STUDIA FORESTALIA SUECICA

Nr 6 1963

Melampsora Pinitorqua (Braun) Rostr. – Pine Twisting Rust

Some Experiments in Resistance-biology

Melampsora pinitorqua (Braun) Rostr.

Några resistensbiologiska försök

by

ALLAN KLINGSTRÖM

SKOGSHÖGSKOLAN

STOCKHOLM

ESSELTE AKTIEBOLAG
STOCKHOLM 1963
312749

Melampsora pinitorqua (Braun) Rostr. — Pine Twisting Rust

The investigations into pine twisting rust made by SYLVÉN in 1917—1918 are still of fundamental importance. Even from an international aspect, very few works as comprehensive as this have been dedicated to *Melampsora pinitorqua* (Braun) Rostr.

In the first place, REGLER (1957) should be mentioned, especially as his paper also refers to some Russian authors whose works have hitherto been difficult to evaluate. TROSCHANIN (1952) has made an extensive study of pine twisting rust; it was written in Russian, but a German translation is also available. Further, MORIONDO has published several papers on his studies of *Melampsora pinitorqua* (1951, 1952, 1954 a and b, 1956, 1957 a and b, 1958, 1961), which are, however, only available in Italian.

What there is further to be found in the literature after SYLVÉN's works (1917 and 1918) comprises short articles and brief references in sundry annual reports and similar publications issued by forest institutions. A brief survey of this literature is, however, still well worth making, because the *Melampsora* problem is extending considerably, *i.e.*, in the first place geographically, but also as a practical economic problem in the forest.

To SYLVÉN the fungus was known to exist in a few localities in central and southern Sweden and in Germany. Nowadays, damage resulting from its attack is being reported from practically the whole of Europe, from Målselv, 69° 10′ north in *Norway* (JØRSTAD, 1953), southern *Lapland* (ELSSMANN, 1952; KARDELL, 1962), the author's own observations from the *Torne* valley, the county of *Medelpad* and from *Finland* (KUJALA, 1950). In *England, Melampsora* has been recorded on several occasions (DAY 1943; PEACE, 1944, 1951, 1957; MURRAY, 1961). In *Germany*, at least two important papers (BÖHNER, 1952, and REGLER, 1957) have been published. The *Melampsora* problem has been the subject of investigation in *Poland* for many years (GARBOWSKI, 1929; ORLOS, 1935; BRENNEJZEN, 1957). *Russia* has also tackled the *Melampsora* question (YOUNITSKY, 1927; GAVRIS, 1939; TROSCHANIN, 1952; see also REGLER above). Pine twisting rust is a regeneration problem in large areas of the *Mediterranean zone* (MORIONDO, see above) down to *Cyprus* (S. G. N., 1923, and PEACE, 1962).

Reports by ZILLER (1961 and 1962), that *Melampsora pinitorqua* has with certainty been found in *North America*, are also of great interest.

The above data are not complete and refer only to the occurrence of the disease on pine. The problem has been further widened by the fact that pine twisting rust will attack several *Pinus* species.

In the following account reference will be made to the names of the species of pines used in the works cited, even though the latin names in the sources may be critizised. The authors' names have unfortunately not been given in the bibliography quoted, which reduces its accuracy, and makes it pointless to insert the names of any authors here.

BIRAGHI (1954) summarizes: *Pinus silvestris, P. montana, P. strobus, P. pinaster* and *P. pinea* (without references to literature). In the case of Italy he mentions: *P. pinea, P. pinaster,* and *P. silvestris.* MORIONDO (e.g., 1951, 1954, and 1957 a and b) has found damage to *P. silvestris, P. pinea, P. pinaster,* and finally to *P. nigra* in Italy. TROSCHANIN (1952) states that *P. strobus* is susceptible to the disease, and BÖHNER (1952) is of the same opinion. TROSCHANIN has further found that *P. murrayana* is susceptible to some extent. It should be noted that *P. murrayana* is considered to be the same as *P. contorta* var. *latifolia,* and that many people think that the use of both these names should be discontinued in favour of *P. contorta* as a cosmopolitan species (HARLOW and HARRAR, 1958). Therefore, with regard to pine twisting rust, *P. contorta* cannot yet be recorded as absolutely resistant, although this has been inferred by some authors. Besides some of the species already mentioned, PEACE (1962) has stated *P. halepensis.* In the statement concerning North America *P. ponderosa* is named as the alternate host.

Various yearly reports from forest institutions have recorded the occurrence of *Melampsora*—e.g., Rep. on For. Res. It is then often necessary to translate Anglo-Saxon names such as maritime pine and Corsican pine; and the definitions given in the leading encyclopaedias do not always correspond. A future list of alternate hosts may well contain other names than the nine species referred to above and which can be given with certainty; it is probably the most comprehensive list now in existence.

As to the occurrence of the disease on *Populus,* REGLER's study of the question (1952) can be mentioned.

To sum up it can be said that Melampsora pinitorqua occurs all over Europe (GÄUMANN, *1959*) *and in parts of Asiatic Russia. Further, there is* *a risk of the disease being spread also in North America. The fungus is known to attack at least nine species of the genus Pinus.*

From the literature cited it can be seen that the distribution of *Melampsora* increased considerably during the twentieth century; so also did its importance as a pathogen. This is undoubtedly due to many factors. More intense forestry, with extensive fellings in conjunction with balanced and rapid reforestation by means of sowing and planting, has assisted this fungus. Further, great interest has been shown in *Populus,* which is the alternate host for the fungus. Great quantities of aspen were felled during two world wars, and this wood is nowadays also used for pulpwood. The number of root suckers will thus naturally increase considerably (cf. RENNERFELT, 1954). There has also been a great interest for genetical improvement work on the genus *Populus.* So far as Swedish conditions are concerned much of the large areas where agriculture has been discontinued, and which cannot be planted at the same rate, is in the first place turned into deciduous forest, much of which is aspen. Natural meadowland in particular has—according to Swedish statistics—decreased considerably (Sveriges off. statistik. Jordbruksräkn. 1956). This is above all the case in the counties of Norrbotten and Västerbotten. In Europe, interest in reforestation has increased, at the same time as structural changes have taken place in the present forest areas due to attacks of *Endothia* on chestnut trees and to some extent on oak, as well as attacks of *Ceratocystis* on elm (KRSTIC, 1960). It has, therefore, been necessary to fell an enormous amount of chestnuts and elms and replace them with conifers and *Populus* species, which may also have influenced the distribution of the pine twisting rust.

It can be seen from the above that there is a good deal of information available in the literature concerning the disease. But, when looking for facts that are of practical importance, hardly anything can be found besides that written already by SYLVÉN (1917 and 1918). By various means it is still necessary to avoid the combination of pine and aspen on fellings and to eradicate aspen near nurseries. Unfortunately, however, this is often easier said than done. Experimental spraying has been suggested in some cases, but for economical reasons this is only possible in nurseries.

In the USSR spraying is recommended (TROSCHANIN, 1952) and is carried out in nurseries and in the field three to four times with four to five days' intervals in May—June. However, too little seems to be known about the biology of the fungus to justify such treatment.

Studies on Resistance to Melampsora

A brief preliminary report on the differences in the attack frequency on different grafted clones of planted pine was made in 1954 (BERGMAN, 1954; RENNERFELT, 1954). The investigation covers eight clones with ten grafts each. The author also had the opportunity of investigating clonal material in the S.C.A. pine seed orchard at Nedansjö in 1953. This work has been followed up by studies of the attack intensity on different progenies of some of the original clones. It is true that differences in the occurrence of a pathogen on grafted clones will give an idea of genetically conditioned resistance, but more reliable conclusions cannot be drawn until a further study of the progeny has been made (cf., e.g., WRIGHT, 1960). The aim of the present paper is to give an account of the investigations at Nedansjö as well as some data on the biology of the fungus and studies on the method of inoculation, etc. GAVRIS (1939) has also given some information concerning resistance to *Melampsora*.

By studying the excellent planting schemes and protocols devised for the plantation at Nedansjö, it was possible to follow the development of each graft, to calculate the distance to aspen root suckers, etc. The result of these investigations can only be that, even if consideration is paid to the distance to aspen, the size of the grafts, etc., certain clones show a greater susceptibility to pine twisting rust than others.

In Table I, data on this natural infection are summarized for 14 grafted clones with at least 17 grafts on each for the year 1952. It will be seen that the attack frequency varies considerably in different years. The values of the very low rate of infection in 1953 are hardly of any importance, but they do not seem to contradict the results of the year 1952. However, the resistance problem cannot in future be solved by making observations of the random occurrence of spontaneous infection. *The aim must be a uniform and experimentally established infection.*

Of the 14 clones examined, it was later decided to select four, which not only included a large number of individuals (28 to 35) but also seemed to differ considerably in their susceptibility to pine twisting rust (see Table I). The intention was to inoculate these test clones with the disease and later see whether the differences between the clones remained.

No method for the inoculation had been described earlier (cf. however REGLER, 1952, concerning inoculation on individual pines,

Table I. Spontaneous infection of *Melampsora pinitorqua* on grafted clones of Scots pine at Nedansjö in 1952.

Clone	Number of individuals	Number of attacks	Seedlings with 0	1	2	3	4	5	6 attacks
Z 66	38	0	38						
Z 2018	27	2	25	2					
Z 4013	29	3	26	3					
Y 4509	28	3	26	1	1				
Z 4012	20	4	17	2	1				
X 4201	39	9	33	4	1	1			
Y 4506	27	7	21	5	1				
Y 4014	19	5	15	3	1				
Z 4005	41	16	28	10	3				
Z 4011	23	9	17	4	1	1			
Y 4015	19	8	12	6	1				
Z 4010	21	10	15	4	0	2			
Z 2017	17	10	9	6	2				
Z 4009	27	34	7	13	4	1	1	0	1

and MORIONDO, 1961). The pine shoots are normally infected by basidiospores, which are formed when teliospores on aspen leaves grow out with basidies. A large-meshed net bag containing aspen leaves with an abundance of teliospores was fastened to each graft to be inoculated. This work was carried out in May, 1957. Provided that the climatic conditions were favourable, the prospects of the grafts being uniformly infected by the fungus should be good. It is generally considered that damp weather, especially if it is also warm, in the spring and early summer will favour the attacks of rust. This is also the case with *Melampsora* on pine (SYLVÉN, 1918). The germination conditions of the teliospores and thus also the distribution biology of the fungus to pine are, however, probably among the least known factors of the ecology of this fungus.

By the end of June, extensive growth of the fungus had occurred on the infected clones, though in a varying degree. This seems to be comparable with the earlier investigations made in 1952 and 1953. It was not unusual to find up to 20 caeomata per shoot—in a few cases as many as 70-odd caeomata were recorded on a single shoot. Such heavy attacks do not generally occur under ordinary circumstances.

The grafts investigated in 1957 had for several years been standing with a planting space of 5 meters; no internal sheltering effect between the plants was therefore feasible. *The attack was very strongly concentrated to the top shoots and the adjacent shoots.* The green annual shoots on the lower branches were seldom attacked.

SYLVÉN has made the same observation but considers the reason to be that the top of the seedlings will be more freely exposed. The reason must, however, be of a more physiological nature, as the difference remains even in the case of the investigations referred to here, in which all grafts were freely exposed. On 130 top shoots, 555 caeomata were found, on 582 shoots surrounding the top shoots there were 690 caeomata, while on 468 annual shoots on the lower branches there were only 33 caeomata (see Table II). The values summarized in this table refer to the four test clones chosen in 1957 and confirm numerically the location of the fungus to the pine tops irrespective of the degree of exposure of the plant. The number of attacks on the top shoot itself is without doubt the factor that varies most among the clones. A simple statistical examination of the data obtained shows that there are significant differences—at least between Z. 4009 which is the most susceptible clone—and the rest. Among the latter the differences are less pronounced.

In some cases no top shoot will develop, or the shoot is destroyed at an early stage. In such cases it seems as if the number of caeomata increases on the shoots surrounding the destroyed top shoot. However, before any of them have replaced the top shoot, they will probably have acquired more of the character of top shoots, thereby influencing their susceptibility to the disease.

Table II. Infection experiments with *Melampsora pinitorqua* on four clones of Scots pine.

Clone	Top shoot caeomata per shoot	Annual shoots surrounding the top shoot	Top shoots + adjacent annual shoots caeomata per shoot	Annual shoots on lower branches — caeomata per shoot	Number of grafts
Z 66	1.4 (± 0.3)	0.9	0.9 (± 0.2)	0.0	28
Z 4013	2.7	0.9	1.2	0.1	32
Z 4005	3.3	0.7	1.2	0.0	35
Z 4009	9.0 (± 1.9)	2.2	3.3 (± 0.7)	0.1	35
	P = 0.001		P = 0.005		
Number of shoots	130	582	712	468	
Number of caeomata	555	690	1245	33	

The fact that the fungus is concentrated to the pine tops infers that the question of resistance in this case is to be found in the metabolism of auxines and growing substances, or in processes which are directed by them (cf. SCHAW and HAWKINS, 1958). It would be of great interest to penetrate this resistance problem further with modern methods and technical means. *Melampsora* will only attack young pines, and the fungus gradually loses its importance for the host tree. It can very well be that this change in susceptibility of the pine has something to do with the change in the auxine spectrum of the host. Further, it would be of interest to study to what degree *Melampsora* is influenced by the pine's incipient ability to flower.

It may thus be of interest in itself to establish that different clones behave differently to *Melampsora*. But evidently the inheritance of the progeny is of greater importance, and the purpose was in this case to study seedlings from the four clones earlier studied by means of methodical infection.

As no cross-breeding had at that time been carried out at the Nedansjö pine seed orchard the only alternative was to arrange the necessary cross-breeding. The method used is described by EKLUNDH-EHRENBERG and SIMAK (1957). Further, there is a paper by HADDERS and ÅHGREN (1958) on the spontaneous cone and seed production at Nedansjö, which may be of some use. Cross-breeding took place in the spring of 1958, but as often happens in pine pollination, some clones did not flower at all. For this reason, the shortage of pollen led to all four clones being pollinated with one and the same clone, i.e. Z. 4011, which was a good pollen producer in 1958. Data referring to Z. 4011 are given in Table I. This clone is of ordinary resistance and can be compared with, for instance, Z. 4005. Cross-breeding was also carried out between Z. 66 and Z. 4009. This cross-breeding scheme is, of course, very limited but it should suffice to give an idea of the resistance of the progeny in F_1 from clones with statistically proved differences. The F_1-material must further be superior to an open pollination in that at least the pollen producer is constant.

While waiting for the result of the cross-breeding, seeds from open pollination in the plantation were collected from the four clones as well as from the pollen producer Z. 4011.

Plantations normally produce an open pollinated progeny for direct use in forestry. It is therefore most important to know whether the progeny of the very susceptible clone Z. 4009, for instance, is considerably inferior to that of the other clones. The difference may never appear in the F_1-material.

In the summer of 1959, there were about 1750 one-year-old seedlings from the above-mentioned five provenances in an ordinary nursery bed in the plantation at Nedansjö. Unfortunately, however, most of the

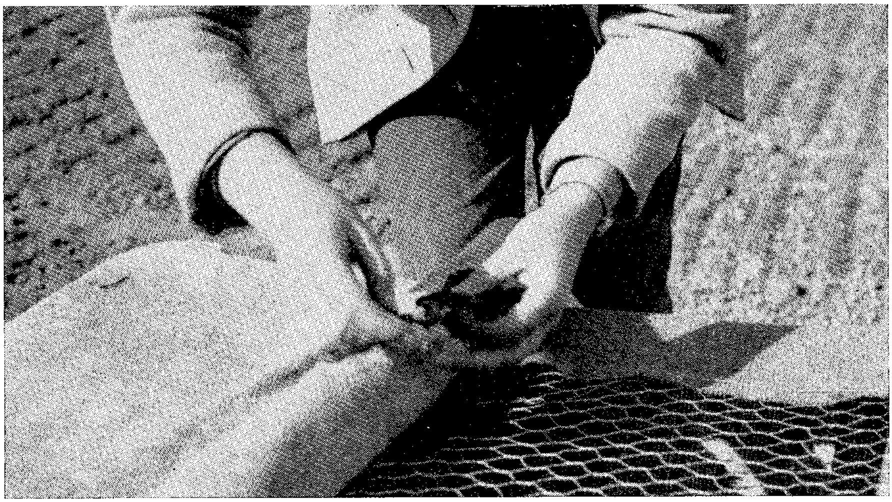

Fig. 1. A method of inoculating in a nursery by using a cage of wire-netting.

seedlings of the very important Z. 66 had been washed away by a current of water during the winter.

No method of inoculating such small seedlings has been described earlier. Bearing in mind the risk of the disease spreading, the wisdom of infecting in a nursery was discussed. A low cage of wire-netting was built erected round the plants (see Fig. 1), and aspen leaves with teliospores were placed on the netting. In order to keep the leaves in place and to prevent the disease from spreading, a damp, coarse cloth was placed on top of the leaves and fastened round the lower edges of the cage by digging it down into the soil. The cloth was kept damp for two days and the cage was then removed. The procedure was repeated twice during the shooting of the seedlings. No damage whatsoever could be discovered in the nursery outside the cage!

On the other hand, there was a loss of about 30 per cent of the inoculated seedlings. The damage was generally not so well defined in the caeomata as in older pines. The entire annual shoots of the damaged one-year-old seedlings were often covered by an unbroken caeoma (see Fig. 2). Even the needle tips could be discoloured by small caeomata. The cotyledons of germinal plants may sometimes be damaged (SYLVÉN, 1917; TROSCHANIN, 1952; ZILLER, 1961 and 1962). The needles of older plants are clearly vulnerable, but not until now has the pine twisting rust been held responsible for such damage (cf. TROSCHANIN, 1952; BÖHNER, 1952; REGLER, 1957, all of whom are of

Fig. 2. The entire shoots of one-year-old seedlings were often covered by an unbroken caeoma.

the opinion that the needles of older plants cannot be infected). GARBOWSKI (1929) points out that the basal parts of the needles may be infected, probably in connection with damage to the shoot.

Table III shows that there are no distinct differences in the progenies in F_1 after open pollination whether the mother clones show a good resistance or not. The progenies of Z. 66 were too few in number to allow an interpretation of their value.

Table III. Seedling progeny test after open pollination.

Mother clone	Number of seedlings 1/0	Number of attacked seedlings	Percentage of attacked seedlings
Z 66	12	0	0 too few
Z 4013	842	217	26
Z 4005	276	67	24
Z 4009	296	81	27
Z 4011	315	113	36

It might be of interest to mention an example of the experiments carried out with *Cronartium ribicola* chiefly on *Pinus strobus.* RIKER (1954) records a case of repeated selection of resistant trees, verified by grafting and testing clones. But on studying the open pollination in F_1 in comparison with plant material in general, no differences were found. In a later work PATTON and RIKER (1958) published an account of an extensive investigation, in which both open pollination and progenies of controlled cross-breeding were studied. It is surprising how seldom the progeny of resistant mother trees show a clear resistance in F_1. Even so, there is today no one who doubts that it is possible to do much to solve the *ribicola* problem with the help of resistance research. In other cases of rust fungi an obvious resistance may be found already in the progeny of open pollination of resistant mother trees. This can, for instance, be the case with *Cronartium fusiforme* (BARBER *et al.*, 1957). This is also thought to be the case with *Peridermium pini* on Scots pine (literature compiled by BOLLAND, 1957).

In 1961, there were 974 one-year-old seedlings from the controlled cross-breeding. These were inoculated in the way described above. It was later found, however, that the damage frequency was surprisingly

Table IV. Seedling progeny test after controlled cross-breeding and, for the completion of earlier investigations, also open pollination.

Mother clone	Number of seedlings	Number of attacked seedlings	Percentage of attacked seedlings
Z 4011 × Z 66	140	1	0.7
× Z 4013	397	42	10.6
× Z 4005	125	12	9.6
× Z 4009	210	4	1.9
Z 4009 × Z 66	102	2	2.0
Z 4009 open pollination	221	12	5.4
Z 66 open pollination	106	6	5.7
Local provenance at Nedansjö	167	26	15.6

low. No conclusions should yet be drawn from this material, as it will be necessary to carry out the infection again. In the meantime, the seedlings will have to be replanted, but the experiment will naturally be followed up.

Brief summary of the above experiment

In grafted clones of Scots pine, which had been under observation several years, it was found that resistance to *Melampsora* varied. It has been shown statistically that the values of the P-factor can amount to 0.001 when a comparison is made between the best and the worst clones after a uniform inoculation has been made. On the other hand, the progeny of an open pollination in F_1 do not show any differences in resistance, nor can the controlled cross-breeding progeny that have been studied in F_1 give a simple answer to the question of heredity. It is most unsatisfactory to have to prophesy the resistance of the progeny in F_1 with no other guidance than the resistance of parental clonal material. This is not to say that it should not be possible in the long run to produce an open pollination material that shows better resistance to *Melampsora*.

Experiments Concerning the Germination Ability of the Teliospore

From the experiments described above it can be seen that some years pine seedlings are not easily infected by pine twisting rust. This inconstancy must be very troublesome if it is intended to investigate the conditions of the resistance in pine. Some years, pine twisting rust will occur with great intensity on fellings and in nurseries. It has then often been possible to correlate this occurrence with a mild and rainy spring. SYLVÉN (1918) has been referred to and he, in turn, has quoted older sources. This relation to the weather is probably quite correct but is too vaguely expressed to make it useful for experimental work in the field.

And how is it possible to compare experiments in which the inoculation were carried out in different years?

If it is at all possible to make any progress with experimental work concerning pine twisting rust on pine, the very biology of the distribution to pine must be further clarified. The conditions of the germination of the teliospores in spring have been insufficiently investigated, see, however, REGLER (1952), and the Russian works referred to by him, especially Schafranskaja (1951). The influence of the weather on the formation of basidies is stressed; partly by emphasizing the necessity of repeatedly exposing the aspen leaves to rain and partly that the temperature is relatively high and the air humidity as high as possible. Further, it is shown that the basidiospores are sensitive to drying.

Regler (1952) also states that particularly during mild autumns and winters a great deal of the teliospores may germinate too early to coincide with the shooting of pine. By wetting the aspen leaves several times during autumn, partly in a green-house, a germination of the teliospores with basidies was obtained.

An account of some experiments carried out under Swedish conditions

If aspen leaves with teliospores from the previous year are collected at about the time of the shooting of pine, it is generally possible to observe the formation of basidies from the teliospores if the leaves are kept wet, e.g. in Petri dishes. In one of the experiments, aspen leaves in humid Petri dishes were put into thermostats with different temperatures: + 5°, 10°, 15°, 20°, 25°, and 30° C. A quick reading was made under a preparator's lens every hour. All preparatory work was carried out at + 4° C.

Already after one hour an abundant formation of basidies developed in + 15°, 20° and 25° C, after another hour also in + 10° C, and after about 24 hours some activity could also be observed in + 5° C. If the dishes that had been kept in + 30° C were moved to a lower temperature, there was still no formation of basidies whatsoever. The higher temperature has evidently a destructive effect on the humid spores. *A quick formation of basidies can thus take place within fairly wide temperature limits.* These values show a considerably quicker germination than that recorded by Regler (1957). However, if aspen leaves were collected very early in spring or even in the autumn and then put into humid Petri dishes, no formation of basidies took place. The time of collecting the leaves therefore seems to be of significance, and germination power in the teliospores seems to be subject to seasonal variations.

In order to get a clearer view of the problem, some experiments were made. In the spring of 1960, aspen leaves were collected once a week from 15 April to 15 June and put into Petri dishes under laboratory conditions at + 20° C (see above). The material was taken from Gransjön in the county of Medelpad; temperature, humidity and rainfall were recorded on the spot. It was found that no spores germinated. The reason for this is not clear. Another fungus occurred abundantly on aspen leaves that year, i.e., *Coccomyces coronatus* KARST. (SCHUMACHER ex FRIES). It cannot be excluded that this was a case of antagonistic influence. No pine twisting rust was found in that locality in 1960.

In 1961 the experiment was repeated in two localities at Gransjön and at Stensängen, to the north of Stockholm. This year a better insight of the biology of the fungus was obtained. In order to make a detailed study possible, the test protocols have been collected in Tables V and VI. From one of the experiments thermohygrograms are also given with comments (see Fig. 3).

Aspen leaves were soaked overnight in cold water (+ 4° C) and then placed in Petri dishes, which were kept at + 20° C (the same procedure as described above).

Germination of basidies was registered after 1, 3 and 5 days and the values 0, 1, 2 and 3 were obtained, whereby 0 naturally stands for no formation of basidies and 3 for a very abundant formation. From the tables it can be seen that the first samples collected showed very little germination power after 24 hours; the formation of basidies, however, gradually increased because of the favourable temperature and humidity conditions. Later during the spring the sample material collected from the Stockholm area showed a considerably quicker formation of basidies, which coincided with the shooting of the pine. Consequentially, therefore, it could also be noted that damage to the young pines was very common that spring.

Some details of the tables are worth special mention. In the material from Medelpad an obvious increase in the germination intensity could be noted after a rainy week (36 mm) with night temperatures that were high for the time of the year (+4° to 5° C). Later three cold and dry weeks followed, during which the germination power of the spores was unchanged. Not until after May 29 did the night temperature pass the freezing-point, the day temperature then rising to between 20° and 30° C. These temperatures in conjunction with some rain resulted in the culmination of the spore germination. However, this seemed to come somewhat too late to coincide with the shooting of the pine.

A comparison with the Stockholm material shows that in that area only the first few weeks were dry and cold (night temperatures). This period was followed by five weeks of rain and high temperatures, and the culmination of the spore germination.

With regard to the germination power of the teliospores, *the following conclusion can thus be drawn.* On the whole, it is difficult to make the teliospores germinate at the time of the autumn when the

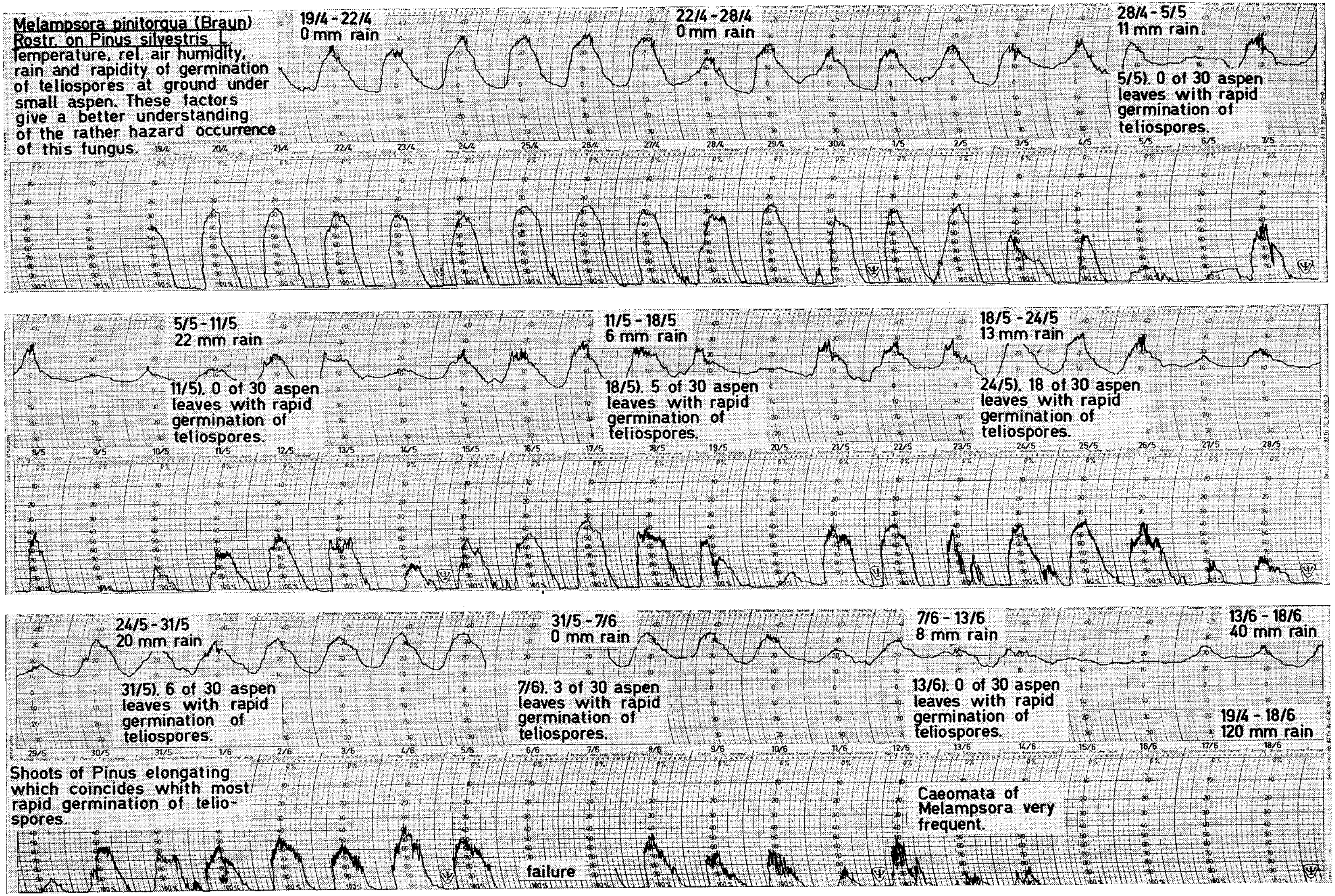

Fig. 3. Thermohygrograms with comments, showing a changing germination ability of the teliospores during the spring.

Tab. V. Registration of teliospore germination of *Melampsora* on aspen leaves after repeated collection of leaves from 22 April to 18 June 1961. The intensity of germination is given in 0, 1, 2 and 3, and registration is made after 1, 3 and 5 days at + 20°C. Material from Bogesund (near Stockholm).

Intensity of germination after 24 hours				72 hours				120 hours				Date	mm rain	Notes
0	1	2	3	0	1	2	3	0	1	2	3			
												22/4—28/4	0	
24	4	2	0	16	8	4	2	10	8	8	4	28/4— 5/5	11	Shoots of *Pinus* elongating with coincide which most rapid germination of teliospores
22	5	3	0	1	8	16	5	7	6	11	*6*	5/5—11/5	22	
10	5	10	5	2	4	8	*16*	12	4	8	*6*	11/5—18/5	6	
1	5	6	*18*	3	1	2	*24*	3	8	4	*15*	18/5—24/5	13	
10	8	6	*6*	10	8	9	3	26	3	1	0	24/5—31/5	20	
17	5	5	3	20	5	3	2	27	1	0	0	31/5— 7/6	0	
22	6	2	0	24	6	0	0	30	0	0	0	7/6—13/6	8	Caeomata of *Melampsora* very common
Clearly decreasing intensity of germination												13/6—18/6	40	

30 leaves of *Populus trem.* gathered at every registration.

Tab. VI. Registration of teliospore germination of Melampsora pinitorqua on aspen leaves after repeated collection of leaves from 30 April to 17 June 1961. The intensity of germination is given in 0, 1, 2 and 3, and registration is made after 1, 3 and 5 days at + 20°C. Material from Gransjön in Medelpad.

Intensity of germination after 24 hours				72 hours				120 hours				Date	mm rain	Notes
0	1	2	3	0	1	2	3	0	1	2	3			
21	6	2	1	18	6	3	3	7	4	16	3	30/4— 6/5	0	
23	5	2	0	20	6	3	1	14	7	7	2	6/5—13/5	36	
9	10	5	6	7	7	10	*6*	8	10	6	*6*	13/5—20/5	1	
6	11	6	*7*	4	8	6	*12*	13	6	6	5	20/5—27/5	1.5	
5	10	8	*7*	10	9	6	5	17	8	4	1	27/5— 3/6	5	Shoots of Pinus elongating which comes a little too early to coincide with most rapid germination of teliospores
3	5	8	*14*	15	5	8	1	21	6	3	0	3/6—10/6	10	
Intensity of germination not yet decreasing												10/6—17/6	12	Rare caeomata of *Melampsora*

30 leaves of *Populus trem.* gathered at every registration.

leaves are shed or in the early spring. Not even under very favourable temperature and humidity conditions can more appreciable activity be registered. It must, therefore, be a case of *germination inhibition*. However, this decreases in the spring under the influence of humidity and rising temperature. As this decrease proceeds, smaller rain quantities and shorter periods of favourable temperature will be required for the formation of basidies. According to the first experiment, one has finally to reckon with a germination time of one hour. Dew and rising temperatures, e.g. on cloudy nights, will be sufficient in the final stage.

It should perhaps be added, that it is quite easy to observe with the naked eye how the little black-brown formations of teliospores germinate and how they are covered by a white layer of basidies (see Fig. 4). In case this is observed on wet aspen leaves when the pine is shooting, there will naturally be a great risk of damage from pine twisting rust that year. The critical time for pine is short, however. When the shoots have developed so far that also the needles begin to show, the main risk has been passed. This is shown by a number of experiments described above in connection with the progeny test. If, at any time, it should be necessary to apply spraying also in Sweden, the critical time when pine may be affected by the disease is thus very short.

There is another phase in the biology of the distribution that is of utmost importance. That is the path of the basidiospore from the aspen leaves on the ground to the one-year-old shoot of the pine. All that is at present considered to be certain, is that the basidiospore is sensitive to drying and high temperatures (25° to 26° C) (SCHAFRANSKAJA, 1940, as cited from REGLER). Higher temperatures are generally followed by

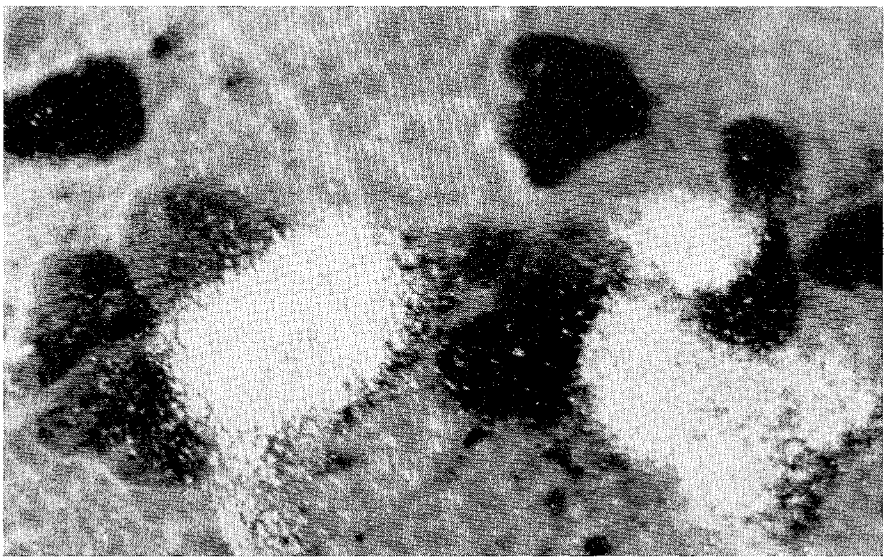

Fig. 4. Crusts of teliospores on the under-side of aspen leaves, some of which have germinated with basidies.

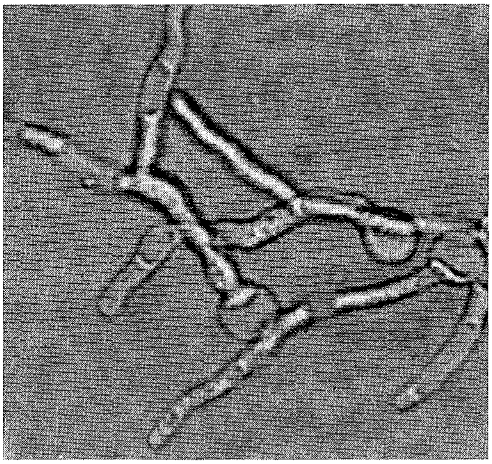

Fig. 5. Germinating basidiospores very much enlarged.

lower relative air humidity, which undoubtedly makes it difficult to distinguish between the influence of temperature and that of relative air humidity. The size of the basidiospores is only 5 to 8 μ. If these spores are to survive and be distributed over a large area—at most 250 meters according to TROSCHANIN (1952) and 200 meters according to SCHAFRANSKAJA (1940, as cited from TROSCHANIN and REGLER)—the humidity conditions must be very favourable.

It is quite possible that the small size of the spores permits them to remain airborne even in rainy weather, which is out of the question in the case of bigger particles, e.g., aeciospores or pollen (cf. McDONALD, 1962).

What remains to be investigated then is the germination of the basidiospores and the penetration of the fungus into the pine shoot, as well as the question of how and when during these processes resistance to the fungus is established. Fig. 5 shows germinating basidiospores very much enlarged.

Summary

It is evident from the review of literature in the beginning of this paper that *Melampsora pinitorqua* is a matter of increasing interest in most parts of Europe. The fungus is found all over Europe and adjacent parts of Asiatic Russia. Recently, there has also been information which makes it very probable that the pine twisting rust is also found in North America.

At least nine species of *Pinus* are more or less suffering from *Melampsora pinitorqua: Pinus silvestris, P. pinaster, P. pinea, P. strobus, P. mugo, P. nigra, P. murrayana, P. ponderosa* and *P. halepensis.*

Fourteen clones (with 17—41 grafts) have been examined after spontaneously occurring disease. There were appreciable differences in their resistance to rust. Some of the clones were chosen for experiments and given a heavy inoculation with the fungus. Even after this standard artificial inoculation there are distinct differences in resistance which have been examined statistically.

Irrespective of whether the small pines are standing alone or in dense spacing, the fungus will only attack the top of the trees.

A progeny test with one-year-old plants after open pollination of the clones does not reveal any differences in F_1 concerning fungal resistance (Tab. I). A number of controlled pollinations were also made. The clones chosen for inoculation experiments were pollinated with the same pollen from one clone only. The progeny test did not show any differences in F_1, when the one-year-old plants were exposed to *Melampsora* (Tab. II).

The germination biology of the teliospores on aspen leaves is a matter of special interest in this paper. The germination must coincide with the development of pine shoots in the spring. A pronounced germination inhibition of the teliospores gradually disappears during the spring which usually coincides with the shooting of the pines (Tab. V, VI). But the fungus may also pass the winter in the aspen buds (cf. Klebahn, 1938; Kujala, 1950; Moriondo, 1956 and 1961). When the germination ability of the teliospores culminates, the germination is very rapid. Finally one hour is quite enough for the development of basidies at 15°—25° C.

Acknowledgements

I wish to express my sincere thanks to Mr. Björn Hagström (Chief Forester) and Mr. Allan Åhgren (Forester) in the Swedish Cellulose Company for their kindness in placing at my disposal the facilities of that important company.

I am also deeply indebted to my chief, Professor Erik Björkman, head of the Department of Forest Botany, at the Royal College of Forestry, Stockholm, for the valuable discussions of the problem dealt with in this paper.

A great debt of gratitude is also due to Mr. Vitalis Carlberg and Mr. Yngve Larsson of the S. C. A. pine seed orchard at Nedansjö for their skilful technical assistance and indeed to the late Dr. Erik Rennerfelt for the initiative he showed in the making of this investigation.

The expenses of the investigation were partly defrayed by a grant from the Royal Academy of Forestry and Agriculture.

REFERENCES

BARBER, J. C., DORMAN, K. W., & BAUER, E., 1957: Slash pine progeny tests indicate genetic variation in resistance to rust. — Res. notes. Southeast. for. exp. stat. 104.

BERGMAN, F., 1954: Om skogsträdens sjukdomsresistens och dess utnyttjande inom skogsträdsförädlingen. — Föreningen för växtförädling av skogsträd. Årsberättelse 1953, 70—98.

BIRAGHI, A., 1954: Some important diseases of conifers in Italy. — FAO plant prot. bull. 2, 166—167.

BOLLAND, G., 1957: Resistenzuntersuchungen über Kienzopf und Schütte an der Kiefer. — Der Züchter 27, 38—47.

BRENNEJZEN, B., 1957: Niebezpieczénstwo zwiększenia szkód powodowanych przez skrętaka sosnowego. [The danger of increase of the damage by spindly shoot on Pines.] — Las polski 31, 7, 10.

BÖHNER, P., 1952: Der Kieferndrehpilz — eine ernste Gefahr für Kiefernkulturen. — Allg. Forstzeitschr. 46, 471—475.

DAY, W. R., 1943: Forest pathology. — Rep. Imp. for. inst., Oxf., 6—7. (Rev. Appl. mycol. 23, 200.)

McDONALD, J. E., 1962: Collection and washout of airborne pollens and spores by raindrops. — Science 135, 435—437.

EKLUNDH-EHRENBERG, C., & SIMAK, M., 1957: Flowering and pollination in Scots pine (Pinus silvestris L.). — Medd. från Stat. skogsforskn.inst. 46: 12, 1—27.

ELSSMANN, H., 1952: Der Kieferndrehpilz auch in Nordschweden. — Allg. Forstzeitschr. 7, 498—499.

GARBOWSKI, L., 1929: Diseases of cultivated plants and of ornamental and forest trees and shrubs recorded in 1926 and 1927 in Great Poland and Pomerania. — Prace wydz chorob roślin w Bydgoszczy państw. inst. naukow. gospod. wiejsk. 7. (Rev. Appl. mycol. 8, 290.)

GAVRIS, V. P., 1939: Selection of immune forms of common pine. — Lesn. hoz. 1939: 8, 5—8. (Rev. Appl. mycol. 19, 375.)

GÄUMANN, E., 1959: Beiträge zur Kryptogamenflora der Schweiz. — Die Rostpilze Mitteleuropas. 12, 136—137.

HADDERS, G., & ÅHGREN, A., 1958: Kott- och fröproduktion samt frökvalitet hos 12-åriga ympträd av tall. — Svenska skogsvårdsfören. tidskr. 56, 455—468.

HARLOW, W. M., & HARRAR, E. S., 1958: Textbook of dendrology. New York.

JØRSTAD, I., 1953: Pucciniastracease and Melampsoraceae of Norway. — Uredineana 4, 91—123.

KARDELL, L., 1962: Svåra knäckesjukeangrepp. — Skogen 49, 341—342.

KLEBAHN, H., 1938: Offene Fragen und neue Beobachtungen über die rindebewohnenden Blasenroste der Kiefern nebst Bemerkungen über einige andere Rostpilze. — Z. Pfl.-Krankh. 48, 369—410.

KRSTIC, M. M., 1960: The significance of introduced diseases from the European point of view. — Proc. 5th world for. congr. 2.

KUJALA, V., 1950: Über die Kleinpilze der Koniferen in Finnland. — Commun. inst. for. Fenniae 38, 1—121.

MORIONDO, F., 1951: La ruggine curvatrice dei germogli sul pino domestric. — Ital. for. mont. 6, 314—317.

— 1952: Ricerche sulla Melamspora pinitorqua Rostr. in Italia. 1. Comportamento dei semanzali di Pinus pinea alle infezioni di M. pinitorqua. — Ann. sper. agr. N. S. 6, 593—611.

— 1954 a: Ossarvazioni sul ciclo biologico della Melampsora sp. del pioppo in Italia. — Ital. for. mont. 9, 259—264.

— 1954 b: Formazione di ecidi interni da porte di Melampsora pinitorqua Rostr. — Ann. Accad. ital. sci. for. 2, 265—271.

— 1956: Ricerche sulla Melampsora pinitorqua Rostr. in Italia. — Ibid. 5, 263—282.

— 1957 a: Osservazioni sulla biologia di Melampsora pinitorqua Rostr. sul litoral tirrenico. — Monti e boschi 8, 31—35 (Rev. Appl. mycol. 37, 189—190).

— 1957 b: Osservazioni sulla diffusione della ruggine curvatrice del pino in Italia. — Ric. sci. 27, 405—411.

— 1958: Alcuni aspetti della epidemiologia della Melampsora pinitorqua Rostr. sulle alpi marittime. — Ital. for. mont. 13, 128—133.

— 1961: Ripetizione sperimentale del ciclo biologico di Melampsora pinitorqua Rostr. — Ibid. 16, 71—77.

Murray, J. S., 1961: Forest pathology. For. comm., Lond. Rep. on for. res. 1960, 60—61.

Orloś, H., 1935: Sprawozdanie z dzialaności Instytutu badawczego w dziedzinie fitopatologji za rok 1933. [Report on the phytopathological activity of the Research institute in 1933.] — Inst. bad. las. Warszawa. państw. Ser. A. 11, 9—19.

Patton, R. F., & Riker, A. J., 1958: Blister rust resistance in eastern white pine. — Proc. 5th northeast. for. tree impr. conf., 46—51.

Peace, T. R., 1944: The occurrence of Melampsora pinitorqua on Scots pine in south-eastern England. — Forestry 18, 47—48.

— 1951: Forest pathology. For. comm., Lond. Rep. on for. res. 1950, 78.

— 1957: Recent observations on the rusts of pine in Britain. — For. comm. Lond. (Rev. Appl. mycol. 36, 799.)

— 1962: Pathology of trees and shrubs with special references to Britain. Oxford.

Regler, W., 1957: Der Kieferndrehrost (Melampsora pinitorqua), eine wirtschaftlich wichtige Infektionskrankheit der Gattung Pinus. — Beitr. zur Pappelforsch. 2, 205—234. (Deut. Akad. d. Landwirtschaftswiss. Wiss. Abh. 27.)

Rennerfelt, E., 1954: Biologische Untersuchungen über den Kieferndreher Melampsora pinitorqua (Braun) Rostr. — Proc. Congr. int. union for. res. organ., Rome 1953, 705—711.

Riker, A. J., 1954: Opportunities in diesease and insect control through genetics. — J. for. 52, 651—652.

S. (G. N.), 1923: Pine branch twist. A fungus disease on pine, Melampsora pinitorqua. — Cyprus agric. j. 18, 19. (Rev. appl. mycol. 2, 529.)

Schafranskaja, W. N., 1951: Der Kieferndreher in Pflanzgärten und seine Bekämpfung. (Cit. from Regler 1957.)

Shaw, M., & Hawkins, A. R., 1958: The physiology of host-parasite relations. 5. — Canad. J. bot. 36, 1—6.

Sveriges off. statistik. Jordbruksräkningen 1956. Stockholm 1958.

Sylvén, N., 1917: Om tallens knäckesjuka (Melampsora pinitorqua (Braun) Rostr.). — Medd. från Stat. skogsförsöksanst. 13—14, 1077—1140.

— 1918: 1917 års knäckesjuka i norra Västergötland. — Medd. från Stat. skogsförsöksanst. 15, 192—204.

Troschanin, P. G., 1952: Der Kieferndreher und seine Bekämpfung. (Transl. by Reinwaldt, Eberswalde). Moskau & Leningr.

Wright, J. W., 1960: Improvement rates through clonal and seedling seed orchards. — Proc. 5th world for. congr. 2. 808—810.

Younitzky, A. A., 1927: Fungi attacking healthy and scorched trees in the forests of the Mari Region, and the damage they cause to young stands arising on areas devastated by fire or bark insects according to the observations of the 1926 expedition. — Kazan inst. of agric. and silvicult. news 3. (Rev. appl. mycol. 6, 700—701.)

Ziller, W. G., 1961: Pine twist rust (Melampsora pinitorqua) in North America. — Plant dis. reptr. 45, 327—329.

— 1962: Melampsora rust on ponderosa pine. Outbreaks and new records. — FAO plant prot. bull. 10, 42—43.

Sammanfattning

Melampsora pinitorqua (Braun) Rostr. — Några resistensbiologiska försök

Av den inledande litteratursammanställningen framgår, att *Melampsora pinitorqua* tilldragit sig ett ökande intresse i de flesta europeiska länder. Svampen uppträder i hela Europa och delar av asiatiska Ryssland. Vidare finns uppgifter från Nordamerika, som synes ge vid handen, att knäckesjukan lyckats sprida sig även till denna kontinent. Åtminstone 9 arter av släktet *Pinus* har visat sig mottagliga.

I detta arbete redovisas olika angreppsintensitet på ympkloner av tall vid spontan infektion. Av dessa ympkloner utvaldes några, som utsattes för intensiv experimentell infektion. Även vid denna likformiga infektion bestod statistiskt säkerställda skillnader i angreppsfrekvensen mellan t. ex. bästa och sämsta klon. Svampen är starkt lokaliserad till själva toppen av tallarna oavsett om dessa står i täta förband eller helt fristående. Vid avkommeprövning efter fri avblomning kvarstår dock ej några skillnader i F_1 vad beträffar angreppsfrekvens. Ett begränsat antal kontrollerade korsningar utfördes även. De utvalda klonerna — vari ingår både den mest och den minst resistenta enligt det refererade infektionsförsöket — pollinerades med samma pollendonator. Förfaringssättet ger intet uttömmande besked om ärftlighetsgången men är ändå ett steg utöver fri avblomning. Ej heller i detta fall uppträdde i F_1 några påtagliga skillnader i resistens.

Teleutosporerna på asplöven har ägnats särskild uppmärksamhet. Deras groningsbiologi är av speciellt intresse för spridningen till tall. En tydligt påvisbar groningshämning reglerar sporernas groningsförmåga. Hämningen minskar under våren på grund av klimatiska orsaker, och sporernas groningsförmåga kulminerar, vilket kan sammanfalla med tallens skottskjutning. Basidiebildningen kan till slut ske på mycket kort tid. En knapp timme är fullt tillräcklig vid $+15$—$25°$ C.