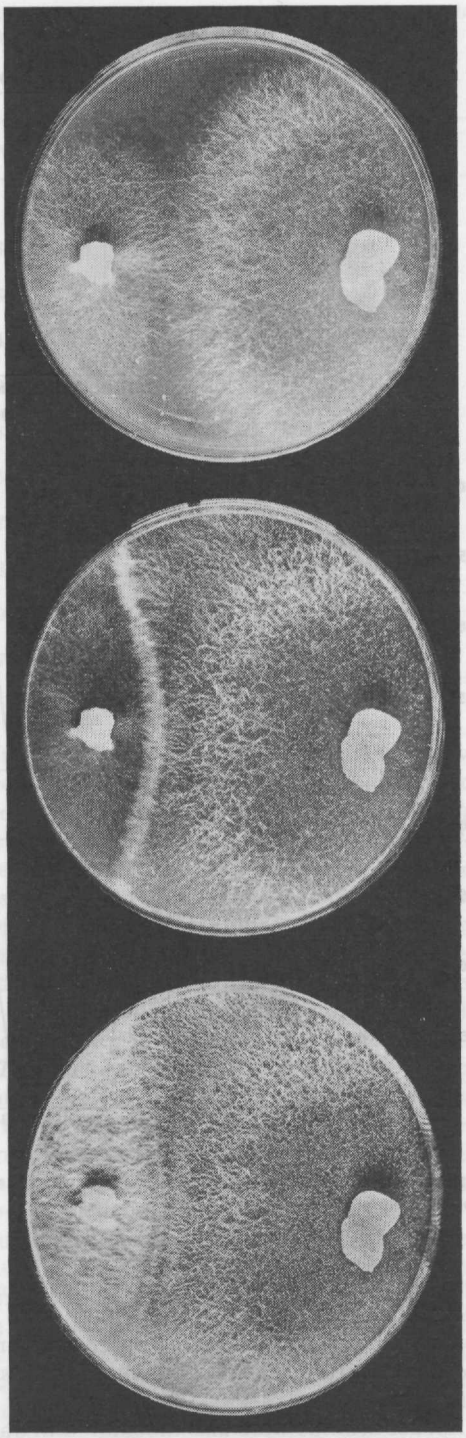

Fig. 4. *Fomes annosus* (left) and *Peniophora gigantea* (right) 8 days (top), 11 days (middle) and 35 days (bottom) after inoculation. In the last plate there are no longer any identifiable conidiophores of *F. annosus*.

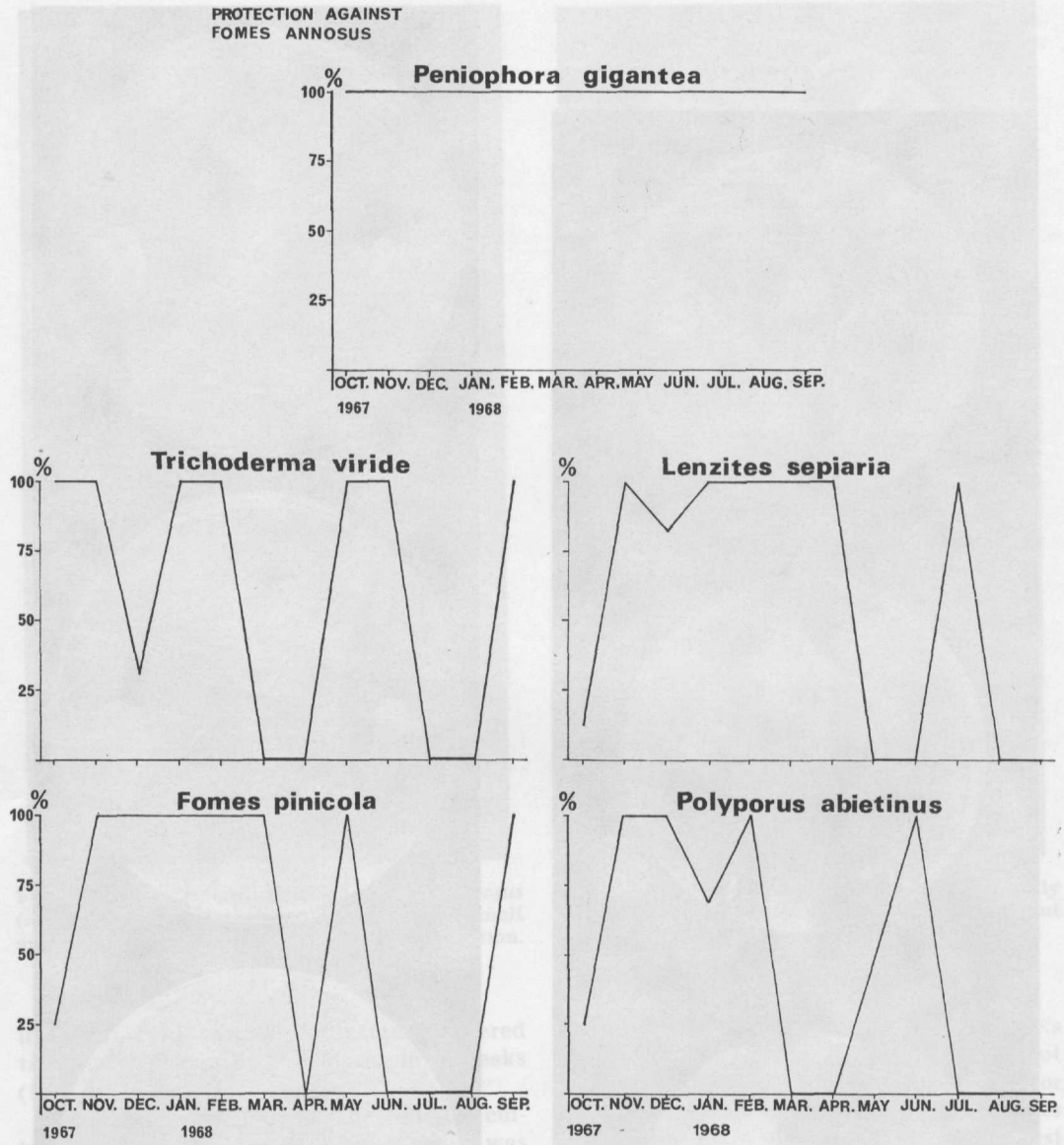


Fig. 5. Effect of different fungus inoculations on the colonization percentage of spruce stumps by *Fomes annosus*.

The present study excluded all stumps where decay had started. The other fungi used to control the root rot did not appreciably protect the cut stump surfaces against *F. annosus* infection.

Spruce stumps were also inoculated in January-September 1968 as shown in Table 1: immediately after felling the stumps were inoculated with the fungi intended to control infection and directly afterwards with *F. annosus*, while other stumps were first inoculated with the fungus intended to control infection and 10 days later with *F. annosus*. Table 1 shows the percentages, a year after the felling, of the areas occupied by *F. annosus* conidiophores in the total cut surface inoculated as described. In April-August, *P. gigantea* almost completely de-

Table 1. *Fomes annosus* present on the stump surfaces one year after inoculation. The area of conidiophores per cent of total stump surface area.

| | 1968 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month of inoculation | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sep. |
| Inoculation with: | | | | | | | | | |
| *Peniophora gigantea* + *Fomes annosus* | 2 | 4 | 13 | 0 | | 0 | 0 | 4 | 27 |
| *Peniophora gigantea* + *Fomes annosus* 10 days later | 3 | 7 | 3 | 0 | 0 | 0 | 4 | 0 | 4 |
| *Trichoderma viride* + *Fomes annosus* | 23 | 86 | 44 | 7 | | 30 | 88 | 37 | 52 |
| *Trichoderma viride* + *Fomes annosus* 10 days later | 43 | 39 | 69 | 20 | 1 | 1 | 0 | 41 | 3 |
| *Fomes pinicola* + *Fomes annosus* | 15 | 30 | 33 | 36 | | 59 | 96 | | 81 |
| *Fomes pinicola* + *Fomes annosus* 10 days later | 2 | 11 | 45 | 17 | 16 | 8 | 11 | 23 | 0 |
| *Lenzites sepiaria* + *Fomes annosus* | 27 | 40 | 27 | 32 | | 53 | 42 | 12 | 33 |
| *Lenzites sepiaria* + *Fomes annosus* 10 days later | 33 | 43 | 23 | 5 | 19 | 41 | 6 | 8 | 2 |
| *Polyporus abietinus* + *Fomes annosus* | 43 | 5 | 89 | 11 | | 45 | 25 | 51 | 26 |
| *Polyporus abietinus* + *Fomes annosus* 10 days later | | 20 | 57 | 1 | 8 | | 3 | 2 | 6 |

stroyed the *F. annosus* inoculated in the stump either at the same time or 10 days later. At other seasons of the year the root rot fungus was more competitive. According to the result obtained, *F. annosus* competes with *P. gigantea* more effectively at low than at high temperatures. Of the other fungi, only *T. viride* and *F. pinicola*, in one case each, were capable of destroying *F. annosus*. This result corroborates that reported from Sweden (Persson-Hüppel 1963), according to which the antagonism of *T. viride* to *F. annosus* also depends on factors other than the temperature.

## Other fungi on cut stump surfaces

A study of other fungi occurring on the cut stump surfaces revealed a certain similarity between stumps inoculated with *Fomes pinicola*, *Lenzites sepiaria* and *Polyporus abietinus*. All stump surfaces inoculated with these fungi became infected by a relatively large number of other fungi. For this reason the results for the said three fungi were combined in Table 2. *Stereum sanguinolentum* was the commonest of the fungi infecting the cut stump surfaces inoculated with the above three fungi, but other species were also found. *F. annosus* was also among the infecting agents, and so were *P. gigantea* and *T. viride*. It seems clear that the ability of *F. pinicola*, *L. sepiaria* and *P. abietinus* to occupy the cut surfaces of spruce stumps at an early stage was relatively limited. Stumps inoculated with these three fungi also became infected by a number of other fungi and, especially after summer inoculations, by bacteria.

*Trichoderma viride* is apparently much more effective than the above three species in occupying the cut surfaces of inoculated spruce stumps. From October to April this fungus inhibited or reduced the infection by several other fungi. During the very low midwinter temperatures, *S. sanguinolentum* was one of the fungi invading inoculated stump surfaces; it was also capable of infecting stumps that, at the time of felling, had been inoculated with *F. annosus*. In the February-March inoculations *T. viride* was relatively predominant, yet, during these months, stumps inoculated with *T. viride* were infected by e.g. *P. gigantea*. In May-July inoculations *T. viride* was still competitive on the cut surfaces of stumps, but in August-Septem-

Table 2. The numbers of the various fungi isolated from spruce stumps one year after inoculation with some wood-inhabiting fungi.

| Stump surface inoculated with: | *Fomes pinicola* *Lenzites sepiaria* *Polyporus abietinus* | | | *Trichoderma viride* | | | *Peniophora gigantea* | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| The time of inoculation | Oct.-Apr. | May-Jul. | Aug.-Sep. | Oct.-Apr. | May-Jul. | Aug.-Sep. | Oct.-Apr. | May-Jul. | Aug.-Sep. | Oct.-Apr. | May-Jul. | Aug.-Sep. |
| Fungi from stumps | | | | | | | | | | | | |
| *Coryne sarcoides* | 10 | 1 | 1 | | | 1 | 2 | 1 | | | | |
| *Armillaria mellea* | | | | 1 | | | 1 | | | | | |
| *Corticium* sp. | | | | | | | | | | 1 | | |
| *Flammula* sp. | | | | 1 | | | | | | | | |
| *Fomes nigrolimitatus* | 1 | | | | | | | | | | | |
| » *pinicola* | 12 | 1 | 1 | 1 | | 1 | | | | 6 | | 1 |
| *Grandinia* spp. | 1 | 1 | | | | | | | | 1 | | |
| *Hypholoma* sp. | | | | | | | | | | 1 | | |
| » *capnoides* | 1 | | | | | | | | | 1 | | |
| *Lenzites sepiaria* | 3 | 2 | 2 | | | | | | | | 2 | |
| *Merulius* spp. | | 1 | | | | | | | | | 1 | |
| » *tremellosus* | 5 | 3 | | | | | 1 | | | 5 | 5 | |
| *Peniophora cinerea* | 3 | | | 1 | | | | | | 9 | | |
| » *gigantea* | 11 | 6 | | 7 | | | 42 | 15 | 8 | 5 | 9 | 2 |
| » *incarnata* | 1 | | | | | | | | | 1 | | |
| » *pithya* | 48 | 1 | 5 | 5 | | 3 | 9 | | | 30 | | 1 |
| *Polyporus abietinus* | 8 | 3 | 3 | | | | | | | 1 | | 1 |
| » *adustus* | 4 | | | | | | | | | | | |
| » *resinosus* | 1 | | | | | | | | | | | |
| » *zonatus* | 2 | | | | | | 1 | | | 1 | 1 | |
| *Poria* sp. | 1 | | | | | | | | | | | |
| » *candidissima* | 1 | | | | | | | | | | | |
| *Stereum purpureum* | 10 | | | 1 | | | 2 | | | 6 | | |
| » *sanguinolentum* | 57 | 9 | 2 | 9 | | | 7 | | | 21 | 16 | 1 |
| *Trametes serialis* | 10 | | | | | | | | | 2 | | |
| *Trechispora brinkmanni* | 4 | 1 | | | | | | | | 2 | | |
| *Alternaria* spp. | | | 1 | | | | | | | | | 1 |
| *Cephalosporium* spp. | 6 | 10 | 1 | | 2 | 1 | | 1 | | 2 | 9 | 5 |
| *Cylindrocarpon* sp. | 1 | | | | | | | | | | | |
| *Phialophora* sp. | 1 | | | | | | | | | | | |
| *Trichoderma viride* | 30 | 8 | | 31 | 7 | | 6 | | | 5 | 9 | |
| *Bacteria* | 3 | 3 | 2 | 2 | 2 | 1 | 2 | | | 3 | 5 | 3 |
| Unknown | 30 | 9 | 3 | 3 | | 1 | 6 | | 1 | 15 | 8 | 1 |

ber its competitive ability vis-a-vis other fungi was no longer so good as it had been earlier in the summer (cf. PERSSON-HÜPPEL 1963).

The most successful infection of the cut surfaces of inoculated stumps was by *Peniophora gigantea*. It often inhibited infection by any other fungi. However, *T. viride* was one of the fungi infecting stumps inoculated in the autumn, and *S. sanguinolentum* one of those infecting stumps inoculated in mid-winter. Not many fungal species were capable of infecting stumps inoculated in February, March and April. In these months *P. gigantea*, however, infected stumps inoculated with *T. viride* and *F. annosus*. Stumps inoculated in May-September with *P. gigantea* were infected by *S. sanguinolentum* which, according to a study by ETHERIDGE (1969) in Canada, has a special capacity for infecting the wood material of *Abies balsamea* (L.) Mill. as long as the relative air humidity is high and the mean diurnal temperature relatively low (7—13° C). *C. sarcoides* also infected the cut surfaces of stumps inoculated with *P. gigantea*. *C. sarcoides* may sometimes be the only decaying agent found in a tree, and it may thus

be antagonistic to other decay fungi (DIMITRI 1968, ETHERIDGE 1970, RICARD 1970).

The most common fungi to infect the control stumps in the autumn were *Peniophora pithya*, *Stereum sanguinolentum* (cf. ETHERIDGE 1970), *Peniophora cinerea*, *Fomes pinicola* and *Trichoderma viride*. *S. sanguinolentum* infected the control stumps at almost any time of the year. Beginning from late winter, *P. gigantea* was a frequent infecting agent in control stumps. In the summer the *Cephalosporium* species were also relatively numerous in the control stumps. The stumps inoculated with *F. annosus* in the winter were found to become infected e.g. by *S. sanguinolentum* and *T. viride*, those inoculated in May e.g. by *P. gigantea*, and finally those inoculated in July e.g. by various *Cephalosporium* species.

The number of the *Fungi imperfecti* species isolated from spruce stumps was relatively low in the present study. The finding agrees with that reported by MEREDITH (1960) concerning *Pinus silvestris* L. stumps in England and disagrees with that reported by ASTIN and DRIVER (1962) concerning stumps of *Pinus elliottii* Engelm. in Georgia, USA.

In summarizing the fungal species infecting stumps, it can be said that *P. gigantea* was conspicuous both as a natural infection on the cut surfaces of spruce and in stumps that had been artificially inoculated. Another fungus which apparently is highly effective at invading stumps through their cut surfaces is *S. sanguinolentum*, also known as a parasite (e.g. HAKKILA and LAIHO 1968). According to the present study, infection by this fungus cannot be confined only to the cold and humid season, a finding also reported from studies of *Abies balsamea* (L.) Mill. in Canada (ETHERIDGE 1970). *S. sanguinolentum* in Finland, in addition to *P. pithya* and *P. gigantea*, is apparently a common species with airborne distribution. Together with *F. annosus*, these four fungi are probably the initial cause of infection on the majority of the cut surfaces of spruce stumps (cf. KALLIO 1965).

## Fungi isolated from excavated inoculated stumps

After the fungal species producing infection through the cut stump surfaces had been ascertained, a few stumps were removed from the ground with their roots, in order to identify the fungi growing in them. Starting from the distal ends, the stumps were cut into lengths of about 20 cm, and the fungi from the discoloured spots of wood were cultured on malt agar for identification.

Stump 12 (Figs. 6 a and 6 b) was inoculated with *Peniophora gigantea* on October 14, 1967. Sample discs of the stump were taken on September 16, 1968, after which the stump was dug from the ground and its fungal species were examined. *P. gigantea* was the only fungus that could be isolated from the discs sawn from the stump. In root II it had caused decay that extended over some 30 cm from the cut stump surface towards the root. The rate of growth was approximately the same as had been reported for this fungus in the stumps of *Pinus echinata* Mill. (KUHLMAN and HENDRIX 1964). From the same root II, bacteria were isolated from discoloured wood material in which discoloration had apparently started from the distal end of the root. In roots I and III the decay, as in root II, extended to a distance of about 30 cm from the cut surface. Root III had bacteria in the discoloured wood where discoloration had started at the distal end of the root. Bacteria were also isolated from the continuous vein of decayed wood in root IV. *P. gigantea* was isolated at point 1, and it had apparently grown for about one metre from the cut surface outward along the root.

Bacteria and unidentified *Fungi imperfecti* were isolated at points 2 and 3; they had caused continuous discoloration down to the distal ends of the roots. The points 4, 5 and 6 indicate spots where bacteria were isolated. A similar result concerning the infection of spruce roots has been reported e.g. by DIMITRI (1969 a, 1969 b).

Stump 184 was inoculated on June 14, 1968, with *P. gigantea* and on June 24, 1968, additionally with *F. annosus*. On May 12, 1969, only *P. gigantea* could be found on a cross section surface of the stump. The decay it has produced extended towards all the roots at an approximate distance of 40 cm from the cut surface of the stump. No other decay fungi were found in the stump.

Stump 18 (Fig. 7) was inoculated on October 14, 1967, with *Polyporus abietinus*, and sample discs were sawn on September 18,

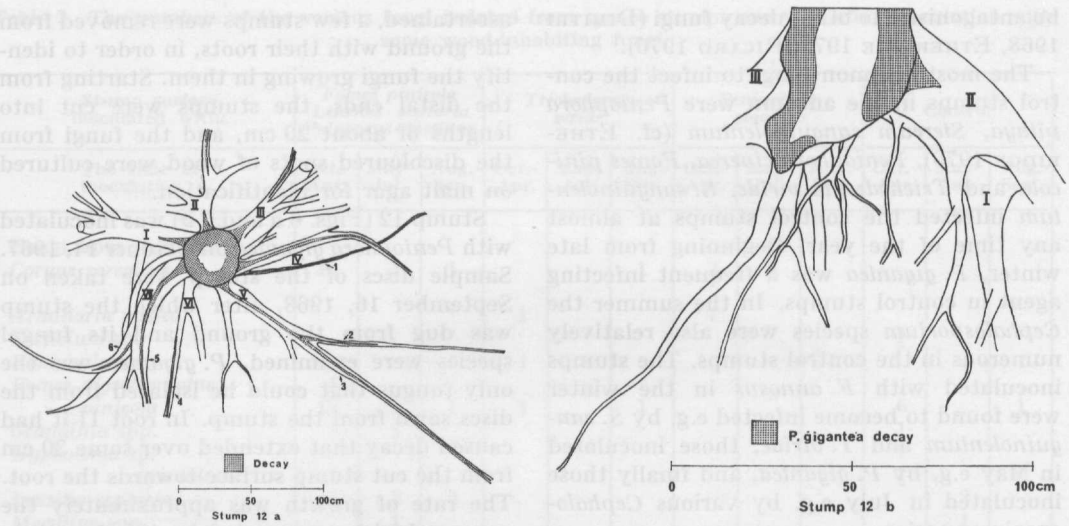


Fig. 6. Stump inoculated with *Peniophora gigantea*. a. top view, b. cross section view.

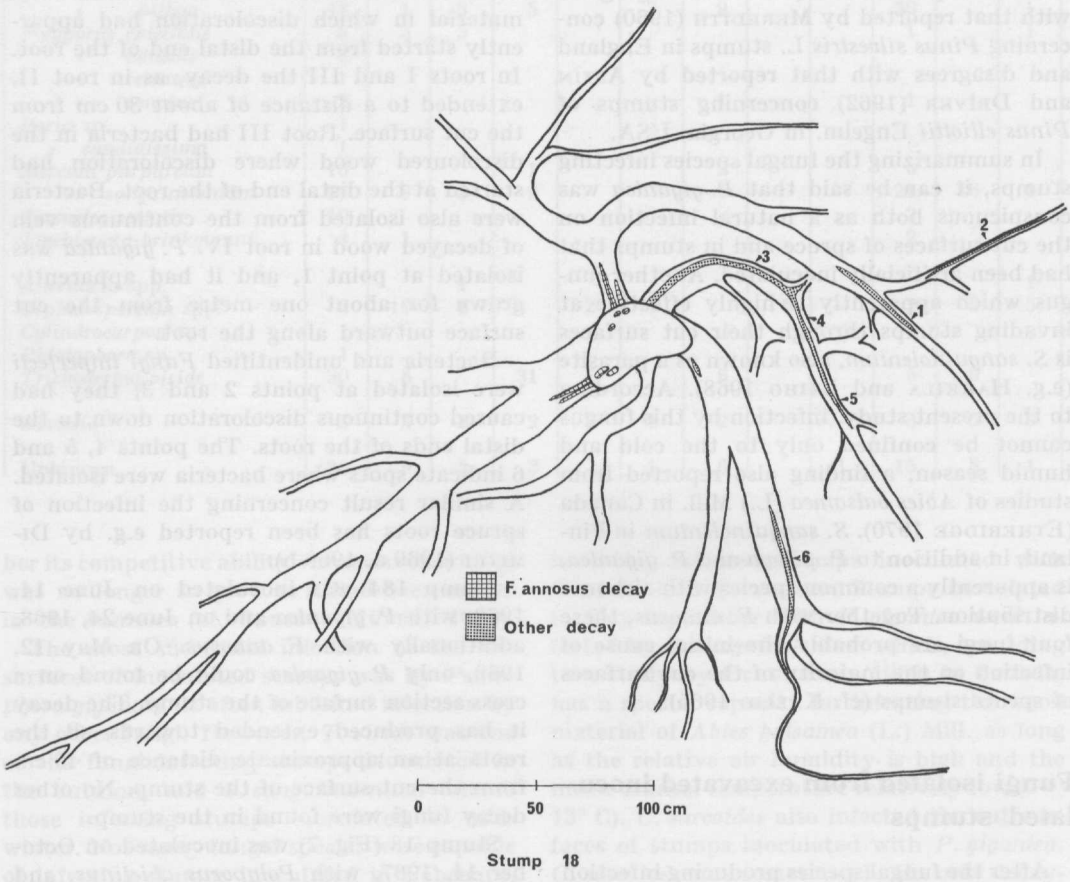


Fig. 7. Stump inoculated with *Polyporus abietinus*.

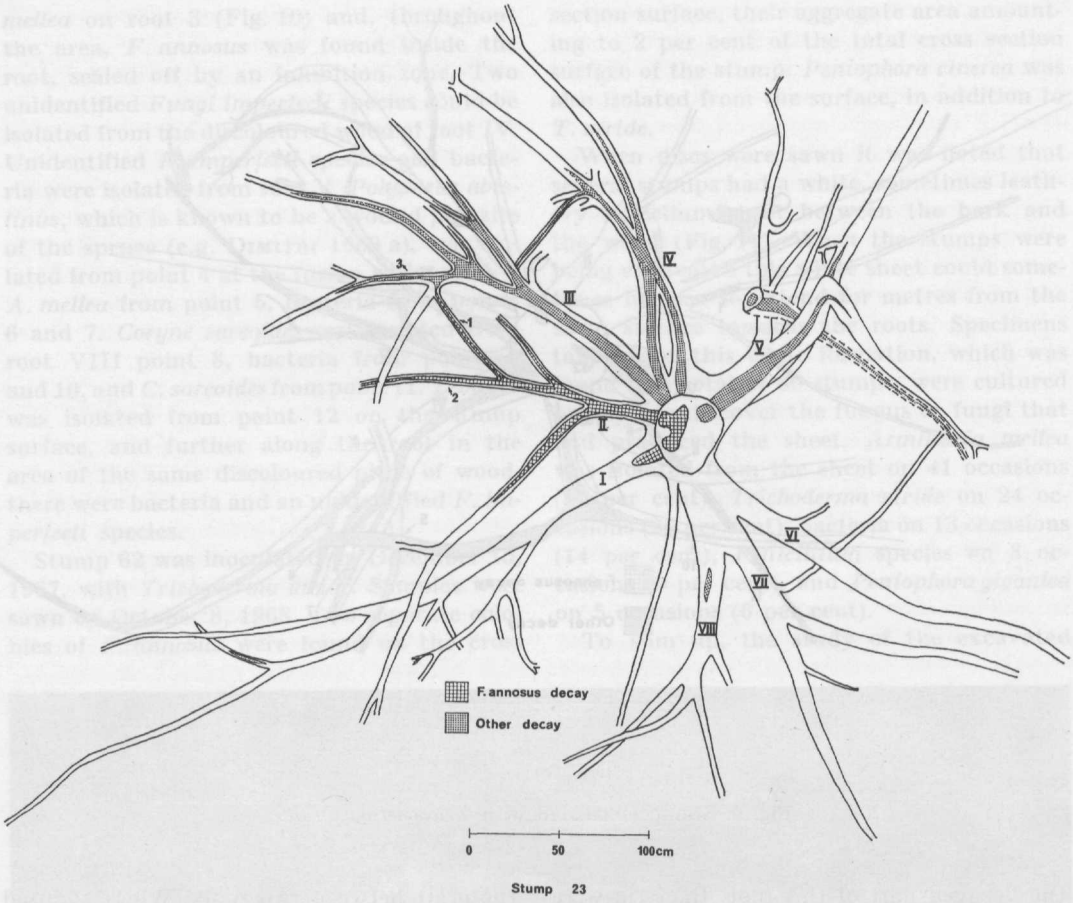


Fig. 8. Untreated control stump.

1968. The disc contained 4 separate colonies of *F. annosus*, with an aggregate area of about 5 per cent of the total surface area of the disc. *F. annosus* had grown about 30 cm towards the roots from the cut surface. *Coryne sarcoides* was isolated at point 1. According to ETHERIDGE (1970), in Canada *C. sarcoides* is one of the commonest fungi infecting the roots of conifers from the soil. No micro-organisms could be isolated from points 1—5, and the specimens remained sterile. DIMITRI (1969 b) also failed to isolate micro-organisms from all discolorations he found in spruce roots. *Armillaria mellea* was isolated from the discoloration at point 6.

Stump 23 (Fig. 8) was quite a large control stump, with a cross section area of 1091 sq.cm. The tree was felled on October 14, 1967, and discs for the study of the root rot fungus were taken from the cut surface on September 18, 1968. *F. annosus* occupied 22 per cent of the total area of the cut surface. In addition, *Peniophora cinerea*, *Stereum sanguinolentum*, *Trechispora brinkmanni* and *Trametes serialis* were also isolated from the surface. In root I, *F. annosus* had grown about 40 cm from the cut surface along the root. In root 1, it had apparently grown both from the cut stump surface and from the ground. It had produced discoloration that extended unbroken over about 1.5 metres. At the point where roots 1 and 2 fused the colour defect could no longer be seen in root 2. Only bacteria could be isolated from the large area of discoloured wood in roots III and IV. Root V had fused with the roots of another spruce. Bacteria and an unidentified species of *Fungi imperfecti* were isolated from

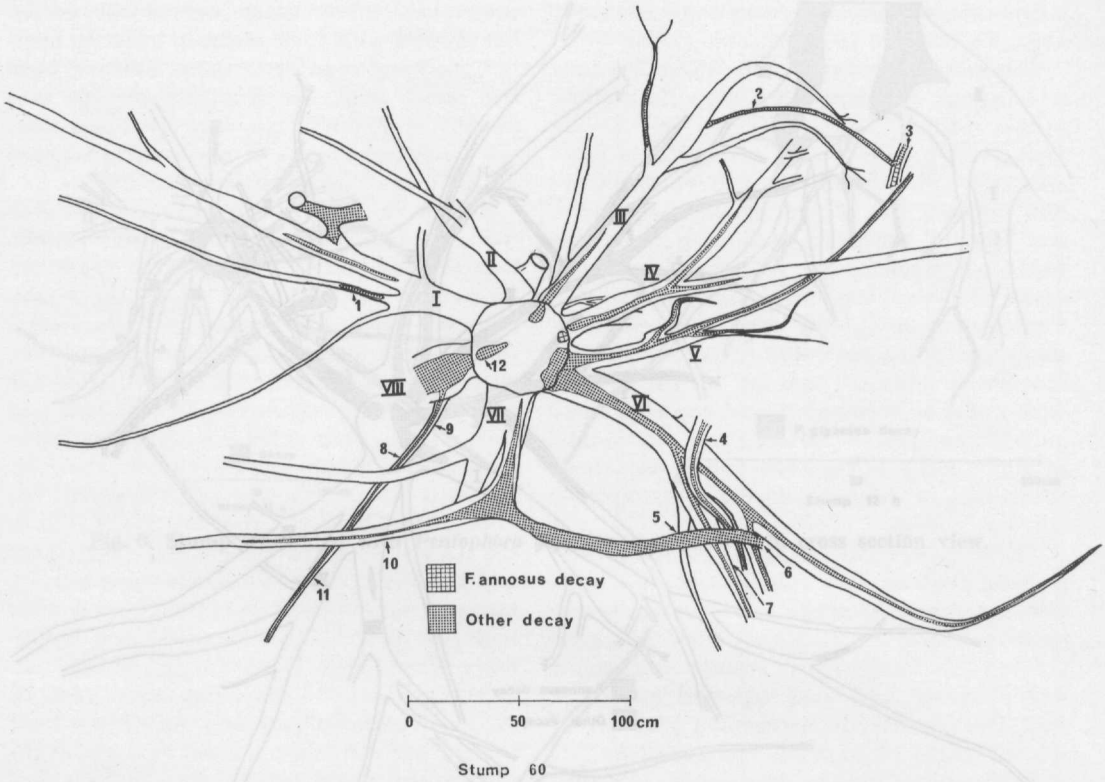


Fig. 9. Stump inoculated with *Trichoderma viride*.

the decayed part of this root. Bacteria were isolated from root VIII.

Stump 33 had been inoculated on November 15, 1967, with *L. sepiaria*, and sample discs had been taken on September 27, 1968. *P. cinerea*, *P. pithya* and *S. sanguinolentum* had been isolated from the disc. No other fungi were found in the stump.

Stump 60 (Fig. 9) was inoculated on December 15, 1967, with *Trichoderma viride*, and the sample discs were taken on October 8, 1968. The cross section surface had one *F. annosus* colony, with an area covering 2 per cent of the total stump surface. *Peniophora pithya* and *T. viride* were also isolated from the disc. The stump was excavated on October 24, 1968. Root I had coalesced with the root of a small decayed spruce tree. Only bacteria could be isolated from the decayed part. No micro-organism was isolated from point 1. The *P. pithya* isolated from root III had grown some 50 cm from the cut stump surface out along the root. Root 2 formed the graft between two roots. Where it fused with root 3, it could be seen that root 2 was infected by *F. annosus*. Beyond the point of fusion, there was an outer rim of *Armillaria*

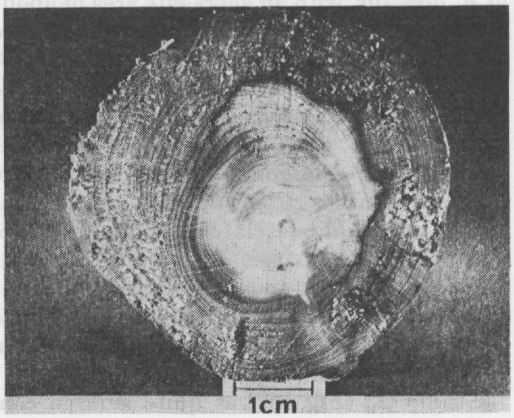


Fig. 10. Stump 60. Cross section of root 3. *Armillaria mellea* outside and *Fomes annosus* inside the inhibitory zone (dark ring).

*mellea* on root 3 (Fig. 10) and, throughout the area, *F. annosus* was found inside the root, sealed off by an inhibition zone. Two unidentified *Fungi imperfecti* species could be isolated from the discoloured wood of root IV. Unidentified *F. imperfecti* species and bacteria were isolated from root V. *Polyporus abietinus*, which is known to be a wound parasite of the spruce (e.g. DIMITRI 1969 a), was isolated from point 4 at the fusion with root VI, *A. mellea* from point 5, bacteria from points 6 and 7. *Coryne sarcoides* was isolated from root VIII point 8, bacteria from points 9 and 10, and *C. sarcoides* from point 11. *T. viride* was isolated from point 12 on the stump surface, and further along the root in the area of the same discoloured piece of wood, there were bacteria and an unidentified *F. imperfecti* species.

Stump 62 was inoculated on December 15, 1967, with *Trichoderma viride*. Samples were sawn on October 8, 1968. Two separate colonies of *F. annosus* were found on the cross section surface, their aggregate area amounting to 2 per cent of the total cross section surface of the stump. *Peniophora cinerea* was also isolated from the surface, in addition to *T. viride*.

When discs were sawn it was noted that several stumps had a white, sometimes leathery mycelium sheet between the bark and the wood (Fig. 11). When the stumps were being excavated this white sheet could sometimes be seen to extend for metres from the sawn surface towards the roots. Specimens taken from this white formation, which was found in a total of 80 stumps, were cultured in order to discover the fungus or fungi that had produced the sheet. *Armillaria mellea* was isolated from the sheet on 41 occasions (45 per cent), *Trichoderma viride* on 24 occasions (26 per cent), bacteria on 13 occasions (14 per cent), *Penicillium* species on 8 occasions (9 per cent), and *Peniophora gigantea* on 5 occasions (6 per cent).

To sum up, the study of the excavated

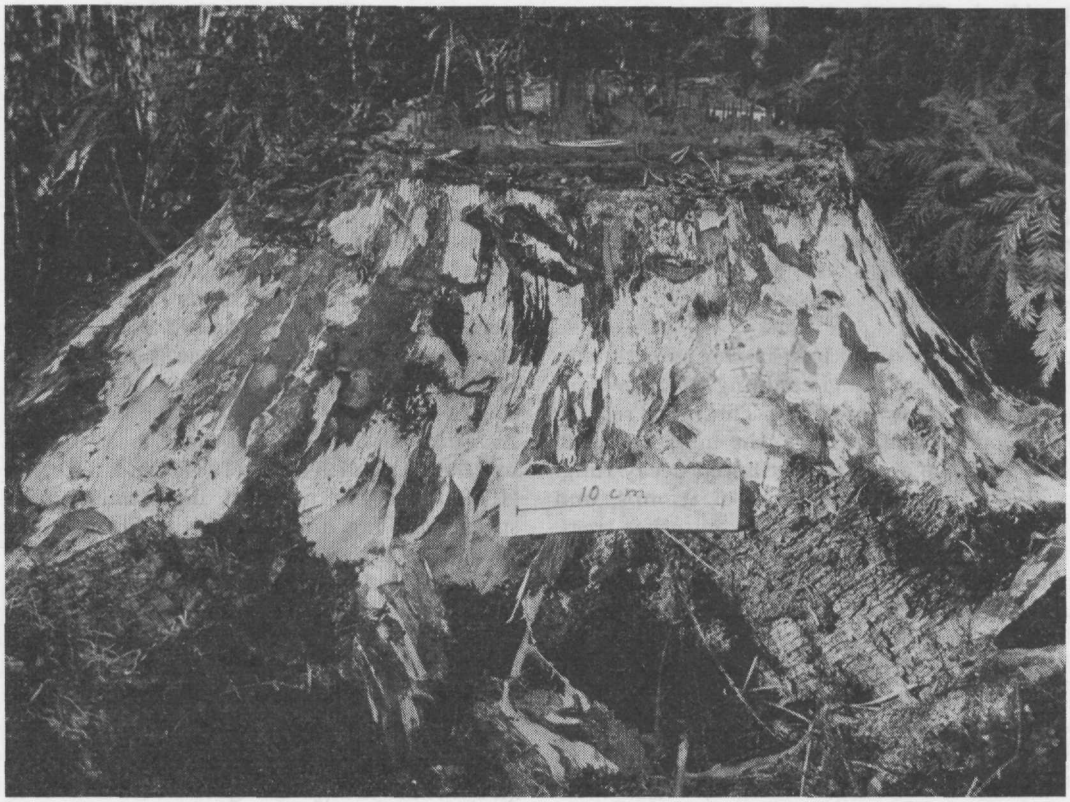


Fig. 11. White mycelium sheets between bark and wood of stump 18. *Armillaria mellea* was isolated from this sheet.

stumps and the fungal species growing in them showed that infection by bacteria and fungi attacks the roots from the soil or from other roots, either with or without grafts. Most of the fungal species obtained in the present study were the same as those isolated in Germany from spruce roots (DIMITRI 1969 b, SCHÖNHAR 1969). In Switzerland (WOESTE 1956) it has been found that spruce roots are easily infected by *Armillaria mellea* and *Fomes annosus*, provided the general conditions for infection are present. On the other hand, it has been shown (LAATSCH 1970) that the spruce can produce agents inhibiting *F. annosus* only as long as the wood contains enough sugars and the water supply is adequate and continuous.

The present study cannot throw much light on the time at which the fungi infected the roots but, at the time the tree was felled, they had probably already been infected by at least some of the microbes found. *Armillaria mellea* was the main fungus isolated from the white mycelium sheet between wood and bark, although other fungi had also grown on this layer, which is rich in nutrients, a year after the felling of the tree. The part played by *A. mellea* is confirmed by observations made about 3 years after the trees had been felled: its sporophores were found even in spruce stumps from which *A. mellea* could not be isolated in the present study.

## Discussion

Stumps provide a long-lasting growth substrate for decay fungi enabling them to compete with other microbes. The more regular and copious the cuttings, the more probable it is that the aerially distributed decay fungi will reach the cut surfaces of stumps, and from there the root systems and other trees. With year-round cuttings, there are always trees being felled whenever a heavy deposition of diaspores from the many tree-inhabiting fungi falls from the air onto the freshly cut stump surfaces. The composition of the fungal species infecting the stumps can be influenced by treating the stump surface with chemicals (RISHBETH 1959 b, 1959 c, 1970). The most efficient way of controlling the composition of the fungal flora of stumps is by inoculating the sawn surface of the stump with selected fungi immediately after felling. Fungal species antagonistic to the patogenic fungi are chosen for the inoculation. In this way, the flora of decay fungi in the forest can be changed to some extent, on a short or long term basis, as desired by man. As proveed by RISHBETH in several studies (1950, 1952, 1959 a, 1959 b, 1959 c, 1961, 1963), inoculation of the cut surfaces of pine stumps with the mycelial suspension of *Peniophora gigantea* restricts the aerial distribution of *F. annosus* through cut stump surfaces. RISHBETH (1970) is doubtful, however, about the possibility of protecting spruce stumps by this method. In the present study it was seen (Fig. 5) that *P. gigantea* did protect spruce stumps. The present study, however, only covered one year, and it cannot be claimed that the results are generally applicable. Weather conditions, for example, may change and fungal strains can also have very different infection capacities and antagonistic qualities. Because the present study was of short duration, it only ascertained those fungal species which take part in the initial phase of decay in spruce stumps. The composition of the fungal species contributing to the decay of pine stumps is known to change as the decay advances (RISHBETH 1951 a, 1951 b, MEREDITH 1959, 1960). Less is known, so far, about the decay process of spruce stumps (DIMITRI 1969 a, 1969 b, RISHBETH 1970). In the present study, a re-examination three years after the felling and inoculation showed that many spruce stumps were occupied by the sporophores of e.g. *Armillaria mellea*, although no isolates of this fungus had been obtained from the wood of the same stumps one year after the felling. *A. mellea* and *F. annosus* have been found, by ZYCHA and KATO (1967) and DIMITRI (1969 b), to be the most important fungal species causing decay in spruce stumps. On the basis of the present study, this opinion can be shared as far as Finland is concerned, but it must be added that the *Peniophora* species and *Stereum sanguinolentum*, at least in the early stages, are even more common in Finland than *A. mellea* and *F. annosus*. NILSSON and HYPPEL (1968) have shown that spruce lesions in Sweden are infected e.g. by *Peniophora gigantea*, *S. sanguinolentum*, and *Cephalosporium* species. If the cut surface of a stump is regarded as a major lesion, the

present study is a confirmation of their findings.

The present study also showed that after, or perhaps even before, the felling of a tree, microbes from the soil infect tree roots. Microbial infection through the soil has been studied e.g. by BAKER (1968), who found that he here faced a complex of problems requiring detailed investigation. The importance of rhizosphere-microflora for pathogenic infection was emphasized by STARKEY (1958) and WOODS (1960). MALOY and ROBINSON (1968) showed that bacteria and non-*Basidiomycetes* fungi were the first to attack the *Abies grandis* Lindl. wood material. The finding of the present study was exactly the same for *Picea abies*, and corroborates the result reported e.g. by SHIGO (1965) on deciduous trees.

## SUMMARY

An attempt was made to restrict the aerial distribution of *Fomes annosus* through the cut surfaces of spruce stumps by inoculating the surfaces, immediately after felling, with mycelial suspension, grown in the laboratory on malt agar, of *Fomes pinicola*, *Lenzites sepiaria*, *Peniophora gigantea*, *Polyporus abietinus* and *Trichoderma viride*. Trees were felled once a month for a year. Samples were taken from the cut surfaces of the stumps approximately one year after the felling and the inoculation.

*P. gigantea* inhibited the infection of cut stump surfaces by airborne *F. annosus* (Fig.5). *P. gigantea* cut down both the total number and the number of the species of fungi infecting the stump through aerial distribution (Table 2). *T. viride* had a parallel but less marked effect. *F. pinicola*, *L. sepiaria* and *P. abietinus* proved to be weak colonizers of spruce stumps. When they were used to inoculate the stumps, the number of fungi infecting the cut surfaces was larger than that infecting the stumps treated with *P. gigantea* and *T. viride*.

A year after the inoculation some stumps were excavated with their roots. Fungi from the discoloured spots of wood in the stumps were cultured for identification. It was found that many different fungal species from the soil and the points of root grafting had infected the roots of the stump in the course of the year. The majority of the identified microbes were non-*Basidiomycetes* fungi, and bacteria.

A year after the felling and inoculation, a white mycelial sheet was seen between the wood and bark of many stumps. Several fungi, including *Armillaria mellea*, *Trichoderma viride*, *Penicillium* species, and *Peniophora gigantea* were isolated from this sheet.

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SELOSTE:

## KUUSEN KANTOJEN MAANNOUSEMASIENI-INFEKTION ESTÄMINEN MUUTAMIA PUUSSA KASVAVIA SIENIÄ KÄYTTÄEN

Vuoden ajan 15. 9. 1967—15. 8. 1968 kaadettiin kerran kuussa metsästä 20—30 kuusta ja niiden kantojen kaatopinnat siveltiin välittömästi kaatamisen jälkeen *Fomes pinicolan* (kantokääpä), *Lenzites sepiarian* (aidaskääpä), *Peniophora gigantean*, *Polyporus abietinuksen* (kuusenkääpä) ja *Trichoderma viriden* laboratoriossa mallasagarilla kasvatetulla rihmastosuspensiolla. Vuoden kuluttua kaatamisesta ja saastuttamisesta sahattiin kannoista näytekiekot. Niistä määrättiin 10 vrk:n säilyttämisen jälkeen maannousemasienen kuromankannattimien kasvualat ja otettiin näytteet muiden sienien viljelemiseksi ja tunnistamiseksi. Muutamia kantoja kaivettiin näytteiden ottamisen jälkeen juurineen maasta ja viljeltiin sekä tunnistettiin niistä puun värjänneet sienet.

Kuvan 5 mukaan *P. gigantealla* siveltyihin kantoihin ei maannousemasieni iskeytynyt. Muut sienet eivät sanottavasti suojanneet kantojen kaatopintoja maannousemasienen infektiolta. Taulukon 1 mukaan *P. gigantea* hävitti huhti-elokuun aikana kuusen kannoista lähes täydellisesti joko yhtaikaa *P. gigantean* kanssa tai 10 vrk myöhemmin kaatopintaan ympätyn maannousemasienen. Muuna aikana vuotta maannousemasieni oli kilpailukykyisempi. *T. viride* ja *F. pinicola* hävittivät kumpikin yhdessä tapauksessa maannousemasienen.

Vuoden kuluttua kaatopintojen saastuttamisesta eristettiin niiden värjäytyneistä kohdista joukko sieniä (Taulukko 2). Taulukon mukaan pystyivät *F. pinicola*, *L. sepiaria* ja *P. abietinus* varsin vähän estämään muiden sienien iskeytymistä kaatopintoihin. Mainituilla sienillä saastutettuihin kaatopintoihin iskeytyivät mm. *Stereum sanguinolentum* (punertuva verinahkasieni), *F. annosus*, *P. gigantea* ja *T. viride*. *T. viride* infektoi kaatopinnat paljon täydellisemmin ja tehokkaammin kuin *F. pinicola*, *L. sepiaria* tai *P. abietinus*. Lokakuusta huhtikuuhun *T. viride* esti miltei kaikkien muiden sienien iskeytymisen kaatopintoihin ja se oli voimakas muiden sienien infektion estäjä myös toukokuusta heinäkuuhun tapahtuneissa saastutuksissa. Elo-syyskuussa sen kilpailukyky muihin sieniin nähden ei ollut yhtä hyvä kuin aikaisemmin kesällä. *P. gigantea* oli kaatopintoihin ympättynä tehokkain ja kilpailukykyisin muihin sieniin verrattuna. Muutamat sienet kuitenkin iskeytyivät *P. gigantealla* saastutettuihin kaatopintoihin. Tällaisia olivat mm. *T. viride*, *S. sanguinolentum* ja *Coryne sarcoides*. Kantojen kaatopinnoista eristettiin vain muutama *Fungi imperfecti*-sieni. Kontrollikantojen kaatopinnoista tehtyjen sienieristysten perusteella ovat *Stereum sanguinolentum*, *Peniophora pithya*, *Peniophora gigantea* ja *Fomes annosus* tavallisimmat kuusen kantoja ilmateitse infektoivat sienet.

Tutkimuksissa kaivettiin noin vuoden kuluttua

saastuttamisesta muutamia saastutettuja kantoja juurineen maasta. Kantojen juuriin todettiin vuoden kuluttua kaatamisesta iskeytyneen sieniä maasta ja toisista juurista käsin. Eristetyistä mikrobeista olivat tavallisimpia bakte eritjaei-*Basidiomycetes*-sienet. Tulos on samansuuntainen useiden ulkomaisten tutkimus tulosten kanssa.

Vuoden kuluttua kaatamisesta tavattiin useiden kantojen kuoren ja puun välistä valkeata kalvomaista muodostumaa. Tästä eristettiin mm. *Armillaria mellea, Trichoderma viride, Penicillium*-lajeja ja *Peniophora gigantea.*

KALLIO, TAUNO O.D.C. 172.8

1971. Protection of spruce stumps against *Fomes annosus* (Fr.) Cooke by some wood-inhabiting fungi. — ACTA FORESTALIA FENNICA 117. 20 p. Helsinki.

The cut surface of spruce (*Picea abies* L.) stumps were inoculated, immediately after felling, with mycelial suspensions grown on malt agar in the laboratory, of *Fomes pinicola*, *Lenzites sepiaria*, *Peniophora gigantea*, *Polyporus abietinus* and *Trichoderma viride*. Samples were taken from the cut surfaces approximately a year after the inoculation. *P. gigantea* inhibited the inflection of root rot fungus on the cut surfaces. Some stumps were excavated with their roots, and the fungi that had infected them from the soil were identified. The majority of the infectious soil microbes were bacteria and non-*Basidiomycetes* fungi.

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## KANNATTAJAJÄSENET — UNDERSTÖDANDE MEDLEMMAR

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