# THE ROLE OF TEMPERATURE IN POLLEN GERMINATION OF PINUS AND ITS BEARING ON CONTROLLED POLLINATICN PRACTICES

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The germination of Pinus pollen in vitro can be accomplished readily by a number of different techniques, all of which call for incubation for approximately t8 hours or more in a liquid, in a saturated atmosphere, or on an agar medium, with or without added nutrients.

Incubation at 25°-26°C. has been used with Pinus pollen by Smith (1939), Righter (1939), Takeuchi (1953), Echols and Mergen (1956), Hellmers and Machlis (1956), and Dillon and Zobel (1957). Tanaka (1955, 1956) has germinated Pinus densiflora pollen over a range of temperatures from 20°C. to 31°C, and has found the germination and tube growth are best at the higher temperatures. He has also shown (Tanaka 1955) that chilling Pinus densiflora pollen in a liquid medium at 8°C. for a short period prior to incubation at 2° C., caused greatly reduced germination capacity, whereas a similar treatment during germination had little or no effect. In general the temperature during incubation does not a ear to be critical, although actually little is known of the behavior of germinating pollen under a wide range of temperature conditions.

Hodgkins (1952) and Watanabe (1953) have subjected pine pollen to high temperatures in an oven, and have determined the minimum sterilization temperature for a number of species. Weger (1938) studied temperatures inside pollination bags of different materials located close to the surface of the ground, and found temperature differences as great as 30°F. between the air temperature inside closed cellophane bags, and the free air outside. More recently Nienstaedt and Kriebel (1955) reported on temperatures inside pollination bags on eastern hemlock, and they found that the high temperature inside certain types of bags at the time of pollination, materially affected the resultant seed yield.

This study has been carried out in an attempt to determine if the temperatures experienced inside pollination bags under field conditions, are in any way detrimental to the germination of pollen. This work was carried out during the regular flowering season (1957) of Austrian pine, Pinus nigra Arnold., in the Valhalla, N. Y. area. Temperatures were recorded inside female strobili isolated in various types of commonly used pollination bags, and the germination of Austrian pine pollen in vitro was followed over a wide range of temperature conditions. The results of the germination studies in vitro have been used to predict the possible limitations imposed on successful controlled pollination by the use of various types of pollination bags.

## I. The Effect of Temperature on Pollen Germination in Vitro

Materials and Methods. The Austrian pine pollen used was collected from male strobili on open grown trees which were shedding pollen at the time of collection. In all cases the pollen was stored at 4°C. and 25 percent R.H. for a short period prior to testing. Two methods of germination were used; (1) the hanging drop culture, as modified from White (1954, p. 100), and (2) a further modification

of this technique by using a small (1 mm. x 10 mm.) block of 0.75 percent BACTO agar in place of the hanging drop. A van Tiegham cell cemented to the glass slide provided the moisture chamber. The germination medium was, in all cases, double distilled water (glass distilled), and the incubation was for a period of 120 hours to ensure complete germination.

Percentage germination was based on the average counts of 150 pollen grains taken at random in each of the five replications set up at each temperature. The criterion for germination was that the length of the pollen tube must be equal to, or greater than, the small diameter of the pollen grain. Pollen tube lengths were based on ocular micrometer measurements (125X) of 10 unbranched pollen tubes, taken at random in each replicate. All averages have been expressed as a percentage of their respective controls, in order to eliminate possible variation between successive experiments.

The temperature treatments were applied in the form of; (a) a 5-day constant temperature exposure, (b) a 5-day alternation treatment, 8 hours per day at the designated temperature, and the remainder at 26°C., the control temperature[1], and (c) dry pollen was taken from the refrigerator and imbibed at a range of low temperatures for 15 minutes prior to incubation at 26°C. These latter treatments were given to simulate more closely the conditions existing in the field. For every temperature treatment, controls were run at 26°C.

Results.

(a) Incubation at Constant Temperature. The results of this experiment are shown in fig. 1. The maximum germination and tube growth occurred at temperatures around 30°C. to 32°C. This substantiated the work of Tanaka (1955), who found a similar temperature optimum for Pinus densiflora. No germination or tube growth occurred below 16°C. or above 42°C, and the fall o ff in this regard was quite sharp as these limiting temperatures were approached. High temperatures caused a reduction in tube length, frequent plasmolysis of the tubes, and often a shrinkage of the protoplasm away from the wall of the pollen tube. In order to see if there was any permanent damage to the living cell at these limiting temperatures, the cultures from the 16°C. and 42°0. treatments were returned to a favorable temperature after spending 120 hours at these constant temperatures. No pollen from the high temperature treatment could be induced to germinate by this means, indicating that this temperature causes a permanent disruption of the cell's function. The pollen from the low temperature treatment, on the other hand, germinated freely after 21. hours at the higher temperature, indicating that their function had been in no way affected by low temperature exposure.

(b) Incubation at Alternating Temperatures. By subjecting the pollen cultures during 8-hour periods to the same temperatures as under (a), intersperced with 16 hours at 26°C., the temperature limits were greatly extended, especially with respect to the lower temperature range (see fig. 2). Under these conditions germination and tube growth ceased at 46°C., however, at the lower end of the temperature scale, 59 percent germination was achieved at a temperature of -4°C , and the tube growth although less, was quite normal. This low temperature tolerance shown by the pollen is quite evident from the shape of the germination and growth curves. The damage to pollen from high temperature treatment was similar to that described in (a).

[1] In all cases the 16-hour treatment was given first.

(c) Low Temperature Imbibition. Pollen imbibed for fifteen minutes at 0°C., 4°C., and 8°C., prior to incubation under control conditions, showed a striking reduction in germination and pollen tube growth (table 1). Permanent damage to the cell resulted from the application of low temperature at this stage, and the severity of the damage increased with the decrease in temperature over this range. Imbibition at 0°C. resulted in the complete failure of germination, and many of the pollen grains revealed a severe shrinkage of the cell protoplasm. This condition was also present in the other treatments, and is probably an indication of a dead cell, as these pollen grains became waterlogged and gave no indication of ever germinating.

Table 1.--The effect of low temperature at the time of imbibition on Austrian pine pollen

| Imbibing temp.°C. | Germination percent | Tube length microns | Germination percent of control | Tube length percent of control |
|---|---|---|---|---|
| 0 | 0 | 0 | -- | -- |
| 4 | 11 | 65 | 13 | 57 |
| 8 | 40 | 94 | 47 | 83 |
| 26(control) | 85 | 114 | 100 | 100 |

II. Pollination Bags, and their Effect on the Enclosed Female Strobile

Materials and Method.

An open grown tree of Austrian pine was selected for the experiment, and to obtain comparable material at an equivalent position on the tree, receptive female strobili were attached to the buds of exposed branch tips on the southern side of the crown and kept turgid by sealing the stalk in a small rubber tube filled with water. These were changed as soon as they appeared to have lost turgidity, usually every 24 hours.

The temperature measurements were made as close to the micropylar openings as possible by means of a small thermocouple placed between the ovuliferous scales on the northern side of the strobilis. The leads from the thermocouple were connected to a s andard ptentiometer, graduated to read in °F. As a check on the accuracy of the readings on these attached strobili, a series of control readings were made on normal strobili over the course of the experiment. No significant difference was detected during this period, and as a result, all temperatures recorded were obtained from strobili affixed to the buds in the manner described. Temperature measurements were recorded from strobili under the following four conditions:

(1) No cover; exposed to direct sunlight.
(2) Enclosed in sausage casing bags (Mergen, et al. 1955).
(3) As for (2), with a thin film of aluminum paint covering the upper two thirds of the bag. A viewi ng window was also retained on the northern face of the bag.
(4) As for (2), plus a brown kraft bag as an outer covering, with ventilation holes cut in the sides and top corners.

Hourly measurements were taken from 9 am. to 4 pm., over a period of five sunny days during the pollination season. Each cover type was represented twice, and all readings were taken during periods of full sunlight, with the exception of the shade temperatures taken in the vicinity of the measurement tree.

Results.

For the five hottest hours of the day from 11 am. to 3 pm., the temperature of the strobili in the sausage casing bags averaged 100°F. Over the entire measurement period the temperature of the strobili in the sausage casing bags averaged 12°F. higher than the sausage casing plus kraft bags, 19°F. higher than the painted sausage casing bags, 20°F. higher than the uncovered strobili, and 25°F. higher than the shade temperature at the base of the tree. The cover bag which consistently gave the lowest reading was the painted sausage casing, which averaged only 1°F. above the uncovered controls. The average hourly temperatures for each cover type and the shade temperatures are presented in fig. 3.

During a brief heat wave in June., temperatures were recorded during the hottest period of the day, and a maximum temperature of 124° F. was measured inside the strobili in unprotected sausage casing bags. The shade temperatures at this same time averaged 94° F.

## Discussion

From the temperature studies conducted in vitro, assuming that they give some indication of the performance of pollen in vivo, it would appear that high temperature is more likely to be a limiting factor in pollen germination than low temperature, unless the low temperature coincides with the imbibition of the pollen following its contact with the fluid in the micropyle. Pollen germinated quite freely even when subjected to a temperature of 25°F. (-1°C.), for periods of up to 8 hours out of every 24 hours, (provided the high temperature preceded the law) however, when this same temperature was imposed on pollen during the process of imbibition, germination was completely inhibited. Temperatures above 108°F. (42°C.) were increasingly detrimental to pollen germination and tube growth when imposed on the same alternating basis, and proved lethal at 115°F. (46°C.).

Using this information it is reasonable to assume that the temperature in pollination bags when used in seasonable early summer weather, will not impose any limitations on the germinating pollen. However, during clear, hot sunny days of above average temperature, there is a real danger of lethal temperatures occurring in unshielded sausage casing of the type commonly used for pollination purposes in this country. Pollination during periods of low temperature would also appear to be inadvisable, as the wetting of the pollen at low temperature by the pollen droplet fluid, may seriously impede its subsequent germinability. Other detrimental side effects arising out of the enclosure of female strobili inside pollination bags have not been considered here.

## Summary

The germination and tube growth of Austrian pine pollen has been studied under a wide range of temperature conditions, and the upper and lower, and also the optimum temperature limits for incubation have been determined. Under conditions of alternating temperature, the limits for germination can be greatly extended, particularly in the low temperature range. high temperatures were more limiting than low temperatures, and caused permanent damage, except in the case of low temperatures applied during the critical phase of imbibition. Temperatures inside strobili enclosed in several types of pollination bags were recorded, and the performance of the pollen under these conditions predicted from observations made in vitro. During hot sunny weather, temperatures which were lethal to pollen when germinated in vitro, do occur inside strobili enclosed in sausage casing bags on the exposed portions of the crown. By protecting these bags from direct insolation, particularly with some type of reflecting paint, the temperatures can be kept within reasonable limits, and closely approximating those of the uncovered strobili.

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**Figure 1.--Germination and tube length measurements of Austrian pine when incubated under conditions of constant temperature.**

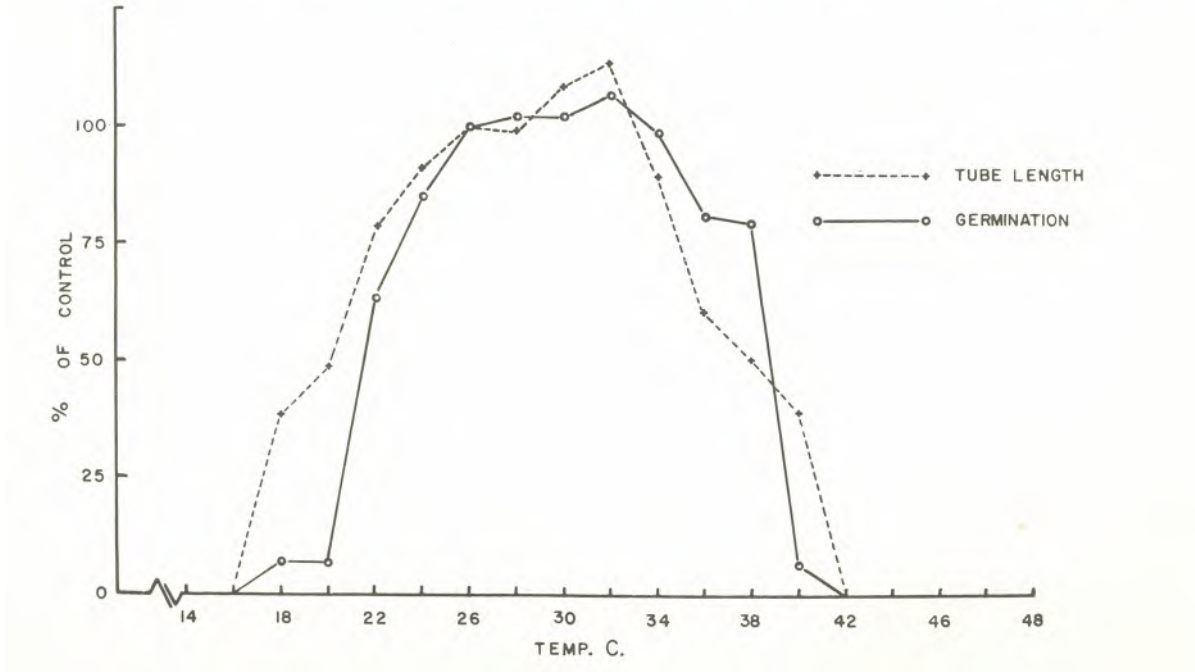

Figure 2.--Germination and tube length measurements of Austrian pine when incubated under conditions of alternating temperature.

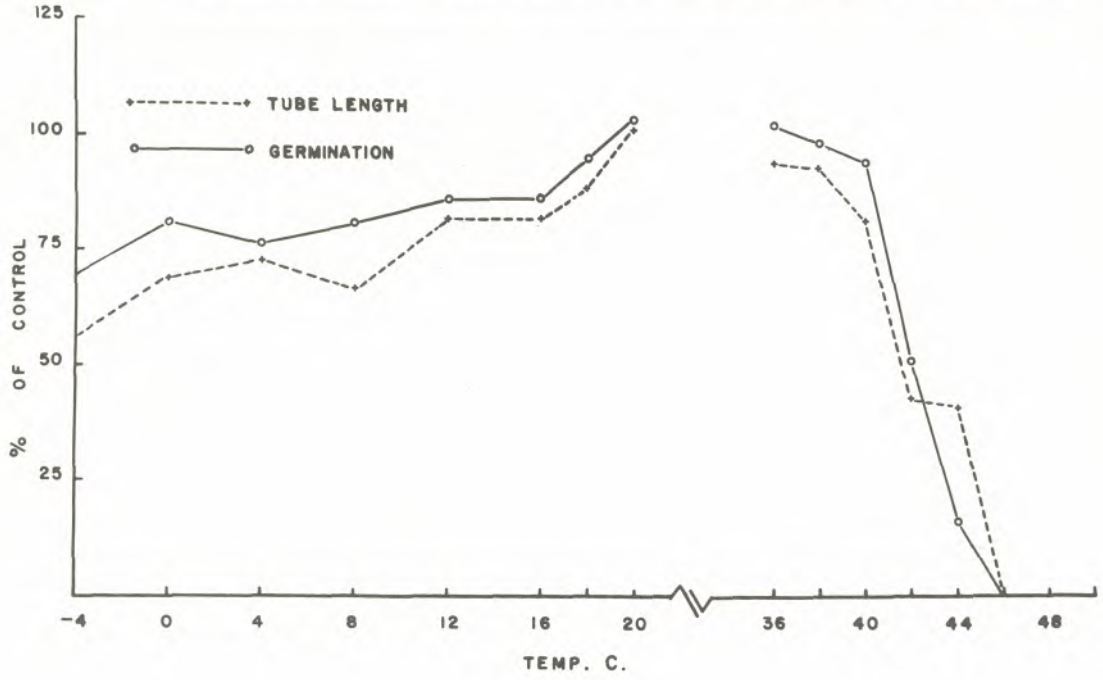


Figure 3.--Average hourly temperatures recorded inside strobili enclosed in various types of pollination bags. The ranges for S.C. and S.C. plus kraft, are shown for the 3 p.m. reading.