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EPIPHYTOLOGY OF *MELAMPSORA* RUSTS OF SCOTS PINE (*PINUS SYLVESTRIS* L.) AND ASPEN (*POPULUS TREMULA* L.)

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PREFACE

Numerous persons helped me during the various stages of this research. The work was initiated in the Finnish Forest Research Institute with the encouragement of Prof. Sakari Saarnijoki and Prof. Risto Sarvas. During the beginning of the work, I received much beneficial advice from Prof. Paavo Juutinen and Mr. Ukko Rummukainen. I have had important discussions concerning the studies with Dr. Allan Klingström of Uppsala University, Sweden, Dr. Veikko Hintikka, Mr. Lalli Laine, and many other colleagues. The encouraging attitudes of these persons, and especially Forest Officers, Mr. Viljo Mattila and Mr. Lauri Rantala, who are working in practical forestry, were decidedly significant in the conduct of the studies.

Mr. Erkki Kaita-aho, Mr. Simo Leinonen, Mrs. Inkeri Erjala, Mrs. Toini Ojala, Mr. Matti Laurila, Mr. Larry Huldén and several other persons assisted me in the field and laboratory work.

The manuscript was read and beneficial criticism given by Dr. Peitsa Mikola, Prof. of Forest Biology, and Dr. Eeva Tapio, Prof. of Plant Pathology, both from the University of Helsinki, and Prof. Paavo Juutinen. Dr. Kim von Weissenberg translated the manuscript, and his wife Joann von Weissenberg revised the English text.

These studies have been included in the research program of the Finnish Forest Research Institute. With a fellowship sponsored by the W. K. Kellogg Foundation (Battle Creek, Mich. U.S.A.) and some financial support from the Society of Forestry in Finland I was able to become acquainted with current research in the corresponding field in the United States and Canada.

I wish to express my sincere thanks to the persons and institutions mentioned above and to all others who have assisted during the course of this study.

Helsinki, October 1973

Timo Kurkela

CONTENTS

INTRODUCTION

The pine twisting rust caused by the fungus *Melampsora pinitorqua* (Braun) Rostr. is economically one of the most important diseases in pine sapling stands in Finland (Kangas 1938, Kujala 1950). In addition to Europe, at least Asia Minor and the western part of Siberia are included in its range (Longo et al. 1970). The main host is Scots pine (*Pinus sylvestris* L.), but the fungus also infects many other pines of the *Diploxylon* group. In order to study the disease in depth, an international working group has been established within IUFRO.

Melampsora larici-tremulae Kleb. causes the needle rust of larch. *M. pinitorqua* and *M. larici-tremulae* can be distinguished from each other only because they develop the aecial states on different hosts, the former on pine shoots, the latter on larch needles. The uredia and telia of both rusts develop on the leaves of aspen. The needle rust of larch is not known to cause any damage of economic importance for forestry.

After the description of the symptoms of the disease (de Bary 1863) and the life cycle of the pathogen (Rostrup 1884), considerable time passed before important publications on the biology of pine twisting rust were published. From the standpoint of epiphytology, the most important of these studies were: Schafranskaja (1940), Troschanin (1952), Regler (1957), Klingström (1963) and Longo et al. (1970). These studies have dealt with i.a. infection of pine, the distance to the source of inoculum, germination of teliospores, and the dependence on climatic conditions for infection of pine.

Although the main features of the cycle of the rust are known, there are no other practical methods of control except eradication of aspen from pine sapling stands. This already was recommended by Hartig (1885). In order to find new control methods, all possible gaps in our knowledge must be eliminated. Detailed studies of i.a. spore dispersal, process of infection, and the rate of development of the rust on pine and aspen have not been made. The occurrence of the rust in the uredial and telial states especially has received little previous attention.

Pine twisting rust had occurred to some extent on various locations in Finland during the early 1960's. In 1964 severe damage was caused almost everywhere in the country but especially in the southwestern and western parts, where, subsequently, the Regional Board of Forestry asked for initiation of this study.

This publication reports on the dispersal of the different spore types of *M. pinitorqua* and the subsequent development of rust on the host plants, pine and aspen, in the field conditions. Previously published results by the author (Kurkela 1973 a) on the release and germination of basidiospores are discussed in the present report in relation to spore dispersal in nature. Data on *M. larici-tremulae* are also included. This is due partly to the fact that observations on *M. pinitorqua* could not be made every year because of the scarcity of the rust, and partly due to the fact that both rusts are almost identical ecologically; therefore the results could be generalized for both species.

MATERIALS AND METHODS

Description of experimental plots

The development of pine twisting rust was followed on locations where the disease previously had caused considerable damage. The locations of the experimental plots of this study are shown in Fig. 1. The most important part of the study was made in Jauli, Ikaalinen township, (N 61°24′, E 23°26′) the Location 1, where observations were made during 1965—1968. The study area was about 160—180 m above sea level. It had been clear-cut and planted with pine. The sapling stands had been beaten up several times

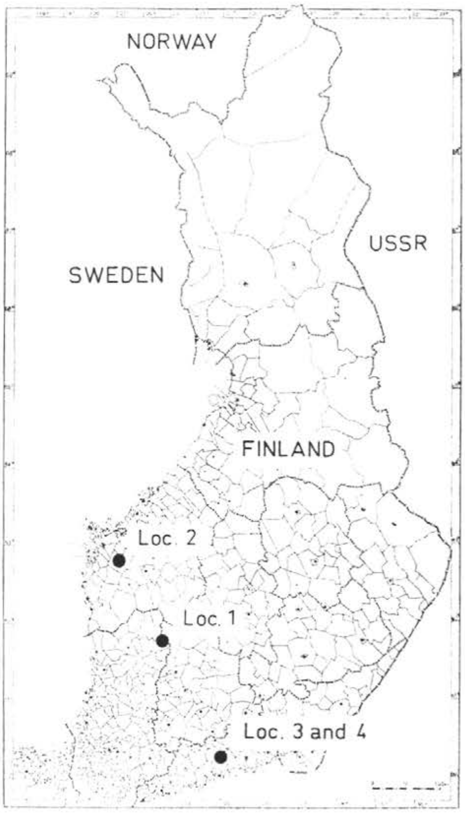

Figure 1. Location of the experiment areas. Ikaalinen, Jauli (N 61°24′, E 23°26′) = Loc. 1. Laihia, Jakkula (N 62°57′, E 22°06′) = Loc. 2. Tuusula, Ruotsinkylä (N 60°38′, E 25°09′) = Loc. 3 and 4.

due to the fail places caused by combined effect of rust and hardwood suckers. At the study site there were 0.5—1 m high pine saplings mixed with some aspen suckers. In 1966 observations were also made in Jakkula, Laihia township, (N 62°57′, E 22°06′) Loc. 2. This area, in a spruce stand, was a North-South clear-cut strip ca. 70 m wide, planted with pine. The pine sapling stand was about 1—2 m high and growing vigorously. The stand was rather densely mixed with aspen suckers.

In 1970 and 1971 the dispersal of *Melampsora* spores was studied in the Ruotsinkylä Experiment Forest of the Finnish Forest Research Institute in Tuusula township (N 60°38′, E 25°09′), Loc. 3. This study area was an opening of 20 m diameter, filled with low (about 0.5 m) aspen suckers, located in a mixed stand of 4—6 m high pine and larch.

In the spring of 1973 the basidiospore dispersal of aspen rust was investigated in the aspen clone bank of the Ruotsinkylä Experiment Forest (Loc. 4). The height of the tallest trees was 6—8 m. When the trees were still without leaves in the spring, insolation, wind, and precipitation could freely affect the fallen leaves on the ground. During the previous summer, the ground vegetation under the trees had been eliminated by using a rototiller.

Measuring of weather factors

The relationship between the pine twisting rust, the aspen rust, and various weather factors were studied by following the dispersal of the pathogen spores, the development of the hosts, and occurrence of the rust on its hosts during various stages of the growing season, and by registering weather factors.

Temperature and humidity. The air temperature was measured 2 m above the ground in a standard weather chamber by means of a thermograph or thermohygrograph. The instrument readings were corrected according to a calibrated precision thermometer in the same weather chamber. The temperature was recorded to the nearest 0.1 °C. Calculations of daily mean temperatures were based on graph readings taken at every odd-numbered hour.

The relative humidity of the air was obtained during June—September from a thermohygrograph (hair hygrometer) placed in the weather chamber. The instrument was calibrated prior to and after use in order to detect any measurement errors. For the present investigation the measurement of high relative humidity was the most important. According to Ahti (1972), it is obvious that when the relative humidity approaches 100 %, the hair hygrometer tends to record too low values, the error being on the average larger in summer than in winter.

Precipitation and dew. The amount of precipitation was measured by a fluviograph with a clock making a complete revolution in one week. In 1971 and 1973, however, the fluviograph used had a clock making a complete revolution in 24 hours. The amount of water passing through the fluviograph was checked with a graduated cylinder. The precision of measurement was to the nearest 0.1 mm.

In 1971 and 1973 a dew scale was used. The amount of condensed water and the duration of dew were measured. The dew scale also recorded minor precipitation when the amount of water was not sufficient to be recorded by the fluviograph. The precision of the dew scale was to the nearest 0.1 g. The dew scale was also used to measure the total length of the wetness period caused by both precipitation and dew. The cup of the scale was adjusted in such a manner that the water that rained into it could flow out trough the cup bottom. Thus, the recorder always deviated from zero as long as the surface of the cup was wet. With the dew scale it was not always possible to distinguish dew from rain, but this was not necessary. It was only important to measure the time during which free water, precipitated or condensed, occurred on the foliage or on vegetation in general. The dew scale was calibrated once a week with weights of 1 and 5 grammes.

Wind. Wind velocity recordings from the climatological station in Kuru, Länsi-Aure, of the Meteorological Institute were used for the studies made in Loc. 1, during 1966—1968. The climatological station was located 7 km from the experimental area. The recordings were made three times a day, at 08.00, 14.00, and 20.00. The daily means of these recordings were used in the present study. This is, as such, a very crude estimate of wind velocity, and does not allow a very detailed analysis of the studies of spore flight.

For the 1970 studies the data on wind velocity from the Helsinki Airport (Vantaa township) climatological station of the Meteorological Institute were used. The airport is located 5 km from the study locations Nr. 3 and 4. The recordings at the airport were made at half-hour intervals.

In 1971 and 1973 the wind velocity was measured at the experimental area in Ruotsinkylä (Loc. 4). The sensor was a Lambrecht Nr. 1467G attached to the recorder, Lambrecht Nr. 1487a. The sensitivity of the sensor was 0.6 m/sec. This device was especially useful for measuring the velocity of wind gusts. The maximum velocity for each 10 minute interval was read from the recorder's paper strip. These values were used for calculation of daily and hourly mean velocities.

Measurement of host tree development

Growth of shoots and needles in pine and larch. The height growth of the pine shoots was measured in order to determine at what phase of growth infection by the pine twisting rust occurs in nature. Growth was measured in Loc. 1 in 1966—1968 and in Loc. 2 in 1966. In the first measurements a total of 50 saplings were included. From each sapling the growth of the terminal leader and two branches formed during the previous year was measured. In 1967 30 saplings were measured. In 1968 the measurements were continued in the same place as previously; but due to the serious attacks by the rust in the previous summer, only 10 saplings could be included.

The reference point for the measurements was marked on each sapling with an insect needle inserted immediately below the base of the terminal bud. The measurements were made daily at noon. The work was begun in mid-May when the terminal bud had started to elongate. The measurements were made with a sliding caliper. Precision of measurement was to the nearest 0.1 mm. One person made all measurements in Loc. 1, and another made those in Loc. 2.

In connection with shoot measurement, the needle length was also measured. From the growing terminal leaders of pine saplings the lengths of the five uppermost and five lowermost needles were measured in 1967. In 1968 the length of 10 marked needles in each of five trees was measured. The length of larch needles in short shoots was also measured during their development. Measurements were made each day, beginning on the day when the tips of the needles emerged from the fascicle, and ceased when no growth could be detected.

Growth of aspen shoots and leaves. Measurement of the short shoots of aspen commenced before the buds began to expand. The reference point was taken to be the scar of the previous year's leaf at the base of the bud, and the measurements were made from this reference point to the tip of the bud using sliding calipers. During the opening of the bud, measurements had to be discontinued until the tip of the expanding shoot clearly could be detected. At this time the growth measurements of the uppermost leaf commenced. The leaf was measured from its base to the tip. Measurements were continued until no growth could be detected. Some 4—5 groups of suckers were selected for the measurements. From each group four buds were marked for the measurements.

Inoculation of pine shoots with basidiospores

For these experiments 2 year-old nursery-grown transplants were used. The transplants had been lifted from the nursery in the spring and placed in cold storage. The growth of the transplants was slowed down in storage until they were used. The transplants were planted in pots in a greenhouse.

The temperature in the greenhouse varied from 14 to 22°C. The relative humidity was kept at 60—95 % with an air humidifier. Of the potted transplants, the most vigorous ones were selected for the experiment and arranged into groups of ten transplants each. The order of inoculation of the groups was randomized. Since temperature has been found to be a very important factor controlling the annual growth of trees (cf. Sarvas 1965, 1972, Hari et al. 1970), the inoculation interval for the transplants was taken to be 50 degree hours. Calculation of the number of degree hours was commenced when the transplants were potted and transferred to the greenhouse. The number of degree hours was calculated from the mean of the temperature minus five degrees at each odd-numbered hour of the day (cf. Sarvas 1965). The method developed by Moriondo (1961) was used for inoculation. The pine shoots as well as the inner side of a test tube of 25 mm diameter was moistened with a spray of water. The growing shoot was inserted into the test tube deep enough that pieces of aspen leaves, bearing sporulating telia fastened to the inner side of the tube, came level with the tip of the shoot. Before application, the aspen leaves had been incubated at 13 °C for 24 hours. It was extremely difficult to standardize the amount of inoculum, i.e. the number of basidiospres formed by the telia, since the number of telia and their ability to germinate on the aspen leaves varied considerably, which facts have been reported also earlier (Klingström 1972, Kurkela 1973 a). As a source of inoculum three pieces 1 × 3 cm, of aspen leaves were placed inside the test tube. The inoculum was made at 15 °C over a period of 16 hours. Subsequently, the transplants were transferred back to the greenhouse. The experiments were made with two lots of transplants planted at two different times. With the first lot, eight groups of inoculations were made; with the second, only three were made. The smaller number of groups in the second lot was due to the fact that these transplants had been kept in cold storage longer and were, therefore, in such poor condition that a larger proportion of them had to be discarded. In both lots, one group of ten transplants was left as an uninoculated control. The height growth of these transplants was measured each day in order to be able to determine at which phase of growth the inoculation had been made. When the transplants were potted, they had already completed about half of their final height growth.

Trapping of spores and handling of material

In order to monitor spore dispersal, two instruments were used: 1) a pollen sampler developed by Sarvas and Wilska (Sarvas 1952, 1962) and 2) a modified Hirst spore trap (Hirst 1952) manufactured by Burkard Manufacturing Company.

The results obtained by the pollen sampler of Sarvas—Wilska indicated the number of spores trapped per unit of time on a tape surface. The trapping surface was cellophane tape covered with vaseline and wrapped around the clock cylinder of a thermograph. The periphery of the cylinder moved 2 mm/hour. The device was equipped with ball bearings and a wing in such a manner that the opening of the cover was always toward the wind. The opening was conical, tapering off toward the inside. At the point nearest the trapping surface, the opening was 2 mm wide. The amount of air moving through the device was dependent on wind velocity. For each period of time the number of spores was counted on 23 microscopic fields corresponding to a total of 4.5 sq.mm. The vaseline-covered ribbons were examined intact directly under a microscope using phase contrast.

The results obtained by the Hirst spore trap indicate the number of spores per unit of air volume passing through the instrument. Using an adjustable air pump the suction was standardized to 10 litres/hour. Due to variation in temperature and air humidity, the suction of the pump varied. Therefore, the pump had to be adjusted at least once in two days. The instrument had ball bearings so that it turned toward the wind. A tape wrapped around the clock cylinder was covered with vaseline and moved 2 mm/hour. The tape was changed every 6 days. For microscopic examination, the tape was cut into sections corresponding to each day. Each section was mounted in polyvinyl alcohol. The mounting medium used was: 35 g polyvinyl alcohol grade 40—20, 100 ml distilled water, 50 ml glycerol or 40 ml lactic acid, and 2 g phenol. Slide preparation was based on the method presented by Downs (1943). The microscopic examination was made with a × 300 magnification. The numbers of basidiospores, aeciospores and uredospores of *Melampsora* trapped on the tapes were counted. Different species of *Melampsora* were not identified since it is completely impossible for basidiospores and also extremely uncertain when single aecio- or uredospores are examined (cf. p. 49).

The observations on spore dispersal commenced in Loc. 1 at the beginning of June when the growth of the pine shoots had just started and the shoot was still covered by the bud scales. In Loc. 3 and 4, the observations commenced at the beginning of May. The dispersal of spores was related to the climate and the variation in weather factors at the time of spore trapping and to the occurrence of uredia on the aspen leaves.

By using multiple regression analysis, the relationship between some weather factors and the daily numbers of uredospores was investigated. The mathematical data processing was limited to uredospores since their dispersal occurs over a much longer time than that of aeciospores and basidiospores. The following variables were used:

x_1 = number of days since the first uredia were observed in the field,
x_2 = dependent variable, daily mean number of uredospores in the air,
x_3 = number of uredia per day on a unit area of aspen leaves calculated by interpolation of the number of uredia per week,
x_4 = average daily temperature,
x_5 = average daily temperature for the coldest period of the day (21.00—08.00 hours) calculated as the average of six temperature readings taken on odd-numbered hours,
x_6 = average daily temperature for the warmest period of the day (09.00—20.00 hours) calculated as above,
x_7 = average daily wind velocity,
$x_8 = x_3/\log_{10} x_1$,
$x_9 = x_3/x_1$,
$x_{10} = x_3/(x_1)^2$.

The calculations were made according to the stepwise regression analysis by Väliaho (1969). By means of the first and third variable and variables 8—10 formed from the former variables, an attempt was made to remove from the model the effect of the changing number of spores due to the increase in uredia. In addition to the daily mean temperature, the average temperatures of the coldest and warmest periods of the day were also included as variables since it is possible that cold nights may in some way affect the development of the fungus, and since most of the spores are released during the warmest period of the day (cf. p. 28). Wind velocity was included especially because the general effect of wind was of interest and since the number of spores trapped in the two types of traps (Sarvas-Wilska and Hirst) may be dependent on wind velocity.

Investigations on the aecial and uredial states of *Melampsora*

In connection with the daily height measurements of the pines, the number of aecia was also counted. Both sporulating aecia and mere yellow spots were counted.

The number of uredia was determined from pressed samples of aspen leaves. The samples were collected at one-week intervals after the first uredia had been observed and continued until the last week of August. At each collection, three branches were taken from each group of aspen suckers marked for growth measurement. Each branch had to bear at least 10 leaves. The number of uredia was estimated on these 30 leaves. Using a binocular stereomicroscope, the uredia on each of three viewing fields on each leaf were counted (cf. Kurkela 1969).

Several investigators have tried to estimate the amount of rust on various species of poplars when assessing their rust resistance (e.g. Schreiner 1959, van der Meiden 1961, Donaubauer 1963). In the paper by Donaubauer (1963) there is also an assessment of the value of previously developed methods. The method used in the present study is surely one of the most elaborate ones for estimating the amount of rust. This method was used since the particular purpose here was to study the development of the pathogen during the growing period, not its effect on the host plant.

In order to determine how far the mycelium spreads in the tissue of the host leaf, the morphology of the diseased leaves was studied. Leaves containing uredia were fixed with a FAA-solution, embedded in paraffin and cut on a microtome. The sections were stained with the method developed by Vagas and Maacz (1960). The size of the infected tissue around the uredia and the distance between the vascular bundles were measured.

RESULTS

Dispersal of basidiospores

The number of airborne basidiospores varied considerably during different years in the Locations 1, 3, and 4. Large numbers of basidiospores were found in the air on rainy days only. The spores were observed in the air 4—6 hours after the beginning of a rain.

In June of 1966, the most important time of spore dispersal and when the pine shoot growth was most intensive, precipitation did not occur in large enough quantities to be registered by the fluviograph (Figure 2). It is possible, of course, that some of the observed maxima of basidiospore dispersal in 1966 occurred due to some local thunder showers. The numbers of basidiospores were quite small, and therefore the results are very unreliable. In the summer of 1966 the same investigations were also carried out in Laihia (Loc. 2), but microscopic studies of the material collected there were not undertaken since no basidiospores were found upon initial examination of the tapes. This lack of basidiospores was apparently due to the fact that only one very small shower of rain occurred during the time of investigation. The shower was not sufficient to cause the telia to germinate and form basidiospores.

The conditions of precipitation in June 1967 were favorable for the formation and dispersal of basidiospores. The number of airborne basidiospores was not very large, however, apparently due to the low air temperature during the periods of rainfall (Figure 3). The average daily temperature during this time was less than 10 °C. The largest number of basidiospores, about 250 spores/sq.cm, was found on June 6.

The crucial importance of rainfall for the dispersal of basidiospores is evident from results obtained in 1968 in Loc. 1 and in 1971 and 1973 in Loc. 3 and 4. The precipitation during May and June of these summers occurred intermittently with very dry days between. The most important maxima of basidiospores occurred during rainy days (Figures 4 and 6). In 1968 the maxima of basidiospores were during June 9 and 24—26. On June 9 the precipitation was 5 mm; on June 24 rain could not be registered, but the day was partly foggy with drizzling rains; and during the two following days, precipitation was several mm. June 27 was rainless, while the precipitation was great on June 29 and when the capacity of the telia to produce basidiospores apparently was exhausted since, during this time, only about ten basidiospores per cu.m. of air could be counted in spite of the very favorable weather. During the previous rains, the number of spores had been about 1 000/cu.m. The hourly maximum amounts were, of course, much higher (Figure 7), about 3 000 spores/cu.m.

In Loc. 3 and 4 during 1970, 1971 and 1973 the species of *Melampsora* rust investigated was apparently *M. larici-tremulae*. In 1970 basidiospores were observed immediately when the investigations began on May 15 (Figure 5). The whole latter part of May was rainy, and therefore no conclusions can be made about the importance of rainfall for formation and dispersal of basidiospores. After June 10 no more basidiospores could be observed in spite of the rains which occurred after that date. From the results obtained during 1971 and 1973 the crucial importance of rain was quite obvious. During both these years, three distinct maxima of spore dispersal were observed (Figure 6). In the intervals between maxima, very few spores were found. Each maximum dispersal occurred during periods of rainfall. After the third maximum, spores were not detected or else the number was small in spite of favorable rainy weather.

The investigations of 1971 and 1973 were undertaken with considerably more accurate instruments for measurement of wind, rainfall and dew. Consequently, the results obtained from the spore traps could be analyzed in more detail than previously. Figures 8—10 show the hourly numbers of basidiospores during periods of maximum spore dispersal in the summer of 1971. These hourly spore quantities can be related to the amount and duration of rainfall, to dew (i.e. occurrence of free water), and relative humidity. The first maximum of basidiospores occurred on May 22 and 23. At this time spores were airborne some 6 hours after the rainfall began; and the maximum number, about 300—400 spores/cu.m, was reached after 12 hours. After the maximum, the number of spores decreased slowly. Spores were airborne for a total of 1.5 days. Rain or fog persisted continuously for 31 hours. A second maximum of basidiospores occurred on May 28. During the previous morning there was a light rain for about five hours, registered only by the dew scale. As a result of this rain, spores could again be detected about 6 hours after the rain had begun. On the morning of May 28 about 2 mm of rain fell during four hours. Between these two days there was some water in the dew scale continuously and thus the aspen leaves on the ground with their telia did not have any chance to dry out during this time. About four hours after the second rain, large numbers of spores were dispersed into the air. The maximum spore density, 1 300 spores/cu.m, was observed seven hours after the rain began. The spore density decreased rapidly after the maximum, due to rapid drying of the air and ground.

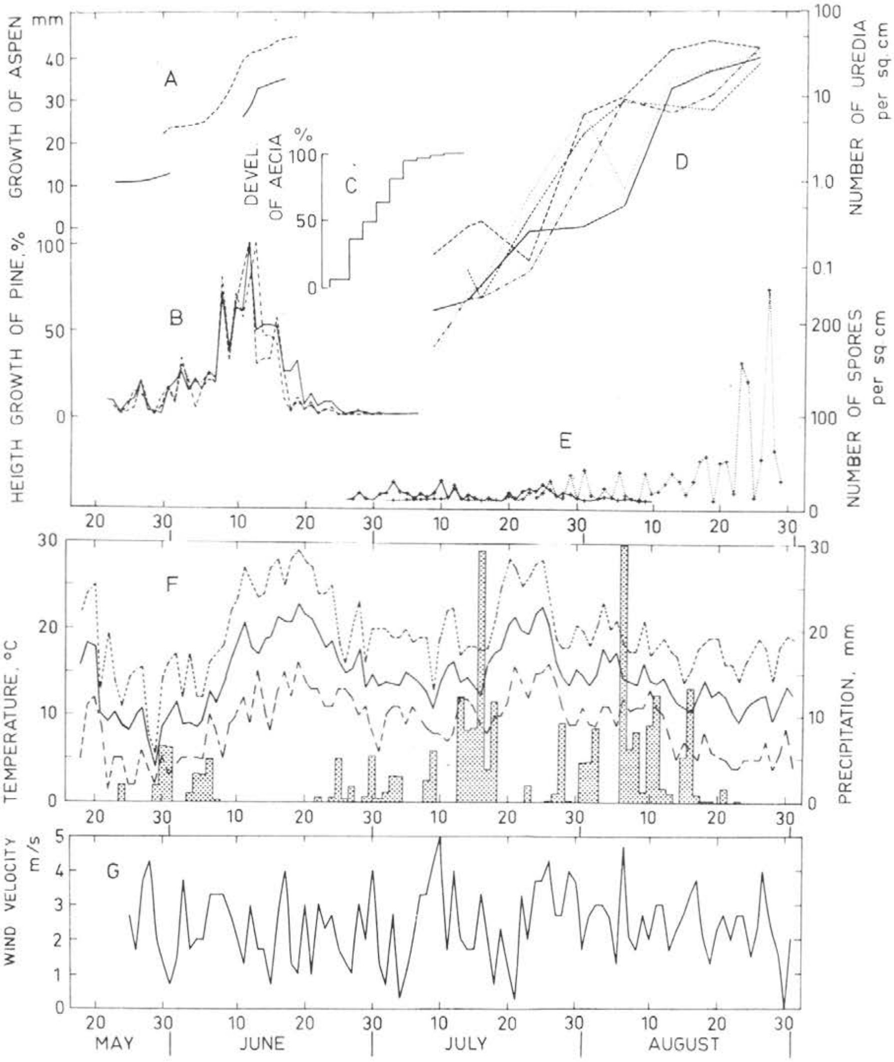

Figure 2. Seasonal development of rust (*Melampsora pinitorqua*) and host trees in relation to some weather factors in Loc. 1 in 1966. A) Cumulative growth (mm) of aspen; lower portion of the solid line indicates the growth of the bud and the upper part, the growth of the short shoot; the dotted line indicates the leaf growth (length of leaf as measured along the central vein). B) The daily height growth of pine as a percentage of total length, the solid line indicates growth of the terminal leader, and the dotted line, growth of the terminals of the previous year's branches. C) Number of mature aecia as a percentage of all aecia. D) Weekly number of uredia per sq.cm leaf surface in five groups of aspen suckers. E) Number of spores per sq.cm. trapped in the spore trap (Sarvas—Wilska), solid line = aeciospores, dotted line = uredospores. F) Daily maximum, average and minimum temperature (°C) and amount of precipitation (mm). G) Wind velocity (m/s), average of observations made at 08.00, 14.00, and 20.00.

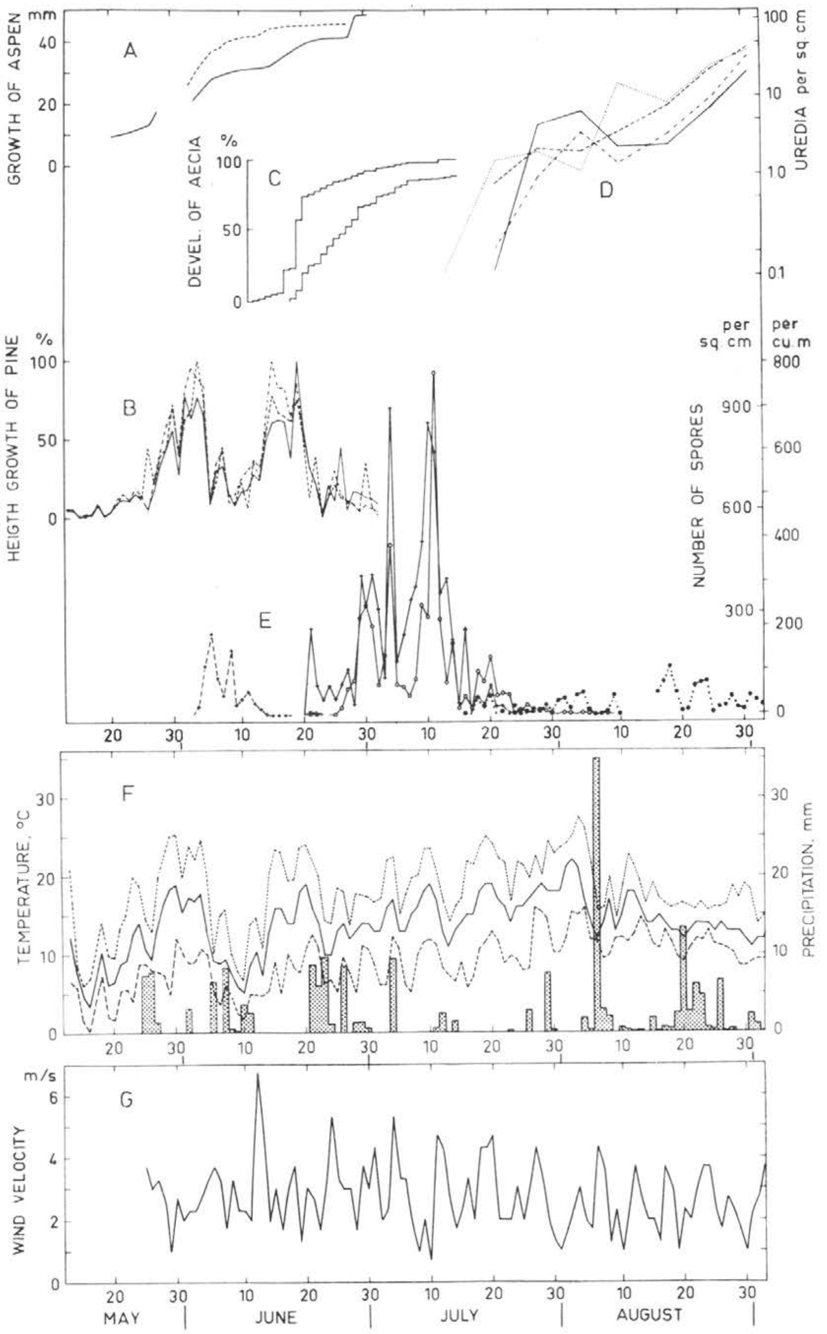

Figure 3. Seasonal development of rust *(Melampsora pinitorqua)* and host trees, Scots pine *(Pinus sylvestris)* and aspen *(Populus tremula)*, in relation to some weather factors in Loc. 1, 1967. A—B) See Figure 2. C) Development of aecia, upper histogram = number of yellow spots, lower histogram = number of mature aecia. D) Weekly number of uredia per sq. cm leaf surface in four groups of aspen suckers. E) Daily numbers of spores, broken line = basidiospores, solid line = aeciospores, dotted line = uredospores. Lines marked with stars indicate results obtained

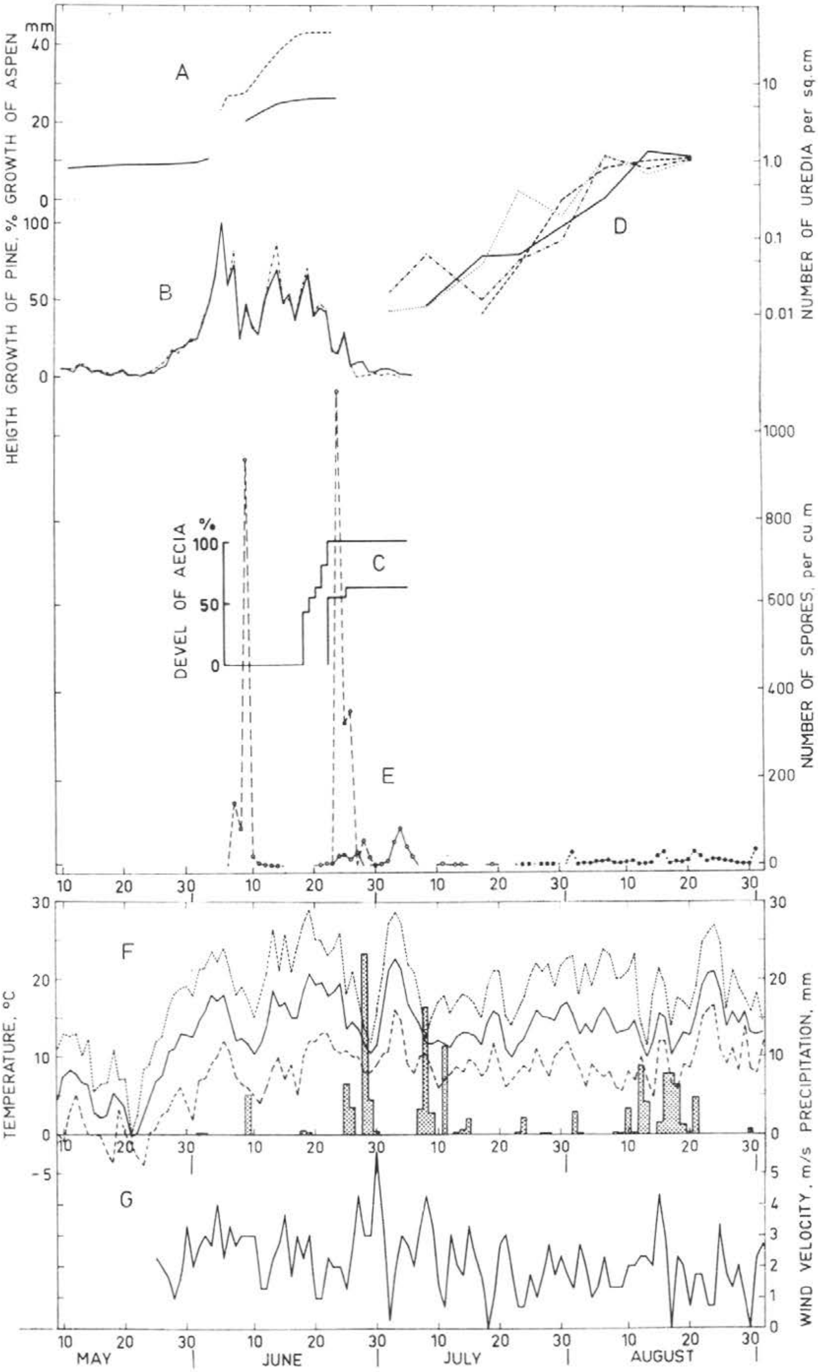

Figure 4. Seasonal development of rust and host trees in relation to some weather factors, Loc. 1, 1968. A—G) As in Figure 3.

with the Sarvas—Wilska pollen trap while lines marked with circles indicate results obtained with the Hirst spore trap. F and G) As in Figure 2.

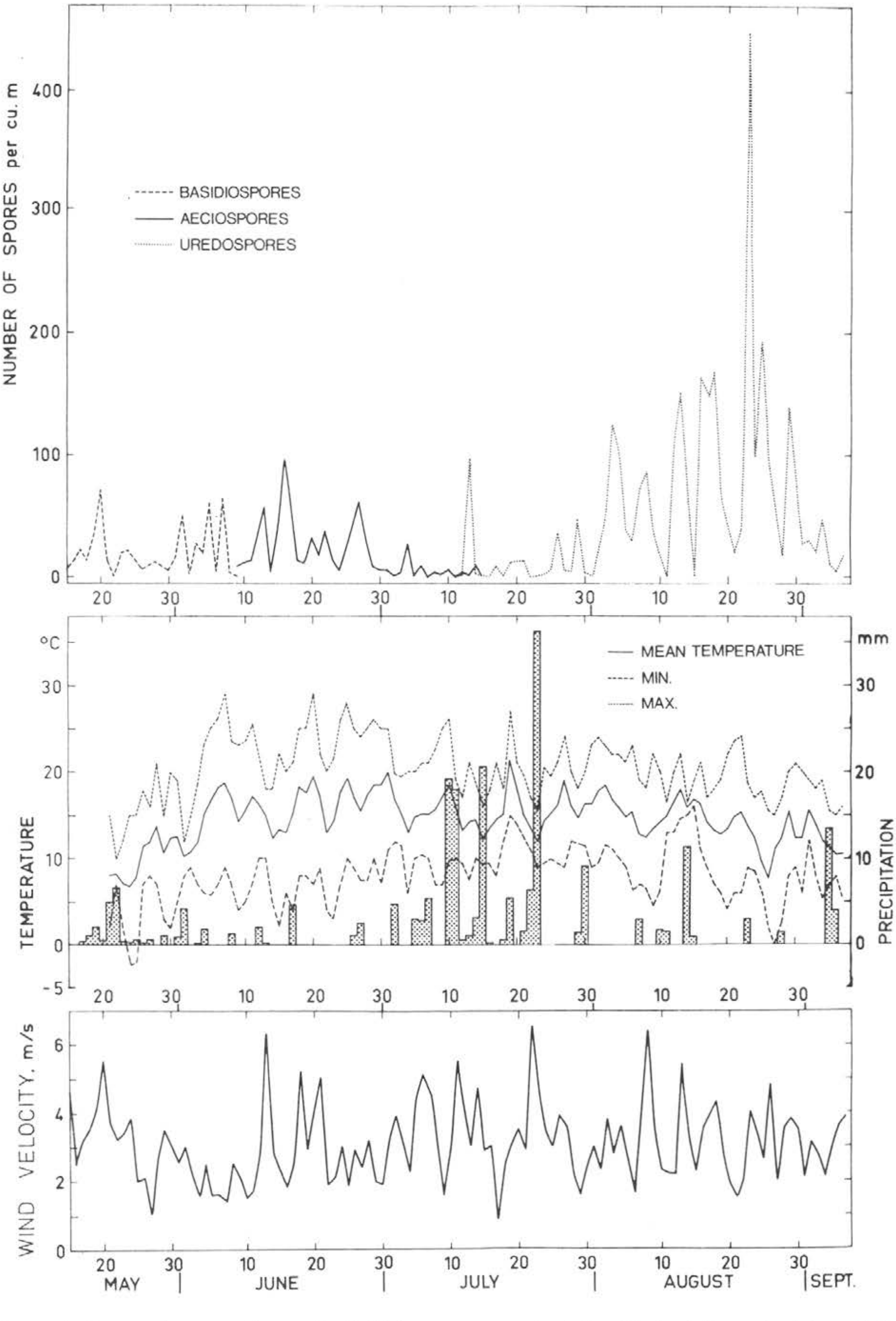

Figure 5. Seasonal spore dispersal of *Melampsora* sp. (mainly *M. larici-tremulae*) in relation to some weather factors in Loc. 3, 1970.

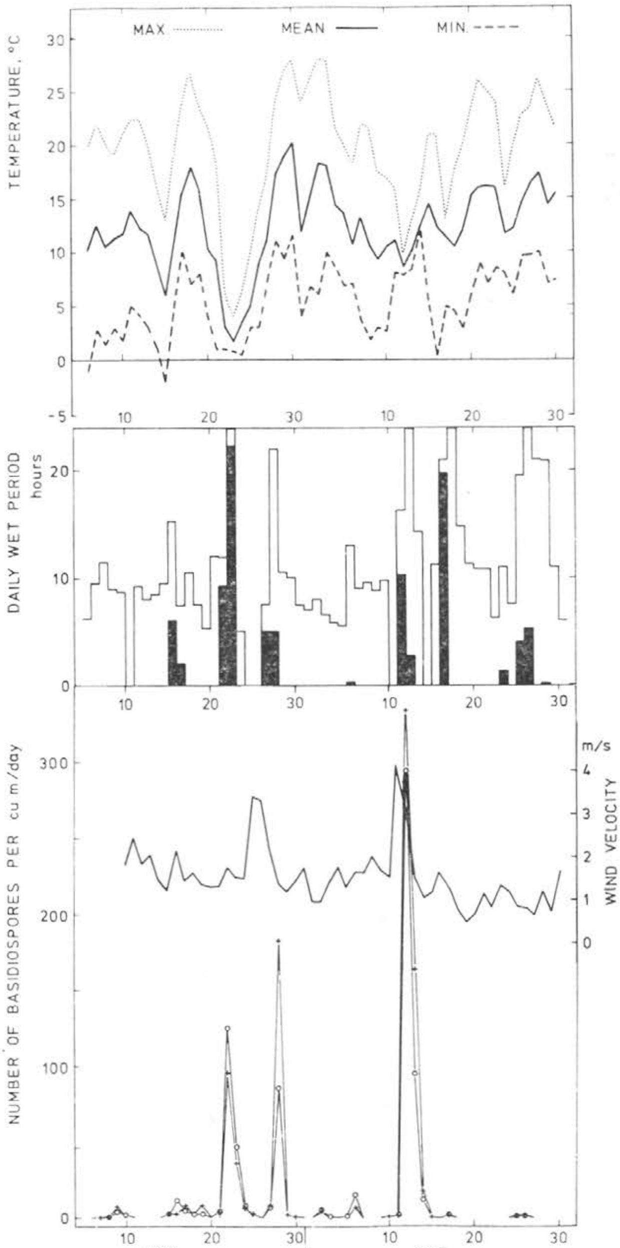

Figure 6. Dispersal of basidiospores of *Melampsora* sp. in relation to some weather factors in Loc. 3, 1971. The spore data were obtained simultaneously with two traps. In the histogram indicating length of daily wet period, the black part indicates duration of rain and the white part, the sum of duration of rain and dew in hours.

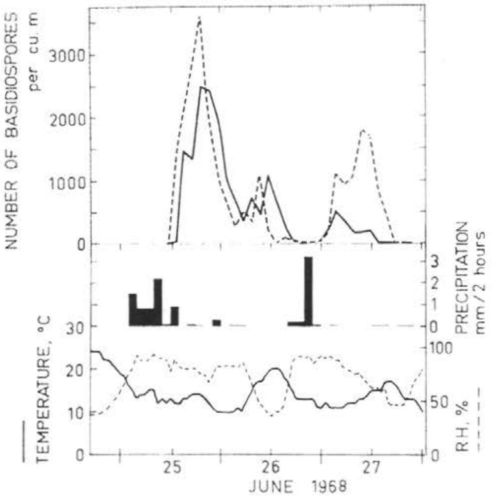

Figure 7. Basidiospore dispersal of *Melampsora pinitorqua* in relation to precipitation, temperature, and relative humidity in Loc. 1, June 25—27, 1968.

The third spore dispersal maximum occurred on June 12 and 13. The first spores were trapped after about 3 hours; after 6 hours the spore density rapidly increased and reached the maximum, ca. 450 spores/cu.m in one trap and over 800 spores/cu.m in the other trap, some 12 hours after the rain began. The almost continuous rain lasted for 41 hours, and considerable numbers of spores occurred in the air for almost two days.

During these three periods of maximum spore occurrence, the capacity of telia to produce spores seemed to be completely exhausted since, after the heavy rain of June 18, only a few spores were trapped.

Results obtained in the spring of 1973 support the conclusions, based on previous years' results, about the most important factors influencing formation and dispersal of basidiospores (Figure 11). In spite of the early spring, the temperatures before the beginning of May were not high enough for formation of spores. During the first days of May, the days became warmer, and after the rain on May 6, the first basidiospores were observed. The second maximum occurred as a result of the rain on May 14. After this, it rained for several consecutive days, and spores seemed to occur less frequently. Apparently, the capacity of telia to produce spores had already decreased considerably. On May 17 a clear maximum of spores was observed, although it was much smaller than the previous ones. The spore maxima on May 6 and 14 were observed 13 and 10 hours after the rains began. During these maxima, the air temperature fluctuated between 5 and 10 degrees.

The presence of water or abundant moisture is crucial for the formation of basidiospores; but, on the other hand, it may be possible that rain inhibits the release of the spores or washes them directly to the ground. In any case,

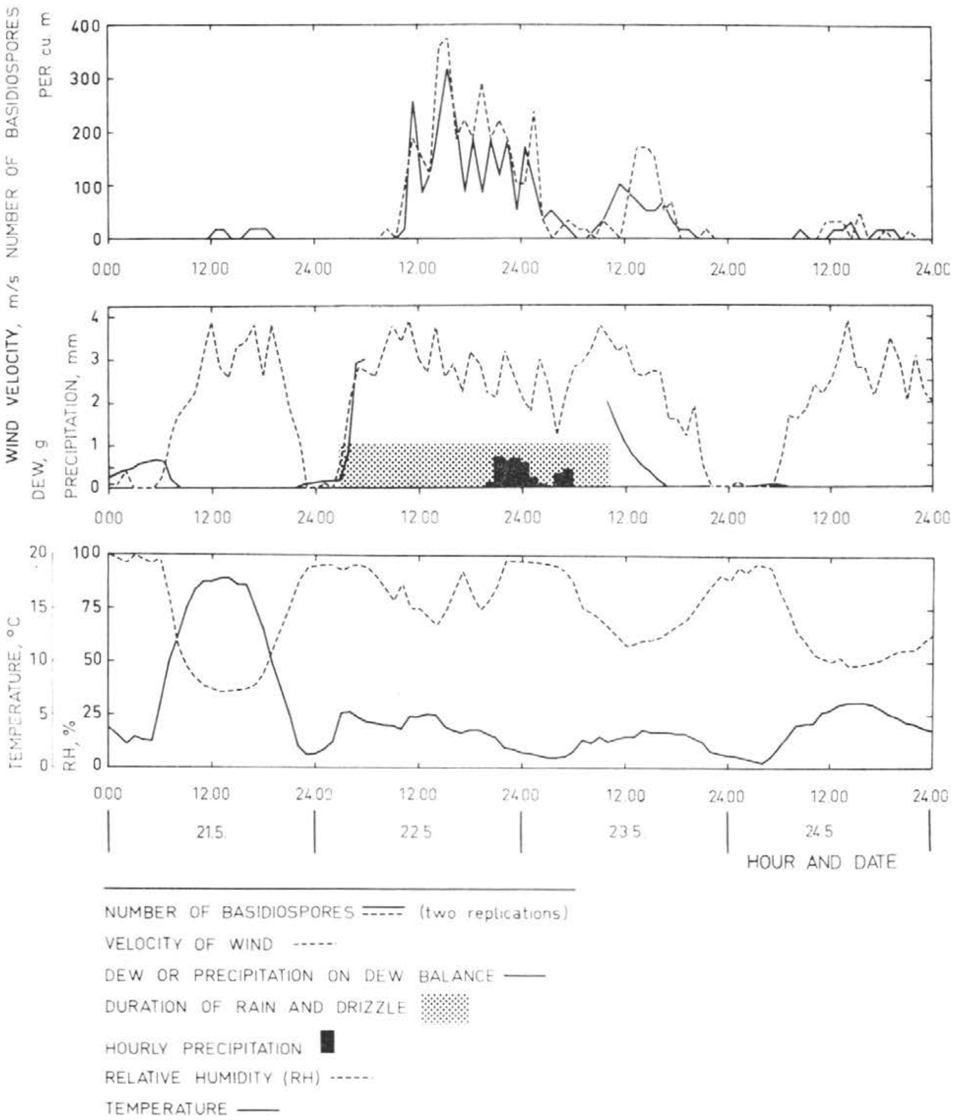

Figure 8. Hourly dispersal of basidiospores (*Meampsora* sp.) in relation to various weather factors in Loc. 3, May 21—24, 1971. The number of basidiospores has been indicated for two replications.

greater numbers of spores have occurred in the air only after rains. The changes in air humidity may have promoted release of the basidiospores.

When the release of spores was at a maximum, the relative humidity of the air was rather high due to the rainy weather. The relative humidity may have varied considerably (100—90 %), however, without appreciably affecting spore density. Apparently, relative humidity, as measured at 2 meters above the ground in a weather chamber, is not a suitable variable

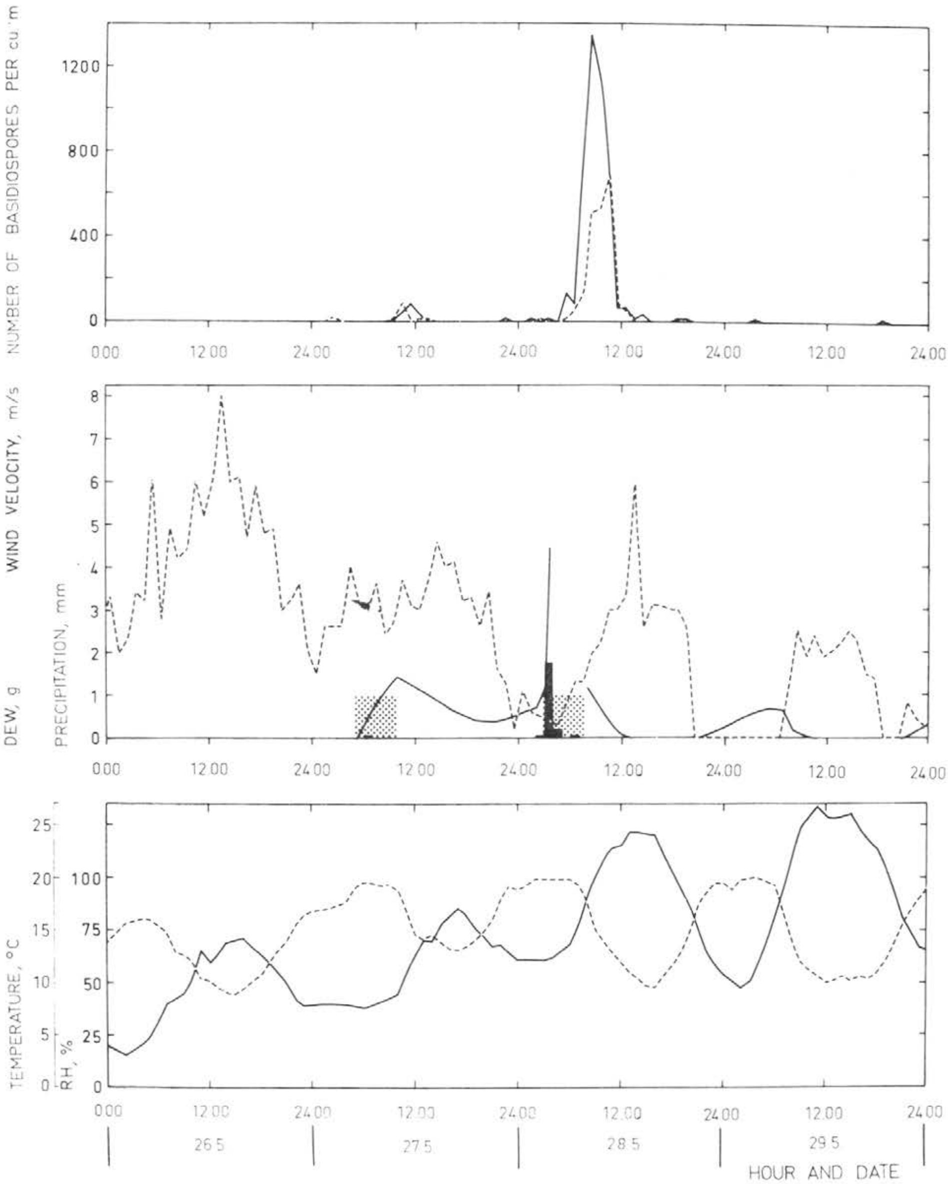

Figure 9. Hourly dispersal of basidiospores (*Melampsora* sp.) in relation to some weather factors in Loc. 3, May 26—29, 1971. For legend see Figure 7.

for explaining formation and release of basidiospores in the ground litter. The same appears to be true for temperature. Although experiments in the laboratory clearly indicated an increase in the rate of spore release (Kurkela 1973 a), the same effect of increased temperature could not be observed in the field. In the field considerable numbers of spores could be released even at very low temperatures (less than +5 °C). This was not ex-

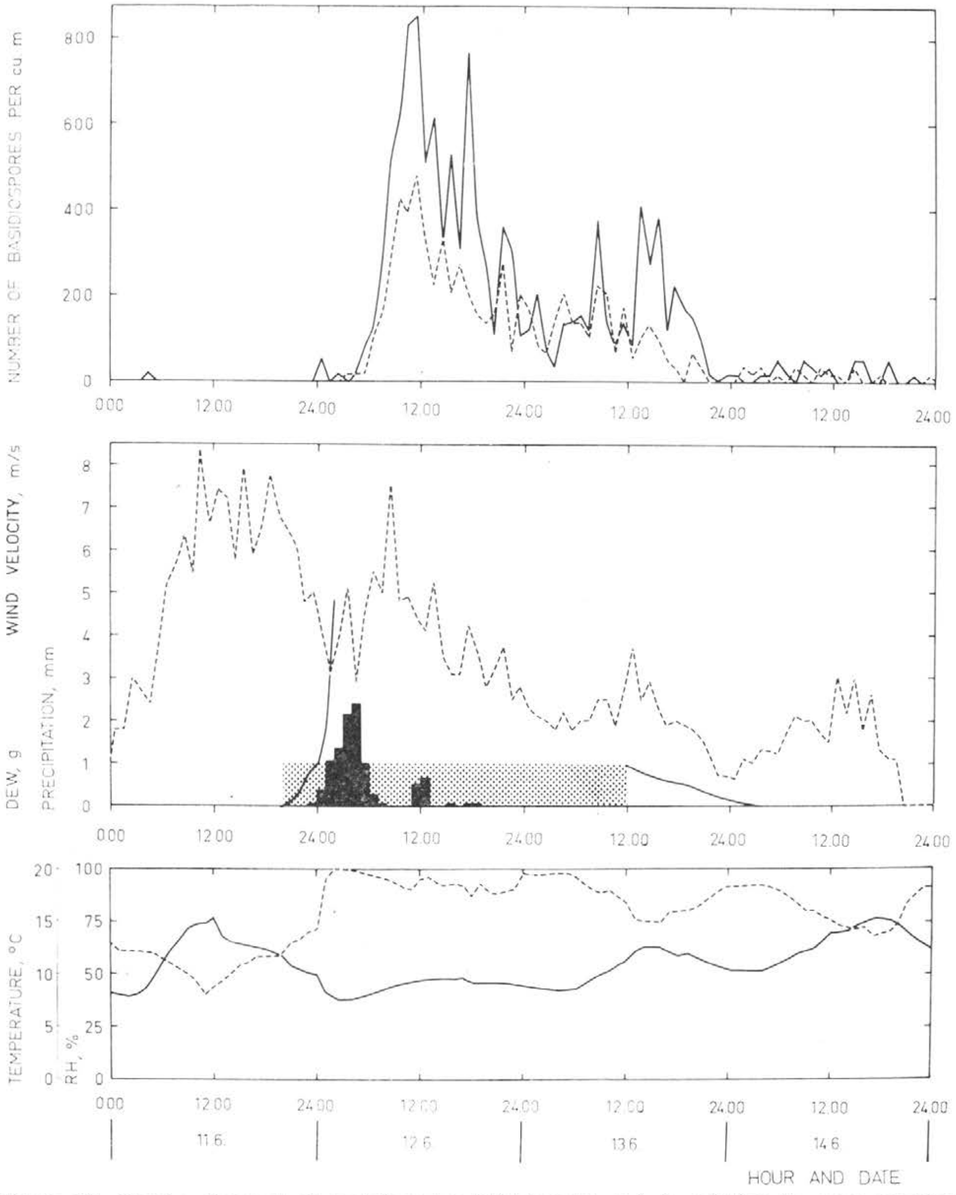

Figure 10. Hourly dispersal of basidiospores (*Melampsora* sp.) in relation to some weather factors in Loc. 3, June 11—14, 1971. For legend see Figure 7.

pected, judging from the results obtained in laboratory experiments. The two results need not be contradictory since the temperature on the ground litter may, sometimes after rains, have been higher than the temperature measured in the weather chamber.

The effect of wind velocity on spore release was even less definite than that of temperature.

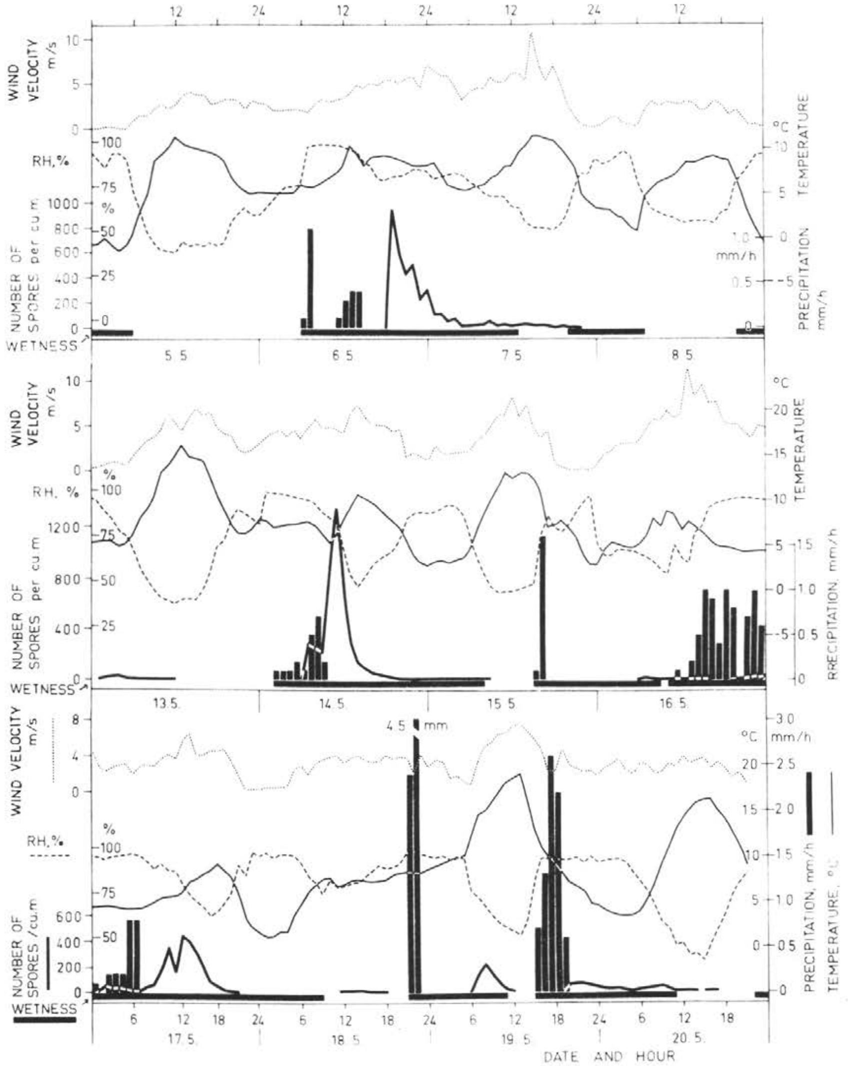

Figure 11. Basidiospore density (spores/cu.m of air) of aspen rust (*Melampsora* sp.), wind velocity (m/s), temperature measured in a weather chamber (°C), relative humidity (RH, %), precipitation (mm/h), and length of wet period caused by rain and dew (h, thick black horizontal line). In Loc. 4 during three four-day periods in May 1973.

Dispersal of aeciospores and uredospores

The number of aeciospores dispersed in the air depended primarily on the extent to which the basidiospores had been able to infect pine shoots or larch needles and, furthermore, on how much the aecia developed after infection. The variation in the numbers of aeciospores from year to year may be regarded as depended primarily on these two factors. Observations on the shoot and needle development of aecial hosts of *Melampsora* in Loc. 1 showed that larch needles and pine shoots reached a susceptible stage about 15th of May and during the first half of June respectively. Aeciospore dispersal started during the second half of June after the first aecia had developed on pine shoots and lasted 20—30 days.

Inspection of the average daily number of aeciospores (Fig. 2—5) shows, especially in the results of 1967, that during high temperatures the numbers of aeciospores have been greatest. Sudden showers can also rapidly increase the number of spores in the air, as happened on July 4 and 11, 1967 (Fig. 12). Since the thundershowers often were preceded by strong gusts of wind, it was usually impossible to separate the effects of wind and rain. The relative humidity of the air was not included as a variable in the graphs (Fig. 2—5) describing the dispersal of spores and development of the disease during the growing season. If the air humidity in general has any influence on release and dispersal of aeciospores of *Melampsora*, then the correlation is clearly negative. The number of spores fluctuated during different periods of the day (Fig. 13). The spore density of pine twisting rust was at a minimum in the middle of the night, during which time there usually is dew and the

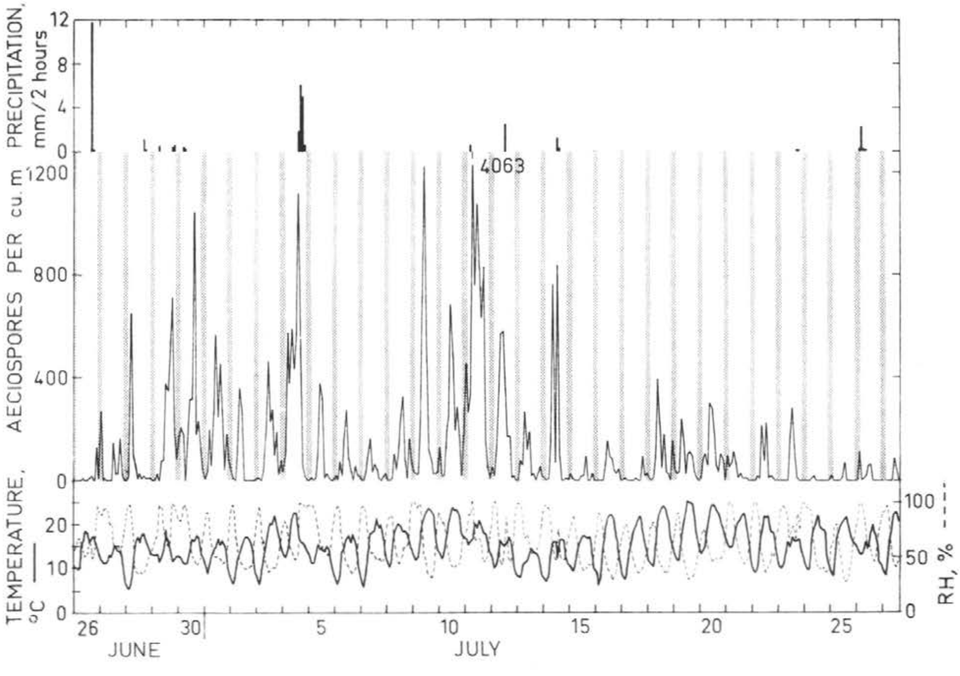

Figure 12. Dispersal of aeciospores of *M. pinitorqua* in the summer of 1967 in Loc. 1 in relation to precipitation, temperature and relative humidity of the air. The shaded areas indicate the time between sunset and sunrise.

air humidity is at a maximum. The number of spores began to increase about 06.00 when the dew started to dry and reached a maximum between 09.00 and 11.00 when the normal dew had completely evaporated. In Tuusula (Loc. 3) the average maximum spore density of the larch rust did not occur until late afternoon at 17.00. The difference between this result and that of Ikaalinen (Loc. 1) may be due to the limited data from Loc. 3, but may also be due to local conditions. In Loc. 1 the aecia of pine twisting rust were located in the tops of 0.5—1.5 m high saplings and thus were exposed immediately to insolation and wind, which dryed the dew rapidly. By contrast, the aecia of the larch rust in Loc. 3 were located on the lower branches of some 6 m high larches which were not reached by continuous insolation.

Dispersal of uredospores commenced in Loc. 1 at the beginning of July and in Loc. 3 at the end of June. The uredospores, similar in this respect to the aeciospores, seem to disperse when dry. Their maximum spore density occurred between 13.00 and 15.00 (Fig. 13). The average maximum of spore density for the whole growing season occurred at 13.00 during 1966 and 1968. On the data from 1967 the variation in spore density was calculated separately for July, August, and September. This was done in order to find out whether the gradual change in various weather components would be accompanied by corresponding changes in the number of uredospores as fall approached. No significant differences between various months were observed. The maximum average spore density for each of the three months occurred at 15.00. The spores were counted at every odd-numbered hour. In Loc. 3 in 1970 the maximum spore density occurred before noon, at 11.00. The minimum density occurred between 24.00 and 06.00. Deviations from the regular pattern of variation were caused by showers accompanied by gusts of wind.

The fact that the daily maximum during the whole dispersal season in Loc. 3 in 1970 occurred at 11.00 is thought to be due to unusually gusty winds and rain showers. On August 23 an exceptionally high spore density occurred. It can be explained only by the coincidence of a very strong wind and a rain shower. The rain, however, could not be registered on the fluviometer. On this day the spore density exceeded 6 000 spores/cu.m/h (Fig. 14) compared to the general daily maximum which was seldom more than 1 000 spores/cu.m/h.

Since the diurnal periodicity of dispersal for both aecio- and uredospores was very obvious, it was rather difficult to distinguish the effects of periodic weather factors on numbers of spores in the air. When the effects of various weather components are examined within a 24 hour period, the air temperature, wind and insolation, which was not measured in this connection, appear to be positively correlated, while relative humidity of the air and dew seem

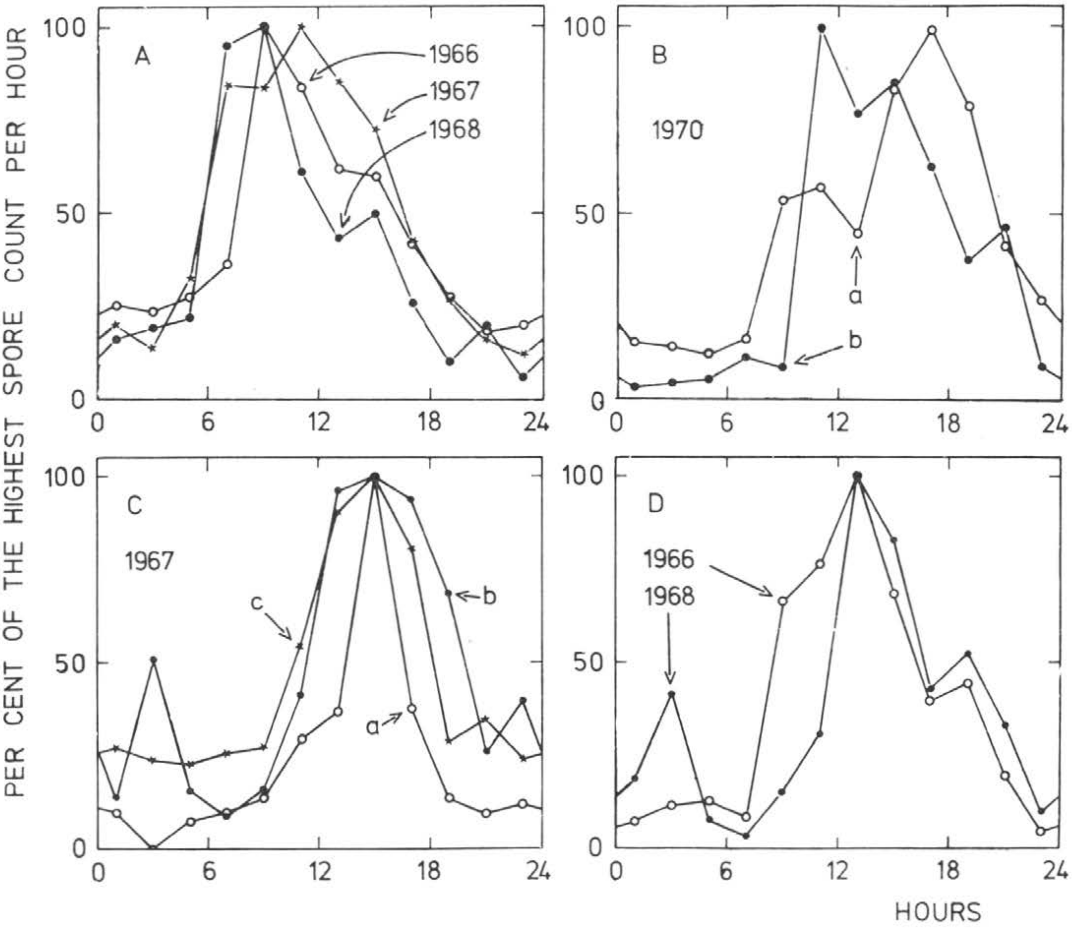

Figure 13. Average daily variation in number of *Melampsora* sp. aecio- and uredospores as a percentage of the highest total count for an hour. A) Diurnal periodicity of aeciospores *(M. pinitorqua)* dispersal in Loc. 1 in 1966—1968. B) Diurnal periodicity of spores *(Melampsora* sp.) in Loc. 3 in 1970, a = aeciospores, b = uredospores. C) Diurnal periodicity of uredospores *(M. pinitorqua)* in Loc. 1 in the summer of 1967, a = July, b = August, c = September. D) Daily average periodicity of uredospores of *M. pinitorqua* in Loc. 1 in 1966 and 1968.

to be negatively correlated with the density of aecio- and uredospores in the air.

Table 1 presents the correlation matrix for the variables used in the regression analysis (except for variable x_{10} which was not statistically significant in any of the models tested). The spore density is most strongly correlated with the number of uredia. This, however, is true only for the data of 1966. For temperature the only significant correlation with the daily spore density was obtained in 1968. In this year the density was positively correlated with the average temperature for the coldest period of the day. To some

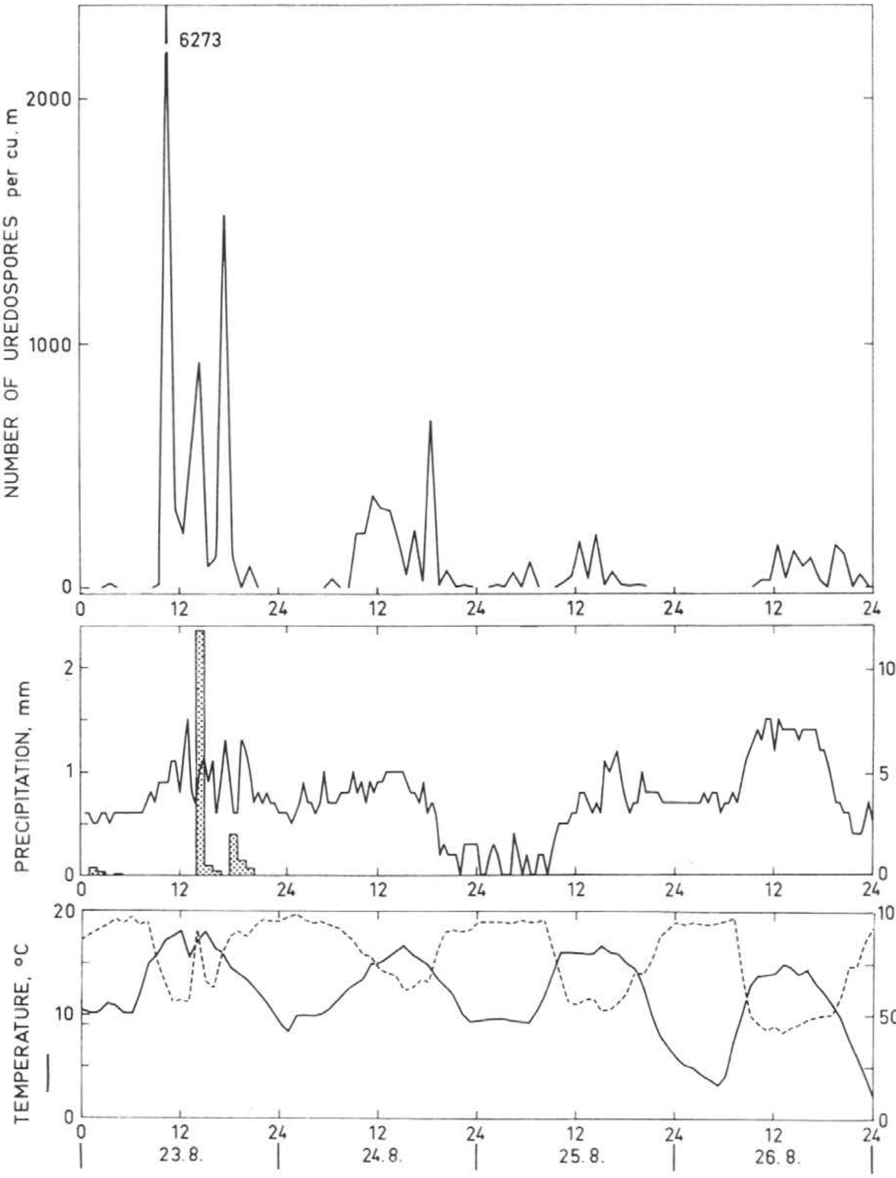

Figure 14. Dispersal of uredospores in relation to rain, wind velocity, air temperature, and relative humidity in Loc. 3, August 23—26, 1970.

extent the density and the temperature appear to be negatively correlated, which simply is due to the fact that, along with the colder weather as fall approached, the number of uredia and uredospores increased logarithmically. A statistically significant correlation between wind and spore density was obtained only in 1968. In addition, the matrix shows the low correlations

Table 1. Correlation coefficients between the number of uredospores trapped, the number of uredia, and some weather factors (for explanations, see pp. 12—13).

		x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8
	1966	0.546							
x_2	1967	0.424							
	1968	0.317							
	1966	0.918	0.560						
x_3	1967	0.841	0.249						
	1968	0.952	0.296						
	1966	—0.336	—0.161	—0.523					
x_4	1967	—0.499	0.041	—0.447					
	1968	—0.429	0.293	—0.444					
	1966	—0.283	—0.240	—0.498	0.795				
x_5	1967	—0.254	0.201	—0.363	0.786				
	1968	—0.171	0.416	—0.280	0.600				
	1966	—0.313	—0.111	—0.451	0.957	0.613			
x_6	1967	—0.631	—0.069	—0.466	0.942	0.575			
	1968	—0.436	0.180	—0.370	0.912	0.269			
	1966	—0.060	0.116	—0.051	—0.124	—0.140	—0.084		
x_7	1967	—0.234	0.130	—0.231	0.126	0.138	0.096		
	1968	0.145	0.375	0.111	0.258	0.127	0.215		
	1966	0.931	0.566	0.999	—0.521	—0.490	—0.454	—0.041	
x_8	1967	0.849	0.253	0.999	—0.440	—0.352	—0.467	—0.239	
	1968	0.937	0.295	0.999	—0.438	—0.291	—0.351	0.111	
	1966	0.954	0.578	0.985	—0.521	—0.470	—0.465	0.001	0.970
x_9	1967	0.855	0.249	0.987	—0.405	—0.304	—0.451	—0.270	0.991
	1968	0.871	0.284	0.976	—0.409	—0.323	—0.284	0.104	0.986

1966 number of observations 41
1967 number of observations 43
1968 number of observations 29

between the temperature at the coldest period of the day and the other temperature variables. This is due to the fact that the average temperature for the coldest part of the day includes temperature readings before midnight of one day and after midnight (i.e. early morning) of next day.

In the regression analysis, data for each year were analyzed separately. The strongest correlations between spore density (x_2) with other variables were obtained with the following equations:

$$1966:\ y = A + Bx_6 + Cx_7 + D^{***}x_9, \qquad r = 0.58,\ \text{d.f.} = 37$$
$$1967:\ y = A^{**} + B^{**}x_5 + Cx_7 + D^{**}x_8, \qquad r = 0.60,\ \text{d.f.} = 25$$
$$1968:\ y = A^{*} + B^{***}x_1 + C^{*}x_6 + Dx_7 + Ex_9, \quad r = 0.55,\ \text{d.f.} = 33$$

*** = statistically significant, at the 0.1 % level of probability.
** = significant, at the 1 % level of probability.
* = significant, at the 5 % level of probability.

As expected, the variables x_8 and x_9, derived from the number of uredia, best explained the variation in number of spores. In 1966 and 1968 the variables x_9 and x_8, respectively, were statistically significant. In 1967 the variables derived from the number of uredia were not significant in the regression equations but the variable x_1, the number of days from the first occurrence of uredia, was very significant.

Relatively little can be explained by the temperature variables. During all years the average daily temperature explained less than the average temperature of the coldest or warmest period of the day (x_5 and x_6). In 1967 the factor of variable x_6, and in 1968 the factor of variable x_5 had some significance. When the regression analysis was made for each month, then the significance of variable x_6 was increased in the equation for 1967. This factor became very significant for July—August, and the correlation coefficient for the whole model was 0.70 (d.f. = 22). In July and early August of 1967 the daily average and maximum temperatures were considerably higher than in 1968 (Figures 3 and 4). The high temperatures seem to have increased the formation and release of uredospores. On the other hand, it is possible that the colder nights of 1968, compared to the previous year, would have been a factor limiting the formation of spores. This explanation also has its weakness in that during 1966 the night temperatures at the end of August were even lower, but the number of airborne uredospores reached its maximum (Fig. 2).

At the end of August in 1968 the weather was very warm and no rain occurred. During this time none of the factors for any of the variables reached high statistical significance. The factor of the wind variable provides the best explanation. It was almost significant, and the correlation coefficient of the whole regression equation was 0.73 (d.f. = 10). The data for these 14 days contained, however, only 1 143 uredospores. An analysis of the total data for the different years did not indicate that the effect of wind on numbers of spores would have been statistically significant. It is understandable that the wind had a low correlation. The wind hardly ever (with, perhaps, the exception of the end of August, 1968) was so low that it would have limited the release and dispersal of the spores. On the other hand, the sudden gusts of wind, which were able to release large numbers of spores, usually occurred in connection with thundershowers. Thus the effect of wind was difficult to determine. The wind velocity used in the calculations was the average of the observations per day, which already by definition excludes the possibility of detecting the effect of gusts of wind in the analysis.

The temperature, in a similar situation to that of the wind, was closely related to the rains. As a result of a rain, the temperature usually dropped, and on the other hand, prior to thundershowers, it was usually very warm. The hourly maximum spore densities usually occurred when there first had been warm and sunny weather followed by sudden thundershowers in connection with strong gusts of wind. Long periods of rain or rain during several consecutive days usually caused a definite drop in the numbers of aecio- and uredospores. This drop may also have been partly due to a drop in the temperature caused by the rain. In order to consider the effect and variation of these short-duration weather components in the regression analysis, the analysis should be based on hourly observations, and the mode of action of each factor should be known in detail.

Twisting rust on pine

Seasonal variation of rust incidence

Tables 2 and 3 indicate the variation in amount of twisting rust in Loc. 1 during 1965—1968. The summer of 1965 was, compared to the previous growing season, more favorable for development of the pine saplings. Aecia of twisting rust occurred on the shoots on an average of 2.2 and 1.5 aecia/dm. During the next year, 1966, there was somewhat more rust per shoot, but the number of aecia per dm was smaller than during the previous year. In 1967 the incidence of rust reached a maximum in Ikaalinen. The amount of rust was about fuorfold compared to the previous year. In 1968 the amount decreased so much that there was not even a tenth of the amount which had been present in 1967. In 1969—1972 no systematic observations were

Table 2. Incidence of pine rwisting rust on the terminal leaders of Scots pine in Loc. 1 during the summers of 1965—1968.

	Year			
	1965	1966	1967	1968
Number of trees surveyed	25	47	30	10
Total length of terminal leaders, dm	77.61	116.50	84.17	18.57
— average length, dm	1.50	2.49	2.81	1.86
Number of aecia				
— total number including yellow spots	94	—[a]	454	11
— aecia matured	55	156	400	6
Number of aecia per terminal leader				
— total number	3.76	—	15.13	1.10
— aecia matured	2.20	3.32	13.33	0.60
Number of aecia per dm				
— total number	2.50	—	5.39	0.59
— aecia matured	1.46	1.34	4.75	0.32
Number of trees				
— with healthy terminal leader	9	—	0	6
— with no matured aecia	12	19	0	6

a) — = no record

Table 3. Incidence of pine twisting rust on shoots of one year old branches of Scots pine in Loc. 1 in the summers of 1966—1968.

	Year				
	1966		1967		1968
	Replications				
	I	II	I	II	I
Number of trees	47	47	30	30	10
Total length of shoots, dm	72.99	76.62	55.51	52.92	16.26
— average, dm	1.55	1.63	1.85	1.76	1.63
Number of aecia					
— total number including yellow spots	—[a]	—	157	146	3
— aecia matured	32	36	135	131	1
Number of aecia per shoot					
— total	—	—	5.23	4.87	0.30
— matured	0.68	0.77	4.50	4.37	0.10
Number of aecia per dm					
— total	—	—	2.83	2.76	0.18
— matured	0.44	0.47	2.43	2.48	0.06
Number of healthy shoots	—	—	2	4	8
— with no matured aecia	32	31	4	4	9

[a]) — = no record

made on the incidence of rust. In the fall of 1969 and 1970 the pine saplings on the experimental plots in Loc. 1 were examined, but no symptoms of rust could be detected. Also, during the following years, no serious epiphytotics occurred, but some rust was observed in various parts of the country.

The annual variation in rust incidence was best explained by the occurrence of rains during the growth of the pine shoots. Figures 2—4 show the daily height growth of the pine in Loc. 1 during 1966—1968. At the beginning of growth, before 10—30 % of the total height had been reached, the shoots were still covered with the fascicle scales. These scales seemed to completely prevent rust infection. Only when the surface of the shoots had been exposed were they susceptible to infection by the basidiospores. In the experiments conducted in the greenhouse the pines became resistant only when height growth was almost completed. Consequently, the pine shoots were susceptible to rust for some 20 days, between June 5—25. The amount of twisting rust was strongly correlated with the rains occurring during these days. During 1964 and 1967 the most important rains in June occurred at a time favorable for rust, causing a very heavy epiphytotic. During 1965, 1966, and 1968 the rains during pine shoot growth were not very heavy, but they were heavy enough to result in formation of basidiospores and a medium to light infection of the pine shoots. June of 1969 was not particularly lacking in rain, but the rains seem to have occurred when the shoots were not susceptible to infection. The next year, 1970, no rains occurred during the critical period, thus causing the pine twisting rust to disappear from most of Finland. Information about the rains during different years and months are available in the Meteorological Yearbook of the Finnish Meteorological Institute (Finnish Meteorological Office 1965—1967, Finnish Meteorological Institute 1968—1970, 1972). In 1966 rain was not registered at all during the time of most intensive growth of pine. The rains during June 3—7 were too light to allow the spread of pine twisting rust. At this time the pine shoots were still protected by the scales of the fascicles. The fact that infection did not take place during these rains can be deduced also from the observation that the first aecia did not occur until June 25. On June 14 and 16 some small thundershowers may have occurred (Finnish Meteorological Office 1966). These, however, were not registered on the fluviometer, at the study area but they could have caused some infection of the pine shoots, which probably resulted in the aecia observed on June 25. Some infection also may have occurred later, e.g. on June 22 when a 0.5 mm rain was registered. The spore counts obtained in 1966 from the Sarvas—Wilska pollen trap were too inaccurate for determining the probable time of infection.

The severe epiphytotic of 1967 was a result of a perfect coincidence of pine shoot growth and rain. It rained on June 1, but the pine shoots could not yet be infected. After this, it was warm for a couple of days when the daily shoot growth almost reached its maximum. On June 5 an almost continuous period of rain began and lasted for seven days. For a long time the average temperature was below 10 °C. The low temperature caused an almost complete cessation of the pine shoot growth (Figure 3). Since the pine shoots remained susceptible to infection for a long time, the infection was unusually intensive even if the temperature was considerably below the optimum for development of the pathogen (cf. Kurkela 1973 a). The first aecia were observed on June 19. If the first infections are assumed to have occurred on June 6, then it had taken 14 days for the aecia to develop. The cold weather could, of course, have slowed their development. No new infections resulted from the rains which started on June 21 since basidiospores were observed in the air at this time.

As was stated in the report on dispersal of basidiospores, two rains, which resulted in the formation of basidiospores and fascilitated infection, occurred in 1968 during the shoot growth of the pines. The first of these rains (5 mm) was on June 9—10. Enough moisture for infection occurred, however, during a relatively short period, resulting in a fairly light infection of the pine shoots (Figure 4, Table 2 and 3). The first observations on aecia formation were made on June 19, and on June 23 mature aecia were found.

On June 18 and 19 small showers, which did not result in measurable numbers of basidiospores, occurred. The rain on June 25 and 26 resulted in intensive formation of basidiospores, but new aecia were not formed. Apparently, the pine shoots had already developed resistance against infection.

Effect of wind on the positions of aecia on pine shoots

The position of aecia on the different sides of the pine shoot was clearly dependent on the wind direction at the time of basidiospore dispersal. Since the rust mycelium is limited to the immediate vicinity of the aecia, the positions of the aecia indicate the directions from which the basidiospores have arrived. Figure 15 indicates the relative proportions of aecia positions on the pine shoots in Loc. 1 in 1965 and 1966. The proportions were calculated on the basis of 500 observations. The predominating directions of basidiospore arrival in Loc. 1 in 1965, as well as in Parkano about 30 km away,

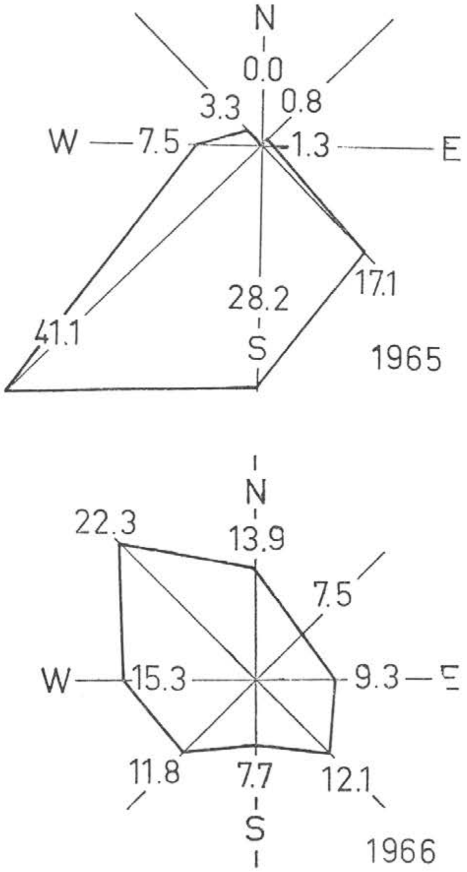

Figure 15. Number of aecia on different sides of pine shoots in percentage of total number, in Loc. 1 in the summers of 1965 and 1966.

were southwest and south. In Parkano some aecia also were located on the northern side of the shoots. The low pressure systems bringing rain to Finland usually arrive from the southwest. During the summer of 1966, at the same location as previously described in Loc. 1, most of the basidiospores had arrived from directions between west and north. During this summer the rains at the time of shoot infection were not caused by low pressure systems. The spores were formed and dispersed only during or after small thundershowers. On the locations examined, aspen leaves bearing telia were found all around the experimental plots. The occurrence of more aecia on some sides of the pine shoots than on others is an indirect indication of how crucially important weather factors of very short duration may be for infection. If such a strict dependence would not exist the aecia would probably be more evenly distributed around the pine shoots. In connection with investigations of this kind, it would be important to register wind direction, but there were no possibilities for such registration during this work.

Growth stage and rust resistance of pine

The infection of pine shoots with basidiospores of *Melampsora pinitorqua* during the pine growth phase resulted in the formation of aecia on the shoots. A relatively large number of aecia also developed on growing needles. When 99.9 % of the shoot growth was completed, aecia were formed only on needles. Inoculations performed later when no shoot growth was observed resulted only in a few yellow spots. These spots were aecial primordia but never did complete their development. In the earlier sets of inoculation the

Table 4. Inoculation of pine saplings with basidiospores of *Melampsora pinitorqua*.

Shoot length, % of final length	Time from planting to inoculation	Number of aecia develped on saplings[a]		Time required for opening of the first aecia, days
		Shoots	Needles	
57.1	0	38	0	11
58.0	0	67	1	11
71.5	3	42	13	11
71.7	4	2	5	14
87.1	6	15	35	12
95.5	9	11	23	12
95.5	14	10	5	11
98.8	14	11	35	13
99.91	18	—[b]	14	13
100	22	0	—	—
100	26	0	0	—

[a]) Every set consisted of 10 seedlings
[b]) Yellow spots only

yellow spots could be detected on the shoots 2—4 days before the aecia opened. Before opening, the yellow spot was surrounded by droplets of liquid with pycnidia forming pycniospores; i.e. fertilizing sporidia (cf. Gäumann 1964). The opening of the aecia occurred 11—14 days after inoculation. The results of these experiments are presented in Table 4.

Correlation between rust incidence, shoot length, and height of pines

Since the basidiospores of a rust fungus land at random on different surfaces, the longer the shoot is, the higher the probability of infection. The correlation between the shoot length and the number of aecia developed on it was studied using the data of 1967. The correlations of shoot length with numbers of aecia (i.e. the number of mature aecia only and counts of aecia which included mature ones and yellow spots) were low ($r = 0.43$ and 0.48) for the data which included terminal leaders. For data on terminals of branches the corresponding correlations were $r = 0.61$ and 0.64, respectively. The combined data resulted in a correlation of 0.75 and 0.77. The highest

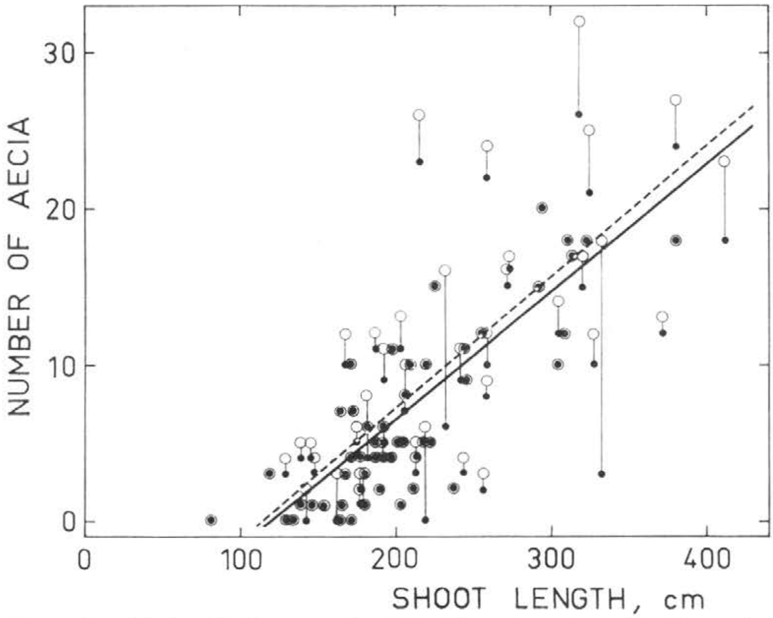

Figure 16. Relationship between shoot length and number of aecia on pine shoots in the summer of 1967. The rings indicate number of yellow spots, and the black dots indicate the number of mature aecia on the shoot. The line connects observations from one shoot. The thick solid line is the regression of number of aecia on shoot length, and the broken line is the regression of number of yellow spots on shoot length.

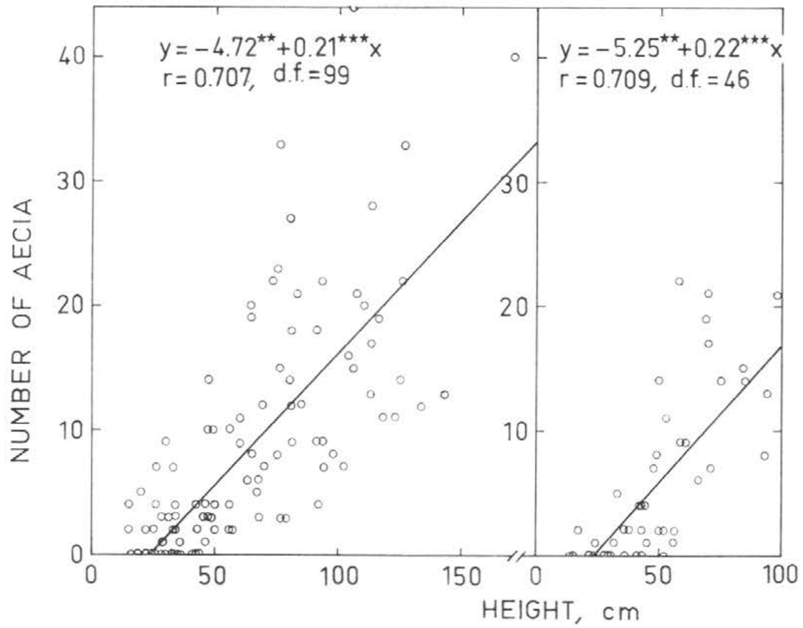

Figure 17. The regression of number of aecia on the two uppermost whorls of branches and the terminal leader on pine sapling height, in Loc. 1 in 1964.

correlation for the combined data was obtained for the regression model $y = A^{***} + B^{***}x$. Figure 16 indicates the relationship between shoot length and number of aecia. Consequently, on the branch terminals the number of aecia seems to be more dependent on length than on stem terminals. The stem terminals were about three times more susceptible than the branch terminals.

In 1965 on some 25 × 25 m large plots in Loc. 1 the number of aecia formed during the previous summer was counted on the main shoot and branches of the previous year as well as on the shoots of the one year old branches. When the saplings were less than 1.5 m high, the number of aecia was linearly correlated with the size of the sapling. Figure 17 presents the results from two plots. This linear relationship was more irregular if no aspen occurred on the plot or in its immediate vicinity.

Interaction of the development of pine and rust

The rust seemed to reduce the growth of the pine shoots to some extent. In the 1966 and 1967 data, the shoots, which later became severely infected, started growth slightly more vigorously compared to those remaining healthy or less infected. After the establishment of the fungus daily relative growth in the infected shoots was less than that in the healthy shoots. For this comparison the relative growth was used, obtained by dividing one day's growth by total length measured the previous day. Even if the difference in average growth caused by the rust seemed clear, no correlation was found between rust severity (number of aecia/dm) and growth of individual trees. Length measurements of severely infected shoots were difficult and more inaccurate due to the twisted shoots as compared to the healthy ones. Since the number of saplings on the experimental areas did not change significantly during 1967 and 1968, the number of aecia per tree could be regarded as an index of the number of aecia in the area (Tables 2 and 3). Since during both years the method of spore trapping was the same, the measured spore densities are comparable. In 1967 the total number of aeciospores was 5 523, and in 1968, 443 spores were trapped. Even though there were ten times more spores in 1967 than in 1968, the number of spores per aecium seems to have been only half the number found in 1968. This difference may have been due partly to the effect of weather factors. It is also possible that in 1967 the aecia may have been too close to each other to get enough nutrients from the shoots for spore production. The sparsely distributed aecia also seem to cause relatively more severe damage than those which are densely distributed. This may be deduced from the fact that the local effect of only one aecium may be sufficient to kill the shoot above the point of infection.

During the severe rust years of 1964 and 1967 in Loc. 1, almost all the terminal leaders of pine were killed on the experimental area as well as elsewhere in western Finland where pine sapling stands with plenty of aspen suckers were found. During other years the damage was less severe.

As a result of the severe rust year of 1967, about 50 % of the pine terminals were dead by August 17. This is thought to be a direct result of the action of the pathogen. During the following winter, the terminals continued to die off so that next spring about 90 % of the terminal leader and branch terminals had died. Death during the winter was often connected with the effects of various weather factors. Many of the shoots infected by the rust in the summer were too weak to endure the strain caused by winter snows. Some shoots which were still alive in the fall died during the winter and spring. The most common reason for death seemed to have been the resin flow and impregnation of the rust-caused wound, resulting in blockage of the phloem connections. It is also possible that the needles on the infected shoots were not resistant enough to desiccation in the winter.

The kind of damage that developed depended quite strongly on the phase of shoot growth at which infection occurred and aecia developed. Early infection resulted in death of the shoot already during the growing season. In some mild cases the shoots were only bent. Infection occurring late during shoot growth usually did not result in twisting or immediate death of the shoots but resulted in wounds healing by the end of the summer. The wounded places, however, seemed to be very susceptible to breaking by snow in the winter.

The recovery of the pines after rust damage and replacement of the dead shoots were very much dependent on the severity of the attacks. A mild infection does not always cause death of the terminal leader. Even if the leader dies, some of the healthy shoots on the current whorl may already partly replace the leader in the same summer. All the shoots at the top of a sapling may die as a result of severe infection. In such a case the terminal may be replaced by a lower branch or by a shoot developed from the bud of a short shoot (Figure 18). In this case two or three growing seasons may pass before the sapling reaches the height to which it would have developed during the year of the rust, had it not been infected.

Figure 18. The top of a pine sapling severely damaged by pine twisting rust. As a result of the intensive infection, all shoots have died. During the growing season following the year of damage, shoots have grown out of the short shoot to replace the leader.

Incidence of rust on aspen leaves

When aeciospores were released from the aecia of *M. pinitorqua* on the pine shoots, the aspen leaves already were fully developed.

In the pine sapling stands or in their vicinity the first uredia were observed on aspen leaves in Loc. 1 at the beginning of July. The primary infection of the leaves seemed to have been caused by the aeciospores developed on pines growing on the experimental plots. Uredia were not observed on aspen leaves before the maturation of aecia but were found only 10—20 days after the aeciospores had started to disperse. Consequently, it is obvious that the rust did not overwinter in the uredial stage. Neither could remote dispersal of uredospores have caused the primary infection of the aspen leaves since the first uredospores were trapped at the same time as the first uredia were observed. When aspen leaves were inoculated during rainy weather with aeciospores obtained from the pine shoots, the uredia developed in 10—14 days. On the other hand, it has to be noted that, depending on weather conditions, *M. rostrupii* Wagner (cf. p. 49) and *M. larici-tremulae* may have been able to cause the uredial state on the aspen 15—30 days earlier than *M. pinitorqua*, since these two species of rust compared to *M. pinitorqua* develop their aecial state earlier.

The number of uredia initially increased geometrically (Figures 2—4) due to infection by the ever-increasing number of uredospores. In both 1966 and 1967 the number of uredia of the aspen leaves at the end of August had reached approximately the same level, about 30—50 uredia/sq.cm. In 1968 the number of uredia was considerably smaller compared to previous years, about 1 uredium/sq. cm. The summer of 1968 was relatively cool and rainy, thus inhibiting the continuous multiplication of the uredial stage on the aspen leaves. According to the observations, aspen rust occurred rather sparsely in the fall of 1968 all over the western and northern parts of Finland. During the following three years (1969—1971), aspen rust was very sparse. It was found only in a narrow zone along the southern coast and sporadically in the vicinity of larch stands. Most likely, the rust observed then in the southern parts of the country was *Melampsora larici-tremulae* or *M. rostrupii*, since the aecial stage of these species was not uncommon during these years, but pine twisting rust could hardly be found at all.

In the fall at the end of the growing season the telia of *Melampsora* developed. Their number was 2—5 times greater than that of the uredia.

The mycelium around the uredium was limited to the yellow leaf area surrounding it. The yellow color of the area was due to the fact that the chloroplasts had disappeared from the cells affected by the mycelium (Figure 19). The veins of the leaves limited the spread of the mycelium in the tissue (Figures 19 and 20). To some extent, the mycelium seemed able to bypass the thinner veins. The distance between veins thicker than 50 % of the leaf thickness varied from 0.36—1.23 mm and averaged 0.7 mm. Consequently, the mycelium which developed from one basidiospore could infect the tissue on an area of 0.4—1.2 sq.mm. From this it can be concluded further that on an area of 1 sq.cm there are about 100—200 areas, delineated by the veins, which can be infected independently by a single spore. Usually all these areas are not infected.

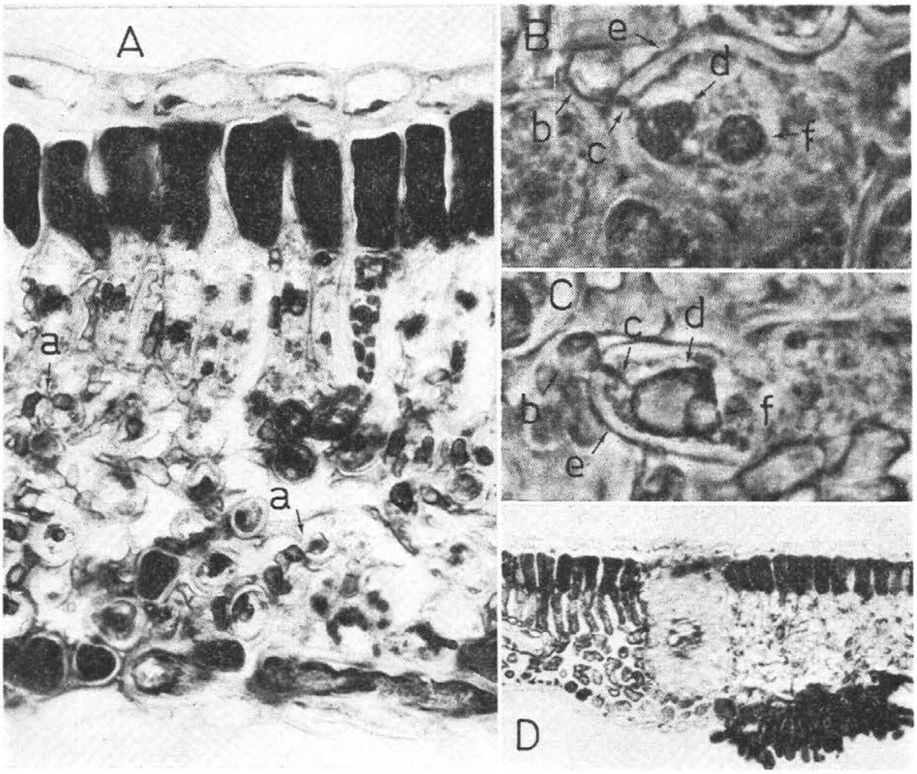

Figure 19. Microscopic sections from aspen leaves infected by the rust. A) Abundant intercellular mycelia in an infected leaf, a) haustorium connected by a thin neck to a mother cell outside the host cell. B and C) Magnifications of the haustorium, b) the mother cell of the haustorium, c) the haustorial neck, d) haustorium, e) wall of host cell, and f) nucleus. D) Cross section of an aspen leaf with a *Melampsora* uredium on the lower surface. The chloroplasts have disappeared from the leaf next to the uredium. In the center of the picture is a vein which has inhibited growth of the mycelium. Healthy tissue to the left of the vein. A) × 300. B and C) × 800. D) × 80.

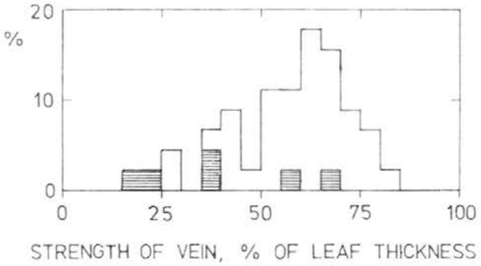

Figure 20. Distribution of the aspen leaf veins of various thicknesses. The thickness of the vein expressed as percentage of the leaf thickness. The shaded areas indicate the number of veins of each thickness class which ones the mycelium had passed.

There was abundant intercellular mycelium in the infected tissue. At places in the tips of the mycelium there were intensively-stained cells which proved to be mother cells of haustoria. In the same host cell there could even be several haustoria, which were connected to the mother cells by a narrow neck (Figure 19). The haustoria appeared to be similar to those studied by Longo and Naldini (1972) and with those of the rust *M. lini* (Ehrenb.) Lév. described by Littlefield (1972).

DISCUSSION

Dispersal of basidiospores and infection of pine

The dispersal of the basidiospores of *Melampsora pinitorqua* was studied during June of three consecutive years: 1966, 1967, and 1968. The study was begun when the buds were still covered with scales, and consequently, the shoots were unsusceptible to infection. Basidiospore dispersal may have occured already before the study began. The maximum spore densities and total numbers of spores were extremely varied during different years. Comparison of the average numbers of spores for different years is complicated by the fact that during 1966 and 1967 the spore counts were obtained with the Sarvas—Wilska pollen sampler, while during later years the counts were obtained with the Hirst spore trap. Since the basidiospores do not have any characteristic features except for the rounded shape, size and a faint, rapidly fading yellow color, their identification was fairly inaccurate on the vaseline-covered, cellophane plates from which one could not make ordinary microscopic slides. During microscopic examination the count might easily have included other particles resembling the spores. This apparently happened in the examination of the material from 1966. When counting spores in 1967 the work was done more critically. Then again, some of the spores may have been uncounted. The numbers of spores counted in 1966 and 1967 were approximately the same, but when considering the abovementioned counting difficulties, it is very probable that the number of spores in 1967 was really much larger at the time when pine was susceptible.

The slight effect of air temperature on the airborne basidiospores concurs with the results of laboratory experiments (Kurkela 1973 a), according to which the formation and release of basidiospores occurs over a wide temperature range. According to present knowledge, the basidiospores are actively released (Klebahn 1904, Dietel 1912, Prince 1943, Savile 1965). and in addition, the spores are so minute (∅ 6—10 μ) that even the lightest air current may move them. Therefore, the correlation between wind velocity recorded on an anemometer and the spore density may be mere coincidence. It may be speculated that the vertical air currents, created when the air becomes warmer, are of importance for dispersal of basidiospores since they are formed on aspen leaves lying on the ground and the basidia are not capable of actively ejecting them very far into the air. The obtained results are not in conflict with such a hypothesis; but, on the other hand, neither can it be proven.

The dispersal of rust basidiospores has been found generally to occur during high relative humidity of the air. In more southern latitudes, where more dew is formed during the night, a diurnal periodicity may be observed with the maximum around midnight (Carter and Banyer 1964, van Arsdel 1967, Snow and Froelich 1968, Pady and Kramer 1971). Even in areas favorable to dew condensation, the formation and dispersal of basidiospores appears to be most intensive when rains have prolonged the moist period (Hirt 1942, Snow and Froelich 1968). In general, the dispersal of basidiospores occurs most intensively, or even exclusively, during nights when there is dew (Hirst 1953, Gregory and Stedman 1958, Sreeramulu 1959, 1963, Wood and Schmidt 1966, Shanmuganathan and Arulpragasam 1966, de Groot 1968). All basidiospores are not, however, dispersed when moist; but, for instance, a reduction in relative humidity may cause release of the spores (McCracken 1972).

Sylvén (1918) already found that the incidence of pine twisting rust was crucially dependent on the rains in May and June. Also, the observations made by Klingström (1963) indicated the importance of rains for spore formation. The germination of telia of *Melampsora* seems to be controlled by an internal rhythm regulated by various weather factors. This results in germination of the telia simultaneously with the height growth of the pine shoots (Klingström 1963, Longo et al. 1970). In the present study the number of airborne basidiospores was reduced to insignificant amounts after mid-June. Exceptional weather may cause irregularities in the germination rhythm of the telia. Regler (1957) found that telia of *Melampsora* may, as an exception, germinate in the fall, which reduces the possibilities for an increase in rust the next spring. Rains have been found to have a crucial influence on formation of basidiospores and the infection caused by several species of *Cronartium* (Hirt 1942, van Arsdel et al. 1956, van Arsdel 1967, Nighswander and Patton 1965, Snow and Froelich 1968, Snow et al. 1968). By means of weather forecasting and by following the development of the telia, it is possible to predict the risk of infection by *Cronartium fusiforme* with a 2—3 day interim (Davis and Snow 1971). For some species of *Gymnosporangium* rains may not be of the same importance as for *Melampsora* and *Cronartium*. The telia of *Gymnosporangium* are often surrounded by a very moist, gelatin-like matrix. Thus, dew during the night may be enough for formation of the spores. This is indicated by the diurnal pattern of release with a maximum at midnight observed by Pady and Kramer (1971). The basidiospores of *Gymnosporangium* can cause infection on host plants even at considerable distances, such as 10—12 km (MacLachlan 1935) and even as much as 23 km (Parmelee 1968), apparently because the spores can be formed during a comparatively short, moist period but possibly also because of their relatively good survival capacity. The basidiospores of *Melampsora* and *Cronartium* appear to die rather easily if the relative humidity of the air decreases below the saturation point (Schafranskaja 1940, Hirt 1942, Nighswander and Patton 1965). Obviously the pine twisting rust cannot spread very far from the source of inoculation, the aspen suckers, due to low survival of basidiospores (Rennerfelt 1954, Schafranskaja 1940, Troschanin 1952, Kardell 1962, Durrieu 1967). The dispersal distance in each case is very much dependent on local conditions. Even during very favorable conditions, rust cannot occur more than 200—250 m from aspen suckers (Schafranskaja 1940, Troschanin 1952).

Dispersal of aeciospores and uredospores

Aeciospores. Dispersal of rust aeciospores has not been studied very extensively (Pady 1971 a). To my knowledge not a single study has been published about the dispersal of the aeciospores of *Melampsora*. Kramer et al. (1968) found in their studies a distinct diurnal pattern for the dispersal of aeciospores of *Uromyces psoralea* Peck and *Puccinia andropogonis* Schw. with a maximum during the night when high relative humidity occurred. Furthermore, they found that the aeciospores of *Phragmidium speciosum* (Fr.) Cooke did not disperse according to a diurnal pattern. The aeciospores of this fungus were released immediately when it rained, regardless of the time of day. Pady et al. (1968, 1969) studied the release of *Gymnosporangium* sp. aeciospores and found that for some species most aeciospores were released in the morning between 06.00 and 08.00 or, for some other species, generally in the daytime during dry periods. In the aecia of *Gymnosporangium* there is a mechanism which keeps them closed during moist weather (Pady et al. 1968). Experiments conducted in dew chambers indicated that the release of spores occurred immendiately at the end of the dew period regardless of the light conditions (Pady et al. 1969). The aeciospores of *Cronartium* species are released according to a diurnal pattern (Powell 1972, Peterson 1973). Powell (1972) showed that the aeciospores of *C. comandrae* Pk. were dispersed mainly in dry weather with the diurnal maximum around noon. Sudden rains may increase the spore density, but during continuous rains only a few spores are released into the air (Powell 1972). The rhythm of dispersal of the aeciospores of *C. comandrae* is very similar to the one observed in the present study for *M. pinitorqua*.

Uredospores. Dispersal of rust uredospores has been investigated much more than that of aeciospores. This is mainly due to the fact that the rusts of cereals are dispersed specifically by uredospores. In general, uredospores have been found to disperse according to a diurnal rhythm with the maximum spore density shortly after noon (Hirst 1953, Sreeramulu 1959, Pady et al. 1965, Kramer and Pady 1966). The uredospore dispersal of *Melampsora* appears to follow the same diurnal rhythm as other rust species. Pady (1971 b) reported that in greenhouse experiments the uredospores of *Melampsora euphorbiae* (Schub.) Cast. and *M. lini* (Pers.) Lev. were more often released during daylight hours. In these experiments the spore density usually had been at a maximum at noon or slightly before noon. Taris (1966, 1968) studied the uredospore dispersal of *Melampsora* species on poplars grown in a nursery. The uredospore density in the air reached maximum in the afternoon. Few spores were dispersed if the temperature was below 10 °C, relative humidity above 80 % or wind velocity less than 1 m/sec. A rain which occurred during the study period increased the spore density markedly. Taris (1966) considered this to be due to the mechanical effect of the rain and wind in releasing the spores.

Factors affecting diurnal periodicity of spore dispersal

The diurnal periodicity of spore release may be a result of one or more weather factors. Several weather factors, including light, total radiation, temperature, relative humidity of the air, dew and wind, vary according to a diurnal periodicity. The rainfall does not usually follow a diurnal pattern in Finnish conditions. Since all weather factors are dependent on one another in one way or the other, it is very difficult on the basis of field observations to determine whether one or several factors crucially influence the diurnal periodicity of release and dispersal of the spores. Several scientists have tried to investigate this problem in different conditions ranging from field conditions to microenvironments created in carefully controlled laboratories. The factors controlling periodicity vary according to the species of fungus.

Temperature seems to be positively correlated with spore formation (Jarvis 1962 a, Smith 1966, Cole and Fernandes 1970, Hammett and Manners 1971), at least when the temperature fluctuates mainly below optimum. For many fungi with dry spores the optimum temperature for spore formation is probably about 20 °C, perhaps even slightly above. On the other hand, the critical temperature above optimum which completely inhibits spore formation may be rather high (e.g. 27 °C for *Alternaria solani*, Waggoner and Horsfall 1969). Wind (Zoberi 1961, Smith 1966, Hammett and Manners 1971) and raindrops (collison with leaves) (Davies 1959, Gregory et al. 1959, Hirst 1961, Bock 1962, Jarvis 1962 b, Hirst and Stedman 1963, Hammett and Manners 1971) are forces releasing the spores. Diurnally varying light often stimulates formation or release of spores (Yarwood 1936, Smith 1966, Cole and Fernandes 1970). Humidity may enhance formation of spores (Jarvis 1962 a) for some fungi, but on the other hand, it may slow down their release (Zoberi 1961, Smith 1966, Hammett and Manners 1971, Powell 1972). A rapid increase in the density of airborne dry spores is usually caused by the initiation of rain (Sreeramulu 1962, Taris 1966, Mills 1967, Hammett and Manners 1971). With the continuing rain the air is rapidly washed free of spores (Hirst 1953, Ludlam 1967, Chamberlain 1967). Structures of the mycelia which form the spores may be destroyed by the rain, causing the spore density to remain low for some time after the rain (Yarwood 1937, Sreeramulu 1964, Waggoner and Horsfall 1969).

Limitations in identifying the spores of *Melampsora* species

Literature on the morphology of spores

The rusts of aspen leaves are collectively known as *Melampsora populnea* (Pers.) Karst., which can be subdivided into several races or species according to the host on which the aecial state occurs (cf. Liro 1908, Hylander et al. 1953). These fungi cannot be distinguished by morphological characteristics. The aecial states of the following aspen rusts have been found in Finland (Hylander et al. 1953):

Melampsora larici-tremulae Kleb. on *Larix*,
M. magnusiana Wagner on *Chelidonium* and *Corydalis*,
M. pinitorqua Rostr. on *Pinus*,
M. rostrupii Wagner on *Mercurialis*.

Obviously, one has to be critical in considering the causal agents of pine twisting rust, *M. pinitorqua*, and the needle rust of larch, *M. larici-tremulae*, as different species. At the time, the separation of the two species was done on the basis of results obtained from inoculation experiments (Rostrup 1884, Klebahn 1897 a, b, 1899, Liro 1906). According to some studies (Longo et al. 1970), *M. pinitorqua* also can infect larch. The rusts of pine and larch can probably occur on aspen leaves as mixed populations. Thus, using the same telial material, it is possible to get aecia to form regardless of which aecial host is used. On the other hand, the aecia of *Melampsora* which occur on larch may belong to species with aspen as alternate host or to species in which the uredial and telial states occur on different species of *Salix*. The name *M. larici* Hartig (cf. Gäumann 1959) has been used as the collective name for these larch rusts. According to Ziller (1959), the lateral walls of the aeciospores of aspen rust generally are expanded inward, whereas the walls of the aeciospores of rusts on *Salix* generally are expanded in the other apex. This special feature of the aeciospore walls has not been studied systematically on the aspen rusts occurring in Finland.

The aeciospores of *Melampsora* can be distinguished from the uredospores on the basis of differences in their surface structure. The surface of the aeciospores is finely verruculose and the uredospores have fairly large spines about 2 μ apart (cf. Gäumann 1959, Wilson and Henderson 1966). This is particularly clear on scanning electron micrographs (Arthaud 1972). According to the literature, the aecio- and uredospores of aspen rusts are of the same size (cf. Gäumann 1959). For all these rusts the uredospores have been reported to be somewhat more oblong than the aeciospores. The size of the aeciospores has been reported as 13—20 $\mu \times$ 12—17 μ and of the uredospores, 15—27 $\mu \times$ 10—18 μ. There are very few descriptions of the rust basidiospores in the literature, including the species of *Melampsora*. This is apparently due to the minute differences between them. According to Klingström (1963), the basidiospores of *M. pinitorqua* are round and measure 5—8 μ in diameter. The figures compiled in Gäumann's (1959) book show basidiospores of *Puccinia* and *Uro-*

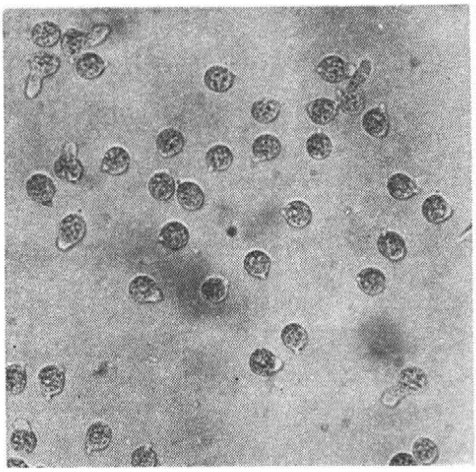

Figure 21. *Melampsora pinitorqua* basidiospores which have been released naturally and deposited on a microscope slide. Some of the spores have an emerging germ tube. × 450.

myces rusts as allantoid in clear contrast to the round ones of *Melampsora*. Some species of rust (e.g. *Melampsoridium betulinum* (Pers.) Kleb.) may have basidiospores of the same size and shape as *Melampsora*, while the basidiospores of e.g. *Thekopsora areolata* (Fr.) Magn. are clearly smaller, about 3 μ (Gäumann 1959). The basidiospores of *Melampsoridium betulium* (Sappin-Trouffy 1896) and many species of *Cronartium* (Hirt 1935, Kais 1963, Krebill 1972) may form secondary sporidia during germination. There do not appear to be any such reports on *Melampsora* in the literature. Figure 20 shows basidiospores of *Melampsora* deposited on glass. Some already have germinated during a release period of one half hour.

Measurements of spores

In order to achieve more accurate identification of spores in examinations of spore trap material, the size distribution of some spore samples of *Melampsora* were measured. Aeciospore from the following species were measured: *M. pinitorqua* on *Pinus sylvestris* L., *M. larici-tremulae* on *Larix sibirica* Lebed., and *M. rostrupii* on *Mercurialis perennis* L. For measurements of uredospores, samples representing *M. pinitorqua* were collected from aspen suckers in a pine sapling stand where twisting rust had occurred abundantly during the same growing season and where hosts for other aecial states were not found in the immediate vicinity. The samples of *M. larici-tremulae* were made on fresh specimens. At least 200 spores per sample of both aecio- and uredospores were measured. The basidiospores of *M. pinitorqua* were obtained in the laboratory from germinating telia depositing their spores directly on microscope slides. The inoculation of pine transplants were made with the same telial material. Five hundred basidiospores were measured. The optics used were ×400. The results of the spore measurements are presented in Table 5. The spore size distributions of the studied species of *Melampsora* were almost identical. Consequently, size measurements are not suitable for identifying individual species. The results corroborate data in the literature, even though the aeciospores of different species appear to be somewhat more rounded and the uredospores somewhat more elongated compared with previous reports. The average diameter of the basidiospores was 7—7.5 μ with extremes of 5.5 and 9.5 μ. When fresh, the basidiospores were yellow but when stored at room temperature this color was lost in about a month. In connection with the measurements, the wall thickness was also observed in order to determine whether this characteristic, reported by Ziller (1959), was suitable for distinguishing the spores of aspen and *Salix* rusts from each other. The observations indicated that the differences in wall thickness varied, depending on the position of the spore. Consequently, this

Table 5. Reults of measurements of aecio-, uredo-, and basidiospores of some *Melampsora* species.

Rust species	Spore form	Dimension	Size class μ																				Total
			9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
			% frequency																				
M. pinitorqua	I[a]	Length						4.0	9.0	9.5	11.0	17.0	18.5	18.0	7.0	5.0	0.5	0.5					100
M. larici-tremulae	I	»		0.5	0.5	1.0	1.5	2.5	9.5	11.0	21.0	15.5	18.0	11.5	4.0	2.0	1.0	0.5					100
M. rostrupii	I	»			0.5		0.5	5.5	3.0	7.0	23.0	24.0	21.0	11.5	1.5	1.0	1.0			0.5			100
M. pinitorqua	I	Width				0.5	4.0	10.5	9.5	12.5	19.0	18.0	18.0	6.0	1.0	0.5							100
M. larici-tremulae	I	»	1.0		2.0	4.5	15.0	19.0	19.0	15.5	10.5	5.5	5.5	1.5	1.0								100
M. rostrupii	I	»			1.0	8.0	15.5	21.5	20.5	16.0	11.0	3.5	1.5	1.0	0.5								100
M. pinitorqua	II[b]	Length							0.5	3.5	3.0	10.1	20.1	20.6	19.1	10.1	7.5	2.0	1.0	2.0		0.5	100
» »	II	»							0.9	1.0	2.8	10.3	15.1	16.4	22.5	13.6	8.0	5.6	1.9	1.4	0.5		100
M. larici-tremulae	II	»							0.9	1.4	6.4	13.3	17.0	24.8	20.6	10.6	1.4	1.8	0.9	0.9			100
» »	II	»									0.4	1.7	8.0	17.2	18.5	19.3	16.4	11.8	4.6	1.3	0.4	0.4	100
M. pinitorqua	II	Width				2.9	5.3	17.4	31.9	31.8	9.7	1.0											100
» »	II	»			1.0	3.9	13.0	14.0	18.3	17.9	19.3	10.2	1.4	1.0									100
M. larici-tremulae	II	»		0.9	2.4	6.5	9.3	15.8	22.3	18.6	15.4	6.5	2.3										100
» »	II	»				1.4	11.5	15.1	21.5	21.6	17.0	9.6	1.4	0.9									100
			Size class, μ																				
			5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5												
			% frequency																				
M. pinitorqua	IV[c]	Diam.	1.4	5.4	14.0	21.6	25.4	17.2	8.4	5.0	1.6												100

[a]) I = aeciospores
[b]) II = uredospores
[c]) IV = basidiospores

characteristic can be used only when examining a large number of spores of the same origin (e.g. when identifying aecia developed on the needles of larch).

Characteristics which determine rust incidence in pine stands

The incidence of rust on the aspen leaves, the germination capacity of the telia, and the weather conditions influencing the pathogen are, of course, the most important properties controlling the infection of the pine shoots in a stand; but the infection also is partly dependent on the structure of the pine sapling stand and the resistance of the pines. Klingström (1969) and v. Weissenberg (1973) isolated from shoots of *Pinus silvestris* L. and *P. taeda* L. some substances that inhibited germination of basidiospores of *M. pinitorqua* and *Cronartium fusiforme* Hedge & Hunt ex Cumm. These substances may, in one way or the other, be related to rust resistance. According to many investigators (Eklundh-Ehrenberg 1963, Schütt 1964, Illy 1966, Kardell 1966, Klingström 1969), the tallest or most vigorously growing saplings commonly have been more intensively infected by the rust. Sylven (1917) and Klingström (1963) observed that the rust occurred more frequently on the terminal leader than on the shoots of the current whorl. This was also observed in the present study. Also, Karu (1937) found that smaller saplings, which often are protected by grass or the branches or larger saplings, were less commonly infected than larger saplings. According to Nabatov (1968), a dense cover of grass will protect the pine saplings from infection by basidiospores of *M. pinitorqua*. The uneven soil surface and the ground cover absorb airbone spores, resulting in a more rapid reduction of spore density with increased distance to the source of inoculum among ground cover than above it (Waggoner 1965). It is, of course, natural that there may even be considerable genetic variation in resistance; and, on the other hand, the terminal leaders are clearly more susceptible to the rust than are the leaders of the branches. The more frequent infection of larger saplings may be partly due to a more favorable environment for trapping infective spores.

Interaction of pine and rust

The capacity of one aecium of twisting rust to produce spores appears to decrease with increasing density of aecia on the shoot. In studying inoculated seedlings of wheat and oats, it has been noted that where large numbers of competing mycelia are crowded in a leaf, the pustules formed are minute and produce few spores (Allen 1923 a). Allen (1923 a) also found that a sparse infection causes greater damage to the host than does the same number of basidiospores infecting the host in closer proximity to each other. The damage caused by the twisting rust is not directly dependent on the number of lesions of infection, since even one aecium alone may be lethal if the infection occurs in an early enough stage of growth. The later the infection occurs, the larger the number of primordial aecia that do not complete their development. When the growth of the shoot is completed, the tissue of the shoot surface appears to grow strong enough not to allow the late-developing aecia to burst open. Moriondo (1954) has found such unopened aecia.

In both the present study and in the experiments by Longo and Naldini (Longo et al. 1970) the development of aecia took 11—14 days from the time of inoculation. Regler (1957) and Moriondo (1961) reported that the development of the aecia took 16 days.

Rust on aspen leaves

The development of aspen rust in the Finnish conditions seems to start when the aeciospores infect the aspen leaves. The overwintering of the uredial state as mycelium may sometimes, however, be possible (Klebahn 1938, Kujala 1950, Regler 1957, Moriondo 1961, Ge et al. 1964). Uredospores may also overwinter in suitable conditions (Chiba and Zinno 1960). The factors influencing the amount of aspen rust are the number of aeciospores infecting the aspen, conditions of rain and temperature when these spores are dispersed and, in addition, the weather in July—August when the uredial state reproduces. The fructification period of the uredial state was found to be 10—14 days when the inoculation was done with aeciospores (cf. Hartig 1885). The same experiment has not been done with uredospores. In the experiments done by Toole (1967) the development of uredia took four days from the time the leaves of *Populus deltoides* Marsh. were inoculated with uredospores of *Melampsora medusae* Thüm. In nature the deposition of a uredospore on a susceptible surface does not invariably result in infection, but often several days may pass and many spores may die before the weather is suitable for infection. Therefore, the reproduction of the disease in nature is much slower than expected on the basis of the fructification or generation cycle.

The summers of 1966 and 1967 with their regularly occurring rains were relatively favorable for the increase of aspen rust. The summer of 1968 was unfavorable for rust development. On the experimental area of Ikaalinen the number of aecia was small, and rainless weather dominated when these spores and the uredospores were dispersed. The increase in the rust population was much slower than during the previous summers.

When the increase is measured by the changes in the total number of uredia, it seems most likely that the progress of the epiphytotic is relatively faster at the beginning of the growing season than at the end. During the progress of the epiphytotic, an ever-increasing portion of the uredia lose their spore-producing capacity when the nutrients of the substrates are becoming utilized or due to secondary fungi or bacteria. When the growing season is suitable for development of *Cladosporium*, this fungus may rapidly infect the newly-developed uredia, apparently inhibiting their spore-producing capacity; and consequently, the number of spore-producing uredia remains small (Kurkela 1973 b). Rainy weather permanently reduces the germination of uredospores formed by the uredia, apparently due to secondary infections (Bier 1965). The same kind of a situation is also observed with *Cronartium comandrae* (Powell 1971), in which case the main secondary colonizing organism in rust galls was *Cladosporium gallicola* Sutton (Sutton 1973). Zadoks (1961) also reports that cereal rust epiphytotics in rainy seasons usually are not very severe.

The reproductive capacity of the fungus is considerably reduced if the mycelium in the tissue of the host cannot expand and form new uredia without new uredial infection (cf. Manners 1971).

Since the network of vascular bundles, which prevent expansion of rust mycelia, is rather dense, it is hardly possible that one infection could result in several uredia. Consequently, the emergence of secondary uredia around the primary uredium probably requires new infections. This view is supported by the observation that the secondary uredia always occur completely at random around the primary uredium, and not in concentric rings around it, which would be the case if the expansion of the mycelium was not limited (Allen 1923 b, 1928).

Possibilities for control of pine twisting rust

The obtained results indicate that the main factors influencing the incidence of pine twisting rust are: 1) the number of overwintering telia on aspen leaves from the previous year, 2) precipitation and duration of rainfall during height growth of pine and 3) the timing of the telial germination activity during pine growth. When these conditions are favorable for rust development, the pines are infected.

Eradication of the aspen, the host of the uredial and aecial states of *M. pinitorqua*, from the pine sapling stands has been considered the most reliable means of controlling pine twisting rust (Hartig 1885, Liese 1923, Böhner 1952). Nowadays the eradication of the aspen is achieved best with chemical methods (Rennerfelt 1954, Bärring 1965, Rummukainen 1969 a, b).

The chemical control of rust on aspen suckers probably is technically feasible today (cf. Aerts 1963, Froiland and Littlefield 1972), but for economic reasons, it cannot be done; and, on the other hand, control of the aspen rust would promote growth of the suckers which would hinder the growth of pine saplings.

Assuming that the aspen, at least to some extent, has the same beneficial effect on the soil as the birch (cf. Mikola 1954) and keeping in mind the value of aspen from the standpoint of game management, the aspen should not be totally eradicated, but its growth should be suitably limited. The growth inhibition caused by the rust (cf. FAO 1958, Lilja 1973) may be considered an advantage. This advantage offered by the rust could be used by spreading in aspen sucker stands a rust which does not have pine as its aecial host. The most suitable rust for this purpose would be the larch rust, *M. larici-tremulae*. It would remain on the area if some larch were planted as mixture in the pine stand. The spread of larch rust to the aspen leaves may already begin about half a month earlier than *M. pinitorqua* (p. 42). This time difference for the start of the epiphytotic may be crucial in establishing the desired proportions of the two rusts in the pathogen population. Assuming that the spore-producing capacity per pustule of each species is the same and that formation of new pustules is not possible in previously infected tissue, the proportion of the two rust species will remain as it was when the aeciospores of the later-arriving rust caused its primary infection. At the end of this rust epiphytotic, when new susceptible leaf tissue is no longer formed, the amount of infected tissue will crucially limit the development of new pustules. If, for example, 50 % of the leaf surface is infected, which may be possible at the end of July if the epidemic started at the beginning of June, only half of the spores deposited on the leaf surface can cause infection. If the number of aeciospores of the two rust species is the same when causing the primary infection and the one rust starts to spread one month earlier than the other and if the amount of rust increases tenfold in one month, the ratio between the two species is 10 : 1. The maximum number of telial pustules (all palisade parenchyma tissue is infected) on an aspen leaf is about 200 pustules/sq.cm, of which pustules of twisting rust, considering a mixed population of *M. larici-tremulae* and *M. pinitorqua*, would be only slightly more than 20 pustules/sq.cm. Since during years of abundant rust, the number of telial pustules of *M. pinitorqua* was on an average not more than 120—150 pustules/sq.cm, the number of telia of twisting rust would be even less than 20 pustules/sq.cm. The drawback of this rust control measure, i.e. using a mixed rust population, is that its effectiveness cannot be measured unless the quantitative identification of the different rusts is possible. So far, no reliable method has been found for this purpose. The determination of the amount of rust in the field does not reveal the effectiveness of the method. The rust can spread over experimental plots located close to each other. Plots located far a way from each other cannot be compared since the differences in the local conditions may greatly influence the amount of rust and the development rate of the epiphytotic. There are a few investigations on the negative effect of a primary infection on the secondary infection. A strong local infection may reduce the intensity of a later, secondary infection by the same species *(Uromyces phaseoli)* (Yarwood 1954). Inoculation with avirulent races of the rust may later reduce the intensity of a virulent race of the same species *(M. lini)* (Littlefield 1967). Two different pathogenic species may prevent each other's development on the host plant (Johnston and Huffman 1958) in the same way as the growth of a non-pathogenic species on the leaves of its host (Yarwood 1956).

Another rust control method may be worth developing: the spread of a secondary parasite or saprophyte in the rustsusceptible aspen sucker or pine sapling stand. Bier (1965) found that some common saprophytes living on the leaves of black cottonwood reduced the rust on them. Infection of the uredial pustules of aspen rust by *Ramularia* (Magnani 1971), and the *Cladosporium* fungi (Kurkela 1973 b) may considerably reduce the dispersal of uredospores. In the Soviet Union Vasiljev et al. (1970) obtained promising results in controlling twisting rust with preparations of some bacteria.

CONCLUSIONS

The conclusions subsequently presented are based mainly on results obtained from the present study but also on results published elsewhere by the author (Kurkela 1973 a, b).

1. The formation of basidiospores occurs only under the influence of rain. The dew does not provide enough moisture for basidiospore formation, and the duration of dew during the night is not long enough. The dispersal of basidiospores commonly begins 4—6 hours after the rain starts. Air humidity does not seem to play an important role in the formation and dispersal of spores if there is free water in the leaf litter. A reduction in air humidity also causes the leaf litter to dry and spore production to stop, resulting in a rapid decrease in the number of airborne spores.

2. Increased air temperature increases the formation of spores and results in early commencement of spore release and larger spore density in the air during maximum dispersal in periods of high temperature. The formation and thus, also the dispersal of the basidiospores occurs over a wide temperature range +5 to +25 °C. The spore formation is at a maximum between 12—22 °C.

3. Wind does not have a crucial influence on the number of airborne spores. The spores are dispersed by the wind, resulting in their deposition on pine shoots according to wind direction.

4. The dispersal of basidiospores may occur in the spring, during May and June, in 2—4 separate periods of rain after which airborne spores are not observed regardless of favorable weather conditions.

5. The formation of aecia on the pine shoots or larch needles is a result of infection caused by basidiospores dispersed during rainy weather. A long rainy period leads to more severe infection.

6. The aecia are formed on the pine shoots within 10—14 days after infection.

7. The infection of the shoots can occur only when the surface of the shoots has been exposed between the fascicle scales. The shoots become resistant when shoot elongation has ceased. In greenhouse conditions with high relative air humidity needles also may be infected.

8. The aeciospores are dispersed when the aecia are mature, beginning at the end of June and continuing through mid-July.

9. There is a clear diurnal rhythm in the dispersal of aeciospores, which most likely is a joint result of all the weather factors following a diurnal rhythm (temperature, relative air humidity, dew, wind, radiation etc.).

10. The formation of aeciospores appears to be at a maximum during high temperature. A high relative air humidity may slow down the release of aeciospores.

11. The number of airborne aeciospores begins to increase from its nightly minimum in the morning at about 06.00, reaching a maximum in the morning or at noon during dry weather. The drop to a minimum is slower than the increase to maximum.

12. A beginning rain is in strong positive correlation with airborne spore density. A maximum may be reached any time of the day as the result of a sudden strong shower, e.g. a thundershower, since it effectively releases spores.

13. As a result of infection caused by the aeciospores on the lower surface of aspen leaves, the uredia develop within 8—14 days. The uredospores cause the multiplication of the rust on the aspen leaves with an initial logarithmic increase. When the dispersal of the aeciospores begins, most of the aspen leaves have already grown to full size.

14. The number of uredia reaches a maximum at the end of August when there may be 40—50 uredia/sq.cm during a year of abundant rust. The telia are developed in August—September and exceed the number of uredia by about 3 times.

15. The production of spores in the uredia may often be reduced by hyperparasites in the uredia or by secondary parasites spreading into the tissue through the uredia.

16. The veins of the leaf inhibit the growth of the rust mycelium in the leaf. The mycelium often stops at the veins. Due to the small size of the areas delineated by the veins, several uredia cannot develop from one infection.

17. The rust mycelium in the leaf tissue is intercellular. Throughout the mycelium are mother cells of haustoria. From the mother cell thin hyphae penetrate the walls of the host cells, and haustoria are formed in the tips of the hyphae.

18. The dispersal of uredospores follows the same pattern as that of the aeciospores. The maximum for airborne uredospores is at the end of August.

19. The damage caused to the pine shoots by the rust infection depends on its severity and time of occurrence. An infection occurring late in the shoot

expansion period usually causes only a rapidly healing scar, while an early infection causes the shoot to bend severely and finally die.

20. The amount of both aspen rust and pine twisting rust varies greatly from year to year. Dry weather unfavorable to rust development during any phase of the cycle may endanger the annual cycle. The *Melampsora* fungus is most sensitive to damage in its telial or basidial stage. If in the spring rain does not occur at a suitable time, basidiospores may not be formed. The basidiospores cannot cause infection unless the host plant is in the proper phase of growth or if the weather rapidly becomes dry when the basidiospores have been formed. Another sensitive phase occurs when the aeciospores are dispersed to the aspen leaves. If it is continuously dry at this time, the aspen leaves are not infected and the cycle is interrupted.

SUMMARY

The dispersal of different spores of *Melampsora pinitorqua* and *M. laricitremulae* (causal agents of pine twisting rust and larch needle rust) was studied using mainly the Hirst spore trap. The growth of pine shoots and development of rust on them was followed by daily observations. Pine shoots also were inoculated artificially.

The development of rust on the aspen leaves was followed by counting the number of pustules on leaf samples which were collected weekly. Also, the growth of aspen shoots and leaves was measured daily.

The basidiospores of *Melampsora* dispersed in the spring during May and June. Dispersal took place under moist weather conditions, starting 4—6 hours after the beginning of a rain, and reaching maximum after some ten hours of rain. When the rainy weather continued for a longer time, infection of pine shoots took place. Those shoots which had terminated their growth were resistant to the rust. The development of aecia on the pine shoots took place within 10—14 days. The aeciospores dispersed in the period June—July, and thereafter the uredial state was formed on the leaves of aspen. The numbers of aeciospores and uredospores in the air usually reached maximum around noon. These spores are both of a type which are distributed dry. High temperature stimulated development of the rust and spore formation. Wind gusts and rain showers occurring during a warm spell may suddenly swell the numbers of these spores at an extremely high rate. Considerable variations could be observed in the occurrence of both pine twisting rust and aspen rust from year to year; this was mainly due to the weather conditions.

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MÄNNYN VERSORUOSTEEN JA HAAVANRUOSTEEN EPIFYTOLOGIA

Melampsora pinitorqua on männyn versoruosteen aiheuttaja. *M. larici-tremulae* aiheuttaa lehtikuusen neulasruosteen. Mainituilla isäntäkasveilla nämä ruostesienet esiintyvät helmi-itiöasteisina. Molempien kesä- ja talvi-itiöaste kehittyy haavan lehdillä. *M. pinitorquan* ja *M. larici-tremulaen* eri itiömuotojen leviämistä ilmassa tutkittiin käyttäen pääasiassa Hirst-itiöpyydystä. Männynversojen kasvua ja ruosteen kehitystä niillä seurattiin päivittäisin havainnoin. Ruosteen kehitykseensä vaatiman ajan määrittämiseksi tehtiin myös keinollisia saastutuksia. Ruosteen kehitystä haavan lehdillä seurattiin viikon väliajoin tehdyin havainnoin sekä mittaamalla haavan versojen ja lehtien kasvua päivittäin. Ruosteiden eri asteiden kehityksessä ja runsaudessa esiintynyttä vaihtelua verrattiin eri säätekijäin vaihteluihin.

Melampsoran basidiosporit levisivät keväällä touko—kesäkuussa. Leviäminen tapahtui kostealla säällä alkaen 4—6 tunnin kuluttua sateen alkamisesta ja saavutti maksiminsa n. 10 tunnin kuluttua. Kostean sään jatkuessa edelleen yhtäjaksoisesti tapahtui männynversojen saastuminen. Kasvunsa päättäneet versot olivat ruosteenkestäviä. Helmi-itiöpesäkkeiden kehitys männynversoissa tapahtui 10—14 päivän kuluessa. Helmi-itiöt levisivät kesä—heinäkuussa, minkä seurauksena tapahtui kesäitiöasteen muodostuminen haavan lehdille. Helmi- ja kesäitiöiden määrä ilmassa oli yleensä runsaimmillaan keskipäivän aikaan. Nämä molemmat ovat ns. kuivana leviäviä itiöitä. Korkea lämpötila edisti ruosteen kehitystä ja itiöiden muodostusta. Lämpimällä säällä sattuneet tuulenpuuskat ja sadekuurot voivat äkillisesti nostaa näiden itiöiden määrät ilmassa erittäin suuriksi. Sekä männyn versoruosteen että haavanruosteen runsaus vaihteli eri vuosina hyvin paljon lähinnä sääsuhteista johtuen.