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THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF MELAMPSORELLA CARYOPHYLLACEARUM AND THE METHOD OF PENETRATION OF STELLARIA MEDIA BY THEFUNGUS

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THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF *MELAMPSORELLA CARYOPHYLLACEARUM* AND THE METHOD OF PENETRATION OF *STELLARIA MEDIA* BY THE FUNGUS

by

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ABSTRACT

The effect of temperature on the development of *Melampsorella caryophyllacearum* and the method of penetration of *Stellaria media* by the fungus.

by

William H. Pawuk

Aeciospores and urediospores of *Melampsorella caryophyllacearum* were placed on 2% water agar (pH 5.5) and incubated from 5 - 30 C. Plates containing the spores were removed every two hr for 24 hr and treated with 5% $CuSO_4 \cdot 5H_2O$ to stop germination. Percent spore germination for each plate was determined by counting 300 spores, and germ tube length was determined by measuring 30 germ tubes. Germination of both spore types was highest at 10 to 25 C. At these temperatures, maximum aeciospore germination of 60-70% was reached in 10, 12, 16, and 18 hr at 20, 25, 15, and 10 C, respectively. At 5 and 30 C, germination of both spore types was reduced, reaching a maximum of 25 to 30%. Using germ tube length as an indicator of vigor, aeciospores were most vigorous from 15-25 C, intermediate at 5 and 10 C, and least vigorous at 30 C. Urediospores were most vigorous at 15-25 C, somewhat less vigorous at 10 C, intermediate at 5 C, and least vigorous at 30 C. After 24 hr, germ tubes of both spore types were still growing vigorously at temperatures between 15 and 25 C.

Infection structures such as vesicles and infection hyphae were formed by both spore types. Urediospores formed vesicles and infection hyphae at all temperatures except at 5 C. Vesicle formation was greatest at 30 C, intermediate at 25 and 20 C, and poor at 15 and 10 C. Production of infection hyphae was greatest at 25 and 20 C, intermediate at 30 C and very poor at 10 or 15 C. Under fluctuating temperatures vesicle formation increased when spores were moved to a higher temperature and decreased when moved to a lower temperature. Infection hyphae forming under fluctuating temperatures were most numerous when moved to a final temperature of 25 or 30 C. Movement from 25 or 30 C to a lower temperature resulted in a decrease in the number of infection hyphae.

Cerastium vulgatum, Stellaria graminea, and S. media were inoculated with aeciospores and urediospores of the fungus and placed at 10, 15, 20, 25, and 30 C for various time intervals. Cerastium vulgatum and S. graminea were resistant to infection under these conditions. Stellaria media was very susceptible and was infected heavily from 15-25 C after 6-12 hr. At 10 C infection occurred within 12 hr and became heavy between 12-18 hr.

Stellaria media was inoculated with urediospores of M. caryophyllacearum and placed at various temperature regimes. With day temperatures of 15-25 C small white flecks began to appear on the leaf surface in 8-10 days. Uredia developed 2-3 days later in the same loci. Urediospores were

produced in 11-14 days after inoculation. Fungus development was slower with a 30 C day and a cool night but was not observed at a constant 30 C. Development at a constant 10 or 27 C was not observed until 18 and 25 days after inoculation, respectively. Subjecting plants to eight hr at 30 C had little effect on symptom development. Plants subjected to 35 C for eight hr a day delayed symptoms. Urediospore viability was not affected by the temperature at which they were produced.

To study the effect of temperature on urediospore production, infected plants were placed at constant 10, 15, 20, 25, and 30 C, and also at a 24 C day and an 18 C night. Spores were collected and counted using a Coulter Counter. Urediospore production was highest at 15, 20, and 24-18 C, intermediate at 10 C, and lowest at 25 and 30 C.

Stellaria media was inoculated with urediospores of M. caryophyllacearum. Infected leaves were collected, stained with lactophenol-cotton blue, and cleared in chloral hydrate. Penetration occurred through stomates. No appressoria were formed but vesicles developed in the substomatal cavity. One infection hypha developed from each vesicle and made haustorial contact with the mesophyll parenchyma cells. Uredia usually started forming in the substomatal cavity.

The Effect of Temperature on Spore Germination and Infection by Melampsorella caryophyllacearum.

Introduction - Melampsorella caryophyllacearum Schroet. (= M. cerastii Pers. Schroet.) is a heteroecious rust that causes a disease known as yellow witches' broom on fir (Abies spp.). In New Hampshire pycnia and aecia are formed in the spring and early summer from overwintering mycelium on fir needles. Infected needles are anatomically distorted and are annual (1,16). Under suitable conditions, aeciospores infect species of Cerastium and Stellaria (11,15). Urediospores develop throughout the summer giving way to teliospore formation in the fall (15) or in the spring (9). Teliospores germinate in spring (11,15) and basidiospores infect young expanding fir stems (6).

The fungus is found wherever fir is present in Europe, Asia, and North America (2). In the United States the disease is common in the Northeast but is most serious in the West (10).

Although artificial inoculation of chickweed with aeciospores has been demonstrated (15), no investigations concerning the environmental conditions necessary for infection have been reported. These studies were conducted to determine the influence of temperature on spore germination and on the infection process.

Materials and Methods - Twigs with aecia were collected from Abies balsamea (L.) Mill. on 28 June 1968 near Errol, N. H. The twigs were stored at 5 C in a closed glass jar

until 15 July. At that time the spores were washed from the aecia by shaking the infected twigs in a flask of distilled water. The spore suspension was passed through several layers of cheesecloth to remove fungus and plant debris. The spores were then washed three times by alternating centrifugation and resuspension in distilled water.

Urediospores used in the study were collected by removing infected leaves from greenhouse-grown Cerastium vulgatum L. and treating them in the same manner as the aeciospores. Urediospores were collected on 15 July and the germination studies on both spore types were done on that day.

Preliminary experiments had shown that aeciospore germination on 2% water agar was best at pH 5.5. Therefore, this medium was used for all germination studies. To determine the influence of temperature on spore germination, suspended spores were placed on the medium and incubated at 5, 10, 15, 20, 25, and 30 C. Every two hr for 24 hr, three plates were removed at each temperature and the germination process stopped by addition of a drop of 5% $CuSO_4 \cdot 5H_2O$. Percent spore germination of each plate was determined by randomly counting 100 spores. In addition, the germ tube length of each of 10 spores per plate was measured using an ocular micrometer at 860X.

When rust spores germinate on agar media, infection structures are often formed (3,6). During these studies, structures resembling vesicles and infection hyphae were formed by both spore types. Since temperature has been

shown to have an effect on formation of infection structures (3), a study was conducted to determine the effect of temperature on formation of vesicles and infection hyphae. Urediospores were placed on water agar as described previously and incubated at 5, 10, 15, 20, 25, and 30 C. Starting at six hr and at two hr intervals for 24 hr, three plates were removed at each temperature. The percentage of vesicles formed was determined by observing 100 germinated spores per plate. The number of infection hyphae that developed from vesicles was also recorded. In conjunction with this experiment 15 plates were removed from each temperature level after six hr of incubation. Three plates from each temperature level were then placed at each of the other temperatures for an additional 18 hr. After that time, the germination process was stopped and urediospores were observed for vesicle and infection hypha formation.

Aeciospores and urediospores used in inoculation studies were collected as described above except that the urediospores were collected from greenhouse inoculated S. media (L.) Cry. With both spore types, plants were inoculated by atomizing spores onto the leaves. The plants were then placed in plastic bags, the bags were closed to prevent drying, and the plants were incubated at 5, 10, 15, 20, 25, and 30 C. The plants were removed from the chambers at desired time intervals, taken out of the plastic bags, and placed in front of a fan to hasten drying of the leaves. Plants were then placed in a greenhouse until the degree of infection could be determined.

Aeciospore infection studies were conducted 9 September using spores collected 28 June. Stellaria media and C. vulgatum were inoculated as described above. Four inoculated plants and one control of each species were placed at each temperature for each time period tested. Inoculated plants were removed after 12, 24, and 36 hr of incubation.

Urediospore infection studies were conducted as described above except that the plants were incubated for 6, 12, and 18 hr after inoculation. Spores used in this study had been collected from S. media and stored at 5 C for 2-4 weeks prior to inoculation. In addition to C. vulgatum and S. media, S. graminea L. was also inoculated.

Results - Both aeciospores and urediospores germinated at all temperatures tested (Fig. 1). Urediospores germinated after two hr at 20 C. Both spore types germinated after four hr of incubation at all temperatures tested.

Germination of both spore types was greatest between 10-25 C. At these temperatures maximum aeciospore germination of 60-70% was reached at 10, 12, 16, and 18 hr at 20, 25, 15, and 10 C, respectively. Maximum urediospore germination of 70-85% occurred at 8, 12, 14, and 16 hr at 20, 25, 15, and 10 C, respectively. At 5 and 30 C, germination of both spore types was reduced, reaching a maximum of 25-30%.

Using germ tube length as an indicator of vigor, aeciospores were most vigorous at 15-25 C, intermediate at 5 and 10 C and least vigorous at 30 C. Urediospores were most vigorous at 15-25 C, somewhat less vigorous at 10 C,

intermediate at 5 C and least vigorous at 30 C. With both spore types germ tubes which formed at 30 C were stunted. At 15-25 C germ tubes of both types were still growing vigorously.

Vesicles formed at all constant temperatures except at 5 C. At temperatures above 5 C vesicle formation increased as temperature increased except for a 12 hr lag at 30 C (Fig. 1). Although the number of germinated spores that produced vesicles at this temperature was greatest at 30 C, spore germination was low and most vesicles were reduced to half size and produced very short germ tubes. Vesicles formed poorly at 10 and 15 C but were abundant at 20 and 25 C. In all cases, if infection hyphae formed, a single hypha developed from each vesicle. Production of infection hyphae was greatest at 20 and 25 C, intermediate at 30 C, and lowest at 15 and 10 C.

Under fluctuating temperature conditions, where spores were moved to a higher temperature after six hr, vesicle formation was increased over that which occurred at the constant-initial temperature level. Movement of urediospores to a lower temperature after six hr of incubation resulted in a decrease in vesicle formation when compared to vesicle formation at a constantly lower temperature level. When the final temperature treatment was 30, 25, and 20 C, the initial temperature treatment had little effect on the number of vesicles that would form when compared to a constant 30, 25, or 20 C. However, when the final temperature treatment was

15, 10, or 5 C, a six hr treatment at a higher temperature increased the number of vesicles formed when compared to the number of vesicles produced at a constant temperature of 15, 10, or 5 C.

At a constant temperature, development of infection hyphae was greatest at 20 and 25 C (Fig. 1). They started to form after six hr and increased throughout the 24 hr of the experiment. At 30 C there was a delay in their initiation (10 hr) and also a decrease in number. In addition, the hyphae were stunted. Very few infection hyphae formed at 15 C and essentially none at 10 C.

Under conditions of fluctuating temperatures, infection hyphae formed best when spores were moved to a final 18 hr treatment of 25 or 30 C. When moved to a final 18 hr treatment of 20 C from 5 or 10 C, there was an increase in infection hyphae production over that at a constant lower temperature. No change was apparent when moved from 15 C, but a decrease in infection hyphae occurred when moved from 25 or 30 C compared to the number produced at a constant temperature of 25 or 30 C.

Cerastium vulgatum and S. graminea proved resistant to infection under the conditions tested. Infection of these plants was very low in all inoculation studies, and when it occurred, only one or two leaves per plant were infected.

Stellaria media, on the other hand, was susceptible. Aeciospore infection of S. media occurred in 12 hr at 15, 20, and 25 C (Table 1). Although one or two leaves on one plant

were infected at 10 and at 30 C at 12 hr, major infection at 10 C did not occur until sometime between 12-24 hr. Major infection at 30 C did not occur, infection was limited to one or two leaves on one plant each at 12 and 24 hr. The amount of infection increased after 12 hr but not after 24 hr at all temperatures in which infection took place. Urediospore infection of S. media occurred at all temperatures tested except that no infection took place at 30 C. Twelve hr were required for infection at 10 C but infection took place in 6 hr at 15, 20, and 25 C. The amount of infection at 10 C after 18 hr increased over that at 12 hr. Similarly at 15, 20, and 25 C there was an increase in the amount of infection in plants incubated for 12 hr. Additional infection occurred if incubation continued for 18 hr.

Discussion - Best germination and germ tube growth of aeciospores and urediospores of M. caryophyllacearum occurred at temperatures of 15-25 C on water agar. This is similar to the results obtained with other forest tree rusts such as Cronartium ribicola (13) and C. comandrae (8). Although vesicle formation was rare at 15 C, incubation of inoculated plants at this temperature for 12 hr resulted in heavy infection. Even after as little as six hr of incubation some leaves became infected. Maheshwari et al. (9) have shown that mineral oil and hydrocarbons isolated from the surface wax of leaves may stimulate formation of infection structures. Increases in their formation have also been attributed to the presence of amino acids (7), various pH and zinc levels (13),

and pelargonaldehyde (4). Perhaps chemical compounds available on or in the leaf stimulate the production of infection structures in M. caryophyllacearum. The presence of such compounds in S. media might help to increase the number of vesicles that form at low temperatures. On the other hand, it is possible that penetration of hyphae at a low temperature and vesicle formation are delayed until the plants are subjected to more favorable temperatures. The increase in production of vesicles by germinating urediospores subjected to higher temperatures during the germination process would support this hypothesis. However S. media proved to be so susceptible, that perhaps few infections are needed to colonize infected leaves rapidly. In such a case low incidence of vesicle production might not be a limiting factor.

The low level of infection at 30 C was probably due to lack of vigor of germination spores. It is unlikely that penetration of the leaf at this temperature would occur unless the spore landed directly on a stomate. Even if this occurred, infection would be unlikely unless water remained on the leaf and a drop in temperature occurred. The occasional leaf infected at 30 C probably was subjected to these conditions when moved to the greenhouse.

For infection to occur following penetration, the vesicles must give rise to infection hyphae which must then give rise to haustoria. Production of infection hyphae was low at a constant 15 C. When the plates were moved to higher temperatures after six hr at 15 C production of infection

hyphae increased. Similar trends were observed with plates moved from 10 and 5 C which would indicate infection could occur sometime following penetration. Again the internal environment of the leaf might also be critical in determining whether or not infection hyphae will develop.

Haustoria-like structures were not observed in culture; therefore the effect of temperature on their formation is not known. Since they are necessary for completion of the infection process, information concerning their development might prove valuable in understanding the requirements necessary for successful infection.

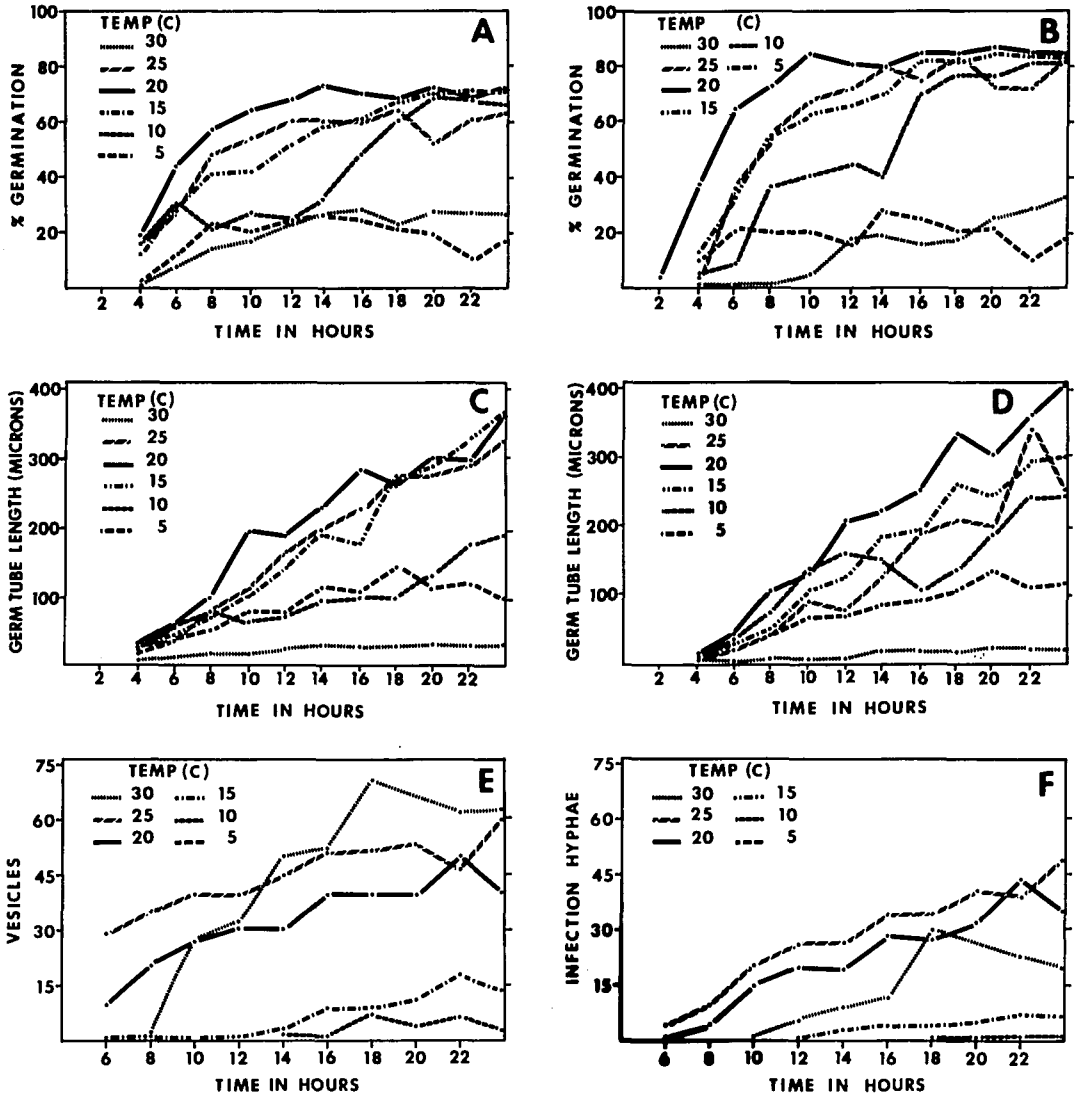

Figure 1. The effect of temperature on the development of Melampsorella caryophyllacearum on 2% water agar. A) germination of aeciospores, B) germination of urediospores, C) aeciospore germ tube elongation, D) urediospore germ tube elongation, E) urediospore vesicle formation, F) urediospore infection hyphae formation.

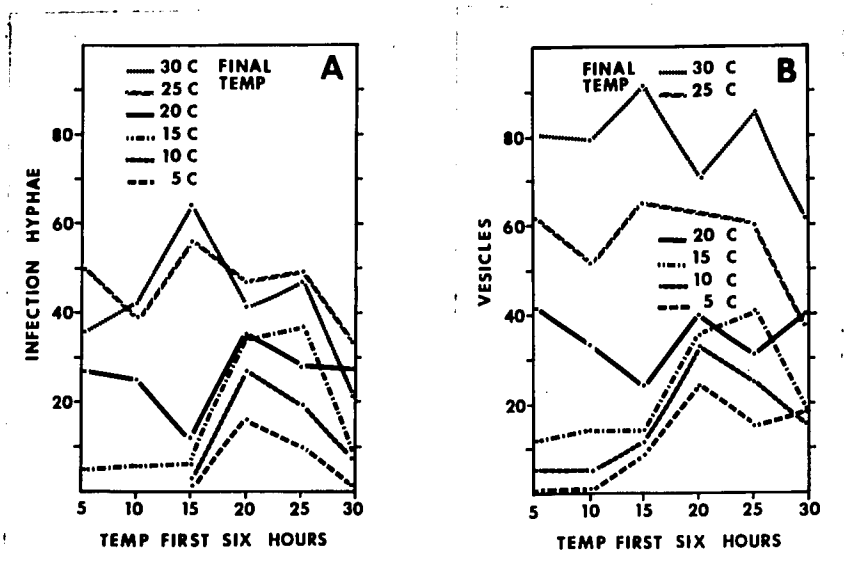

Figure 2. Effect of fluctuating temperature on the production of infection structures on 2% water agar by Melampsorella caryophyllacearum. A) infection hyphae, B) vesicles

Table 1. Effect of time and temperature on the percentage of leaves of Stellaria media infected by aeciospores and urediospores of Melampsorella caryophyllacearum.

Temp	AECIOSPORES 12 hr	24 hr	36 hr	UREDIOSPORES 6 hr	12 hr	18 hr
10	x	70	60	0	20	50
15	40	70	60	5	65	75
20	35	70	75	5	50	70
25	5	25	20	5	30	65
30	x	x	0	0	0	0

x = 1-2 leaves on one plant

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The Effect of Temperature on the Development of *Melampsorella caryophyllacearum* on *Stellaria media*.

Introduction - *Melampsorella caryophyllacearum* Schroet. (*M. cerastii*, *M. elatina*) is widespread where fir (*Abies*) is found in conjunction with either chickweed (*Stellaria media*) or mouse-ear chickweed (*Cerastium vulgatum*). In Germany on silver fir the fungus frequently causes stem cankers and branch infections which result in witches' brooms (2). Decay fungi gain entrance through canker sites (2). In North America the disease is common in the East on balsam fir (1) and serious outbreaks sometimes occur (9). However, the disease is most serious on subalpine fir in the Rocky Mountains (3). Numerous branch infections have resulted in spike tops and death of trees. However, decay columns are rarely initiated at canker sites (7). During certain periods, infection seems to be more severe than in others. In one case Peterson (7) examined 300 brooms and found fewer than 10 were more than 30 years old. However, no studies have been conducted to determine what environmental conditions lead to such epiphytotics. Temperature is one environmental factor which is important in the development of other rust diseases (9). This study was initiated to determine the effect of temperature on the development of *M. caryophyllacearum*.

Materials and methods - Greenhouse grown plants of *S. media* were placed at 15 C and inoculated with urediospores as described by Pawuk (6). After 36 hr, one uninoculated control and four inoculated plants were moved to various temperature

regimes with 16 hr of daylight. The plants were observed daily and the development of fungus recorded. The effect of exposure to high temperatures for part of a day was also studied by subjecting infected plants to eight hr treatments of 30 or 35 C. For the remainder of the day the plants were placed at 24 C for eight hr and at 18 C for the eight hr of night. A control and four inoculated plants were removed periodically from the eight hr high temperature treatment and placed at a continuous 24 C day and 18 C night regime. This consisted of removing plants from the high temperature treatments after exposure times of 1, 2, 4, 6, 8, 10, 12, and 14 days.

To study the effect of temperature on sporulation plants were grown at a constant 15 C for 10 days. At this time urediospore production was initiating. On each plant one heavily infected leaf was selected from which to collect spores. Five randomly selected plants were removed from the 15 C treatment and placed at each of the following temperatures: 10, 15, 20, 25, 30, and at a 24 C day with an 18 C night. A 50-ml beaker was placed below each selected leaf to collect spores as they fell. At each urediospore collection the leaf was tapped with a dissecting needle to aid in the release of mature spores. Urediospores were collected at four-day intervals for 16 days, at which time sporulation had ceased. Following collection and prior to counting the spores were suspended in 20-ml of a 0.5% NaCl solution containing two drops of Tween-20 per liter added as a wetting agent. The

spores were allowed to stand overnight at 5 C, then resuspended and counted using a Coulter Counter. Each sample was counted three times, the mean calculated, and the number of spores collected was determined.

Results - The first visible evidence that infection had taken place was the development of small white flecks on the leaf epidermis. One or two days later, at most emperatures, these flecks turned yellow, giving rise to uredia. In a few more days mature urediospores were produced. In most cases two to three days following initiation of urediospore production, chlorotic spots began to appear around the uredia. A few days later the chlorotic tissue started to become necrotic. The time required for initiation of each step was dependent upon temperature (Figure 1).

Urediospores were produced in 11-14 days following inoculation at day temperatures of 15-25 C regardless of the night temperature. However, urediospore development on plants at a 5 C night was usually two days behind that on plants grown at a higher night temperature. Twenty-five C seemed to be the upper threshold for rapid fungus development for at a constant 25 C the fungus developed well and sporulation began 14 days following inoculation. However, at a constant 27 C, sporulation was delayed until 28 days following inoculation and was restricted to a few loci on a few leaves of each plant. No spores developed at a constant 30 C but sporulation did occur at a 30 C day when accompanied by a cooler night temperature. In such cases sporulation began 15-19 days following inoculation and was relatively sparse.

The low temperature threshold for rapid fungus development was 15 C. Below this temperature, at 10 C, the sporulation process was delayed until 28 days following inoculation. Uredispores produced at this temperature and at higher temperatures demonstrated a high degree of germinability. This would indicate that the temperature at which the spores were produced had little effect on their viability.

Subjecting infected plants to eight hr at 30 C had little or no effect on initiation of uredium and urediospore production regardless of the number of days the plants were subjected to the eight hr treatment (Figure 2). Urediospore production with no exposure to 30 C began in nine days while it began in 12 days on plants which had been subjected to 30 C for 14 consecutive days.

Infected plants subjected to 35 C for eight hr a day demonstrated delays in all processes of infection when compared to plants not placed at 35 C. The greater number of days the plants were held at 35 C the longer it took for each process to develop. For example, sporulation began in 11-12 days after 1-2 days exposure to eight hr of 35 C while 13-15 days were required after 4-8 days exposure, and 19 days were required with 14 days exposure. Continued exposure to 35 C resulted in delays not only in sporulation but also delays in the onset of chlorosis and necrosis. Exposures of about 8 days reduced the number of uredia-producing leaves to a few per plant. In contrast, plants with only a few days at 35 C had numerous uredia on almost all leaves.

Differences existed in spore production on plants grown at the temperature levels tested (Figure 3). At the .05 level of confidence, mean spore production at 24-18, 20, and 15 C was greater than at 25 or 30 C. Urediospore production at 10 C was lower than at 24-18 C, the same as at 20 or 15 C, and higher than at 25 or 30 C. Spore production at 25 C was greater than at 30 C.

Over the period sampled spore production at a given temperature was not constant from one sampling day to another. At 24-18 C, urediospore production was highest at days 5-8 and then began to decline (Figure 4). A similar trend existed at 25 C although spore production at this temperature never approached that which occurred at 24-18 C. At 25 C, by the eighth day, sporulation had stopped on three plants and by the twelfth day it had stopped on all plants. Plants at 30 C developed poorly and the fungus ceased to produce spores by the eighth day.

Spore production increased to the eighth day and then leveled off at 15 and 20 C. Low and constant spore production occurred at 10 C until after the twelfth day when it increased quite rapidly. During days 1-4 at 24-18 C, spore production was greater than at all other temperatures. No differences in spore production existed at the other temperatures. During days 5-8, spore production at 24-18 C was greater than all other temperatures. Production at 20 C was greater than at 15, 25, 10, and 30 C while production at 15 C was greater than at 25, 10, and 30 C. No differences existed in spore production at 25, 10, or 30 C.

During days 9-12, differences in spore production at the temperatures tested began to diminish. At 24-18 C production was no longer different from that at 20 C and production at 20 C was no longer different from that at 15 C. Production at 10 and 25 C was lower than at 24-18, 20 and 15 C. By this time sporulation had ceased at 30 C.

The most noticeable changes in spore production occurred during days 13-16. Production at 24-18 declined rapidly and was now lower than at 20, 15, or 10 C. No differences existed in spore production on plants grown at 20, 15 or 10 C during this period. No sporulation took place at 25 C during this period.

Discussion - Temperature has a marked effect on the development of M. caryophyllacearum. This effect was observed on spore germination, infection structure formation, germ tube elongation, and infection in a previous study (6). In this study temperature had an effect on symptom development and sporulation on S. media. The fungus developed most rapidly at day temperatures of 15-25 C. These temperatures also coincide with the optimum temperature for germination and germ tube elongation. Reduced rates of fungus development occurred above or below these temperatures and no visible development occurred at 30 C unless combined with a cooler night temperature. Because fir is a cool climate species, these data suggest that temperature would not be a major limiting factor in the development of M. caryophyllacearum on S. media within the natural range of Abies in this country.

Brief exposures to high temperature might slow the rate of fungus development but it is doubtful if these exposures would be of sufficient duration to have much impact.

Low numbers of urediospores produced at a constant 25 C or higher might indicate that sporulation would be reduced at these temperatures. However, since the plants were grown at 15 C for 10 days before being placed at 25 C, the abrupt change to the higher temperature might have been detrimental to the development of the plant or the fungus. This may account for the low sporulation at the 25 C treatment at least. When inoculated plants were placed at a constant 25 C 36 hr after inoculation, urediospore production was observed to be of longer duration and appeared to be more abundant than that observed in the sporulation study. However, further studies would be needed to confirm this observation statistically.

Since all studies were carried out in controlled environmental chambers at relatively low light intensities, the effect of sunlight on fungus development is not known. Van Arsdel et al. (11) found that teliospores of Cronartium ribicola produced in a greenhouse in sunny weather were lower in viability than those produced at the same or higher temperatures in cloudy weather. Since S. media usually inhabits shaded sites, infected plants would not normally be subjected to high light intensities. However, many other species of Stellaria and species of Cerastium are susceptible to M. caryophyllacearum (1). Some species, such as C. vulgatum,

develop well in full sunlight. In areas where S. media is absent the development of the rust on other species might be influenced significantly by sunlight. Because of the difficulty in infecting C. vulgatum and S. graminea L. (7), the development of the fungus on these species was not studied.

Because of its high susceptibility, Stellaria media could play an important role in the build up of inoculum levels. With a generation time of 10-12 days under most temperature conditions moisture would probably be the most limiting factor in its spread to other suscepts. The role S. media plays in overwintering of the fungus is not known. Reports of overwintering and teliospore production fail to mention S. media as an overwintering host (5,11). No teliospores were produced on S. media under the conditions tested. Furthermore, mature plants of S. media rarely if ever overwinter in New Hampshire. However, infections on fir are rather common in the forest. This would indicate other chickweed species are important in the overwintering role. Because teliospores were not produced, and because infected leaves always died in a few weeks, we have no data concerning the effect of temperature on teliospore production and germination. Consequently what environmental conditions are necessary for infection of fir are not known. Better success in infection of other species of Cerastium and Stellaria might lead to an understanding of these requirements.

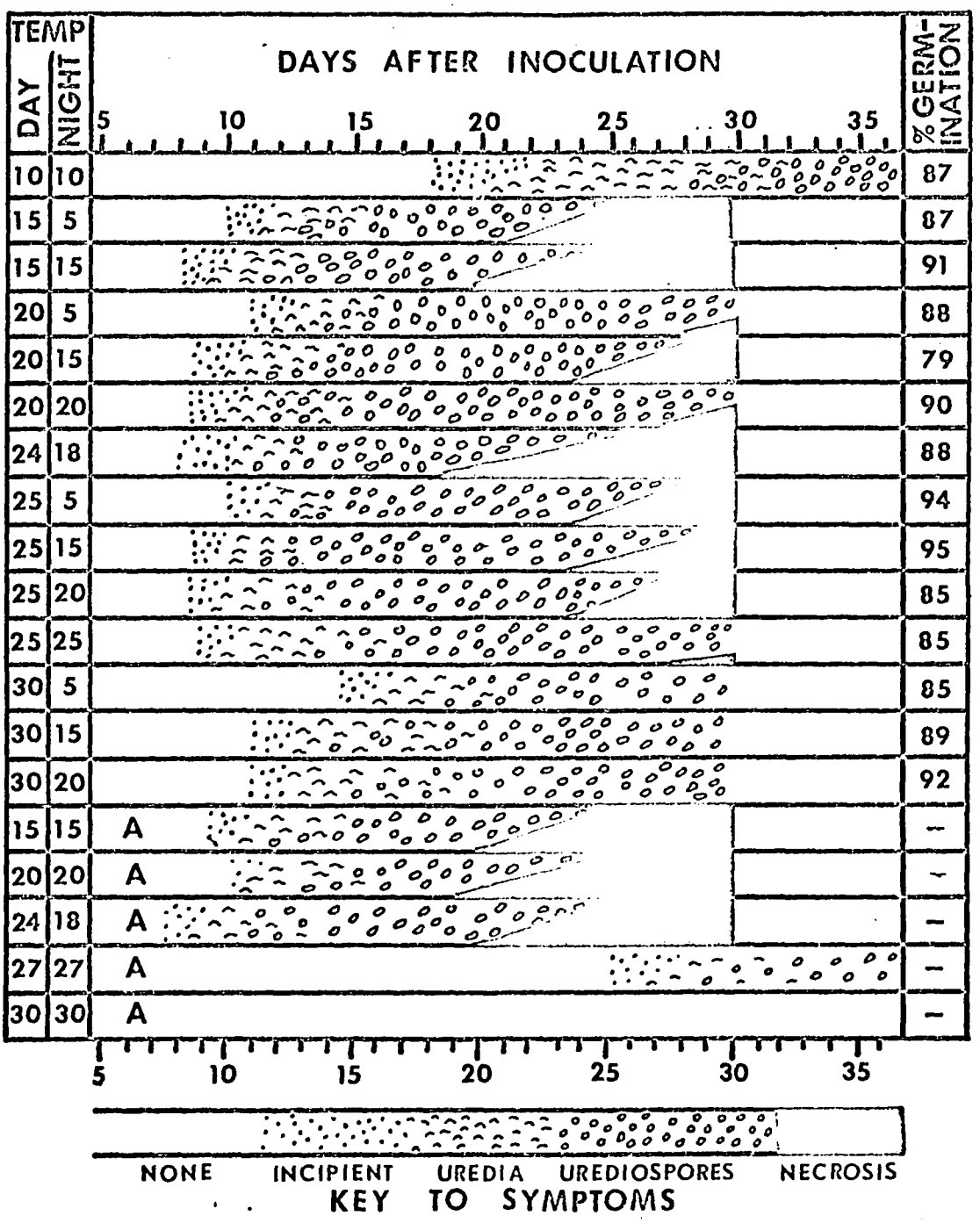

Figure 1. The effect of temperature on the rate of symptom development on Stellaria media caused by Melampsorella caryophyllacearum.

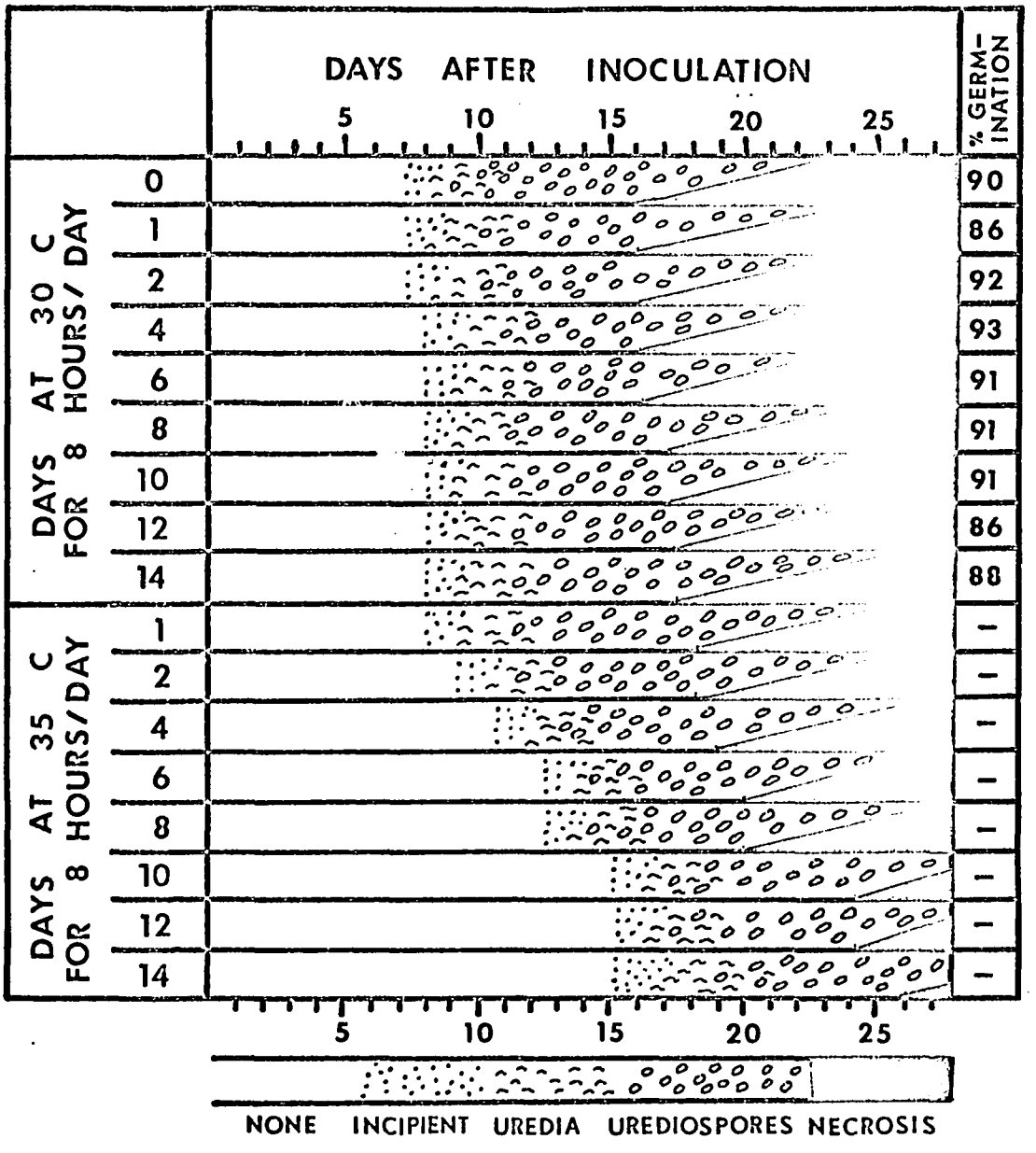

Figure 2. The effect of high temperature in delaying symptom development on Stellaria media infected with Melampsorella caryophyllacearum.

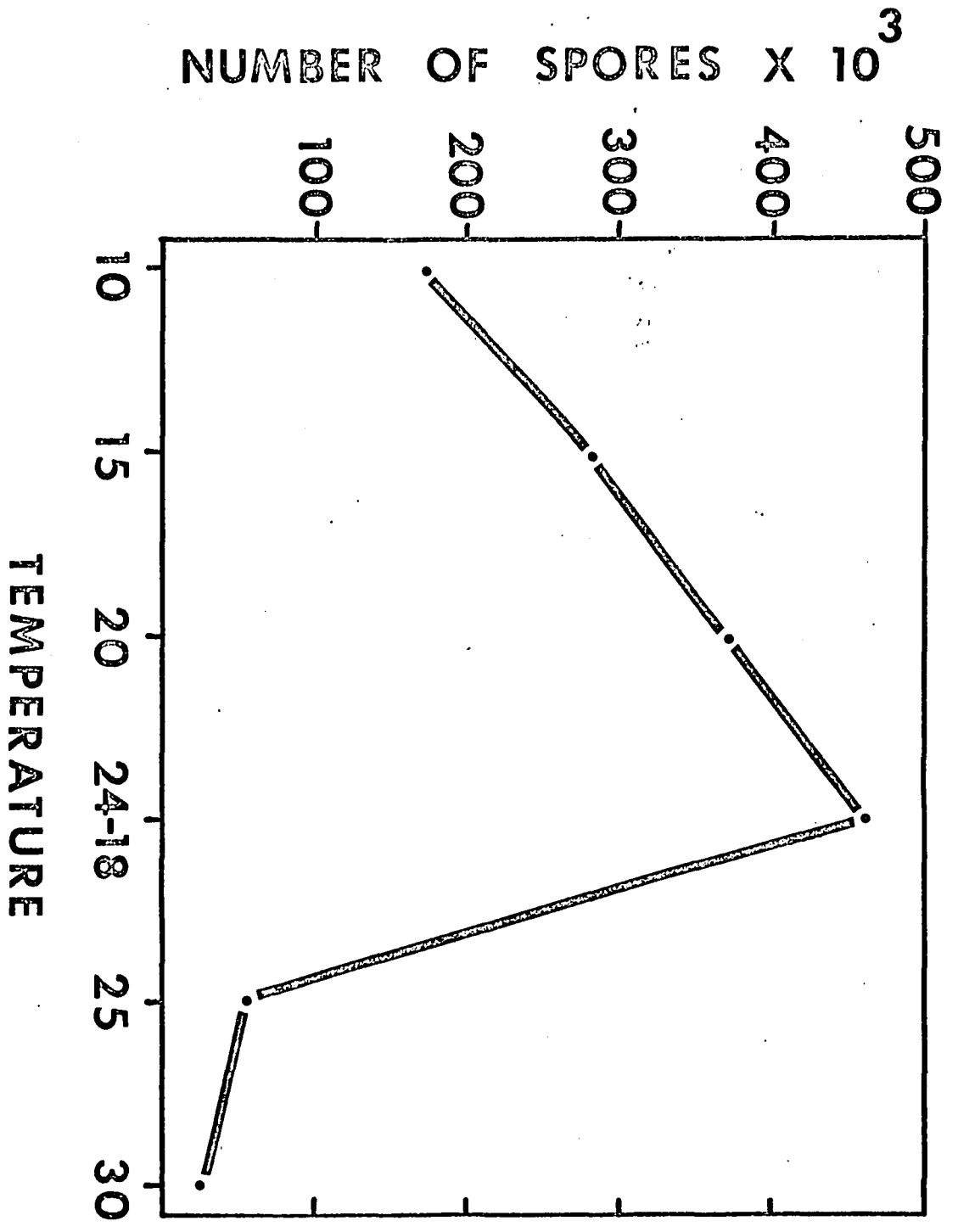

Figure 3. The effect of temperature on urediospore production by Melampsorella caryophyllacearum on Stellaria media.

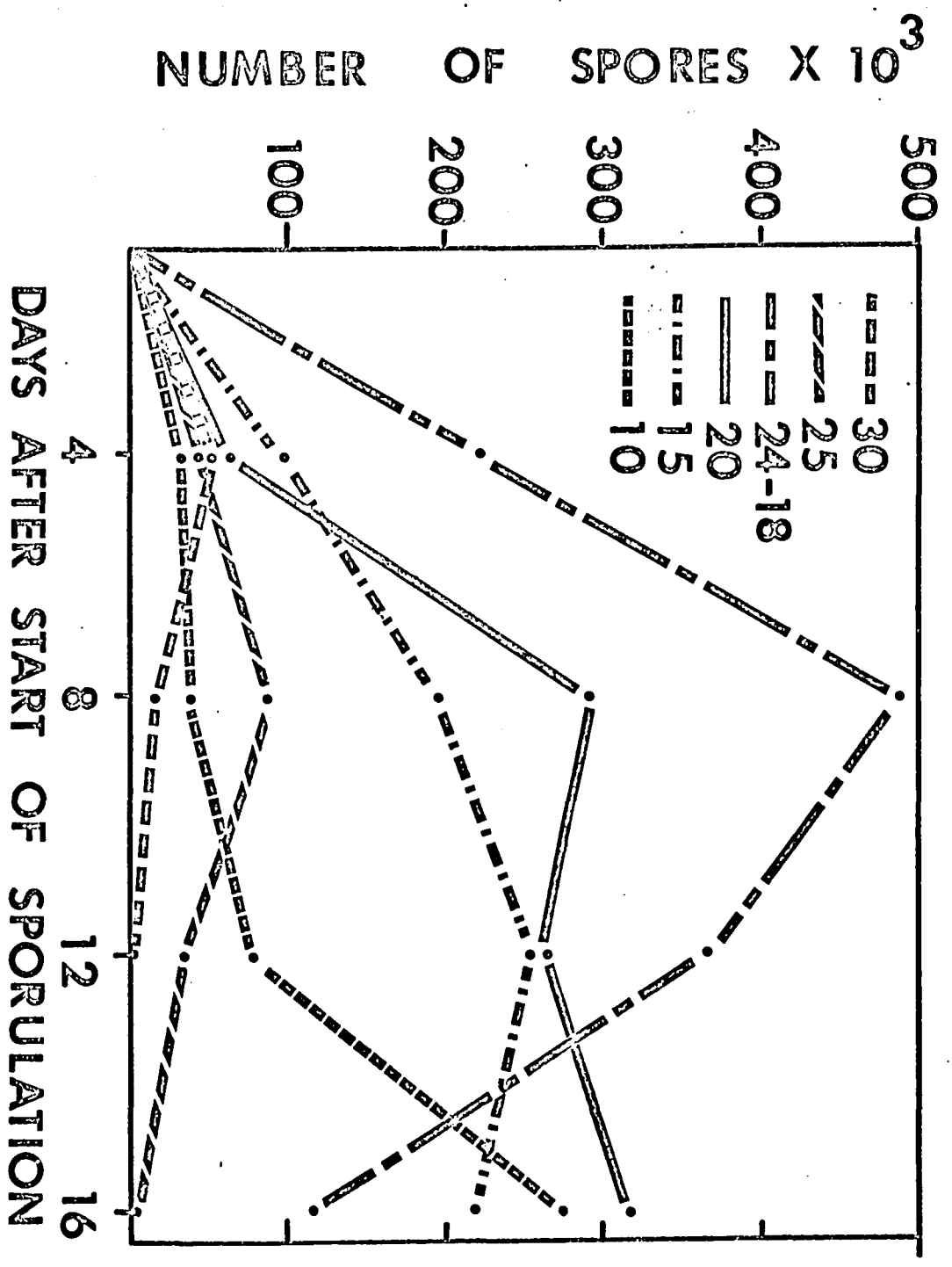

Figure 4. Urediospore production by Melampsorella caryophyllacearum on Stellaria media at different time and temperature levels.

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Penetration of *Stellaria media* leaves by *Melampsorella caryophyllacearum* and early development of the fungus.

Introduction - The pathological histology of *Melampsorella caryophyllacearum* Schroet. (= *M. cerastii*, *M. elatina*), the rust fungus that causes the yellow witches' broom disease of fir (*Abies*), has been studied in detail on fir (6) and on the uredial hosts (*Stellaria* and *Cerastium*) (2). However, no observations of penetration and early development of the fungus in the uredial hosts has been reported. This study was conducted to determine the mode of penetration and the early development of the fungus in *Stellaria media* (L.) Cry.

Materials and Methods - Greenhouse grown *Stellaria media* plants were placed at 20 C and inoculated on the adaxial surface with urediospores as described by Pawuk (3). After 36 hr, the plants were returned to the greenhouse. Leaves were removed every two days starting with placement in the greenhouse, stained in lacto phenol-cotton blue solution, and cleared in chloral hydrate (5). After clearing, the leaves were mounted in glycerine and observed for evidence of the fungus.

Results - Urediospores germinated readily on the leaf surface and penetrated through the stomates. Germ tube growth over the leaf surface seemed to be random. There appeared to be no particular attraction of germ tubes toward stomates. Often, germ tubes were observed to be growing past or across stomates without entering them. When penetration was observed

no appressoria were formed. The germ tubes grew directly through the stomates and formed vesicles in the substomatal cavity of the leaf. Usually only one germ tube entered through any one stomate. However, it was not uncommon to observe penetration of a stomate by two germ tubes and on one occasion three germ tubes had penetrated through one stomate and formed vesicles in the substomatal cavity.

A single infection hypha developed from each vesicle that was observed. Growth of the infection hyphae was intercellular and directed toward the mesophyll parenchyma cells. Within 36 hr after inoculation haustoria had developed from the infection hyphae and penetrated the mesophyll parenchyma cells. After this, the fungus started to branch and develop intercellularly throughout the leaf. Four to five days after inoculation the fungus began to form uredia. This usually occurred in a substomatal cavity following the clumping of hyphae. Uredia were produced under both the adaxial and abaxial leaf surfaces. Upon maturation, the uredia ruptured the leaf epidermis.

Discussion - The random growth of the urediospore germ tubes over the leaf surface suggests that there is no chemical stimulus given off from S. media stomates that would attract the fungus to them. The multiple penetration of some stomates by the fungus might indicate that certain stomates may be emitting some chemical or chemicals which promote penetration. On the other hand, this may be due to chance alone.

Formation of infection structures in vivo was similar to that found in vitro by Pawuk (3). In both cases no structures resembling appressoria were formed. Development of the fungus after penetration and vesicle formation was similar to that described by others in that the fungus produced infection hyphae and developed intercellularly with haustoria (1,4).

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