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# SCOTS PINE DISEASES

## PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM KÓRNIK, POLAND, 16—20, MAY 1989

Edited by **Timo Kurkela** and **Ryszard Siwecki**



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Jaostusosasto

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Joint meeting: 7th European Colloquium of Forest Pathologists and the members of two IUFRO Working Parties

**S2.05.04 Resistance of pines to *Melampsora pinitorqua***

**S2.06.02 Canker and shoot blight of conifers**

Edited by

**Timo Kurkela** and **Ryszard Siwecki**

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# PREFACE

In many European countries Scots pine (*Pinus sylvestris* L.) is an important conifer tree species. Over the past 10 to 15 years an increasing damage of Scots pine has been observed in many areas, particularly in Central Europe. There are results that Scots pine decline can be considered at least partly as reaction to air pollution effects. Nevertheless, also other biotic factors, diseases or pests can contribute to the damage. Therefore, detailed knowledge is needed about all biotic factors as well as about the biology of biotic agents, symptom and disease development, and interaction between host and parasite. To present and to discuss recent research results was the aim of a meeting on "Scots pine Diseases". The meeting was organized by the two International Union of Forest Research Organizations Working Parties "Canker and Shoot Blight of Conifers" (S2.06.02) and "Resistance of pines to *Melampsora pinitorqua*" (S2.05.04) as 7. European Colloquium for Forest Pathologists in connection with the Editorial Board of the European Journal of Forest Pathology. At the kind invitation of Professor Dr. W. Bugała, Director of the Institute of Dendrology of the Polish Academy of Sciences the meeting was held at Puszczykowo near Kórnik, Poland, from May 16 to 20, 1989.

The meeting was attended by more than 60 participants of 11 countries. The excellent local organization by Professor Dr. R. Siwecki and his coworkers of the Institute of Dendrology, the great hospitality, and the very good facilities of the Forest Hostel, where all participants were accommodated, made the meeting so enjoyable and memorable.

In the proceedings most papers presented at the meeting on "Scots Pine Diseases" are given in full length. It is hoped that the papers provide a sound basis and stimulus for further research on this significant subject, in order to get a better insight into processes of forest ecosystems and to contribute to the conservation of our forest resources.

B.R. Stephan
Chairman WP S2.06.02

T. Kurkela
Chairman WP S2.05.04

# WELCOMING ADDRESSES

Ladies and Gentlemen;

In the name of the leadership of the Polish State Forests and of Polish foresters in general I have the honour of welcoming you heartily here at the international symposium on "Scots Pine Diseases".

Scots pine is the main forest species of Poland covering 70 % of our forest area. It may be said that Polish foresters belong to those who can be considered as experts on the breeding and silviculture of Scots pine.

In recent years our pine stands have suffered previously unknown calamities caused by great accumulation of injurious biotic and abiotic factors. The most serious of these was the occurrence in the years 1979-1985 of a epidemic attack by Gypsy moth (*Lymantria monacha*) which we had to combat by chemical and biological agents over an area of 5 million hectars of pine and spruce forests. In these years there were also local epiphytotics of *Gremmeniella abietina* in young pine stands of central and northern Poland on a scale previously unknown to us, which we had to deal with. For several years now we observe an increase in the occurrence of fungal diseases of Scots pine caused by *Heterobasidion annosum*, *Armillaria mellea*, *Lophodermium seditiosum* or *Melampsora pinitorqua*. I am sure you will get detailed information about the occurrence and injury extent of all these diseases during this symposium. I am sure that the results of studies that will be presented here by all our eminent guests, famous forest pathologists from over a dozen countries participating in this meeting, will help in the development of prompt and effective methods of combating these diseases.

I would like to stress also, that thanks to the extensive and proficient scientific work conducted by employees of the Forest Research Institute in Warsaw, the Institute of Dendrology of the Polish Academy of Sciences in Kórnik and the three Forestry Faculties in Warsaw, Poznan and Kraków, we generally have a good knowledge of the fungal diseases affecting our forests and limiting the cultivation of Scots pine in our country.

Another great problem of the forests in Poland is the most severe industrial pollution in Europe. About 4 million ha of Polish forests, thus almost one half of the forest area, is under the influence of injurious levels of sulphur dioxide, oxides of nitrogen, fluoric compounds and heavy metals. Industrial pollution occurring on such a scale originates both from our own industry and from sources in neighbouring European countries. Examples of the tremendous devastation in forests affecting their very existence are to be found in the spruce forests of southern Poland, primarily in the Sudety and Izerskie Mts. As the results of macroscale inventories made every two years the sanitary state of our forests is decidedly improving but the health continues to decline and is far from what we would like to see in our forests. The extreme industrial

pollution effects in our forests are amplified by unfavourable climatic conditions in some years and they can have a significant effect on the increased occurrence of dangerous diseases and injurious insects. As an example we can quote two fungal diseases, *Scleroderris lagerbergii* and *Melampsora pinitorqua*, which will be the subject of special interest for this symposium.

In spite of all these great dangers we have in Poland rich and valuable Scots pine stands known by foresters around the world. Among these we can particularly include the Masurian pine from the Pisz Forest and the pine from the southern regions of Wielkopolska and northern Opole region.

Finally, in my own name, and in the name of the Poznan forests, I wish you fruitful discussions, a wide and detailed exchange of information concerning serious diseases of Scots pine and many pleasant impressions from the short stay in Poland. At the same time I would like to assure you that our foresters will do everything to make your stay in our country pleasant and that the fruits of your presence here be utilized for the benefit of better protection of pine forests in Europe against serious fungal diseases.

Ryszard Dzialuk
Director General
Polish State Forests

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Ladies and Gentlemen;

Opening this symposium devoted to the topic Scots pine diseases I have the pleasure of welcoming the participants in the name of the employees of the Institute of Dendrology of the Polish Academy of Sciences in Kórnik and in my own. I extend a particularly warm welcome to the foreign participants of the symposium.

In recent years in many countries of Europe we observe the distressing phenomenon of the decline of an increasing number of forest trees. Until recently the classical example of this phenomenon was the famous dying of elms caused by a fungus attacking vascular tissues. This has led to an almost complete disappearance of elms from cultivation and particularly of some species of the genus. Presently we witness an attack of various fungal pathogens and the decline of other species, among those some which are very important to forest economy representing the main constituents of European forests. Everyone heard about the decline of firs, oaks and beeches. However also the most common species of our forests, Scots pine is affected to an increasing degree by various diseases, which lead to severe losses in yield and not infrequently to the dying of trees. In many countries intensive studies are conducted aimed at determining the causes of the suddenly progressing tree decline. It does not suffice to find one or a few species of fungi that occur in the dying trees. All seems to indicate that the dying of trees has other causes and that there are many causes. In general these are factors that in a relatively short time reduce the biological resistance of trees to fungal pathogens. Among the injurious factors mentioned most commonly are the toxic substances emitted by industrial plants. They pollute air, soil and water in increasing concentrations. It is also increasingly commonly indicated that unfavourable climatic

changes occur in Central Europe. Particularly worrisom is the increasing yearly severity of drought, the declining precipitation and serious lowering of the ground water table.

The symposium beginning today will consider the most serious pathogens of Scots pine. I wish to express the hope that the papers presented here and the following discussions will permit a closer identification of the causes for the spreading Scots pine decline in our country and in neighbouring ones. May your deliberations at this symposium prove fruitful in this respect.

Władysław Bugała
Director
Institute of Dendrology
Kórnik

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Ladies and Gentlemen;

As Chairman of the Polish National IUFRO Committee I have the honour and pleasure of welcoming you here at the International Symposium on Scots pine diseases.

This meeting is sponsored by two IUFRO Working parties WP S2.06.02 Canker and Shoot Blight of Conifers and WP S2.05.04 Resistance of Pines to *Melampsora pinitorqua*. The organizer of this Symposium, Prof. Ryszard Siwecki, is the current co-chairman of WP P2.05.06 Genetic aspects of air pollution. In the previous term, before the IUFRO Congress in Ljubljana, Prof. Siwecki was also co-chairman of WP S2.05.03 Resistance of poplars to diseases and insects. The planning for the current Symposium was started during his previous term of office as a IUFRO functionary.

The Institute of Dendrology, in whose name Prof. Bugala just spoke, has been very active in organizing symposia under the sponsorship of the International Union of Forest Research Organisations. The first IUFRO meeting was held here in 1973 on "Dormancy in Trees", followed shortly in the same year by the Symposia on "Genetics of Scots pine". In 1980 there were again two symposia, the IUFRO/FAO Joint Symposium on "Resistance Mechanisms in Poplar Diseases" and the IUFRO Symposium on "Scots Pine Forestry of the Future". In 1985 a IUFRO Symposium was held here on "Flowering and Seed-Bearing in Forest Seed-Orchards".

At all these meetings Scots pine was prominently considered, even at the meeting on Poplar diseases, in view of the interest in *Melampsora pinitorqua*, a parasite of both pine and poplars, on which much scientific work has been done at the Institute. Now we have a meeting on "Scots Pine Diseases".

IUFRO is an organisation that lives by the activity of its members. It is a viable organisation and it is viable in Poland, thanks to the meetings being organized here and thanks to the willingness of you, visiting scientists, to participate these meetings. I hope you will find the current meeting interesting and time spent here useful.

Maciej Giertych
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# DISEASES OF *PINUS SYLVESTRIS* IN POLISH FORESTS

## ABSTRACT

Some of the main diseases of Scots pine in Poland are characterized and discussed. They are damping-off, needle-cast, Scots pine die-back, *Heterobasidion* root and butt rot, *Armillaria* root rot and red ring rot.

## DAMPING-OFF

The proportion of Polish forests consisting of *Pinus sylvestris* is relatively high (at least 70 %), and the number of Scots pine seedlings that has to be produced is correspondingly high, too. The production of seedlings has, however, been almost continuously plagued by the damping-off problem. The first major contribution on this question was by Kozlowska (1957) who, however, was interested at the time in only one pathogenic fungus, namely *Fusarium oxysporum* Schl. Since then, Gierczak (1963, 1967), Manka and Gierczak (1971) and Kluge (1971) have shown that other fungal pathogens may also play a major role in attacking pine seedlings in Poland. The main one is *Rhizoctonia solani* Kühn (especially at higher soil temperatures), and to a much smaller extent, *Fusarium* species other than *F. oxysporum*, *Cylindrocarpon destructans* (Zinssm.) Scholten, *Phytophthora cactorum* (Leb. et Cohn) Schröt. and *Pythium* spp.

In naturally regenerated pine stands the disease is nearly always absent. In contrast, naturally regenerated pine seedlings in some Polish yew stands (e.g. in the district of Wierzchlas) have been killed off completely by *Cylindrocarpon radicicola* (Manka et al. 1968). The control measures applied in Poland are: sowing at the proper time into well prepared moist and warm soil, various chemical treatments (i.e. seed dressing with the systemic preparations Oxafun T or Funaben T, spraying after emergence of the seedlings when needed etc.), and still rather experimentally, the use of biopreparations containing fungi such as *Mycelium radicis atrovirens* (mycorrhiza forming strains) or mixtures of antagonistic saprophytic soil fungi.

## NEEDLE CAST

Needle cast has for a long time been a very well documented disease, in Poland as well as in other European countries. It is at its most dangerous form in nurseries and young plantations, with its characteristic symptom of premature cast of diseased needles in the spring time. The causal fungi nowadays accepted in Poland are *Lophodermium seditiosum* Minter, Staley et Millar (Kowalski and Budnik 1976 and 1977), *L. pinastri*

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(Schrad.) Chev. (to a limited extent) and also possibly *Cyclaneusma minus* (Butin) DiCosmo, Peredo et Minter. The problem of needlecast in Poland became more complicated in the last 25 years owing to the appearance of a completely new needle cast disease on pines older than about 5 years that results in the premature casting of 2-year-old needles in the autumn. The species most frequently present among the fungal isolates obtained from the diseased needles were *Sclerophoma pithyophila* (Corda) v. Höhn, *Cyclaneusma minus* (Butin) Di Cosmo, Peredo et Minter and *Lophodermium pinastri* (Manka et al. 1979). *Sclerophoma pithyophila* was isolated from green needles and from dead needles. Owing to the seasonal nature of the fall of diseased needles, some researchers are inclined to call the phenomenon autumn needle cast (in contrast to the spring needle cast discussed earlier). The disease is probably promoted by air pollution and a water deficiency in the soil. Control measures against spring needle cast are practicised only in nurseries, although sometimes also in the very young plantations, by means of traditional spraying (twice) with 0.4 % Cynkotox 65 or other preparations. Autumn needle cast is not controlled at the moment at all. It is a serious problem that needs to be solved as soon as possible.

## SCOTS PINE DIE-BACK

This is shoot dying caused by *Gremmeniella abietina* (Lagerb.) Morelet. The first large-scale outbreak of the disease in Poland occurred not long ago, namely at the turn of the 1970's and 1980's (Manka 1983), in the northern parts of Poland, especially the forest districts of Gdansk, Szczecin, Szczecinek and some others. It primarily attacked the youngest pine stands, sometimes completely destroying large parts of them. Nevertheless, many of the other affected stands recovered (starting around 1983) and are now becoming more and more normal. However, following the collapse of the epidemic, it was still possible to isolate the pathogen from formerly diseased trees in some years.

To protect the stands against the disease in the future the following procedures have been accepted: 1) keeping to the silvicultural cultivation rules, 2) introducing tree species other than *P. sylvestris* (especially broadleaved trees), 3) avoiding planting young pines in places shaded by older pine stands, as well as in places with a higher air humidity, for instance near large water reservoirs (riversides, shores etc.), depressions etc., 4) a monitoring system permitting early eradication of primary infection centres.

## *HETEROBASIDION* ROOT AND BUTT ROT

This is nowadays the major tree disease in Polish forests, affecting an area of about 120,000 ha of pine stands, that needs to be controlled. Younger stands (up to 30 years old) are mainly attacked, but older ones can also be affected especially on abandoned agricultural land. Control nowadays primarily consists of treating stumps with a water suspension of *Phlebia gigantea* oidiospores, as well as to some extent also (still experimentally only) carrying out thinnings at times and in areas with an expected, natural profuse production of *P. gigantea* oidiospores (Manka et al. 1972), or by special types of thinnings that make control of *H. annosum* and *G. abietina* possible (Manka 1986): removing the thinnest trees only (diameter up to 7-8 cm in the lower part of

trunk) from the young stands so that natural stump infection by *H. annosum* would be strongly reduced because of the small stump surface. Unpublished material (entitled: "The influence of thinnings in young pine stands on soil microfungi communities and in turn on root pathogens") of the author and coworkers indicates that neglecting the thinning procedures in pine stands results in a distinct diminishing of the role of the saprophytic soil mycoflora that normally protect the roots against *H. annosum*.

## *ARMILLARIA* ROOT ROT

The Armillaria root rot in Poland mainly occurs on *Picea abies* and *Pinus sylvestris*, and in both cases is caused primarily by *Armillaria obscura* (Schaeff.) Herink, as well as much more rarely by *A. bulbosa* (Barla) Kile et Watling and *A. borealis* Marxmüller et Korhonen. *Pinus sylvestris* is attacked most heavily at a young age (from a few years up to a dozen or so). After this phase infection is rather rare, and only at an advanced age does it again attack intensively (Domanski 1953). The economic importance of the disease is at the moment probably about 10 times less than that of *Heterobasidion annosum*. *Armillaria* root rot is particularly damaging in lowland pine stands, especially when the young trees grow close to each other. Reducing the stand density by means of cultivation cuttings, connected with the removal of stumps and roots, is (according to Manka, Kwasna, Kazimierczak and Babkiewicz, unpublished) not only a good silvicultural practice that diminishes the susceptibility of pine to *Armillaria*, but also increases the biotic resistance of the soil against the pathogen. An additional effect has also been obtained by introducing deciduous trees into pine plantations as admixtures, although, according to old experience, the same admixtures can cause similar problems if composed of badly planted and badly growing or even dead trees.

## RED RING ROT

The disease has been well known in Poland for a long time. It is considered a very serious problem, and is very difficult to control at the same time. The causal agent, *Phellinus pini*, is prevalent in older pine stands all over the country, attacking the most valuable lower parts of trunks (Manka & Chwalinski 1961). In contrast to 5-needle pines and Douglas fir, infected *Pinus sylvestris* usually have almost no external symptoms apart from the presence of *Phellinus pini* conks on the trunk. Apart from the conks, one potential diagnostic tool would be the acoustic effect obtained by banging at the trunk with a hammer or axe, and the presence of holes in the trunks denoting the places from where old conks have dropped off. Swellings, sometimes called "blind conks", are extremely rarely found on diseased trunks (Manka & Chwalinski 1961). According to our findings (Zaleski & Manka 1939), the greatest destruction of wood by this fungus usually occurs in the lowest part of the trunk (0 - 5 m), the highest being at a height of 10 to 15 meters.

The following control measures are usually stressed: removing trees with conks from the stands, the gradual pruning of lower branches in well-formed, 25 to 35-year-old trees to get an 8 to 10 m long trunk without sites for infection. The latter procedure, not yet very popular in praxis, seems to be of great prophylactic value.

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# FUNGI INFECTING THE SHOOTS AND STEMS OF *PINUS SYLVESTRIS* AND *P. NIGRA* IN SOUTHERN POLAND

## ABSTRACT

Observations on 12 fungal stem and shoot parasites of *Pinus sylvestris* and *P. nigra* in Poland during the period from 1975-1987 are summarized. The symptoms of the diseases are briefly described, and the role of predisposing factors discussed.

## INTRODUCTION

In Poland *Pinus sylvestris* L. and *P. nigra* Arn. play an important, although not the same role. *Pinus sylvestris* occupies over 70 % of the forest area, and is one of the most important tree species from the economical point of view. *P. nigra*, being rather tolerant to air pollution, is of importance in industrial regions. In Upper Silesia, where the conversion of stands dying as a result of air pollution was initiated earlier than elsewhere, some of *P. nigra* stands are already 40 to 70 years old. In the 70's the proportion of *P. nigra* in these stands amounted to 8 %, and was twice as high as that of *P. sylvestris*. It is thus fully justified to keep track of all the factors which lower the vitality of both these pine species. *P. nigra* is not native to Poland, and the information available about this tree species in the regions where it occurs naturally cannot be indiscriminately applied here. Among other things, its susceptibility to diseases may change outside its natural range (Roll-Hansen 1972).

This paper describes the fungi which may be a serious threat to *P. sylvestris* and *P. nigra* owing to shoot and stem infection. Some attention is also paid to factors which modify the appearance of these fungi, as well as to those fungi which secondarily inhabit sick and dead trees.

## MATERIALS AND METHODS

This paper is based on the results obtained since 1975. The studies were conducted in Southern Poland on permanent and temporary experimental plots in stands contaminated by industrial emissions to a varying degree (mainly in the Upper Silesia Industrial District: Brynek, Chrzanów, Katowice, and Swierklaniec management areas)

(Domanski et al. 1981, Latocha 1975), and in stands free of such contamination (Herby, Krynica, Lezajsk, Lubliniec). The investigated pine stands were mainly growing on moist coniferous and moist mixed coniferous forest sites. Only in Krynica did *P. sylvestris* represent the first generation of trees on former agricultural land.

The field studies consisted of observation of the disease symptoms and estimating their intensity according to a predetermined scale. This estimation included all the trees on permanent plots, and 100 trees on temporary plots. In the laboratory the fungi growing on damaged and dead organs were identified on the basis of fructifications and the mycelium isolated on suitable medium, utilizing standard sterilization methods (Kowalski 1982a). In order to distinguish the fungi causing the diseases from fungi secondarily inhabiting the dying tissue, organs with various stages of the disease were investigated, and the inocula for the isolations taken from various points including the border region between dead and living tissues.

## FUNGI INFECTING THE SHOOTS AND STEMS OF *PINUS SYLVESTRIS* AND *P. NIGRA*

### Die-back of developing shoots of *P. sylvestris*

*Melampsora pinitorqua* Rostr.

*M. pinitorqua*, pine twist rust, is widely distributed throughout almost the whole of Europe and south western Asia. Under favorable conditions it causes great damage to pine plantations (Klingström 1969, Kišpatic 1961, Kurkela 1973), which is also true for Poland (Łukomski 1963). During 1960-1972 it was the cause of the damage that affected an area of 3000 to 10000 ha of young pine stands (Siwecki 1974). Its secondary host, mainly *Populus tremula* L., is being eradicated from pine plantations, but suckers and new seedlings form an adequate site for the pathogen to complete its life cycle. The situation may be different in industrial regions. In Upper Silesia, for instance, *Populus tremula* is very vigorous when young (Latocha 1975), and it is recommended for plantations replacing old dying stands. Since 1985 many plantations of *Pinus sylvestris* in Southern Poland have been severely attacked again. An example of this is shown in Table 1. The data represent ten 5 to 10-year-old plantations randomly selected in the Chrzanów management area.

Some shoots had symptoms typical of infection by *M. pinitorqua* (Krutov 1971, Kurkela 1973). In cases where there were several infection sites on a single shoot, the shoot did not twist, and one year after infection the wounds healed leaving marks resembling hail damage (Fig. 1). However, most of the trees showed symptoms different from those associated with attack by *M. pinitorqua*, and identical to those reported by Domanski & Kowalski (1988). Current year shoots with local necrosis caused by *M. pinitorqua* and caeomata present at the same points, were not distorted into an S-shape but died, and their apices were bent downwards (Fig. 2, 3). It was also characteristic that the 20-50 cm long shoot sections below the sites of *M. pinitorqua* attack also died. Besides *M. pinitorqua*, seven species of fungi were isolated from the dead shoots (Table 2). *Botrytis cinerea*, and to a lesser degree *Sphaeropsis sapinea*, were credited with intensification of the damage *caused by M. pinitorqua* and the appearance of other disease symptoms (Domanski & Kowalski 1988).

Table 1. Frequency of *Melampsora pinitorqua* on *Pinus sylvestris* in ten 5 to 10-year-old plantations in the Chrzanów management area in 1986.

| Plantations age | Trees (%) with local necrosis on living shoots | with dead shoots |
|---|---|---|
| 7 | 89 | 24 |
| 7 | 73 | 0 |
| 5 | 68 | 22 |
| 7 | 34 | 2 |
| 7 | 24 | 2 |
| 6 | 22 | 2 |
| 6 | 8 | 0 |
| 8 | 6 | 1 |
| 10 | 0 | 2 |
| 6 | 0 | 0 |
| mean | 32.4 | 5.5 |

Table 2. Secondary fungi on shoots of *Pinus sylvestris* attacked by *Melampsora pinitorqua* during 1985-1987.

| Fungi | Frequency* within local necrosis | on dead shoots |
|---|---|---|
| *Alternaria alternata* (Fr.) Keissler | + | + |
| *Botrytis cinerea* Pers.: Fr. | ++ | +++ |
| *Cladosporium cladosporioides* (Fresen.) de Vries | + | + |
| *Epicoccum purpurascens* Ehrenb. ex Schlecht. | + | + |
| *Fusarium* sp. | + | + |
| *Sclerophoma pithyophila* (Cda) Höhn | + | +++ |
| *Sphaeropsis sapinea* (Fr.) Dyko et Sutton | - | + |

* fungus colonized less than 20 % (+), 21-70 % (++), or more than 70 % (+++) of investigated shoots

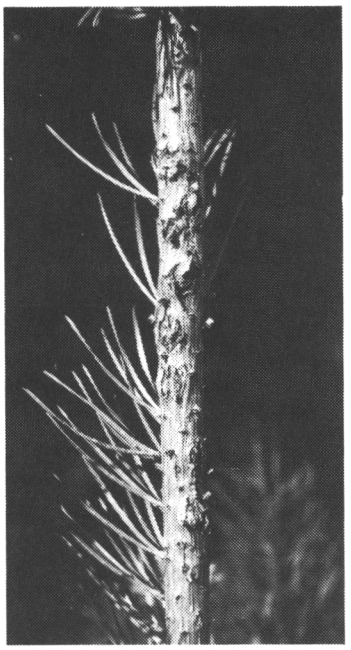


Fig. 1. Fragment of stem of 6-year-old *Pinus sylvestris* with numerous healed wounds caused by *Melampsora pinitorqua*.

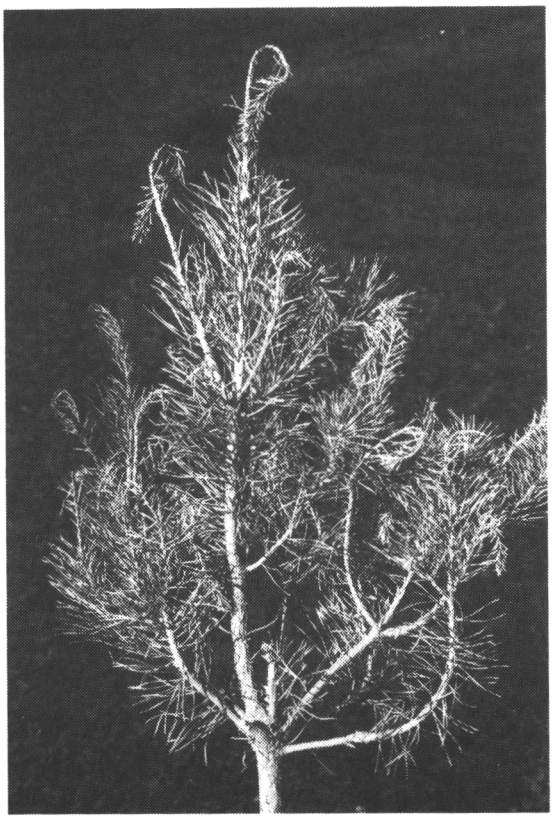


Fig. 2. 6-year-old *Pinus sylvestris* with dead shoots due to attack by *Melampsora pinitorqua* and *Botrytis cinerea*.

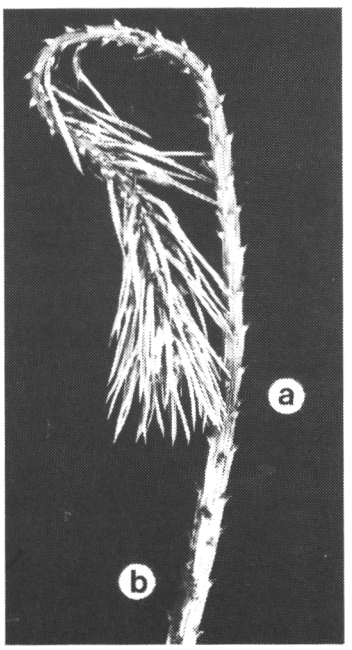



Fig. 3. Dead pine shoots: a) infection point of *M. pinitorqua*, depressed bark and caeoma; b) site of ample sporulation of *B. cinerea*.

*Botryotinia fuckeliana* (de Bary) Whetzel
Anamorph: *Botrytis cinerea* Pers.: Fr.

Pines, contrary to spruce, larch, and Douglas fir, are considered to be relatively resistant to *B. cinerea* attack. Extensive attack took place on the living shoot sections, however, after colonization of tissues killed by *M. pinitorqua*. This characteristic of *B. cinerea* has been known for a long time (see Domanski & Kowalski 1988). The rate of shoot mortality was high, and the symptoms appeared on current year shoots that were not fully developed already at the beginning of July. *B. cinerea* produced abundant spores on the attacked shoots, and was also isolated from the border area between dead and living shoot sections (Fig. 3). Besides the described phenomena, no other harmful activities of *B. cinerea* have been observed on pines in young plantations or in older stands.

*Sphaeropsis sapinea* (Fr.) Dyko et Sutton

This species, similarly to *Botrytis cinerea*, caused necrosis in shoots, both downwards and upwards, starting from the points of primary infection by *Melampsora pinitorqua*. It was less important than *B. cinerea* because it did not infect shoots so frequently (Table 2), and the rate of attack on living tissues was lower. Ripe pycnidia appeared about 4 weeks after the death of the shoot at the earliest. Up to the present time, *Sphaeropsis sapinea* has not been observed to infect shoots of *Pinus sylvestris* or *P. nigra* as a primary pathogen, as has happened in Holland or Germany (Butin 1984, van Dam & de Kam 1984). It was also only sporadically observed on shoots killed by *Gremmeniella abietina* (Tab. 4). On the other hand, it is common as a saprophyte on branches left in the forest after logging, and on the needles on such branches (Kowalski 1988).

Table 3. Some fungi isolated from strangulations present at the base of the stems of 3 to 4-year-old *Pinus sylvestris* and *P. nigra*.

| | Trees (%) | |
|---|---|---|
| | *P. sylvestris* | *P. nigra* |
| *Fusarium sambucinum* Fuck. | 7.1 | 12.2 |
| *Pestalotia hartigii* Tub. | 5.7 | 5.4 |
| *Pezicula livida* (Berk. et Br.) Rehm | 18.4 | 8.1 |
| *Phacidiopycnis pseudotsugae* (Wilson) Hahn | 2.8 | 2.7 |
| *Phomopsis occulta* Trav. | 15.1 | 20.3 |
| *Rhizoctonia solani* Kühn | 13.7 | 9.5 |
| Number of investigated trees | 212 | 74 |

### Necrotic symptoms and strangulation on the butt and root collar of young *P. sylvestris* and *P. nigra*

Strangulation following necrosis involving the entire stem circumference was observed on 3 to 4-year-old *P. sylvestris* and *P. nigra*. These symptoms were particularly numerous in 1975. They appeared one year after mechanical cultivation of the soil in the plantations when the lower part of the stems of pine seedlings became covered with a layer of soil several centimetres thick. As can be seen in Figure 4, location of the strangulation varied according to the soil level. On some seedlings the strangulation involved a short section of the stem (Fig. 4 a-e), and on others it was more extensive (Fig. 4 f-h). Altogether 31 species of fungi were isolated from strangulations. The most important ones are listed in Table 3. In general, formation of strangulations at the root collar is attributed, although not definitely, to *Pestalotia hartigii*, while the strangulation of seedlings planted at too great a depth or with the lower part of the stem covered with soil to *Phacidiopycnis pseudotsugae*. Thus, if we do not take into account the fungi that cause damping off (*Fusarium*, *Rhizoctonia*), then the frequent presence of *Pezicula livida* and *Phomopsis occulta* within the strangulations is a new phenomenon (Kowalski 1983a).

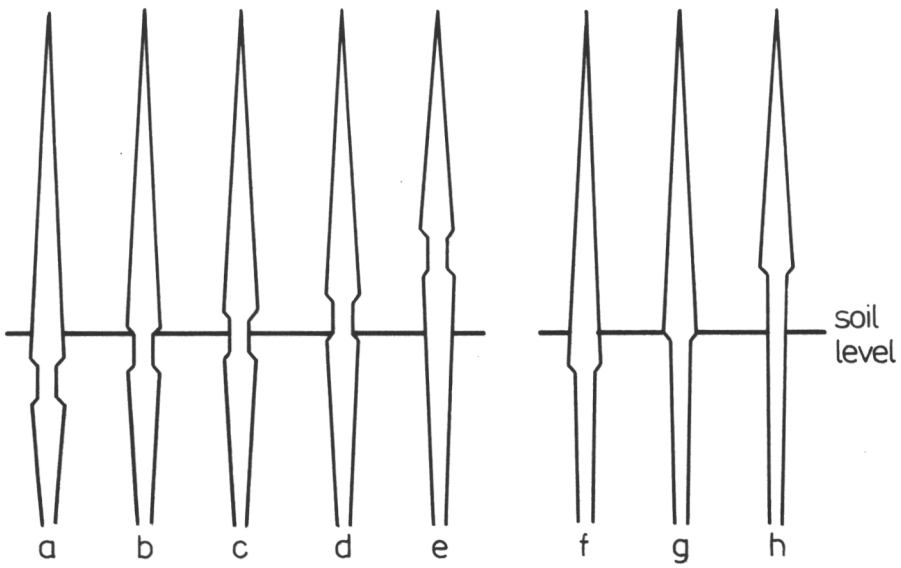



Fig. 4. Schematic presentation of the parts affected by local (a-e) and extensive (f-h) necrosis and strangulations on 3 to 4-year-old *Pinus sylvestris* and *P. nigra*.

Table 4. Fungi on dead and diseased pines in some of the experimental plots* during 1979-1986.

| Fungi | Trees (%) *P. sylvestris* dead | *P. sylvestris* living with dead shoots | *P. nigra* dead | *P. nigra* living with dead shoots |
|---|---|---|---|---|
| *Alternaria alternata* (Fr.) Keissler | | 0.3 | | 0.2 |
| *Apiospora montagnei* Sacc. | | | | 0.1 |
| *Aspergillus* sp. | | 0.1 | | |
| *Aureobasidium pullulans* (de Bary) Arn. | | 0.1 | | 0.1 |
| *Cenangium ferruginosum* Fr. | 9.7 | 13.6 | 9.9 | 4.8 |
| *Ceuthospora pinastri* (Fr.) Höhn. | | 0.1 | | |
| *Chaetomium globosum* Kunze ex Steud. | | 0.1 | | |
| *Coniothrium fuckelii* Sacc. | | 1.0 | 0.1 | 1.0 |
| *Crumenulopsis sororia* (Karst.) Groves | 1.6 | 0.7 | 8.7 | 0.6 |
| *Cytospora pinastri* Fr. | | | | 0.4 |
| *Dothiorella* sp. | | 0.1 | | |
| *Epicoccum purpurascens* Ehrenb. ex Schlecht. | | | | 0.2 |
| *Fusarium* sp. | | 0.1 | | |
| *Gremmeniella abietina* (Lagerb.) Morelet | 38.4 | 46.4 | 69.6 | 81.9 |
| *Lachnellula* sp. | | 0.2 | | |
| *Nectria cinnabarina* (Tode) Fr. | | | | 0.1 |
| *Pezicula livida* (Berk. et Br.) Rehm | 26.4 | 1.2 | 16.1 | 0.9 |
| *Phomopsis occulta* Trav. | 1.6 | | 4.1 | 0.1 |
| *Sarea resinae* (Fr.: Fr.) Kuntze | | 1.2 | | 1.9 |
| *Sclerophoma pithyophila* (Cda) Höhn. | 17.7 | 14.6 | 1.3 | 3.6 |
| *Scoleconectria cucurbitula* (Tode: Fr.) Booth | 8.0 | 7.0 | 2.2 | 2.6 |
| *Seimatosporium lichenicola* (Cda) Shoemaker et Müller | | | | 0.1 |
| *Sphaeropsis sapinea* (Fr.) Dyko et Sutton | 4.0 | 0.1 | | 0.1 |
| *Stereum sanguinolentum* (Alb. et Schw.) Fr. | 4.8 | | 0.6 | |
| *Thelephora terrestris* Ehrb.: Fr. | | | 1.3 | |
| *Therrya pini* (Alb. et Schw.) Kujala | | 0.1 | | |
| *Trichaptum abietinum* (Fr.) Ryv. | | | 0.3 | |
| *Trimmatostroma* sp. | | | | 0.7 |
| *Tympanis pinastri* Tul. | | 1.0 | 1.7 | 0.1 |
| *Valsa pini* (Alb. et Schw.) Fr. | 3.2 | 1.7 | 1.9 | 0.3 |
| *Valsa* sp. | | 0.1 | 0.6 | |
| Number of investigated samples | 125 | 1288 | 529 | 2054 |
| Number of isolations | | 450 | 500 | 225 |

* explained in text

*Pezicula livida* (Berk. et Br.) Rehm
Anamorph: *Cryptosporiopsis abietina* Petr.

*P. livida* is common in Poland as a saprophyte on various species of coniferous trees. It is one of the first microorganisms to colonize the bark and wood of branches that die as a result of insufficient light. Thus, it plays an important role in natural pruning (Butin & Kowalski 1989, 1990). It is also common on diseased and dying coniferous trees in stands damaged by industrial emissions (Domanski et al. 1981). Investigations have shown, however, that under certain conditions this harmless, and even useful saprophyte may attack living tissues.

As has been mentioned already, *P. livida* frequently caused necrosis and strangulations on young pines whose lower part of the stem were covered with soil as a result of mechanical soil cultivation. On *P. sylvestris* it caused such necrosis more than twice as frequently as on *P. nigra* (Table 3). *P. livida* was many times isolated from the bark and wood within strangulations already in the initial stage of the disease, i.e. when the sections above and below the strangulation were still alive. No other species of fungi were found in the necrotic tissues that could be suspected of causing this necrosis. However, it was not found in strangulations below the soil level (Fig. 4 a,f). Similarly to the case for pines, necrosis with strangulations or cankers containing *P. livida* was observed on 5 to 7-year-old larches injured by industrial emissions and frost (Kowalski 1982a). The entire part of the trees above the cankers or strangulations had died as a result of this necrosis .

*P. livida* also attacked trees already infected by other pathogens thus accelerating their death. Domanski et al. (1976) observed this fungus on young conifers already infected by *Armillaria* sp. or *Heterobasidion annosum* (Fr.) Bref. It frequently appeared on the butt of pines when their shoots were infected by *Gremmeniella abietina* (Table 4). This resulted in the death of the entire aboveground parts of the trees. *P. livida* subsequently penetrated the roots killing them and causing rot in their wood. Laboratory investigations conducted according to the standard block method showed that *P. livida* is able to produce such rot. After four months action on the wood, the decrease in dry weight ranged from 0.1 to 13.5 % (Domanski unpubl.).

These examples show that the pathogenic activity of *P. livida* is manifested only when trees are weak. It is highly probable that infection may originate from peripheral parts of the bark where, in living trees, this species sometimes lives as an endophyte (Kowalski unpubl.).

The observations on *P. livida* made in Poland agree with reports of other authors. Spaulding (1961) frequently observed this species on pathological strangulations at the base of thin stems which caused the death of the seedlings. Also observations made by other authors provide certain evidence of its weak parasitic power (Ferdinandsen & Jørgensen 1939, Wilson 1928). However, artificial infection has so far been unsuccessful (Gregor 1931, Johansen 1949, Murray 1954, Smerlis 1973). On the basis of observations carried out in Southern Poland, the role of *P. livida* on coniferous trees may be compared to the role of *P. cinnamomea* on broadleaved trees. Investigations on this species have shown that, under certain conditions, it is able to infect living tissues and cause their necrosis (Kehr 1988).

*Diaporthe conorum* Niessl.
Anamorph: *Phomopsis occulta* Trav.

*Diaporthe conorum* commonly occurs on conifers, especially in vegetative form (Gremmen 1960, Grove 1935, Kowalski & Lang 1984). It is considered to be a weak parasite, sometimes causing bark necrosis on injured trees, or a fungus which accompanies other pathogenic fungi (Dodge 1932, Hahn 1930, 1943). The results reported above indicate that covering the lower part of the stems of pine seedlings with soil during mechanical cultivation produces favourable conditions for infection. *D. conorum* was frequent, similarly to *Pezicula livida*, in pathological strangulations within the root collar (Table 3). It was also found in butts of pine with shoots infected by *Gremmeniella abietina*, although not as frequently as *Pezicula livida* (Table 4).

Besides pines, *D. conorum* frequently infects the butts of young larches injured by industrial emissions and frost, or attacked by other fungi (Kowalski 1982a).

The investigations carried out up to the present have not indicated that this fungus causes a local necrosis or the death of trees not already weakened.

## Cankers on stems and branches

*Crumenulopsis sororia* (Karst.) Groves
Anamorph: *Digitosporium piniphilum* Gremmen

This fungus has been reported in Poland since 1979, although only from the Upper Silesia industrial region (Kowalski & Domanski 1983). Infection of *P. nigra* occurs five times more frequently than that of *P. sylvestris* (Table 4). The stems are mainly infected, while infection on the shoots is rare. Infection on stems is restricted to the whorls, usually on the butt. The infection is followed by the development of cankers and ample resin outflow (Fig. 5). Eccentric growth increment of the annual rings and dark gray coloration of the wood may be observed on cross-sections (Fig. 6). Trees several years of age usually die within 1-3 years after infection. The pathogen produces pycnidia and apothecia in canker cracks in the bark of living as well as dead trees.

*Sarea resinae* (Fr.: Fr.) Kuntze
Anamorph: *Pycnidia resinae* (Ehrenb.: Fr.) Höhn.

It occurs on various conifers throughout Poland, although not very frequent. It has been sporadically isolated from infection spots on living needles (Kowalski 1987a) and from the bases of branches died as a result of insufficient light (Butin & Kowalski 1990). It sometimes accompanies other pathogens, e.g. *Lachnellula willkommii* on larch (Butin & Kowalski 1990), *Cronartium ribicola* on white pine (Kowalski unpubl.). Contrary to *Cenangium ferruginosum*, *Sclerophoma pithyophila* or *Scoleconectria cucurbitula*, it rarely occurred secondarily on shoots killed by *Gremmeniela abietina*. It was generally more frequent on *P. nigra* than on *P. sylvestris* (Table 4). Apothecia or pycnidia were usually present on the resin outflow within local cankers, outgrowths and bark cracks (Fig. 7). Sometimes there were no injuries or pathogenic fungi that could have produced such symptoms. Only *S. resinae* was isolated from the bark and wood, which indicated that these symptoms could have been a result of activity by this fungus. These observations made under natural conditions confirmed the results obtained by Smerlis (1973) who successfully conducted artificial infection on *Abies*, *Juniperus*, *Larix* (2 species), *Picea* (4 species), and *Pinus* (5 species). Accepting *S. resinae* as a harmless saprophyte on ejected resin (Kujala 1950) may not be always fully justified.

*Cronartium flaccidum* (Alb. et Schw.) Wint.

It was only sporadically encountered in the stands under investigation. The symptoms agreed with those recognized as typical for this pathogen.

*Lachnellula* sp.

It occurred sporadically within cankerous deformations on shoots several years old accompanied with ample resin outflow. Only a vegetative form (*Naemospora* sp.) was found in bark cracks, and thus no species identification was posssible.

### Dying back of current year shoots and branches

*Gremmeniella abietina* (Lagerb.) Morelet.
Anamorph: *Brunchorstia pinea* (Karst.) Höhn.

This pathogen occurred for the first time in Poland on an unparalleled scale during 1979-1983. Pine stands were attacked over an area of 100,000 ha, especially severely in the northern and southern parts of the country (Kowalski 1983b, Smykała 1987).

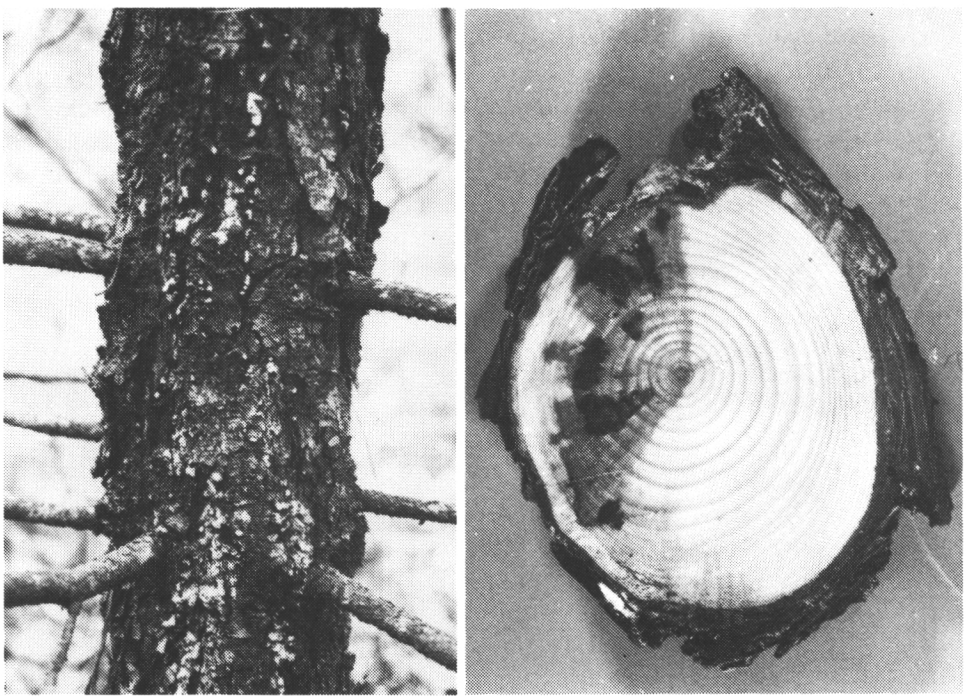


Fig. 5-6. Butt of 21-year-old *Pinus nigra* attacked by *Crumenulopsis sororia*: 5) external symptoms, 6) cross section.

The disease process and its effects differed in the south and in the north. In northern Poland, stands of *P. sylvestris* were attacked. Stands, especially of age classes I and II, covering an area of over 3000 ha were killed. It was hard to determine the precise area affected since attack by *G. abietina* in part of the area occurred simultaneously with an outbreak of *Lymantria monacha* L. In southern Poland, on the other hand, the investigations conducted on one of the permanent experimental plots (7.6 ha) showed that during the same period *P. nigra* was most severily attacked (25.7 %), while *P. sylvestris* and *P. strobus* were attacked to a much lesser degree (2.7 % and 1.5 % respectively). In the case of plantations established on former agricultural land in Krynica, 25.9 % of the *P. sylvestris* and 35.2 % of the *P. strobus* were attacked (Kowalski & Domanski 1983). The damages due to this disease in southern Poland was much smaller than that in the north. On pines planted in the open area mainly the current year shoots or whole branches, especially in the bottom part of the crown were dying (Fig. 8). On older shoots cankerous wounds were formed (Fig. 9). Only single trees died in such plantations. On the other hand, mortality was high in case of *P. nigra* planted under the canopy of old pine stands or in their immidiate vicinity. The high susceptibility of *P. nigra* to attack of *G. abietina* is of major concern because this tree species has been introduced as a species tolerant to industrial air pollution. Analysis of the attack by *G. abietina* on *P. nigra* was carried out during 1985-1986, i.e. 2-3 years after the end of the epidemic. In 45 stands aged 4 to 70 years in Upper Silesia, the 11 to 25 -year-old of age stands were most severely attacked (67 % of trees with symptoms on the average). The shoots in the lower part of the crowns were affected in $^2/_3$ of the case, and the number of dead shoots did not exceed 25 %.

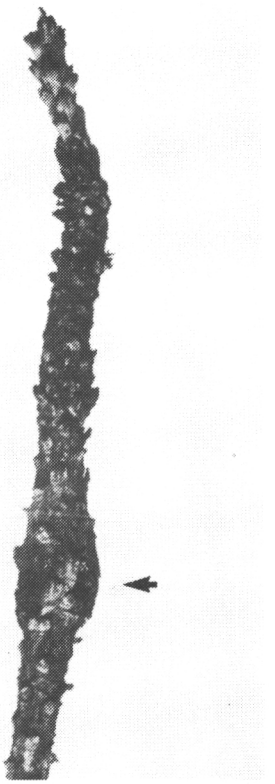


Fig. 7. Symptoms on 2-year-old shoot of *Pinus nigra* infected with *Sarea resinae*.

This fungus thus did not threaten the life of the trees on the whole. However, an extremely large reservoir of infectious material of this pathogen was maintained on the shoots (Kowalski 1987b). The symptoms and extent of damage in northern Poland suggest that the European race of the pathogen had appeared there, while the symptoms in the southern part of the country were more characteristic for the American race (Dorworth 1981). More investigations are required on this aspect (Butin & Kowalski 1984).

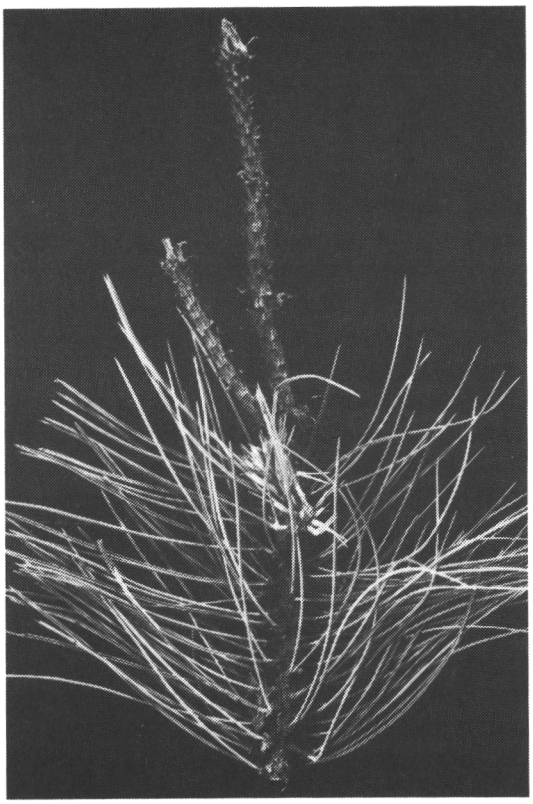


Fig. 8. 1-year-old shoots of *Pinus nigra* with symptoms of *Gremmeniella abietina* infection.

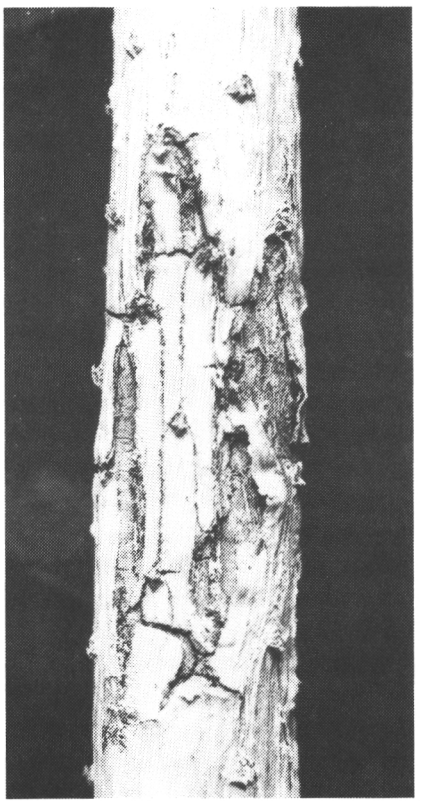


Fig. 9. Cankerous wound on 2-year-old shoot of *Pinus sylvestris* caused by *Gremmeniella abietina*.

*Cenangium ferruginosum* Fr.: Fr.

*C. ferruginosum* in Poland, similarly as in other countries, occurs commonly on branches which are dying, as a result of insufficient light (Butin & Kowalski 1990), and on branches in the slash left after logging. It is also sometimes found quite numerously in infection spots on the living needles of *P. sylvestris* (Kowalski 1982b and 1987a, Kowalski & Lang 1983). In the beginning of the present century it was accepted as a cause of epidemic dieback of pine twigs which, however, was a result of the erroneous acceptance by Schwarz (1895) that *Brunchorstia pinea* (= *B. destruens* Eriks.) is a conidial stage of *C. ferruginosum*.

Contemporary opinion on the role of this pathogen is not uniform. Some authors consider it to be a saprophyte, others make it responsible for the dieback of pine shoots, especially in the presence of certain factors weakening the trees (review by Donaubauer 1974). In Poland, Łukomski (1968) worked on the causes of pine shoot dieback during 1959-1960. He accepted *C. ferruginosum* as the causal agent of this disease.

During the present investigations, two different situations showing evidence of parasitic activity by *C. ferruginosum* on pine were encountered. In one of them *C. ferruginosum* turned out to be a secondary pathogen. It frequently (Table 4) attacked the lower part of those shoots whose tops were attacked by *Gremmeniella abietina* and, on

moving downward, caused the dieback of older shoots. Also adventitious shoots, produced at the base of shoots killed initially by *G. abietina*, were dying (Fig. 10).

Other cases indicated that *C. ferruginosum* may be a primary pathogen. It infected 2-year-old shoots on branches free of other pathogens. In the initial stage of the disease the necrotic spots appeared only on these shoots. The needles on these 2-year-old shoots were also changing colour, starting from their bases. Later on 1-year-old shoots were dying as a result of secondary infections. Such activity of *C. ferruginosum* was observed in young as well as in older (about 70 years of age) stands. These field observations agree with results of artificial infections conducted by Smerlis (1973). He obtained positive results on various species of pine and spruce, and negative ones on *Abies*, *Juniperus*, and *Larix*.

*Scoleconectria cucurbitula* (Tode: Fr.) Booth
Anamorph: *Zythiostroma pinastri* (Karst.) Höhn.

It is frequently a saprophyte on branches which have died as a result of insufficient light (Butin & Kowalski 1990), and on slash (Kowalski 1988). The fungus is only sporadic in the necrotic spots on needles of *P. sylvestris* (Kowalski 1982b and 1987a). It frequently occurred saprophytically on shoots killed by *Gremmeniella abietina* especially on *P. sylvestris* (Table 4). Sometimes, similarly to *Cenangium ferruginosum*, it infected the lower part of the shoots, the apices of which were attacked by *G. abietina*, and it could have contributed to the death of longer sections of shoots (Fig. 10). Only a few cases were registered indicating that *S. cucurbitula* could alone, similarly to *G. abietina*, kill the current year shoots of *P. sylvestris* on which shortly after death, orange pycnidia (*Zythiostroma pinastri*) appeared on the needle scars and between the scales on long shoots.




Fig. 10. Crown of 5-year-old *Pinus nigra* (growing under the canopy of a 90-year-old stand of *P. sylvestris*) which died as a result of the disease initiated by *Gremmeniella abietina*: a) shoots killed by *G. abietina*, b) adventitious shoots, c) shoots which died due to the infection caused by secondary pathogens (such as *Cenangium ferruginosum*, *Scoleconectria cucurbitula*, rarely *Tympanis pinastri* or *Valsa pini*).

## Other fungi

During 1979-1986 a total of 31 species of fungi were found on the stems and branches of infected and recently died *Pinus sylvestris* and *P. nigra* in the plots where infection by *Gremmeniella abietina* dominated, excluding those stands where *M. pinitorqua* was a main species or where strangulation at the stem's base mainly occurred. Only the species having some connection with stem or branch diseases have been characterized in the above. The frequency of those species and the remaining ones that usually secondarily colonized the organs killed by other pathogens, is given in Table 4.

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# DISEASES OF SCOTS PINE IN FINLAND

Diseases of Scots pine in Finland are mainly the same as those to be found, e.g., in Central Europe. The northern boreal forests have also some special problems. Of course, some diseases which are harmful for Scots pine in its southern range of occurrence are lacking in Finland.

In the nurseries, we have damping off caused by *Rhizoctonia* spp. and *Pythium* spp. These groups of fungi can also cause root rot at a later stage in the growth of seedlings. Scleroderris canker (*Gremmeniella abietina*) has caused the most serious losses in the production of Scots pine seedlings over the last 20 years. Needle cast (*Lophodermium seditiosum*) is temporarily harmful (Kurkela & Lilja 1983). Snow blight (*Phacidium infestans*) causes a considerable loss of seedlings in the absence of chemical control (Jamalainen 1961).

The most important root disease in Finnish Scots pine forests is *Heterobasidion annosum*. It occurs abundantly on pines in southeastern Finland (Laine 1976). The P-group of the fungus is specialized on pines, while the S-group seems to be less serious, although it is common in young pine stands growing on previous spruce sites (Korhonen 1978a). *Armillaria obscura* (species C; Korhonen 1978b) kills young pines, but the fungus is rare and occurs only on dry esker sites in southern Finland. *Rhizina undulata* is a common root rot fungus on pine seedlings regenerated artificially or naturally after prescribed burning or wild fires (Laine 1968).

As in the nurseries, *Gremmeniella abietina* has also been the most serious and problematic disease agent in Scots pine forests in Finland since the 1960's (Aalto-Kallonen & Kurkela 1985, Uotila 1985). During the 1980's several thousands of hectars of Scots pine stands have had to be clearcut, and the area of stands with retarded growth is even greater. In certain years, *G. abietina* can be found in almost every Scots pine stand.

*Phacidium coniferarum* infects pruning wounds if pruning of Scots pine is done in late autumn (Räisänen et al. 1986). *Lachnellula pini* causes cankers at the base of young pines mainly on dry heathlands but only in northern Finland (Kurkela & Norokorpi 1979). Needle casts, *Lophodermium seditiosum* and *Lophodermella sulcigena* cause temporary epidemics in young stands (Kurkela 1979a, Jalkanen 1986). Although these diseases are not a threat to pine forests, they can cause considerable growth losses. Another important needle disease on Scots pine in the northern forests is snow blight. In addition to the nurseries, it kills small pines specially in eastern and northern Finland (Rummukainen 1971) where the snow cover is thick almost every winter.

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Other frequently occurring diseases of Scots pine are twisting rust (*Melampsora pinitorqua*), and resin top caused by *Cronartium flaccidum* or *Peridermium pini.*

The effects of air pollution in Finnish forests have not been as crucial as in some parts of Central Europe. Of course, acidic deposition continuously affects our trees and forest soils. In some industrial areas pines are badly affected. Owing to the northern position of Finland, the naturally adverse climatic factors can damage Scots pine. For example, summer frost appears to make pines more susceptible to infection by *G. abietina.* Heavy summer frost can also kill the current growing shoots of pines. Winter drought is common in pines growing at higher altitudes and close to the boreal limit of pine forest. On peatlands an imbalanced mineral nutrient status makes pines more susceptible to the effects of cold weather (Kurkela 1979b).

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# FUNGI HAVING AN IMPACT ON SCOTS PINE

## ABSTRACT

Investigations have been carried out on the spread, biology and effects of some fungi on Scots pine in Bulgaria. The most important fungi are *Lophodermium pinastri* complex (*L. pinastri*, *L. seditiosum* and *L. conigenum*), *Melampsora pinitorqua*, *Cronartium flaccidum* and *Heterobasidion annosum*.

## INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is one of the main tree species in Bulgarian forests, occupying 49.6 % of the coniferous forest area. The climatic conditions in Bulgaria, as well as the biological characteristics and ecological demands of this species, have resulted in Scots pine being one of the fastest growing and most productive species (after *P. peuce* Gris.) in our country.

Average timber volumes for higher age classes reach up to 450 solid cubic meters/ha, and the mean annual increment is more than 4 $m^3$/ha for age classes II - IV. As a result of intensive forest management, the percentage of Scots pine forest areas has increased every year and is now 54.8 % of the total area of all coniferous plantations. This demanded a serious consideration of the health conidition of this species, as well as considerations of its variety in form , the disease resistance of particular provenances etc.

The more important fungi having an impact on Scots pine have been studied in our country. Following their identification and investigation, and development of control methods, their effects have been limited to certain levels. However, the changes in the ecological situation in Bulgaria, as elsewhere in Europe, have aggravated the physiological condition of the forest ecosystem. It has been necessary to continue investigating the state of health of the forest ecosystems, under these new conditions.

## *LOPHODERMIUM* SP.

*Lophodermium* fungi have considerable impacts on the production of planting materials in forest nurseries, as well as on young stands. Investigations have shown that there are three species of this genus that parasitize the needles: *L. pinastri* (Schrad.) Chev., *L. seditiosum* Minter et al., and *L. conigenum* (Braunaud) Hilitz. These species have been observed on 1 - 2 % of the primary needles, and on 14-15 % of the secondary ones. The

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total spread of these fungi in Bulgaria, the broad range of their effects on young pine seedlings, as well as the difficulty of estimating them individually has meant that studies have had to be carried out on the three species complex simultaneously.

The studies that were carried out on the sporulation of *L. pinastri* complex during 1982-1985 showed that, under the conditions prevailing in Bulgaria, there were 2 periods of maximal sporulation (Petkov 1989). The first period occurred at about the end of May and the beginning of June. This was the period when the mean diurnal temperatures remained for a relatively longer time at about 15-16 °C, and humidity was about 70 %. A sudden decrease in humidity irrespective of favourable temperatures, could make sporulation cease. The second period of maximal sporulation was around the end of August and the beginning of September, when the average temperature during our studies was about 16 °C or more, but the relative humidity about 72 %. These maximal sporulation periods were only aproximate ones, and could occur two or three weeks earlier or later depending on the temperature and relative humidity. The results of the studies on the sporulation of *L. pinastri* complex agreed with those carried out by Kozlowska (1968), but differed considerably from the data reported from many other European countries.

As Scots pine is a important primary tree species, detailed studies were carried out on its resistance to the *L. pinastri* complex (Petkov 1988). Nine native Scots pine provenances that were growing in the Govedartsi ecological plantation at on altitudes of 1250 m were investigated.

Their resistances were determined in two ways, and later compared:

1. During the period 1983-1987 we made a macroscopic estimation scale of the sanitary condition of fallen needles, as well as the dead needles still attached in trees, according to provenances and particular individuals.
2. The buffering capacity (B.C.) of an extract of homogenized needles from the same trees was determined according to the method of Scholz and Stephan (1974).

The results were compared with the increment in height and diameter.

The macroscopic estimations and laboratory studies carried out showed that there were some differences in the susceptibility of the provenances to the disease. The provenances of higher altitudes (Malyovitza - 1700 m, Banderitsa - 1900 m, as well as Dospat - 1350 m, and Hvoyna - 1600 m) were found to be less vulnerable to *L. pinastri* complex under the conditions prevailing in the plantations. These same provenances could retain their needles for a longer period and, in most cases, showed insignificant symptoms of the disease up to the age of 3 or 4 years. According to the height and diameter increments, the provenances from Dospat, Hvoyna, Velingrad (1200 m alt.), and Velingrad (850 m alt.) were most productive under the conditions prevailing in the plantations. Correlation was found between the resistance of particular provenances determined by macroscopic estimation. The buffering capacity ($r = 0.68$) and pH ($r = 0.59$) of the needles were also determined. The buffering capacity appeared to change in two different ways for different provenances and for specific individuals. These changes provided considerable information about the behaviour of particular individuals or provenances each year, although some other features also had to be considered in order to specify resistance.

## *MELAMPSORA PINITORQUA*

Another serious problem associated with the production of planting material in forest nurseries, as well as with young forest plantations is pine twisting rust (*Melampsora pinitorqua* Rostr.). It has spread throughout almost the whole country where Scots pine is cultivated. More severe damage was reported by Dimitrof (1922, 1923) and Tsanova (1960, 1967) when the area of infected sites reached up to 7000 ha. The increase in this disease was connected with the increase in the areas of young pine plantations on sites where rapid invasion of the pioneer species *Populus tremula* had occurred. The rust was found to have developed less on seedlings with the largest increment, and that it had developed on the top and lateral shoots irrespective of whether the seedlings were growing singly, or in plantations.

The emergence of teliospores on the leaves of *P. tremula*, and the appearance of basidiospores in the spring (April), were directly dependent on the temperature and relative humidity of the air.

The *P. tremula* forms, most sensitive to the fungus were those with grey-white and grey-green bark, as well as young seedlings of the hybrids *P. tremula* × *P. tremuloides*, and *P. alba* × *P. tremula* (Tsanova 1967). In Bulgaria, *Melampsora pinitorqua* attacks both Scots and Austrian (*Pinus nigra* Arn.) pines. Observations indicated that the rate of infection of Scots pine ranged from 15 to 42 %, and of Austrian pine from 7 to 14 %. Scots pine was damaged the most, and nonrecoverable damage occurred on 9 to 26 % of the trees. The corresponding percentage for Austrian pine was 12 %. Studies on the resistance of middle mountain Scots pine, and low mountain Scots pine against this disease showed that inoculation resulted in infection of 37 to 72 % of the former trees, and 43 to 76 % of the latter. Nonrecoverable damage occurred on 9 to 13 % and 12 to 26 % of the trees, respectively. The disease decreased along with an increase in age. The climatic factors in spring were very important for the development of both the fungus and disease since they influenced the growth and development of both host and pest. These results were of both theoretical and practical importance in the afforestation with both Scots and Austrian pines that either contained the secondary host or were situated close to it.

## *CRONARTIUM FLACCIDUM*

The disease caused by the rust *Cronartium flaccidum* (Alb. et Schw.) Wint. (= *C. asclepiadeum* (Wild) Fr.) was less important. The effects of this fungus were reported in our country in 1961 by Vâtov (1961), who pointed out that the disease had spread over areas of 6 to 70 ha, and damaged 30 to 52 % of the trees. The fungus developed on the stems of 33 - 55 % of the trees, causing deformation and decline. Our observations made in Loukovit National Forest in 1987 showed that the fungus was a serious threat to some young Austrian pine stands (10 to 15 years old). In contrast to the results obtained in other countries, the studies carried out on *C. flaccidum* in Bulgaria showed that it mainly attacked Austrian pine, with little effect on Scots pine.

Conditions favoring the development of this fungus are: Austrian and Scots pine growing on poor and arid sites in monocultures within the oak forest belt (from 500 up to 700 m alt.), where herb hosts such as *Vincetoxicum officinale* Moench, *Pedicularis palustris* L., *Impatiens* sp. etc. are abundant. Sanitary felling, if carried out in time,

helps to limit the disease. Since about 12 % of the young pine stands grow on sites that are favourable for the development of this pathogen, more studies must be carried out on this disease.

## *HETEROBASIDION ANNOSUM*

The root fungus *Heterobasidion annosum* (Fr.) Bref. is the most important pathogen from the point of view of both the scientific and practical aspects of the health of Scots pine in Bulgaria. Its importance is determined by its propagation range, and by the tremendous amount of damage it causes. Only plantations become infected, while in natural pine forests its development is only saprophytic.

Studies carried out in man-made forests have shown that 53.3 % of the trees in Scots pine plantations are damaged, - 15 % in Austrian pine plantations, and 23 to 27.5 % in Macedonian pine (*Pinus peuce* Gris.) plantations. These figures are of course relative since fungal development is dependent on tree age, sites, site index, forestry activities etc.

The effect of stand age is significant. The fungus was most frequent in plantations ranging from 20 to 60 years of age. With this age range the groups of withered or dead trees formed centers covering several hectares each. Site quality is also very important. The studies showed that the impact was greatest and most severe on sites of indices $B_1$, $C_2$, $B_2$, and their intermediate forms. The fungus frequency was as high as 25 to 50 %. Pine plantations are dominant on these sites in our country. The fungus was less frequent (up to 25 %) on sites of indices $C_2$, $B_1$, $C_1$, and (up to 10 %) on sites of indices $B_1$, $A_1$, $A_2$, and their intermediate forms. On very moist or very arid sites the fungus occurred only on individual trees.

The relationships found between root fungus development, environmental conditions, and their changes, permitted direct commercial losses for the plantations to be determined. The losses were very different for age class II stands (20 to 40 years old) and for age class III stands (41 to 60 years old).

Withering in the age class II stands was from 5 to 26.5 % depending on the site type. This was almost the same as the proportion of trees that had died due to natural biological self-thinning. However, the pathogen did not only attack trees that had been suppressed, but also developed disease centres. In this case the damage represented an absolute loss of current increment of 0.54 to 2.57 $m^3$/ha for age class II, and 1.37 to 1.93 $m^3$/ha for age class III. Related to the normal current increment this loss was 4.9 to 23.3 %, and 27.9 to 32.7 % for the two age classes respectively.

This demanded development of a number of prophylactic measures designed to limit the propagation of the fungus and the losses caused by it.

## CONCLUSIONS

Scots pine is one of the main tree species in the forests of Bulgaria. Its state of health has a considerable impact on stand productivity and other forest functions (sheltering, protection etc.). The diseases caused by the *L. pinastri* complex, *M. pinitorqua*, *C. flaccidum* and *H. annosum* appeared to be the main factors determining the health of Scots pine forests in Bulgaria. The studies carried out on the biology, ecology, and

frequencies of these pathogens, on the damage they cause, and on the resistance of particular species and forms to them, have suggested measures for limiting their impact, and how to improve the condition of man-made forests in our country. Austrian pine has also been partially considered because it is a very important tree species for Bulgaria - it grows together with Scots pine, and can be used in recreating forests in regions situated at lower altitudes.

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# THE MAIN PINE DISEASES IN THE NETHERLANDS IN THE 1980'S

In the Netherlands a serious epidemic of *Sphaeropsis sapinea* occurred from 1982 to 1986 both in *Pinus sylvestris* and *P. nigra*. The epidemic was most serious in areas with high ammonium depositions originating from nearby intensive livestock farms. It was shown experimentally that an excess of ammonium-nitrogen induced a lower water potential in 5-year-old potted *P. nigra* var. *maritima* plants and that it caused *S. sapinea* to grow faster in those plants. It is likely, therefore, that the *Sphaeropsis* epidemic has been triggered by high ammonium depositions coinciding with dry summers.

An increased activity of *Armillaria* species has been observed. Also in this case drought might have been the predisposing factor. *Ascocalyx abietina* (anamorph: *Brunchorstia pinea*) caused an epidemic in 1981 and 1982 in *P. nigra*, after two severe winters. The epidemic stopped abruptly after the mild winter of 1982/1983. The epidemic for the first time occurred also in the southern part of the country. There is some concern about the possibility of increased frost sensitivity of *Pinus* spp. caused by excessive nitrogen depositions. This might render also *P. sylvestris* more susceptible to *Brunchorstia*. To date *P. sylvestris* has not been seriously attacked by *Brunchorstia* in the Netherlands.

Other pine diseases in the Netherlands are at a normal level; the main diseases being *Heterobasidion annosum*, *Lophodermium seditiosum* and *Endocronartium pini*.

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# MAIN DISEASES OF *PINUS SYLVESTRIS* L. IN ITALY

In Italy the distribution area of *Pinus sylvestris* L. covers some 45 000 ha. The tree grows mainly at lower and middle altitudes of the Alps, but isolated stands are also found in the northern Appennines.

Pure stands are mainly to be found in the Central Alps (South Tyrol) and in some parts of the moorlands and the Alps of Lombardy. In the Piedmontese Alps and the Maritime Alps, on the border with France, *P. sylvestris* almost always grows in mixed stands with larch and Norway spruce, while near the coast it sometimes grows with cluster pine, *P. pinaster*.

In the Alps, *P. sylvestris* mostly grows in naturally regenerating stands. In some areas, however, it is the species normally chosen for reforestation, especially where the soil conditions are not suitable for other species.

Observations made during the last 20 years have revealed that *P. sylvestris* is threatened by a number of parasitic fungi, particularly in reforestation and naturally regenerating stands. The most important are: *Brunchorstia pinea* (Karst.) Höhn., *Cenangium ferruginosum* Fr. ex Fr., *Heterobasidion annosum* (Fr.) Bref., *Lophodermium seditiosum* Minter et al., *Melampsora pinitorqua* Rostr., *Sclerophoma pithyophila* (Corda) Höhn., *Sphaeropsis sapinea* (Fr.) Diko and Sutton, (syn. *Diplodia pinea* (Desm.) Kickx.).

The damage caused by these fungi is conditioned by a number of factors, especially those related to climate. *B. pinea* has been recorded mainly in Alto Adige (South Tyrol) (Moriondo 1963, Barbacovi et al. 1979) where it usually attacks pine seedlings in naturally regenerated stands as well as places where Scots pines is growing outside its natural distribution area, usually above 1500 m a.s.l. Mature trees are attacked in valley bottoms or other humid areas. The situation is similar in the Appennines (Barbacovi et al. 1979, Capretti 1984).

*Lophodermium seditiosum*, too, requires a very humid environment. It occurs in the moorlands of Lombardy and in some nurseries in the Alps and the Appennines. It is particularly active during the tree's vegetative period. *L. seditiosum* infections have reached serious proportions on seedlings (below 1.5 m level) in some plantations.

Besides the needle discolouration caused by by *B. pinea*, some fungi cause needle cast on *P. sylvestris* growing in some central Alpine forests: they are *Hendersonia montana*, *Lophodermella* (*Hypodermella*) *sulcigena* (Moriondo 1963, Capretti et al. 1985) and *Naemacyclus minor*.

After an exceptionally dry winter and spring, *Cenangium ferruginosum*, *Sclerophoma pithyophila* and *Sphaeropsis sapinea* (Capretti 1956) sometimes cause

dieback in *P. sylvestris* (Biraghi 1963), particularily on the lateral branches of young trees.

More important than these, however, is *Melampsora pinitorqua*, which in the sixties and seventies caused epidemics on various pine species including *P. sylvestris*. This species was particularly affected in the naturally regenerated stands in Liguria (Longo et al. 1970, 1980). These authors suggest that *M. pinitorqua* emerges and spreads on *Populus tremula* in Italy only when the weather is favourable, with short humid spells in winter and spring.

During the last few years root rot fungi have been studied. Isolated attacks of *Heterobasidion annosum* (P group) against *P. sylvestris* have been recorded on a number of occasions in the Central Alps (Biraghi 1963, Capretti and Moriondo 1983, Capretti 1986, Korhonen et al. 1989).

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# DISEASES OF SCOTS PINE IN CANADA

## INTRODUCTION

Scots pine (= Scotch pine), *Pinus sylvestris* L., is one of the least demanding exotics introduced into Canada from Eurasia in the 19th century for ornamental use. It is now one of the most widely scattered pines in Canada.

Scots pine is not a major species, but it is recommended as a windbreak in Prairies because of its hardiness. It is also valued for its picturesque form and color among pines. It is one of the most popular pines to be used as Christmas trees in both eastern and western provinces. In some western provinces, it is also used for making kraft pulp. It has been accepted for reforestation on certain sites because of its hardiness and fast growth. Although there are several plantations of this species in different age groups in most provinces, none of them are of any major economic importance.

For this report, we did a survey of diseases noted in the Compendium of plant disease and decay fungi in Canada (Ginns 1986) and the Canadian Forestry Service Reports published annually on forest insect and disease conditions in Canada. To our knowledge, this is the first attempt to bring together all the Scots pine diseases in Canada.

## DISEASES OF SCOTS PINE

Although Scots pine is known as a hardy softwood species, it does fall prey to a large number of fungal enemies, most of which are not of major importance. As the objectiv of this paper is to have an overview of Scots pine diseases, we shall discuss only major disease problems of the species; other common disease problems are just listed.

**Shoot blight and canker.** Scleroderris canker, caused by *Gremmeniella abietina* (Lagerb.) Morelet, is one of the most important diseases of Scots pine in Canada. It was first reported on that species in 1968 at Valcartier (Quebec) and later on in Ontario, Nova Scotia, New Brunswick and Newfoundland. All these provinces are located in eastern Canada and in most cases, the disease was observed in Christmas tree plantations or on ornamental trees. Shoot symptoms and canker are more difficult to detect on Scots pine than on red pine, the most severely infected species in Canada. The disease was known to be present for at least 15 years in a plantation although old cankers were detected on the trunk when the 45 year old Scots pines began to die. Both

the North American and the European races of the fungus have been found on Scots pine in Canada; the latter one was identified for the first time in 1981, near the New York state and the Quebec border. The disease was responsible for the destruction of thousands of seedlings in tree nurseries. The disease is also known to lower the quality of the Christmas trees even though the infected trees are not necessarily killed. Management of the disease in some plantations infected with the European race was accomplished through complete destruction of the infected plantations so as to eliminate this race from the area. Both races, however, can easily be managed by pruning and sanitation when the rate of infected trees is low (Laflamme 1988).

**Twig blight.** Another disease, sometimes mistakenly identified as Scleroderris canker, is a twig blight caused by *Cenangium atropurpureum* Cash and Davids and *Cenangium ferruginosum* Fr. ex Fr. These two species are very difficult to differentiate. They do not cause any tree mortality but they are often associated with canker, branch and top mortality. They have been reported only in eastern Canada (Ontario and Quebec).

*Diplodia canker. Sphaeropsis sapinea* (Fr.) Dyko and Sutton (= *Diplodia pinea*) is a relatively new disease in Canada. It was first reported on Scots pine in 1970 in Lachute (Quebec). Damage and mortality caused by this fungus have also been reported since 1983 from southern Ontario. Scots pine and other exotic pines are apparently severely affected by this fungus.

* * *

*Caliciopsis pseudotsuga* Fitzp., *Cenangium acuum* Cke and Pk., *Lachnellula calyciformis* Willd. ex Fr., *Nectria cinnabarina* (Tode ex Fr.) Fr., *Scoleconectria cucurbitula* (Tode ex Fr.) Booth, *Sydowia polyspora* (Bref. and Tav.) E. Müller, *Tympanis confusa* Nyl., *T. hypopodia* Nyl. and *Valsa pini* have been reported on few occasions as fungi associated to canker or shoot blight.

**Rust fungi and needle cast.** Five rust species are causing gall or blister rust to the branches or the stem of Scots pine. They are: *Cronartium coleosporioides* Arth., *Cronartium comandrae* Pk., *Cronartium comptoniae* Arth., *Cronartium quercuum* (Berk.) Miyabe ex. Shirai, *Endocronartium harknessii* (J.P. Moore) Y. Hitarsuka. This last fungus is the gall rust most commonly found on *P. sylvestris* but it has caused mortality in few occasions only. Damages are usually reported in Christmas tree plantations. The other four rust species are seldom reported on *P. sylvestris*.

The needle rust, *Coleosporium asterum* (Diet.) Syd. is affecting pine needles throughout Canada without great damage; Scots pine is one of the numerous pine species affected by this disease. *Melampsora medusae* Thüm. is a poplar rust having many conifers species as alternate host. It has been successfully inoculated on *P. sylvestris*.

**Needle cast.** The following needle cast fungi have been identified on Scots pine needles in Canada, but none of them had caused great damage up to now. They are: *Cyclaneusma niveum* (Pers. ex. Fr.) Dicosmo, Peredo and Minter, *Lophodermella concolor* (Dearn.) Darker, *Lophodermium pinastri* (Schrad.) Chev., *Meloderma desmazierii* Duby) Darker and *Sclerophoma pithyophila* (Cda.) Höhn.

**Root rot.** Singh (1981) reported that *Armillaria* spp., then known as *A. mellea*, are known to reduce volume in Scots pine through tree mortality, wind throw and growth loss both as a primary and secondary pathogen. However, the damage is generally low and scattered. The fungus when in combination with root weevil is causing some initial damage, but is mainly predisposing the trees to infection by *Armillaria* (Singh 1981).

Scots pine is reported as a host species for *Heterobasidion annosum* (Fr.) Bref. only in the province of Ontario.

**Seedling diseases.** On Scots pine seedlings, the fungi known to cause a damping-off are: *Fusarium* sp., *Pythium debaryanum* Hesse and *Rhizoctonia solani* Kühn. The gray mold fungus (*Botrytis cinerea* Pers.) and the smothering fungus (*Thelephora terrestris* Ehr. ex Fr.) are affecting older seedlings.

**Decay and stain fungi.** These wood decay and wood discoloration fungi are reported on Scots pine: *Ceratocystis minor* (Hedgc.) Hunt, *Dichomitus squalens* (Karst.) D.Reid, *Fomitopsis cajanderi* Karst., *Fomitopsis rosea* (Alb. and Schw. ex Fr.) Karst., *Ganoderma applanatum* (Pers.) Pat., *Trichaptum abietinus* (Pers. ex. Fr.) Ryv., *Gloeophyllum sepiarium* (Wulf. ex. Fr.) Karst., *Onnia tomentosa* (Fr.) Karst., *Tyromyces sericeomollis* (Rom.) Bond. and Singer, *Poria subacida* (Pk.) Sacc., *Peniophora pini* (Burt.) Weres. and Gib. and *Sistotrema brinkmannii* (Bres.) Eriksson.

* * *

The following fungi have been classified as pathogens of *P. sylvestris* after artificial inoculation: *Biatorella resinae* (Fr.) Mudd., *Lachnellula aqassizii* (Berk. and Curt.) Dennis, *L. arida* (Phill.) Dennis, *L. gallica* (Karst. and Har.) Dennis, *L. occidentalis* (Hahn and Ayers) Dharne, *Nectria fuckeliana* var. *macrocarpa* (Wollenw.) Booth, *Potebniamyces coniferarum* (Hahn) Smerlis (= *Phacidiopycnis pseudotsugae*), *Pragmopara amphibola* Massal., *P. pithya* (Fr.: Fr.) Groves and *Valsa friesii* (Duby) Fckl. (Smerlis 1973).

In conclusion, many fungi have been identified as pathogens of Scots pine. Although few pathogens are very virulent, the relatively low number of Scots pine plantations reduce the importance of these diseases. Abiotic problems such as road side damages, snow and ice damages, winter drying, are often reported on different pine species, but they are not well enough documented on *P. sylvestris* to be analysed here. Before ending this report, we have to point out a devastating animal of Scots pine plantations: the porcupine (*Erthizon dorsatum* L.). In a plantations of mixed pine species for example, this animal chooses Scots pine first and causes tree mortality by eating the bark of the trees.

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# PATHOGENIC FUNGI ON *PINUS SYLVESTRIS* IN HOKKAIDO, JAPAN

The Tokyo University Forest, situated in Hokkaido, northern Japan (40-43° N), contains excellent natural stands and various trial plantations. The main coniferous species are *Abies sachalinensis* and *Picea jezoensis*. There are also many trial plantations of foreign tree species in the forest. *Pinus sylvestris* is one of the important tree species in the plantations. Planted *P. sylvestris* grows very quickly in the young stage. Southern variety, especially, has a similar growth rate to *Pinus strobus*. However, it suffers from vole damage. Voles eat the bark at the base of the pine stem under the snow and kill the pine. The density of mature standing trees is therefore low. More than 10 species of pathogenic fungi have been recorded during a 12-year survey (Saho and Takahashi 1973). No new findings of fungi have since been reported.

## PATHOGENIC FUNGI

The pathogenicity of the pathogens is denoted as (X) and (XX).

1. ***Armillaria mellea*** (XX)
   *A. mellea* has recently been reclassified in accordance with the new taxa, but this report is made under the name *A. mellea*. Susceptibility to *A. mellea* is the same as for *P. strobus*. Following heavy damage by voles, *A. mellea* invades Scots pine and kills it. Severe infection by a needle rust fungus, *Coleosporium phellodendri*, affects the growth of the pines and *A. mellea* subsequently kills them.
2. ***Cenangium acuum*** (X)
   The needles are affected, especially the young ones. In the heaviest cases of infection, almost all the needles carry fruiting bodies of the fungus. However, this fungus does not kill the pine.
3. ***Coleosporium campanulae*** (X)
   The needles are affected. In Hokkaido we have to cut back the ground vegetation before planting. Sasa, a small type of bamboo, is the dominant species in the ground vegetation. If we do not cut it back, the planted seedlings become completely covered. No plants can grow under sasa, because it is too dark, and sasa grows up to one meter or so in height within a year. Sasa therefore grows much quicker than the planted seedlings, and it is considered the most severe threat to plantations of all tree species. Weeding has to be done after planting, too. In general, weeding has to be done once or twice a year for at least three years.

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Weed control is an absolute necessity when establishing new plantations in Hokkaido.

On the next stage, *Campanula*, *Eupatorium*, *Petasites* and *Stellaria* are the dominant species in the ground vegetation instead of sasa. These species pose other problems to planted young seedlings. The main problem is rust fungi, of which *Coleosporium campanulae* is one.

4. ***Coleosporium phellodendri*** (XX)

The alternate host is a woody plant, *Phellodendron sachalinensis* (Saho 1963). The effective range of infection by the rust onto pines is more than 100 m from a tree to the seedlings. Seedlings planted close to naturally regenerated forest are commonly affected. After severe damage by the rust, *A. mellea* attacks and kills the pines.

5. ***Cronartiun quercuum*** (X)

This rust is common on pines. The small branches are most severely affected. The susceptibility appears to be about the same as for *Pinus densiflora*, a common Japanese red pine.

6. ***Naemacyclus niveus*** (X)

Damage caused by this fungus is not so severe. Last summer which was extremely dry, this fungus appeared on two-year-old needles.

7. ***Pezicula livida*** (X)

The lower side branches are affected. It appears to be a weak parasite on branches and twigs.

8. ***Scoleconectria cucurbitula*** (X)

The same as for *P. livida*.

9. ***Lachnellula calycina*** (XX)

This disease causes serious problems when the seedlings are planted in wet soil after snowmelt. The fungus is supposed to gain entry through wounds made by frost.

10. ***Tryblidiopsis pinastri*** (X)

Weakened twigs, mostly those broken by snow, are affected.

11. ***Tympanis hypopodia*** (X)

This is a weak parasite of pine. However, it occurs quite often on pruned and fallen branches lying on the ground. When side branches were debarked at the basal part to main stem, this fungus colonized these weakened twigs. Therefore, it appears to be a weak parasite.

12. ***Tympanis pithya*** (X)

The same as for *T. hypopodia*.

Of the 12 pathogens listed, *Armillaria mellea*, *Coleosporium phellodendri* and *Lachnellula calycina* are the most serious.

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# THE CURRENT SCLERODERRIS SITUATION IN EUROPE

## ABSTRACT

The ascomycete *Gremmeniella abietina* causes one of the most serious canker and dieback diseases of conifers in Europe, North America and Japan since about 20 to 30 years. The fungus is distributed over nearly whole Europe. Areas with extensive damage were and are still the coastal regions of Central Europe, higher elevations of the Alps and some regions in south and particularly north Scandinavia. In most European regions the Scleroderris situation has more or less stabilized, and epidemics occur only locally. Pine and spruce species are the main hosts. Most susceptible are *Pinus nigra*, *P. cembra*, and locally *P. contorta*. *P. sylvestris* and *Picea abies* are lesser attacked under normal conditions. *Gremmeniella abietina* is a variable organism, and several races or forms are distinguished. They differ in morphological, physiological and pathogenic characters. The epidemic outbreak of the disease is favoured by various environmental factors, which stress the host plant. In a final paragraph the need for future research work is indicated.

## INTRODUCTION

The Scleroderris canker and dieback disease is one of the most serious fungal diseases of conifers during the last 20 to 30 years. The causal organism is the ascomycete *Gremmeniella abietina* (Lagerberg) Morelet (syn. *Scleroderris lagerbergii* Gremmen, *Ascocalyx abietina* (Lagerberg) Schläpfer; anamorph: *Brunchorstia pinea* (Karsten) v.Höhnel). Although the fungus was already known for about 100 years, it became a significant dieback and canker disease since about 1965, when in the Netherlands and in northern Germany many Austrian black pine stands (*Pinus nigra* Arnold) were killed. In eastern North America a very severe epidemic occurrence of the so-called European strain of *G. abietina* was observed since 1972, mainly in red pine stands (*Pinus resinosa* Aiton), but also on other conifers. And on the north Japanese island Hokkaido the fungus caused extensive damage in native Sachalin fir stands (*Abies sachalinensis* (Fr. Schmidt) Mast.) since about 1970. The causes of the sudden outbreak of Scleroderris epidemics in temperate conifer forests of different parts of the world during the last 25 years are still unknown. Fungus, disease symptoms, epidemiology and control measures were studied in many investigations. More than 85 % of all papers on the Scleroderris canker disease were published since about 1965 (Stephan & Schulze 1987, Stephan

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1988). The present-day knowledge about Scleroderris research can be found in the proceedings of Scleroderris meetings (Manion 1984, Donaubauer & Stephan 1988). In the following some remarks on the current situation in Europe are given.

## DISTRIBUTION OF *GREMMENIELLA ABIETINA*

The fungus is distributed over nearly whole Europe, and can be found from France and Great Britain to the northwestern Soviet Union, and from northern Turkey, Bulgaria, northern Greece and northern Italy to northern Scandinavia and even to Iceland (Fig. 1). In some countries *G. abietina* is relatively rare and known from only a few locations, e.g. in France, east Germany, Poland or Hungary. In other countries it is a frequent fungus in many pine and spruce stands, e.g. in the Netherlands, in western Germany, Austria or in Scandinavia. The frequency does not correlate necessarily with the economic significance of the Scleroderris disease in the respective countries or areas. The disease is also widespread in relation to the elevation and occurs from sea level (e.g. in the Netherlands and in northern Germany) to over 2000 m in the Austrian and Swiss Alps. In general, the Scleroderris disease can develop an epidemic particularly in maritime climates and in higher elevations with cool and wet summer.

## HOST RANGE OF *GREMMENIELLA ABIETINA*

*Gremmeniella abietina* has been reported from 47 species within 7 genera of the conifer family Pinaceae. Detailed lists are given by Donaubauer (1972), Dorworth (1971) and Stephan (1970). With the exception of *Abies sachalinensis* and a few *Picea* species, which were attacked up to now only in Japan and North America respectively, the other pine and spruce species and also Douglas fir are known as hosts of *G. abietina* in Europe. Among the hosts *Pinus nigra* is the most susceptible one. But sometimes also other species are heavily attacked by *G. abietina*, so e.g. *Pinus cembra* in the Alps at higher elevations or the introduced north American *Pinus contorta* in northern Sweden. Sometimes attacked, but less susceptible are *Pinus sylvestris* and *Picea abies*. The latter one is mostly damaged when grown in the understory of diseased Scots pine stands. The damage of the many other hosts is limited mostly to few locations. With respect to the exotic species the Scleroderris canker disease is restricted to small groups or individual trees in collections, arboreta or provenance trials. In general, it can be stated that heavy attack occurs when species or provenances are planted outside its natural range, or when not adapted populations are used. This is presumably the case with *Pinus cembra*, where material from lower elevations was taken for afforestations at higher elevations (Donaubauer 1984). The most susceptible stage of the trees is in the nursery and in plantation age up to 30 years.

There are differences in the resistance or susceptibility to *G. abietina* between and within host species (Roll-Hansen 1972, Stephan et al. 1984, Dietrichson & Solheim 1987). Resistance seems to be correlated with specific physiological and/or biochemical traits, and is obviously controlled genetically.

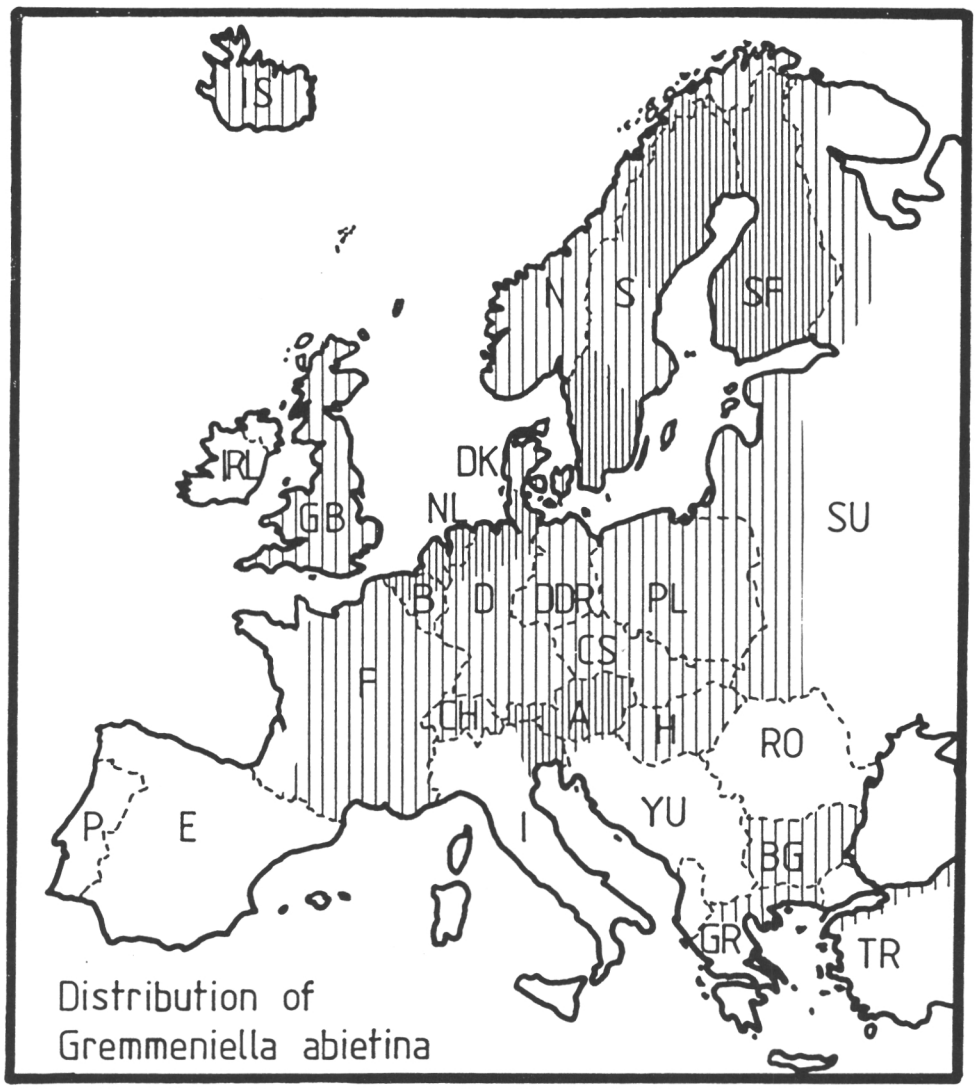



Fig. 1. Distribution of *Gremmeniella abietina* in Europe (hatched area). Regions with extensive damage by the Scleroderris disease are hatched narrowly. State of 1989.

## VARIATION OF *GREMMENIELLA ABIETINA*

The fungus shows a great variation in relation to its sexual and asexual form. Apparently, there occur in Europe two fungal types which differ in the frequency of apothecia formation: Apothecia can be found abundantly in the Alps at higher elevations and in Scandinavia with exception of southwest Norway. On the other hand apothecia are rare in Italy, France, Great Britain and Germany. Whether these differences are governed genetically, or whether apothecia are more common in areas

with a continental climate, is not yet clear. Such differences are also known from the two main fungal strains in North America, where the so-called north American strain produces abundant apothecia, whereas they are rare in the European strain.

In Europe, the fungus is also known in two different anamorphous forms based on the size and the number of septa of the conidia. The most common form has shorter conidia with an average of 3 septa. This form can be found on most of the hosts. The other form has larger conidia with 5 to 7 septa and is called *Brunchorstia pinea* var. *cembrae* (Morelet 1980). It occurs on fewer host species, mainly on *Pinus cembra* in the Alps (Donaubauer 1974), but is also distributed on *Pinus sylvestris* throughout Finland (Uotila 1983). In Switzerland both anamorphous forms can occur on the same host and even on the same twig (Heiniger & Kanzler 1988). The average number of septa of conidia is likely to be determined genetically, because the same type of conidia is produced in nature as on artificial medium (Uotila 1983).

There are known three continentally disjuncted races of *G. abietina* in North America, East Asia and Europe, which differ in morphological, biochemical and cultural characters as well as in pathogenicity to various hosts and in symptom expression. But one can also find pronounced differences in virulence among isolates from different geographical areas in Europe and from different hosts (Donaubauer 1974, Capretti & Dorworth 1988). So far, it has not yet been studied in detail, if the morphological distinct anamorph *Brunchorstia pinea* forms differ also in pathogenicity.

## ENVIRONMENTAL FACTORS AND DISEASE

The influence of various environmental factors was object of many investigations (see e.g. papers in Manion 1984, and in Donaubauer & Stephan 1988, Uotila 1988, Barklund 1989). Summarizing one can state that cool weather, long periods of rainfall and low light intensities favour spore release and germination, host colonization and at least disease development. The latent phase changes over to symptom expression during onset of flushing. Stress by frost or damage by voles during winter resulted in higher attack of Scots pine seedlings or *Pinus contorta*, respectively, by *G. abietina* (Petäistö & Repo 1988, Karlman 1988, and this volume).

There are also pronounced site effects on the epidemiology of *G. abietina*. So the disease is in general most serious when the trees are shaded (Gremmen 1965, Butin & Hackelberg 1978, Gibbs 1984). The disease is typical for dense *Pinus nigra* stands, although also free standing trees can be affected. Very interesting is the behaviour of *G. abietina* during its long latent or symptomless phase in pine or spruce shoots. Twigs of apparently healthy trees of these host species are inhabited by a number of endophytic fungi (Barklund 1989), which are normally not pathogenic. An examination of the relationship between the endophytic mycoflora and *G. abietina* showed that the higher the number of endophytes was and the more optimal the growth conditions for them were, the lesser was the symptom expression by *G. abietina* (Barklund 1989). Worser growth conditions for both the host plant and the endophytes favoured *G. abietina*, which seems to be more tolerant to environmental stress. In this connection *G. abietina* is obviously more tolerant also to acid precipitation than the other endophytes, which results in greater Scleroderris disease damage in stands polluted by immissions (Barklund 1989). Donaubauer (1984) did not find this relation for damage in *Pinus cembra* and ozone concentrations in the Austrian Alps.

## NEED FOR FUTURE RESEARCH

Although we have a good knowledge on the Scleroderris disease of conifers and its causing pathogen *Gremmeniella abietina*, particularly in Europe, many questions are still open. The solution of many problems can be done in future only by a close cooperation between pathologists, physiologists and scientists of other fields. There are still gaps of knowledge on the biology of *G. abietina* and its life cycle in various parts of Europe. Of great significance and help for future work will be studies on population genetics of the fungus and studies in the laboratory. First results in this relation are given by studies of Uotila (this volume) who analyzed monospore cultures of *G. abietina*. It will also be necessary to stimulate studies on the resistance of host plants, on substances involved, and on the inheritance of resistance by crossing experiments. Here also in vitro experiments can be helpful (Skilling et al. 1988).

Often the results of infection experiments of various authors can not be compared, because they used different material and methods. Therefore, a standardization of methods, particularly for inoculation experiments, is necessary. And in this connection the interaction between host and fungus, as well as the interaction, competition or antagonism between *G. abietina* and natural occurring endophytes are worth to be investigated thoroughly.

Finally, there is still a lack of knowledge on the causes of the increase of the Scleroderris disease during the last decades. Is it a fault of changes in the forestry management or of pollution effects or of any other causes?

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# *GREMMENIELLA ABIETINA* IN SWEDEN: HISTORICAL BACKGROUND AND SYMPTOMATOLOGY OF THE DISEASE

## INTRODUCTION

There has recently been an increase in interest in the dieback diasease caused by *Gremmeniella abietina* (Lagerb.) Morelet in conifers. Much of this concern stems from the fact that serious outbreaks of this disease in Scots pine (*Pinus sylvestris* L.) occurred in Sweden in 1981, 1985, and 1988 (Barklund 1988). Stands of all ages were damaged, but the disease was most prevalent in stands 20 - 40 years old. It is well established that this pathogen tends to attack the same stands repeatedly, whenever weather conditions are favourable. In 1988, the most extensive damage yet to be reported in Sweden was apparently the result of a cool, rainy summer followed by a warm winter (Barklund 1988). There is also great concern regarding the heavy attacks of *G. abietina* in young stands of the introduced lodgepole pine, *Pinus contorta* Dougl. ex Loudon, which have caused considerable mortality (Karlman 1987).

Although *G. abietina* usually only attacks Norway spruce (*Picea abies*) growing in the understory of Scots pine stands, widespread dieback occurred in pure, young stands of Norway spruce in 1977 (Barklund and Rowe 1981, Barklund 1983). Since then, similar types of attacks have been noted sporadically, suggesting that infections in such stands had earlier been overlooked.

## DISTRIBUTION OF *G. ABIETINA*

The disease first drew attention in plantings of exotic trees in the latter part of the 19th century. Afforestation efforts in heathlands with Scots pine of German origin and *P. nigra* ssp. *nigra* failed because of attacks by *G. abietina* (Nilsson 1901).

In Norway spruce, top-dying caused by *G. abietina* was recorded at Kolleberga Kronopark in 1893, and Lagerberg (1913) wrote that top-dying in Norway spruce occurred occasionally, but that it was overlooked because of its sporadic appearance. In 1910-12 the disease occurred in southwestern Sweden on Norway spruce, mostly in understory trees in Scots pine stands, but no damage to Scots pine in the same stand was reported.

In heathlands in northern Sweden, Lagerberg (1912) described a common canker disease on stems of Scots pine. In contrast to the shoot dieback in southern Sweden, this disease showed a perennial character. The difference in symptoms possibly caused Lagerberg to wrongly identify the pathogen; in any case Roll-Hansen (1964) thoroughly

argued that *G. abietina* was the causal organism, as did Ettlinger (1945) and Kujala (1950).

Still, Lagerberg (1945) wrote that although *G. abietina* was a pathogen of *Pinus* species over wide areas of western Europe it was unlikely that the disease would cause considerable damage in Sweden, owing to the limited introduction of exotic pines in our forests. About 50 years after the first report of *G. abietina* killing Scots pine of German origin, heavy damage to such trees, which were by now 30-50 years old, occurred in the province of Småland (Kohh 1964). Kohh also reported that in southern Sweden native Scots pine, often in dense stands, was moderately damaged and that some damage also occurred on Norway spruce. In northern Sweden, also during the 1950's, the potential of *G. abietina* to damage Scots pine was demonstrated, again natural regeneration in heathlands was attacked (Kohh 1964). Moreover, during the 1950's, the fungus had killed about 75 million Scots pine seedlings in nurseries (Björkman 1959, Kohh 1964). These disasterous attacks led to the use of fungicides against *G. abietina*, which is still going on today.

Obviously it was long believed that *G. abietina* did not pose a threat to Scots pine. Björkman (1961) described the top-dying of Norway spruce in Sweden in an IUFRO meeting, wrote that pathologists had no reason to suspect that heavy damage occurred on *Pinus sylvestris*.

During 1967-69, Scots pine stands in southwestern Sweden were attacked, which resulted in an inventory being made by the Swedish Forest Survey (Ohlsson 1970 unpub.).

Top-dying in pure Norway spruce stands occurred in 1977 (Barklund and Rowe 1981) in about the same area in south and southwest of Sweden where Lagerberg (1913) had registered the disease in the understory of Scots pine stands. Top-dying in Norway spruce forming the understory of Scots pine stands is nowadays very common in the southern half of Sweden.

Lagerberg (1945) was of the opinion that as long as exotics were not planted on a large scale, *G. abietina* would not cause much damage in Sweden. However, the exotic *Pinus contorta* Dougl. ex Loudon has been extensively introduced into northern Sweden, and lately, about 50 million seedlings have been planted every year. Karlman (1981) thoroughly described the various potential threats to *P. contorta*, and a few years later she reported serious damage by *G. abietina* in southern and coastal provenances of *P. contorta* in stands less than 10-year-old (Karlman 1986). Aggressive attacks on *P. contorta* in 1987 (Karlman 1987) led to a change in the Swedish policy of planting *P. contorta*, and the earlier figure of 35 000 ha per year was reduced to about 27 000 ha per year as areas at high elevations or otherwise subjected to a severe climate were withdrawn. This change in policy was based on the finding that most damage occurred in these severe environments.

As has been the case for *P. contorta* the severity of attack on Scots pine has been increasing during the 1980's, but in contrast to *P. contorta*, most attacked Scots pine were more than 10 years old. In 1981 and in 1985, damage was noted by foresters in many parts of the country. As a consequence, an inventory, in the form of a questionnaire, was made, which showed that at least 40 stands of Scots pine in southern and middle Sweden were being attacked. Trees of all ages, but especially those in unthinned, 20 to 40-year-old stands were diseased (Fristrom 1988). In 1988, we witnessed the most extensive damage ever reported in the country. The high damage

levels were attributed to weather conditions that were unusually suitable for the pathogen (Barklund 1988). However, it still seems as if *G. abietina* disease has increased in both Scots pine and Norway spruce over the last 50 years. Owing to the lack of reliable records during much of this period, the validity of this assumption cannot be tested.

## SYMPTOMATOLOGY OF THE DISEASE CAUSED BY *G. ABIETINA* IN SWEDEN

Three types of diseases caused by *G. abietina* have been distinguished in Sweden.

One type occurs in nurseries and in newly planted areas, where Scots pine seedlings are killed (Björkman 1959, Kohh 1964). Norway spruce seedlings planted in the field have occasionally been reported showing the same disease symptoms (Roll-Hansen 1967), as exemplified by one severe outbreak that occurred in Jämtland in 1988 (Beyer-Ericson, pers. comm.). During early spring, needles on the diseased seedlings loosen and become pale, greyish-green. The disease can be detected at an early stage by pulling on the needles which will easily detach. In this way the planting of infected seedlings can be avoided. Such diseased seedlings soon show the typical symptoms: The needle bases on shoots developing the previous year turn brown, the buds die and no flushing occurs (Laflamme 1986). Snow cover probably creates suitable winter temperatures for disease development (Marosy and Patton 1988). Fungicide treatments are necessary to keep infected nurseries free from damage.

Another form of the disease is characterized by perennial cankers. The infection begins in a current shoot of Scots pine and proceeds into older parts of the branch, invading the stem, where a canker starts to develop. After a few years the tree may be girdled and killed (Roll-Hansen 1964; Kurkela and Norokorpi 1979). Girdling can take place at all heights in the tree, but it seems to occur typically in the lower branches of trees 1 - 2 m tall growing at high elevations in northern parts of the country, where snow can cover these low branches. In a number of studies, it has been concluded that snow cover can favour disease development (Kujala 1950, Wilson and Rudolph 1970, Dorworth 1971, Yokota 1975).

Apparently, in regions with severe winters, the temperature above the snow is below the developmental threshold of the fungus. In contrast, temperatures within the snow are during long time suitable for the development of the fungus.

The third type is characterized by shoot dieback. Most commonly, the current shoots of the lowest branches of pole-size are attacked. In these cases the fungus is probably taking part in the natural pruning process. Under certain conditions, for example, in dense stands, the attacks can lead to the development of somewhat shorter than average crowns. However, if weather conditions favour the fungus the dieback may spread over the entire tree, and trees of all ages can be attacked. Small cankers on the branches are common. Infections of this type may be perennial, and the canker disease can develop (Kurkela 1981). Top-dying of Norway spruce can also be included in this category (Barklund and Rowe 1981). The dieback is favoured by warm winters, helping to explain why this form of the disease is most common in the southern half of Sweden.

The disease development was favoured by ambient temperatures between -6 °C and +5 °C during the winter or by snow cover to maintain a similar temperature on branches within the snow (Marosy and Patton 1988). In infection experiments in

climate chamber, mycelial growth inside the shoot was faster at +5 °C than at +20 °C (Barklund and Unestam 1988). Thus, a relatively mild canopy temperature during the winter appears to favour disease development in the southern half of Sweden. On the other hand in the northern half of Sweden sustained snow cover might be a primary factor for disease to establish.

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# *GREMMENIELLA* INFECTION ON LODGEPOLE PINE IN NORTHERN SWEDEN

## ABSTRACT

Severe infection by *Gremmeniella abietina* has been recorded in conventional plantings of *Pinus contorta* at high elevations in northern Sweden. Since 1987 new areas have been infected 50-100 m below the National Board of Forestry's restricted altitude zone for the planting of *P. contorta*. Topographic depressions with poor cold air drainage, which were severely infected in 1987, have functioned as sources of infection and quickly spread the infection throughout the plantations. There is a strong correlation between the *Gremmeniella* infection and the temperature sum at the site. *Pinus contorta* that has been planted on fine-textured fertile soil, which formerly supported spruce, often have a high degree of *Gremmeniella* infection. However, in large areas in northern Sweden with a more favourable climate, *P. contorta* plantations are healthy and productive.

## INTRODUCTION

The planting program of lodgepole pine (*Pinus contorta* var. *latifolia*) in Sweden is evaluated every fifth year. During the past five-year period the total planting area has reached 35.000 ha per year and regeneration with lodgepole pine has, as previously only been permitted north of 60° N latitude - with a few exceptions (cf. Karlman 1981). So far more than 400.000 ha have been planted with lodgepole pine in northern Sweden. The introduction of this North American conifer species has been considered very successful - especially in the southern and central parts of northern Sweden. Even in the very north of Sweden, in areas with a severe climate, where regeneration with the indigenous conifer species had failed more than once, the young lodgepole pine plantations seemed surprisingly healthy and productive. In the early 80s the National Board of Forestry thus recommended planting of lodgepole pine especially in those areas in northern Sweden where a satisfactory regeneration with the native conifers was difficult to achieve.

In 1986, before the new decision, the National Board of Forestry and the Forest Service made surveys of the condition of a large number of conventional lodgepole pine plantations in northern Sweden. The results of their investigations seemed very promising at first. However, during 1987 a complementary study of pathogens was made (Karlman 1987) in the same plantations that the Forest Service investigated in

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1986, owing to the fact that the infection by *Gremmeniella abietina* (Lagerb.) Morelet in provenance and progeny tests - studied by the author since 1976 (Karlman 1984, 1986) - was considered alarming in 1986. During the summer of 1987 it was found that we had a similar situation in our high-altitude stands as in the progeny tests (Karlman 1987b, c). In November 1987 severe restrictions in the planting program of lodgepole pine were implemented by the National Board of Forestry. Up to 1990 the total planting area must not exceed 27.000 ha per year and the planting of lodgepole pine was forbidden for a two-year-period within 2.7 million ha of forest land in northwestern Sweden.

**A fast-growing exotic with a wide ecological amplitude.** *Pinus contorta* exhibits an unusually wide ecological amplitude with regard to climate. It is one of the most widely distributed pine species in the world, with native stands occurring from Baja California to the Central Yukon Territory and from sea-level on the Pacific Coast up to 3 900 m above sea-level in the Sierra Nevada. In its natural habitats populations of *Pinus contorta* are clearly adapted to tolerate widely different climatic conditions. Even in B.C. and the Yukon, the regional climate varies appreciably, depending on site altitude and distance from the coast. *Pinus contorta* has, however, been transferred 4-6 latitudes further north to other daylight conditions in northern Sweden.

**Trial and error - a question of correct provenance choice.** The first trials in Sweden were established in the early 20th century. In northern Sweden the oldest plantation of *Pinus contorta* dates from 1928. Initially it was not so easy to know where in Canada the most suitable provenances for northern Sweden were to be found - Umeå and Dawson City are situated at the same latitude (64° N). During the first years of plantation, provenances that originated too far south were chosen, which often resulted in a high frequency of damage. A few excellently growing old plantations with *Pinus contorta* of southern origin can, however, be found in northern Sweden as well. During the 70s it became clear that, in order to get a satisfactory result in the regeneration of lodgepole pine, it was necessary to use provenances of more northern origin. Recommendations for a provenance transfer of more northern Sweden were drawn up by Hagner and Fahlroth (1974), Remröd (1977), and the Natinal Board of Forestry (1979, 1987). These guidelines have later been revised by Lindgren et al. (1988). From a pathological point of view, the situation was under good control until 1987, when the first *Gremmeniella*-infected plantations were observed in high-altitude stands in northern Sweden (Karlman 1987 b).

## INCREASING *GREMMENIELLA* INFECTION

**Vole damage - a predisposing factor.** Up to 1986 parasitic fungi were only of minor concern in the provenance trials. Mainly southern provenances were infected, and the commercial plantings seemed healthy and productive. The most severe threat to *Pinus contorta* during this period was not from pathogens, but from vole damage (Hansson & Lavsund 1982, Karlman 1982, Hansson 1985). Experiences are similar in Norway (Roll-Hansen & Roll-Hansen 1977) and in Finland (Annila et al. 1983, Rousi 1983). Severe vole damage was recorded during the winters of 1977/78 and 1980/81 in northern Sweden. *Pinus contorta* has a great capacity to recover even from severe vole

attacks. A correlation has, however, been found between vole-damaged trees and *Gremmeniella* infection (Karlman 1984, 1987 b). The explanation is simple. A tree with a reduced vitality is often attacked by secondary pathogens. The wildlife researchers expected two other peaks in the vole population to occur during the winters of 1984/85 and 1987/88, but thanks to very extreme weather conditions the vole populations crashed during both winters. Thus *Pinus contorta* has been saved from more serious vole attacks during the 80s. Locally, severe damage has been recorded.

**Alarming results from progeny tests in 1986.** In the autumn of 1986 the increase in *Gremmeniella*-infection in the progeny tests was, however, considered alarming (Karlman unpublished data). In these trials, which were laid out in northern Sweden in 1976 - in high altitude areas with a severe climate, the most northern and hardy population samples of *Pinus contorta* were tested. By studying in detail every single tree within the trial plots from year to year, it was found that trees that were slightly infected in 1983 were severely infected or killed by *Gremmeniella abietina* three years later (Karlman 1987 b, Figs. 1 a,b). The logical conclusion was: If the most northern progenies of lodgepole pine could be infected by *Gremmeniella* in tests with exclusively northern material (no mixture of *Pinus contorta* of southern and northern origin to provide a typical infection buildup), then this infection could also happen in conventional plantings sooner or later. The Board of Forestry was so informed in 1986.

**A large scale survey in 1987.** A complementary large-scale survey of the condition of about 120 lodgepole pine plantations in northern Sweden was made in 1987 (Karlman 1987 b,c) - the same randomly selected plantations as those investigated by the Forest Service in 1986. Permanent sample plots were laid out in 1987 and followed up one year later. Furthermore, 80 lodgepole pine plantations in the province of Västerbotten (64-66° N) were studied in 1988, to investigate conditions of following the very extreme amounts of snow in that area during the winter of 1987/88. In 1988 45.000 trees were studied individually in 191 lodgepole pine plantations, along with an additional 20.000 trees in provenance and progeny trials.

Results from the survey in 1987 indicated that the investigated *Pinus contorta* plantations must have already been infected in 1986 because apothecia of *Gremmeniella* were observed on infected trees in 1987. The incidence of infection was greatest in high-altitude stands with a harsh climate in northern Sweden. The most severe infection was found in topographic depressions lacking cold air drainage (cf. Pomerleau 1971, Dorworth 1972, 1973, Luley & Manion 1984, Aalto-Kallonen & Kurkela 1985). Slopes having N and NW aspects were generally more infected than those with a southern aspect (cf. Reid 1966, 1968). North of 64° N latitude, the most northern provenances were not resistant enough. South of 64° N the incidence of infection was highest in *Pinus contorta* plantations of the more southern provenances.

**Alarming results from commercial plantations in 1988.** In 1988, the frequency of *Gremmeniella* was much higher than in 1987 in a great number of *Pinus contorta* plantations in northern Sweden. Severe damage was recorded in regeneration areas 50-100 m below the limit prescribed by the Board of Forestry in 1987. Infected plantations were also recorded closer to the coast. Infection was especially frequent in those parts of northern Sweden (64-66° N) that were subjected to an extreme amount of snow

during the winter of 1987/88. In areas with a more normal snow cover the infection had increased only slightly. It is, however, important to point out that most lodgepole pine plantations in areas in northern Sweden with a more favourable climate were healthy and productive in 1988.

**Introduced conifers susceptible.** It is more than 100 years since the first scientific papers about *Gremmeniella* were published in Europe (Karsten 1884, Brunchorst 1888). Since then the fungus has changed names several times. Some of them are *Crumenula abietina*, *Scleroderris lagerbergii*, *Lagerbergia abietina* and *Ascocalyx abietina*. According to Müller and Dorworth (1983) *Ascocalyx abietina* is just now the correct name. I prefer to use the better-known generic name *Gremmeniella* in this paper. In 1987 more than 600 papers had been published about *Gremmeniella* in the world (Stephan & Schulze 1987). The main theme in the European literature about *Gremmeniella* is the great susceptibility of introduced or transferred conifers. Even Brunchorst (1888) pointed out that introduced conifers (*Pinus nigra*, *P. mugo*) were more susceptible to *Crumenula* infection than the indigenous Scots pine. Severe infection was also recorded in southern Sweden on German provenances of Scots pine after the severe winter of 1952/53 (Björkman 1961, Kohh 1964). Stefansson (1957) described serious attacks by this patogen in 20- to 24-year-old plantations of lodgepole pine, growing at altitudes 600 m above sea level in northwestern Jämtland (64° 30'N). These heavy attacks were apparently related to the bad weather conditions in the summer of 1952 and to the severe winter of 1952/53. Plantations at lower altitudes were not infected.

Provenance choice is of utmost importance for the resistance of trees to parasitic fungi (cf. Karlman 1981, 1986). For the first plantations of Scots pine of the early 50s in Sweden, provenances from too far south were chosen. This resulted in severe weather damage in high-altitude stands in northern Sweden after a few unusually cold winters in the 50s and the early 60s (Eiche 1966). The weather-damaged trees were highly susceptible to *Gremmeniella* infection. The cankers on the stems were described as "strangulation damage" and the weather damage as "the Red Devil". Stem girdling and production of bark necrosis on trees 1-2 m high have been carefully described by Lagerberg (1912) and by Roll-Hansen (1964), who usually found girdling to occur at the base of the stem - often initiated by frost damage. Visible damage is, however, not necessary for successful infection (cf. Kurkela 1981).

**Favourable weather conditions - for parasitic fungi.** The most hardy provenances of lodgepole pine have been used north of 66° N. All plantations of these are not infected. As long as the plants are covered with snow the *Gremmeniella* infection is usually low. In 1987 the most severely infected trees were 1.5-2 m high. These were trees that had been exposed to the extreme weather conditions above the snow cover in high-altitude stands in northern Sweden. A significant correlation has been found between severe weather damage and *Gremmeniella* infection (Karlman 1984, 1986). The weather conditions during the past years (1984-88) were in fact very favourable for the spread of pathogens. Even our native conifers were infected by a lot of parasitic fungi in 1988. Scots pine in young plantations and in 30- to 40-year-old stands in southern and central Sweden was infected by *Lophodermium seditiosum* Minter et al. and *Gremmeniella abietina*, respectively (Barklund 1988). *Melampsora pinitorqua* Rostr., *Phacidium*

*infestans* Karst. and *Lachnellula pini* (Brunch.) Dennis were very frequent on Scots pine in northern Sweden and *Chrysomyxa abietis* (Wallr.) Unger similarly was on Norway spruce (*Picea abies* (L.) Karst.) - (Karlman 1988).

After four years with severely cold winters, cold rainy summers and great variations in temperature during November 1984-86 and in March 1987, a mild winter followed with an extremely deep snow cover in parts of the provinces of Västerbotten and Norrbotten. *Pinus contorta* is not adapted to such extreme snow conditions. Our most hardy lodgepole pine provenances derive their origin from southern and central Yukon, and have been transferred 4-6 degrees of latitude further north to other daylight conditions in northern Sweden. We know that the hardening process is controlled by photoperiod and temperature. The photoperiod differs markedly at 62° N compared to 67° N. The period of active growth is longer for southern provenances. Dormancy induction occurs later and therefor they are not able to develop a satisfactory hardiness during an autumn with poor hardening conditions. The first hard frost occurred in northern Sweden in August in 1987.

**The importance of snow.** In these northern parts of Sweden there is an oceanic influence on the climate. This may be compared with a dry very continental climate in the Yukon, where the snow is more powdery and lighter than that falling in most parts of northern Sweden. Adding to this adaptation problem, the root development of lodgepole pine is poor in relation to its rapid rate of early growth and its long needles tend to collect much ice and snow. The trees were bent over into the snow, which gave the fungus more favourable temperature and moisture conditions on such toppled and bent trees than obtained on upright straight trees.

A relationship has been observed between *Gremmeniella* infection and snow depth (Martin 1964, Yokota 1975, Yokota et al. 1974). In this respect Marosy and Patton's (1988) study of the effect of winter temperature and snow cover on the development of *Gremmeniella* infection on red pine (*Pinus resinosa* Ait.) seedlings in northern Wisconsin and Michigan is of great interest. They found that at least 50 days with an air temperature between -6 °C and +5 °C for the entire day was necessary for symptoms to develop. A deep snow cover enabled the fungus to colonize the host tissue independent of the ambient air temperature (cf. the results from northern Sweden).

**The importance of correct site choice.** On sites in northern Sweden where Scots pine has a high mortality, an incorrect choice of species is presumably a strongly contributing factor. Scots pine has often been planted on fine-textured fertile soils, where Norway spruce would have been the correct alternative (cf. Aalto-Kallonen & Kurkela 1985, Jalkanen 1987). This seems to hold for lodgepole pine as well - but damage on lodgepole pine will appear in a somewhat later stage of development than on Scots pine. Studies in Finland (Lähde 1974) suggest a relationship between good sites and *Gremmeniella* infection, which will increase on fertilized soils (Kurkela 1984, Kallio et al. 1985). *Pinus contorta* planted on former agricultural land is highly susceptible to infection (Kujala 1950, Donaubauer 1972, Karlman 1988 b).

**The effect of high temperature.** Like most other fungi *Gremmeniella* is sensitive to high temperatures (Skilling 1968, Sletten 1971, Dorworth 1972). In culture the growth of *Gremmeniella* has been found to be inhibited at temperatures between +18 °C and

+25 °C (Ettlinger 1945, Barklund 1989). Blenis et al. (1984) showed, however, that the survival of the fungus was more closely related to average daily temperature than to extreme daily temperature. Thus the fungus was able to survive daily exposure to +34 °C for 4 weeks on condition that the interceding average temperature was between +24 °C and +28 °C. Of course there is no guarantee, that the same results are achieved under field conditions (cf. Blenis et al. 1984).

The early summer of 1988 was unusually warm in Sweden, which resulted in increased vitality of the trees. The slightly infected lodgepole pine plantations may have recovered from the disease during that period. However, the winter of 1989 (from January to April) was exceptionally mild with temperatures just around zero, which presumably will favour disease development. *Pinus contorta* has been tested in northern Sweden during a five-year-period with very extreme weather conditions. The widespread *Gremmeniella* infection indicates that extensive use of this exotic still must be questioned on sites with a harsh climate in northern Sweden until unequivocal results of more long-term investigations are presented.

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# VARIATION IN UNIASCUS MONOASCOSPORE CULTURES OF *ASCOCALYX ABIETINA*

## ABSTRACT

Monoascospore cultures originated from a single ascus were isolated from 12 asci. These cultures were used in studying the growth of mycelium, the production of conidia, conidium type and pathogenicity in the phloem of Scots pine. A number of genetically identical pairs of uniascus monospore isolates were separated. Strains from southern Finland mainly produced 4 -celled conidia, and northern strains 4 to 8 -celled conidia. The southern strains were more pathogenic in phloem inoculations than the northern strains. Uniascus monoascospore isolation is a useful tool in studying the mating system or apothecia production of *Ascocalyx abietina*.

## INTRODUCTION

The variation among *Ascocalyx abietina* (Lagerb.) Schläpfer-Bernhard strains is wide (Dorworth & Krywienzyk 1975, Skilling 1981, Uotila 1983, Akimoto 1984). The fungus has been divided into races on the basis of disease symptoms and physiological characteristics (Skilling 1981). However, the way in which these characteristics are inherited has not been investigated. Another open question is the mating system of the fungus, which is difficult to study because the fungus does not produce apothecia in cultures. The purpose of this study was to investigate the variation among single-ascospore isolates of *Ascocalyx abietina*.

## MATERIAL AND METHODS

The apothecia were mainly collected in July in southern Finland, and in August in northern Finland. The samples were stored dry at 5 °C. Three strains were collected from young pine stands (I-A 1, J 1, Virtasalmi), and the others were collected from seedlings. One strain was collected from lodgepole pine (PCSI 1).

The apothecia were placed in drops of sterile water on the underside of the lid of a Petri dish containing malt extract agar medium. After incubation for 6-24 hours at 15 °C, distinct groups of eight ascospores originating from a single ascus were looked for (Fig. 1). The study material consists of monoascospore cultures isolated from 12 asci, from each of which at least 7 ascospores germinated (Table 1). Single ascospores were

separated according to the method of Korhonen and Hintikka (1980). The ascospores were transferred to a 1 % malt agar containing pine needle extract. The colony diameter of the original monoascospore cultures was measured at the age of 42 days. Then the mycelia were transeferred to 150 ml Erlenmeyer flasks containing autoclavized barley grains and milled pine needles. Conidia were produced in light at 15 °C after incubation for two to six weeks. One hundred conidia per strain were measured (length, septation). A strain was classified as the 4 to 8 -celled type if there was one or more conidia containing at last 7 cells (Uotila 1983). Conidia production was estimated semiquantitatively by counting lumps of conidia in the Erlenmeyer flasks.

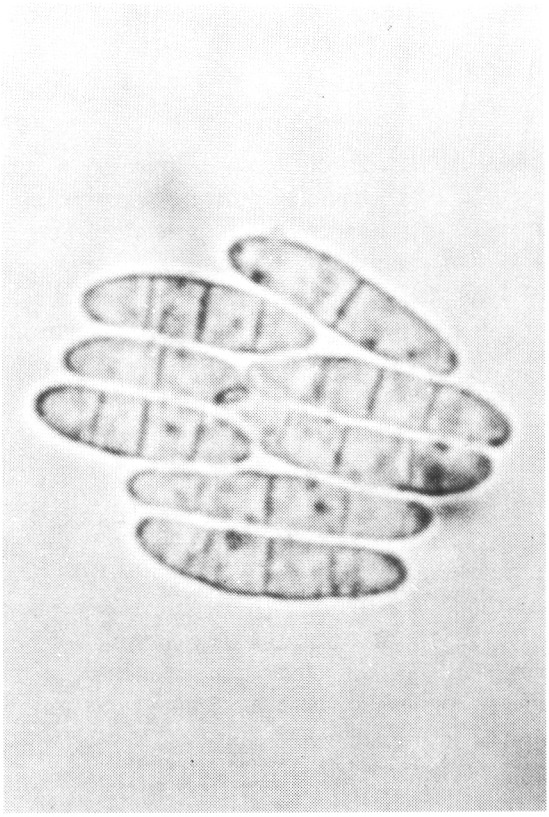


Fig. 1. Eight ascospores of *Ascocalyx abietina* ejaculated from a single ascus onto the agar surface. ×1900.

Two monoascospore cultures (Eno and Virtasalmi) were inoculated in the phloem of *Pinus sylvestris* (ten replicates) at Tuusula (grid reference N 670, E 39) once a month from April 1985 to March 1986 (Uotila 1989). In October 1987 31 monoascospore cultures from four asci were inoculated in the phloem of two pine stands (Padasjoki N 680, E 39 and Vilppula N 688, E 39). Two isolates (L 2.1 and L 2.2) had become contaminated during the 5-year storage period before inoculation. Each strain was

inoculated in 8 trees, and a control hole also bored in each tree. The bark was surface sterilized with ethylether. The size of the cankers was measured in June 1988.

Table 1. List of *Ascocalyx abietina* samples used in this study.

| Code | Place | Coordinates | Date |
|---|---|---|---|
| 1. I-A 1 | Kuru | E 31 N 688 | 1.7.1984 |
| 2. J 1 | Jämsä | E 40 N 686 | 20.8.1984 |
| 3. PCSI 1 | Ruovesi | E 35 N 686 | 14.7.1984 |
| 4. PSSI 1 | Ruovesi | E 35 N 686 | 14.7.1984 |
| 5. L 2 | Rovaniemi | E 48 N 735 | 27.9.1982 |
| 6. S 1 | Rovaniemi | E 48 N 736 | 14.6.1985 |
| 7. SU 2 | Suomussalmi | E 59 N 720 | 1985 |
| 8. PU 2 | Puolanka | E 53 N 719 | 8.9.1987 |
| 9. KIMA 1 | Rovaniemi | E 48 N 735 | 5.8.1986 |
| 10. KIMA 2 | Rovaniemi | E 48 N 735 | 5.8.1986 |
| 11. PASE 1 | Rovaniemi | E 49 N 736 | 4.8.1986 |
| 12. PAKU 1 | Posio | E 51 N 735 | 6.8.1986 |
| 13. Virtasalmi | | E 51 N 689 | 22.8.1984 |
| 14. Eno | | E 67 N 695 | 9.7.1984 |

## RESULTS

The growth rates of the uniascus ascospore cultures varied considerably (Table 2). The colour and the type of growth also varied. Genetically identical pairs resulting from mitotic division after meiosis appeared to be present in the monoascospore cultures of some asci. Vegetative incompatibility was visible in the pairings of some monoascospore cultures. However, the reaction was not always clear.

When the production of conidia was abundant, slimy, pink clumps of conidia were produced without pycnidium formation. The production of conidia varied greatly even among uniascus ascospore cultures (Table 2). For example, four of the monoascospore cultures in strain Paku 1 produced conidia abundantly, whereas the remaining three strains did not produce any conidia. The ability to produce conidia abundantly in culture was more common in the strains from northern Finland. The conidial type also varied among the monoascospore cultures originating from a single ascus (Table 2). There were both 4-celled and 4 to 8 -celled conidial types among the isolates from four asci. The southern strain usually represented a 4-celled type, and the northern strains a 4 to 8 -celled type. There were also differences in length of the conidia, and some of the strains produced deformed conidia.

Table 2. Mycelial growth (colony diameter) of *Ascocalyx abietina* on needle extract malt agar (42 days), the production of conidia (number of clumps per bottle), conidium type (number of cells), mean canker height in the phloem of artificially infected *Pinus sylvestris* trees, and the range of canker heights.

| Culture | Diameter mm | Conidial production | Type | Canker cm | Range cm |
|---|---|---|---|---|---|
| I-A 1.1 | 35 | 6 - 11 | 4 | 13.9 | 8.8 - 22.8 |
| 1.2 | 43 | 1 | 4 | 13.0 | 8.6 - 22.6 |
| 1.3 | 42 | 0 - 4 | 4 | 12.2 | 8.4 - 19.2 |
| 1.4 | 35 | 0 (+) | 4 | 13.6 | 7.8 - 22.5 |
| 1.5 | 39 | 3 - 16 | 4 | 12.7 | 7.3 - 22.5 |
| 1.6 | 37 | 0 - 1 | 4 | 12.5 | 7.1 - 18.5 |
| 1.7 | 42 | 0 (+) | 4 | 13.3 | 8.0 - 21.4 |
| 1.8 | 41 | 4 - 5 | 4 | 13.8 | 8.3 - 23.2 |
| J 1.1 | 44 | 0 (+) | 4 | | |
| 1.2 | 40 | 6 | 4 | | |
| 1.3 | 47 | 8 | 4 - 8 | | |
| 1.4 | 47 | 1 | 4 | | |
| 1.5 | 46 | 1 | 4 | | |
| 1.6 | 43 | 5 | 4 - 8 | | |
| 1.7 | 49 | 15 | 4 | | |
| 1.8 | 46 | 0 (+) | 4 | | |
| PCSI 1.1 | 45 | 3 - 5 | 4 | 14.0 | 8.2 - 28.6 |
| 1.2 | 40 | 1 - 20 | 4 | 10.2 | 5.5 - 15.7 |
| 1.3 | 42 | 90 - 100 | 4 | 13.1 | 9.2 - 16.8 |
| 1.4 | 41 | 3 - 30 | 4 | 17.6 | 9.0 - 28.9 |
| 1.5 | no germination | | | | |
| 1.6 | 45 | 90 - 100 | 4 | 14.6 | 8.0 - 22.1 |
| 1.7 | 42 | 1 | 4 | 12.2 | 7.0 - 20.5 |
| 1.8 | 41 | 0 (+) | 4 | 10.8 | 5.8 - 20.2 |
| PSSI 1.1 | 44 | 0 | | | |
| 1.2 | 35 | 1 - 3 | | | |
| 1.3 | 41 | 0 | | | |
| 1.4 | 40 | 0 | | | |
| 1.5 | no germination | | | | |
| 1.6 | 41 | 0 (+) | | | |
| 1.7 | 41 | 1 - 6 | | | |
| 1.8 | 33 | 10 - 50 | | | |
| L 2.1 | 8 | 0 - 1 | 4 - 8 | - | - |
| 2.2 | 32 | 25 - 34 | 4 - 8 | - | - |
| 2.3 | 34 | 2 - 4 | 4 - 8 | 10.9 | 6.9 - 16.2 |
| 2.4 | 34 | 2 - 3 | 4 - 8 | 9.4 | 6.4 - 12.5 |
| 2.5 | 39 | 25 - 29 | 4 - 8 | 8.2 | 4.4 - 13.4 |
| 2.6 | 37 | 1 - 12 | 4 - 8 | 11.7 | 6.7 - 25.2 |
| 2.7 | 34 | 3 - 6 | 4 - 8 | 3.8 | 0,5 - 6.5 |
| 2.8 | 31 | 2 - 25 | 4 - 8 | 8.7 | 6.5 - 12.5 |
| S 1.1 | 37 | 100 | 4 - 8 | | |
| 1.2 | 37 | 1 | 4 - 8 | | |
| 1.3 | 36 | 80 | 4 - 8 | | |
| 1.4 | 38 | 1 | 4 | | |
| 1.5 | 38 | 0 (+) | 4 | | |
| 1.6 | 38 | 0 - 2 | 4 - 8 | | |
| 1.7 | 38 | 80 | 4 - 8 | | |
| 1.8 | 39 | 100 | 4 - 8 | | |

Table 2. (cont.)

| Culture | Diameter mm | Conidial production | Type | Canker cm | Range cm |
|---|---|---|---|---|---|
| SU 2.1 | 33 | 90 | 4 - 8 | | |
| 2.2 | 35 | 100 | 4 - 8 | | |
| 2.3 | 44 | 90 | 4 - 8 | | |
| 2.4 | 55 | 80 - 100 | 4 - 8 | | |
| 2.5 | 50 | 70 - 90 | 4 - 8 | | |
| 2.6 | 31 | 70 | 4 - 8 | | |
| 2.7 | 30 | 40 - 60 | 4 - 8 | | |
| 2.8 | 44 | 90 | 4 - 8 | | |
| PU 2.1 | no germination | | | | |
| 2.2 | 42 | 0 | | | |
| 2.3 | 45 | 40 - 80 | | | |
| 2.4 | 35 | 90 | | | |
| 2.5 | 64 | 80 - 90 | | | |
| 2.6 | 61 | 80 - 90 | | | |
| 2.7 | 50 | 50 - 70 | | | |
| 2.8 | 44 | 90 | | | |
| KIMA 1.1 | 36 | 90 | 4 - 8 | | |
| 1.2 | 29 | 30 | 4 | | |
| 1.3 | 37 | 100 | 4 - 8 | | |
| 1.4 | 38 | 100 | 4 | | |
| 1.5 | 41 | 50 - 70 | 4 - 8 | | |
| 1.6 | 37 | 60 - 100 | 4 | | |
| 1.7 | no germination | | | | |
| 1.8 | 38 | 50 - 90 | 4 | | |
| KIMA 2.1 | 30 | 15 - 30 | 4 | | |
| 2.2 | 41 | 35 - 80 | 4 - 8 | | |
| 2.3 | 33 | 100 | 4 | | |
| 2.4 | 37 | 30 - 50 | 4 | | |
| 2.5 | 41 | 35 - 60 | 4 - 8 | | |
| 2.6 | no germination | | | | |
| 2.7 | 37 | 15 - 20 | 4 | | |
| 2.8 | 25 | 100 | 4 | | |
| PASE 1.1 | 44 | 50 - 60 | 4 - 8 | | |
| 1.2 | 49 | 60 - 80 | 4 - 8 | | |
| 1.3 | 46 | 60 | 4 - 8 | | |
| 1.4 | 46 | 90 | 4 - 8 | | |
| 1.5 | 48 | 50 - 70 | 4 - 8 | | |
| 1.6 | 49 | 5 | 4 - 8 | | |
| 1.7 | 50 | 8 - 20 | 4 - 8 | | |
| 1.8 | 48 | 90 | 4 - 8 | | |
| PAKU 1.1 | 18 | 0 | | 8.3 | 5.1 - 13.1 |
| 1.2 | 42 | 70 | 4 - 8 | 11.6 | 7.3 - 18.0 |
| 1.3 | 44 | 70 - 80 | 4 - 8 | 11.1 | 6.1 - 16.0 |
| 1.4 | 43 | 80 - 90 | 4 - 8 | 10.3 | 0.6 - 15.0 |
| 1.5 | 30 | 0 | | 11.7 | 8.8 - 15.0 |
| 1.6 | 59 | 80 - 90 | 4 - 8 | 11.5 | 6.8 - 18.8 |
| 1.7 | 17 | 0 | | 8.8 | 5.2 - 12.6 |
| 1.8 | 32 | 0 | | 10.8 | 7.3 - 18.1 |

It is possible to find some identical pairs of genotypes in Table 2. PAKU 1.1 and 1.7 represent identical genotypes on the basis of mycelium growth, lack of conidia production on the medium and canker height. Cultures J 1.3 and 1.6 are identical on the basis of conidial type. In this case one pair out of four is a 4 to 8 -celled type, which means that this characteristic is determined by at least two genes. The growth of monoascospore cultures in the phloem also varied. The southern strains (I-A 1 and PCSI 1) seem to be more pathogenic than the northern ones (L 2 and PAKU 1) (Table 2). The range of canker height was wide, but the largest values and means of the northern strains were smaller than those of the southern ones. Monoascospore cultures known to be identical pairs caused cankers of the same size.

In an inoculation experiment carried out at Tuusula the monoascospore isolate Virtasalmi, representing the southern strain, also caused cankers in the August and September inoculations, which the northern strain Eno only caused cankers in the dormant period inoculations (Fig. 2).

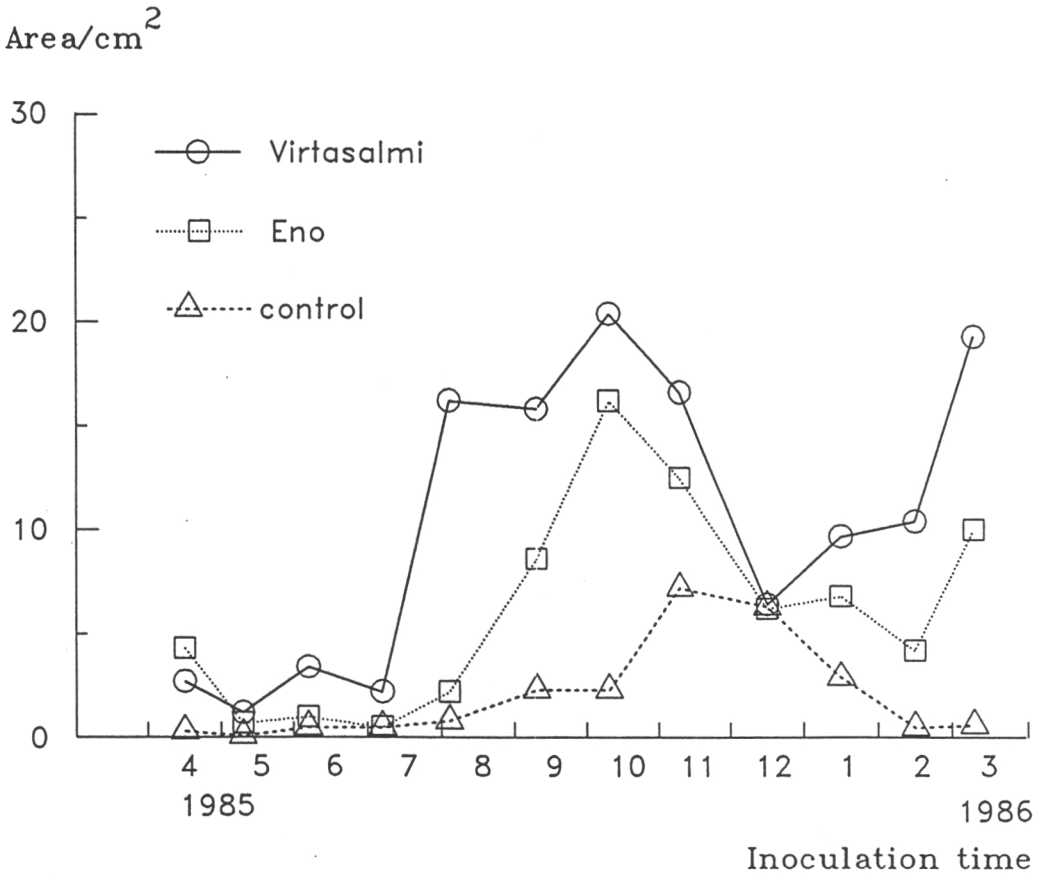



Fig. 2. Canker areas of two *Ascocalyx abietina* monoascospore isolates and controls at Tuusula. Mycelia were inoculated once a month in Scots pine phloem. The size of the cankers was measured in June 1986.

## DISCUSSION

Monoascospore isolation method used in this study is a cheap and easy means of studying the genetic variation of *Ascocalyx abietina*. Combined with biochemical methods, it can provide useful knowledge about the strains and races of this fungus. Hudler et al. (1984) observed that light intensity and temperature affected sporulation, but there were no differences among eight of his isolates. In this study the production of conidia clearly varied between the isolates. This discrepancy may be due to the different media used in the two studies. It can be assumed that, in nature, all strains produce conidia. Those isolates which do not produce conidia might lack the enzymes needed for the production of conidia on artificial medium.

The variation in conidia septation has been simplified into two types (4-celled and 4 to 8 -celled) in this study. In fact this could be a continuous distribution, which is quantitatively inherited. One possibile way of producing apothecia is to inoculate compatible mycelia in the phloem. However, this has not been attempted yet. The role of microconidia in the development of apothecia is not known, but microconidia could offer means of producing apothecia artificially.

In inoculation experiments with mycelium it is important to note that the differences in pathogenicity are not the same throughout the year. At the same inoculation time, the more pathogenic strains can be pathogenic, whereas the host can defend itself against less pathogenic strains. Further research is needed on the genetic variation of *Ascocalyx abietina*, because genetic variation could be one explanation for the different disease symptoms in southern and in northern Finland.

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# SUSCEPTIBILITY OF JACK PINE FROM QUEBEC SEED SOURCES TO SCLERODERRIS CANKER

A comparative plantation was established in 1983 to test for the susceptible of 41 *Pinus banksiana* Lamb. provenances from Québec to Scleroderris canker. These provenances were distributed between the 67° 11' and the 78° 56' of Longitude West and the 46° 20' and the 50° 11' of Latitude North.

Containerized seedlings were planted during the summer of 1983 in a young jack pine stand where the disease was present. A randomized complete block design was used. Each one of the 4 blocks contained 41 plots. Each plot consisted of 25 seedlings of the provenances assigned to that plot.

The two-year-old seedlings were artificially inoculated in 1984 and two years later in 1986 by exposing them to infected branches. None of the provenances tested were resistant to the disease but three levels of susceptibility were distinguished based on the rate of infection after three years. Eleven provenances, all from the northeastern part of Québec, were identified as the least susceptible. The rate of infection was significantly related to the geographical origin of the provenances suggesting a clinal variation for the susceptibility of *P. banksiana* to Scleroderris canker.

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# CLIMATIC FACTORS CAUSING ASCOSPORES RELEASE OF *GREMMENIELLA ABIETINA* VAR. *BALSAMEA*

## ABSTRACT

The objectives of this study were: 1) to measure the sporulation of *G. abietina* on an hourly basis, 2) to measure climatic factors such as rain, relative humidity, air temperature and leaf wetness, on an hourly basis, 3) to analyse these observations in regard with climatic factors.

## MATERIAL AND METHODS

The study area was located in the Réserve faunique des Laurentides, one hundred km north of Québec City, Québec (Canada). The infected tree was a black spruce (*Picea mariana* (Mill.) B.S.P.) situated in an 20-year-old natural stand composed of black spruce and larch (boreal forest region).

*Gremmeniella abietina* var. *balsamea* Petrini et al. (1989) was chosen because of its late starting period of ascospore release (Smerlis 1968). Young apothecia were located late June in 1986. Mature fruiting bodies were first observed on July 9. The instrumentation was installed on July 11 and the recording started at 16.00 hr. The sampling area was not accessible after September 5, which was the closing date at 12.00 hr. Data have been recorded every hour for 1339 hours.

A Burkard's 7-days volumetric spore trap was installed with its opening 30 cm away from apothecia and oriented in their direction. The adhesive-coated tape was treated as recommended by the Burkard Manufacturing Co. it was changed every week and brought to the laboratory. The tape was then cut in seven bands (one per day) and tapes were mounted on microscope slides, stained with lactophenol cotton blue, covered with a 24×50 mm microscope cover glass and identified (date and starting hour of trapping). Later on, spores were counted on the entire surface inside a two mm bands for each of the 24 hours of the day. This method was used instead of partial counting because the apothecia were in the vicinity of the trap and spores were not completely dispersed in the air: many 8-ascospores bundles were observed which could have biased the data. A Polyvar light microscope with a magnification of 400× was used for spore counting.

A data logger (Easy Logger Recording System from Omnidata International) was used to record the following parameters on an hourly basis: air temperature (°C) and relative humidity (%) measured in a shelter (1.5 m from the ground) and in the crown of a spruce (0.5 m from the ground) next to the infected one, leaf wetness (%) inside the

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crown of the same spruce and in an open area (0.5 meter from the ground), and rain (mm) in an open area. The instrumentation was located in a radius of two meters from the infected spruce. Temperature and relative humidity were measured with a TH 15 Physchem Temperature and Relative Humidity Probe; a LWS 10 Leaf Wetness Sensor and a tipping bucket rain gauge were used to record leafwetness and rain. Except for rain, the parameters were measured every 5 minutes and the means were recorded every hour.

**Three types of events were defined.** 1 - A sporulation event: at least three consecutive hours of sporulation with a minimum of 25 ascospores (more than 2 ascus) per hour. 2 - A non-sporulation event: at least three hours without any ascospore. 3 - An undetermined event: less than three consecutive hours, or less than 25 ascospores per hour for any time period.

## RESULTS

**Sporulation period.** Few ascospores were captured the first and the last days of the studied period. Smerlis (1968), using coated slides as spore traps, noted a period of sporulation extending from the second week of July to the second week of October. This is in accordance with our observations.

The peak of sporulation was observed during the first three weeks of August when more than 1000 ascospores per hour were recorded 5 times: July 31, August 6, August 9, August 11, August 21. Smerlis (1968) reported only the first week of August as the peak period of sporulation. This difference could be attributed to differences in climatic conditions between the two studies.

**Comparison of parameters.** Relative humidity and temperature measurements made in a shelter did not differ significantly (T test, $p=0.28$ and 0.31) from those made in the crown of the tree. The Canadian standard measurements of relative humidity and temperature are carried out in a shelter at 1.5 m from the ground. For this reason, measurements in the shelter were used for statistical analysis. There was no significant difference (T test, $p=0.04$) between leaf wetness measured inside and outside the crown. As there is no standard for leaf wetness measurement and as usually apothecia are located on defoliated shoots in the outer crown, we thought that leafwetness measured in open air was appropriate. This measurement was used for statistical analysis.

When all measurements were considered (1339 hours), we observed that the strongest correlation coefficient was between leaf wetness and sporulation ($r=0.63$, $p<0.001$). There was also a positive correlation between sporulation and relative humidity ($r=0.25$, $p<0.001$), and between sporulation and rain ($r=0.17$, $p< 0.001$). Temperature and sporulation were not significantly correlated ($r=0.05$, $p=0.08$).

A total of 140 events were identified (Table 1); the 25 sporulation events and the 46 non-sporulation events were used for statistical analysis. They represent 954 hrs or 71 % of the total duration of the study, and 51 % of the total number of events. The mean of each climatic variable was computed for each event. These means were then used to obtain the event type means (Table 2). There was a significant difference (T test, $p<0.001$) between leaf wetness recorded during the sporulation events (68.1 %) and the non-sporulation events (12.9 %) (Table 2). Rain and humidity were significantly

higher during the sporulation events as compared to the non-sporulation events (T test, p=0.001 and p<0.001). There was no significant difference (T test, p=0.98) in temperature between the sporulation and the non-sporulation events. Using the means of each event, high positive correlations were observed between sporulation and leaf wetness (r=0.63, p<0.001), rain (r=0.39, p=0.006), and relative humidity (r=0.47, p<0.001). Sporulation and temperature were not significantly correlated (r=0.1, p=0.43).

Table 1. Frequency and duration of events

| | Frequency | | | Duration (hours) | | |
|---|---|---|---|---|---|---|
| Event type | No | % | Total | Mean (*) | Range | % |
| Sporulation | 25 | 17.9 | 250 | 10.0 (1.3) | 3-34 | 18.7 |
| Non-sporul. | 46 | 32.8 | 704 | 15.3 (1.9) | 3-62 | 52.6 |
| Undetermined | 69 | 49.3 | 385 | 5.6 (0.7) | 1-29 | 28.7 |
| Total | 140 | 100 | 1339 | 9.6 (0.8) | 1-62 | 100 |

(*) Standard error of the mean.

Table 2. Climatic variable means of the sporulation and non-sporulation events. ()=standard error of the mean.

| | Climatic variables | | | |
|---|---|---|---|---|
| Event type | Temperature (°C) | Rain* (mm) | Leafwetness* (%) | Relative Humidity* (%) |
| Sporulation (n=25) | 12.5 (0.6) | 1.1 (0.4) | 68.1 (2.3) | 90.7 (0.5) |
| Non-sporulation (n=46) | 12.4 (0.7) | 0.1 (0.1) | 12.9 (2.5) | 76.8 (1.3) |

* Significantly different at the 0.05 level (T test)

**Leaf wetness.** Leaf wetness was the best indicator of sporulation for *G. abietina* var. *balsamea*. Even if the sensor was not designed to measure leaf wetness on conifer foliage, it did perform quite well. Leaf wetness was more usefull than the other climatic factors because in most cases, non-sporulation events were clearly distinguished from sporulation events. For example, on July 20, leaf wetness readings were 0 % at 15.00 hr (the end of a non-sporulation event) and 84 % at 16.00 hr (the beginning of a sporulation event). For the same hours, relative humidity was respectively 85 % to 88 %, and no rain was recorded.

**Rain, dew, and drizzling.** We found a strong correlation between ascospore release and rain but correlation between ascospore release and leaf wetness was stronger. In many occasions (Figure 1) no rain was recorded during sporulation events while leaf wetness readings were high due to free water deposition. Dew or drizzling may have been responsible for this free water deposition.

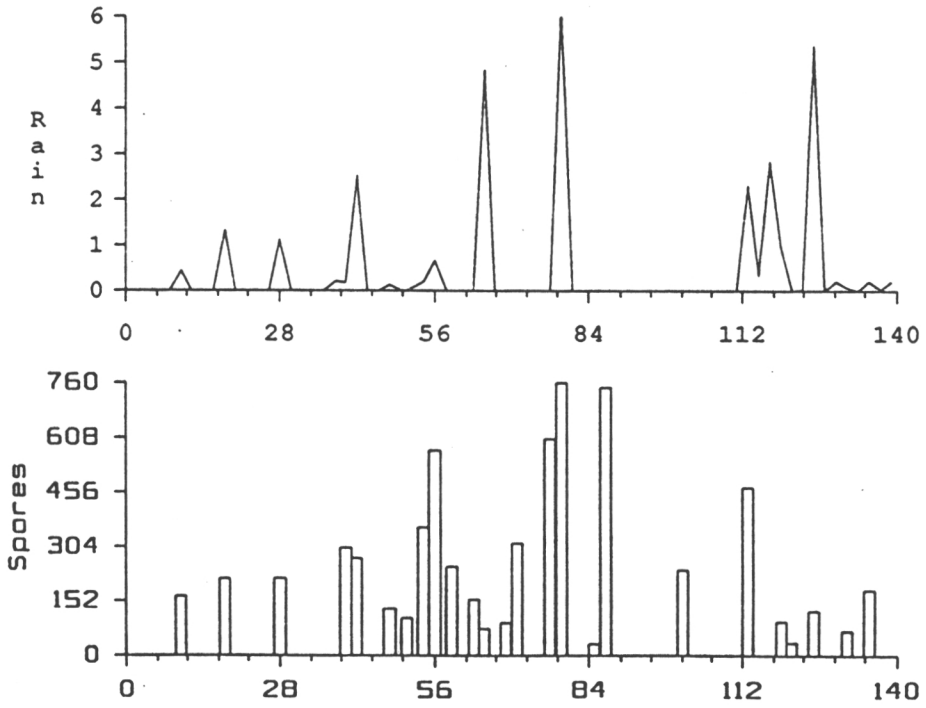



Figure 1. Number of spores and quantity of rain (mm) for each event.

Table 3. Dew pattern on leaf wetness readings (July 23-24)

| Time | L.W. | Rain | Spores |
|---|---|---|---|
| 22.00 | 0 | 0 | 0 |
| 23.00 | 2 | 0 | 0 |
| 24.00 | 3 | 0 | 0 |
| 01.00 | 6 | 0 | 0 |
| 02.00 | 8 | 0 | 0 |
| 03.00 | 15 | 0 | 0 |
| 04.00 | 20 | 0 | 0 |
| 05.00 | 24 | 0 | 0 |
| 06.00 | 28 | 0 | 0 |
| 07.00 | 36 | 0 | 0 |
| 08.00 | 25 | 0 | 0 |
| 09.00 | 19 | 0 | 0 |

On data sheets, dew patterns could be recognized by an increase in leaf wetness late at night with a maximum reading between 3.00 and 5.00 hours and leaf wetness reading of approximately 0 between 7.00 and 9.00 hr (Table 3). Eleven nights with such dew patterns have been identified; no sporulation event was recorded during these nights. It seems from these observations that dew was not responsible for ascospores release.

Drizzling is most probably the cause of free water deposition on apothecia when no rain is recorded. Drizzling was not measured directly but could be inferred from leaf wetness readings. On August 11, for example, a sporulation event was characterized by four hours with more than 1000 ascospores per hour, without any rain recorded. For this period leaf wetness readings were 74 %.

In two occasions, we observed a recovery of the apothecia in its capability to produce ascospores in less than two days. For example, August 9, an important rain caused the production of a large quantity of spores. After 14 hours of important sporulation, including 4 hours with more than 1000 ascospores per hour, rain continued (12 mm per hour for 5 consecutive hours) with practically no sporulation, indicating a run out of spore. Twenty eight hours after the end of sporulation, a new important sporulation event started.

**Relative humidity.** We did not find that relative humidity was a good predictor of sporulation. Many non-sporulation events were recorded under very high relative humidity. In fact, high relative humidity does not necessarily mean that free water will be deposited on foliage. It would be the case if it reached 100 % but we never recorded this value. The sensors do not perform well at the upper end of the scale. Therefore, the lack of accuracy of the sensor may be partly responsible for the weak relation between sporulation and relative humidity.

**Temperature.** Sporulation occurred in a wide range of temperature, from -1.0 to 26 °C, but sporulation of more than 100 ascospores per hour was limited when temperature varied from 7 to 16 °C. Air temperature was not a factor acting directly on sporulation. Air temperature over 0 °C could contribute to the maturation of ascospores, but not to the sporulation itself.

## CONCLUSION

1 - Rain and drizzling (free water deposition) are responsible for sporulation of mature ascus.
2 - Dew is not responsible for important sporulation.
3 - Temperature has no direct effect on sporulation.
4 - Relative humidity sensor was not efficient over 95 %.
5 - Leaf wetness is the best reading to identify sporulation event.

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# HOW ENVIRONMENTAL FACTORS AFFECT *BRUNCHORSTIA PINEA* (KARST.) HÖHN. INFECTION ON PINES

## INTRODUCTION

After a series of serious attacks of *Brunchorstia pinea* (Karst.) Höhn. on a number of pine species (Biraghi 1963, Moriondo 1963, 1967, Barbacovi et al. 1979, Capretti 1984), our Institute decided to study the diffusion and patogenicity of this fungus. During 1983 to 1988 various conifer species were inoculated with a number of *Brunchorstia pinea* isolates under a variety of environmental conditions in order to test the susceptibility / resistance of these species.

The present study is a continuation of this research project, and examines how variation in light exposure affect *B. pinea* inoculations on pines.

## MATERIALS AND METHODS

A). The first test described here, was carried out on ca. 8-year-old *Pinus nigra* trees growing in a nursery in the Apennine Mountains. The trees were inoculated with a suspension of 100,000 conidia/ml of the *B. pinea* isolate I-8 (Capretti & Dorworth 1988). Half the trees in the study were kept in constant shade from May 15 to October 15, and the other half kept in constant light.

Groups of 20 trees were inoculated on four occasions during their growing period: May 30, June 15, July 19 and August 23, 1984. Each trial was carried out on a group of shaded trees and a group of exposed trees. There were 3 replications of each trial, and a control group for shaded trees and one for exposed trees.

Inoculation was made by spraying conidia that had been dispersed in water with 0.1 % agar.

The trees were examined the following spring. Infections were judged positive when the characteristic discolouration of the needle bases could be seen. Terminal shoot infections and lateral meristem infections were recorded separately.

B). A second test was carried out in June 1984 on seedlings also grown in the nursery. The inoculation procedure was the same as that in Test 1. Groups of 30 trees were given doses of *B. pinea* conidia varying from 100,000 (1) to 5,000,000 (6) conidia/ml (0 = control).

Each trial was carried out on 30 exposed trees and 30 shaded trees. There were three replications in each trial.

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## RESULTS AND CONCLUSIONS

In general, inoculations on the main shoot of young *P. nigra* individuals are more often successful when the trees are dominated than when they are free growing and their main shoots fully exposed to light.

The predisposing effect of shade was evident in the first test on trees that were artificially shaded with netting.

On the lateral branches, however, the difference between shaded and exposed trees was much less pronounced because these branches tended to receive some shade even when the trees were exposed to light.

The trials show that the best time for inoculation in the Apennine region is during the first half of June, when the trees have completed their height growth but the shoots are not yet lignified.

Here too, the infection rates were higher on shaded trees (73.3 %) than on exposed ones (28.3 %).

The second test revealed that increasing the conidial dose had a clear effect on the shaded trees, on both the main shoots and the lateral shoots. On the exposed trees, in contrast, increasing the dose increased the number of infections somewhat, but not enough to reach statistical significance.

The trials confirm field observations that *Brunchorstia pinea* is more frequent on pine plantations with a northerly exposure that receive less light than on those with a south only exposure. The highest infection rates of all occurred on the crowns of shaded trees.

It also seems clear that the spreading of *Brunchorstia pinea* is inhibited by the sub-mediterranean climate of the Apennines: the higher temperatures and lower precipitation during the July-August period inhibit both the diffusion of the inoculum to new sites and the successful infections.

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# THE EPIPHYTOTIC OCCURRENCE OF *GREMMENIELLA ABIETINA* IN SCOTS PINE FORESTS OF NORTHERN POLAND DURING 1982-1984

A mass outbreak of needle browning and the dying of apical buds, followed by the death of all the trees, was observed on young Scots pine stands in spring 1982. The first symptoms of abnormal growth occurred on the trees after the hard winter of 1979/1980 and the wet autumn of 1981. This phenomenon was observed for the first time in the Lipusz Forest District in autumn 1981, and in the Kościerzyna Forest District in spring 1982.

In July, 1982 an unknown epidemic disease spread extensively over areas primarily consisting of stands of age 10 to 40 years. In 1982 the disease was estimated to cover approximately 1100 ha, and by 1983 it had affected up to 24400 ha. In 1982 the occurrence of the disease was only estimated in two forest districts, and in 1983 it spread with varying intensity over the whole area of Scots pine forest belonging to the Gdańsk Regional District .

In 1984 the activity of the disease ceased and it did not spread over new areas. After these three years, more than four hundred hectars of young stands of Scots pine were removed in whole area of the Regional District of Gdańsk.

**Symptoms and diagnosis of the disease.** The dying of pines began from the top of shoots (Fig.1). It was preceded by resin flow from the buds in late autumn. A longitudinal cut revealed that the buds were brown inside. In the spring the affected trees lost their needles, and the top buds and shoots died. New shoots frequently developed from secondary buds on the affected trees (Fig.2). Sometimes small cankers were found on the shoots and stems (Fig.3).

Following studies carried out by scientists from the Forest Research Institute in autumn 1982, the pathogen was identified as *Gremmeniella abietina*. Two species of Acarina were also found on trees killed by the fungus: *Oligonychus ununguis* and *Diapterobates oblongus*. The Scots pine forest areas were inspected several times. The weakened stands were later attacked by insects e.g. *Blastophagus piniperda*, *Blastophagus minor* and *Pissodes* sp. As soon as the disease was fully diagnosed, foresters from Gdansk planned control measures against the disease. Information was also provided about the scale of the disease and the estimated damage (Table 1).



Fig. 1. 12-year-old trees heavily attacked by *Gremmeniella abietina* in spring 1983.



Fig. 2. Regeneration of new shoots from secondary buds on 12-year-old trees in autumn 1983.



Fig.3. Canker on the shoot of an infected tree in 1983.

Table 1. Results of four inspections on young Scots pine stands severely damaged by *Gremmeniella abietina* in the Gdansk Regional District.

| Inspection dates | Area affected in % | | | | Total |
|---|---|---|---|---|---|
| | 5 % | 6% - 30% | 30% - 60% | over 60 % | |
| | ha | | | | |
| 1982.12.31 | 364 | 300 | 337 | 232 | 1121 |
| 1983.03.30 | 785 | 1437 | 301 | 143 | 2666 |
| 1983.06.30 17731 | 7032 | 9053 | 1219 | 406 | |
| 1983.10.30 24481 | 9888 | 10808 | 3122 | 609 | |

Several silvicultural methods were undertaken to control the disease in the areas with stands most severely damaged by the fungus . The trees were removed from totally affected stands . Individual dead trees were also cut from the less affected stands. All cut branches from the diseased trees were burnt or sprayed with lime sulphur and carbolic acid. In the afforested areas the new planted stands consist mostly of seedlings of *Betula* sp., *Quercus rubra*, *Prunus padus*, and *Tilia cordata*.

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# INOCULATION OF SCOTS PINE SEEDLINGS WITH *ASCOCALYX ABIETINA* DURING A SIMULATED GROWING SEASON

Scots pine seedlings were inoculated with *Ascocalyx abietina* during a simulated growing season. The effect of inoculation time and frost stress on the result of inoculation was studied.

The photon flux density during the growing season was 90 mol $m^{-2}s^{-1}$. The day length was regulated to correspond to the natural day length. The summer temperature was about +12.5 °C and the temperature sum of the growing season 928 d.d. (Fig. 1). The relative humidity was 85 - 100 %, and the shoots were moistened by a mist blower during the experiment. The winter was three months long (temperature 0 - 1 °C).

The frost hardiness ($LT_{-10\ m}$, °C) was measured during the growing season and at the beginning of winter (Fig. 1). The method is based on the difference in impedance of the shoots after and before the frost treatment.

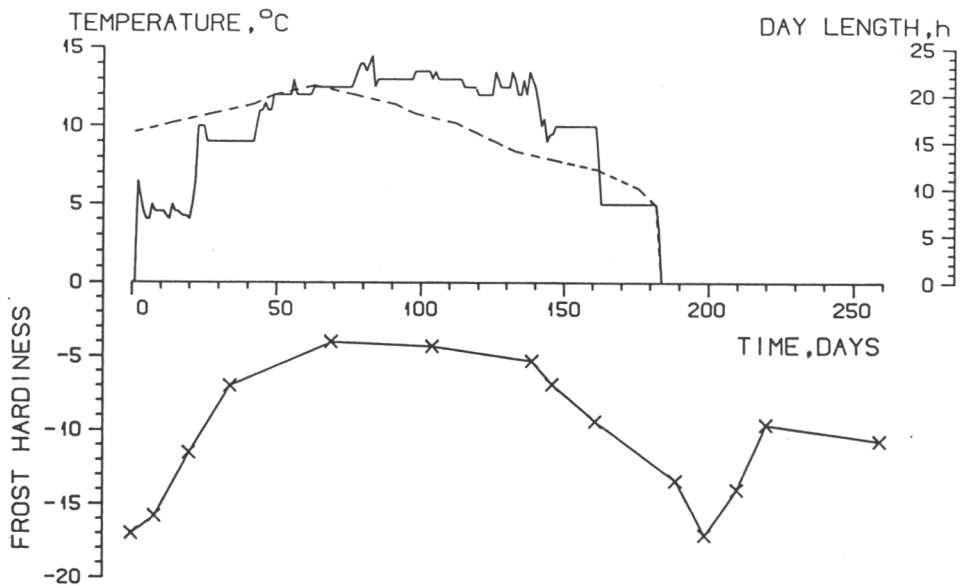



Fig. 1. The day length (- - -) and the temperature (———) of the growing season and the frost hardiness (—x—) of the seedlings during the growing season.

Scots pine seedlings were inoculated in the spring or in the fall. By the end of the spring inoculation period the new shoots of the seedlings had attained 65 % of their total length for the growing season.

Inoculations during the spring resulted in development of the disease, but not inoculations in the fall. Inoculations in early spring resulted in more dead seedlings than later inoculations in the spring (Fig. 2). None of the 60 control seedlings developed the disease (Fig. 3).

The frost stress (-5 °C) applied in the spring had no clear effect on the result of inoculations done before and after the spring frost stress (Fig. 3). When the stress was applied in the fall the seedlings became susceptible to inoculations carried out before and after the fall frost stress (Fig. 3). Frost stress without inoculations had no or very little effect.

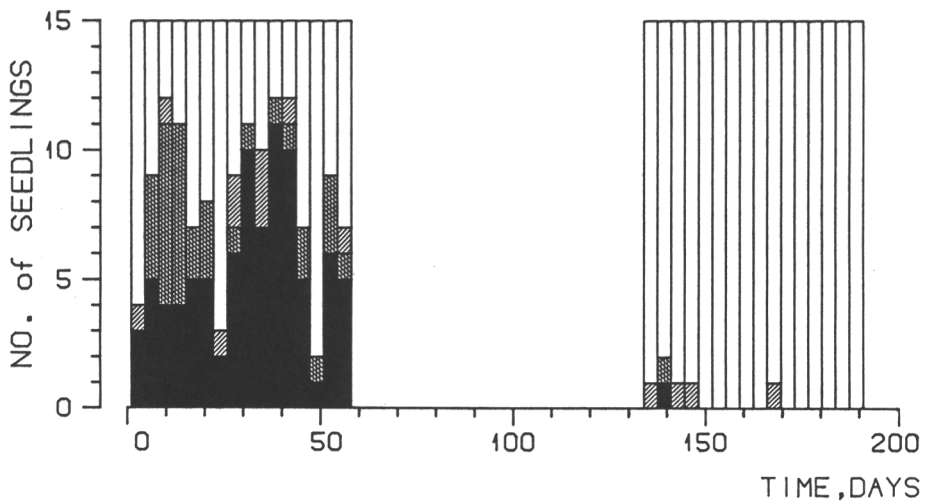



Fig. 2. The inoculation period in spring (8 weeks) and fall (8 weeks) and the results of inoculation.

=diseased

=dead

=weakened and

=healthy seedlings

The control (c) in Fig. 3.

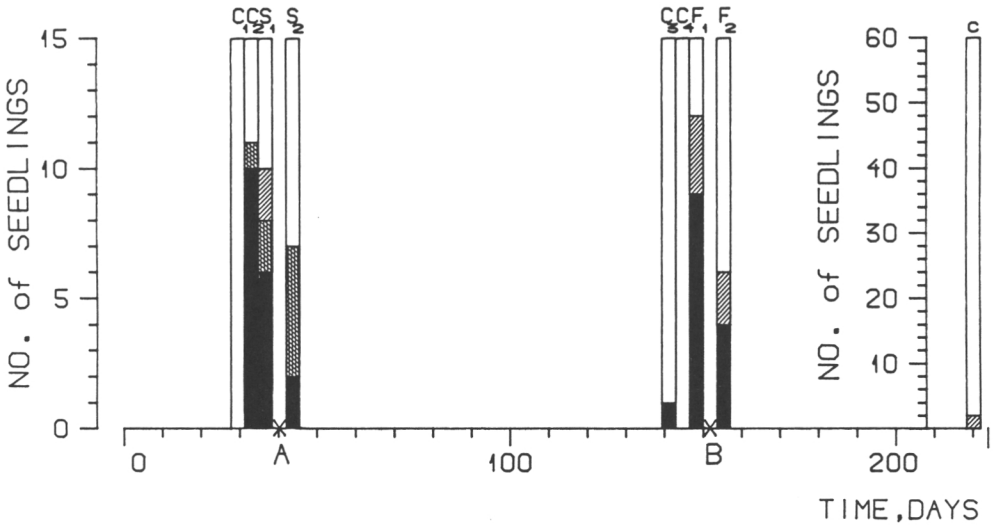



Fig. 3. The effect of frost stress in the spring (A) and in the fall (B).

$S_1$, $S_2$ = inoculation before and after the frost stress in the spring
$C_1$ = no inoculation, frost stress in the fall
$C_2$ = no frost stress, inoculation in the spring
$F_1$, $F_2$ = inoculation before and after the frost stress in the spring
$C_3$ = no inoculation, frost stress in the fall
$C_4$ = no frost stress, inoculation in the fall
c = no inoculation and no frost stress

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# A SERIOUS EPIDEMIC OF *SPHAEROPSIS SAPINEA* IN THE NETHERLANDS AND THE ROLE OF AMMONIUM DEPOSITION AS AN EPIDEMIOLOGICAL FACTOR

## ABSTRACT

Research on *Sphaeropsis sapinea* in the Netherlands from 1985 to 1988 is reviewed. Disease symptoms, population studies and inoculation experiments are discussed. The effect of fertilization with ammonium sulphate on the disease resistance is reported.

## INTRODUCTION

In 1982 shoot blight of pine caused by *Sphaeropsis sapinea* (Fr.) Dyko and Sutton (syn.: *Diplodia pinea* (Desm.) Kickx) was found for the first time in the Netherlands (van Dam & de Kam 1984). A serious epidemic subsequently developed, peaking in 1985 (de Kam & van Dam 1987). Preliminary research made clear that *S. sapinea* had been present in Western Europe for over 100 years, but in the Netherlands the fungus seems never to have caused any economic damage until the 1980's (de Kam 1985). The aim of the studies we have been carrying out since 1985 was to explain how *S. sapinea* which had been endemic so long without causing any damage in the Netherlands, could suddenly develop into an epidemic. Two working hypotheses were adopted: the first was that a strain to which our pines had no resistance genes might have been introduced; the other was that excessive ammonium depositions could have rendered the trees more susceptible to the existing *Sphaeropsis* population. As the final results of our studies are not yet ready for publication, this paper reviews the preliminary results of our work, leaving the details for future publication.

## LIFE CYCLE

*S. sapinea* is a Coelomycete and has no known teleomorph. The conidia are produced in pycnidia on conidiogenic cells arising from the inner cell wall. They are 1- to 4-celled, becoming brown at maturity. The conidia are dispersed with rain throughout the year, with a peak during spring. The conidia may infect newly developed current year's shoots, second year's cones, older branches and the main stem. After the tissue has been killed, the pycnidia that provide the new inoculum are produced. Shoot blight is only

produced in spring, infection of older branches may occur during the whole year, with a peak in summer (Chou & MacKenzie 1988).

## FIELD OBSERVATIONS

**Symptoms caused by *Sphaeropsis*.** In the Netherlands the following symptoms have been observed.

- Shoot blight: attack of the current year's shoot, resulting in local lesions, deformation or death. If the top shoot is killed (leader dieback) shoot blight may cause deformation of the tree.
- Bark necrosis: attack of bark tissue of older branches on the stem; leads to dieback of large branches and may, in serious cases, cause the death of the tree.
- Crown wilt: a sudden wilt and dieback of the whole crown was often observed and is considered to be the result of girdling of the main stem.
- Blue stain: after colonization of the bark the fungus grows into the wood causing wedge-shaped blue-black staining of the wood. Blue stain is often most pronounced at the nodes.
- Excessive resin production: branches attacked by *Sphaeropsis* produce excessive amounts of resin dripping from the branches.
- Attacks of seed cones: Cones of *Pinus* are very vulnerable to attack by *S. sapinea* in the second year of their development. The seeds in these cones usually germinate normally, except when the attack takes place in an early state of development of the cone, in which case the seeds do not develop.

The symptoms described here confirm earlier research (Waterman 1943, Peterson 1977, Currie and Toes 1978, Chou 1984).

**The hosts of *S. sapinea* in the Netherlands.** *S. sapinea* has been found to attack *Pinus nigra* var. *maritima* and *P. nigra* var. *nigra* most seriously. Only during the peak of the epidemic in 1984 and 1985 *Pinus sylvestris* was heavily attacked. The fungus has occasionally been found on Douglas fir without doing much damage and on *Picea omorica* in a Christmas tree plantation. We consider *P. sylvestris* just as susceptible to *Sphaeropsis* as the black pines, but in the Netherlands Scots pine is more resistant to adverse factors such as low temperatures and attack by *Gremmeniella*. This might render Scots pine somewhat less attractive to *Sphaeropsis* which, as we will see below, eventually may act as a weak parasite.

**Development of the epidemic in the Netherlands.** The first record in 1982 originated from "Zwartwater", a forest area in the southeast of the country. One year later we found the fungus in various localities in the southeast, and also in the "Veluwe", in the centre of the country. In 1984 a survey was carried out by the Dutch Forestry Service (Vitaliteit 1984). It was then concluded that the fungus caused shoot blight and bark necroses everywhere in the country where *Pinus* was grown, but it was especially serious in regions where trees were grown on poor sandy soils with marginal availability of potassium, magnesium and phosphorus. Moreover, on these soils high ammonium depositions occur, because the majority of the intensive livestock farms in the Netherlands are situated in these areas. In 1986 a more detailed survey was carried out in the Peel area, in the southeast of the country, which was most seriously affected. About 25,000 hectares were surveyed; in about 900 ha more than 40% of the side

branches of the trees were killed; in nearly 2,300 ha more than 20% of the branches were dead (Staatsbosbeheer 1986).

## VARIABILITY OF *SPHAEROPSIS*

If the epidemic had been initiated by the introduction or the development of a new strain of *Sphaeropsis*, we might expect at least two different *S. sapinea* populations to occur in the Netherlands. To study this we compared a limited number of isolates from the Netherlands, from other European countries, and from other continents. The optimum temperature for growth on malt agar in pure culture of the Dutch isolates varied between 26.6 and 31.7 °C. It was not possible to group the isolates on the basis of their optimum temperature for growth in vitro (Smits 1986). The size of the conidia varied considerably between isolates, but we will probably not be able to distinguish populations in *Sphaeropsis* on the basis of their spore size. Differences in conidial cell wall morphology among isolates, as suggested by Wang et al. (1985), could not be confirmed in our study: all isolates had pycnidia containing both smooth and pitted spores. The colony characteristics depend greatly on the growth medium; no groups of isolates could be distinguished on the basis of their growth characteristics in vitro. We conclude that *S. sapinea* is highly variable in vitro. A detailed study including many isolates from over the world is urgent and highly recommended. To date our studies give no evidence that the epidemic in the Netherlands has been caused by a new aggressive strain of *S. sapinea*. However, as it is practically impossible to prove its absence, the question remains open.

## THE NATURE OF *S. SAPINEA*

The nature of *S. sapinea* has long been unclear: is it a primary pathogen or a facultative parasite? Another point of discussion was whether it needed a wound to penetrate the host. The answers to these questions have been given in the publications of Brookhouser and Peterson (1971), and Chou (1976a, 1976b, 1978, Chou & Mackenzie 1988). From those studies it is clear that *Sphaeropsis* sometimes acts as a primary pathogen and sometimes as a weak pathogen, and that it may penetrate the young current year's shoot without wounding, but needs a wound to penetrate older bark tissue. In a number of pilot studies we have confirmed those results by artificial inoculations. Newly developing shoots sprayed with a conidial suspension without previous wounding developed symptoms within a week wounding such shoots did not increase the level of damage. Healthy, vigorously growing shoots easily became diseased by *S. sapinea* if weather conditions were favourable to the fungus. In contrast, on bark tissue of branches older than 1 year necroses only developed after previous wounding, and grew faster in branches that had previously been stressed. For instance, in branches that had been excised from the tree immediately before inoculation, *Sphaeropsis* caused much bigger necroses than on branches that were left on the tree (de Winter 1986), and in young potted plants seriously stressed by water deficiency the fungus developed much bigger necroses than in plants that were watered normally. We concluded that the epidemic of *Sphaeropsis* in the Netherlands, with extensive necrosis of old branches and crown wilt, occurring over a large area could only be explained by assuming that some stress factor was involved. A stress factor, moreover, that was probably not a

traditional one like frost damage or drought, because those stress factors have periodically been present in the past seem never to have given rise to such *Sphaeropsis* epidemic in the Netherlands.

## NITROGEN EXCESS AS A PREDISPOSING FACTOR

Circumstantial evidence that high ammonium depositions might be an epidemiological factor was provided by Roelofs et al. (1985), Boxman and van Dijk (1988) and Ronse et al. (1986). They claimed that the nitrogen content of pine needles in stands seriously attacked by *Sphaeropsis* was significantly higher than in stands with little or no *Sphaeropsis*. In regions with many intensive livestock farms the ammonium input in forest soils has been shown to be 120 to 270 kg N/ha/y (Roelofs & van Dijk 1987). The $NH_3$ volatilizes from animal manure, and reacts in the air with acid aerosols originating from $SO_2$ emissions, to form ammonium sulphate (van Breemen et al. 1982).

In a fertilization experiment carried out with 2-year-old potted *Pinus nigra* var. *maritima* plants under semi-controlled conditions in a greenhouse we studied the effect of ammonium sulphite (AS) and potassium sulphate (PS) on the sensitivity of the plants to *S. sapinea*. The plants were fertilized for 2 consecutive years with 5 levels of AS, ranging from 0 to 600 kg Nitrogen per ha in the first year, and 0 to 300 kg in the second year. In the third year an overall gift of 100 kg AS was given. In each level half of the plants were fertilized with 100 kg PS in the third year, the stems of 93 of the then 5-year-old plants were wounded and inoculated with mycelium of *Sphaeropsis* grown in pure culture. Forty other plants served as controls.

The size of the bark necroses that developed as a result of the inoculations was measured 15, 20 and 25 days after inoculation. The average lenghts of the necroses in the various treatments varied from 17 to 103 millimeters. It was found that *Sphaeropsis* caused longer necroses on plants fertilized with AS ($p=0.012$ on log-scale), whereas fertilization with PS caused smaller necroses ($p=<0.001$ on long-scale). In some treatments AS fertilization caused a threefold increase in the mean length of the necroses. The mineral content of the pine needles was analysed periodically. The analyses showed that excess of AS disrupted the mineral balance of the plants: serious deficiencies of K, Ca and Mg were induced. Moreover, high gifts of AS resulted in a significant decrease of the needle water potential of the plants. This means, that in our experiment AS fertilization also induced drought stress. It is concluded that *Pinus nigra* growing in poor sandy soil becomes more susceptible to *Sphaeropsis*, when fertilized with an excess of AS.

## DISCUSSION

Our results suggest that the epidemic of *S. sapinea* in the Netherlands was the result of a breakdown of the resistance of *Pinus nigra* and *P. sylvestris* to the fungus, and not caused by the introduction or the development of a more virulent or aggressive *Sphaeropsis* population. The mechanism through which the resistance was broken is not yet totally clear; the results of our experiments suggest that potassium, calcium and magnesium deficiencies, and some micro-elements, e.g. Fe (van den Burg et al. 1988) may play a role, as well as water stress. These deficiencies can be induced by excessive

ammonium depositions. This may expain why the epidemic was most serious in areas with high ammonium deposition on dry soils with marginal availability of K and Mg.

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# INFECTION OF *PINUS RADIATA* BY *SPHAEROPSIS SAPINEA* IN NEW ZEALAND - AN OVERVIEW

## ABSTRACT

The symptoms and incidence of 2 types of infection of *Pinus radiata* by *Sphaeropsis sapinea* in New Zealand, namely shoot blight and woody stem infection, are briefly described.

## INTRODUCTION

Although *Sphaeropsis sapinea* (Fr.) Dyko et Sutton comb. nov. (= *Diplodia pinea* (Desm) Kickx has been known in Europe for over 150 years (Sutton 1980), the earliest, detailed studies of pine disease associated with this fungus apparently came from New Zealand (Curtis 1930, Birch 1936) and Australia (Young 1936) where *Pinus* species are exotic.

Birch's study of *S. sapinea* coincided with the first wave of large-scale planting of *P. radiata* in New Zealand from around 1925 to 1936 when more than 300,000 ha of plantations were established. There was no further study of this pathogen in this country until the planting boom of the 1960s which has pushed up the total acerage of *P. radiata* today to one million hectars.

Interest in *S. sapinea* was reviewed by increasing reports of leader dieback and stem malformation of *P. radiata* seen to be associated with this pathogen. Finally a severe incident of shoot dieback in an area where large-scale establishment of *P. radiata* was being considered raised the question of the risk of possible disease spread.

Although literature about this fugus was extensive, there were conflicting views about its pathogenic role. Consequently, in late 1960s, it was decided to undertake studies to clarify the threat posed by *S. sapinea* in New Zealand.

It is now clear to us that, so far as *P. radiata* is concerned, there are two different types of *S. sapinea* infection: (1) shoot blight or infection of green stem (Chou 1976a), and (2) crown wilt resulting from woody stem infection (Chou 1987). The two types of infection occur under quite different conditions and have different diagnostic features, as will now be briefly described.

## SHOOT BLIGHT

Shoot or tip blight results from *S. sapinea* infection of current-season green stem. The earliest indication of infection is a purplish, water-soaked necrotic spot or patch which appears during the period of rapid shoot elongation; subsequent lesion extension

eventually leads to death of a part or the whole of the green stem. Lesion extension often halted at the lower part of the stem where the tissue is more mature, and advance of the pathogen beyond last-season growth was rare. Depending on conditions, infection may result in a restricted stem lesion, tip diebacks, or death of the whole current-season growth. Blighted shoots covered with massive pycnidia can persist on affected trees for several years. Repeated leader infection can lead to stem malformation resulting in volume loss from the valuable butt log (Currie & Toes 1978).

Infection mainly occurs during the spring/summer flush, but a minor wave of infection is often evident during the autumn flush. Infection usually occurs in young stands under 10 years of age (Chou 1977), and severe incidence of the disease seem to occur only on particular sites. For example, in New Zealand a very severe outbreak of shoot blight occurred from 1968 to 1973 on a valley site in the eastern part of the North Island, henceforth known as "death valley" (Chou 1976a, Currie & Toes 1978). Observations on that site showed infection to be extremely sensitive to changes in topography, slope aspects, and tree age (Chou 1977). In Chile severe shoot blight was observed in a young stand at Fundo Colicheu, a site seemingly susceptible to persistent standing mists arising from the Itata River (Chou 1980-unpub.).

That wounding is not required for infection is apparent from field observations and has been confirmed by inoculation studies (Chou 1976a,b). Microscopical studies show that hyphal penetration of host epidermis can occur within 6 h following inoculation (Chou 1978). The formation of a "hyphal aggregate" is essential for host penetration. However, in one study only about 5 % of germinating spores formed such structures under presumably favourable infection conditions, suggesting that a high inoculum density is required for successful infection (Chou 1976a, 1978). Spore germination was often observed to be greatly suppressed on the host surface as compared with that seen on slides (Chou 1981). Spore germination was inhibited by vapours of several of the monoterpenes from *P. radiata* cortical oleoresin, but the role of these toxicants in disease resistance is still unclear (Chou and Zabkiewicz 1976).

Lesion extension can be extremely limited under certain conditions and infection then becomes inconsequential. Lesion extension occurs under a much narrower range of post-inoculation temperature and shoot wetness conditions than that of host penetration (Chou 1982a). Pre-inoculation temperature and photoperiod regimes also affect lesion extension by modifying host resistance (Chou 1982b). Lesion extension is also controlled by the degree of maturity of host tissue (Chou 1976b). Wide differences in host susceptibility appear to exist in the field, and have been demonstrated in inoculation studies (Burdon et al. 1982). However, further field tests of trees selected for the above study were frustrated by a lack of natural infections, even though the trial was set up on a valley site ("death valley") where the disease had been severe.

## WOODY STEM INFECTION (CROWN WILT)

In 1978 death of the upper crown of *P. radiata* trees down to several year-old stems was observed apparently associated with *S. sapinea*. However, our previous finding that this fungus seemed unable to invade woody stem tissues made us doubtful about its primary role in this type of dieback. Extensive examinations of diseased trees in New Zealand and also in Chile enabled us to construct a picture of the sequence of disease development. It appeared that infection of woody stems occurred under drought stress, i.e. under conditions likely to cause a temporary breakdown of host resistance, and

evidence for this was later provided by inoculation tests (Chou 1987). Subsequent studies showed that the high resistance of woody stems can also break down under stress imposed by heavy pruning that leads to *S. sapinea* infection (Chou & Mackenzie 1988). The term whorl-canker was used in an earlier description of such infection (Gilmour 1964).

The earliest sign of infection is wilting of the upper crown or a branch, as shown by chlorosis and loss of succulence of the foliage. This wilting can be traced to *S. sapinea* infection somewhere lower down the stem where discolouration and necrosis of the inner bark tissue as well as blue staining and drying of the wood can be observed; as a result of this water conduction to the upper crown can be totally obstructed. Soon the fungus can move up to invade the wilting shoots, causing typical tissue discolouration and necrosis, and producing massive pycnidia in the bark. Infection of woody stems does not always produce crown symptoms or dieback. Infection can be localised, presumably because of restoration of host resistance when stress factors are relieved. If water conduction is not seriously hampered, then obvious crown symptoms will not be present. Other stress factors that can bring about a reduction of stem resistance and encourage *S. sapinea* infection may include hail storms (Young 1936) and perhaps insect damage (Hadow & Newman 1942).

Drought stress appears to affect trees over 10 years of age. In the very severe drought of 1978-79 in Chile, some stands 10 to 15 years of age were badly affected, while neighbouring 3-year-old stands were virtually intact (Chou 1980-unpub.). In New Zealand most affected stands were also over 10 years of age. From inoculation studies with 2-year-old *P. radiata*, the threshold needle water potential for infection was estimated to be -2.5 MP which represents a very severe water stress, but is still above the wilting point of around -3.5 MP (Chou 1987). An earlier inoculation test using one-year-old trees had failed to produce any infection under the same stress level (Chou and Wright - unpub.). Thus older trees seem more vulnerable to water stress than younger trees.

It was also found that resistance level along stem varies with stem age; resistance to infection increases towards the base of stem but decreases towards the top (Chou 1987, Chou & Mackenzie 1988). Field observations showed 2-3 year old stems to be more commonly infected than 4-5 year old stems, and little infection resulted when inoculations were made into pruning wounds near the base of the tree even under heavy pruning intensity (Chou & Mackenzie 1988).

## CONCLUSIONS AND DISCUSSION

There are no accurate data on overall disease loss caused by this fungus in *P. radiata* plantations. Presumably infections caused by *S. sapinea* represent typical endemic diseases which flare up locally from time to time, but one likes to think that the overall damage is something that can be lived with. However, severe outbreaks do tend to cause considerable panic, with fears that an epidemic such as occurred with Dutch elm disease or chestnut blight is developing. Thus far, the prognosis and advices given based on our present understanding of *S. sapinea* has worked to allay fear and dissuade unnecessary actions, but for how long? No doubt the very high host resistance in the current population of *P. radiata* in New Zealand is the key to limiting infection damage, but will this situation hold forever, and what are the factors that might upset such a balance? These questions cannot be answered from our present knowledge of the fungus/host

relationships. Virtually nothing is known about the genetics and mechanism, of host resistance, nor about pathogen variability. We also know very little about the epidemiology of either shoot blight or crown wilt (dynamics of infection increase, site and climatic factors affecting infection etc.). My question is: do we still need to continue the study of this fungus?

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# *SPHAEROPSIS SAPINEA* SHOOT BLIGHT OF *PINUS NIGRA*, *P. PONDEROSA*, AND *P. JEFFREYI* IN KÓRNIK ARBORETUM

## ABSTRACT

The first symptoms of shoot blight were observed in the Kórnik Arboretum on *Pinus ponderosa* and *P. jeffreyi* ten to twenty years ago. More extensive damage appeared after 1985. *P. nigra* growing close to *P. ponderosa* showed the same symptoms last year. Studies on the pathogenicity of isolated *Sphaeropsis sapinea* (Fr.) Dyko et Sutton were carried out on seedlings of *Pinus sylvestris* and *Picea abies*.

## INTRODUCTION

*Sphaeropsis sapinea* (Fr.) Dyko et Sutton (= *Diplodia pinea* (Desm) Kickx) belongs to the Fungi Imperfecti, and seriously damages coniferous trees from the genera: *Abies*, *Cupressus*, *Larix*, *Pinus* and *Pseudotsuga*. This fungus appears primarily on pines, on both young seedlings and mature trees. It has been isolated from the bark of trunk and branches, and from the needles and cones of 33 different species (Palmer et al. 1987, Sutton 1980). Damage caused by *S. sapinea* on trees more than 30 years old is more evident than that on seedlings (Peterson & Wysong 1968). Infection of seedlings is followed by top mortality, as well as by collar rot of thicker and thinner roots (Palmer & Nicholls 1985). On mature trees the pathogen causes yellowing and necrosis of current-year shoots, and an elongated, depressed area on branches or stems (de Kam & van Dam 1987, Brookhouser & Peterson 1971). These are conflicting opinions about the way in which *S. sapinea* infects trees. Brookhouser and Peterson (1971) showed that the pathogen penetrates the needles of Austrian pine, Scots pine and Ponderosa pine through the stomata. De Kam and van Dam (1987) also obtained positive results with artificial infection by spraying spore suspensions on the developing, unwounded shoots of two year old pines. On the other hand, some authors have suggested that infection of this fungus takes place through trunk wounding caused by pruning (Gilmour 1964). Resistance of the trees against *S. sapinea* infection depends on certain abiotic factors. Water stress, caused by a prolongated drought, plays an important role (Chou 1987). This paper presents some of the results of an investigation aimed at clarifying the causes of the browning of current-year shoots on three pine species, *P. jeffreyi*, *P. ponderosa* and *P. nigra*, in the Kórnik Arboretum.

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## MATERIAL AND METHODS

The symptoms of a disease were observed on certain pine species in Kórnik Arboretum during recent years. Symptoms resembling shoot blight caused by the fungus *Sphaeropsis sapinea* appeared on pines several years old, of the following species: *P. jeffreyi* (inventory No 114), *P. nigra* (i.N. 115) and *P. ponderosa* (i.N. 4175).

**Isolation of** *S. sapinea.* Samples from shoots with attached needles were collected in September 1988 and March, April 1989, and placed in wet Petri dishes. After 3-4 days cultivation, spores were gathered from the pycnidia and transferred onto potato dextrose agar (PDA). The fungus was incubated at room temperature in daylight.

**Pathogenicity test.** Healthy cuttings from *P. ponderosa* twigs, as well as two-year-old seedlings of *P. sylvestris* and *Picea abies*, were artifically infected with *S. sapinea*. The inoculation was performed in a greenhouse by: 1) placing a two-week-old culture of the fungus on a wound where part of the covering tissue had been removed, 2) spraying both injured and uninjured twigs and seedlings with a water suspension of spores. Inoculated twigs and seedlings were placed in a climatic chamber at 28 °C and R.H of 80 %.

## RESULTS

**Symptoms of the disease.** Yellowing of the current-year shoots was observed on *P. jeffreyi* and *P. ponderosa* trees more than ten years ago. Intensification of the disease

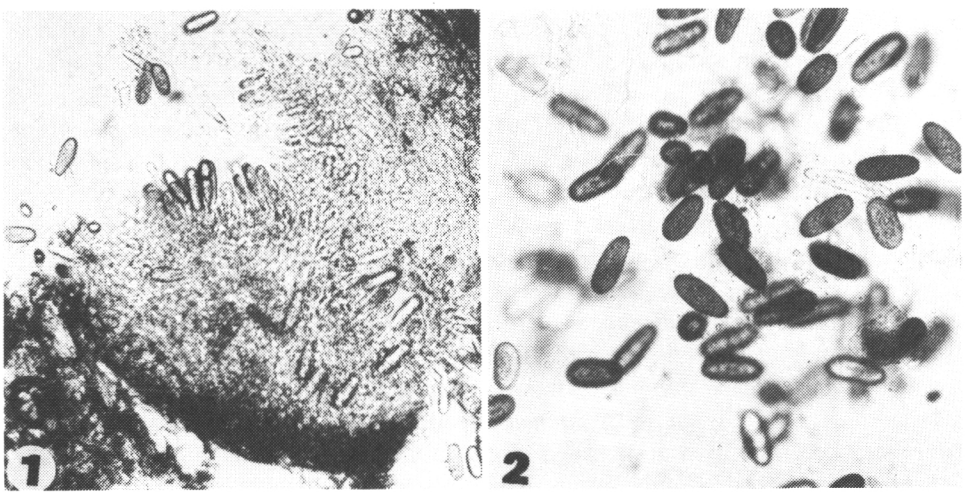



Fig. 1. Transverse sections of unilocular pycnidia of *S. sapinea.*
Fig. 2. Conidia of *S. sapinea.*

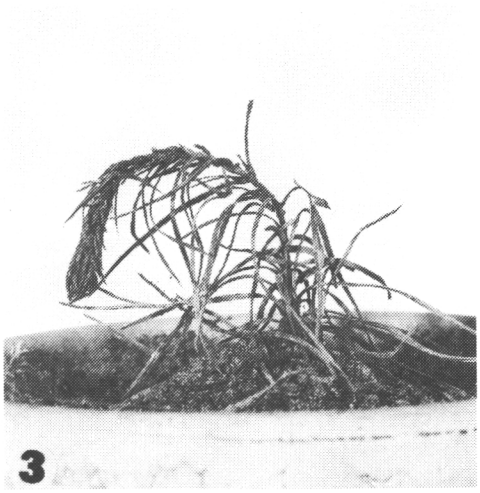






Fig. 3. Dieback of *Pinus sylvestris* seedling artificially infected with *S. sapinea*.
Fig. 4. Shoot blight of *Picea abies* artificially infected with *S. sapinea*.

occurred after 1985. The first disease symptoms were observed on a *P. nigra* specimen growing next to the diseased pines in recent years. In the early development of the shoot blight, moist, light-brown spots at the base of the needles were observed already in the early spring. They were followed by necrosis of the current shoots and their needles. Brown, necrotic spots appeared on the twigs below the infected tops. They were especially visible after the epidermis had been removed.

**Morphology of the pathogen.** Black fruiting bodies, 250 μm in diameter with a central ostiole were present on and below the epidermis of both dead needles and shoots. The length of the conidiogenous cells varied between 15 and 20 μm. The conidia, oblong cylindrical, apically rounded, aseptate or 1-septate, thick-walled, hyaline or yellowish and dark brown when mature, varied in size within 30-50 × 10-16 μm (Figures 1 and 2).

**Pathogenicity test.** Necrotic spots on *P. ponderosa* needles inoculated with mycelium of *S. sapinea* placed on the wounded shoot were observed after 7 days. Serious yellowing and browning of the needles occurred after one month. Needles sprayed with a suspension of spores became spotty 2 months after being infected. Full necrosis of the needles occurred, however, after 4 months. In the case of both inoculation variants applied here, moist necrotic spots were observed on the shoots at the stage of the disease when serious needle yellowing took place. Seven days after the wounded seedlings of *P. sylvestris* and *Picea abies* were artificially infected with the fungus, needle discoloration appeared around the inoculation point. Seedlings of *P. sylvestris*

wilted completely within one month after infection (Fig. 3). The top of the shoot of one of the *Picea abies* died at the same time, bending to form a "bishops crozier" (Fig. 4). The disease manifested itself on seedlings sprayed with the suspension of spores in the form of yellow spots on many needles two months after infection. Complete browning of the needles, followed by the death of the entire plant occurred within 3 or 5 months after inoculation on pine or spruce respectively.

## DISCUSSION

*S. sapinea* caused shoot blight of three species of pines, *P. jeffreyi*, *P. nigra* and *P. ponderosa*, in the Kórnik Arboretum. No symptoms were observed on specimens of *P. sylvestris* growing there. According to the literature, however, this disease should be common on this species (Brookhouser & Peterson 1971, Peterson 1977, de Kam & van Dam 1987). The author has observed the disease on single trees of *P. sylvestris* in other parts of Poland. Artificial inoculation of the seedlings of *P. sylvestris* confirmed the pathogenicity of *S. sapinea* against this pine. It has been also shown that the fungus causes the disease on two-year-old seedlings of Norway spruce. There are no reports of any form of shoot blight on older spruce trees. Neither has it been observed on this species growing in Kórnik Arboretum. First case of shoot blight on the Jeffrey and Ponderosa pines appeared very probably after the 62/63 winter described by Zielinski (1974) as "catastrophic" for the health of trees. The disease developed on the same trees after 1985. Also on *P. nigra* the symptoms of the disease were observed to develop in approximate time. Manifestation of the disease caused by *S. sapinea* and its intensification observed in Kórnik Arboretum are tightly connected with the effect of unfavourable climatic conditions on the trees. These were the severe winters and/or long periods of drought that occurred in the years 1982-1984 (Chylarecki & Filipiak 1987).

## ACKNOWLEDGEMENT

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# *CRUMENULOPSIS SORORIA* IN BASAL CANKERS OF SCOTS PINE ON AFFORESTED ABANDONED FIELDS

## INTRODUCTION

*Crumenulopsis sororia* (Karsten) Groves was first described on Scots pine (*Pinus sylvestris* L.) in Finland by Karsten (1871) as *Crumenula sororia*. Later on Kujala (1948, 1950) found the same fungus on lodgepole pine (*Pinus contorta* Loud.). In Britain, Batko and Pawsey (1964) found it on *P. nigra* Arn. (Corsican pine), and *P. griffithii* McClel.. In the Netherlands, Gremmen (1955) reported it on *P. sylvestris*, *P. nigra*, and *P. cembra* L. More recently *C. sororia* and its cankers has been investigated on lodgepole pine in Britain (Hayes 1973, 1975, 1977, 1980, Hayes et al. 1975, Graig and Hayes 1981), and in Germany (Stephan and Butin 1980). Hanso (1969) reported the fungus in Estonia.

Ettlinger (1945), van Vloten and Gremmen (1953), and Gremmen (1960) re-examined the genus *Crumenula* de Not., and made a more clear morphological delimitation between *C. pinicola* Karst. and *C. sororia* Karst. *C. pinicola* usually occurs on pine bark, but is seldom associated with cankers, while the apothecia of *C. sororia* occur on the bark on or around resinous cankers. The size of the asci and the form of ascospores are also differentiating characteristics between these two species. Groves (1969) gave a new generic name, *Crumenulopsis* for these two fungi.

*C. sororia* causes irregular cankers on pine twigs and stems. Resin flow and blackish staining on the bark usually indicate infected sites on pine stems. The wood under the dead bark has also blue or almost black staining.

*C. sororia* has never before been a problem in practical forestry in Finland. During the 1940's, the fungus caused serious damage in experimental lodgepole pine stands established in the 1920's or early 1930's. During the 1970's, interest was again shown in the cultivation of lodgepole pine. These stands are now coming to the age when infection and canker development can be expected.

In this paper, some observations on the occurrence of *C. sororia* on Scots pines growing on abandoned fields are presented. The relationship of nutritional status and infection is also discussed.

## RECENT OBSERVATIONS IN FINLAND

In July 1988 seriously cankered Scots pines were observed in a 17-year-old stand in Rautavaara, Mäntymäki, 63°20'N, 28°40'E. The development of the cankers primarily started from the branch whorls, as well as from internodal bark cracks

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(Figures 1-2). The cankers showed all characteristic symptoms of *C. sororia* infection; resin flow, blackish stained bark, irregular form of cankers, black stain in the wood, and even mature apothecia of *C. sororia* were present. A slowly growing grey mycelium, which produced typical branched conidia of *Digitosporium piniphilum* Gremmen (an anamorph of *C. sororia*), was later on isolated from the cankers. The height of the infected trees was about 9 m, and the annual height increment during the preceding five-year period was about 55 cm, with a slight decrease in 1988. About one third of the trees in the stand had one canker or more at the base of the stem. During the same summer and next winter, more infected trees were observed in several young pine stands growing on abandoned fields in southern Finland.

Scots pine stands established on fertile agricultural land often exhibit resin bleeding at the base of the stems. This phenomenon has not been investigated earlier, but was assumed to be caused by vigorous radial growth of the xylem, which produces cracks in the cork bark and in the outer living bark tissue. Such superficial cracks apparently become a liability for the trees after the canopy closure stage. At this stage the relative humidity increases drastically inside the stand, thus creating favourable conditions for *C. sororia* infection. During the 1970's, about 10,000 hectares of agricultural land were afforested with planted Scots pine. These stands are now in danger.

## RELATIONSHIP BETWEEN INFECTION AND THE SOIL FERTILITY

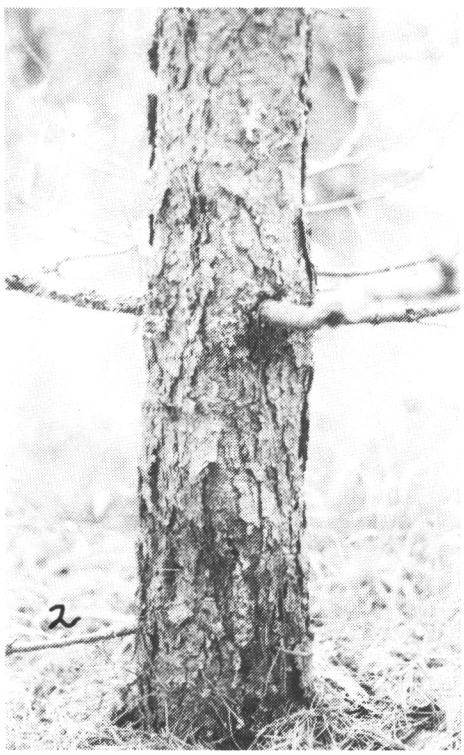
Cankers with *C. sororia* are usually found on poorly growing pines that have most likely been suffering from a deficiency of mineral nutrients, e.g. Scots pine on moor sites (Kujala 1950) or Corsican pine on sites with a low nutrient status, especially phosphorus, or inadequate drainage (Batko and Pawsey 1964). Unfavorable climatic conditions are also considered to predispose pines to *C. sororia* (Gremmen 1968). Anthropogenic factors, e.g. air pollution through decreasing growth, could predispose pines. Kujala (1950), Kowalski and Domanski (1983), and Kowalski (in this volume) found the fungus on slowly growing pines in urban areas. It is doubtfull whether the fungus could benefit from the effects of air pollution or acidic deposition. For example, the pH range for the successful germination of its ascospores is from 4.5 to 7 (Hayes and Manap 1975).

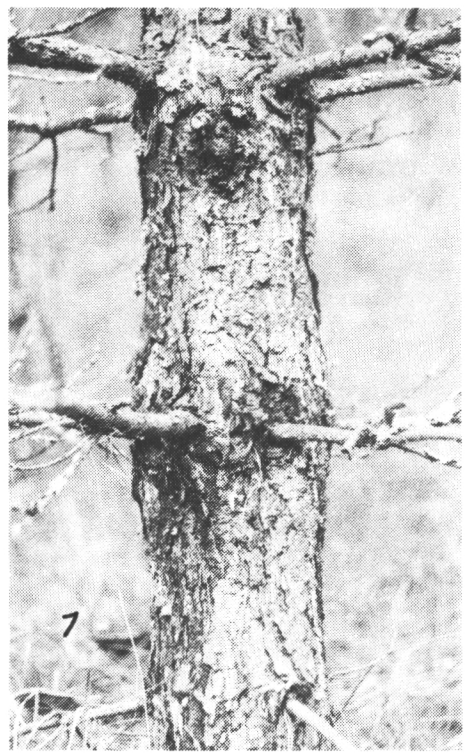
*C. sororia* seems to be common in environments exposed to certain growth decreasing factors. No abiotic factor alone may cause the susceptibility, but it could be physiologically connected with poor growth. However, cankers have also been found in some cases in well growing stands, e.g. on lodgepole pine (Kujala 1950) and Corsican pine (Batko and Pawsey 1964). Even on sites with well- growing pines, the nutritional status may not be optimal for the development of resistance against *C. sororia*. The canker development on pines on abandoned fields could have been affected by the availability of mineral nutritients exceeding the requirements of Scots pine.

## IMPORTANCE OF *CRUMENULOPSIS SORORIA*

The canker disease of pines caused by *C. sororia* may not be economically important, e.g. by Phillips (in Smith et al. 1988). Still cankers are common on lodgepole pine in

Scotland (Hayes et al. 1975). During the coming five years the disease becomes much more important in Finland. The problem has not yet been recognized by Finnish forestry, and more fields are being afforested in order to cut back agricultural overproduction. Fortunately, birch is now more favored than pine, and this will help avoid a lot of the *Crumenulopsis* problems.




Figures 1 and 2. Cankers at the base of Scots pine caused by *Crumenulopsis sororia*. 1) Blackish stain and resin flow at the base of branches in infected nodes. 2) Two cankers, the lower of which may have an internodal origin.

## MORE RESEARCH NEEDED

In Scotland, Hayes et al. (1981) developed multiple regression models for predicting the extent and severity of attack by *C. sororia* on different provenances of lodgepole pine. They used environmental variables related to geography and weather, and stand variables such as provenance and age. In mountainous country, as Scotland, the geographical variables may explain a high proportion of the disease incidence. In non-mountain areas such as Finland, however, geographical variation is less and hence these variables may have less explaining value. Significant variables for the model in Finland could be provenance, topography, and soil quality.

In Scotland, the original latitude of the provenances was not related to the disease incidence (Hayes et al. 1981), although there were significant differences between provenances. In contrast with that, lodgepole pine stands in Germany showed a clear

northward increase in resistance according to the original latitudes of the provenances (Stephan and Butin 1980).

Scots pine provenances from southern Finland are not likely to have a marked variation in resistance, although, such variation may exist at the level of individual trees. Thus, the reasons for the susceptibility of certain stands may be due to site factors, most likely the soil.

Internodal bark cracks and the base of branches are apparently suitable sites for infection. The pathological histology of these entries should be investigated. Why do the cracks develop? What is the reason for the imbalance between the growth of xylem and bark? Despite the good growth, an incorrect nutritional status in the soil could be one reason. In sites with infected stands, earlier agricultural practices may have changed the proportions of individual mineral nutrients to a situation that is not favorable for pines. Some results from recent investigations on *Lophodermella sulcigena* needle cast (Kurkela and Jalkanen 1981) point to the importance of optimum levels of macro- and micro-nutrients.

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# DIFFERENTIATION OF PINE SHOOT TISSUE IN RELATION TO THE DEVELOPMENT OF AECIA BY *MELAMPSORA PINITORQUA*

## INTRODUCTION

The aecia of rust fungi occurring on conifers develop on growing or newly grown tissue. According to the biotrophic type of growth, rust fungus does not kill the cells from which it takes nutrients. In pine shoots the cells close to the aecium are often separated from each other by an abundant growth of mycelium. Being dependent on the host tissue, the rust mycelium apparently tries to keep host cells alive.

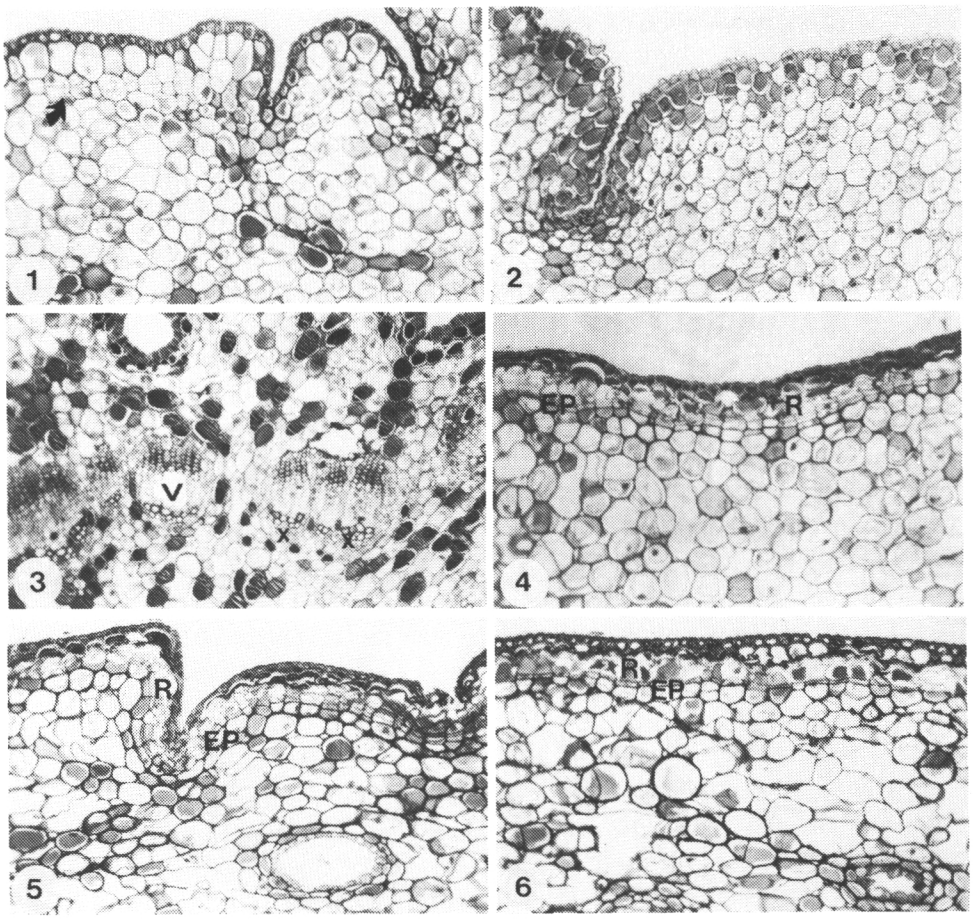
The elongation of pine shoots in Finland mainly takes place in June. During the elongation period the shoots are susceptible to infection by *M. pinitorqua*. Production of aeciospores of the rust may continue until the second half of July (Kurkela 1973)

During pine shoot elongation the primary vascular bundles are separated, and the xylem cells are weakly or not lignified at all. In the epidermis, some suberization may occur only at the bases of the developing short shoots, where some collenchymatic tissue also appears. During the period of elongation, the zone of elongating tissues moves from the base to the tip of a shoot. Cessation of elongation is associated with increased secondary growth in the cambium, and lignification of the xylem fibers. At the same time the development of phellem is initiated in the primary bark parenchyma under the epidermis.

The aim of this study was to observe the timing of these processes during the elongation and susceptibility period of Scots pine shoots. Observations were also made on the effect of rust infection on the development of defence structures.

## MATERIAL AND METHODS

New leader shoots of pine were collected in a five-year-old stand in Tammela (++Grid reference $27^{o}$ E) during June and July with about one weeks intervals. The first set of samples, at least four shoots at each time, were taken on June 4th, and the last set on July 17th, 1981. Two pieces were cut from the tip of each shoot, two from the middle part, and two from the base and fixed in FAA (formaldehyde - acetic acid - alcohol). After dehydration the pieces of shoot were embedded in paraffin, cut with a microtome, and stained with the safranin-fast-green method. The slides for microscopy work were embedded in euparal.




Figs. 1-3. Transversal cut of a pine shoot at the beginning of elongation, June 4. Fig. 1. The base of the shoot. Initial cell divisions for the phellogen development (arrow). Fig. 2. The middle part of the shoot The development of the phellogen has not yet initated. Fig. 3. The middle part of the shoot. The primary vascular bundles (V) are still separated by parenchymatous cells, primary xylem (x). Magnification: ×125.

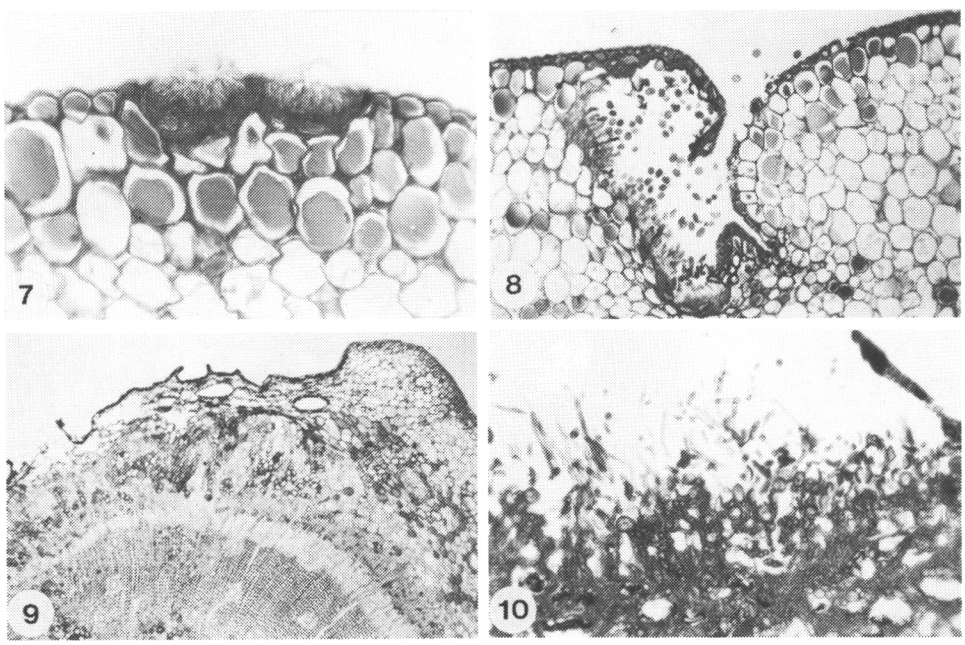
Figs. 4-6. Tranversal cuts of the bark tissue of a Scots pine shoot, on July 17, exophylactic periderm completely developed. Fig. 4. The base of the shoot. Fig. 5. The middle section of the shoot. Fig. 6. The section close to the shoot apex. EP; exophylactic periderm, R; ruptured cell layers outside the EP. Magnification: ×125.

## RESULTS

The growth of pine shoots had begun on June 4th, and the base of the shoots was just appearing from under the bud scales of short shoots. Acording to earlier observations, pine shoots become exposed to infection by basidiospores of *M. pinitorqua* at this time (Kurkela 1973).

**The development of healthy shoot tissues.** On June 4 the shoots had grown by less than one fourth of their final length. Secondary growth at the base of the shoots had already connected the primary vascular bundles to form a complete ring of xylem and phloem. Some initiation of phellogen was also observed (Fig. 1). Phellogen, which sequentially developed to form exophylactic periderm, usually initiated in the second or third layer of parenchyma cells below the epidermis. The tissues in the upper part of the shoots had not hardened being stil soft and flexible (Fig. 2). Only some of the tracheids in the primary xylem had a red colour in the slides, indicating the onset of lignification. The primary vascular bundles were separated from each other by parenchymatous tissue (Fig. 3).

Nine days later, on June 13, although elongation had advanced the middle section of the shoots was still flexible and lacked complete xylem ring. Phellogen was initiated only at the base of the shoots. On June 19th elongation had obviously ceased in the middle part of the shoots. About half of the existing xylem was already lignified. Some



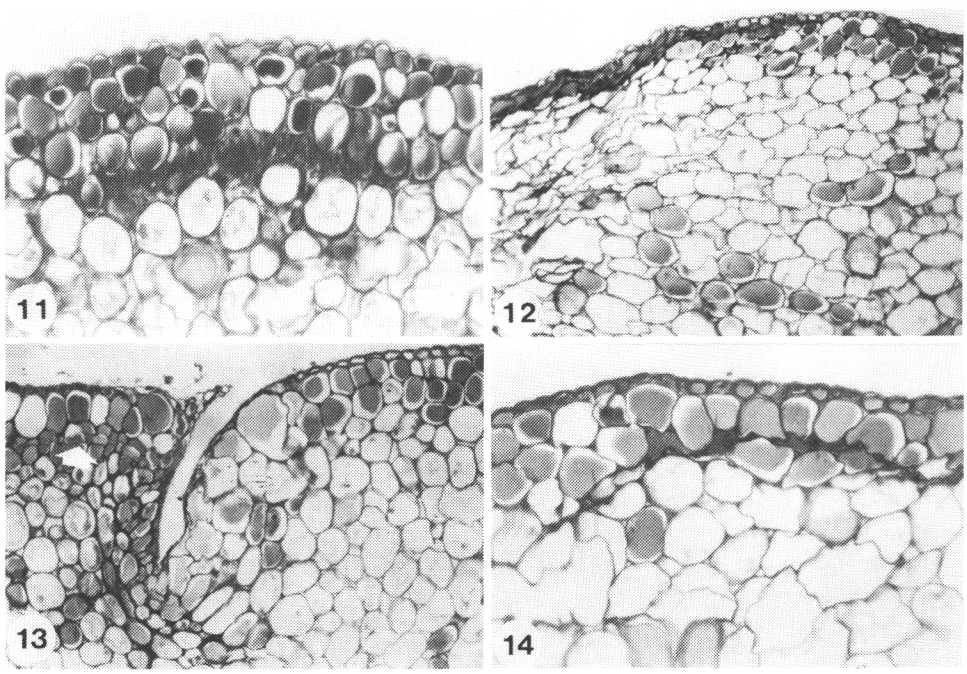

Figs. 7-10. Fig. 7. Spermogonia of *Melampsora pinitorqua* (×250). Fig. 8. A mature aecium (×125). Fig. 9. Collapsed bark tissue at the site of an aecium after the cessation of spore production (×40). Fig. 10. Abundant hyphal growth and production of conidia by *Cladosporium* sp. on a senescent aecium of *Melampsora pinitorqua* (×250).

tangential cell divisions were observed in the bark parenchyma cells, showing initial development of exophylactic phellogen. Elongation still continued at the tip of the shoots without any hardening of the tissues.

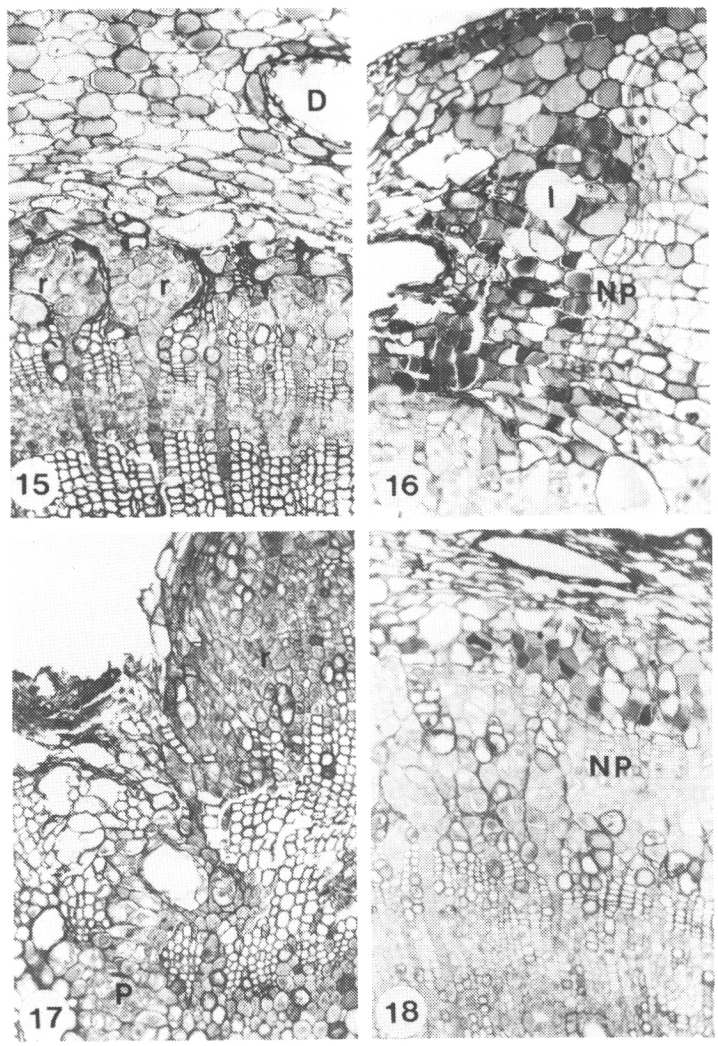
On the 27th of June elongation had ceased throughout the whole shoot. Lignification and periderm formation had reached the tip of the shoot, even though the periderm was not complete in each shoot. On the later dates, July 7 and 17, exophylactic periderm completely covered the healthy parts of the shoots (Fig. 4-6). The epidermis had started to loose owing to rupture of the parenchyma cells between the epidermis and periderm, which is a natural sequence after the development of the periderm. Most of the secondary xylem was also lignified.

***Melampsora* infection.** Twist rust developed only on the middle part of the shoots in the investigated trees. Infection must have occurred during the period between June 7-15 when it rained several times in the area. In the samples taken on June 13 or 19, some yellow lesions and spermogonia were found (Fig. 7). Eight days later, on June 27, the aecia were fully developed producing aeciospores (Fig. 8).

By July 7 some of the aecia were already non-productive (Fig. 9). On July 17 spore production was almost over and some secondary fungi, e.g. *Cladosporium* sp., had invaded aecial lesions (Fig. 10).




Figs. 11-14. Fig. 11. Rust mycelium (m) separates the host cells from each other (×250). Fig. 12. Collapse of bark parenchyma without the development of the exophylactic or necrophylactic phellogen (×125). Fig. 13. Inhibited phellogen formation in the bark tissue close to an aecium (right). The phellogen formation initiated (arrow at left) (×125). Fig. 14. Collapsed cells at the site of the initiation of phellogen and exophylactic periderm (×250).




Figs. 15-18. Fig. 15. Transversal section of a Scots pine shoot injured by *Melampsora pinitorqua*. Resin duct (D) with collapsed epithelial cells, expanded rays (r) as a result of an undirected division of the parenchymatous cells. Fig. 16. An impervious tissue (I) between the bark tissue killed by the rust and the wound periderm (NP). Fig. 17. Expanded ray parenchyma (r) going to form a phellogen, activated pith cells (P). Fig. 18. Wound periderm (NP) formed between the bark parenchyma and phloem. Magnification: ×125.

**Effect of rust infection on the histology of pine shoots.** The rust was found to have caused no hypertrophic growth reaction in the host tissue, neither did it kill the host cells in colonized tissue as long as it was producing aecidiospores. Somehow the rust is able to keep the host cells alive, even though they are separated from each other by intercellular mycelium (Fig. 11). In the investigated shoots, *M. pinitorqua* seemed to be

able to prevent or delay the development of exophylactic periderm. This was clearly seen in the infected shoots taken on July 7 and 17. In these samples the exophylactic periderm was also well developed at the top of the shoots. The epidermis had started to loosen as a result of rupture of the parenchyma cells remaining between the periderm and epidermis (Figs. 4-6). In the infected middle part, close to the aecia, the periderm or phellogen were not present or were incompletely developed (Figs. 8, 11-12).

During its active period the rust was also able to prevent the development of wound periderm (also called necrophylactic periderm, NP) (Fig. 11). After the production of aeciospores had ceased, necrosis spread rapidly in the tissue around the aecia. The cells where phellogen should be initiated (Fig. 14) as well as the epithelial cells in the resin canals, often collapsed first (Fig. 15). After necrosis, an impervious zone (Fig. 16) apparently developed in the bark parenchyma between living and necrotic tissues. In the shoots injured by rust, the development of NP had initiated anywhere in the bark parenchyma, phloem, or in the meristematic tissue in the cambium (Fig. 16-18). According to Srivastava (1963) probably all the living cells in the bark are capable of giving rise to phellogen cells. Prior to the phellogen formation in the phloem, its parenchymatous cells often activated to an undirected expansion (Fig. 15). Pith parenchyma could also be activated, but it was not observed to form an organized wound periderm. Mostly the shoots, where the rust injury reached the pith, died later. In Italy Moriondo (1954) found the wound periderm formation also in the pith of the shoots of *Pinus pinea* injured by *M. pinitorqua*.

Mechanical wounds, not caused by rust, were apparently soon separated from the living tissue by NP, even when exophylactic periderm had not yet formed.

## DISCUSSION

*M. pinitorqua* does not produce any abnormal growth reactions in the tissues it colonizes. It appears that the twisting of attacked shoots is caused by expansion of the bark parenchyma tissue. This is not a growth reaction, but is merely caused by intercellular intrusion of the rust mycelium. *M. pinitorqua* may produce pectolytic enzymes to make more space for mycelial growth, as is reported to be the case with *Cronartium ribicola* growing in white pines (Welch & Martin 1974 a, b). Although *M. pinitorqua* mycelium may separate the host cells from each other, they do not die while the rust is still active. Perhaps, the rust uses or intoxicates derivatives harmful to the host produced e.g. in enzymatic degradation of pectic substances (Martin 1980).

Some twisting may also be due to the death of tissue on one side of the shoot while the tissue on the opposite side continues its growth expansion. In such cases, the rust infection would have had to have occurred in a very early stage of shoot growth.

The formation of exophylactic phellogen in new shoots is closely connected to the development of resistance to *M. pinitorqua*. At the time of phellogen initiation, the parenchyma cells below the epidermis may become impervious,thus forming the first barrier against infection. Non-suberized impervious tissue usually develops as the first protective layer in wounds before the development of NP (Mullick 1975). The hypersensitive dying of cells at the site of penetration, which is not connected to the defence structures in tissues, and which may be an expression of genetic resistance (Jonsson et al. 1978), was not found. However, it is possible that some of the very early wounds at the base of the shoots were caused by rust infection. If so, this kind of hypersensitive reaction may be more connected to the specific time and site of

infection, than to the genetic resistance causing differences in the rate of infection between individual trees.

The delay in formation of NP around active aecia is natural as long as the host cells are alive and initiation of phellogen is assumed to require some stimulation from necrotic cells. When the rust mycelium become inactive, NP developed in the way described by Mullick (1977). Rather well-defined non-suberized impervious tissue (Fig. 16) usually separated NP from collapsed necrotic tissue.

It can be concluded from the results of this investigation that: 1) The development of structural resistance against *M. pinitorqua* in Scots pine shoots coincides with the initiation of phellogen and exophylactic periderm, 2) rust infection causes a delay in the formation of exophylactic periderm and NP, and 3) this delay may prolong the time of susceptibility in the shoots once they are infected.

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# VARIATION BETWEEN HALF-SIB PROGENIES OF *PINUS SYLVESTRIS* L. IN CROWN DEFORMATIONS BY *MELAMPSORA PINITORQUA* ROSTR.

## ABSTRACT

A small 25-year-old half-sib progeny trial of *Pinus sylvestris* was scored for stem and branch twists characteristic of attack by *Melampsora pinitorqua* Rostr. Both single tree and family heritabilities of this trait were found to be substantial. Susceptibility appears to be higher on the more productive progenies but is unrelated to stem crookedness of the mother trees.

## INTRODUCTION

When looking through our Scots pine experiments for symptoms of diseases in preparation for the visit to our experimental forest by the International Symposium on Scots pine diseases, we noticed that on a small 25-year-old progeny trial there were crown deformations characteristic of attack by *Melampsora pinitorqua*. An evaluation was made of the intensity of the damage and this paper is a report from these observations. An attempt was made to evaluate the heritability of susceptibility to this fungus.

Klingström (1963) reported that clones of Scots pine had different susceptibility both to natural and artificial inoculations with *Melampsora pinitorqua* but their half-sib and full-sib progenies did not demonstrate the variation. Illy (1966) working with *Pinus pinaster* has shown that half-sib progenies differ significantly in the severity of attack by *Melampsora pinitorqua*. The more vigorous plants were more readily attacked. Martinsson (1980) has shown that full-sib Scots pine families differ significantly in resistance to artificial inoculations with the fungus. All of the above studies were conducted on very young seedlings.

## MATERIALS AND METHODS

In the fall of 1963 in Forest District Dłużek of northeastern Poland seeds were collected from 9 standing trees randomly selected along a transect through a very good mature stand of Scots pine (*Pinus sylvestris* L.) in Compartment no. 301d. The trees were assessed for various traits including an estimate of stem crookedness on a 1 to 3

score (Table 1). For the sake of comparison seeds were also collected from a population of not less than 10 trees on a felling in a nearby stand (Comp. no. 246d).

The seeds were sown in a nursery in Kórnik nr. Poznań and the seedlings were outplanted in the spring of 1965 as 1-year-old material onto a small experimental area in Forest Range Zwierzyniec, Kórnik, belonging to the Institute of Dendrology. The experiment was layed out in a complete block design with 4 replicated blocks, 20 trees per plot, in 5 rows spaced 1.20 cm with 4 trees per row spaced 0.60 cm. This was the standard spacing used in Scots pine afforestations at the time.

Since establishment, the area was thinned silviculturally twice. Currently, at 25 years, there are 2 to 6 trees per plot (av. 4.08).

In the spring of 1989 all trees were assessed for the characteristic twisting of stems and branches caused by *Melampsora pinitorqua* Rostr. attack (Table 1). Completely straight stems were classified as 1 and those most affected as 5. The data was subjected to an analysis of variance and the family variance component was used to calculate single tree heritability defined as:

$$h_s^2 = 4\ V_F/V_{Ph}$$

and family heritability defined as:

$$H_F^2 = \frac{V_F}{V_F + V_{FB}/b + V_E/nb}$$

The results for trees which represent the population from Comp. 246d of Forest District Dłużk were both included and excluded from the analysis of variance.

## RESULTS AND DISCUSSION

Table 1 gives the scores for stem crookedness for the mother trees, the average scores for stem and branch twists in the 25-year-old progeny and the basal area (BA) in $m^2$ per ha for 23-year-old progenies (from latest assessment). Table 2 shows the analysis of variance of the results obtained for the score of pine twist rust damage in the 25-year-old progenies and the calculated heritability values:

The actual average damage scores for the 9 different progenies ranged from 2.15 to 3.08 (Table 1) on the adopted scale (1-5) and as can be seen from the calculated F value (Table 2) they were not significantly different. However, the heritability values are sufficient to merit selection for resistance to the rust.

The population included with the progeny trial had an average performance of 3.23 and thus was higher than for any of the progenies. It presents therefore a more susceptible stand. When the analysis of variance is performed to include both the 9 progenies and the population, the F for genotypic elements raises to 1.92 becoming significant at 0.10 level. This indicates that the differences we observe between progenies are unlikely to be purely accidental and therefore are genetic in nature.

There appears to be no relation between the observed crown deformations on the experimental area and the original score of stem crookedness. The correlation coefficient is practically nonexistent, r = 0.06. In the maternal stand in Forest District Dłużk there were no poplars and probably few in the vicinity. Thus stem straightness is not determined there by *Melampsora pinitorqua*. In Kórnik however, especially in

the Zwierzyniec Forest Range, there are numerous poplar experiments so the incidence of this rust is likely to be high and pines damaged.

There is negative correlation between the basal area of the progenies and the score of stem and branch twists (r = -0.675). Almost the same value is obtained (r = -0.671) if population no. S-07-78 is included in the regression analysis. This indicates that the more productive progenies are less susceptible to the pine twist rust. Thus the relation with vigor seems to be opposite to that reported for *Pinus pinaster* (Illy 1966).

Table 1. Stem deformations on mother trees and progenies

| Mother tree number | Score of stem crookedness | Progeny number | Score of stem and branch twisting | BA $m^2$/ha |
|---|---|---|---|---|
| K-07-32 | 2 | S-07-77 | 2.15 | 41.88 |
| K-07-33 | 2 | S-07-79 | 2.84 | 33.63 |
| K-07-34 | 3 | S-07-80 | 2.35 | 36.46 |
| K-07-35 | 3 | S-07-81 | 2.64 | 26.76 |
| K-07-36 | 3 | S-07-82 | 2.47 | 38.68 |
| K-07-37 | 1 | S-07-83 | 2.60 | 31.72 |
| K-07-38 | 3 | S-07-84 | 3.08 | 28.98 |
| K-07-39 | 1 | S-07-85 | 2.73 | 37.85 |
| K-07-42 | 1 | S-07-86 | 2.50 | 39.96 |
| | | Population | | |
| | | S-07-78 | 3.23 | 30.96 |

Table 2. Results of the analysis of variance

| Source of variation | d.f. | SS | MS | Variance components | F |
|---|---|---|---|---|---|
| Families | 8 | 8.34 | 1.043 | VF = 0.0255 | 1.58 |
| Blocks | 3 | 0.71 | 0.237 | VF = 0 | - |
| Families x blocks | 24 | 9.70 | 0.404 | VFB = 0 | - |
| Residual | 111 | 73.25 | 0.660 | VE = 0.660 | |
| Total | 146 | 92 | | VPh = 0.6855 | |

$h_s^2 = 0.149$ $h_F^2 = 0.367$

Otherwise the present study based on observations on 25-year-old progenies confirms those observed on young seedlings (Klinström 1963, Illy 1966, Martinsson 1980) as discussed in the introduction. Our stand is in fact of a very good quality and the pine twist rust damage is not a major feature. The stand was already deprived of all badly damaged individuals by two successive thinnings, however the rust damage is still observable in the crowns and as shown above appears to be under genetic control.

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# PROPAGATION AND BIOLOGY OF *MELAMPSORA PINITORQUA* ROSTR. IN BULGARIA

## INTRODUCTION

Continously increasing demand for wood, both regional and on a world wide scale, encouraged the establishment of intensive forest plantations oriented at the accelerated production of raw material. Several thousand hectars of plantations have recently been established in Bulgaria. The plantations comprise both deciduous and coniferous tree species, and an important role is played by representatives of the genus *Pinus*: *P. sylvestris*, *P. nigra* ssp. *austriaca* and *P. nigra* ssp. *corsicana*. One consequence of this action was that the species were moved to sites far away from their natural area. This was decisive as regards their increased susceptibility to certain diseases, e.g. *Melampsora pinitorqua*. The aim of this paper is to determine how propagation takes place, and to investigate certain bio-ecological aspects of this fungus in our country, especially in intensively managed, coniferous cultures plantations.

## MATERIAL AND METHODS

The investigation was focused on determining how the disease is propagated in the intensive forest plantations established in the lower forest zone (elevation of up to 600 m) in northern Bulgaria. Field trials consisted of six coniferous species planted in randomized blocks with 3 replications. Not less than 60 seedlings were planted per replication.

In order to study the propagation mechanisms of the fungus, as well as to identify certain aspects of its bio-ecology, observations were carried out every 15 days beginning from April up to the end of June, and every month beginning from July up to October. These observation intervals were selected on the basis of earlier results obtained by Tsanova (1967) in studies on this fungus.

External symptoms of the disease were described in the field. A study was carried out simultaneously on the propagation of this fungus in poplar plantations, as well as in the nurseries located close to the pine stands and/or single pine trees.

## RESULTS AND DISCUSSION

The results obtained in this study (Table 1), as well as those presented by Tsankov and Naidénov (1984) or Naidénov (1984), show that *Melampsora pinitorqua* propagates to a

Table 1. Propagation of *Melampsora pinitorqua* in the intensive experimental plantations according to region, year and host species (in percentages of infected plants).

| Tree species | Experimental fields | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Michailovgrad | | | Vratsa | | | Dermantsi | | |
| | 1979 | 1986 | 1988 | 1979 | 1986 | 1988 | 1979 | 1986 | 1988 |
| *Pinus sylvestris* | 19.2 | 35.3 | 47.2 | 17.5 | 36.8 | 51.3 | 23.4 | 43.5 | 53.8 |
| *Pinus nigra* | 12.1 | 26.5 | 30.2 | 15.6 | 34.8 | 43.5 | 20.5 | 43.1 | 52.4 |
| *Pinus corsicana* | 10.3 | 23.4 | 32.1 | 16.7 | 38.3 | 42.6 | 19.9 | 42.8 | 45.6 |
| *Pinus strobus* | 6.5 | 17.3 | 22.5 | - | - | - | 14.6 | 29.9 | 38.4 |

massive extent in the intensive forest plantations established in the lower forest zone in our country.

Annual precipitation varied between 500 and 670 mm and the temperature during the growing season was as high as 30 - 32 °C (occasional extreme temperatures of 40-41 °C). The winters were rather mild and snowless during the study period.

The results showed that both local pines (*P. sylvestris* and *Pinus nigra* ssp. *austriaca*) and introduced ones (*P. nigra* ssp. *corsicana* and *P. strobus*) were attacked by the fungus. It is interesting to note that uredospores and teleutospores were observed not only on the leaves of white poplar, aspen and grey poplars, but also on the leaves of certain American balsam poplars (*Populus tacamahaca*) and Euro-American hybrids which had been planted in the proximity of the experimental plantations. Such an observation indicates a high degree of plasticity of the fungus with respect to its potential host plant. It can be seen from Table 1 that the pines in the experimental plot at Dermantsi were more attacked than those at the other sites. This could be due to the fact that the field trials established at Dermantsi also included white poplar, aspen, grey poplar and American balsam poplars and Euro-American hybrids. They represented a natural and optimal base for pathogenicity, and the trees became intensively infected. These trials differed from those at the other locations in that almost all the terminal and lateral shoots on the attacked trees were infected.

The field trial at the Michailovgrad Experimental Station had been planted at a distance of about 1 km from an aspen stand. This proves that both basidiospores and teleutospores, growing on the dry leaves scattered on the soil, can be transported through much greater distances than has been reported in the literature (Lanier et al. 1976, Tsanova et al. 1979).

The presence of the disease has also been recorded on some roadside sites and in parks considerably far removed from sites with poplars. We can thus conclude that the infection is possible even without the presence of the alternative host plant i.e. poplars. The opposition situation has also been reported. Infection on white poplar, aspen, and grey poplars in the absence of pines also needs to be demonstrated. Such a hypothesis has been proposed by Zachev (1963) and by Tsanova (1967).

The symptoms of the disease are much clearer on white pine (*Pinus strobus*) than on other pines. The length of the necrotic wound can reach up to 5 or 6 cm. This is the

result of a much more sensitive bark structure. Despite this, the experiment showed that this pine species is considerably more resistant than double-needled pines. The similarity of the form of attack on *Pinus nigra* ssp. *austriaca* and on *P. nigra* ssp. *corsicana* should also be stressed. This is due to the fact that they are systematically so closely related, that many authors consider them to be different ecotypes of the same species. In contrast to the opinion expressed by Lanier et al. (1976) we cannot include them in the group of resistant pines. This is further proof that the resistance or susceptibility of one type or species at a specific locality is highly dependent on the prevailing growing conditions.

Investigations of the sort presented e.g. by Tsanova et al. (1967) confirm that there are specimens which are susceptible to the disease and those resistant when growing under identical conditions. The vegetative propagation of resistant clones would permit the establishment of highly productive intensive forest plantations, that are resistant to the rust.

As was to be expected, the density of the plantations played an important role in determining the intensity of attack by *Melampsora pinitorqua*. The results presented in Table 2 show that the plants most severely attacked were growing in the plantation with a spacing of about 3.6 x 1.2 m (60 to 70 %), and those least affected in the plantation with a spacing of 3.6 x 3.6 metres. This could be explained by the creation of a specific microclimate in a dense plantation that is more favourable for the rust. Primary contamination in such a stand would affect almost all the terminal and lateral shoots.

Our studies show that the percentage of infected trees increases with age. We can thus conclude that infection by *M. pinitorqua* is not exclusively attributed to the young age of the plants, but depends directly on the specific growing conditions.

It has been established that the germination of teleutospores and the formation of basidiospores coincides with the development (flush) of the buds. This indicates that the fungus must be perfectly adapted to the life cycle of the host plant. It is quite natural that such a development stage would be determined by the climatic conditions. It varies in different years from the beginning of April up to the middle of May.

This can be used in practical forestry in recommending when chemical control of the disease should be carried out.

A complementary study performed in a way similar to that of Tsanova (1967, 1979) showed that the fungus attacks plantations located at elevations ranging from 300 up to 450 m a.s.l. However, owing to the fact that during the past 15 years the lower limit of the natural distribution of Scots pine has fallen and that white and grey poplars have been used on a massive scale in afforestation work also at lower elevations, means that almost all species of pine currently planted in Bulgaria are threatened to a varying

Table 2. Intensity of the attack by *M. pinitorqua* according to tree spacing (in percentages of infected trees).

| Spacing (m) | Years | | | | |
|---|---|---|---|---|---|
| | 1979 | 1980 | 1982 | 1986 | 1988 |
| 3.6 x 1.2 | 22.4 | 26.8 | 47.6 | 64.6 | 67.5 |
| 3.6 x 1.8 | 18.4 | 21.5 | 41.2 | 58.1 | 60.3 |
| 3.6 x 2.4 | 14.3 | 15.2 | 25.6 | 30.4 | 45.2 |
| 3.6 x 3.6 | 10.2 | 12.7 | 25.2 | 26.8 | 28.3 |

degree by *Melampsora pinitorqua* follows in various degrees almost all species of pines planted presently in Bulgaria.

## CONCLUSIONS

The results of these studies permit us to state that *M. pinitorqua* is a parasite which propagates in all parts of our country. It can seriously damage pine plantations. The fungus attacks not only bifoliated pines of local origin (*Pinus sylvestris*, *P. nigra* ssp. *austriaca*), but also the five-needled white pine (*Pinus strobus*) which has been introduced.

Germination of the teleutospores, as well as formation of basidiospores, coincides perfectly with the development of the pine buds. This demonstrates the good adaptation of the parasite to the life cycle of the host plant.

Preliminary results show that the alternative host is not indispensable for primary infection of both the pines and the poplars.

It has been established that the fungus can also live as a parasite on American Balsam poplars and the Euro-American hybrids. This poses certain problems in the establishment of poplar plantations next to coniferous forests.

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# SELECTING AND BREEDING PINES FOR RESISTANCE TO *CRONARTIUM QUERCUUM* f. sp. *FUSIFORME*

## ABSTRACT

Planting resistant strains of pines is the most effective way to control fusiform rust on plantation-grown southern pines. Research on selecting and breeding for resistance to this disease has been carried out for over 30 years. Progress of this research was accelerated by the development of an effective, standardized inoculation system that can test large numbers of seedlings for resistance at any time of the year. Gall development is the major symptom in evaluating results from this inoculation test, but other symptoms such as stem lesions without swelling and specific gall characteristics are also helpful. Disease-free survivors from artificial inoculation tests have been planted in seedling seed orchards. Seeds produced in these orchards are the most rust resistant currently available. Present crops of seedlings from such seedling orchards have reduced rust infections by 50 % in field tests, and further significant reductions are anticipated.

## INTRODUCTION

Fusiform rust of southern pines, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, is one of the most important forest tree diseases in the North America. It probably ranks first in terms of economic losses. This native disease, which was first reported in 1896 (Underwood & Earle 1896), originally had very little effect on the management of southern pines. This situation continued with only a gradual increase in disease severity until about 1950. During the past 40 years, however, there has been a dramatic increase in rust incidence across the southern United States. It is generally agreed that this increase in severity, which has reached epidemic proportions in some areas, is largely the result of more intensive forest management. The changes in forest management that caused increasing rust incidence, especially planting, site preparation and fertilization, have been reviewed in an earlier paper (Powers et al. 1981). The most extensive survey on the prevalence of fusiform rust in the southeastern United States was carried out by Phelps (1973). This survey showed that rust is most severe in a band from South Carolina across Georgia and into central Louisiana (Fig. 1). By far the highest level of rust injury is in a zone across central Georgia. It is for this reason that the Southeastern Forest Experiment Station of the USDA Forest Service has headquartered its research on fusiform rust in Athens, Georgia. The disease is causing losses of $128 million annually in product degrade alone (Anderson & Mistretta 1982).

alone (Anderson & Mistretta 1982). If mortality and costs of replanting were included, the figures for annual losses would be much higher.

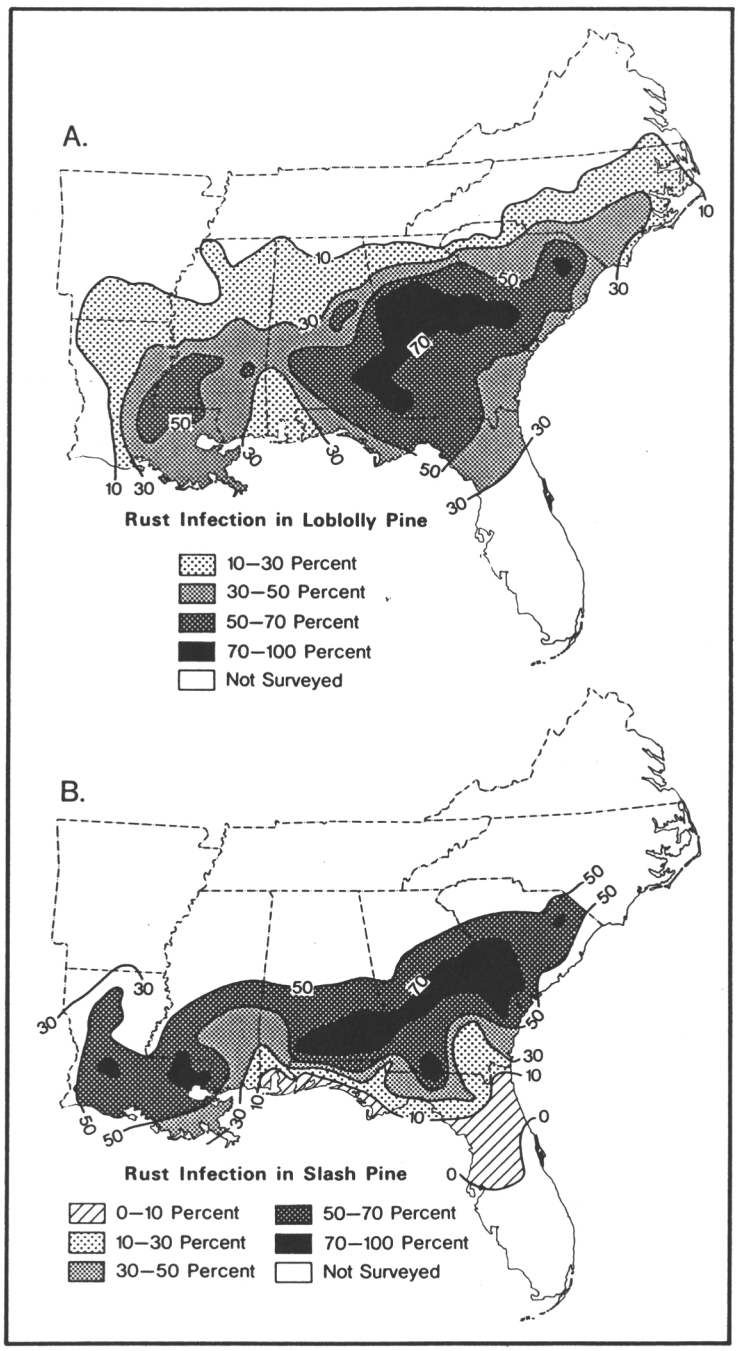



Figure 1. Incidence of fusiform rust in loblolly pine (A) and slash pine (B) plantations in the southern United States.

## DEVELOPMENT RESEARCH

During the past 25 years, studies on various aspects of fusiform rust have been among the most intensive forest tree disease research in the United States. Since spraying with fungicides for control under forest conditions is not economically feasible, much of the research has focused on development of resistant selections or families of southern pines. The work on selection and breeding of resistant slash (*Pinus elliottii* var. *elliottii* Engelm.) and loblolly pines (*P. taeda* L.) began during the 1950's, when Barber et al. (1957) found that certain clones or families of slash pine had inherent rust resistance. Testing for disease resistance was slow at that time, because researchers were limited by the lack of an efficient inoculation technique that could be utilized at any time of the year. This limitation was very obvious to those involved in the research, and efforts were made to develop an inoculation procedure that could screen large numbers of seedlings, give reproducible results, and be used during any season of the year. The procedure that evolved, the concentrated basidiospore spray (CBS) system (Matthews & Rowan 1972), is now the standard method used at the Resistance Screening Center in Asheville, North Carolina (Laird & Phelps 1975). In this system, aeciospores are collected from pines and processed for storage under vacuum in glass ampoules. In this manner, aeciospores viability can be maintained for 15-20 years. Rehydrated aeciospores are used to inoculate leaves of oaks, the alternate host for the rust. Basidiospores produced on oak leaves, the inoculum for pine seedlings, can be obtained at any time of the years for screening tests.

While the research was being carried out to develop these screening procedures, some excellent work was being done on the histology of rust infections in relation to resistant responses of the pine host. Jewell and Snow (1972) observed that some 6-month-old seedlings initially judged to have rust galls recovered and exhibited no gall symptoms at 1 year of age. Another type of resistance was expressed by localized zones of dark staining, tanninized host cells that limited the spread of the rust mycelium. This type of response in slash pine was reported by Jewell and Speirs (1976) and Miller et al. (1976). External to these areas of cell necrosis are purple lesions on the stems. This condition is referred to as symptoms without swelling (SYMNOS). This type of symptom, as well as others, such as short galls, galls with rough cortical tissue, or seedlings lacking any evidence of infection have been studied in relation to eventual disease development (Griggs et al. 1984, Kuhlman 1988, Kuhlman & Powers 1988). These symptoms are now being used to evaluate seedlings at the Resistance Screening Center to improve correlations between inoculation test results and field performance (Walkinshaw et al. 1980). The SYMNOS type of response is more indicative of resistance in slash than in loblolly pine. Within each species, and particularly in the case of loblolly pine, SYMNOS vary greatly by family. For example, in one highly resistant loblolly pine family seedlings with SYMNOS are relatively rare while in other families with lower resistance a higher proportion have SYMNOS. In general, the responses of slash pine to fusiform rust infections are easier to interpret than those of loblolly pine. Variable responses in loblolly are probably attributable to its more heterogenous population, which contains a vast array of natural hybrids with many other species of southern pines.

In most tree improvement programs in the southern U.S., silvicultural characteristics such as growth and form were the first consideration in making superior tree selections. Fusiform rust resistance was of secondary importance. As a result, many

of the first-generation selections now included in state and industry forest tree seed orchards are quite susceptible to rust. Highly rust resistant selections are in a distinct minority. Clones in the first-generation orchards were cross pollinated, and the resulting progeny tested in field plantings. Results from these tests indicated which families were the most promising for increasing rust resistance. Gains are much greater when family background rather than individual tree performance is the basis for selection.

## DEVELOPMENT OF RUST RESISTANT ORCHARDS

In our rust resistance program, the first step in selection was to identify the most promising families, both for rust resistance and other characteristics. Family performance was observed in progeny test plantings at age 5 to 8 years. Within the best families, one or two trees were selected for our program, based on both growth and rust resistance. Scion material was obtained from these superior tree selections, grafts were made, and ortets planted in clone banks. Several years are required before the ortets begin to produce cones. In general, loblolly pines must be at least age 10, and slash pines age 12 before they produce cones. As soon as seeds were available, either from parent selections or clone bank trees, the resulting seedlings were tested with the CBS system.

By 1974, it was obvious that many of these selections were producing seedlings far superior in rust resistance to those commercially available from other state and industry orchards. We therefore decided to establish, in cooperation with the Georgia Forestry Commission, a 24.3 ha (60 acre) production orchard of these disease-resistant selections (Powers & Kraus 1983). This orchard was equally divided between slash and loblolly pine. Because of the relatively low number of resistant selections available, half of the acreage for each species was designed as a standard clonal orchard and the other half as a seedling seed orchard. The clonal orchards were established with a standard spacing of 9.1 x 9.1 m (30 x 30 ft) with each 0.4 ha (1 acre) block containing 49 grafts. Most trees were grafts of second-generation selections, but a few of the best first-generation selections were included to produce 49 different clones per block.

One completely new aspect was the use of survivors of the CBS tests to form a seedling seed orchard. Each seedling seed orchard block contained 50 replicates. Each replicate included a randomly placed single tree of each family included in the block. All seedlings used in these plantings were survivors of artificial inoculation tests that were gall free 9 months after inoculation. Seedlings of 12 to 16 families were usually included in each block, and 93 and 76 families are represented in the loblolly and slash orchards, respectively. These families were rated as resistant after inoculation tests and in some cases also on the basis of field progeny test data. Seedling seed orchard trees were planted closely - 1.5 x 4.6 m (5 x 15 ft). As selection proceeded, heavy thinning was needed to reach an eventual spacing of about 9.1 x 9.1 m (30 x 30 ft). More than 90 % of the seedlings originally planted were or will be removed. The first removals were trees that became diseased by natural infection in the field. Subsequent removals were based on growth and form, and on family histories of rust resistance. Data on rust infection, height, and growth were taken the third year after planting and every year thereafter.

The first loblolly pine seedling seed orchard blocks, planted in 1975, were survivors of greenhouse inoculation tests from 16 wind-pollinated families rated

intermediate to good for rust resistance. After 3 years of exposure to natural field infection, the seedlings in these families ranged from 2 to 56 % infected (Table 1). A similar block of slash pine ranged from 21 to 44 % infected. No susceptible checks were included in the orchard blocks, but adjacent plantings of nursery-run trees had 78 % infection at 5 years of age. Therefore, the incidence of rust in this particular area is moderately heavy.

There was a good correlation (r = 0.755) between the results from the artificial inoculation tests and subsequent natural infection on the survivors in the orchard. For example, the first 10 families listed in Table 1 have been consistently among the more resistant in several artificial inoculations, and all of these families had 16 % or less infection after a 3-year exposure to natural infection. The next 5 families have been intermediate in resistance in greenhouse tests and ranged from 18 to 48 % infection in the orchard. Family 10-31, the most heavily infected family in the orchard, was a marginal candidate for the orchard because in two artificial inoculation tests it had only 25 % less infection than susceptible checks.

Mean heights of the tallest 10 trees in each loblolly family are shown in Table 1. These trees are most likely to be the group from which eventual seed producers will be selected. The average height at age 5 ranged from 4.5 m (14.9 ft) for an open-pollinated family from an Arkansas seed source growing in central Georgia to 5.7 m (18.6 ft) for another open-pollinated family from Marion County, Florida. The mean height for all loblolly families was 5 m (16.4 ft), which is excellent growth for loblolly pine. The

Table 1. Fusiform rust infection at age 3 years and height at age 5 years of 16 loblolly pine families in a rust resistant seedling seed orchard.

| Family | Rust infection (%) | Height[a] (Meters) | (Feet) |
|---|---|---|---|
| SML-9 | 2 | 4.7 | 15.5 |
| 29-R X 1495-35 | 4 | 5.0 | 16.5 |
| 10-6 | 4 | 4.8 | 15.7 |
| TDR | 6 | 5.1 | 16.7 |
| TFS | 8 | 5.3 | 17.4 |
| 1495-35 | 9 | 4.5 | 14.9 |
| 11-9 | 10 | 4.8 | 15.9 |
| 11-20 | 11 | 4.8 | 15.8 |
| 42-R | 13 | 4.8 | 15.7 |
| 10-5 | 16 | 5.5 | 17.9 |
| 15-42 | 18 | 5.3 | 17.3 |
| 2318 | 28 | 5.0 | 16.4 |
| T-605 | 32 | 5.3 | 17.3 |
| T-601 | 41 | 5.7 | 18.6 |
| 29-R | 48 | 4.6 | 15.0[b] |
| 10-31 | 56 | 5.0 | 16.4[c] |
| Mean | 19 | 5.0 | 16.4 |

[a]Mean height of tallest 10 trees.
[b]Based on 6 remaining trees.
[c]Based on 9 remaining trees.

correlation between levels of rust resistance as expressed by percent infection and the mean height of the 10 tallest trees was not significant ($r = 0.185$), indicating that the intense selection pressure for rust resistance has not had a large affect on the growth of the progeny from the rust resistant orchard.

## COMPARISON OF RUST RESISTANT SEED SOURCES

The orchards are now 14 years old and producing quantities of seeds. Enough seeds were collected in October 1987 to produce 5 million seedlings for planting during the winter of 1988-89. Eventual production will be at least 15 million seedlings annually. We compared the rust resistance of our U.S. Forest Service-Georgia Forestry Commission (USFS-GFC) seedling and clonal orchard material with that from other rust resistant seed sources. We used a CBS greenhouse inoculation test for this evaluation. Results of artificial inoculation tests have been closely correlated with field performance in several studies (Dinus & Hare 1974, Miller & Powers 1983). Seedlings inoculated in this study were obtained from six seed sources: (1) a second-generation seedling seed orchard established with progeny of selected first-generation trees that had survived an artificial inoculation test (USFS-GFS seedling orchard); (2) a second-generation clonal orchard composed of rust resistant selections from first-generation progeny tests (USFS-GFC clonal orchard); (3) and (4) two forest industry first-generation clonal orchards containing progeny-tested rust resistant trees; (5) a rogued first-generation clonal orchard of trees selected for multiple traits; and (6) the rust resistant Livingston Parish geographic seed source.

Seeds from each of the 6 sources were germinated and seedlings transplanted into plastic planting tubes 4 cm in diameter and 21 cm long (volume approx. 150 cc), one seedling per tube. The medium was a 5:4:1 mix of peat moss, vermiculite, and perlite. Basidiospore inoculum was produced by inoculating northern red oak (*Quercus rubra* L.) seedlings with a mass collection of aeciospores from rust galls in north-central Georgia. Pine seedlings were inoculated at age of 4 weeks by spraying them with a suspension containing basidiospores at $50 \times 10^3$/ml (Matthews et al. 1978). Ninety-six seedlings per seed source in each of 4 replications, or 2,304 seedlings, were inoculated. The seedlings were grown in the greenhouse for 9 months before being examined for infection. Infection data were collected and the percentage of seedlings with typical galls was determined. Data were analyzed by a 2-way analysis of variance; means were separated according to Duncan's multiple range test (Hicks 1964).

There were highly significant differences in resistance to fusiform rust among the 6 different seed sources of loblolly pine (Table 2). Average percentages of rust-free seedlings from the various sources ranged widely. Resistance of seedlings from the GFC-USFS second-generation seedling seed source in the study. Rust resistance of seedlings from both the Livingston Parish and the GFC-USFS second-generation clonal orchard sources was significantly higher than those of industry orchards and the controls. Seedlings from industry orchard No. 1 were significantly better than seedlings from both the control and industry orchard No. 2, whereas seedlings from rust-resistant industry orchard No. 2 were not significantly different from the commercial control.

Table 2. Comparison of the rust resistance of seedlings from 6 loblolly pine seed sources.

| Seed source | Percent rust free[a] |
|---|---|
| USFS-GFS (seedling orchard) | 61 a |
| USFS-GFC (clonal orchard) | 44 b |
| Livingston Parish | 44 b |
| Rust-resistant industry orchard No. 1 | 34 c |
| Rust-resistant industry orchard No. 2 | 19 d |
| First-generation loblolly production orchard (commercial control) | 13 d |

[a]Values followed by the same letter do not differ significantly at the 0.05 level as determined by Duncan's multiple range test.

We are currently testing the progeny of each potential crop tree in each seedling seed orchard block. By this method, the best 50 trees per 0.4 ha block can be selected, and levels of resistance improved even more. The current levels of resistance reduce rust prevalence by at least 50 % in comparison with first generation orchard-run seedlings. Eventual production from this rust resistant orchard will reduce infection levels by at least 75 %. Seedlings with this type of resistance can be planted in areas of very high rust hazard with good expectations of little loss to this disease. One additional bonus from using the seedling seed orchard approach has been the broadening of our genetic base, since every crop tree is a new genetic combination representing a wide assortment of male parents. New selections are now being made among these crop trees for grafting and use in next-generation clonal orchards to continue the program of rust control in the southern United States.

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# FUSIFORM RUST RESISTANCE IN WESTERN SOURCES OF LOBLOLLY PINE

## ABSTRACT

Western seed sources of loblolly pine have performed well for fusiform rust resistance in southern portions of Mississippi, Alabama, and Georgia for over 50 years. Possible explanations are presented as to why fusiform rust resistance has remained stable in these trees. Factors considered are a very favorable climate for the disease in the areas that the trees originated, a relative constant virulence of the pathogen throughout the southern pine region, and heterogeneity of the tree populations that were established with bulk seed lots.

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Loblolly pine (*Pinus taeda* L.) from western Mississippi, Livingston Parish in Louisiana, and all natural forests west of the Mississippi River has moderate to high levels of resistance to *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* compared to other sources of loblolly pine (Grigsby 1973, Wells & Wakeley 1966, Wells et al. 1982). Select trees from these high-resistance areas provide excellent sources of resistance for seed orchards. Also, stock from bulk seed collected in Livingston Parish has been planted extensively in the southern pine region because of its resistance to fusiform rust and good growth characteristics (Wells 1985). Wells (1969) recommended three seed collection areas for loblolly pine and the zones where the seed could be moved to take advantage of inherent qualities for growth, survival, and rust resistance. One of the seed collection areas defined was Livingston Parish, Louisiana (LPL). The LPL stock was recommended for an area encompassing the southern two-thirds of Mississippi, Alabama, Georgia, and northern Florida.

During the period of 1971-1980, 19 forest industries reported planting 329,603 acres with rust-resistant geographic sources of loblolly pine (Wells 1985). For the most part, the stock was planted in the zone recommended and the performance of the trees for survival, growth and rust resistance was good. Rust levels were greatly reduced after 1974 in northern Florida and Georgia and this was due to the extensive use of resistant sources of loblolly pine (Schmidt et al. 1986). The only exception to this was noted in Madison County, Florida, where LPL stock was heavily infected and the more resistant Texas sources are being used in that area (Pait & Draper 1983). Further, there are no reports of other pests (insects or diseases) that have been unusually severe on LPL stock that were planted in the areas Wells recommended.

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The use of exotic trees in large scale forestry operations has almost invariably resulted in serious pest problems (Zobel 1980). One might expect that similar problems would have occurred when the western sources of loblolly pine were moved up to 800 miles eastward from their origin. That such problems have not been encountered with these trees bears considering because this stock will likely continue to be used, if not as bulk seed lots, as components of tree breeding programs and production seed orchards.

I believe that there are at least three reasons that the resistance to fusiform rust in western loblolly pine has remained stable:

1. Weather is as favourable for the disease in the areas where the seed originated as it is elsewhere in the South.
2. The rust population in western Mississippi, Louisiana, and Texas is as virulent on western loblolly pines as any form of the pathogen that has been found.
3. Most western loblolly that has been moved eastward on a large scale has been from bulk seedlots. These heterogenous tree populations have at least two different types of resistant to *C. q. fusiforme* and as a result are strongly buffered against specialization of the pathogen.

Long periods of mild, wet weather are required for the infection process of *C. q. fusiforme* on both oaks and pines. Southeast Texas, southeast Louisiana, and western Mississippi are in a belt that receives the highest annual rainfall in the Gulf Coastal Plain during March, April, and May; the season when rust infection occurs in that area. The terrain is low and wet with many small streams and a high water table which causes standing water after rains on most forest sites. Consequently, it is unlikely the trees from these areas have encountered a climate more favourable for fusiform rust than the one in which they originated. One possible exception to this is northern Florida where dense fogs are common during the spring months. These conditions undoubtedly contribute to the poor performance of the LPL in Madison County, Florida.

When Wakeley first documented the resistance of LPL trees, he and others speculated that LPL stock might not be resistant to strains of the rust in states such as Georgia and Florida (Wakeley 1944). Although considerable pathogenic variation in *C. q. fusiforme* has since been documented on western loblolly pine trees, there is no evidence that the rust population in any part of the southern pine region is unusually virulent on these trees. We have compared rust inocula from Madison County, Florida with other sources of *C. q. fusiforme* and thus far no evidence has been found that it possesses higher virulence than inocula collected in Georgia and Mississippi[1]. There is a tendency for the rust from southeast Louisiana to be more capable of infecting LPL stock than are rust inocula from elsewhere in the southern pine region (Powers and Matthews 1980). This is thought to result from long term adaptation of the pathogen to the trees on which it has evolved and should not be interpreted as evidence that the resistance of LPL trees is eroding because of recent specialization of the pathogen (Snow 1982).

There are at least two types of resistance in western loblolly pine trees to *C. q. fusiforme* that present a strong deterrent to fusiform rust. Lack of gall development has been recognized as a type of resistance by all who have worked with these trees and

[1] Unpublished results by G. Snow and Fred Matthews.

most research results are presented as percent of trees galled. Round gall formation has more recently been considered as another type of resistance and this trait appears to be inherited separately to lack of gall development (Snow et al. 1980). There is possibly a third type of resistance that is not as well defined as the first two. One often observes that field grown trees develop less rust than would be expected from seedling inoculation tests. Whether this phenomena is related to phenology of shoot development or some physiological process not active in juvenile tissues is not known. When one considers that each type of resistance is likely controlled by an array of different genes, the probability of the rust becoming specialized to surmount all of these obstacles seems remote.

Some hard pines in eastern North America appear to have evolved to an equilibrium with the pine oak rusts (Snow 1986). Shortleaf (*Pinus echinata* Mill.), Virginia (*P. virginiana* Mill.), and sand (*P. clausa* [Chapm. ex Engelm.] Vasey ex Sarg.) pine are not severely damaged by *C. quercuum* and infection results in small round galls which affect only a small part of the tree. This situation is what would be expected in a system in which the host and pathogen are in equilibrium. The pathogen exists but is relatively innocuous.

There is a similarity between the host pathogen systems on shortleaf, Virginia, and sand pine and the western populations of loblolly pine. The frequency of resistant trees, including those with the tendency for round gall development, suggests that they too are approaching an equilibrium with *C. quercuum*. This equilibrium is likely the result of a long association of loblolly pine and the rust pathogen in natural forests. Similar equilibria no doubt exist in our southern forests for other host-pest systems.

Research to better understand how the natural host-pest systems have reached and are reaching a balance should help us do a better job of tree breeding and of managing southern pine forests.

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# SOME REMARKS ON THE PRESENT SITUATION IN FORESTRY AND THE ROLE OF FOREST PATHOLOGY

ABSTRACT

During the last few decades forest ecosystems have been severely stressed by anthropogenic air pollution. As a result the vitality of the ecosystems has decreased and the susceptibility of trees to biotic stresses has correspondingly increased. In the complex interaction of the "host-parasite-environment", remedial silvicultural measures (e.g. liming) may have very complicated, many-sided effects. Forest pathology should take an increasingly role in interdisciplinary research work aimed at elucidating the important and complex questions of forest decline.

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In many parts of the world man-made forests have primarily been established large areas with one or a few fast-growing tree species - often as pure plantations with short or medium rotation periods. This makes the stands more susceptible to frequent and extensive outbreaks of damage. The forests are world-wide strongly subjected to intensive utilization as a source of raw-materials, and as a place of recreation. Both forms of use pose a threat.

A return to natural production forest (e.g. only mixed-stand plantations, no clear-cutting etc.) is taking place only in developed and densely populated countries where the social functions of the forests are more important than those of yield production.

Numerous biotic and abiotic factors can affect forest ecosystems at the same time. Of these, anthropogenic air pollution is currently the main stress factor, especially in the northern hemisphere (Fig. 1). The increased stress brought about by air pollution means that

a) the ecological and nutrition-physiological conditions deteriorate (Rehfuess 1983, Ullrich 1981, 1983),
b) the composition of and the relationship between the species change (e.g. less or other species, respectively; saprophytic or symbiotic species become parasitic etc.)

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c) the host-plants vitality/susceptibility and
d) the aggressivity/pathogenity (virulence) of the causative-organisms (virus, bacteria, fungus) change,
e) the choice of species as host-plant increases (i.e. stenoxene species become plurivor (polyphag),
f) possibly enhancement of occurrence (i.e. regional species become cosmopolitan as a result of intensive exchange of propagation or raw material, respectively).

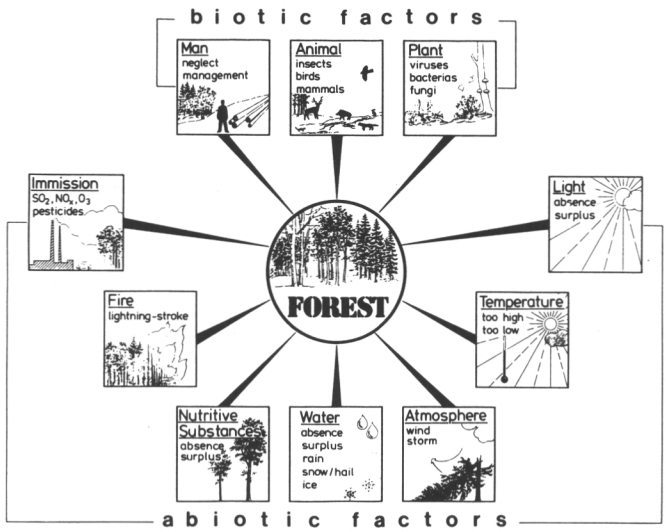



Fig. 1. Numerous biotic and abiotic factors effect the forest in a given area at the same time: emissions are currently the most important.

As a result of this situation, clear symptoms of forest decline were observed in 1988 on 25.2 % of the forest area covered by stands older than 60 years in Federal Republic of Germany (26 % in Hessen).

In this connection there is considerable discussion about whether wet and dry deposition or the biotic stresses are the main reasons for forest decline. In my opinion it is a question of a dynamic process (Fig. 2).

As the deposition of various pollutants, e.g. $SO_2$, $NO_x$, $O_3$, increases over time, the vitality of the trees/stands decreases to certain level - e.g. to point B - which can be considered as the point of no return. The absence of preventive measures before this point - e.g. liming - deposition normally leads to very serious damage in forest ecosystems.

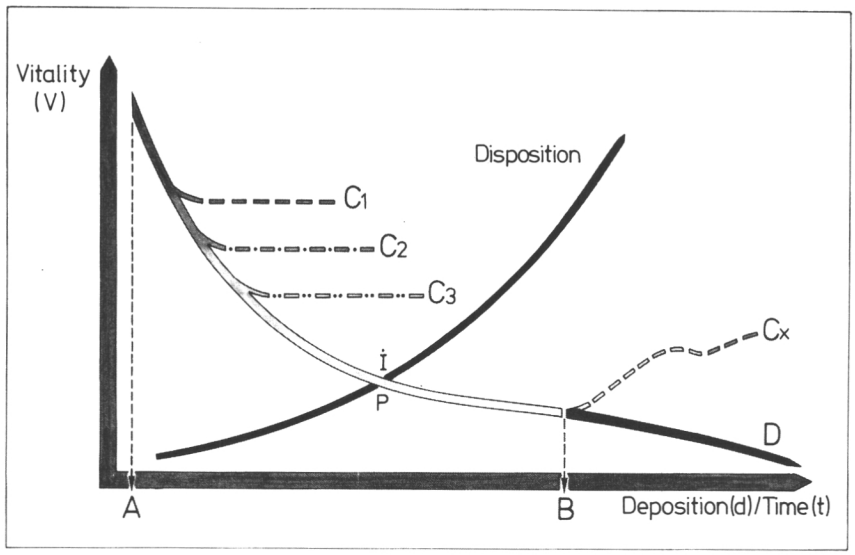



Fig. 2. As a result of increasing anthropogenic air-pollution the vitality of forest ecosystems is decreasing or the susceptibility of trees is increasing.

Along with the decreased vitality the disposition for biotic stress factors (e.g. insect pests or fungi) is rising very rapidly. At a certain level they may infect and kill the tree. In such a situation a much smaller population density of insects or infection pressure of fungi will cause serious damage. When such damage is caused by biotic factors at such a low vitality level, they are often held to be the prime cause of the diseases. However, this is only very conditionally the case.

In the complex interaction of the "host-parasite-environment" the primacy falls to the one of the three factors that deviates most strongly and for the longest time from the norm! But in a situation where these are substantial changes in the environmental conditions (e.g. very low pH values of rain, fog, and soil: leaching of nutrients, soil compaction etc.) and in host-vitality, the occurrence and virulence of the pathogens may be the decisive factors! Under these conditions forest pathology has an enhanced significance and plays an important role in the prevention and control of forest diseases.

Conifers especially suffer from a range of root and stem diseases - e.g. *Heterobasidion annosum* and *Armillaria*. We know for example that the finest roots of Norway spruce are located in the soil layer close to the surface. They can be easily damaged or infected, but we can also improve the conditions by fertilization or liming.

Liming especially frequently leads to a higher infection ratio and to the fast spreading of the mycelium of *H. annosum* in Norway spruce stems (Fig. 3 a,b; Dimitri & Schumann 1989).

Thus, in this connection the relationships are very complicated and many-sided. Forest pathology should therefore take an increasingly active role in interdisciplinary research work aimed at elucidating these important and complex questions.

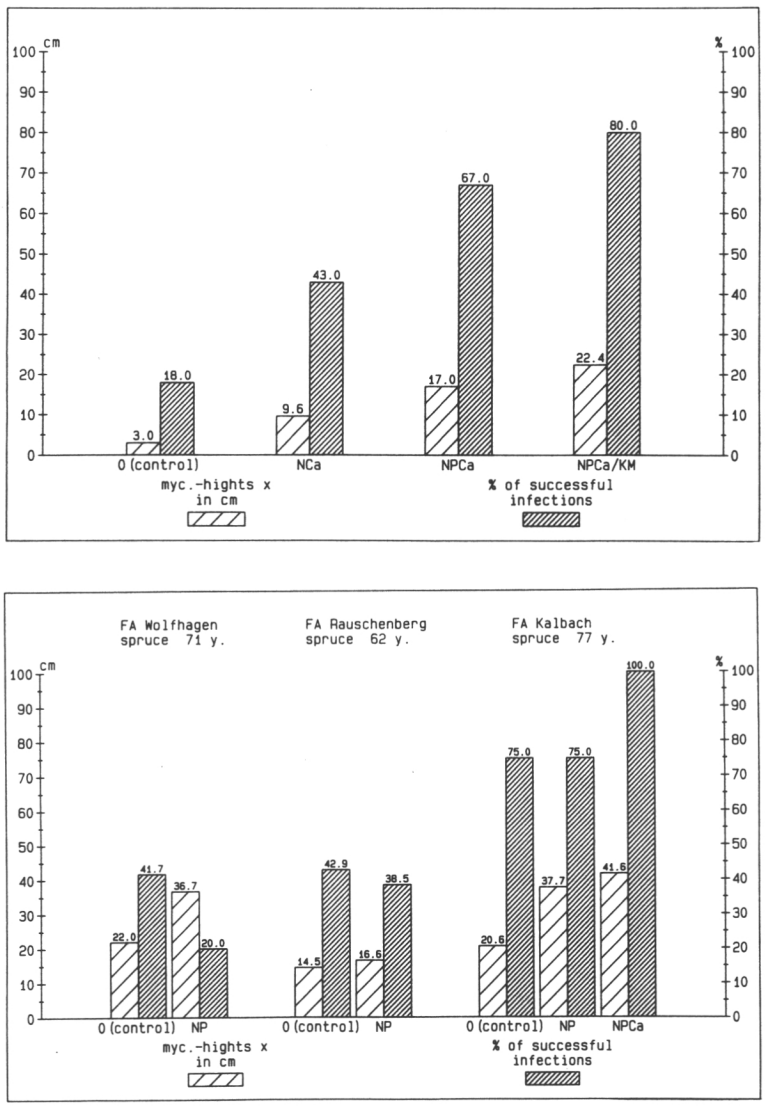



Fig. 3. The ratio of successful infection and the spread of *Heterobasidion annosum*-mycelium in trees is increased by fertilisation - especially liming - of Norway spruce stands.

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# STUDIES ON THE GENETIC STRUCTURE OF GROUPS OF SCOTS PINE TREES RESISTANT AND SUSCEPTIBLE TO *HETEROBASIDION ANNOSUM*

## ABSTRACT

Isoenzymatic studies on the genetic structure of the resistance and susceptibility to *Heterobasidion annosum* in Scots pine (*Pinus sylvestris* L.) showed statistically significant differences in the frequencies of alleles and genotypes between the groups of trees. Genetic similarity indicated that the trees differed in the genetic structure, while at the same time considerable genetic similarity was observed within the resistant or susceptible groups of Scots pine, especially when locus GOT B was taken into consideration.

## INTRODUCTION

The observed difference in the mortality of individual Scots pines attacked by *Heterobasidion annosum*, suggests that resistance and susceptibility are conditioned genetically. However, the resistance is most likely conditioned by many genes via a number of metabolic pathways. The genetic structure of the phenomenon was studied in two groups of Scots pine using isoenzymatic methods. Our material appeared to be suitable for determining the enzymatic loci of inheritance and the genetic basis of resistance and susceptibility in *Pinus sylvestris*. The second aim of our investigation was to determine whether there is correlation between the resistance or susceptibility to the pathogen and specific alleles or isoenzymatic genotypes, that could be used as markers associated with the phenomenon.

## MATERIAL AND METHODS

The plant material used in the study consisted of Scots pine trees resistant to the pathogen, selected in 1967. Two years later the experiment was established with open pollinated progeny of these trees (Białobok & Siwecki 1968, Siwecki 1983). The first

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stage of our work consisted of studies on plant material collected from 41 mother trees (M) resistant to *H. annosum*, and from 20-year-old progeny of which 50 were resistant (Pr) and 16 were susceptible (Ps) to infection. The second stage involved analysis of the genetic structure of two groups of trees from a population experiment established in Zwierzyniec, near Kórnik, and represented by 5 resistant populations and 5 susceptible ones from Kleka (K), Mragowo (M), Pniewy (P), Suleczyno (S) and Wronki (W). There were 15 individuals in each group, totalling 75 trees from the resistant populations and 75 from the susceptible ones.

Isoenzymatic analyses were conducted on extracts from winter buds on starch gel using the electrophoretic method and genetic interpretation reported by Rudin (1975), Rudin and Ekberg (1978), Yazdani and Rudin (1982), Szmidt and Yazdani (1984), Gullberg et al. (1982). The variability of 12 enzymatic loci was studied: fluorescent esterase (Fest) E.C. 3.1.1.2., 6-phosphogluconate dehydrogenase (6PGD) E.C.1.1.1.44., alkoholdehydrogenase (ADH) E.C.1.1.1.1., glutamate dehydrogenase (GDH) E.C.1.4.1.3., malate dehydrogenase (MDH) E.C.1.1.1.37 (two loci), shikimate dehydrogenase (ShDH) E.C.2.5.1.1. (two loci), diaforase (Diaf) E.C.1.6.2.2. (two loci).

The following coefficients were calculated for determining of the genetic structure of the susceptible and resistant trees: expected heterozygosity (He), observed heterozygosity (Ho) - (Nei & Roychoudhury 1974), fixation index (F) - (Jain & Workman 1967) to show whether the group of individuals is in Hardy-Weinberg equilibrium, genotypes polymorphism index (Pg) - (Khaler et al. 1980) and the genetic similarity coefficients according to Nei and Hedrick ($S_N$ and $S_H$) (Hedrick 1975) based respectively on the allelic frequencies and frequencies of genotypes in various groups of individuals. Dendrities and dendrograms were constructed on the basis of the genetic similarity coefficients, taking into consideration the genetic distances derived from these coefficients ($D_N$ and $D_H$) defined as $D = 1 - S_{(H,N)}$. The statistical significance of the differences in the frequencies of alleles and genotypes, as well as the differences in the heterozygosity indices and indices of genotypic polymorphism in the studied groups of trees, were estimated using the $chi^2$ test and U test (Gren 1972).

## RESULTS

### Genetic structure of the group of maternal trees and their progenies

In the maternal trees (M) and progeny groups (Pr = resistant and Ps = susceptible), 39 enzymatic alleles were identified in 12 loci. Only in the case of allele Diaf B2 were there statistically significant differences in the frequency of occurrence between the progenies of resistant and susceptible trees (Table 1). The most common genotypes are listed in Table 2. Out of a total number of 63 identified genotypes, 6 of them (6PGD11, 6PGD12, GOT B11, GOT B22, MDH A12 and Diaf A12) showed a statistically significant difference of occurrence between the maternal trees and resistant progenies on the one side, and susceptible progenies on the other (P = 0.01 or 0.05).

The mean observed heterozygosity (Ho) in the studied groups of individuals did not differ significantly (U-test), the values being Ho(M) = 0.319, Ho(Pr) = 0.322 and Ho(Ps) = 0.334. The value for the progeny as a whole was Ho(Pr + Ps) = 0.315 (Table 3). For individual loci, statistically significant differences were noted for 6PGD, ADH,

GOT B, ShDH A and Diaf B. In these cases the fixation index F also showed a deviation from the Hardy-Weinberg equilibrium in the progeny susceptible (Ps) to *H. annosum*. The mean genotype polymorphism indices (Pg) were about 12 % lower in the group of susceptible trees (Ps) than in resistant ones. The values of this index in this group of trees were distinctly lower for loci ShDH A and Diaf B, and at the same time higher for locus Diaf A.

Genetic similarity coefficients according to Nei ($S_N$), as well the coefficients of Hedrick ($S_H$), indicated that the studied groups of trees differed in genetic structure (Fig. 1), the greatest similarity being between maternal group of (resistant) trees (M) and the resistant progeny (Pr).

## Genetic structure of the resistant and susceptible tree groups from populations experiment

As regards allele frequency, there were significant differences by $chi^2$ test in locus B of diaforase (Diaf B) for allele Diaf B2. In the resistant population the allele was found about 4 times as commonly as in the group of trees susceptible to the pathogen. Considerable differences in the allele frequencies were also found for 6PGD3. However, in this case the statistical significance of the differences was not confirmed by the $chi^2$ test (Table 1). As regards of frequency of genotypes, statistically significant differences were found for 5 genotypes. Genotypes GOT B11 and Diaf 13 were more common in the group of resistant trees, genotypes 6PGD11 and GDH12 less common. For genotypes 6PGD 12 and GOT B12, as well as for Diaf B12 considerable differences were observed between the groups of resistant and susceptible trees. In view of their small numbers, however, the significance of the differences could not be confirmed by the $chi^2$ test (Table 2, Fig. 2). The mean observed heterozygosity (Ho) for 9 of the studied loci was very close in both groups of trees. On the other hand, considerable differences were observed when individual enzymatic loci were considered. The susceptible and resistant populations differ in heterozygosity at the following loci: F Est, GOT B, GDH and 6PGD.

The mean fixation index (F) was close to zero in both groups of individuals, indicating that they were in Hardy-Weinberg equilibrium when all studied loci were considered together. When the individual loci were considered separately, however, the value of this index deviated from zero, demonstrating an excess of heterozygotes at loci F Est and 6PGD for the resistant group of trees and at GOTB and Diaf B for the susceptible population. In the case of locus ShDH the excess of heterozygotes was observed in both the compared groups (Table 3). Genotype polymorphism indices (Pg) considered separately for individual loci indicated a lower index only for locus 6PGD in the resistant group of trees (Ppr), and a higher one in all other cases (Table 3) compared to the population susceptible to the pathogen (Pps). On the average, the resistant population had a genotype polymorphism index that was about 10 % higher than the susceptible group of trees.

Table 1. Frequencies of alleles in the groups of Scots pines. M - maternal group of trees, Pr - resistant progeny, Ps - progeny susceptible to the pathogen, Ppr - group of resistant and Pps - group of susceptible trees from the population experiment. xxx - Statistically significant differences in frequency of alleles at the level 0.05, x - differences, where significance was not confirmed by the $chi^2$ test.

| | | FREQUENCY ( 1986 ) | | | | | FREQUENCY ( 1987 ) | | |
|---|---|---|---|---|---|---|---|---|---|
| LOCUS | ALLEL | M n=82 | Pr n=100 | Ps n=32 | Pr+Ps n=132 | $chi^2$ | Ppr n=75 | Pps n=75 | $chi^2$ |
| Est F | 01 | 0.012 | 0 | 0 | 0 | | 0 | 0 | |
| | 1 | 0.720 | 0.750 | 0.750 | 0.750 | | 0.733 | 0.767 | |
| | 2 | 0.098 | 0.010 | 0.094 | 0.054 | | 0.100 | 0.100 | |
| | 3 | 0.122 | 0.090 | 0.031 | 0.060 | | 0.153 | 0.113 | |
| | 4 | 0.049 | 0.060 | 0.125 | 0.092 | | 0.013 | 0.020 | |
| 6 PGD | 1 | 0.634 | 0.750 | 0.625 | 0.688 | | 0.570 | 0.629 | |
| | 2 | 0.305 | 0.230 | 0.344 | 0.287 | | 0.394 | 0.364 | |
| | 3 | 0.061 | 0.020 | 0.031 | 0.025 | | 0.035 | 0.007 | X |
| ADH | 1 | 0.622 | 0.663 | 0.594 | 0.628 | | --- | --- | |
| | 2 | 0.378 | 0.337 | 0.406 | 0.372 | | --- | --- | |
| GDH | 1 | 0.658 | 0.660 | 0.625 | 0.643 | | 0.587 | 0.560 | |
| | 2 | 0.342 | 0.340 | 0.375 | 0.358 | | 0.413 | 0.440 | |
| GOT A | 1 | 0.976 | 0.990 | 1.0 | 0.995 | | 0.911 | 0.947 | |
| | 2 | 0.012 | 0 | 0 | 0 | | 0 | 0 | |
| | 3 | 0.012 | 0.010 | 0 | 0.005 | | 0.89 | 0.053 | |
| GOT B | 1 | 0.598 | 0.490 | 0.531 | 0.510 | | 0.640 | 0.547 | x |
| | 2 | 0.354 | 0.440 | 0.406 | 0.423 | | 0.293 | 0.447 | x |
| | 3 | 0.049 | 0.040 | 0.063 | 0.052 | | 0.067 | 0.007 | x |
| | 5 | 0 | 0.030 | 0 | 0.015 | | 0 | 0 | |
| MDH A | 1 | 0.951 | 0.930 | 0.938 | 0.934 | | --- | --- | |
| | 2 | 0.049 | 0.070 | 0.062 | 0.066 | | --- | --- | |
| MDH C | 1 | 0.744 | 0.750 | 0.781 | 0.766 | | 0.529 | 0.529 | |
| | 2 | 0.244 | 0.230 | 0.219 | 0.225 | | 0.400 | 0.355 | |
| | 3 | 0 | 0.020 | 0 | 0.010 | | 0.071 | 0.116 | |
| | 4 | 0.012 | 0 | 0 | 0 | | 0 | 0 | |
| ShDHA | 1 | 0.829 | 0.800 | 0.875 | 0.837 | | 0.845 | 0.877 | |
| | 2 | 0.110 | 0.150 | 0.094 | 0.122 | | 0.134 | 0.116 | |
| | 3 | 0.024 | 0.020 | 0.031 | 0.025 | | 0 | 0 | |
| | 4 | 0.012 | 0.010 | 0 | 0.005 | | 0 | 0 | |
| | 5 | 0.024 | 0.020 | 0 | 0.010 | | 0.021 | 0.007 | |
| ShDHB | 1 | 0.927 | 0.940 | 0.938 | 0.939 | | 0.951 | 0.950 | |
| | 2 | 0.073 | 0.060 | 0.062 | 0.061 | | 0.049 | 0.050 | |
| Diaf A | 1 | 0.695 | 0.688 | 0.625 | 0.657 | | --- | --- | |
| | 2 | 0.280 | 0.302 | 0.219 | 0.260 | | --- | --- | |
| | 3 | 0 | 0.010 | 0.156 | 0.083 | | --- | --- | |
| | 4 | 0.024 | 0 | 0 | 0 | | --- | --- | |
| Diaf B | 1 | 0.902 | 0.820 | 0.906 | 0.863 | | 0.809 | 0.850 | |
| | 3 | 0.024 | 0.050 | 0 | 0.025 | | 0.007 | 0.100 | X |
| | 2 | 0.073 | 0.130 | 0.094 | 0.112 | XXX | 0.184 | 0.050 | XXX |

Table 2. Frequencies of more common genotypes in the studied groups of Scots pine trees. See in Table 1 for explanation of Pr, Ps, Ppr, and Pps . xxx - statistically significant difference in frequency at the level 0.05, xx - at the level 0.01, differences not confirmed by the $chi^2$ test.

| GENOTYPE | | FREQUENCY 1986 | | | | $chi^2$ | FREQUENCY 1987 | | $chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | | M | Pr | Ps | Pr+Ps | i=2 | Ppr | Pps | i=2 |
| Est F | 11 | 0.561 | 0.560 | 0.563 | 0.561 | | 0.480 | 0.587 | |
| | 33 | 0 | 0 | 0 | 0 | | 0 | 0.013 | |
| | 12 | 0.122 | 0.140 | 0.188 | 0.164 | | 0.187 | 0.160 | |
| | 13 | 0.122 | 0.120 | 0.063 | 0.092 | | 0.307 | 0.200 | |
| | 14 | 0.049 | 0.120 | 0.125 | 0.122 | | 0.013 | 0 | |
| | 24 | 0 | 0 | 0 | 0 | | 0.013 | 0.040 | |
| 6PGD | 11 | 0.439 | 0.560 | 0.375 | 0.467 | XXX | 0.225 | 0.400 | XX |
| | 12 | 0.342 | 0.340 | 0.500 | 0.420 | XX | 0.620 | 0.443 | X |
| | 22 | 0 | 0 | 0 | 0 | | 0.085 | 0.143 | |
| ADH | 11 | 0.390 | 0.490 | 0.375 | 0.433 | | --- | --- | |
| | 22 | 0.146 | 0.163 | 0.188 | 0.167 | | --- | --- | |
| | 12 | 0.463 | 0.347 | 0.438 | 0.392 | | --- | --- | |
| GDH | 11 | 0.415 | 0.400 | 0.375 | 0.390 | | 0.440 | 0.293 | |
| | 12 | 0.488 | 0.520 | 0.500 | 0.510 | | 0.293 | 0.533 | XXX |
| | 22 | 0.097 | 0.080 | 0.125 | 0.102 | | 0.267 | 0.173 | |
| GOT A | 11 | 0.951 | 0.980 | 1.000 | 0.990 | | 0.822 | 0.893 | |
| | 13 | 0.049 | 0.020 | 0 | 0.010 | | 0.178 | 0.107 | |
| GOT B | 11 | 0.366 | 0.220 | 0.250 | 0.235 | XXX | 0.676 | 0.700 | |
| | 22 | 0.122 | 0.160 | 0.063 | 0.111 | XXX | 0.059 | 0 | |
| | 12 | 0.415 | 0.520 | 0.563 | 0.539 | X | 0.250 | 0.100 | XXX |
| | 13 | 0 | 0.049 | 0 | 0 | | 0.015 | 0.020 | X |
| MDH A | 11 | 0.902 | 0.860 | 0.875 | 0.870 | | --- | --- | |
| | 12 | 0.098 | 0.140 | 0.125 | 0.132 | XX | --- | --- | |
| MDH C | 11 | 0.610 | 0.600 | 0.625 | 0.612 | | 0.543 | 0.594 | |
| | 22 | 0.122 | 0.100 | 0.063 | 0.081 | X | 0.129 | 0.058 | |
| | 12 | 0.244 | 0.260 | 0.313 | 0.286 | | 0.257 | 0.232 | |
| | 33 | 0 | 0 | 0 | 0 | | 0.071 | 0.116 | |
| ShDH A | 11 | 0.683 | 0.640 | 0.813 | 0.726 | X | 0.690 | 0.754 | |
| | 12 | 0.171 | 0.220 | 0.125 | 0.148 | X | 0.268 | 0.232 | |
| | 15 | 0.040 | 0.049 | 0 | 0.025 | | 0.042 | 0.014 | |
| ShDH B | 11 | 0.878 | 0.900 | 0.875 | 0.892 | | 0.901 | 0.900 | |
| | 12 | 0.098 | 0.080 | 0.125 | 0.102 | | 0.099 | 0.100 | |
| Diaf A | 11 | 0.415 | 0.458 | 0.375 | 0.416 | X | --- | --- | |
| | 12 | 0.513 | 0.438 | 0.188 | 0.313 | XXX | --- | --- | |
| | 13 | 0.021 | 0 | 0.313 | 0.155 | | --- | --- | |
| Diaf B | 11 | 0.829 | 0.660 | 0.813 | 0.736 | X | 0.676 | 0.700 | |
| | 13 | 0.098 | 0.220 | 0.188 | 0.204 | X | 0.250 | 0.100 | XXX |
| | 12 | 0.049 | 0.100 | 0 | 0.050 | | 0.015 | 0.200 | X |

Table 3. Heterozygosity observed (Ho) and expected (He), fixation index (F), and genotype polymorphism indices (Pg) in the groups of Scots pine trees. The other denotations are as in Table 1.

| LOCUS | M | | | | Pr+Ps | | | | Pr | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | He | Ho | F | Pg | He | Ho | F | Pg | He | Ho | F | Pg |
| F Est | 0.455 | 0.390 | 0.143 | 0.646 | 0.416 | 0.394 | 0.053 | 0.635 | 0.426 | 0.400 | 0.061 | 0.637 |
| 6PGD | 0.501 | 0.463 | -0.076 | 0.672 | 0.415 | 0.424 | -0.022 | 0.586 | 0.384 | 0.380 | 0.011 | 0.566 |
| ADH | 0.470 | 0.463 | 0.015 | 0.612 | 0.457 | 0.369 | 0.193 | 0.622 | 0.447 | 0.347 | 0.223 | 0.613 |
| GDH | 0.451 | 0.488 | -0.082 | 0.580 | 0.454 | 0.515 | -0.134 | 0.571 | 0.449 | 0.520 | -0.159 | 0.563 |
| $GOT_A$ | 0.047 | 0.048 | -0.021 | 0.094 | 0.016 | 0.015 | 0.063 | 0.030 | 0.019 | 0.020 | -0.010 | 0.039 |
| $GOT_B$ | 0.515 | 0.513 | 0.004 | 0.674 | 0.561 | 0.606 | -0.080 | 0.645 | 0.564 | 0.580 | -0.029 | 0.653 |
| $MDH_A$ | 0.093 | 0.098 | -0.054 | 0.177 | 0.127 | 0.136 | -0.071 | 0.235 | 0.130 | 0.140 | -0.075 | 0.241 |
| $MDH_C$ | 0.387 | 0.268 | 0.308 | 0.552 | 0.374 | 0.303 | 0.190 | 0.549 | 0.384 | 0.300 | 0.219 | 0.561 |
| $ShDH_A$ | 0.299 | 0.293 | 0.020 | 0.498 | 0.312 | 0.287 | 0.080 | 0.493 | 0.337 | 0.320 | 0.049 | 0.537 |
| $ShDH_B$ | 0.135 | 0.098 | 0.274 | 0.219 | 0.115 | 0.091 | 0.209 | 0.192 | 0.113 | 0.080 | 0.291 | 0.183 |
| Diaf. | 0.438 | 0.561 | -0.281 | 0.562 | 0.467 | 0.469 | -0.004 | 0.650 | 0.435 | 0.458 | -0.052 | 0.591 |
| Diaf. $^{A}_{B}$ | 0.180 | 0.147 | 0.183 | 0.300 | 0.277 | 0.288 | -0.040 | 0.463 | 0.308 | 0.320 | -0.038 | 0.506 |
| - | 0.331 | 0.319 | 0.036 | 0.466 | 0.333 | 0.315 | 0.054 | 0.473 | 0.333 | 0.322 | 0.033 | 0.474 |

Table 3. (cont.)

| | Ps | | | | Ppr | | | | Pps | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOCUS | He | Ho | F | Pg | He | Ho | F | Pg | He | Ho | F | Pg |
| F Est | 0.412 | 0.375 | 0.090 | 0.624 | 0.429 | 0.520 | -0.213 | 0.640 | 0.389 | 0.400 | -0.028 | 0.588 |
| 6PGD | 0.490 | 0.563 | -0.149 | 0.601 | 0.518 | 0.690 | -0.333 | 0.553 | 0.472 | 0.457 | 0.032 | 0.623 |
| ADH | 0.482 | 0.438 | 0.092 | 0.632 | --- | --- | --- | --- | --- | --- | --- | --- |
| GDH | 0.469 | 0.500 | -0.067 | 0.594 | 0.485 | 0.293 | 0.395 | 0.805 | 0.493 | 0.533 | -0.082 | 0.600 |
| $GOT_A$ | 0 | 0 | --- | 0 | 0.162 | 0.178 | -0.098 | 0.293 | 0.101 | 0.107 | -0.056 | 0.191 |
| $GOT_B$ | 0.549 | 0.688 | -0.253 | 0.601 | 0.500 | 0.453 | 0.093 | 0.683 | 0.502 | 0.587 | -0.170 | 0.582 |
| $MDH_A$ | 0.116 | 0.125 | -0.076 | 0.219 | --- | --- | --- | --- | --- | --- | --- | --- |
| $MDH_C$ | 0.342 | 0.313 | 0.085 | 0.507 | 0.556 | 0.543 | 0.023 | 0.617 | 0.581 | 0.594 | -0.023 | 0.577 |
| $ShDH_A$ | 0.225 | 0.188 | 0.163 | 0.319 | 0.268 | 0.310 | -0.158 | 0.410 | 0.218 | 0.246 | -0.132 | 0.377 |
| $ShDH_B$ | 0.116 | 0.125 | -0.076 | 0.219 | 0.094 | 0.099 | -0.052 | 0.178 | 0.095 | 0.100 | -0.053 | 0.180 |
| $Diaf._A$ | 0.537 | 0.500 | 0.069 | 0.710 | --- | --- | --- | --- | --- | --- | --- | --- |
| $Diaf._B$ | 0.170 | 0.188 | -0.104 | 0.304 | 0.312 | 0.265 | 0.151 | 0.477 | 0.265 | 0.300 | -0.132 | 0.460 |
| - | 0.326 | 0.334 | -0.025 | 0.444 | 0.369 | 0.372 | -0.009 | 0.517 | 0.346 | 0.369 | -0.067 | 0.464 |

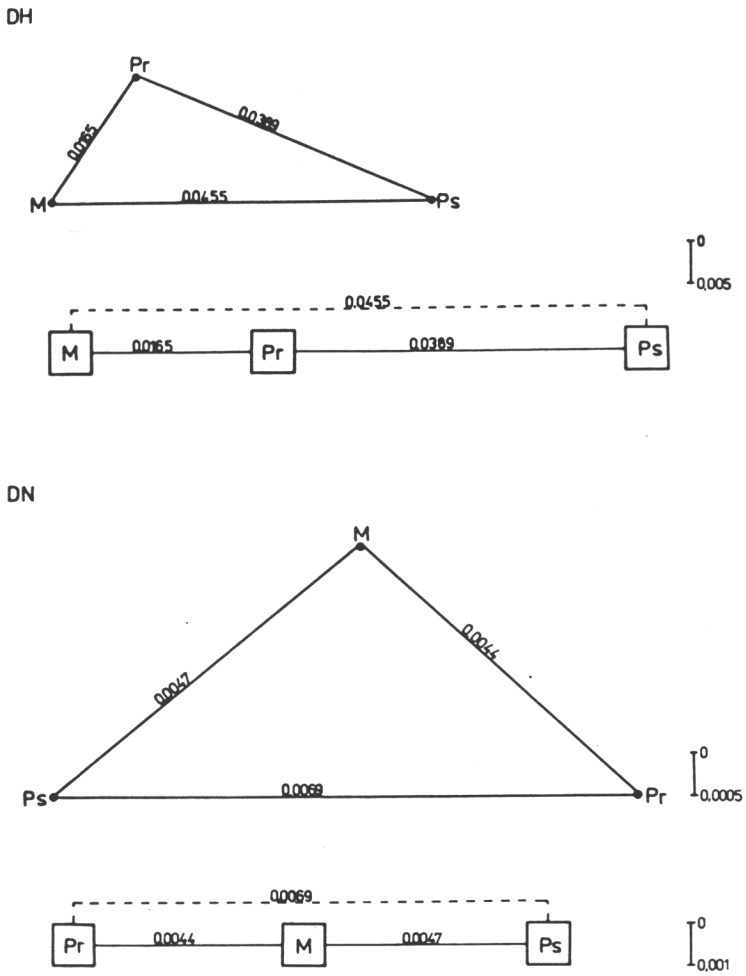



Fig. 1. Genetic distances according to Hedrick (DH) and according to Nei (DN) between the groups of trees. M - maternal group of trees, Pr - resistant, and Ps - progeny susceptible to the pathogen.

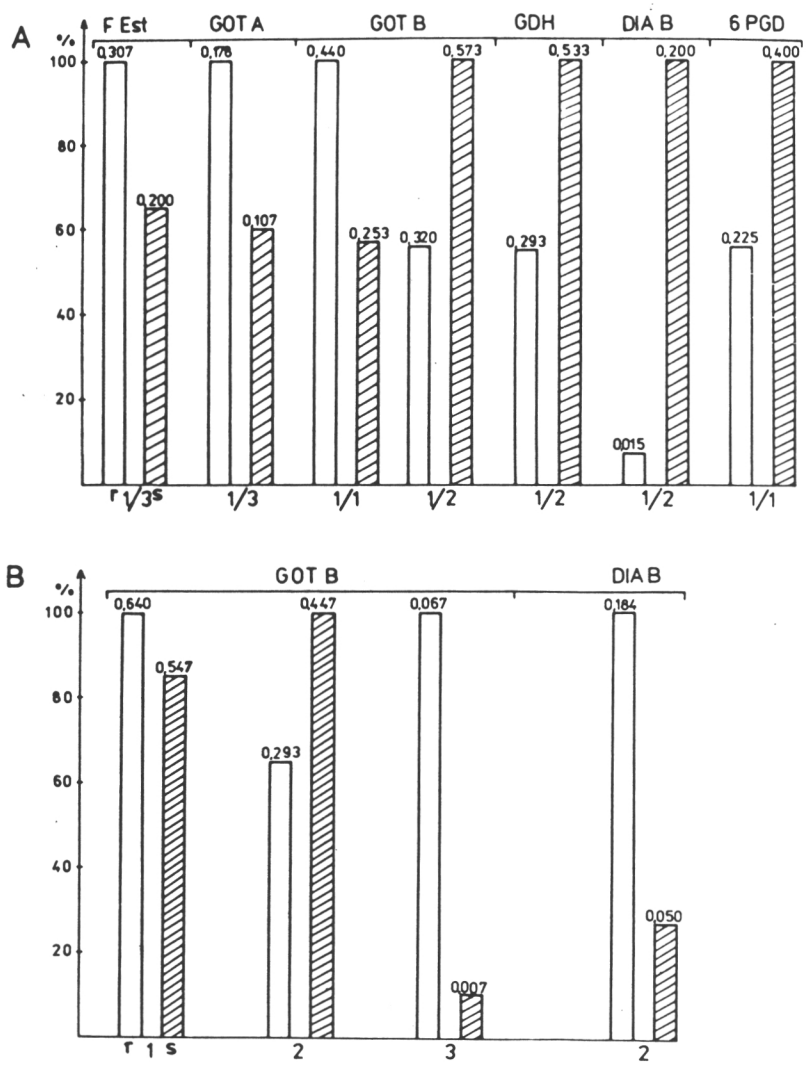



Fig. 2. Differences in frequencies of some isoenzymatic genotypes (A) and alleles (B) in the groups of resistant (r) and susceptible pines (*Pinus sylvestris*).

The genetic similarity coefficients for 9 loci indicated that the susceptible and resistant groups of trees differed in genetic structure. This also suggests that there is a tendency of resistant or susceptible trees to group together. The most distinct grouping was observed in the case of locus F Est and GOT B on the basis of Nei's genetic similarity coefficients, i.e. on allele frequencies (Fig. 3 and Fig. 4).

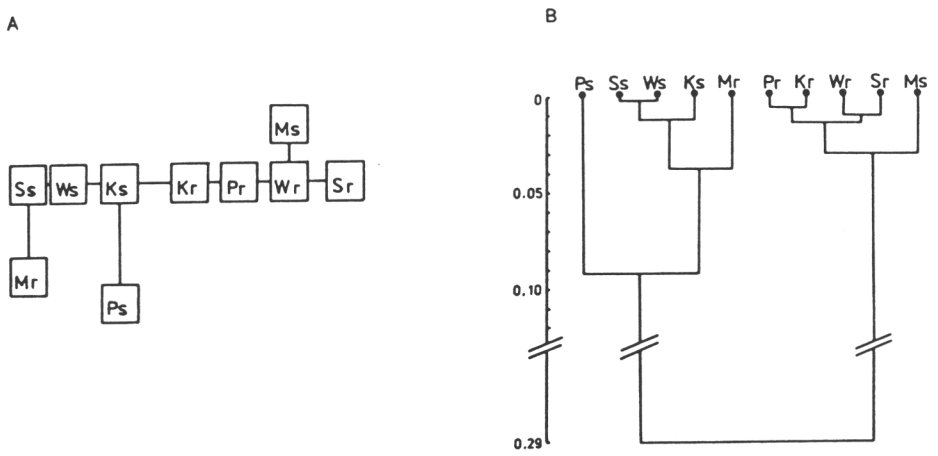



Fig. 3. Dendrites and dendrograms showing the genetic distances according to Nei between the resistant (r) and susceptible (s) groups of trees from K - Kleka, W - Wronki, P - Pniewy, M - Mragowo, S - Suleczyno. Based on frequency of alleles in locus GOT B.

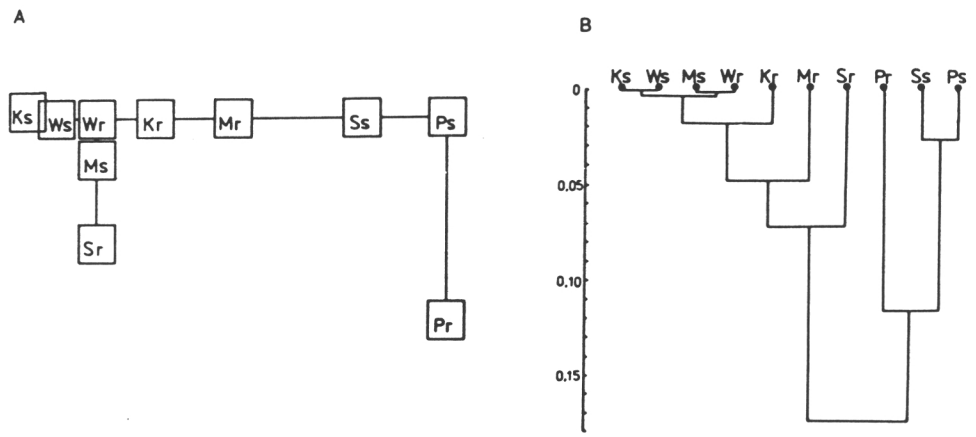



Fig. 4. Dendrites and dendrograms showing the genetic distances between resistant (r) and susceptible (s) groups of trees from different localities according to Nei, based on the frequency of alleles in locus F Est. See Fig. 3. for explanation

## DISCUSSION AND CONCLUSIONS

Studies on the genetic structure of susceptible and resistant groups of trees to pathogenic agents and environmental pollution are important not only from the point of view of understanding the adaptation processes in plant populations where such factors act. The practical implications of such studies may be important to silviculture (Siwecki & Prus-Głowacki 1988, Prus-Głowacki & Nowak-Bzowy 1989). While we are aware of the fact that resistance mechanisms are most likely conditioned by the action of many genes and that they occur at many levels of organismic organization and in various metabolic pathways, searching for correlation between specific genetic parameters, such as allele and genotype frequencies, heterozygosity and genotype polymorphism on the one hand, and susceptibility or resistance to pathogenic agents on the other, appears to be very important. Our studies, even though not conducted on a very extensive material, seem to indicate that at least some of the studied enzymatic loci can be used as markers correlated with resistance to *H. annosum*. In two experimental series the allele and genotype frequencies involving locus GOT B and Diaf B significantly differentiated between the groups of resistant and susceptible trees to *H. annosum* (Tables 1 and 2, Fig. 3).

The genetic similarity indices ($S_N$ and $S_H$) indicate that the groups of trees differ in this genetic structure, while at the same time there is considerable similarity within groups of trees resistant or susceptible to the pathogen. This similarity is particulary striking when we take into consideration locus GOTB (Fig. 3). This phenomenon may indicate a differential process of selection within the compared groups of individuals in the Zwierzyniec experimental area. Conformation of our observations requires, however, further studies on wider material using additional gene markers.

## ACKNOWLEDGEMENTS

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# TISSUE RESPONSES OF THIN ROOTS OF SCOTS PINE TO INFECTION BY *HETEROBASIDION ANNOSUM*

## ABSTRACT

Histological studies were conducted on Scots pine seedlings infected by different strains of *Heterobasidion annosum*. The penetration and colonization of the roots were associated with alterations in the structure of the cortical cells: The changes consisted of vacuolization and accumulation of polyphenols that interfere with similar changes connected with the natural process of senescence of the root cortex. The seedlings differed from each other in the intensity and extent of these reactions.

The process of compartmentalization in response to infection by *H. annosum* in field condition was described.

## INTRODUCTION

Older trees are highly tolerant to rot due to the mechanism of compartmentalization (Shigo 1982, 1983) which enables them to restrict the spread of the rot to the center of the trunk. This mechanism enables a tree to continue to grow until the period of reproduction is reached. The defence system seems to be so efficient that the development of other systems designed to ensure the survival of the species against rot fungi should not be expected during phylogenesis. If the above hypothesis is correct, it would be important to look for useful resistance factors in the root that could be utilized in plant breeding against the disease.

According to Hodges (1969) and Garret (1970) the susceptibility of *Pinus* spp. to *H. annosum* first appears only in the root system. Both fine roots and feeder roots show a relatively high resistance to infection. However, it can be overcome by a higher infection potential (Dimitri 1963, Braun and Lulev 1969, Zycha 1976). This suggests that resistance against *H. annosum* is conditioned by the activity of the living tissues.

Absence of the disease symptoms that are usually identified with heartrot formation, does not necessarily prove that the root zone of young trees is not infected. Manka (1970) found differences in the functional structure of the fungal communities operating in forest stands. In healthy pine stands microorganisms had a strong antagonistic effect on the growth of *H. annosum*, whereas in stands heavily attacked by the pathogen the influence of microorganisms was quite the opposite. He concluded that the functional structure of the fungal community influenced the growth of *H. annosum* in the rhizosphere of thin roots. Anticipating the existence of the fungus on the pine roots (Orłos and Dominik 1960), an explanation is needed about what part of the root

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system is colonized and under what conditions can the fungus survive in the younger or older roots before breaking through into the trunk.

The properties of the root environment, root anatomy and physiology are certainly of great importance for the process of infection. Unfortunately there is little information available about the physiological effects of fungal invasion in roots, and about the interactions between the fungus and root tissues during the earliest stages of root infection. The reason for this lack of knowledge is the difficulties involved in establishing a controlled environment where the influence of the soil and root microflora could be eliminated.

Werner (1987) demonstrated the differences in the reactions of *in vitro* grown pine seedlings to infection by four strains of *H. annosum*. Investigations on the resistance of seedlings growing in pure cultures on agar medium enable the process of infection to be studied under controlled conditions, as well as to avoid the stress caused by wounding. Many variable factors that influence and modify the resistance of the host can be eliminated in this way. Owing to the young age of the plant material, the infection of the roots corresponds to the feeder roots of older trees. The possibility of *H. annosum* penetrating these roots has not been investigated satisfactorily yet, and it is very difficult to make observations of this process under natural conditions.

## MATERIALS AND METHODS

The material of the present study represented open-pollinated, half-sib families of resistant and susceptible Scots pine individuals growing in forest stands at Wronki and Kleka (Białobok and Siwecki 1969, Siwecki 1983, Werner 1987).

**Scots pine seedlings.** The seeds were sterilized with 2 % $HgCl_2$ for 4 min. and subsequently washed three times (15 min. each) in sterile water. The seeds were germinated in Petri dishes on 0.6 % agar medium, in darkness at 24 °C. The germinated seeds were then transferred under sterile conditions to 300 x 30 mm test tubes containing starvation agar medium (15 g agar x 100 µg thiamine in 1 l $H_2O$) according to the method of Pachlewska (1968). The seedlings were grown in controlled conditions at 20-24 °C with 1800 lux light (14 hour per day), 80 % RH. After one month the seedlings were inoculated with four *H. annosum* isolates obtained from diseased trees growing at four localities.

The seedlings for the histopathological studies were taken each week during the early stages of the experiments, and then each month.

Fragments of the roots were fixed in FAA. After embedding in paraffin and cutting on a microtome, the sections were stained with Conant's quadruple stain. Polyphenols were detected with toluidine blue (O'Brien et al. 1964).

For the ultrastructural studies small fragments of the seedling roots were fixed in 4 % glutaraldehyde in 0.05 M cacodylate buffer, at 4 °C for 24 h. After being rinsed in buffer, the tissue was secondarily fixed with 2 % $OsO_4$ in 0.1 M cocadylate buffer (4 °C for 2 h) and contrasted in 2 % uranyl acetate. Dehydration was performed by sequential treatment with ethanol, acetone and propylene oxide. After embedding the root fragments in an eponic mixture, they were cut on an LKB microtome. The sections were stained in uranyl acetate and lead citrate according to Reynolds (1963). The microphotographs were made under a JEM 7 b electron microscope.

**Thin roots of 20-year-old trees.** The thicker roots were taken from 20-year-old Scots pine trees growing in an experimental plot in Zwierzyniec forest, near Kórnik. The trees represented open pollinated $F_1$ progeny from trees selected at 5 localities in Poland and artificially inoculated with *H. annosum* in 1973 (Siwecki 1983). Roots of between 0.8 and 1.5 cm in diameter were collected from trees with visible signs of resin flow and a general appearance suggesting advanced progress of the disease. Similar roots from healthy trees were taken as controls.

Fragments of discoloured or necrotic roots were placed on a selective agar medium (Kuhlman and Hendrix 1962) in order to identify *H. annosum*.

Part of the root samples was cut by hand with a razor blade and stained in 0.05 % toluidine blue 0 at pH 4.5 in order to detect the presence of polyphenols. For light microscopy, the roots were fixed in FAA and embedded in paraffine. Sections 10-20 µm thick were stained with safranine and fast green. Suberine was localized using Sudan black B in 70 % ethanol.

## RESULTS AND DISCUSSION

**Infection of seedlings by *H. annosum*.** The infected seedlings were divided into three groups based on the symptoms of the disease and ethiological features.

The seedlings in the first group died within a few days to several weeks after inoculation. The tips of the cotyledons of dying seedlings were initially discolored greyish-green, yellow and later brown. The brown colour then subsequently spread over the whole plant. Some seedlings wilted early on even before these symptoms became apparent. The roots were overgrown by the mycelium in form of a fine weft. In the case of seedlings from the second group the fungus covered the roots at varying intensity. They were generally able to survive for several months after inoculation. The third group was formed of seedlings classified as resistant. In most of these seedlings the fungus induced abundant dichotomy of short roots. This phenomenon was observed almost at the same as growth resumption after a period of dormancy. In cases where many repeatedly dichotomous roots were produced, the seedlings were in good condition and developed as satisfactorily as the control seedlings.

The cells of the root cap, as well as the cells of the superficial layer of the cortex, are continuously sloughing off. They are a source of nutrients for epiphytic mycelium (Fig. 1). With an appropriate inoculum potential, penetration into the cortical cells took place (Fig. 2). The sites of primary infection occurred at random and thus were not easy to determine. Histopathological studies revealed, however, that the period between inoculation and penetration varied from several hours up to more than ten days. It depended on the pathogenicity of the isolate and the defensive ability of the host, since the experimental conditions were identical. In cases where the amount of phenolic substances in the layer of sloughing cortical cells was higher, the growth of the mycelium was clearly slower and the infection started at a later date.

Generally the fungus entered the susceptible plants at the root-collar. The hyphae spread successively from one cell to another through the walls and the pits. This type of growth enabled rapid spread of the fungus towards the phloem and xylem elements, causing the death of the lower parts of root without any visible signs of colonization by

the hyphae. As a result of this kind of attack, the seedlings died early and showed typical wilting symptoms.

The presence of the mycelium on the roots of the more resistant seedlings was associated with changes in the metabolically active cortical cells. The changes consisted vacuolization and the accumulation of polyphenolic compounds and a simultaneous decrease in the starch content (Fig. 3). As a result of the penetration of the pathogen and its radial spreading these alterations also affected deeper layers of the parenchyma. The seedlings were characterized by the intensity and extent of these reactions. The speed at which the mycelium spread in and on the roots depended to a great extent on the intensity of the necrotic reactions.

In the case of short roots, as well as old parts of the lateral roots of resistant seedlings, the hyphae usually colonized only the second and eventually the third layer of the cortical cells, where their growth terminated (Fig. 4). The presence of highly degenerated hyphae suggests that some lytic processes can take place in that area. The cells of these layers of the parenchyma contained more phenolic compounds compared to those lying outside. Sequential changes in the structure of the polyphenols, followed by their disappearance, as observed in the superficial cortical cells, suggests the active participation of the fungal enzymes (Fig. 5).

It can be concluded from these observations that the accumulation of polyphenolic compounds in the parenchymatic cells resulted from two overlapping processes. The first was closely related to the process of senescence of the cortical cells. The second depended on the activity of the pathogen, and displayed some characteristics of a hypersensitive reaction. As the final products of these two processes are similar, it would appear that the parasite intensified the normal ageing processes of the root parenchyma and, in this way, participated in the formation of induced resistance factors.

Metacutinization in the slowly growing roots was much faster than that in the fast growing roots. This had a significant effect on the rate and way in which the fungus colonized both types of roots. The importance of the process of metacutinization in the infection of roots by microorganisms has been emphasized many times, most recently by Kottke et al. (1986).

Term "Mycorrhizal Infection Zone" (abbrev. MIZ) is used in the investigation of mycorrhizae (Marks and Foster 1973). It corresponds anatomically with the section of "white tissue" lying between the growing root apex and the point where the cortical cells begin to display symptoms of ageing and turn brown due to the accumulation of polyphenols. It is of particular interest that an association considered to be pseudomycorrhizae was most frequently observed just within this zone in the roots of the resistant seedlings. It was characterized by thick hyphae that grew mostly inside the cells into which they usually had entered through the pits. No cell wall damage was observed, and there was only a small quantity of polyphenols in the cells occupied by the hyphae (Figs. 6 and 7).

Digestion of the cell walls observed in older parts of the roots indicates an increase in the cellulolytic ability of the fungus in the later stages of the host/pathogen association.

Horton and Keen (1966) presented the opinion that the suppression of cellulase activity is dependent on the ability of the host to keep the fungus supplied with carbohydrates, and the ability of the fungus to extract and metabolise them. In the case of the pseudomycorrhizae it seems very probable that the development of an apparent

balance, followed by transition of the fungus into the stage of destructive activity in the older parts of the roots, is dependent on the same factor. One indirect evidence for this was the fact that the pseudomycorrhizae were not widespread in all types of root system branching, but were confined to those cells which contained sufficient amounts of sugar that had not been converted into phenolic compounds. This means that there are different conditions for the fungus in different parts of the root system, depending on the age of the roots and on the amount of sugars and phenolic compounds. Some undetermined relationship between these factors could be a precondition for the appearance of the pseudomycorrhizae.

The duration of the apparent state of balance is therefore dependent on the physiological stage of the root, host resistance and the pathogenicity of the fungus. The mechanisms responsible for maintaining this equilibrium between the two organisms were overcome by the increase in the amount of infecting material, followed by increased production of toxins and the activity of fungal enzymes. The toxicity of the oxydated and subsequently polymerized phenolic compounds due to the activity of laccase, could be a decisive factor controlling further stages of infection.

**Responses of woody thin roots to infection by *H. annosum*.** Study of the anatomy of thin roots invaded by *H. annosum* in field conditions resulted in the recognition of certain characteristics of the wood formed after the time of infection that may help to explain the resistance of these roots.

According to the CODIT concept, decay in the stems or roots is restricted to compartments (Shigo 1975, Shigo and Marx 1977). The sheats of tissue that act as boundaries between the wood undergo discoloration, and the healthy wood formed prior to the infection is known as walls 1, 2 and 3. The weakness of these walls is responsible for radial and longitudinal spread of the parasite, and consequently for the formation of heartrot. Creation of wall 4, known as the "barrier zone" (Shigo and Larson 1969) is connected with the cambium activity at the time of infection or injury.

Infection of the small roots (about 0.5 cm in diameter) took place when the roots were 2-year-old. This is confirmed by the lack of concentricity in the structure of the wood. The number of growth rings on the side opposite the point of infection indicates the duration of the process of infection. The first cell layers of the "barrier zone" produced after infection were longitudinal parenchyma that formed a capsule around the tracheids occupied by the hyphae. The parenchymatic cells on the xylem side of the vascular cambium evidently differentiated and formed the structure of "protective nature", that led to localization of the fungus inside the damaged wood (Fig. 8).

In general the barrier zone in thin roots should be regarded as a modified complete growth ring, which plays a protective role in limiting the spread of toxins and fungus into the wood formed after the time of infection. It included longitudinal parenchyma, numerous rows of radial parenchyma, many resin ducts and disordered abnormal tracheids. The contents of the parenchyma cells stained blue-green with toluidine blue, thus indicating a high concentration of polyphenols. The dark stained material inside the lumens of the dead cells lying close to the necrosis indicates that the death of these cells is the result of a hypersensitive reaction (Fig. 9).

Parenchymatic cells may play an important role in restricting the diffusion or spread of autolytic or toxic substances from resin-soaked wood into new wood formed after the fungal invasion.

The walls of tracheids in the wood invaded by fungi are more lignified. Some of the tracheids are resin - impregnated (Fig. 10). These are the symptoms of the higher production of phenolics due to the shift in oxidative metabolism from glycolysis and TCA cycle pathways to acetate and pentose shunt shikimic acid pathways (Kuc 1967).

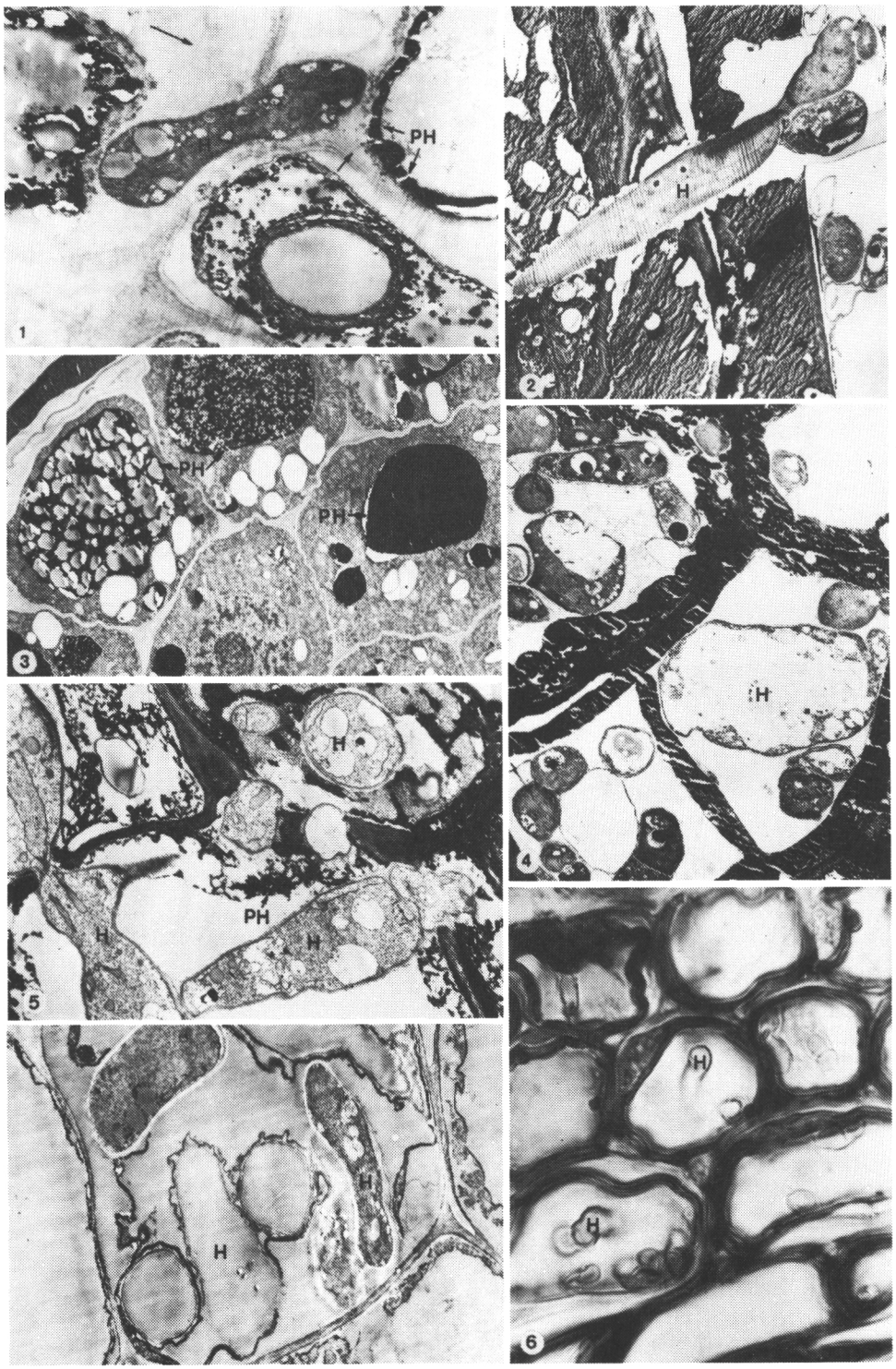
In the case of thicker roots (up to 1.0 cm in diameter), the barrier zone included scattered cells of longitudinal parenchyma formed as the first layer. The protection wood was composed of compacted, misshapen and misaligned tracheids, more numerous vertical resin ducts and an increased number of rows of radial parenchymatic cells (Fig. 11). Many tracheids occluded by resin or oxidized polyphenols were observed in wood formed prior to the barrier zone. Such blocking of tracheids was common in areas adjacent to the decay and inside the barrier zone (Fig. 12).

In the case where the fungus invaded the roots directly through the periderm the hypersensitive reaction of the bark cells resulted in the formation of an extensive necrosis. Below the necrosis, on the border of the dead and healthy bark, the phellogen was restored. Initially it formed the cells of the necrophylactic periderm that walled of the dead tissue. The cork cells formed later on were identical to the exophylactic periderm. These cells were optically dense due to the presence of flobaphenes (Fig. 13). When the extent and intensity of the necrotic reactions in the bark were sufficient to restore the phellogen, the parasite failed to enter the cambium and the wood.

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Fig. 1. A hypha (H) developing in the walls of the cells of the root cap. The cell walls are decondensed and the microfibrils (arrows) are visible. Vacuoles are filled with phenolics (PH). (9,000×).
Fig. 2. Hypha (H) crossing over superficial layers of cortical cells filled with polyphenols; (6,700×).
Fig. 3. The process of vacuolization and accumulation of polyphenolic compounds (PH) occuring in the parenchymatic cells of lateral roots; (4,200×).
Fig. 4. Hyphae (H) inside the lumens of cortical cells filled with polyphenols; (4,000×).
Fig. 5. Destruction of the phenolic compounds (PH) due to the activity of fungal enzymes; (4,500×).
Fig. 6. Pseudomycorrhizae. The presence of thick hyphae (H) inside cortical cells does not cause any apparent alterations in the structure of their protoplasts; (1,000×).
Fig. 7. Pseudomycorrhizae. The hyphae (H) are surrounded by the invaginated host membranes. Note the small quantity of polyphenols; (7,000×).


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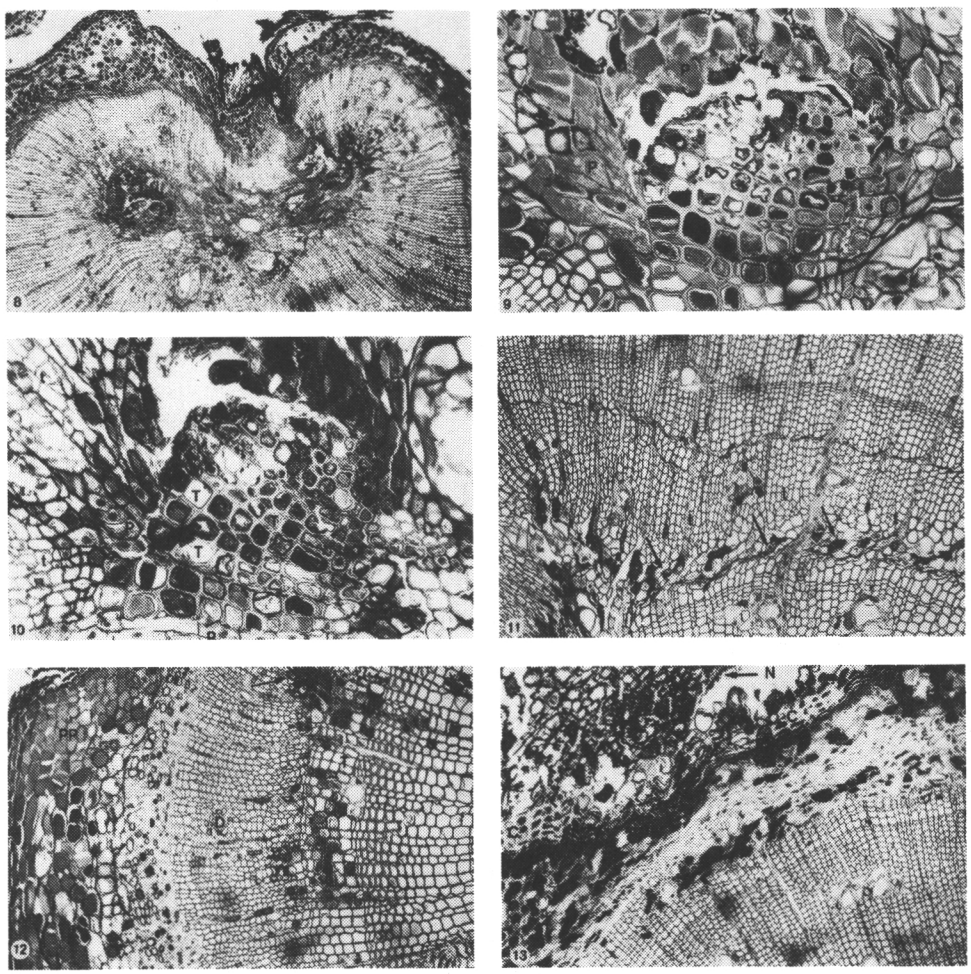



Fig. 8. Transverse section of a root invaded by *H. annosum*; (32×).

Fig. 9. Necrotic part of a root surrounded with parenchyma (P). The living cells are filled with polyphenols. On the border of the necrosis some dead, dark stained cells are visible; (200×).

Fig. 10. Fungus invaded section of wood. The walls of the tracheids (T) are thicker and their lumens are resin-plugged. Polyphenol rich parenchyma separates the necrose from misshapen, disordered tracheids (t) differentiated after parenchyma (P); (200×).

Fig. 11. Transverse section showing new growth rings formed as a reaction to invasion by the pathogen. The barrier zone exhibit scattered cells of parenchyma (P). Numerous rows of radial parenchyma (arrows) and abnormal tracheids of protection wood (t); (100×).

Fig. 12. Hand-cut transverse section of thicker root (1,5 cm in diameter) showing the barrier zone. Arrow indicates parenchyma cells (P) lying close to the injury. Parenchyma cells stained dark blue-green, indicates the presence of polyphenols (toluidine blue pH 4,5). PP, phloem parenchyma; T, tracheids formed before the fungal invasion (arrows), part of them are resin-plugged (R); t, tracheids of protection wood; D, resin ducts; (100×).

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# INTERRELATIONS OF *PINUS SYLVESTRIS* SEEDLINGS WITH *HETEROBASIDION ANNOSUM* AND "MYCORRHIZA FRANK" AT THE CELL LEVEL

## INTRODUCTION

A biological control method for protecting Scots pine seedlings against the root-rot fungus *Heterobasidion annosum* has been assessed at the All-Union Research Institute of Silviculture and Forestry Mechanization in Pushkino. The method involves inoculating the soil with a mycorrhizal fungus "Mycorrhiza Frank" (class Fungi Imperfecti; Gundaeva et al. 1981) at the time of sowing.

The aim of this study is to investigate the mode of infection of these fungi in the roots of Scots pine seedlings by electron microscopy.

## MATERIAL AND METHODS

The Scots pine *(Pinus sylvestris* L.) seedlings were grown in the laboratory on Melin-Nilsson synthetic medium (Shemakhanova 1962) under sterile conditions at 20-22 °C. The light conditions were typical for the summer months. The various experiments included simultaneous inoculation of the seedlings with "Mycorrhiza Frank" and *H. annosum*, propagated on 4 % wort agar, and a separate inoculation of these fungi. The control seedlings were inoculated with sterile wort agar. At the moment of inoculation the seedlings were 1.5 months old.

One month later the vitality of the seedlings was determined and found to be "slightly weakened". The root tips of the seedlings were fixed with glutaraldehyde and 2 % $OsO_4$, stained with uranylacetate, and examined by electron microscopy.

## RESULTS AND DISCUSSION

A preliminary investigation of the pure cultures of the fungi under study showed that their hyphae can be identified on the basis of their ultrastructure. The hyphae of "Mycorrhiza Frank" are dark-coloured, have more electron-dense cytoplasm, and the septal pores are of the simple type (Fig. 1 A, B). A distinguishing characteristic of *H. annosum* is the presence of thickenings in the pore structure, i.e. dolipores which are typical for the basidiomycetes (Fig. 1 C).

The micrographs of the cells of artificially inoculated seedlings showed that, at the moment of sampling, both fungi occupied the slime around the root, as well as the

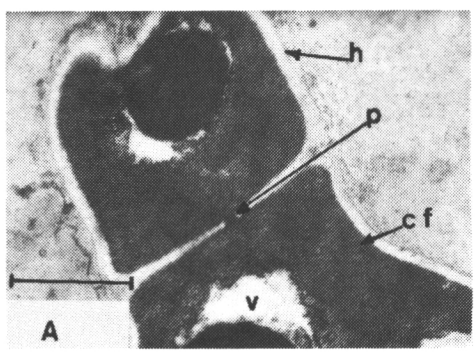
intercellular living tissues and degraded cells of the root bark. Both fungi had preserved their characteristic ultrastructure (Figs. 2 and 3). Where simultaneous inoculation had been made, both fungi were detected in the roots of the same seedlings but were never found in the same cell or adjacent cells.

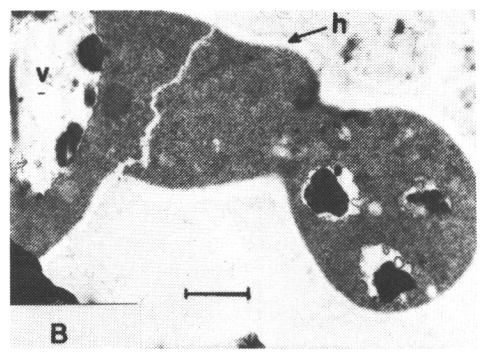
All inoculated seedlings exhibited a protective response to the intrusion of both fungi, manifested by tannin exudation accumulating along the vacuole edge and at the cytoplasm membrane of the root cells (Fig. 4).

The micrographs (Figs. 2 and 3 D, E) show the mycelium of *H. annosum* and "Mycorrhiza Frank" in the intercellular space of the plant tissue. The same structural peculiarities are typical of the pine ectotrophic mycorrhiza, the so called Hartig net, formed by certain basidiomycetes. The thin walls of the fungi are typical of an attacking fungus. The attack of the fungus is reflected in the degraded cells of the plant (Fig. 3 B, C). Fungi displaying similar slightly parasitic behaviour, as observed in the present experiment, are described by Meyer (1984) as being unstable and unhealthy for the plant. Meyer calls this phenomenon an ectoendotrophic mycorrhiza.

Thus, under the same artificial conditions, both a pathogenic and a mycorrhizal fungus behave in the same way, i.e. by penetrating the root of the seedling they cause a similar response in the plant, creating a situation similar to the ectoendotrophic mycorrhiza.

It would be of interest to carry out comparable experiments under field conditions.



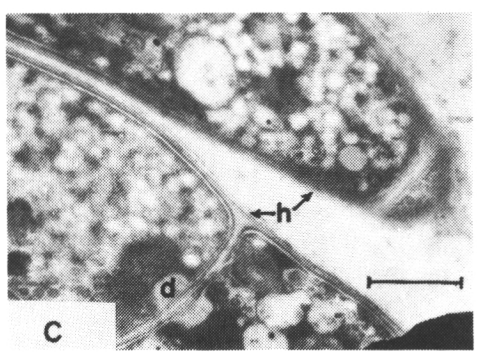




Fig. 1. The hyphae in pure culture: A and B - "Mycorrhiza Frank". Cells with electron-dense cytoplasm, characteristic vacuoles and a septal pore of the simple type. C - *H. annosum*. Organelles, granules of stored nutrients and a dolipore. Abbreviations to all figures: h - hyphae; v - vacuole; p - pore; d - dolipore; cf - cytoplasm of the fungus; w - cell wall of the plant; cp - cytoplasm of the plant; t - tannins. Scale 1 μm.

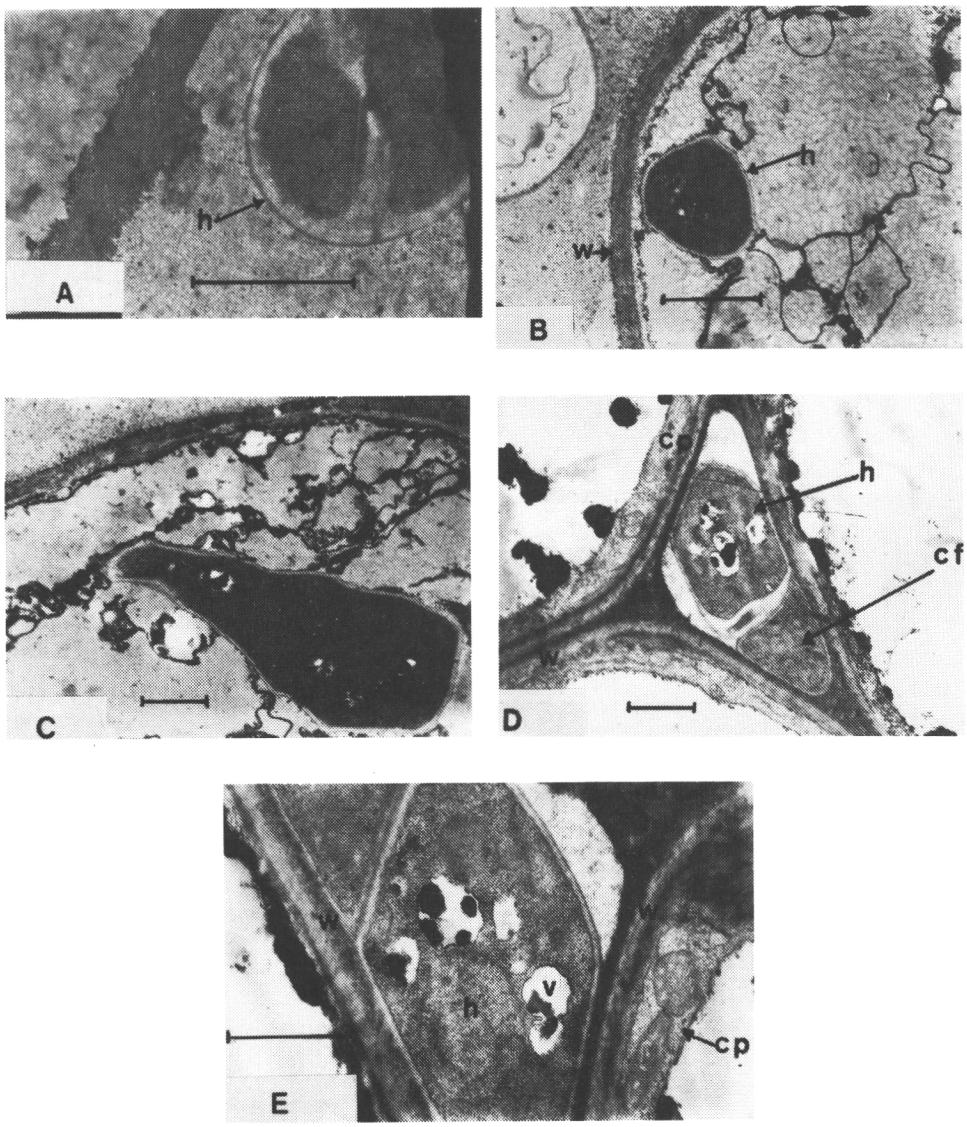



Fig. 2. Hyphae of "Mycorrhiza Frank": A - in the slime of the root cap (with a pore); B and C - in degraded cells of the root bark of the seedling; D - in intercellular tissues; E - between the plant cell walls.

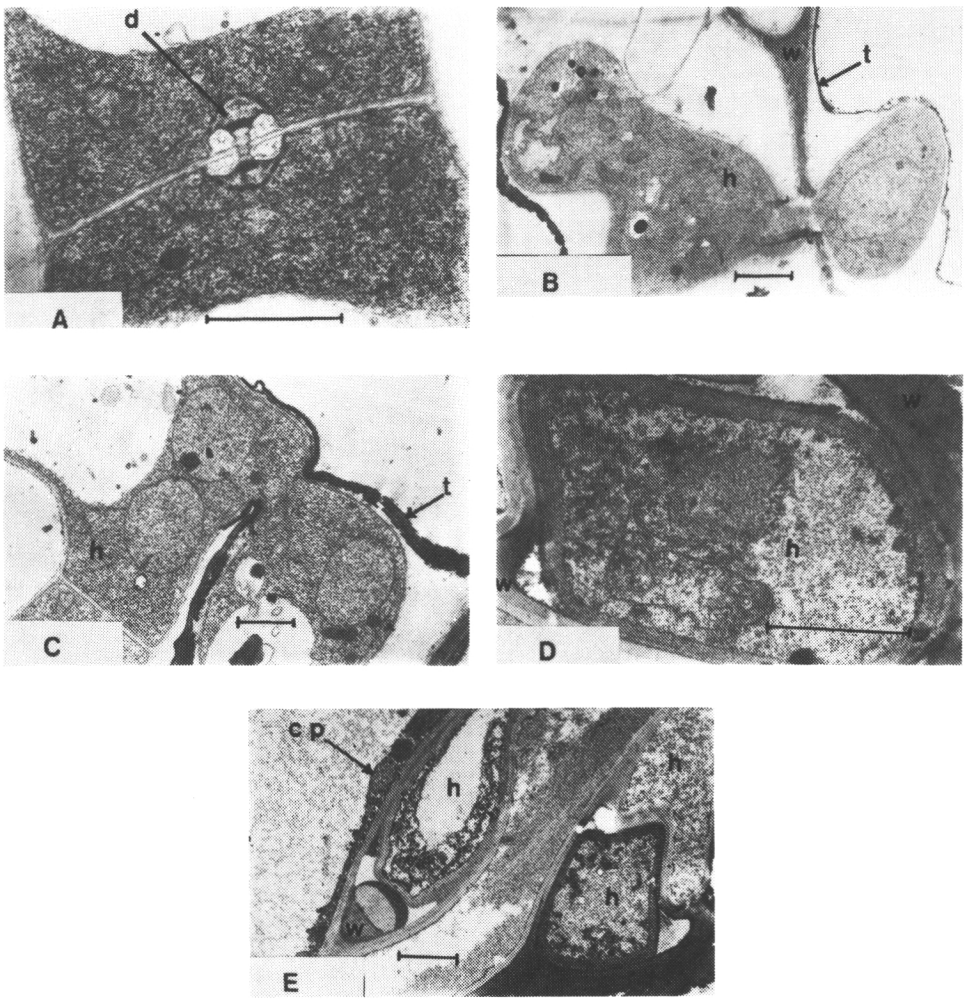



Fig. 3. Hyphae of *H. annosum* A - in the slime of the root cap; a dolipore; B - in degraded cells of the pine root; the hypha runs through the cell wall; C - the hypha runs through protective components (tannins); D and E - the fungus between the walls of the bark cells of the seedling.

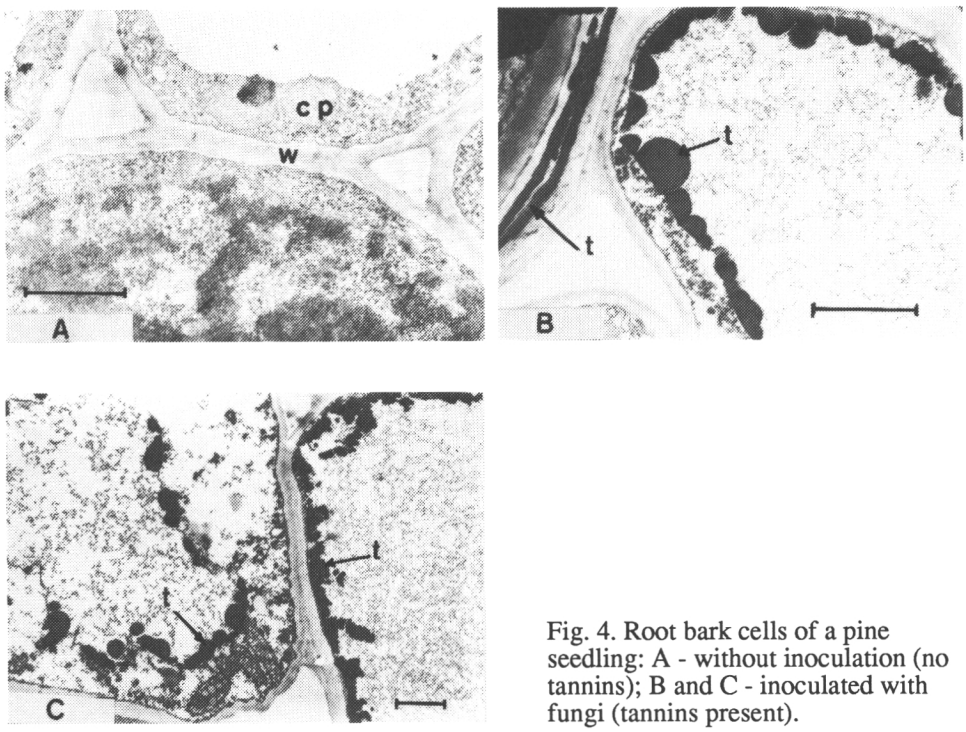



Fig. 4. Root bark cells of a pine seedling: A - without inoculation (no tannins); B and C - inoculated with fungi (tannins present).

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# INHIBITION OF ATP-ase OF SCOTS PINE ROOTS BY TOXIN PRODUCED BY *HETEROBASIDION ANNOSUM*

## ABSTRACT

The toxin produced by *Heterobasidion annosum* is not soluble in ethanol. At an aqueous concentration of 1 % it inhibited plasmalemma functioning and tonoplast ATP-ase. Glucose and maltose acted in a similar way. Both toxin and the sugars at the above concentration also inhibit germination of Scots pine seeds.

## INTRODUCTION

ATP-ATP phosphorylase (ATP-ase EC. 3.6.1.3) is the main enzymatic system regulating the energy processes living organisms. This system regulates transport, growth activity, resistance and all metabolic processes. The ATP-ATP-ase reaction is:

$$ATP + H_2O \xrightarrow[EC.\ 3.6.1.3]{ATP\text{-}ase} ADP + Pi + energy$$

Many natural cofactors such as $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$ regulate this reaction. The system exhibits varying sensitivity to specific inhibitors, e.g. ouabaine, $NO_3^-$ and others. Tipton et al. (1975) and Macri et al. (1980) observed that toxins isolated from *Helminthosporium maydis* and *Cercospora beticola* inhibit ATP-ase activity in maize and beet root. The question posed in this study was whether the *Heterobasidion annosum* toxin is active and influences ATP-ase activity.

## MATERIAL AND METHODS

**Pathogen and toxin isolation.** *Heterobasidion annosum* was isolated from a Scots pine tree in Wronki Forest District. The isolate was more pathogenic than three others currently being studied in our laboratory, and also produces considerable more toxins than the others. The isolation of this fungi and its pathogenicity have been described in details by Werner (1987). The fungus was grown in 1 l Roux boutles in 200 ml synthetic medium with maltose as the carbon source. After six weeks' cultivation the toxin was isolated from the filtrate by precipitation with ethanol (1:1).

**Isolation of enzymes.** The enzyme was isolated from Scots pine seeds germinated on water or treated with toxins. One gram of seeds was used for the analysis. After deep freezing and homogenisation, the enzyme was extracted in 50 mM tris-malate buffer

(pH 7.0) with 200 mM sucrose and 1 mM EDTA. After 10 min centrifugation (1500 g) the supernatant (fraction I), was centrifuged for 15 min at 15,000 g. The supernatant is fraction II, and the pelet fraction III (Buczek 1981).

**Enzyme assay.** ATP-ase activity was measured as the rate of Pi liberation in 3 ml containing 3 mM ATP (Tris salt), 100 mM Tris-maleate (pH 6.59), 25 mM KCl, 250 mM sucrose and 0.2-0.4 cm of the enzyme extract containing 0.1 mg protein. The reaction was stopped by the addition of trichloroacetic acid (10%), and after removal of the protein, the inorganic phosphate released from ATP was determined by the method of Fiske and Subbarow (1925). The activity of ATP-ase was expressed in micromoles of Pi liberated per mg of protein. Soluble protein was determined according to the method of Lovry et al. (1951).

**Reagents.** ATP (trisodium salt) was purchased from Sigma Chemical Company, and Tris from Calbiochem-Behring Corp. Other reagent-grade chemicals were obtained from POCh, Gliwice.

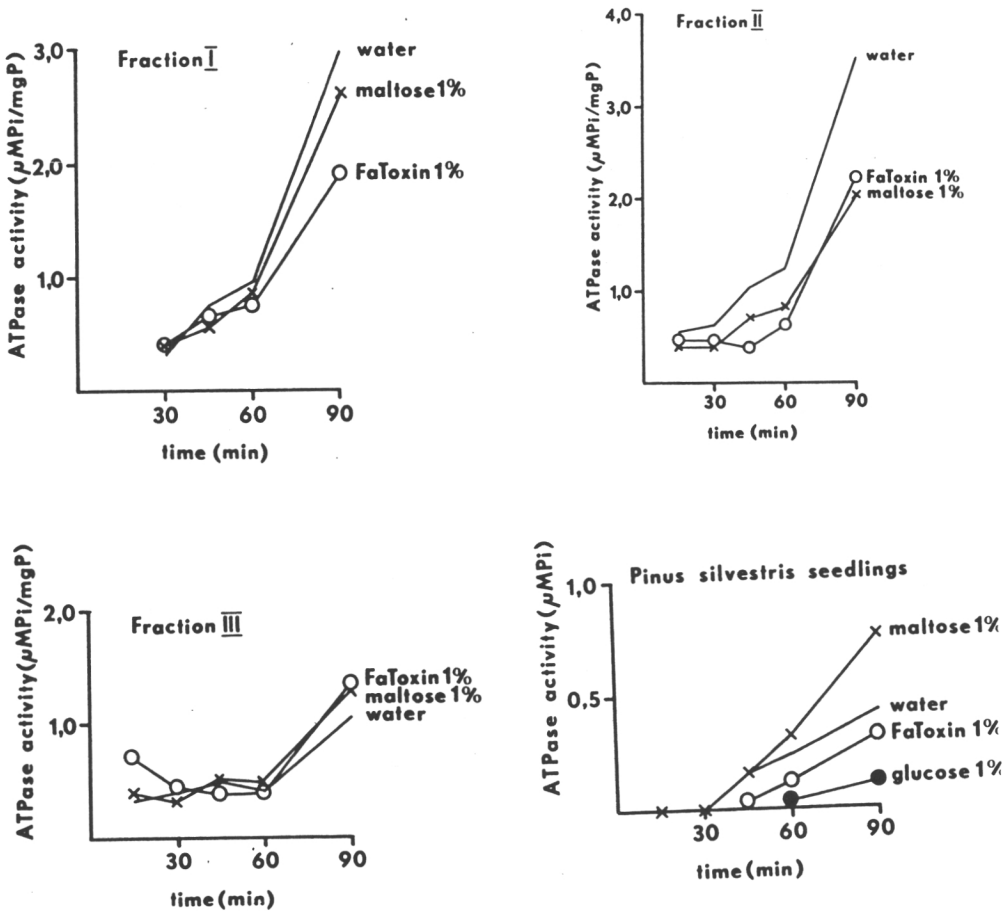
## RESULTS AND DISCUSSION

The toxins were isolated from the filtrate of the *H. annosum* cultures that had been growing for four weeks on standard nutrient medium with maltose as the sole carbon source. The pH of the medium fell during cultivation from 5.6 to 4.6. The filtrate was treated with 96 % ethanol and the toxin precipited after centrifugation for 15 min at 3000 g. ATP is the main energy carrier in all living organisms. The level of ATP and ATP-ase activity determine the metabolic activity of tissues (Bowley and Black 1982). The ATP level in tissue is regulated by the ATP-ase activity.

According to data in the literature (Table 1), dry seeds contain only traces of free ATP. After 48 hours inhibition the level of ATP is 10 times higher and continues to increase during germination. In a number of investigations it has been shown that the activity of plant ATP-ase may be stimulated by mineral ions ($Mg^{2+}$, $Ca^{2+}$) depending on their ratio in the incubation medium (Buczek 1981). However, mineral ions are not the only agents capable of modifying ATP-ase activity. In the *in vitro* experiments of this study ATP-ase from Fractions I and II were inhibited by the *H. annosum* toxin (Figs. 1 and 2). When the Scots pine seeds were treated with 1 % maltose, ATP-ase was also inhibited. The ATP-ase activity of Fraction III (Fig. 3) was not modified by the toxin or the monosaccharides. The inhibitory effect of toxin or maltose on the ATP-ase activity correlated with the inhibition of Scots pine seed germination caused by this toxin or sugars.

Table 1. The ATP content of different Scots pine organs.

| Organ | ATP content mg/100 g | Author |
|---|---|---|
| needles | 2 - 4 | Tomaszewski 1977 |
| seeds - dry | 52 | Tesche 1969 |
| germinated | 520 | Bartels 1968 |
| radicles | 112-250 | Bartels 1968 |




Figs. 1-4. The activity of ATP-ase isolated from Scots pine seeds or root surface. (1) fraction I; (2) fraction II; (3) fraction III; (4) root surface.

**Modification of ATP-ase activity present on the root surface.** Five to seven - day-old Scots pine seedlings were used *in vivo* experiments. The roots of the seedlings immersed in the specified solution with 3 mM ATP or ATP and toxin or sugars (glucose or maltose) in 1% concentration for the enzyme assay, decomposed ATP. The ATP-ases present on the surface of cells were sensitive to toxins and other modifying substances (Fig. 4). When a 1 % glucose solution was tested instead of the toxin, similar inhibition was observed. The above observation permits the following conclusions to be made:

1. The *H. annosum* toxin isolated from the fungal filtrate is not soluble in ethanol (1:1). At an aqueous concentration of 1 % the toxin inhibits the functioning of the plasmalemma and tonoplast ATP-ase (Fraction I). Glucose and maltose act in the same way as the toxin at the concentrations applied. Both the toxin and glucose also inhibit germination of Scots pine seeds. This inhibition is probably the result of ATP-ase inhibition.

2. The ATP-ase present in the cytoplasm (fraction II) is sensitive and inhibited by toxin and maltose.
3. The ATP-ase associated with the cellular structure, mitochondria and ribosomes (Fraction III) is resistant to toxin and monosaccharides.
4. The *H. annosum* toxin and glucose inhibit *in vivo* the ATP-ase present on the surface of the roots of germinated seedlings of Scots pine. A similar situation probably prevails in nature when the cells of Scots pine seedling roots are attacked by pathogenic fungi.

## ACKNOWLEDGEMENT

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# PATHOGENICITY OF FOUR *HETEROBASIDION ANNOSUM* ISOLATES AND THEIR ATP-ase ACTIVITY

## ABSTRACT

Four different isolates of *Heterobasidion annosum* were studied. Toxin production by the isolates correlated with their pathogenicity and ATP-ase activity.

## INTRODUCTION

Scots pine seedlings growing in pure culture and inoculated with *Heterobasidion annosum* have varying resistance to the different pathogen strains. In cases where seedlings were inoculated with less pathogenic isolates of the pathogen, the formation of mycorrhiza occurred (Werner 1987). In the present, preliminary study, attempts were made to determine the correlation between pathogenicity and ATP-ase activity.

## MATERIAL AND METHODS

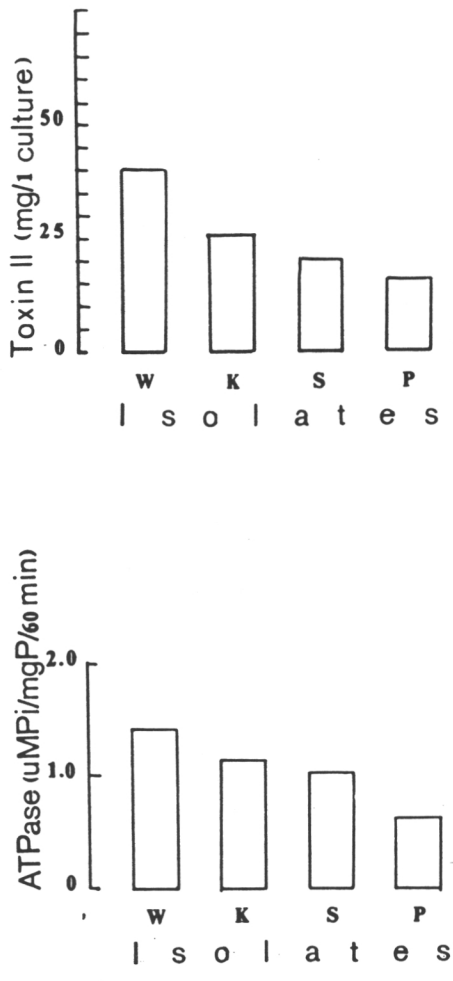
**Pathogen and methods.** Details of the isolation of the fungi and their pathogenicity have been described by Werner (1987). Four different isolates of the pathogen were isolated from diseased trees growing in Wronki (W), Kleka (K), Suleczyno (S) and Pniewy (P). ATP-ase was isolated from a four-week-old culture of *H. annosum*. After deep freezing and homogenisation, the enzyme was extracted in 50 mM tris-malate buffer (pH 7.0) with 200 mM sucrose and 1 mM EDTA. After 15 min centrifugation of 15,000 g, the supernatant was used as ATP-ase.

**Enzyme assay.** ATP-ase activity was measured as the rate of Pi liberation in a 3 ml solution containing 3 mM ATP, 100 mM Tris-malate (pH 6.59), 25 mM KCl, 250 ml sucrose and 0.2 - 0.5 ml of enzyme extract. The reaction was stopped by the addition of trichloroacetic acid (10%) and, after the removal of protein, the inorganic phosphate realeased from ATP was determined by the method of Fiske and SubbaRow (1925). Soluble protein was determined according to the method of Lovry et al. (1952).

## RESULTS AND DISCUSSION

**Pathogenicity and toxin production.** The four different strains of *Heterobasidion annosum* had different growth vigour and pathogenicity (see Werner 1987). Two different precipitate fractions were obtained from the fungal filtrate following the

addition of ethanol (1:1). Fraction I, collected from the surface of the solution has no bioassay activity. Fraction II, obtained after centrifugation (15 min. in 3000 g), was active in the bioassay. This toxin inhibited the germination of Scots pine seeds at a concentration of one percent. After precipitation and drying, the amount of toxin was weighed, and expressed in mg per culture (Fig. 1). Gel filtration on Sephadex was used for purification. The size of toxic fraction can be measured refractometrically, and the amount expressed in units of refraction. The results obtained by both methods were very similar. Comparison of the results obtained by Werner (1987), who assessed the pathogenity of the pathogen as the mortality of Scots pine seedlings, with toxin production by the different strains, shows correlation between pathogenicity and toxin production.




Figs. 1 and 2. Amount of *Heterobasidion annosum* toxin, and ATP-ase activity of four different isolates: Wronki (W), Kleka (K), Suleczyno (S) and Pniewy (P).

**Pathogenicity and ATP-ase activity.** The ATP-ATP-ase system present in all living cells regulates many metabolic processes. The ATP pool in growing fungal mycelia, particularly *H. annosum*, has been determined by Johansson and Hägerby (1974). The maximal ATP levels registered for *H. annosum* were 8-11 µmol/g dry matter, during the phase of maximal growth rate. In the present work the mycelium was in a state of intensive growth when used for ATP-ase isolation. ATP-ase was isolated from four different *Heterobasidion* strains: Wronki (W), Kleka (K), Suleczyno (S) and Pniewy (P). There were morphological differences between the strains as regards rate of growth and toxin production. ATP-ase was isolated from the mycelium using Buczek's (1981) method, and tested with an *in vitro* system. The results (Fig. 2) show differences in ATP-ase activity, and correlation between pathogenicity and ATP-ase activity. These preliminary results allow the following conclusions to be made:

1. More pathogenic *H. annosum* strains produce more toxin, and have more active ATP-ase. Production of toxin and pathogenicity are probably correlated with the growth vigor of the fungus and ATP-ase activity.

2. Many fungitoxic plant compounds are known to affect oxidative phosphorylation (Johansson and Hägerby 1974), and the ATP-ATP-ase toxin system is probably the main system in the host-pathogen relationship.

## ACKNOWLEDGEMENTS

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# MONITORING THE STATE OF HEALTH OF SCOTS PINE FORESTS IN BYELORUSSIA

## ABSTRACT

The general principles of the systems used in monitoring the state of health of Byelorussian forests are described. Special attention has been directed to damage caused by *Heterobasidion annosum* and *Armillaria* in Scots pine forests. The two main methods of data collection are visual inspection in the forest and remote sensing by aerial photography.

## INTRODUCTION

A clear decline in forest vitality has been recorded in highly industrialized countries during the past few years. This problem is now also prevalent in some parts of Byelorussia, especially in industrial areas and in areas which were exposed to the radioactive fall out from the Chernobyl nuclear accident. The most common tree species in Byelorussia is Scots pine (*Pinus sylvestris* L.), which dominates in 57.6 % of all the forests in the republic. Mixed forests, where present, usually contain an admixture of birch (*Betula pendula* Roth.). A large proportion (52.8 %) of the pine stands are young plantations. The most widespread and damaging disease of Scots pine in Byelorussia is root rot, caused by *Heterobasidion annosum* and *Armillaria*. The infection centers of these fungi cause openings, or "windows", in the canopy of the stand. In monitoring the state of health of the Byelorussian forests, special attention has so far been directed at the damage caused by root rot on Scots pine.

## METHODS

The main purpose of forest monitoring is to make a prognosis of the changes which are taking place in the biogeocenosis. The problem of monitoring can be solved in different ways by linking together present knowledge about forest condition: its composition, structure, productivity, and economical use (Isaev & Kondakov 1986). The main questions to be solved before conducting the observations are as follows (Mozolevskaya 1986): the placement of the main experimental plots and observation stations, the optimum size and type of experimental plots, the time of and period between observations, the parameters characterizing the condition of the forests and changes therein, simple and reliable methods for data collection and analysis, the comparison of

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the results of long-term observations and their combination with meteorological data and information about the dynamics and cycles of natural processes.

Before the monitoring system for the Byelorussian forests was selected, the methods which are used in the Soviet Union were tested in the field. Some new methods were also developed. The infection foci of *Heterobasidion annosum* and *Armillaria* cause openings, or "windows", in the canopy of the stand. The most acceptable system for recording the occurrence of these diseases was found to be a combination of ground monitoring made in the forest, and remote sensing aerial photography. Aerial photography is used for effective investigation of large forest areas. Aerial photography enables location of the infection foci at the beginning of their development. By comparing the photographs taken from the same forest over a number of years and from different forests, it may be possible to make conclusions about the dynamics of the disease. A general map can also be prepared from the photographs showing the health status of the pine forests. Where a more precise evaluation is necessary, this is done in the forest. Ground monitoring is made at two levels. At the first level, the foresters and forest surveyors, in connection with their other work, make observations on small permanent plots and along permanent lines. At the second level, the research workers make more thorough investigations on larger permanent plots. It is very important to know the history of the stand in connection with the ground monitoring. The number and diameter of the infection foci in a stand compartment are assessed and the percentage area of the foci out of the total area is calculated. The volume of timber which is lost as a result of root rot is estimated on the basis of the infected area and the timber volume in healthy parts of the stand. The methods used in monitoring the effect of air pollutants and radioactive fallout on the forests are in principal the same as the ground methods described above, and are carried out together with root rot monitoring.

## CONCLUSIONS

The practical application of this monitoring work was started in summer 1989. So far it has been mainly conducted in the neighbourhood of large cities and areas contaminated by the Chernobyl accident. Monitoring the state of health of the forests makes it possible to receive information quickly, to discover the diseases in their early stage, to get information about the dynamics of the disease and its dependence on different factors, to cut the damaged stand at the right time, to conduct control measures, and to restore the forests. The accumulation of knowledge makes it possible to build mathematical models describing the changes in the state of health of the forests. Prognose of the sanitary state of the pine forests, together with the forest inventory data of the Byelorussian Republic, ensures better control of forest diseases and the optimal use of forest resources.

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# A NOTE ON THE DIVERSITY OF *ARMILLARIA* SPECIES IN POLAND

## ABSTRACT

The division of the *Armillaria mellea* complex into intersterile groups has been studied in mating experiments. Pure cultures of unknown species belonging to the genus *Armillaria*, obtained from material collected in Poland, have been compared with Korhonen's testers. Three species from the *Armillaria mellea* complex have been identified.

## INTRODUCTION

Although *Armillaria mellea*, known as a tree root pathogen, has been studied for more than 150 years, only quite recently was the genus *Armillaria* found to consist of several species differing both genetically and ecologically.

Until 1970, all species belonging to the genus *Armillaria* appeared in the phytopathological literature under the common name *Armillaria mellea* (Vahl. ex. Fr.) Karst. or *A. mellea* (Vahl) Quel. or *Armillariella mellea* (Vahl) Karst. Later, as more and more species have been described, the name "biological group of *Armillariella*" was proposed by Romagnesi (1973) and, subsequently the "*Armillariella* (*Armillaria*) *mellea* complex" by Korhonen in 1978.

Cytogenic studies based on mating experiments, i.e. mating between mycelia of monosporous origin in pure cultures (Hintikka 1973), has made it possible to distinguish a series of intersterile, i.e. genetically uniform, biological groups or "biological species", within the *Armillaria mellea* complex in North America and Europe (Ullrich & Anderson 1978, Korhonen 1978, Anderson & Ullrich 1979).

In Europe, 5 of such groups have been distinguished and denoted by the first letters of the alphabet: A, B, C, D and E (Korhonen 1978).

The current nomenclature of the European species in the *Armillaria mellea* complex is as follows (Guillaumin et al. 1985):

| | |
|---|---|
| *Armillaria borealis* (Marxmüller et Korhonen) | - A group |
| *Armillaria cepestipes* (Velenovsky) | - B group |
| *Armillaria obscura* (Secr.) Herink, syn. *A. ostoyae* Romagn. | - C group |
| *Armillaria mellea* (Vahl. ex Fr.) Kummer | - D group |
| *Armillaria bulbosa* (Barla) Romagnesi | - E group |

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The occurrence of different species of the genus *Armillaria* has been studied in Poland along with the development of research on these fungi throughout the whole of Europe. Although the taxonomy of the above genus is still an open question, the common name *Armillaria mellea* is widely applied in Poland to denoting different species of honey mushroom.

The first attempts to identify the Polish species of the genus *Armillaria* were undertaken at the Laboratory of Forest Pathology of the Forest Research Institute, within the framework of Polish - Finnish cooperation in 1978. At the time, three Polish isolates were tested in mating experiments using testers of 5 intersterile biological groups distinguished by Korhonen (Rykowski 1984). The following isolates were obtained:

1. Isolate $S_1$ - from the stump wood of an oak left in a pine plantation in Miłomłyn Forest District,

2. Isolate $S_2$ - from the root wood of a 5-year-old pine in a pine plantation in Sekocin Forest,

3. Isolate N - from a basidiocarp found in a root-stem junction in Sekocin Forest.

Tests have shown that all the isolates under study belonged to the group distinguished by Korhonen (1978) corresponding to the species *Armillaria ostoyae*. Consequently it was recognized that the species name of the root pathogen in young pine was *Armillaria ostoyae* and not *Armillaria mellea*. In this connection, a need has arisen to continue investigating the taxonomy of the fungus.

Efforts to identify the Polish strains of *Armillaria* were subsequently undertaken at the Finnish Forest Research Insitute under the guidance of Dr. K. Korhonen in 1988.

## MATERIAL AND METHODS

Eight Polish isolates were used in the form of monosporous cultures. They were tested in a mating system with mycelia of five intersterile biological groups according to Korhonen. The matings were performed in Petri dishes on agar medium containing 1 % malt extract. The Polish isolates were established using spores collected in deciduous (Milomlyn Forest District) and mixed (Sekocin Forest and Pulawy Forest) forest stands.

## RESULTS

The tests resulted in the identification of 3 species of fungi of the genus *Armillaria* which are undoubtedly encountered in this country, i.e.:

| | |
|---|---|
| - *Armillaria borealis* (Miłomłyn) | - A group |
| - *Armillaria ostoyae* (Sekocin) | - C group |
| - *Armillaria bulbosa* (Puławy) | - E group |

## CONCLUSIONS

So far there are no Polish names for the above species. Furthermore, the remaining European species, including *Armillaria mellea*, have not so far been found in Poland. Although until recently, *A. mellea* has widely been recognized.

The interspecific differentiation of each fungus has far reaching phytopathological consequences, such a differentiation being of great importance for both theoretical and practical reasons. The latter reason deserves special attention since there is a possibility of a differentiated response of *Armillaria* species to external factors, including the methods that are used to limit the occurrence of this fungus.

It should be stressed that the results obtained so far point to the necessity of carrying out further studies on the identification of *Armillaria* species on a national scale, as well as on the scale of specific ecosystems and selected ecological niches.

## ACKNOWLEDGEMENT

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# SOME MECHANISMS OF RESISTANCE OF MYCORRHIZAE TO PATHOGENIC INFECTIONS

## ABSTRACT

Research on the metabolites produced by ectomycorrhizal fungi, ectomycorrhizae and by the host plant resulting from mycorrhizal infection is reviewed. Attributes of ectomycorrhizae in relation to limitation of several feeder root diseases are underlined. Mycorrhizae as an alternative biological control agent in relation to expensive, physical or chemical soil treatment is proposed.

## INTRODUCTION

Mycorrhizal plants have several advantages over nonmycorrhizal plants. The ability of ectomycorrhizae to protect conifer seedling roots against pathogens belongs to the most important. Marx (1972) and Zak (1964) suggested a number of mechanisms by which mycorrhizal fungi might protect roots against soil pathogens: physical protection by the mantle, competition with the pathogen for available nutrients and stimulation of an antagonistic microflora associated with the mantle. Ectomycorrhizae may afford protection by also secreting antibiotics originating from the fungal symbiont or by stimulating host root cells to secreate antimicrobial metabolites which may inhibit or kill potential pathogens. This paper is not intended to be an exhaustive review of the literature on the mechanism of resistance of mycorrhizae to pathogenic infection. An excellent general review on this subject was made by Marx (1972). In this review only the latter mentioned mechanisms by which ectomycorrhizae afford protection from disease to feeder roots will be considered: antibiosis against the pathogen and induction of antimicrobial metabolites production in the mycorrhizal roots.

**Production of antibiotics by fungal symbionts.** It is important to distinguish between production of antibiotics by the fungal symbionts in pure culture or in basidiocarps and in mycorrhizal association.

**Production of antibiotics by fungal symbionts in pure culture.** Marx (1972) listed ectomycorrhizal fungi reported to produce antibiotics either in pure culture or in basidiocarps; the list contains 101 species of symbionts able to produce of antibiotics inhibitory to bacteria, in most instances *Staphylococcus aureus* and *Escherichia coli*. This is misleading, however, since in a large proportion of the reports, an examination for antifungal activity was not attempted. It is difficult to extrapolate production of

antibacterial antibiotics in limiting feeder root diseases since most of them are caused by fungi. Therefore several workers have looked for antifungal activity in ectomycorrhizal fungi using fungi pathogenic on feeder roots as bioassay organisms. For example, Šašek (1967) and Šašek and Musilek (1968 a,b) determined whether mycorrhizal fungi form antibiotics specially active against the parasites of their host plants. A total of 35 strains of mycorrhizal fungi were tested against 15 parasitic strains. The mycorrhizal fungi were frequently antagonistic against *Rhizoctonia solani* and then in descending order against *Phythium debaryanum*, *Nectria cinnabarina*, *Phaeolus schweinitzi*, *Fomes annosus*, *Fusarium oxysporum*. The highest antibiotic activity was detected with cultures of *Tricholoma saponaceum* and *Rhizopogon luteolus*. Rudawska (unpublished results) tested several strains of ectomycorrhizal symbionts of Scots pine for their antagonism against two pathogens: *Fusarium* sp. and *Rhizoctonia solani* that can cause damping-off and root rot in young pine seedlings (Schwerdtfeger 1981). Most strains (Table 1) produced antifungal compounds against *R. solani* (except *R. luteolus* and *Amanita citrina*). A slight antagonistic response against *Fusarium* sp. was also detected. Several strains of *Paxillus involutus* appeared especially effective against *Rhizoctonia solani*.

Table 1. Antagonism of ectomycorrhizal symbionts of Scots pine to *Rhizoctonia solani* and *Fusarium* sp. in agar medium.

| Mycorrhizal fungus | Test pathogens | |
|---|---|---|
| | *Fusarium* sp | *Rhizoctonia* sp |
| *Suillus granulatus* | - | +++ |
| *Suillus bovinus* 1. | ++ | ++ |
| *Suillus bovinus* 2. | - | + |
| *Rhizopogon luteolus* | + | - |
| *Pisolithus tinctorius* | ++ | +++ |
| *Amanita citrina* | - | - |
| *Amanita rubescens* | - | + |
| Mrgx-ectendomycorrhizal strain | - | +++ |
| *Paxillus involutus* 2. | ++ | +++ |
| *Paxillus involutus* 3 | + | +++ |
| *Paxillus involutus* 4. | ++ | ++ |
| *Paxillus involutus* 5. | + | ++ |
| *Paxillus involutus* 6. | - | +++ |
| *Paxillus involutus* 7. | - | +++ |
| *Paxillus involutus* 8. | + | ++ |

Antagonism : strong +++, moderate ++, weak +, no antagonism -

Although *Fomes annosus* is not considered to be a feeder root pathogen, several workers have found it to be inhibited by antibiotics produced by certain mycorrhizal fungi. Hyppel (1968) tested 85 isolates of some 42 different species of mycorrhizal fungi and found that over 40 % of them inhibited *F. annosus* in dual cultures. Marx (1969a) also found that *F. annosus* was weakly inhibited by mycorrhizal fungus

*Leucopaxillus cerealis* var. *piceina*. In addition, he reported that this mycorrhizal fungus strongly or very strongly inhibited 24 species of *Pythium*, 5 species of *Rhizoctonia* and especially 9 species of *Phytophthora*. Marx devoted a great amount of effort in the identification of antifungal and antibacterial antibiotics produced by this ectomycorrhizal fungus *Leucopaxillus cerealis* v. *piceina*. The antibiotic was identified as diatretyne nitrile (Marx 1969b). Maximum production of this antibiotic occurred during the rapid and incipient autolytic growth phases in liquid medium (Fig. 1). Diatretyne antibiotics from culture filtrates of few known ectomycorrhizal associates of trees here originally identified by Anchel et al. (1962).

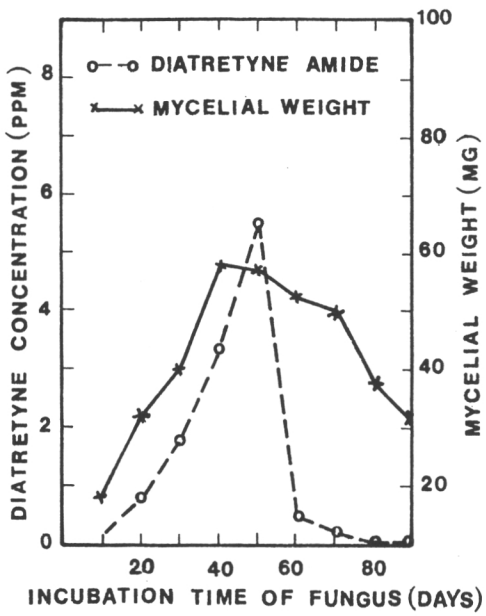



Fig. 1. Pattern of diatretyne synthesis and degradation by *Leucopaxillus cerealis* var. *piceina* in Melin-Norkrans medium during 90 days incubation at 25 °C. From D.H.Marx 1969.

In addition to reporting antibiotic production by *L. cerealis* var. *piceina* Marx (1969a) also found other symbionts of pine e.g. *Laccaria laccata*, *Lactarius deliciosus*, *Pisolithus tinctorius*, *Suillus luteus* which to different degrees could inhibit several root pathogens from the genus of *Pythium* and *Phytophthora*. An interesting aspect of this report was the difference in biological activity of the inhibitors produced by various symbionts. It is obvious that numerous mycorrhizal fungi have the capacity to produce antibiotics in pure culture or in basidiocarps. Antifungal activity also has been found in certain mycorrhizal fungi by Rypaszek (1960), Anchel et al. (1962), Park (1970), Pratt (1971), and Eghbaltalab et al. (1975). However, a prerequisite for implicating these antibiotics in feeder root disease control is to show that these antibiotics are produced by the symbionts while in mycorrhizal association with their hosts (Marx 1972).

**Production of antibiotics in mycorrhizal association**. Only a few mycorrhizal fungi have been examined for production of antibiotics while in mycorrhizal association.

Marx and Davey (1969) extracted diatretyne nitrile and diatretyne 3 from mycorrhizae formed by *L. cerealis* var. *piceina* and from the rhizosphere substrate of the mycorrhizae on aseptic *Pinus echinata* seedlings. Neither short roots nor the substrate adjacent to short roots on nonmycorrhizal pine seedlings contained the diatretynes (Table 2). Marx and Davey (1969a) were able to demonstrate that the diatretynes present in mycorrhizae formed by *L. cerealis* var. *piceina* were functional in the resistance of feeder roots to infection by *Phytophthora cinnamomi*.

Table 2. Diatretyne chromophores and their relative concentration in extracts from mycorrhizae of *Leucopaxillus cerealis* var. *piceina*, mycorrhizal rhizosphere, and nonmycorrhizal short roots of short leaf pine seedlings grown in aseptic culture. From Marx and Davey (1969).

| Extract | Diatretyne chromophore[a] | Concentration (µg/g)[b] |
|---|---|---|
| Mycorrhizae | DN | 1,5 |
| | D3 | 2,5 |
| Mycorrhizal rhizosphere | DN | 5,5 |
| | D3 | 4,5 |
| Nonmycorrhizal short roots (control) | - | - |
| Nonmycorrhizal short root rhizosphere | - | - |

[a] DN=diatretyne nitrile; D3=diatretyne 3.
[b] based on 1 g wet weight of sample.

**Induction of antimicrobial metabolite production in mycorrhizal roots.** As a result of pathogenic attack most plant cells are capable of elicit of defensive reaction in the root thus weakening or halting the further development of pathogen invasion. Phenols, quinones, various phytoalexins, and numerous other compounds have been found in tissues of a variety of plants during pathogenesis. Many of these were found to be inhibitory to the pathogen and are considered by many authors to be important in disease resistance.

Mycorrhizal fungi may stimulate host roots to produce or accumulate sufficient concentrations of substances inhibitory to root pathogens. Krupa and Fries (1971) found chemical evidence in support of this conclusion. They found volatile compounds, primarily terpens and sesquiterpens presented in the root system of Scots pine seedlings grown in pure culture with and without the fungal symbiont. Infection of the roots by the fungus resulted in production and (or) accumulation of volatile compounds in concentration two to eight times greater than that of non-inoculated controls (Table 3).

Many of these terpenes and sesquiterpenes are fungistatic and are considered by these investigators to be produced as a nonspecific response of the host cells to

symbiotic infection. These substances when present in sufficient concentrations, may restrict the growth of the mycorrhizal fungi within the host tissue, resulting finally in the symbiotic state and furthermore, volatile and nonvolatile substances may inhibit pathogens in the rhizosphere. Indeed, Krupa and Nylund (1972) found inhibitory effect of these volatile organic constituents of ectomycorrhizal root system of *Pinus sylvestris* on the extension growth of *Phytophthora cinnamomi* and *Fomes annosus*. Krupa et al. (1973) analyzed root system of *Pinus echinata* after the infection of feeder roots by the ectomycorrhizal fungi. Symbiosis with *Pisolithus tinctorius* resulted in an approximate 40-fold increase in the level of 3-carene, and with *Cenococcum graniforme* induced an approximate 30-fold increase in β-phellandrene (Table 4). Vapors of these volatile compounds affected the vegetative growth of such root pathogenic fungi like *Phytophthora cinnamomi* and *Fomes annosus* in laboratory experiment.

Table 3. Some volatile organic compounds in ether extracts of mycorrhizal and nonmycorrhizal root systems of *Pinus sylvestris* grown in pure culture with and without *Boletus variegatus*. From Krupa and Fries 1971.

| | Relative amounts | |
|---|---|---|
| Major constituent | Nonmycorrhizal | Mycorrhizal |
| α-pinene | 270 | 780 |
| 3-carene | 290 | 1200 |
| β-phellandrene | 5 | 18 |
| γ-terpinene | 1 | 8 |
| Terpinolene | 30 | 122 |

Table 4. Some monoterpenoids in nonmycorrhizal and ectomycorrhizal roots formed by *Pisolithus tinctorius* and *Cenococcum graniforme* on *Pinus echinata*. From Krupa et al. (1973).

| | mg/g Dry Weight of Tissue | | |
|---|---|---|---|
| Monotrerpenoid | Nonmycorrhizal Roots | Mycorrhizal Roots Formed by | |
| | | *P.tinctorius* | *C.graniforme* |
| 3-carene | < 0.01 | 0.43 | < 0.01 |
| β-phellandrene | 0.02 | 0.03 | 0.60 |

Besides volatile terpenoids, substances which may be synthesized in the roots as a result of mycorrhizal infection also include phenolics. Phenolics accumulate rapidly during other host-parasite interactions and can mediate disease suppression through inactivation of fungal enzymes or strengthening of plant structural components. Gäumann et al. (1960) were the first to demonstrate nonspecific induction of phenolics

and disease resistance in plants by mycorrhizal fungi. Sinclair et al. (1982) and Sylvia and Sinclair (1983 a) found that the mycorrhizal strain *Laccaria laccata* and its metabolites, can protect primary roots of seedlings of Douglas fir from lethal root rot by *Fusarium oxysporum*, by inducing an accumulation of osmiophilic materials in cortical cells of roots. When roots were challenged with fungal pathogens following incubation with *L. laccata*, invading hyphae (*Fusarium*) were least abundant and often absent in tissue with the greatest accumulation of osmiophilic materials. Histochemistry of fresh sections showed that induced materials were primarily phenolic (Sylvia and Sinclair 1983b). Authors suggest that phenolics induced by *L. laccata* in the primary root may be the basis for root protection. Chakravarty and Unestam (1986) also reported the elicitation and production of antimicrobial metabolites of phenolic origin in the root as a result of infection of *Pinus sylvestris* seedlings with mycorrhizal fungi *L. laccata*, *P. tinctorius*, *Hebeloma crustuliniforme* and a mixture of naturally occurring mycorrhizae (Table 5).

Table 5. Amount of total phenols (catechol equivalents ) of root extract in mycorrhizal, mycorrhizal plus pathogen treated, pathogen treated and control seedlings. From Chakravarty and Unestam (1986).

| Seedlings infected with: | Total phenol (mg/g dry wt. of root) |
|---|---|
| Control (without any infection) | 1.2 |
| *Rhizoctonia* sp | 0.0 |
| *Fusarium* sp | 0.0 |
| *Laccaria* | 3.8 |
| + *Rhizoctonia* sp | 3.0 |
| + *Fusarium* sp | 2.8 |
| *Hebeloma* | 2.5 |
| + *Rhizoctonia* sp | 2.1 |
| + *Fusarium* sp | 2.1 |
| *Pisolithus* | 3.8 |
| + *Rhizoctonia* sp | 2.5 |
| + *Fusarium* sp | 3.0 |
| Naturally occurring mycorrhizae | 3.7 |

Disease severity and plant mortality caused by either pathogen were drastically reduced in the presence of the mycorrhizal fungi. All mycorrhizae, except the association with *Hebeloma*, remained highly protective throughout the year (Chakravarty and Unestam 1987) as a result, partly at least, releasing of antimicrobial compounds in the roots of tested seedlings.

In a greenhouse experiment with *Pinus sylvestris* seedlings (Rudawska, unpublished results), a severe reduction of disease symptoms caused by *Fusarium* and *Rhizoctonia* was achieved when mycorrhizal *Paxillus involutus* fungus was used (Fig. 2). In this short term experiment disease severity and plant mortality caused by either pathogens were drastically reduced in the presence of *Paxillus involutus* mycelium and

its metabolites even before mycorrhiza formation. It was demonstrated that in dual culture several strains of *Paxillus* inhibited both pathogens (Table 1). Although in the described experiment *P. involutus* did not enter into ectomycorrhizal association with the seedlings due to the short duration of the study, probably antifungal metabolite produced by the fungal symbiont as well as synthesized in the host plant itself considerably reduced seedlings mortality caused by the tested pathogens.

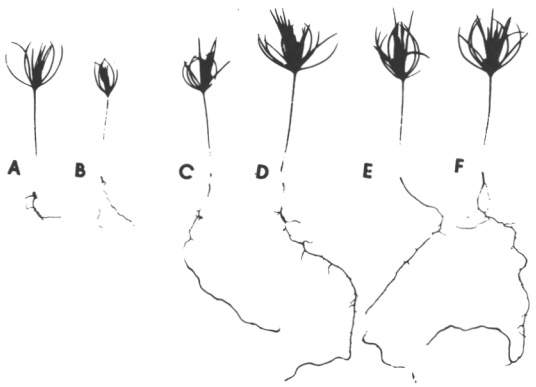



Fig. 2. Growth responses of one-month-old seedlings of *Pinus sylvestris* as influenced by different treatments: *Rhizoctonia* only (A), *Fusarium* only (B), inoculation with mycorrhizal *P. involutus* strain (C), *P. involutus* plus *Rhizoctonia* (D), *P. involutus* plus *Fusarium* (E), and control (no fungus F).

## CONCLUSIONS

The answer to: can mycorrhizae control root disease? - remains still as a formidable challenge. However, as presented in the review of the past and current research, there is a clear indication that mycorrhizal fungi can deter or significantly reduce the effects of some pathogens on the host plant. The data from literature as well as some of my preliminary results of the laboratory and greenhouse studies look promising and justify further investigations. Experiments to date have focused on pure culture of mycorrhizal fungi or isolated ectomycorrhizal root systems. However, there is a great need of research which integrates these observed effects and applies them to whole tree systems. Although several species of ectomycorrhizal fungi were found to be effective against a feeder-root pathogen, no one species can be applied to all trees in all situation. Great variability of physiological reactions was revealed between fungi in relation to the host plant, environment, soil type and location. Artificial inoculation is one means of utilizing mycorrhizal fungi more fully. Cultural practices should be manipulated to increase the incidence of the most effective mycorrhizal fungi in field soil to enhance their efficiency on diseases. To date several root pathogens are controlled using very expensive and ambiguous physical and chemical soil treatment. Ectomycorrhizae creates opportunity for biological control of feeder root pathogens and should be utilized more effectively in the near future.

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# COMPARISON OF SOME MORPHOLOGICAL FEATURES OF NEEDLES OF PINE GROWING UNDER ABIOTIC OR BIOTIC STRESS

ABSTRACT

Some morphological features of needles from upper, middle and lower parts of crown of trees in four classes of crown density were evaluated. The examined trees were growing in two mature pine stands under abiotic (air pollution) or biotic (*Heterobasidion annosum*) stress. A great variability of tested parameters was found. The dry weight of needles correlated with the visual evaluation of the trees. The index of the dry weight of 1 mm of needle could be useful comparative criterion on indirect evaluation of physiological status of trees.

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# DIRECT EFFECTS OF SULPHUR DIOXIDE ON SCOTS PINE

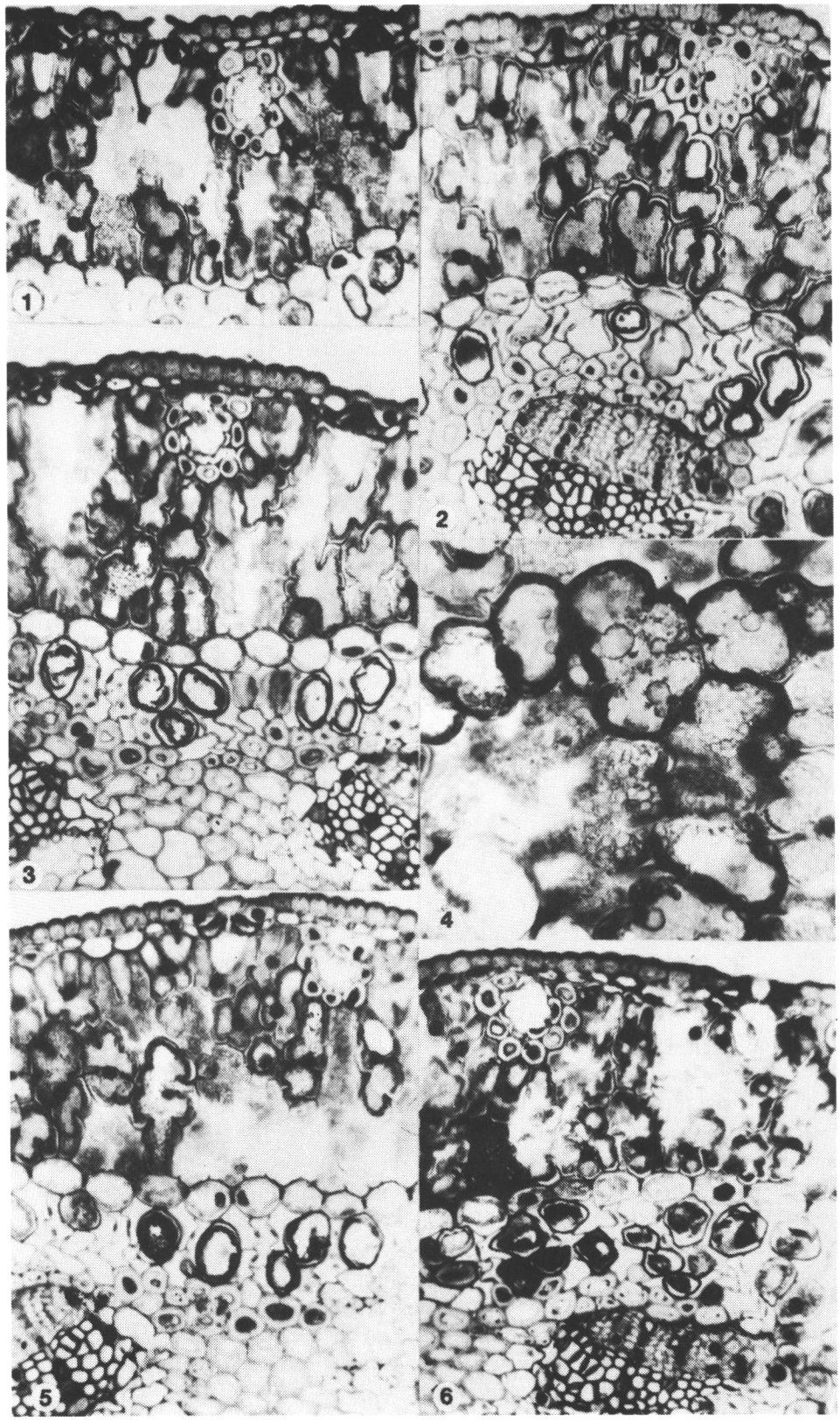
Scots pine is the basic forest species in the state forests of Poland. The rapid development of industry has resulted in a major part of Poland seriously polluted by $SO_2$. Our forests are heavily polluted by $SO_2$ derived from neighbouring countries. The damage caused by $SO_2$ in pine stands appears as a change in needle colour and crown shape, as the death of side branches and the apex, a decline in volume production.

The biomass production of trees growing in polluted areas is one of the most important questions. However, such production is markedly decreased by disorders in a number of physiological processes and by structural and ultrastructural changes in the needle tissues caused by $SO_2$. Such disturbances depend on the exposure time and gas concentration, and on the degree of susceptibility of the Scots pine trees to $SO_2$. The extent of changes in physiological processes and the structure of needle tissues brought about by $SO_2$ varies in accordance with the growing season and the physiological age of the shoots.

The results of studies conducted at our institute indicate that the first reaction of Scots pine needles to $SO_2$ is the plasmolysis and degeneration of the protoplasts of the mesophyll cells, leading to the complete collapse of these cells (Figs. 1 - 6). After fumigation with $SO_2$, the endodermis and conductive elements of the phloem and xylem remains for a relative long time in an unchanged state. The cells of the endodermis undergo deformation only after the whole mesophyll has already collapsed.

These are numerous invaginations of the plasmalemma in many of the mesophyll cells of needles from trees more sensitive to $SO_2$. $SO_2$ caused numerous changes in the mesophyll ultrastructure, i.e. swelling and degradation of the thylakoides, granulation of the cytoplasm and plastic matrix, degradation of the ribosomes and endoplasmic reticulum, agglutination of chromatine and cell plasmolysis.

Changes in the rate of $CO_2$ exchange in light and darkness showed a positive, statistical significant correlation between the rate of net photosynthesis, photorespiration and dark respiration and damage to Scots pine needles caused by $SO_2$. The inhibition of photosynthesis and photorespiration, and the stimulation of dark respiration, are dependent on the degree of susceptibility of the tree, the gas concentration, the exposure time and season of the year. Sulphur dioxide fumigation of Scots pine seedlings throughout the entire growing season reduces the production of Calvin cycle compounds, mainly PGA, whereas there is a relative enhancement of products derived from PEP carboxylation such as malate, aspartate and glutamate (Fig. 7). Regardless of the $SO_2$ concentration used and the duration of the time elapsed after termination of fumigation, the transport of photoassimilates from the needles to the


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2
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roots of Scots pine seedlings underwent stronger disturbances due to the action of $SO_2$ than $CO_2$ assimilation (Fig. 8).

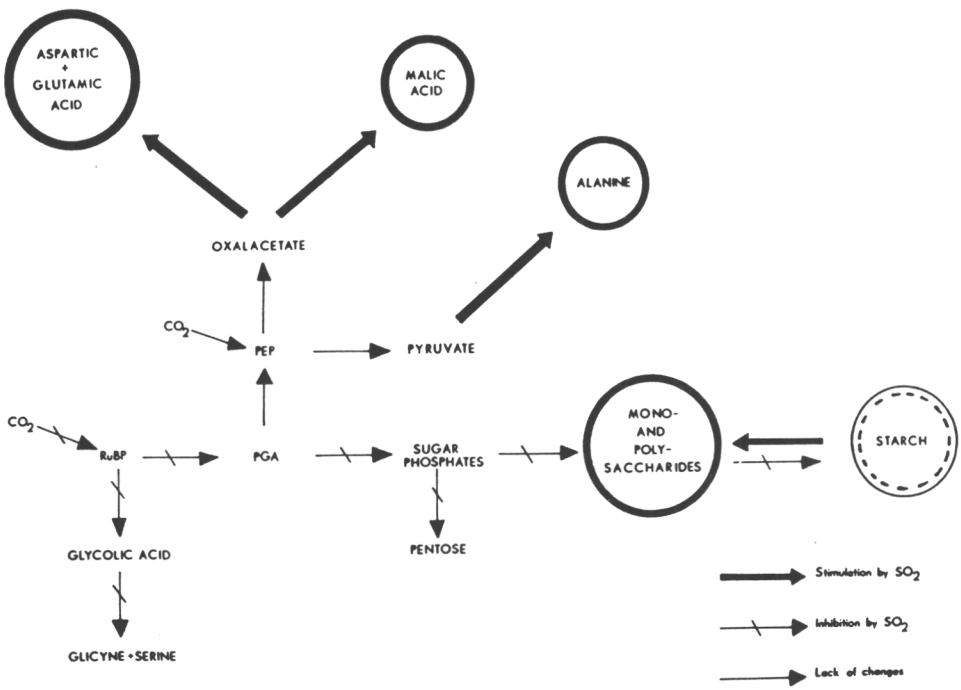



Fig. 7. Scheme of carbon metabolism in the photosynthesis process of Scots pine shoots exposed to $SO_2$.

Figures 1 - 6. Fig. 1. Cross section of a needle of Scots pine - tolerant clone, three days after exposure of the shoots to $SO_2$. The mesophyll cells surrounding the substomatal chamber have collapsed. Fig. 2. Fragment of a needle of a sensitive clone showing plasmolyzed cells of the mesophyll, endodermis and vascular parenchyma. Besides the conductive elements of the xylem, the cells of the stele are deformed. The epithelial cells of the resin canals are not enlarged due to hypertrophy. Fig. 3. Alterations in the needle structure of the tolerant clone 5 days after exposure to $SO_2$. The slightly collapsed cells of the mesophyll extend from the epidermis to the endodermis. Fig. 4. Plasmalemma invaginations in the mesophyll cells of the more sensitive pine 5 days after fumigation. Fig. 5. Cross section of a needle of a tolerant pine 10 days after fumigation. The needle structure resembled those collected 5 days after the $SO_2$ treatment. Fig. 6. Collapsed tissues of a needle from the sensitive pine 10 days after $SO_2$ exposure. Considerable deformations of the mesophyll and transfusion tissue are visible.

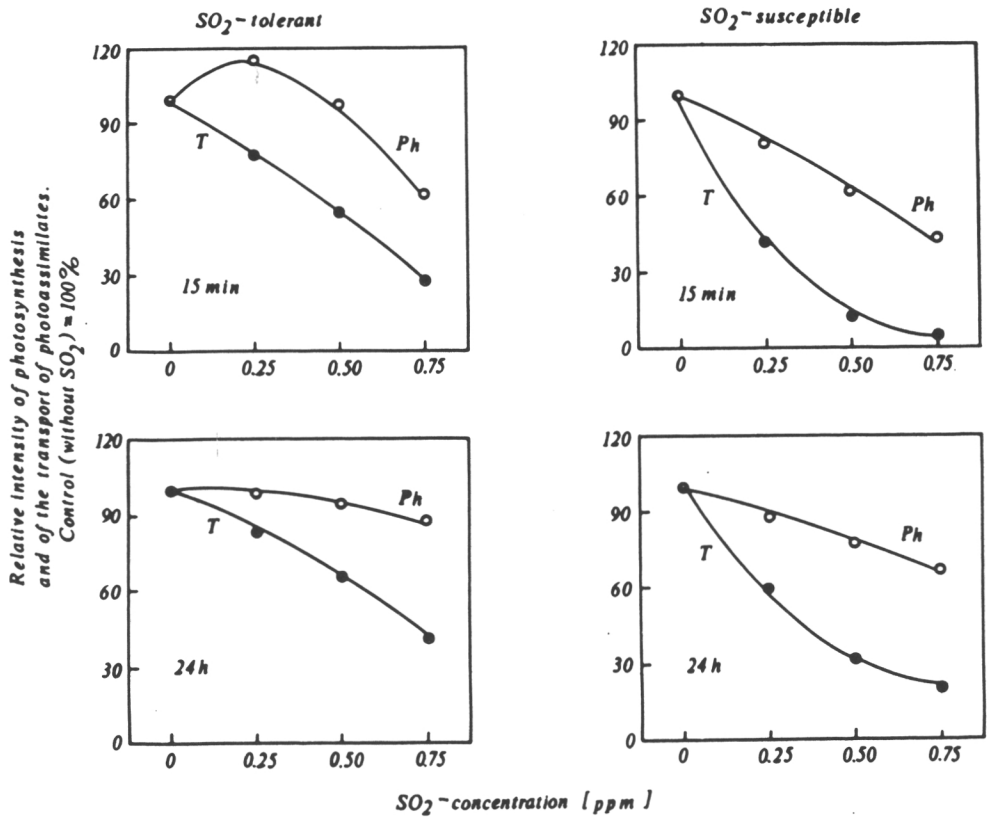



Fig. 8. Net photosynthesis and transport of photoassimilates in Scots pine seedlings extremely susceptible or relatively tolerant to the action of $SO_2$. Immediately (15 min) after the termination of fumigation (5 h, 0-0.75 ppm $SO_2$) or 24 hrs later, the needles were exposed to $^{14}CO_2$ for 25 min. Transport of photoassimilates was measured 24 hrs after exposure to $^{14}CO_2$.

The inhibition of the translocation of assimilates following exposure to $SO_2$ is reflected primarily as an increased retention of carbohydrates in the leaves and, is a consequence of disturbances in sugar uptake into the transport element (i.e. phloem loading process), and not due to the direct inhibition of long distance transport itself (Fig. 9).

Some of the studies currently being carried out on the effect of $SO_2$ on Scots pine concern changes in free proline levels in needles (Fig. 10). During the initial period of $SO_2$ fumigation there is an absence or only a slight increase in the content of free proline in Scots pine needles. This is caused by the utilization of proline in protective measures against $SO_2$ and regeneration processes. The higher concentration of sulphur dioxide, or a longer fumigation period, cause a rapid accumulation of free proline in the needles as a result of the binding of toxic ammonia. The final effect of $SO_2$ on the

needles is a decrease in the content of free and bound proline (Fig. 10). This indicates the degradation processes, proteolysis and desamination of amino acids.

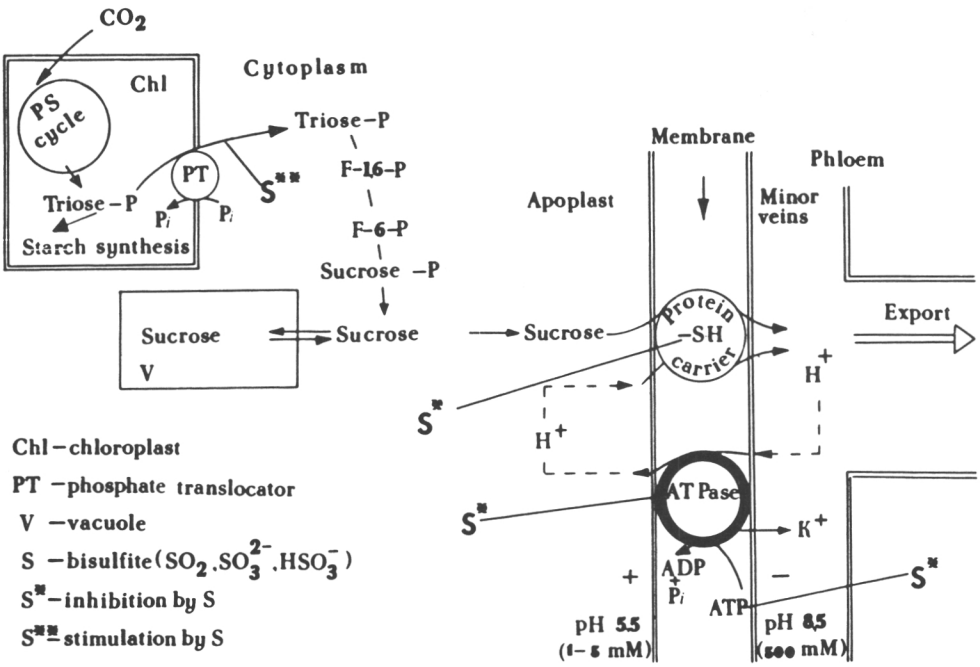



Fig. 9. The scheme of bisulfite action on the sugar transport.

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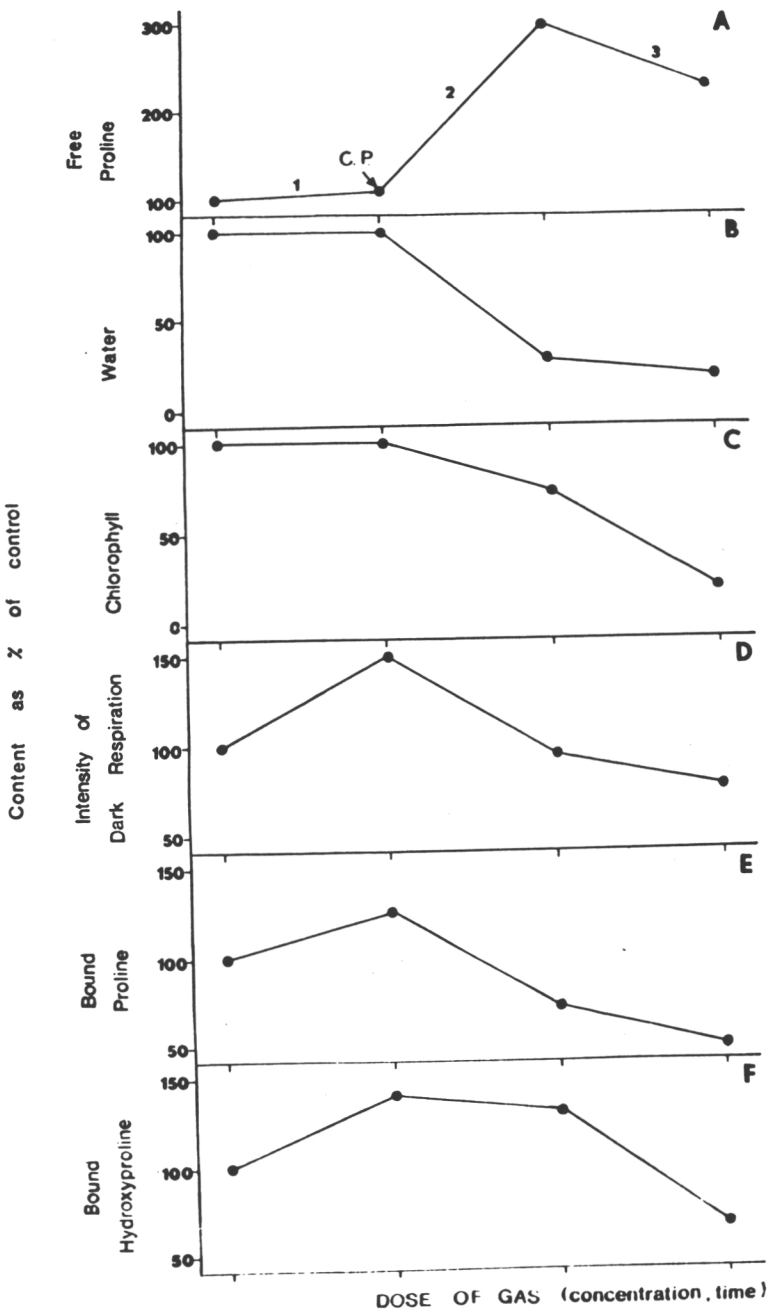



Fig. 10. Scheme of the changes in levels of free proline (A), water (B), chlorophyll (C), dark respiration (D), bound proline (E) and hydroxyproline (F) in the needles of Scots pine during exposure to $SO_2$.

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# FIELD SELECTION AND CONSERVATION OF SCOTS PINE INDIVIDUALS LESS SENSITIVE TO AIR POLLUTION

## ABSTRACT

Individual trees were selected from 40 to 90-year-old Scots pine (*Pinus sylvestris* L.) stands located around a lead-zinc and copper smelters and their sensitivity to the long-term effects of a mixture of air pollutants ($SO_2$ + heavy metals) tested. Vegetatively propagated trees were used to establish experimental plots in a polluted area and an unpolluted one. Exposure of the grafts to the effects of $SO_2$ and heavy metals in field conditions differentiated the studied clones in extent of needle necroses and in growth.

## INTRODUCTION

During the past few decades some forest areas in Central Europe have been threatened by air pollution. These pollutants causing a severe and extensive forest decline, even in areas located far away from industrial regions. In some regions the irreversible destruction of forests has occurred. Such damage is already evident, especially in stands of *Abies alba*, *Picea abies*, *Pinus sylvestris* and other tree species in the central European countries.

The responses of woody plants to the action of industrial pollutants, as well as the possibility of selecting less sensitive species, populations and individuals, have been the object of numerous studies (Gerhold 1977, Reinert et al. 1982, Rachwał & Oleksyn 1987, Oleksyn 1989, Siwecki & Rachwał 1989). Some of these studies have also been performed on Scots pine. This species is characterized by a high sensitivity to industrial pollution (Wentzel 1963, Antipow 1979, Jeffree 1980). The results obtained so far indicate that there is variation in the sensitivity of Scots pine trees to the action of toxic gases (mainly $SO_2$) on the population, progeny and individual levels.

The decline of pine stands (caused primarily by industrial emissions) has also reached our country in recent years. For this reason studies on the responses of populations, progenies and individual trees of Scots pine to the action of air pollution were initiated already several years ago (Białobok et al. 1980, Siwecki & Rachwał 1989). This paper presents some of the results obtained so far.

Metsäntutkimuslaitoksen tiedonantoja 360: 209-215, 1990

## MATERIAL AND METHODS

The first selection of less sensitive Scots pine individuals was done during 1973-78 in the vicinity of the zinc smelter at Miasteczko Slaskie, and the "Czarna Huta" chemical works at Tarnowskie Góry (Upper Silesian Industrial Region). The zinc smelter at Miasteczko Slaskie has been emitting pollutants consisting of $SO_2$ and dust containing heavy metals (Zn, Pb, Cd, Mn, Cu and others) at very high level since 1968. The average daily levels of $SO_2$ amounted to 0.18-0.35 mg $m^{-3}$ during the vegetation period (April - September), and ranged between 0.02-0.81 mg $m^{-3}$ (Latocha 1975). The total zinc content in the forest soil biological accumulation level amounted to 1703.8 ppm and the lead content reached 6000 ppm.

"Czarna Huta" chemical works has emitted pollutants since 1919. The type of pollution has changed several times during this period. During the last few years the following gaseous pollutants have been emitted into the atmosphere: $SO_2$, $NO_x$, $H_2S$ as well as dust containing barium sulphate, zinc and boron sulphides, heavy metals and others. In recent years the average concentration of sulfur dioxide for the whole year amounted to 0.22 mg $m^{-3}$ per day, and during the vegetation period (April - September) 0.18 mg $m^{-3}$, ranging between 0.07 and 0.49 mg $m^{-3}$ (Latocha 1975). Deposition of dust amounted to 171.1 mg $m^{-3}$ per year. The total zinc content in the forest soil biological accumulation level ranged between 348 and 663 ppm and was dependent on the distance from the source of emissions. The corresponding figure for lead reached 1800 ppm.

**Description of the emissions from the Głogów copper smelters and the deposition levels at a nearby field experiment.** The experimental area with Scots pine clones was established in spring 1987 at a distance of 3.5 km to the NE from the main emission sources (Głogów smelter I and II). At least a few grafts from each clone were planted. In 1987 the emission of $SO_2$ from the copper smelter "Głogów I" alone was 69,060 tonnes, CO 109,703 tonnes and dust 1,684 tonnes. The total emissions of gaseous and dust pollutants rank this smelter in 6th or 7th place among the most noxious industrial plants in Poland (Anonym 1988). The mean annual concentration of $SO_2$ measured near the experimental area varied from 0.02 to 0.07 mg $m^{-3}$, with maximal daily concentrations of 0.13 to 3.06 mg $m^{-3}$ (M. Wierzbicki - personal communication). The soils in the vicinity of the smelters are characterized by high concentrations of Cu, Pb, Zn and other elements. The concentration of Cu and Pb in the upper layer of the soil (0-20 cm) has reached very high levels near the experimental area. The total Cu content varies within the limits of 250-300 ppm, but in places reaches as high as 3200 ppm. The total concentration of Pb is equal to 450-500 ppm with a maximum in some places of up to 1000 ppm.

The average concentration of $SO_2$ in the investigated areas markedly exceeded the IUFRO maximum permissible levels (0.05 mg $SO_2$ $m^{-3}a^{-1}$) for coniferous forests (Wentzel 1981). The average concentration of toxic heavy metals in the soil also markedly exceeded the permissible levels.



Fig. 1. Air pollution causes considerable damage to the Scots pine stands around nonferrous metallurgical smelters.

**Selection, propagation and establishment of the clone archive in Kórnik, as well as the experimental area in Głogów.** Ten less sensitive Scots pine individuals were selected from the heavily damaged stands (Fig. 1) surrounding the above mentioned industrial works. Selection was carried out with regard to phenotypic properties of the investigated trees (Figs 2 and 3). A three-class scale was used for rating the resistance or susceptibility of the investigated trees (Table 1):

(+,++,+++) - trees of high vitality, with dense and compact crowns, long annual shoot increments, shoots with 1 and 2-year-old needles, without (or only with slight) visible needle damage (Figs. 2 and 3);

(±) - trees with decreased vitality, thinned out crowns with round or flat tops, short annual shoot increments, shoots with 1 or 2 (only in some cases) -year-old needles, with intermediate visible needle damage;

(-,--,---) - poorly growing trees, loose crowns, short annual shoot increments, shoots with only one-year-old needles, with severe visible needle damage. Trees of this type were selected and propagated for a comparative investigation of the mechanisms of resistance only (Fig. 2 left, Fig. 3 left and right).



Fig. 2. 70-years-old pines which have survived in a polluted environment could be selected for the investigation and breeding purposes.

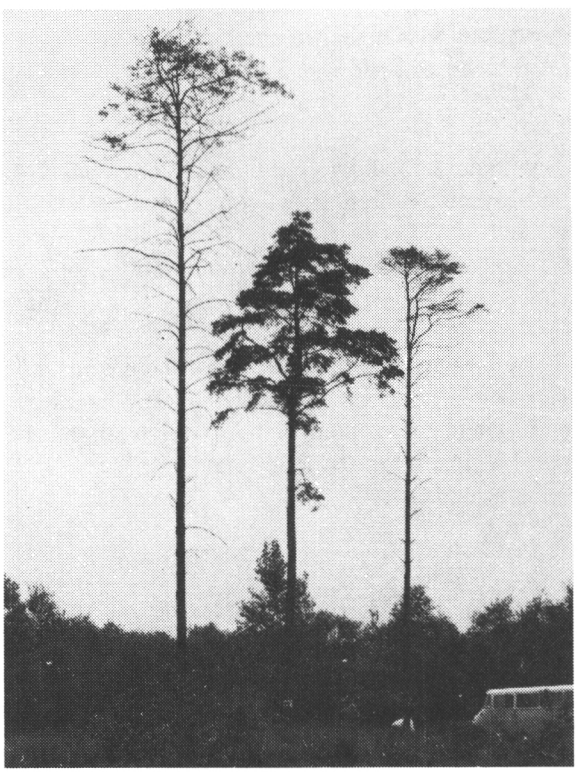


Fig. 3. Example of the field selection of Scots pine individuals in the polluted regions. In the front the less sensitive tree, on the left and right susceptible ones which were also collected for investigations on the tolerance mechanisms and for breeding purposes.

Table 1. List of less sensitive (+,++,+++) and susceptible (-,--,---) Scots pine individuals selected in the vicinity of industrial works in the Upper Silesia and Legnica-Głogów copper region, as well as some results of laboratory and field studies.

| No | Species | Tree No | Forest and Range District No | Results of: Field tested mother trees | Laboratory tested shoots from grafts | Field tested drafts at Głogów Copper smelter | Age of mother trees | Height | Distance (m) and direction from the source of emission |
|---|---|---|---|---|---|---|---|---|---|
| | | | CHEMICAL WORKS "Czarna Huta " | | | | | | |
| 1. | Pinus sylvestris L. | K-03-82 | Repecko 133p | + | ± | -- | 60-65 | 15 | 1000 W |
| 2. | " " | K-03-83 | " 133p | + | ++ | -- | 60-65 | 10 | 1000 W |
| 3. | " " | K-03-84 | " 133p | + | + | ++ | 60-65 | 11 | 1000 W |
| 4. | " " | K-03-85 | " 133p | + - | +++ | + | 60-65 | 12 | 1000 W |
| 5. | " " | K-03-86 | " 133p | + - | ++ | + | 60-65 | 11 | 1000 W |
| 6. | " " | K-03-87 | " 134p | ++ | ± | + | 65-70 | 18 | 1500 W |
| 7. | " " | K-03-89a | " 75K | +++ | - | - | 65-70 | 17,5 | 2000 NW |
| 8. | " " | K-03-89b | " 75K | - | | | 65-70 | 16,5 | 2000 NW |
| 9. | " " | K-03-90 | " 101b | (±) - | --- | --- | 50-60 | 15 | 800 NW |
| 10. | " " | K-03-91 | " 101b | + | --- | -- | 50-60 | 11 | 1000 NW |
| 11. | " " | K-03-91A | " 101b | ++ | | | 50-60 | 12 | 1000 NW |
| 12. | " " | K-03-92a | " 102f | + | | | 45-50 | 10 | 1500 NWW |
| 13. | " " | K-03-92b | " 102f | - | | | 45-50 | 9 | 1500 NWW |
| 14. | " " | K--03-159/1 | Żyglinek | + - | ± | | 40 | 5,5 | 1000 E |
| 15. | " " | K-03-159/2 | " | + - | ++ | + | 40 | 5 | 1000 E |
| 16. | " " | K-03-159/3 | " | + - | | | 40 | 5 | 1000 E |
| 17. | " " | K-03-160a | Repecko | + - | | | 40 | 5 | 1000 W |
| 18. | " " | K-03-160b | " | + - | | | 40 | 5 | 1000 W |
| | | | ZINC SMELTER "Miasteczko Śl. | | | | | | |
| 19. | Pinus sylvestris L. | K-03-96 | Żyglinek 134 | +++ | | +++ | 90 | 23 | 600 NW |
| 20. | " " | K-03-97 | " 134 | + | | | 90 | 22 | 800 NW |
| 21. | " " | K-03-164 | " 134 | + | | | 90 | 24 | 700 NW |
| 22. | " " | K-03-165 | " 134 | + | | | 90 | 20 | 600 NW |
| 23. | " " | K-03-166 | " 134 | + | - | | 90 | 21 | 700 NW |
| 24. | " " | K-03-167 | " 135 | - | | | 85-90 | 20 | 500 N |
| 25. | " " | K-03-168 | " 135 | + | | | 85-90 | 20 | 500 N |
| | | | LEGNICA-GŁOGÓW COPPER REGION (LGOM) | | | | | | |
| 26. | Pinus sylvestris L. | K-15-27 | Lubiń/Koźlice 229 | +++ | | +++ | 85-90 | 25 | 300 E |

Selection was made from among the tens of thousands of trees over an area of several thousand hectars. The selected trees were propagated vegetatively by grafting onto 2 to 3-year-old Scots pine stocks. Propagation has been done twice every year: in spring (April) and in the summer (end of August) in the Zwierzyniec forest nursery of the Institute of Dendrology. The grafting ability was strongly dependent on the properties of individual trees. Grafts of propagated trees were kept for 2-4 years in the nursery and then replanted in the clone archive at Kórnik (Fig. 4) and to the experimental plot in a polluted area close to the copper smelter at Głogów.

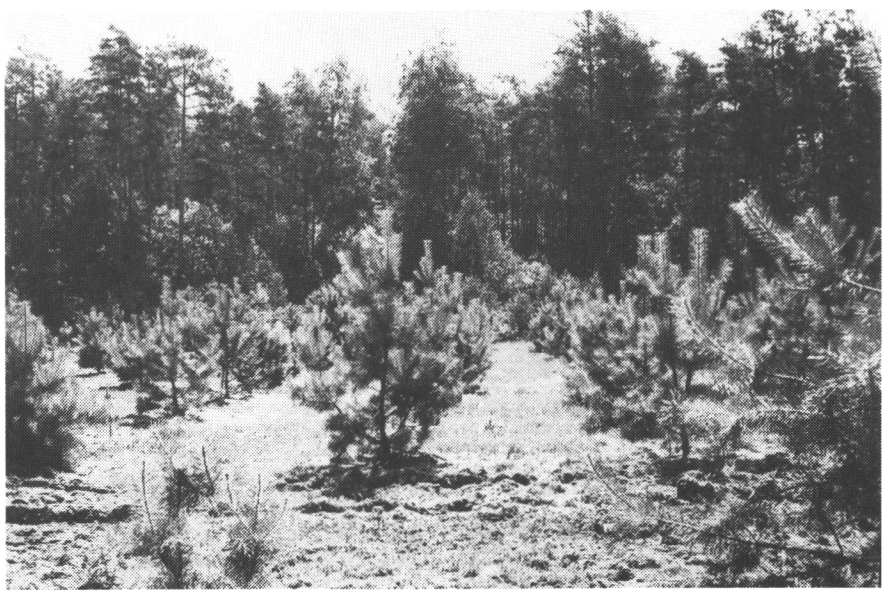


Fig. 4. The clone archive could supply improved seeds for pine plantations in industrial regions.

## CONCLUSIONS

Selected and propagated individuals of Scots pine from industrial regions serve as material for further studies into the mechanisms of various susceptibility to the action of air pollution. The $SO_2$ susceptibility of some selected clones, as tested in the laboratory and field conditions, show great differences between studied clones in extent of needle necrosis and in growth (Table 1). Trees originally classified as being more tolerant to the effect of air pollution were less susceptible in field conditions than Scots pine seedlings from a provenance experiment established in the neighbourhood (Oleksyn et al. 1988, Siwecki and Rachwał 1989). Since the progenies of the trees less susceptible to $SO_2$ indicated strong inheritance of this character (Białobok et al. 1980), improved plant material characterized by a greater tolerance to air pollution could possibly be obtained from the next generations of plantations of less sensitive clones.

However, even though we have now selected some individual trees less sensitive to the effects of air pollution and collected them in special archive clones, the only real solution for the future lies in diminishing industrial pollution to levels below the maximum ones tolerated by trees.

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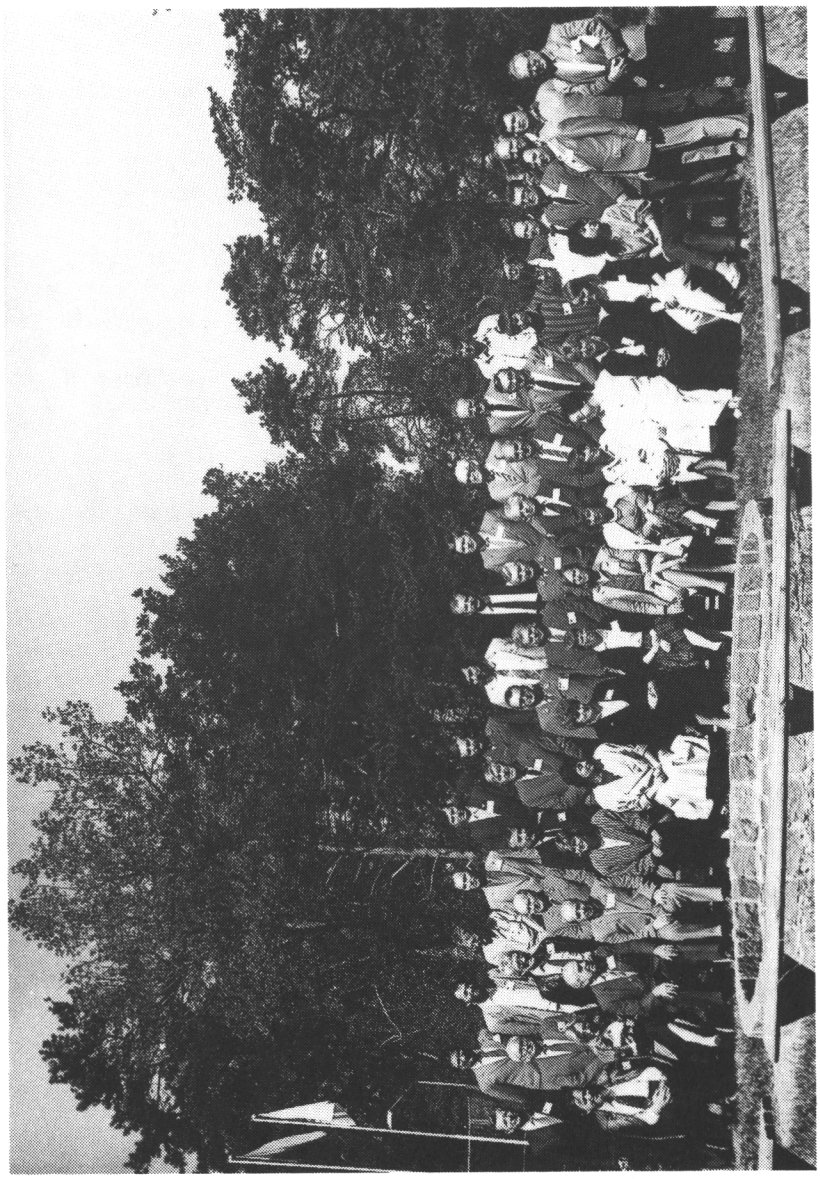
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