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## PROCEEDINGS OF THE MEETING OF THE IUFRO WORKING PARTY S2.05—05, RESISTANCE IN PINES TO *MELAMPSORA PINITORQUA,* JUNE 1979, SUONENJOKI, FINLAND

## IUFRO:N TYÖRYHMÄN S2.05—05, VERSORUOSTEENKESTÄVYYS MÄNNYSSÄ, KESÄKUUSSA 1979 SUONENJOELLA PIDETYN KOKOUKSEN ESITELMÄT

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# PROCEEDINGS OF THE MEETING OF THE IUFRO WORKING PARTY S2.05—05, RESISTANCE IN PINES TO *MELAMPSORA PINITORQUA,* JUNE, 1979, SUONENJOKI, FINLAND

## IUFRO:n työryhmän S2.05—05, Versoruosteenkestävyys männyssä, kesäkuussa 1979 Suonenjoella pidetyn kokouksen esitelmät

edited by

Kim von Weissenberg and Timo Kurkela

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Papers were presented by six persons. Literature on breeding *Melampsora* rust-resistant poplars was reviewed and results were presented about the crucial effects of temperature and humidity on poplar-rust epidemics. Research made on *M. larici-populina* and *M. pinitorqua* in Poland was reviewed. Serological tests were suggested as a means for indentifying aspen rusts. In Italy it had been observed that good aeration without shading by vegetation promoted telial germination and also infectivity of basidiospores of *M. pinitorqua.*

Results obtained on testing resistance in Scots pine and on dormancy, activation and long-term storage of teliospores were presented. Specific recommendations for future research were adopted.

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Kokouksessa pidettiin seitsemän esitelmää. J. Gremmen selosti kirjallisuuskatsauksessaan poppelien ruosteenkestävyysjalostuksessa saatuja kokemuksia. Z. Krzan esitti tutkimustuloksia lämpötilan ja kosteuden vaikutuksesta poppelin ruoste-epidemiaan. Muusta Puolassa suoritetusta *Melampsora*-tutkimuksesta hän kertoi yhdessä R. Siweckin kanssa laatimassaan esitelmässä.

T. Kurkela ehdotti serologisia testejä mahdollisesti haavan ruosteiden määrittämiseen soveltuvana keinona. B. Naldini Longo työtovereineen esitti tuloksia tutkimuksista, jotka käsittelivät talvehtimispaikan laadun vaikutusta talvi-itiöiden itävyyteen ja kantaitiöiden infektiokykyyn. Männyn ruosteenkestävyystutkimuksissa Ruotsissa saatuja tuloksia esitteli O. Martinsson ja Suomessa saatuja tuloksia K. von Weissenberg. Jälkimmäinen esitti myös tuloksia talvi-itiöiden dormanssista, aktivoitumisesta ja pitkäaikaisesta varastoinnista sekä versoruosteen maantieteellisestä vaihtelusta. Kokouksessa laadittiin eräitä suosituksia tulevan tutkimustoiminnan painopistesuunnista.

Helsinki 1980. Valtion painatuskeskus

# PREFACE

The second meeting of the IUFRO Working Party, Resistance in Pines to *Melampsora pinitorqua,* was held during June 27—29, 1979 in Suonenjoki, Finland. Professor Tauno Kallio and Agr. For. Lic. Paavo Pelkonen welcomed the participants to the Experiment Station for Reforestration of the Finnish Forest Research Institute. The meeting was opened by the Chairman of the Working Party, Prof. Francesco Moriondo of Florence, Italy. Dr. Kim von Weissenberg of Finland was asked to serve as chairman.

During the meeting seven papers were presented. Participants also visited some field experiments in the surroundings of Suonenjoki and made a field trip to Scots pine seed orchards and progeny tests in Toivakka.

The possibility of broadening the field of the Working Party was discussed. It was considered better, however, to concentrate on pine twist rust also in the future. It was further agreed that the results of the international species trial initiated in Florence in 1974, should later be published jointly by those participating. The following recommendations were unanimously adopted by the Working Party:

1. To improve methods for testing field resistance.
2. To improve laboratory and greenhouse methods for measuring resistance of the host and pathogenicity of the fungus.
3. To pay more attention to the high variability of the pathogen in relation to all aspects of resistance breeding.
4. To intensify host-range studies in Melampsoraceae.

All the participants enjoyed the warm reception by Finnish friends, especially the ''sauna break'', and agreed to come together again within a few years for the next meeting.

In Florence, October 1979

Prof. *Francesco Moriondo*
Chairman of the Working Party

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# PROBLEMS AND PROSPECTS IN BREEDING *MELAMPSORA* RUST-RESISTANT POPLARS[1)]

John Gremmen[2)]

The large number of *Melampsora* species and their wide and shifting ranges of alternate hosts show that the pathogen has a capacity to adapt to new conditions. Therefore, rust resistant cultivars of poplars have repeatedly been attacked by new or other races of the rusts as soon as the cultivars have been used on a large scale. The only escape from this dead-lock is the use of multilines of a wide variety of poplars mixed with other hardwood species.

## STATUS OF *MELAMPSORA* ON POPLARS

The world's distribution of *Melampsora* species attacking poplar and willow has been studied in detail by a number of mycologists, among them Arthur (1934), Fresa (1936), Gäumann (1959), Hiratsuka (1927, 1933), Jørstad (1953), Klebahn (1914), Marchionatto (1937), Matsumoto (1919) and Ziller (1974).

Geographic data have been used by van Vloten (1944), in mapping the occurrence of *Melampsora* species in Europe, Asia and North America, as well as the dissemination of ''European'' species of *Melampsora* on poplars in South America and South Africa. For a long time the southern hemisphere remained free from poplar rust because poplars did not occur in this part of the world. However, since 1972 *Melampsora medusae* Thüm. and *Melampsora larici-populina* Kleb. also reached Australia (Walker et al. 1974) and New Zealand (van Kraayenoord et al. 1974), both rapidly spreading in clonal stands of poplars recently established in these new areas.

In Western Europe including the Netherlands, various *Melampsora* species among them *M. larici-populina, M. allii-populina* Kleb. and *M. populnea* (Pers.) Karst. are widespread (Gremmen 1954). They represent the most important rust species causing serious leaf attack followed by early defoliation. Especially in localities where larch and rust-susceptible poplar clones are closely planted together, a disastrous effect caused by *M. larici-populina* was noticed (van der Meiden and van Vloten 1958).

How these rust fungi were distributed from continent to continent remains a point of controversy. Some scientists claim that uredospores are carried by air currents over thousands of miles, even over oceans.

Nobody will dispute that these spores may travel so far, since they have been demonstrated on high altitudes, but the dilution effect over long distances is so important that only few of them will have a chance to land on a susceptible host. In addition Omar (1978) in his study on poplar rust informed that ultraviolet radiation may well be a factor which prevents rust uredospores initiating disease after a very long distance of dispersal, since sunlight and ultraviolet radiation significantly reduce germination.

Other mycologists, however, demonstrate with stronger arguments that rusts were spread by man himself together with the host plant. Savile (1973) discussing *Melampsora aecidioides* (D.C.) Schröt. informed that it is painfully clear that the ability of uredospores to overwinter in dormant buds is shared by other *Melampsora* species on poplars. He suspects that

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[1)] Presented at the meeting of the IUFRO Working Party S2.05—05, Resistance in Pines to *Melampsora pinitorqua,* June 1979, Suonenjoki, Finland.

[2)] Dorschkamp Research Institute for Forestry and Landscape Planning, Bosrandweg 20, Postbus 23, Wageningen, The Netherlands.

*M. medusae* and *M. larici-populina* were introduced both in New South Wales and New Zealand by planting stock.

Nowadays poplars are used in many parts of the world as fast growing tree species for the increasing demand for wood and pulp. The interest for this tree species is still increasing, since special research institutes are now producing new selections and clones with special qualities for large-scale planting.

However, simultaneously with the increase of poplar stands all over the world, it was clear that disease problems became more and more important, forcing researchers to perform detailed work on pathogenic organisms, such as *Melampsora* rust, *Marssonina* leaf blotch, *Xanthomonas* canker a.o. Forest pathology research further proved that still more diseases are just waiting in other continents to get a foothold in the new plantations in other parts of the world.

Beside chemical control of some leaf diseases, such as rust and *Marssonina,* mainly executed in nurseries on planting stock, breeding resistant trees became an increasing field of research and even today it is still considered to be one of man's best weapons to overcome these noxious diseases.

However, breeding rust-resistant poplars proved to be far more complicated than was assumed. Van Vloten (1944, 1949) commencing research in poplar rust was well aware of these problems. One of his opinions was that rust-resistant poplars can only be bred with a detailed background knowledge of the *Melampsora* species and races and the technical possibility to apply this knowledge.

This is not a simple goal, since many *Melampsora* species cannot be distinguished in the field. Some of them are even hard to recognize by microscopic examination, but can merely be identified by inoculation experiments on special testplants. The same is true for distinguishing races.

In the Netherlands research on *Melampsora* rusts has been performed for more than 30 years, mainly dealing with *M. larici-populina, M. allii-populina* and *M. populnea* s.l.

The general picture of their life cycle is as follows:

Resting telia germinate in spring when weather conditions are favourable, producing basidia in which meiosis produces 4 haploid nuclei of two mating types. The basidium becomes 4-celled and each cell forms a basidiospore of one of the two mating types, arbitrarily designed ''plus'' or ''minus''. Basidiospores infect the alternate host, producing a monokaryotic mycelium and a fruiting body termed pycnium, which produces pycnospores, receptive hyphae and nectar. This nectar attracts insects which transfer the pycnospores from one pycnium to another. When these spores adhere to the receptive hyphae of the opposite mating type, this will result in the formation of a caeoma (aecium). The caeomaspores infect poplars or willows, producing uredinia with uredospores, followed by formation of telia.

However, apart from these general characteristics in the life cycle of poplar rust organisms, each ''species'' shows minor variations, e.g.:

1) *Melampsora larici-populina:* Uredinia and telia are formed on poplars in the sections Aigeiros Duby and Tacamahaca Spach. Pycnia and caeomata develop on larch, which is, as far as known, the only alternate host plant. However, numerous races have been demonstrated by van Vloten (1944, 1949).

2) *Melampsora allii-populina:* Uredinia and telia are formed on poplars in the section Leuce Duby. Tacamahaca. Pycnia and caeomata develop on *Allium* spp. and *Arum* spp.

3) *Melampsora populnea* s.l.: Uredinia and telia are formed on poplars in the section *Leuce* Duby. Pycnia and caeomata are formed on pine shoots, so-called ''*Melampsora pinitorqua* Rostr.'', on larch needles, so-called ''*Melampsora larici-tremulae* Kleb.'', or on leaves of *Mercurialis perennis* L., so-called ''*Melampsora rostrupii* Wagner''. Additional research work done in Italy demonstrated that beside the occurrence of *''M. pinitorqua''* on *Pinus pinea* L., *P. sylvestris* L., *P. nigra* Arn., *P. pinaster* Ait. and *P. halepensis* Mill., seedlings of 5-needle pine species, larch and Douglas-fir can be infected (Moriondo 1974).

Years ago the present author investigated a provenance of *M. populnea* from a Leuce crossing with the aim to identify the alternate host. In the inoculation tests an abundance of pycnia developed on larch needles, as well as a small number on Douglas-fir needles. Unfortunately the work had to be discontinued but evidence was obtained that a ''Douglas-fir race'' could be isolated by the use of Douglas-fir as a test plant.

It is known that other *Melampsora* species, such as *M. medusae* and *M. occidentalis* Jacks., occurring in North America alternate on *Pseudotsuga menziesii*

(Mirb.) Franco. The explanation for this phenomenon may be that certain *Melampsora* forms developed 0 and 1 stages on Douglas-fir in North America, since this tree is native on this continent, whereas for the same reasons 0 and I stages developed on larch trees on the Eurasian continent, hence the result depends on the available tree species.

However, there is not only variation in alternate host plants, but also a difference in reaction on a variety of poplars (Table 1) when inoculated with *Melampsora populnea,* using caeomaspores from pine and larch.

Table 1. Reaction of various poplar species and provenances upon inoculation with two different *Melampsora populnea* provenances.

| Host plant | *Melampsora* from pine | *Melampsora* from larch |
|---|---|---|
| *P. tremula* nr. 9 | + | + + |
| *P. tremula* nr. 23 | + | + + |
| *P. canescens* nr. 34 | + | – |
| *P. alba* nr. 31 | ± | – |
| *P. alba* nr. 37 | – | ± |
| *P. grandidentata* nr. 85 | – | – |
| *P. grandidentata* nr. 88 | – | + |
| *P. grandidentata* nr. 99 | – | – |
| *P. tremuloides* nr. 78 | + | – |
| *P. tremuloides* nr. 79 | – | – |
| *P. tremuloides* nr. 96 | + | + |

This phenomenon is not restricted to the genus *Melampsora* Castagne, since very embarrassing observations were made in blister rust research by Hiratsuka and Maruyama (1976) while working with a rust provenance from *Pinus monticola* Dougl. Fresh caeomaspores of *Cronartium ribicola* J.C. Fischer ex Rabh. produced telia on *Castilleja miniata* Dougl.ex Hook, the Indian paint brush, a common member of the Scrophulariaceae, widely distributed in North America.

This discovery had great significance, since it again demonstrates the dynamic development in the Uredinales.

In this connection it is interesting to reflect for a while upon the intentions to eradicate *Ribes* species in order to eliminate the blister rust fungus. This was not only unrealistic, but it would have stimulated the development of special races of *C. ribicola* on the Indian paint brush.

We have sufficient evidence now that rust fungi have the possibility to survive on other hosts and even to adapt to new hosts of totally different plant families. Such a change in host plant may well have occurred several times during the existence of certain rust fungi, e.g., as a result of the extinction of its specific host.

## DISCUSSION

Detailed methods have been developed to culture *Melampsora* species and their races, e.g., by use of a leaf-disc method (van Vloten 1944) which enable us to produce sufficient uredospores for inoculation experiments of poplars.

In this way a good estimation of the susceptibility of a certain poplar clone tested for certain rust races can be obtained. Even resistant clones for special rust races can be selected.

Such new promising resistant clones may be, however, attacked again as soon as:

1) other rust species or races are introduced from abroad. This is an oppressive reality when we recollect the story of *M. medusae,* a rust spreading from North America to Europe, recently invading the southern hemisphere.
2) New rust races develop as the result of re-combination of gene material when poplars and the alternate hostplants of the rust are closely growing together. Van Vloten (1949) was able to demonstrate by artificial crossings in *M. larici-populina,* that new rust races may show up which differ in pathogenicity and even in colour of the spores.
3) Unknown races, forming part of the natural pool of races in the field, show up as the result of selection pressure due to breeding new clones.

While breeding for resistance in potatoes against the fungus *Phytophthora infestans* (Mont.) de Bary, Müller (1928, 1931, 1933) observed that the new resistant W-variety became attacked after several years of healthy growth. This occurred simultaneously in various localities of Germany. It could be demonstrated that *P. infestans* consists of various physiologic races. Three possibilities for explanation of this phenomenon were discussed, viz. 1) mutation in the pathogen, 2) selection in an already existing population of *P. infestans*, and 3) introduction of an unknown race from abroad.

Van Vloten (1944, 1949) however, assumed that this ''new'' race was already present as part of the population of the fungus, but that it showed up as the result of extensive breeding and selection work.

This is a particularly interesting point appropriate for broader discussion, since it could give new evidence for the recent development of the ''new'' race in *Ophiostoma ulmi* (Buism.) Nannf., called the ''agressive form'', characterised by increased pathogenicity compared with the ''semi-agressive'' form.

There is still a lot of confusion regarding the question whether this ''agressive race'' was introduced into Europe or not. According to Brasier and Gibbs (1973) ''It would thus seem that the agressive and nonagressive strains were originally present in both Europe and America''.

It seems therefore quite acceptable that this''new''race of *Ophiostoma ulmi* merely showed up as the result of the breeding work against the so-called ''semi-agressive'' race of this pathogen.

A similar development of more agressive forms has been recently noticed in *Xanthomonas populi* subsp. *populi* (Ridé) Ridé & Ridé, the cause of bacterial canker in poplar (Ridé and Ridé 1978). This will be discussed in a separate paper.

## CONCLUSIONS

Breeding rust-resistant poplars has no prospect as long as monoclonal plantations of this tree species are established, mainly because:

a) numerous *Melampsora* species and races occur in Europe, Asia, North-America, as well as ''European'' species in South America, South Africa and even now in Australia and New Zealand. They are continuously spread by man as result of planting poplars in new areas of the world.
b) *Melampsora* rusts and in general the Uredinales have a dynamic life cycle as has been discussed in detail in this paper. For survival, they may even change alternate host plant.
c) New rust races are formed by re-combination of gene material, especially when the principal host and alternate host are growing closely together.
d) Selection pressure on rust populations in the field by breeding new clones. This activity implies the possibility for selection of other races which may be more virulent than the existing ones.
e) Poplars are constantly replaced by new resistant selections which are the result of continued breeding work.

The only escape from this dead-lock is the use of poplar plantations consisting of multilines of selected, heterogenous clones in which a great proportion of good and healthy-growing semi-resistant clones are included, or even better, a variety of poplars mixed with other hardwood species.

This is not merely for reasons of attack by *Melampsora* rusts, but also for other pathogenic organisms, threatening poplar cultures.

In such new situations there is less build-up of pathogenic organisms and less probability that certain virulent rust species or races will kill trees on a large scale.

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Folia Forestalia 422:10 — 13.1980

# EFFECT OF CLIMATIC FACTORS ON DEVELOPMENT OF THE DISEASE CAUSED BY *MELAMPSORA LARICI-POPULINA*.[1][2]

Zbigniew Krzan[3]

Detached poplar leaves were inoculated with urediniospores of *Melampsora larici-populina* and incubated in growth chambers where either humidity or temperatures were kept constant at 100 % RH and +16 °C, respectively, while the other factors varied within a wide range. In field experiments the effects of these 2 factors on disease development were studied for 4 years. It was concluded that for germination, the optimum RH was 100 % and temperature +16 °C. Below RH 65 % germination stopped. The final extent of disease development in the field was determined by the pattern of preceding weather conditions and especially relative air humidity.

## INTRODUCTION

In studies of poplar leaf rust caused by *Melampsora larici-populina* Kleb. conducted at the Institute of Dendrology of the Polish Academy of Sciences in Kórnik, problems of the influence of some climatic factors on disease development were also included. These studies were based primarily on the uredinium stage of the pathogen, because of the great importance of this stage in disease severity. Abundant occurrence of uredinia of *M. larici-populina* resulting in heavy infection of poplar leaves seems to be closely connected with higher relative air humidity (RH). The significant roles of RH and air temperature in distribution and germination of urediniospores of rust fungi has been previously discussed by Taris (1966), Turel (1969), Kurkela (1973), Omar and Heather (1975) and Manners (1978). These studies were done on fungi from the genera *Melampsora* and *Puccinia* both including important pathogens of trees and agricultural plants.

The purpose of the present study was to investigate in laboratory tests the humidity and temperature conditions optimal for germination of *M. larici-populina* urediniospores on poplar leaves and to determine how relative air humidity and air temperature influence disease development in the field.

## MATERIALS AND METHODS

The studies were conducted in laboratory conditions where detached leaves of *Populus trichocarpa* Torr. & Gray, *P. 'Robusta'* and *P. nigra 'Italica'* were infected with a water suspension of urediniospores of the average concentration 4 × $10^5$/ml. The leaves were then incubated for 6 days in growth chambers, in different air humidity and a constant temperature of +16 °C (experiment 1) and in different temperatures and a constant air humidity of 100 % (experiment 2). In order to facilitate observations in a light microscope the urediniospores were stained with cotton blue diluted in lactofenol and taken off the leaf surface using fixative spray for cytodiagnosis — "Mercofix". The percentage of urediniospores that had germinated in different conditions demonstrated the magnitude of the influence of air humidity and temperature on germination.

The influence of these two climatic factors on rust severity in the field was studied

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during 4 years of observations in a field experiment. In statistical analyses monthly means of relative air humidity and air temperature were used. Rust severity was characterised by Mean Infection Index (MII) calculated as an arithmetical mean of degrees of infection (from 0 = no infection to 5 = heavy infection) of about 30 poplar clones growing in replications.

Correlation coefficients (r) between rust severity and relative air humidity were calculated separately for individual months and then converted to Fisher's z-values by the formula:

$$z = 1/2 \ln \frac{1 + r}{1 - r} = \text{arc tan } r$$

These z-values were then averaged for all 6 months tested (May — October) and converted back to r. Degrees of freedom were calculated by the formula: $[\Sigma(n-2)]-1$ that is $[6 \times (4 - 2)] - 1 = 11$. Such a transformation was necessary because correlation coefficients do not have a normal distribution and therefore cannot be directly averaged (Fisher 1956). As a result a single correlation coefficient was calculated based on 11 degrees of freedom.

## RESULTS

As a result of these tests it was concluded that the optimal air humidity for germination of urediniospores of *M. larici-populina* is 100 % (Table 1), and the optimal air temperature is 16 °C (Table 2). When the air humidity was below 65 % the germination practically stopped. This was probably associated with the rapid evaporation of water from the leaf surface in these conditions. Temperatures higher than optimum inhibited germination of urediniospores much more effectively than low temperatures (Table 2).

Table 1. Percentage of germinated urediniospores of *M. larici-populina* on the leaf surface after 6 days of incubation in different air humidities.

| Relative air humidity % | 100 | 85 | 75 | 65 | 55 |
|---|---|---|---|---|---|
| Germination % | 56 | 30 | 23 | 7 | 0 |
| Time of water evaporation from the leaf surface hrs. | — | 10,0 | 4,5 | 1,5 | 0,5 |

Table 2. Percentage of germinated urediniospores of *M. larici-populina* on the leaf surface after 6 days of incubation in different air temperatures.

| Temperature °C | 3 | 16 | 19 | 22 |
|---|---|---|---|---|
| Germination % | 22 | 33 | 16 | 4 |

We also found qualitative differences in germination of urediniospores in various conditions. In optimal conditions spores germinated very quickly, producing long, ramose germ tubes with the cytoplasm migrating towards their ends. When RH or temperature conditions were unfavourable for urediniospores, they produced only short, simple germ tubes within the same incubation period.

During 4 years of observations of disease development in the field it was established that its final extent (i.e. the severity of infection at the last observation every year) was determined by the pattern of preceding weather conditions. Influence of some climatic factors, especially relative air humidity, was also dissimilar in the consecutive months of the growing season (Table 3).

Table 3. Correlation coefficients of the rust severity on poplar leaves with monthly mean relative air humidity ($r_{1,2}$) and with monthly mean air temperature ($r_{1,3}$) calculated on the basis of 4 years of observations.

| Months | Correlation coefficients | |
|---|---|---|
| | $r_{1,2}$ | $r_{1,3}$ |
| May | 0,676 | 0,281 |
| June | 0,914* | −0,055 |
| July | 0,913* | −0,882 |
| August | 0,477 | 0,019 |
| September | 0,297 | −0,355 |
| October | 0,440 | 0,944* |

*) Significant at $P < 0,1$

It is striking that relative air humidity was always positively correlated with the infection severity and the magnitude of the correlation was decidedly greatest and statistically significant in June and July (Table 3). This indicates that the values, which relative air humidity attains during these 2 months have had the greatest effect on the level of infection of poplar leaves by the rust in the tested 4 years.

A negative or positive correlation of the disease severity with air temperature depends on whether in the given month air temperature was higher or lower than optimum for germination of urediniospores. Thus in June and July, when air temperatures usually exceeded 16 °C, the correlation was negative, while in May and October when air temperatures usually were lower than optimum, the correlation was positive. Additionally in October when average air temperature was about 8—9 °C, this correlation was significant ($P < 0{,}1$).

Above results were based on only 2 degrees of freedom (4 years of observations) and therefore we calculated the "r" coefficient of correlation of rust severity with relative air humidity totally for 6 months tested. As a result we obtained the $r_{1,2} = 0{,}486$ which was statistically significant, and had 11 degrees of freedom.

These results suggest that during the studied period RH had a significant influence on rust severity in field conditions. On the basis of these observations Figure 1 was prepared in which graphically a relationship is shown between the magnitude of rust infection and the pattern of RH and air temperature attained in June and July during 4 consecutive years of observations. On the graph the relationship, particularly with RH is apparent.

## DISCUSSION

The results of our studies showed that some climatic factors have a significant influence on the development of *M. larici-populina* and therefore on the extent of damage caused by the pathogen on poplar leaves. It turned out that a pattern of these factors and in particular the amount of relative air humidity in the early propagation phase of the pathogen may facilitate or seriously restrict development of the disease. The important role of high RH in the germination and growth of the germ tubes of urediniospores was earlier stressed by Omar and Heather (1975) who, when studying the germination of urediniospores of *M. larici-populina* in Australia had established that the optimum RH is 100 %. At optimal conditions of humidity and temperature lasting for a minimum of 3 hours, germ tubes can already infect stomata.

Taris (1966) found however, that for rapid spread of urediniospores of *Melampsora* sp. it is necessary to have a relative air humidity not higher than 80 %. This observation had been confirmed by Kurkela (1973) who had shown that a high RH as well as abundant and long-term rains may substantially reduce possibilities for aeciospores and urediniospores of *M. larici-tremulae* and *M. pinitorqua* (Braun) Rostr. to spread.

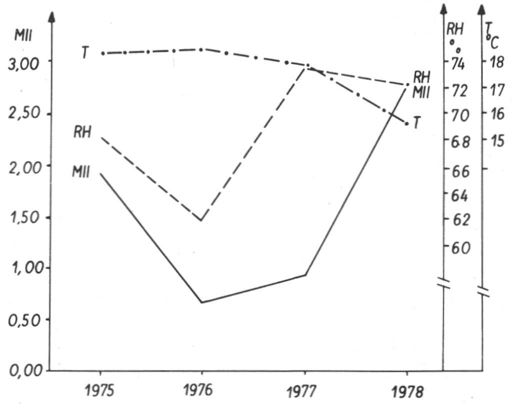

Figure 1. The relationship between severity of rust infection on poplar leaves (MII), relative air humidity (RH) and air temperature (T) in June and July during 1975—1978.

In our climatic conditions during summer and fall there exist, within the diurnal cycles, conditions which guarantee possibilities for the spread of urediniospores during daytime when RH is usually below 80 %, and germination during night-time when RH is close to 100 %.

Our results also indicated a positive correlation between rust infection and RH. In practice this means that whenever there is higher RH one should expect greater losses in poplar plantations as a result of stronger rust infection.

We stated that for germination of urediniospores optimal temperature was about 16 °C, which is in complete agreement with the results obtained earlier by Turel (1969) for the germination of urediniospores of *M. lini* (Schum.) Lév. in axenic cultures. Studying wheat rust caused by *Puccinia striiformis* Westend., Manners (1978) has shown that temperatures lower than 7 °C and especially higher than 20 °C had considerably limited spore development and that the optimum was 15 °C.

Different results were obtained in Australia by Omar and Heather (1975) who have determined an optimal germination temperature for urediniospores of *M. larici-populina* to be 25 °C. Possibly these results indicate that there exists in Australia a race of the pathogen that is adapted to the local climatic conditions.

Taris (1966) established that for good spread of urediniospores of *Melampsora* sp. air temperature should be higher than 10 °C. In our climatic conditions, during the time of disease propagation air temperature is usually close to the optimum for germination and therefore does not inhibit spread of urediniospores.

One should consider therefore, that in Poland, except for the short periods of summer heats, air temperature is not a factor that would hinder the spread of *M. larici-populina*. Thus the role of this factor in the development of the disease, though significant, is much less than that of relative air humidity.

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# RECENT STUDIES ON *MELAMPSORA LARICI-POPULINA* AND *MELAMPSORA PINITORQUA* IN POLAND[1)2)]

Zbigniew Krzan and Ryszard Siwecki[3)]

Recent research on *Melampsora larici-populina* in Poland is reviewed. Effects of saprophytic mycoflora and climatic factors on disease development have been studied and infection processes in tissues of resistant and susceptible hosts have been followed. Poplar clones with varying degree of resistance have been selected. Field experiments on infection of open pollinated pine progenies with *Melampsora pinitorqua* have recently been initiated. The role of air pollution in epiphytotics of *M. pinitorqua* in pine stands has also been studied.

## INTRODUCTION

Basic research on resistance breeding of poplars and pines at the Institute of Dendrology in Kórnik is being conducted on two species of fungi from the genus *Melampsora* namely *M. larici-populina* Kleb. and *M. pinitorqua* (Braun) Rostr., which in Poland are important as causal agents of serious diseases of poplar and pine. Other fungal species from the genus *Melampsora* such as *M. allii-populina* Kleb., *M. larici-tremulae* Kleb., *M. magnusiana* Wagner and *M. rostrupii* Wagner occur only sporadically in our country and are not of economic importance.

The first reports on the occurrence of *Melampsora* rusts in Poland come from the beginning of the present century: Namyslowski (1911), Wodzicko (1911), Wroblewski (1915, 1925).

*M. pinitorqua* occurs almost throughout the country, except for the southern mountain part of Poland where the aecial host — *Pinus sylvestris* L. is lacking. *M. larici-populina* is common in the whole country.

## STUDIES ON *MELAMPSORA LARICI-POPULINA*

*M. larici-populina* forms pycnia and aecia on needles of *Larix decidua* Mill. It does not cause any serious damage to this host. The disease is, however, extremely dangerous to the alternate host *Populus nigra* L., which still occurs naturally in carr forests, or to the various poplar cultivars from the sections Aigeiros and Tacamahaca which are used in plantations. Uredinia and telia of the pathogen occurring massively on their leaves cause heavy infection and premature, pathological leaf fall which may even lead to the death of young trees.

A stool bed with about 300 poplar clones and a field experiment with poplars and larches growing in 6 replications were used in our studies of *M. larici-populina.* Rust severity was estimated by a 6-point scale and then averaged as Mean Infection Index (MII) for individual clones or totally for groups of clones. In the stool bed observations were made thrice a year (always at the end of July, August and September) and in the field experiment they were made at weekly intervals from the beginning of June to the end of October. Use of MII permitted a comparision of the severity of the disease on poplar leaves both between various clones and different years of observations.

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2) This work has been partially supported by grant FG-Po-298 from the US Department of Agriculture under PL-480, and partially by grant II/15 from the Polish Academy of Sciences.

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Over the last 4 years the biology and morphology of *M. larici-populina* has been investigated, and the effect of some climatic factors and saprophytic mycoflora on the development of the disease has also been studied. In these studies much attention has been paid to the uredinium stage, which as a propagation phase has a decisive influence on the severity of the disease and on the injuries it inflicts.

On the basis of these studies poplar clones characterized by considerable resistance to rust were selected (Krzan 1976, 1980). The mode of infection of the leaf by aeciospores and urediniospores was studied in laboratory tests. Similarly, further development of the pathogen in tissues of both susceptible and resistant hosts was also studied (Werner 1979). Studies on the role of saprophytic fungi on the inhibition of the development of the pathogen are in progress. General statements of studies on *M. larici-populina* have been published (Siwecki 1978).

## STUDIES ON *MELAMPSORA PINITORQUA*

*M. pinitorqua* is dangerous to both aecial and alternative hosts (Siwecki 1976). The aecial host is Scots pine *(Pinus sylvestris* L.) commonly occurring and growing in our forests. Aecia of the fungus develop on young, non-lignified pine shoots causing their twisting and breaking and thus deforming the tree. According to data collected by the Forest Research Institute in the years 1960—1972 the disease occurred intensively on an area of 3—10 thousand hectars of young plantations and stands of pine in Poland (Siwecki 1974). *M. pinitorqua* causes also injuries on the leaves of its secondary host, namely *Populus alba* L., *P.* × *canescens* (Ait.) Smith and *P. tremula* L. *P. alba* and *P.* × *canescens* occur in Poland in river valleys and *P. tremula* as a supplementary species in pine stands. Some detailed results of studies on *M. pinitorqua* have been presented earlier (Siwecki and Werner 1974).

In Kórnik a special field experiment was established in 1977 to study biology, morphology and occurrence of *M. pinitorqua,* where in 3 replicates 2-year-old pine seedlings were planted. The seedlings originated from seeds of individual, open pollinated trees from 5 of the best Polish pine provenances from Bialowieza, Hajnówka, Bolewice, Rychtal and Karczma Borowa. Between the rows of Scots pine young plants of aspen from Kórnik were planted to provide a natural infection for the pines. In 2 border rows local Scots pine was planted.

The amount of damage on the pine is every year estimated as the number of affected seedlings and determined as the number of shoots with the aecium stage per seedling. Percentage expression of these observations is the measure of the severity of the disease. The amount of damage on poplar leaves are estimated by the 6-point scale averaged to Mean Infection Index (MII).

In the first year of observations no infection of pine shoots was found. This was probably caused by the lack of inoculum. Late in the summer the first uredinia appeared on the aspen leaves. On the basis of morphological characters these uredinia have been tentatively identified as uredinia of *M. pinitorqua.* In the autumn some telia of the fungus appeared on infected leaves. These studies are continued.

Interesting results were obtained recently in Poland in connection with studies of communities of pathogenic fungi in forests growing under influence of industrial air pollution. It was found that in these regions injuries to young pines caused by *M. pinitorqua* were about 50 % less than in the forests which were not under the influence of air pollution (Grzywacz 1973, Grzywacz and Wazny 1973). Domański (1976) found however that in the case of close proximity of both hosts and a dense canopy of a young pine — aspen stand in Dulowa near Kraków, even within the zone of strong air pollution, an epiphytotic occurrence of *M. pinitorqua* on pine was possible. In Domański's opinion the cause of such a strong development of the disease may be specific microclimatic conditions which have facilitated pathogen development and have largely protected the inner parts of the stand from air pollutants.

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# SEROLOGICAL TESTS FOR IDENTIFYING SPECIES OF *MELAMPSORA* ON ASPEN[1]

Timo Kurkela[2]

Serological tests were suggested as a means of indentifying aspen rusts, which are morphologically similar.

## INTRODUCTION

In Finland 4 species of *Melampsora* occur on aspen (*Populus tremula* L.) leaves in the uredial or telial stages, with a different host for the aecial stage (Liro 1908). The species are:

| | Aecial host |
|---|---|
| *Melampsora pinitorqua* (Braun) Rostr. | *Pinus sylvestris* L. |
| *M. larici-tremulae* Kleb. | *Larix* spp. |
| *M. rostrupii* Wagner | *Mercurialis perennis* L. |
| *M. magnusiana* Wagner | Papaveraceae |

Only the first species, when it causes pine twist rust, is economically important in Finland. Aspen is less important than pine in forestry and often grows as a weed in pine regenerations. On aspen leaves the 4 *Melampsora* species, collectively called *M. populnea* (Pers.) Karst., cannot be identified with certainty using morphological characters. Reliable identification, however, is needed for epidemiological studies and for testing resistance of pines to *M. pinitorqua*.

## SEROLOGICAL TESTS

Serological tests, due to their high specificity, may give a possibility for accurate identification. Therefore, attempts were made to produce antiserum in rabbits by injecting them with an aeciospore extract. Ground spores of crude homogenates of aspen leaf tissue infected by *M. populnea* were used as test antigenes, or alternatively, antigene was extracted using the method described by Gooding and Powers (1965). Double-diffusion tests on purified agar gel were used to indicate immunological precipitation (Growle 1961, Ouchterlony 1969).

Final results are not available for this paper, but some interesting preliminary results have been obtained (Fig. 1). Anti-

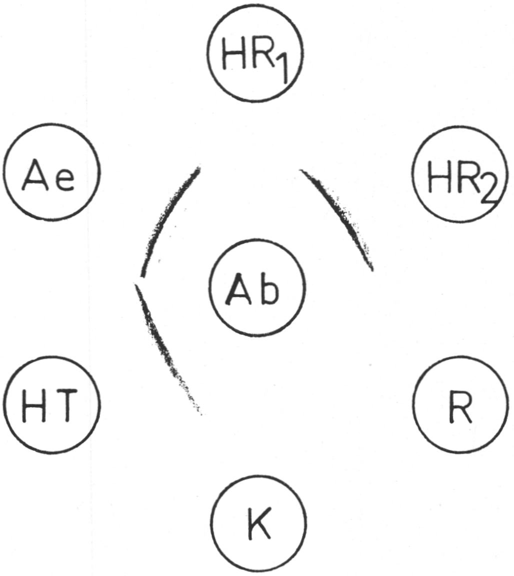

Fig. 1. Precipitation lines obtained in a serological double-diffusion test on agar gel. Ab) Rabbit-blood antibodies obtained with (Ae) extract from aeciospores of *Melampsora pinotorqua,* (HR$_1$, HR$_2$, and HT) extracts from aspen leaves with *Melampsora populnea,* (K) extract from leaves of *Betula pendula* with *Melampsoridium betulinum,* and (R) extract from leaves of *Salix caprea* with *Melampsora caprearum.*

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[1] Presented at the Meeting of the IUFRO Working Party S2.05—05, Resistance in Pines to *Melampsora pinitorqua,* June, 1979, Suonenjoki, Finland.

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serum obtained with aeciospores of *M. pinitorqua* reacted strongly, but differently, with antigene from the same aeciospores and with extracts from aspen leaves bearing the uredial and telial pustules of *Melampsora.* In contrast, the same antiserum produced very weak precipitation lines when reacting to extracts from leaves of *Betula pendula* Roth or *Salix caprea* L. infected by *Melampsoridium betulinum* (Fr). Kleb. or *Melampsora caprearum* (DC.) Thüm., respectively. During some summers it may be difficult to collect aeciospores in sufficient quantities, and spores are often contaminated with bacteria, other fungi, insect eggs, and mites. In the purifying process many of the spores may be lost. Very probably, different *Melampsora* species have the same type of contamination, which can cause confusing precipitation lines in double-diffusion tests.

Basidiospores can be used as alternative material for producing antiserum, but one should be very careful when identifying the telial stage on overwintered aspen leaves. Existence of only one aecial host at the location where material is collected does not exclude the possibility of a mixed *Melampsora* population on the aspen leaves. Results from inoculation tests may also be confusing; in greenhouse tests especially, cross infection seems possible, e.g., one may obtain infection on both pine and several species of larch with the ''same'' telial material (Longo et al. 1975, Weissenberg, pers. commun.).

As this serological work continues the main objective will be to find specific reactions differentiating between different species or races of aspen rusts.

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# OVERWINTERING AND GERMINABILITY OF TELIOSPORES OF *MELAMPSORA PINITORQUA*[1]

Nicola Longo[2], Francesco Moriondo[3] and
Biancamaria Naldini Longo[4]

The authors studied the influence of different overwintering sites on the germinability of teliospores of *Melampsora pinitorqua.* They observed that germinability was higher when telia overwintered in uncovered sites, both at ground level and above.

Environmental features that increase germinability of teliospores are possibly aeration and irradiation. Ultimately, teliospores which overwintered in uncovered sites produced basidiospores with higher infectivity on seedlings of *Pinus pinea* L.

## INTRODUCTION

The teliospores of *Melampsora pinitorqua* (Braun) Rostr. differentiate in autumn on the leaves of *Populus tremula* L. and, under natural conditions, acquire the germination ability in the following spring.

According to Kurkela (1973) several authors (Dietel 1912, 1921; Regler 1957; Klebahn 1914) found that teliospores of *Melampsora* and *Puccinia* varied in germinating activity. This they attributed to the environmental conditions during overwintering. Similarly previous observations carried out at different sites in the area of Monticiano (Siena) (Longo et al. 1976) showed that the environmental conditions could affect overwintering and germinability of teliospores. Particularly it was proved that germinability was affected by the type of soil cover; teliospores which had overwintered on uncovered soil showed a very high germinability, whereas those which had overwintered in the underbrush showed a very low one.

The objectives of this study were to investigate: 1) the influence of different soil covers and the influence of different overwintering sites on the germinability of teliospores; 2) how the infectivity of basidiospores was affected by the conditions in which teliospores overwinter.

In order to provide experimental data for the first objective 3 trials were carried out. The first was made in 1976 at Monte Quoio (Monticiano, Siena). The purpose of this trial was to compare the germinability of teliospores wich had overwintered on covered and uncovered soil, but with a type of vegetation different from that of 1973 (Longo et al. 1976). The second trial was carried out in 1978 in Firenze to study the effects of shading. The third one in 1979 at Antella (Firenze) and Luriano (Monticiano, Siena) had the purpose to evaluate how overwintering at ground level or at some distance from the ground could affect germinability of teliospores.

Experiments concerning the second objective were carried out as follows: 2 separate sets of *Pinus pinea* seedlings were infected by germinating teliospores wich had overwintered at Farma (Monticiano, Siena) on 2 types of soil-cover similar to those in the trial of 1973.

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1) Presented at the Meeting of the IUFRO Working Party S2.05—05, Resistance in Pines to *Melampsora pinitorqua,* June, 1979, Suonenjoki, Finland.

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## MATERIALS AND METHODS

All the tests were performed with leaves of *Populus tremula* carrying telia of the rust and collected from the ground in the area of Monticiano (Siena) in the autumn of the preceding year. The leaves were immediately divided into as many lots as were the replicates of each test; then they were arranged in wide-mesh nets and placed in different overwintering sites.

Sampling of leaves for controlling the germinability of teliospores started in the following spring at random during the germination period (after the middle of March). Then teliospores on aspen leaves were placed in petri dishes on moist filter paper and incubated at 15 °C for 50 hours. Intensity of germination was observed and evaluated by the methods previously adopted (Longo et al. 1970, 1976).

In the trial of 1976 the intensity of germination estimated under the stereomicroscope was checked by an approximate count of the basidiospores produced by germinating teliospores. The basidiospores fallen from a fixed number of telia on a determinated amount of physiologic solution were counted for each replicate. For this purpose small petri dishes (5,5 cm in diameter) were used, each containing on the bottom 3 cc of physiologic solution and on the cover 3 cc of water-agar with 1000 adhering telia. The count of basidiospores, performed under a light microscope at 100 × magnification, was relative to a surface of 1,14 mm$^2$ and was averaged over 50 surfaces.

The overwintering sites are presented in Table 1.

The trial of 1976 at Monte Quoio consisted of 4 replicates on uncovered soil and 4 replicates in the surrounding underbrush made up of scrub (*Erica scoparia* L.), a few scattered pines *(Pinus pinaster* Ait.) and strawberry-trees (*Arbutus unedo* L.).

The trial of 1978 in Firenze consisted of 4 replicates under a shading net (75 %) and 4 uncovered replicates next to the preceding ones.

The trial of 1979 was arranged in the following way. At Antella: a) 12 replicates were placed on the ground at the foot of as many trees of *Pinus pinea* L., 4 m tall and 10 years old in a small plantation with a spacing of 0,5 × 1 m. 12 more replicates were placed on the top shoot of the pine trees. b) 3 replicates were placed on the ground at the foot of 3 young planes (*Platanus* sp.), 3 m tall, which grew in a row on agricultural soil. 3 more replicates were placed on the top of the planes. c) 4 replicates were placed under polyethylene bags, 2 of them just above the

Table 1 — Key of trials on the germinability of teliospores of *Melampsora pinitorqua* Rostr. which had overwintered in different sites.

| Number of trial | Year | Location | Number of sets | Number of replicates for each set | Type of overwintering site |
|---|---|---|---|---|---|
| 1st | 1976 | Monte Quoio | 2 | 4 | A) uncovered soil<br>B) underbrush: shrub, a few scattered pines and strawberry trees |
| 2nd | 1978 | Firenze | 2 | 4 | A) uncovered soil<br>B) covered soil under a shading net (75 %) |
| 3rd | 1979 | Antella a) | 2 | 12 | A) on the ground under pines<br>B) on pines |
| | | —"— b) | 2 | 3 | A) on the ground under planes<br>B) on planes |
| | | —"— c) | 2 | 2 | A) under polyethylene bags on the ground<br>B) under polyethylene bags suspended above the ground |
| | | Luriano | 2 | 1 | A) on the ground under a pine sapling<br>B) on the pine sapling |
| 4th | 1978 | Farma | 2 | 4 | A) uncovered soil as in 1973<br>B) underbrush |

ground and 2 at 2 m. At Luriano: 1 replicate was placed on the ground at the foot of a *Pinus pinaster* sapling 4 m tall, and 1 on its top shoot.

In order to verify the second objective, in the autumn of 1977 at Farma (Table 1, 4th trial) 4 replicates were placed on uncovered soil and 4 in the underbrush; 2 types of soil cover as for the trial of 1973 were used. In the spring of 1978 telia which had overwintered on the different soil covers were collected and incubated. Then 80 seedlings of 1-year-old *Pinus pinea* were inoculated, in 2 series of 8 replicates each, with an approximately equal number of germinating teliospores.

The intensity of rust infection was estimated by calculating the percentage of infected shoots. The rate of infection was indicated with (1) or (2) according to whether the yellow zones with pycnia and aecia were few and only on the primary needles, or many and on the stem too.

The method used for the statistical comparison of results was the Student's t-test for coupled samples.

## RESULTS AND DISCUSSION

Results obtained in 1976 at Monte Quoio (Fig. 1) show an opposite trend in the intensity of teliospore germination. In the first 2 collections the intensity of germination was lower for material collected in the underbrush as compared to the material overwintered on uncovered soil. In the next 2 collections the trend was exactly the opposite and the maximum germination intensity of teliospores overwintered in the underbrush never reached that of teliospores overwintered on uncovered soil as it was obtained in the first 2 collections. Also the statistical analysis (not performed for the number of basidiospores) showed that the difference between intensities of germination was not significant (Table 2). The difference between the result of this test and that of 1973 may be explained taking into account the difference of vegetation on the two sites. In 1973 the underbrush cover was made up of scrub *(Erica scoparia)* and broadleaf trees (*Quercus cerris* L., *Q. pubescens* Willd., *Castanea sativa* Mill.), and it provided a rather continuous cover, whereas in 1976 it was sparser and more discontinuous (Table 1). This situation may account for the reduced difference between overwintering conditions in the underbrush and on uncovered soil.

Figure 1 shows the intensity of germination estimated with a stereomicroscope and the number of basidiospores produced by teliospores of the same sample. The 2 series of values seem to coincide proving the reliability of the estimate made with the stereomicroscope commonly used by us.

Although the graphs from the test of 1978 do not reveal a clear trend (Fig. 2), the statistical analysis shows that the intensity of germination of the teliospores overwintered in the shade and that of those overwintered on uncovered soil is significantly different (Table 2). Shading is probably one of the conditions which could affect overwintering of teliospores.

The trial of 1979 gave the following results (Fig. 3).

At Antella:

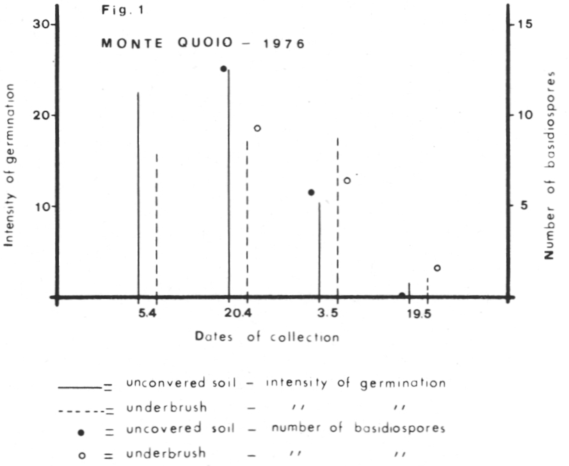
a) Although the teliospores overwintered on the ground at the foot of the pines had germinated rather abundantly, the statistical analysis showed that the intensity of germination of those overwintered on the top shoots of the pines was significantly higher (Table 2).
b) Teliospores overwintered both on the ground at the foot of the planes and on the planes germinated very abundantly. The difference between their intensity of germination was not significant (Table 2).
c) Germination of teliospores overwintered under a bag of polyethylene is to be considered practically negligible (especially if compared with that of the contemporaneous tests) both for teliospores overwintered near the ground and for those placed at 2 m from the ground.

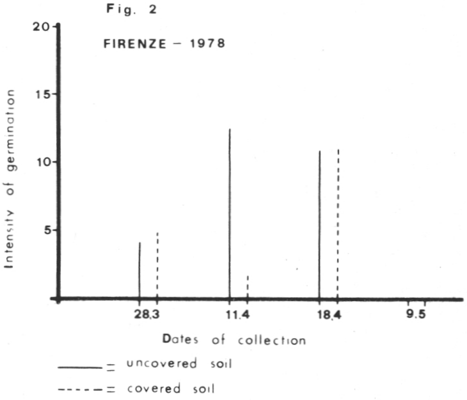
At Luriano:

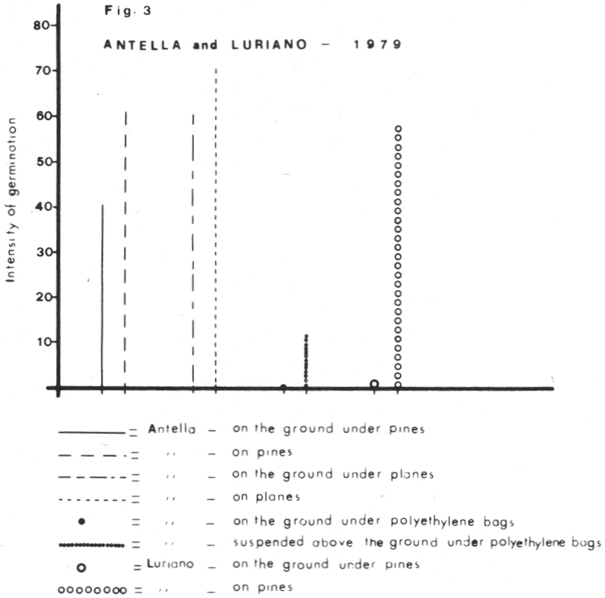
Germination of teliospores overwintered on the ground under the pines was negligible, whereas teliospores overwintered on the pines germinated abundantly and the result was quite comparable to the highest values in the other tests.

We wish to point out that no statistical analysis was performed for the last 2 tests because the examined material was too few in number; in spite of this the results appear rather significant, especially if they are compared to those of the parallel tests.

A comparison of the results of the 4 tests carried out at Antella and Luriano in 1979 showed that a certain distance from the ground of the overwintering teliospores could affect their germinability in that it is related to the environment conditioned by the type of soil cover. In fact telia overwintered on agricultural soil at the foot of plane trees were as uncovered as those on the planes, whereas telia overwintered on the ground at the foot of pine trees were under cover, while those on the pines were not. Likewise the polyethylene bags affected negatively both sets of leaves, the one just







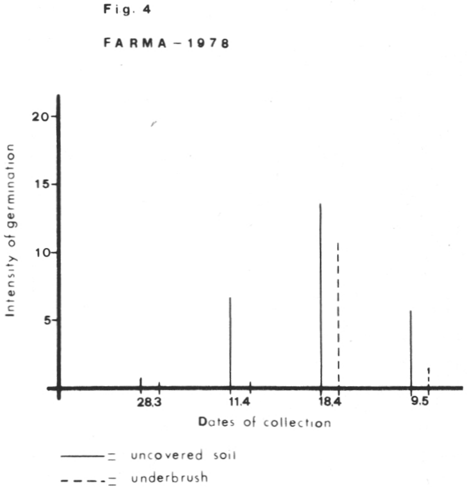


Fig. 1 — Germination of teliospores overwintered at Monte Quoio in 1976 on uncovered soil and in the underbrush at different dates of collection. The two series of values derive from the estimate made with the stereomicroscope and from the number of produced basidiospores.

Fig. 2 — Germination of teliospores overwintered in Firenze in 1978 on uncovered and covered soil at different dates of collection.

Fig. 3 — Germination of teliospores overwintered at Antella and Luriano in 1979 in different sites both at ground level and at some distance from the ground.

Fig. 4 — Germination of teliospores overwintered at Farma in 1978 on uncovered soil and in the underbrush at different dates of collection.

above the ground and the one 2 m above the ground (Antella, c); the same occurred at Luriano with the vegetation covering the set of leaves on the ground.

Some differences in germinability of teliospores due to the influence of the overwintering sites were found also by Heagle and Moore (1970) in *Puccinia coronata* f.sp. *avenae* Fraser and Ledingham. These authors obtained no germination from teliospores which had overwintered in polyethylene bags or in a refrigerator; instead a good intensity of germination was observed in teliospores which had overwintered outside. Moreover tests carried out at ground level and at 60 cm above the ground showed that teliospores overwintered on the ground and covered by snow and ice for a long time in winter germinated much better than those overwintered at 60 cm above the ground where the snow cover lasts for a shorter time. We are of the opinion that here too the distance from the ground of the overwintering telia may be responsible for a variety of environmental changes.

The intensity of germination of teliospores overwintered at Farma (Fig. 4) on uncovered soil and in the underbrush was similar to that of 1973; the difference between the 2 intensities of germination is evidenced by the statistical analysis (Table 2). This behaviour was to be expected because the 2 types of soil cover used in this test were similar to those of 1973.

The statistical analysis carried out on the percentage of the infected shoots showed that the amount of rust infection on *Pinus pinea* seedlings produced by telia overwintered in the underbrush was significantly lower than that obtained from uncovered telia (t = 7,223 highly significant for 7 degrees of freedom). Similarly the rate of infection on seedlings inoculated with telia from the underbrush was lower than that on seedlings inoculated with telia from the uncovered soil (Table 3). The 2 series of inoculations were carried out with an approximately equal amount of germinating telia and therefore with more or less the same number of basidiospores (Fig. 1); the results obtained reveal that infectivity of the basidiospores from the above overwintering sites is different.

Our investigations and the results obtained lead us to the conclusion that particular environmental conditions during overwintering are most favorable to germinability of teliospores of *Melampsora pinitorqua*. They involve good aeration and effective irradiation causing rapid alternation of moistening and drying conditions and temperature changes. (About the influence of the alternation of moistening and drying conditions on certain germination-inhibiting agents of teliospores see literature cited by Kurkela 1973). These environmental features usually occur on uncovered sites or, at any rate, in places characterized by sparse and discontinuous vegetational cover.

Table 2. Tests of differences in intensity of teliospore germination overwintered in different sites on 4 locations. The t-test for coupled sites (A vs. B, Table 1) was made on the summation of results from different dates of collection.

| Trials | degree of freedom | t-value | significance |
|---|---|---|---|
| Monte Quoio 1976 | 3 | 0,8140 | – |
| Firenze 1978 | 3 | 4,5296 | + |
| Antella 1979 a) | 11 | 5,2080 | + + |
| —''— b) | 2 | 2,8574 | – |
| Farma 1978 | 3 | 2,9624 | + |

– = not significant (P > 0,05)
\+ = significant (P < 0,05)
\+ + = highly significant (P < 0,001)

Table 3 — Results of the inoculations of *Pinus pinea* seedlings with germinating teliospores overwintered at Farma in 1978.

| Overwintering site of teliospores | Number of inoculated seedlings | % of infected seedlings | Number of inoculated shoots | % of infected shoots | % of infected shoots according to degree of infection |
|---|---|---|---|---|---|
| Uncovered soil | 82 | 93,9 | 717 | 36,9 | 8,2 (1)<br>28,7 (2) |
| Underbrush | 78 | 70,5 | 685 | 14,0 | 5,1 (1)<br>8,9 (2) |

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Folia Forestalia 422: 25 — 31.1980

# TESTING SCOTS PINE FOR RESISTANCE TO PINE TWIST RUST[1]

Owe Martinsson[2]

Full-sib families of Scots pine were tested for resistance against pine twist rust. Different test techniques were used for testing the same material.

The results showed that there was a genetically dependent resistance to the disease. On very young seedlings resistance was influenced by seed weight. A large-scale test method is outlined.

## INTRODUCTION

Pine twist rust is caused by *Melampsora pinitorqua* (Braun) Rostr., a heteroecious rust fungus alternating between certain hard pines and some species of *Populus.*

The aecial stage of the fungus develops on unlignified green shoot tissue or primary needles of pine. Therefore small seedlings suffer more from an attack than taller saplings or trees.

Pine twist rust is dependent on climatic conditons (Sylvén 1917, Kardell 1966, Kurkela 1973b). Therefore the damage in forestry varies from year to year. Another reason for more or less severe attacks is of course the density of aspen, i.e., in Scandinavia *Populus tremula* L, in, or in the vicinity of regenerations of Scots pine, *Pinus sylvestris* L.

The work now reported covers a period of 5 years including the preliminary tests. The objective was to compare the relative level of resistance against pine twist rust in full-sib families of Scots pine. The original methods used in these tests appeared unreliable. The research was therefore at the end of this period concentrated on improving the test methods with the objective of developing a large scale method for artificial inoculation.

## INOCULATION TESTS WITH SPORE-PRODUCING LEAVES

### Preliminary tests of one-year-old seedlings

A large number of full-sib families of Scots pine were tested for resistance against pine twist rust (Martinsson 1973, 1975, 1976 and Löfmark 1976). These tests were performed in plastic greenhouses on 1-year-old seedlings during the second growing season. The inoculum, aspen leaves producing basidiospores, were distributed as evenly as possible between the seedlings. The control of quality and quantity of inoculum was poor. Each experimental unit contained 150—200 full-sib families and 25—50 seedlings per family. Significant differences of resistance were shown between full-sib families and general combining abilities of parent trees were calculated (Martinsson l.c.).

During the test work in 1972—1976 several methodological shortcomings showed up. It was not possible to repeat the conditions under which the inoculation was performed due to the varying quality of inoculum and the difficulties in controlling the environmental conditons in a big plastic greenhouse. Another problem was to decide at which age the seedlings should be tested and to keep all seedlings in the proper physiological condition at one moment. Finally it was a problem to assess the damage due to fungus attack in such a way that the assessment corresponded to the developing capacity of the seedling.

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In order to control environmental conditions the tests should be carried out in growth chambers or greenhouses with good climatic control. This would also make it possible to work around the year which would be advantageous from a practical point of view. Tests of very young seedlings would imply another practical advantage. The need of space per seedling and time for each experiment would be reduced. Also, the risk for unfavourable environmental effects would be minimized the shorter the time between sowing and the end of the experiment would be.

## Tests of 2-month-old seedlings in inoculation boxes

The influence on the resistance of seed weight and the quality and quantity of inoculum was investigated in two experiments. In both experiments seedlings were raised from those seed lots which had been previously tested for resistance to pine twist rust as 1-year-old seedlings.

*Influence of seed weight:* Several authors have found that the seed weight has an influence on several properties of seedlings (Stromeyer 1938, Rohmeder 1972). Preliminary results by von Weissenberg indicated that the seed weight may have an influence also on the resistance to pine twist rust (Weissenberg 1978 and pers. commun.).

On the basis of the test performed in 1973 (Martinsson 1973) 16 full-sib families were selected, 8 with high resistance and 8 with low resistance to pine twist rust (Tab. 1). The experiment had a complete randomized block design with 5 replications and 9 seedlings of each family per block. The seeds were sown with the spacing of 3 cm in squared plots, 3 × 3 seedlings, in plastic boxes filled with peat. All 16 plots of one block were surrounded with two border rows of seedlings. At the inoculation, 9 weeks after sowing, each block was contained in a ca 1 m³ wooden box suggested by von Weissenberg (l.c.) (Fig. 1). In the boxes spore producing leaves of aspen were distributed on a network 20 cm above the seedlings. The experiment was carried out in high humidity in a greenhouse. The air temperature was kept below 25 °C. After 4 days the seedlings were taken out from the boxes. Six weeks later the first assessment was done. Each seedling was then classified as either severely attacked,

Fig. 1. Inoculation boxes used in the experiment

Table 1. Full-sib families of Scots pine tested for resistance against pine twist rust.

| Family | % severely attacked seedlings 6 weeks after inoculation | % seedlings killed by rust 9 months after inoculation | 1000-grain weight, gr. | Degree of attack on 1-year-old seedlings |
|---|---|---|---|---|
| 411—42 | 42 | 67 | 5,86 | 3,86 |
| 411—48 | 44 | 73 | 5,98 | 0,91 |
| 411—4 | 40 | 78 | 6,71 | 0,99 |
| 411—109 | 40 | 69 | 4,90 | 1,05 |
| 411—37 | 44 | 76 | 7,23 | 4,31 |
| 411—73 | 42 | 89 | 5,43 | 1,21 |
| 411—130 | 16 | 51 | 7,13 | 1,24 |
| 411—115 | 42 | 82 | 6,35 | 3,80 |
| 403—61 | 55 | 80 | 5,48 | 0,72 |
| 403—112 | 33 | 80 | 6,89 | 3,70 |
| 403—160 | 29 | 76 | 5,40 | 3,13 |
| 403—147 | 31 | 82 | 6,08 | 1,04 |
| 403—44 | 69 | 82 | 5,36 | 3,35 |
| 403—97 | 60 | 78 | 4,70 | 1,46 |
| 403—113 | 31 | 69 | 6,70 | 3,35 |
| 403—34 | 24 | 65 | 5,72 | 1,45 |
| 403—111 | — | — | 5,90 | 3,57 |

i.e. probably not surviving, or healthy to slightly attacked, i.e. surviving.

After dormancy out-of-doors the seedlings were moved into a greenhouse. Nine months after inoculation during the second growing season a second assessment of the experiment was done. By this time there was no doubt whether a seedling had survived the attack or not.

At the first assessment the most resistant family had 16 % and the most susceptible 69 % severely attacked seedlings (Tab. 1). At the second assessment, however, it appeared that of these families 51 % and 89 % respectively of the seedlings were dead. The mutual rank order of the 16 families at the first assessment corresponded, however, very well to that of the second assessment. In contrast there was a bad correlation between the result of this test and that performed on 1-year-old seedlings. On the other hand there was a significant correlation between the seed weight of the 16 families and the result of this early test (Fig. 2).

In order to eliminate the influence of seed weight the percentage of severely attacked seedlings was multiplied by a factor, corr. = 1000-grain-weight — 4,5. (The value 4,5 was close to the value of the lowest seed weight and chosen empirically after several trials with other values.) A good correlation was then obtained between these corrected seed weights and the values obtained from the test of 1-year-old seedlings (Fig. 3).

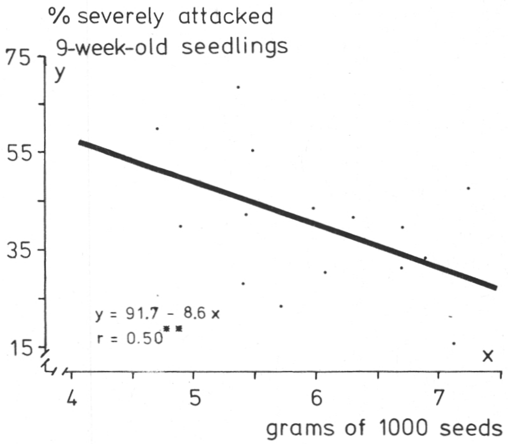

Fig. 2. Correlation between 1000-grain weight and percentage of severely attacked seedlings 6 weeks after inoculation.

This small experiment suggests that the seed weight probably influences resistance to pine twist rust of 9-week-old seedlings. The experiment also shows that ranking of resistance of 9-week-old seedlings is possible to do 6 weeks after inoculation.

*The influence of quality and quantity of inoculum:* In this experiment two full-sib families, 403—111 and 403—34, were used. The former family had in an earlier experiment on 1-year-old seedlings showed extreme

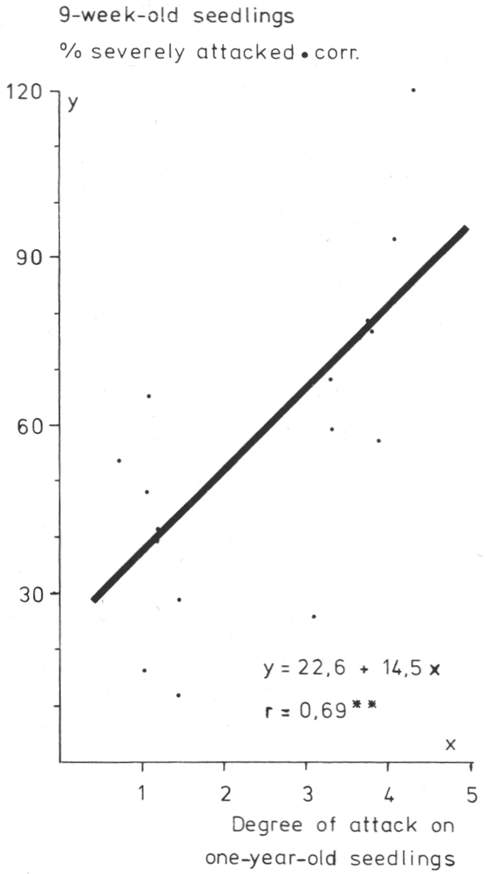

Fig. 3. 16 full-sib families of Scots pine attacked by pine twist rust. Relationship of the percentage of severely attacked 9-week-old seedlings to degree of attack on 1-year-old seedlings. Corr. = 1000-grain-weight — 4.5.

susceptibility and the latter extreme resistance to pine twist rust (Tab. 1).

The experiment was carried out in a greenhouse and the seedlings were contained in the inoculation boxes. Ten boxes were used and each box contained 60 2-month-old seedlings per family. The spacing between the seedlings was 3 × 3 cm. In each inoculation box was a device for estimation of the number of spores per mm$^2$ fallen on the seedlings. This device was composed of a lath with 3 microscope slides. The lath was placed horizontally 10 cm above the seedlings. On each slide were printed 3 squares, 9 × 9 mm each. Within these squares the number of fallen basidiospores were counted every evening for 4 days. The total number of basidiospores fallen on the seedlings in a box was then estimated on the basis of those counted within the 9 squares. The inoculum for this experiment had been collected in two places, Bogesund close to Stockholm and Habo in the county of Västergötland. Five boxes were provided with inoculum from one place and five from the other. Different amount of aspen leaves were put into the ten boxes in order to obtain different infection pressures (Fig. 4).

During 4 days the experimental material was exposed to high air humidity (as close as possible to saturation) and a temperature between +15 °C and +25 °. After the inoculation period the boxes with the inoculum were removed. Six weeks after that the seedlings were classified as either severely attacked, i.e. probably not surviving or as undamaged to slightly damaged, i.e. probably surviving.

The difference in mortality between the two families varied between inoculation boxes with different spore production but the resistance of family 403—34 was higher than 403—11 in all 10 boxes which was corresponding to the resistance test made on 1-year-old seedlings (Fig. 4).

From these experiments the following conclusions can be drawn:

- It is important to have a very even distribution of inoculum within an experiment if variations of genetically dependent resistance should be distinguished.
- An adequate spore density for this sort of test could be 10-30 spores/mm$^2$ (cf. Weissenberg 1978).
- The relative differences in resistance did not seem to be influenced by the fact that the inoculum in this experiment was collected in 2 places.

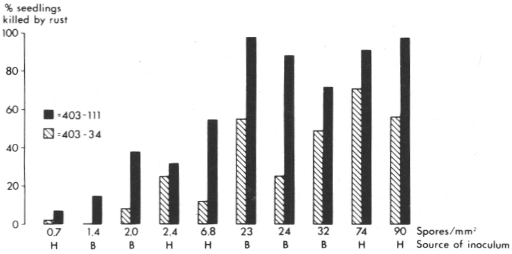

Fig. 4. Resistance against pine twist rust of two full-sib families of Scots pine, 403—11 (low resistance) and 403—34 (high resistance) at different levels of infection pressure and two sources of inoculum (H) Habo and (B) Bogesund.

## INOCULATION TESTS WITH SPORE SUSPENSIONS

It would be desirable to check quality and quantity of inoculum in advance of a resistance test. A method for testing resistance of fusiform rust in loblolly and slash pine is being developed and applied by the U.S.D.A. Forest Service at the test centre in Asheville, N.C., USA (Laird et al. 1973).

A modification of this method for test of resistance against pine twist rust on Scots pine should be possible to develop. To make that method applicable on tests of pine twist rust resistance of Scots pine, two main problems must be solved:

1. How to trap and store basidiospores.
2. How to control dispersal of basidiospores over the pine seedlings.

Basidiospores develop from telia on aspen leaves in high air humidity. Optimum temperature for spore development is 12—22 °C (Kurkela 1973a). The basidiospores are released and germinate in a few hours after the dispersal. With artificial spore trapping the germination process must be stopped until the moment the spores are used for inoculation. This could possibly be done through refrigeration. Dispersal of basidiospores over the seedlings could be done by means of spraying of spores suspended in water.

In a pilot study this artificial inoculation was carried out on a small scale. Leaves of aspen with telia were attached to a wet filter paper which was attached to the inner side of the lid of a water bowl. In the bowl was sterilized water onto which the basidiospores fell. The bowl was in room temperature but placed on a refrigerated surface which kept the temperature of the water close to ±0 °C. After 48 hours basidiospores had developed and fallen onto the water surface. The water was sucked up from the bowl and the spores were separated from the water on a millipore filter. The germinability of the spores was checked. After that the spores were suspended in water and sprayed by means of a simple hand mist sprayer on 2-month-old seedlings of Scots pine.

The germinability of the basidiospores on water agar was only 5—10 %. At the preparation of the suspension the spores had a tendency to cluster. Furthermore the collected amount of spores was probably too small. These may have been the reasons why only 2 seedlings out of 100 got a rust attack. Yet this pilot study demonstrated the principle of using a spore suspension.

For a long time there have been attempts in the laboratory at the Faculty of Forestry in Umeå to construct technical equipment for collecting and dispersing basidiospores

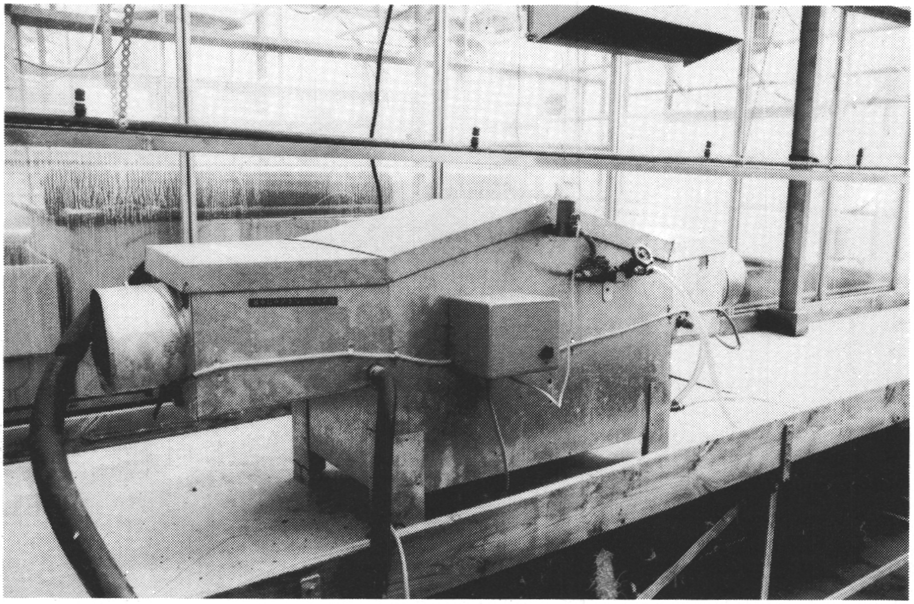

Fig. 5. Facility for collecting basidiospores of *Melampsora pinitorqua*.

of *Melampsora pinitorqua*. This work has so far not been completely successful and is still not brought to and end. A prototype of a device for collection and another for spraying of basidiospores have been constructed. These equipments have to be developed and improved before they can be used in practice.

The spore collection facility (Fig. 5) is a ''box'' with a perforated bottom on which the aspen leaves are put. In the box is a nozzle for production of mist of water. Through the perforated bottom and out through tubes, one in each end of the box, is a slow air stream moved by means of 2 propellers. The water-saturated air stream in which ripe basidiospores are transported, pass through 2 refrigerated lattice works in which the water is condensed and frozen together with the basidiospores. The spores are kept frozen until they are used in the spraying facility.

The spraying facility (Fig. 6) is a conveyor on which boxes with growing seedlings are placed. The speed of the conveyor belt can be adjusted. Over the conveyor belt 2 mist nozzles are connected to a small container for spore suspension. The nozzles are also connected to an adjustable air pressure. The distance between the nozzles and the conveyor can be changed.

A lot of work remains before these facilities can be used in practice. Among other problems appeared the difficulty to keep the temperature of the lattice works in the spore collector low enough at the same time with a temperature high enough on the bottom of the box for basidiospore production.

## DISCUSSION AND CONCLUSIONS

All tests of resistance to pine twist rust here reported on have been carried out in a greenhouse habitat. Furthermore the seedlings have grown up in a greenhouse and no seedlings older than 2 growing seasons have been tested. This may have had an influence on the validity of these tests.

Since it is most interesting to know the resistance in a natural habitat, it is important to find the correlation between a test result in an artificial habitat and the one that would have been achieved in a forest habitat under strong infection pressure. If there is no correlation the test in an artificial habitat is useless and have to be substituted by experiment in natural habitats only.

This work on tests of resistance against pine twist rust in Scots pine has indicated:

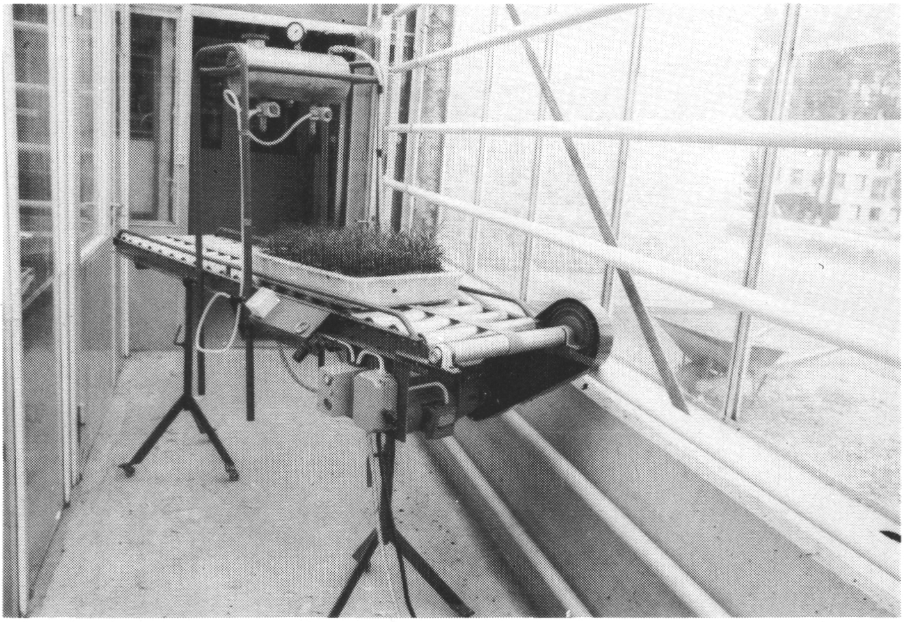

Fig. 6. Facility for spraying basidiospores of *Melampsora pinitorqua* on pine seedlings.

- That it is possible to show genetically dependent variation in susceptibility to pine twist rust among full-sib families of Scots pine.
- That a correlation is achieved between the test results obtained from 1-growing-season and 2-growing-season old pine seedligs if the former result is adjusted with a correction factor based on seed weight.
- That it may be possible to develop a large scale test method in which quantity and quality of inoculum is controlled.

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Folia Forestalia 422: 32 — 36.1980

# EFFECT OF TEMPERATURE ON DORMANCY, ACTIVATION AND LONG-TERM STORAGE OF TELIOSPORES OF *MELAMPSORA PINITORQUA*[1]

Kim von Weissenberg[2]

Incubated teliospores of *Melampsora pinitorqua* on aspen leaves collected in the spring produced germinating basidiospores during a year when they had been stored previously at temperatures from +20 °C to −16 °C. Teliospores of leaves collected in the fall did not germinate until early the next May and only if they were stored outdoors. Teliospores stored outdoors or indoors at −2 °C could, however, be activated by repeated incubation in April. Activation was only partially successful for leaves stored indoors at +20 °C. The differing responses of teliospores on leaves collected in the spring and fall to storage conditions are discussed.

## INTRODUCTION

The spring timing of basidiospore release by *Melampsora pinitorqua* (Braun) Rostr. in nature has been studied by, among others, Schafranskaja (1951, ref. Regler 1957), Klingström (1963) and Kurkela (1973 a). Some environmental factors affecting teliospore germination (Regler 1957, Klingström 1963, Kurkela 1973 a, b, Longo et al. 1976, 1980 and infectivity of basidiospores (Longo et al. 1980) have also been studied. Kurkela (1973 a, b) has reviewed extensively the literature in this field.

More complete understanding of various time-related factors controlling dormancy and activation of teliospores and properties of the basidiospores they produce is required for efficient experimental research on resistance of the hosts and pathogenicity of *M. pinitorqua.*

The objectives of this study were to elucidate 1) the effect of temperature during storage of aspen (*Populus tremula* L.) leaves on germination of telio- and basidiospores, 2) the date in the spring when dormancy breaks naturally, and 3) whether dormancy can be artificially broken earlier than in nature by temperature and wetting treatments.

## MATERIALS AND METHODS

Aspen leaves with telia were collected in May 1977 in Suonenjoki (N62°36, E27°03′) in central Finland and in October 1978 in Ilomantsi (N63°09′, E31°05′) in eastern Finland. The leaves were taken from the ground under aspen suckers growing near pine (*Pinus sylvestris* L.) heavily infected with *M. pinitorqua.*

### Leaves collected in the spring

The leaves were spread on the floor to dry at room temperature. Leaves with abundant telia were selected, thoroughly mixed, and divided into 4 batches each containing 120 leaves. Each batch was enclosed loosely in a plastic bag and stored at one of 4 temperatures: −16 °C, −4 °C, +5 °C and +20 °C from July 1977 to late June 1978. For technical reasons, the means of these target temperatures varied during the storage period with standard deviations

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[1] Presented at the Meeting of the IUFRO Working Party S2.05—05, Resistance in Pines to *Melampsora pinitorqua,* June, 1979, Suonenjoki, Finland.

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Table 1. Water content in leaves stored at 4 temperatures. Prior to storage all leaves were uniformly dried at room temperature.

| Storage temperature | Sampling dates | | | | |
|---|---|---|---|---|---|
| | 1977 | | 1978 | | |
| | July | Sept. 21 | March 1 | April 13 | June 27 |
| | (Water content, %) | | | | |
| −16 | 13,2 | 12,6 | 13,0 | 14,0 | 14,0 |
| −4 | 13,8 | 14,4 | 15,0 | 15,0 | 15,0 |
| +5 | 11,4 | 10,9 | 13,0 | 12,0 | 12,0 |
| +20 | 9,0 | 6,9 | 8,0 | 9,0 | 8,0 |

of 0,7—2,4 °C. Water content in the leaves was determined 5 times during the storage period: Each time 10 leaves were taken from each storage room, weighed, dried at 105 °C for 24 hours, and weighed again (Table 1).

Germination of basidiospores produced by teliospores on these leaves was determined at intervals of about 1 month during the storage period. No determinations were made in November — January.

Basidiospores were produced as follows: Leaves with telia were soaked in tap water at +20 °C for 2 hours and adhered with water to the inner side of lids of petri dishes, the bottoms of which were covered with wet filter paper. The dishes were incubated at +20 °C in plastic boxes lined with wet paper towels.

The percentage of germinated basidiospores in the petri dishes was determined as follows: After 20 hours of incubation, an agar-covered (2 % water agar) slide was inserted into the petri dish for 1 hour. The leaf was removed, but the slide remained in the dish for 5 hours at +20 °C. Using a microscope (200×), 100 basidiospores were counted and scored as germinated if the length of the germ tube was ≥ spore diameter (Klingström 1969). The mean of 4 leaves (=400 basidiospores) was taken as a measure of percentage of germination for each date of determination.

## Leaves collected in the fall

The leaves were spread on sandy soil without vegetation near the nursery of the Experiment Station for Reforestration in Suonenjoki (N62°38′, E27°04′). The leaves were covered with galvanized network to keep them on the ground. A portion of these leaves, from which non-related litter and leaves with only few telia were removed, was divided into 3 equal batches: Two batches were enclosed in large plastic bags, one of which was stored during the winter in a temperature chamber set at +20 °C and the other in a cold-storage room set at −2 °C. The 3rd batch was divided into sets of 10 leaves, each set was enclosed in a bag made of galvanized network and returned to the outdoors storage place. These 3 storage methods were started on October 23, 1978, at which time the first snow fell on the leaves outdoors. These were continuously under a cover of snow from November 16, 1978 to April 27, 1979. The number of degree days to which leaves had been exposed during the study was calculated according to the method described by Sarvas (1967) using a threshold temperature of +5 °C. For the leaves stored outdoors temperature was recorded on a thermohygrograph in a nearby weather chamber 2 m above ground. The amount of rain was recorded in an automatic gauge next to the weather chamber.

Two experimental series were carried out to determine teliospore germination:

*Series 1:* New sets of 10 leaves from each storage place were examined at about 1-month intervals starting from October 23, 1978 and with usually about 2-week intervals starting from March 15 till June 8, 1979. At this time the experiment was discontinued since rains wetted all leaves outdoors and, due to the warm weather at that time, teliospores stored outdoors started to germinate naturally.

*Series 2:* Two sets examined first in Series 1 on April 1 (Sets A) and April 11, 1979 (Sets B), were examined 3—5 additional times at about 2-week intervals. Treatment was as described above except that between examinations the leaves were stored in their petri dishes at −16 °C and then thawed at room temperature for 2 hours before incubation. These leaves were exposed to 15 or 30 degree days during each incubation period.

Teliospores were germinated for 44 hours during incubation with the method described for production of basidiospores from leaves collected in the spring.

The percentage of germinated teliospores was determined using a stereomicroscope (50×) equipped with an ocular grid containing 100 squares. On each leaf 4 telia-bearing areas, delineated by the grid to 4 mm², were examined: 2 from the central part of the leaf, 1 from its tip, and 1 from its base. Teliospores were counted at each of the 100 crosses of hair lines in the grid and scored as germinated if a yellow basidium was seen under the cross. No counts were made if a cross was outside a telial pustule. For each examination date a set of 10 leaves from each method of storage was used.

## RESULTS AND DISCUSSION

### Germination of basidiospores from leaves collected in the spring

Germination remained at a fairly constant level during the whole storage period except for rapid reduction in September and an increase in February. None of the storage temperatures consistently influenced germination (Figure 1). Throughout the storage period and at all temperatures the telia seemed to produce about the same number of basidiospores (cf. Kurkela 1973 b) although the spores were not counted in relation to number of telia on the leaves. In contrast, Heagle and Moore (1970) found that teliospores of *Puccinia coronata* f. sp. *avenae* Fraser et Ledingham did not produce basidiospores after long-term storage at +15 °C and +30 °C.

Reduction in germination from September to February may be related to the observations made by Kurkela (1973 b): Spores cast in September germinate more slowly than spores cast in June. Since, however, Kurkela (l.c.) had defined germination more conservatively and had used a shorter incubation time than in the present study, slower germination may not explain the results obtained in the present study.

### Germination of teliospores on leaves collected in the fall

The new method used in this study for quantitative assessment of teliospore germination produced accurate counts suitable for parametric statistical analysis. The method may be, however, somewhat slower than the classification methods used by Klingström (1963), Longo and Naldini (1970), and Longo et al. (1976).

*Series 1.* No teliospores on the leaves collected in the fall germinated before May 9, 1979 when degree days started to accumulate at the nearby weather chamber (i.e., the daily mean temperature > 5 °C (Figure 2). At the time of a more rapid increase in the number of degree days but only few and light rains, the teliospores stored outdoors became more active (*sensu* germinable, Heagle and Moore 1970) and reached about 80 % germination when ca. 220 degree days had accumulated. This

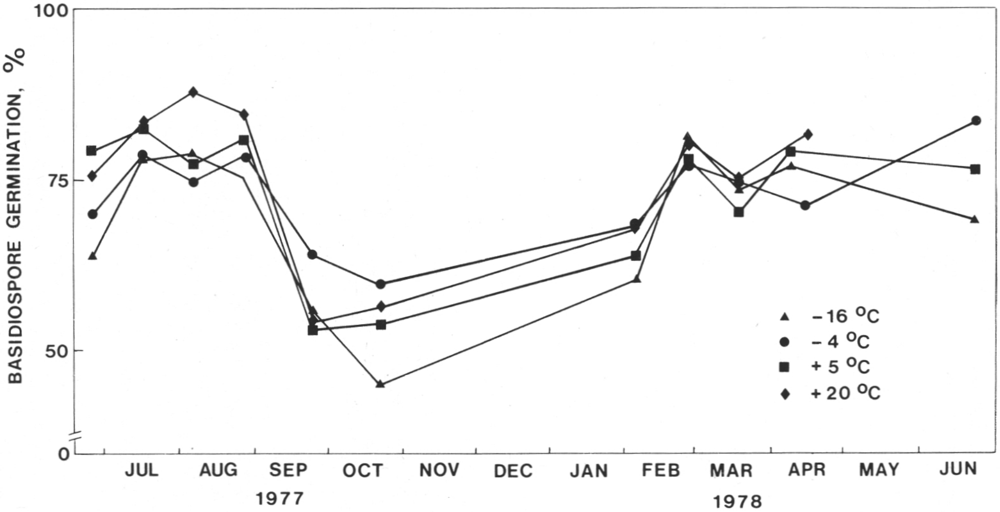

Figure 1. Germination of basidiospores cast on water agar from teliospores on leaves collected in the spring, stored at 4 temperatures and incubated uniformly for 20 hours.

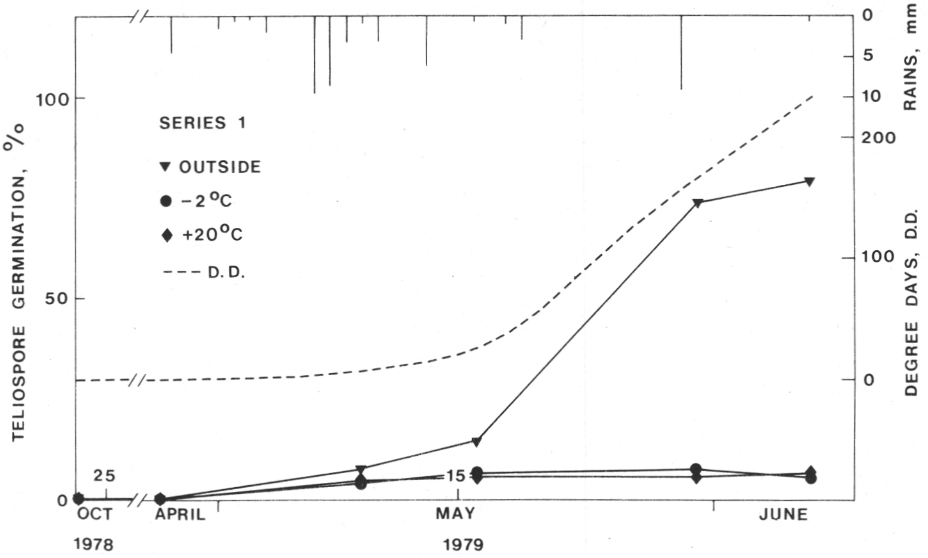

Figure 2. Germination of teliospores on leaves collected in the fall, stored in 3 places and uniformly incubated for 44 hours. The broken line indicates the number of degree days accumulated near the outdoors storage location. Vertical lines indicate amount of rains received by leaves stored outdoors.

timing of natural activation was similar to timings observed by Schafranskaja (1950, ref. Regler 1957) in the Soviet Union, Klingström (1963) in Sweden and Kurkela (1973 a) in Finland, but Longo et al. (1976) have reported that in Italy activation may start already in February.

Teliospores stored indoors at +20 °C and −2 °C until they were incubated did not have more than a few active teliospores regardless of the date when they were incubated. Similar results have been obtained for teliospores of *P. coronata* f.sp. *avenae* overwintering in plastic bags at −5 °C (Heagle and Moore 1970). The poor germination of teliospores stored indoors may also be due to lack of repeated wetting prior to incubation. The teliospores stored outdoors had been periodically wetted by melting snow and several minor rains before germination increased in mid-May (Figure 2).

*Series 2:* Teliospores stored outdoors and indoors at −2 °C were activated more by repeated incubation than those stored indoors at +20 °C (Figure 3) and earlier than teliospores stored outdoors (cf. Figure 2). The earliest time that teliospores can be activated was probably not indicated by these experiments. In Germany, Regler (1957) was able in Nowember to activate teliospores on leaves collected in the fall by wetting and drying the leaves twice and then incubating the re-moistened leaves in a humidity chamber. In the present study, the earlier activation series was started on April 1, but germination started only during the 4 th incubation and did not reach the same final level as the activation series started 10 days later on April 11 (Figure 3). Thus, in central Finland artificial activation may be possible later than in Germany.

The effects of repeated incubation (soaking and storage at +20 °C) were usually cumulative and finally resulted in nearly the same percentage of germination (Figure 3, Sets B, outdoors storage) as did the conditions where the leaves were stored outdoors in nearly natural conditions (Figure 2). Periods at −16 °C between incubations did not necessarily reverse the trend of increasing percentage of germination. See Regler (1957), Kurkela (1973 b) and Snow (1968) for effects of other methods of pre-conditioning and activation of teliospores of rusts.

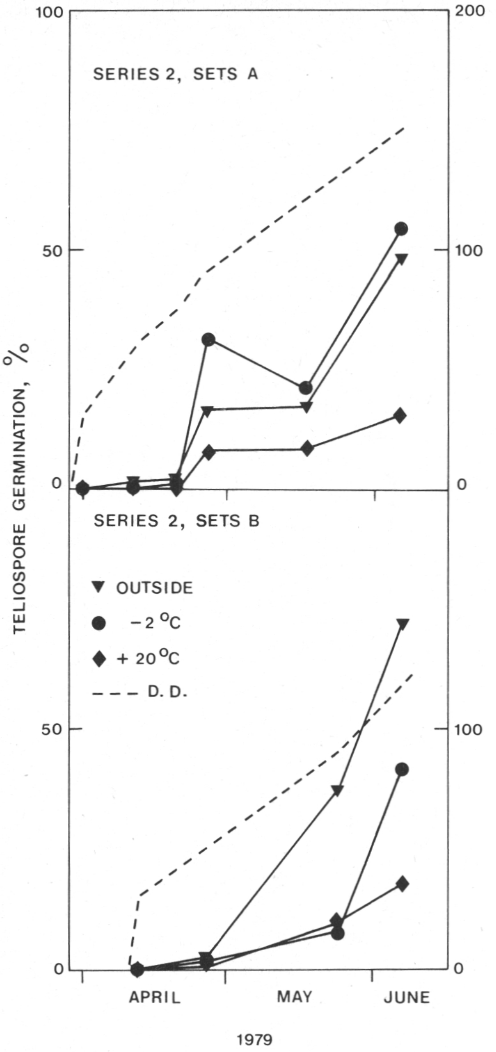

Figure 3. Germination of teliospores on leaves collected in the fall, initially stored in 3 places and uniformly incubated for 22 or 44 hours at ca. 1—2 week intervals, during which the leaves were stored at —16 °C. The broken line indicates the number of degree days accumulated in the leaves at the end of each incubation period.

The present results revealed a remarkable difference between the ways teliospores on leaves collected in spring and fall reacted to temperature: The high storage temperature (+20 °C) had an adverse effect on dormant teliospores collected in the fall but the active teliospores collected in the spring continued to produce viable basidiospores for nearly a year, even when stored in this temperature. Similarly, Kurkela (1973 b) found that brief exposure to +25 °C did not destroy activated teliospores. Thus, dormant teliospores are susceptible to high temperatures while the activated ones are less so. In the spring teliospores of *M. pinitorqua* presumably are in a different physiological condition from that existing in the fall. The nature of this condition may give information on, e.g., mechanisms regulating spore metabolism in relation to karyogamy, meiosis, and breaking of dormancy (cf. Sussman and Halvorson 1966, Petersen 1974).

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SELOSTE

# IUFRO:N TYÖRYHMÄN S2.05—05, VERSORUOSTEENKESTÄVYYS MÄNNYSSÄ, KESÄKUUSSA 1979 SUONENJOELLA PIDETYN KOKOUKSEN ESITELMÄT

Kansainvälisen Metsäntutkimuslaitosten Liiton (International Union of Forestry Research Organizations) työryhmän S2.05—05 2. kokous pidettiin Suonenjoella 27—29. kesäkuuta 1979. Työryhmän englanninkielinen nimi on *Resistance in pines to Melampsora pinitorqua* ja sen tehtävänä on välittää tietoa tutkijoiden välillä sekä koordinoida eri maissa suoritettavaa männyn versoruostetutkimusta. Työryhmä on IUFRO:n pienimpiä, mikä johtuu siitä, että männyn versoruoste on lähinnä eurooppalainen ilmiö, tosin tautia esiintyy myös Lähi-Idässä ja Siperiassa. Vain osassa esiintymisaluettaan versoruoste on taloudellisesti merkittävä.

Suonenjoen metsänviljelyn koeasemalla pidetyssä kokouksessa oli osanottajia Hollannista, Italiasta, Kiinasta, Puolasta, Ruotsista ja Suomesta, yhteensä 11 tutkijaa. Kokouksen aikana tutustuttiin kenttäkokeisiin koeaseman ympäristössä ja Toivakassa, missä käytiin myös männyn siemenviljelyksillä. Kokouksessa kuultiin seitsemän esitelmää.

Kirjallisuuskatsauksessa poppelien ruosteenkestävyysjalostuksesta J. Gremmen varoitti ruostesienten vaihtelukyvystä. Yleensä patogeeniset sienet jakautuvat fysiologiansa ja patogeenisuuteensa suhteen joukkoon toisistaan erottuvia rotuja. Nämä olemassa olevat rodut voivat tuottaa edelleen uusia geneettisiä kombinaatioita, jotka vuorostaan saattavat sopeutua uusiin ihmisen muuttamiin olosuhteisiin.

Poppelinkasvatuksessa uudet lupaavilta näyttäneet taudinkestävät kloonit ovat miltei poikkeuksetta saastuneet lyhyessä ajassa sen jälkeen, kun ne on otettu laajempaan käyttöön. Tämä voi johtua jo alueella olevien patogeenien rotujen runsaussuhteiden muutoksesta tai uuden geneettisen kombinaation seurauksena syntyneestä rodusta. Alueen ulkopuolelta voi saapua myös rotuja, jotka ovat jo valmiiksi kykeneviä sopeutumaan paikallisesti muuttuneisiin olosuhteisiin.

Z. Krzanin mukaan sekä lämpötila että ilman suhteellinen kosteus vaikuttavat *Melampsora*-epidemian kehitykseen. Lämpimänä kesäkautena poppelinruosteet leviävät nopeimmin, kun ilman suhteellinen kosteus on korkea. Syksyisin, kun keskimääräinen suhteellinen kosteus lisääntyy ja keskimääräinen lämpötila laskee alle ruosteen optimin, lämpimien kausien merkitys korostuu ruosteen kehityksessä.

Krzanin ja R. Siweckin yhteisesti laatimassa esitelmässä todettiin Puolassa tehtyjen *Melampsora*-tutkimusten kohdistuneen aiemmin lähinnä poppelinruosteisiin. Männyn versoruostetutkimuksia on kuitenkin viime aikoina lisätty. Versoruostetta esiintyy kaikkialla Puolassa Karpaattien aluetta lukuunottamatta.

Suomessa haavan ruosteesta esiintyy neljä erilaista fysiologista ''lajia'', joiden helmiitiöasteet kehittyvät eri isäntäkasveilla. Epidemiologisissa tutkimuksissa sekä testattaessa männyn ruosteenkestävyyttä näiden lajien tarkka määrittäminen on välttämätöntä. Morfologisesti näitä ruostesieniä ei voida erottaa toisistaan. Mahdollisesti määritykseen soveltuvana keinona T. Kurkela esitti immunodiffuusiotestit, joita ei ole aiemmin sovellettu *Melampsora*-sieniin.

O. Martinsson testasi Ruotsissa männyn versoruosteenkestävyyttä erilaisin menetelmin täyssisarusjälkeläistöissä. Hän totesi männyssä esiintyvän genettisesti määriytynyttä versoruosteenkestävyyttä. Lisäksi hänen tuloksensa vahvistivat K. von Weissenbergin havaintoa, että aivan nuorissa männyn taimissa siemenen paino saattaa vaikuttaa taimen ruosteenkestävyyteen.

K. von Weissenberg esitteli Suonenjoella suorittamissaan tutkimuksissa käyttämiään menetelmiä sekä joitakin saamiaan esituloksia mm. männynversoruosteen talvi-itiöiden dormanssista, aktivoitumisesta ja pitkäaikaisesta varastoinnista, männyn täyssisarperheiden kestävyyseroista sekä versoruosteen maantieteellisestä vaihtelusta.

Työryhmä hyväksyi seuraavat suositukset tulevia tutkimuksia varten:

1. Kenttäkestävyyden mittaustekniikkaa kehitettävä.
2. Kehitettävä isäntäkasvin kestävyyden ja sienen patogeenisuuden mittaamiseen soveltuvia laboratorio- ja kasvihuonemenetelmiä.
3. Kiinnitettävä enemmän huomiota patogeenin suureen vaihtelevuuteen kaikessa ruosteenkestävyysjalostuksessa.
4. Selvitettävä tarkemmin Melampsoraceae-ruosteiden isäntäkasvit.

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WEISSENBERG, K. von & KURKELA, T. (Eds.) 1980. Proceedings of the meeting of the IUFRO Working Party S2.05—05, Resistance in Pines to *Melampsora pinitorqua,* June 1979, Suonenjoki, Finland. Seloste: IUFRO:n työryhmän S2.05—05, Versoruosteenkestävyys männyssä, kesäkuussa 1979 Suonenjoella pidetyn kokouksen esitelmät. Folia For. 422:1—38.

Literature on breeding *Melampsora* rust resistant poplars was reviewed and results were presented about the effects of temperature and humidity on poplar-rust epidemics. Research made on poplar rusts in Poland was reviewed. Serological tests were suggested as a mean for identifying aspen rusts. Good aeration without shading by vegetation promoted telial germination and infectivity of basidiospores of *M. pinitorqua* in Italy. Results on testing resistance in Scots pine and on dormancy, activation and long-term storage of teliospores were presented.

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