

Effect of Humidity during Artificial Extraction on the subsequent Vigor of Pine Pollen

by

RUSSELL A. RYKER

Central States Forest Experiment Station
U.S. Forest Station
Carbondale, Illinois

Controlled pollination of pines generally has been disappointing because cones contain too few seeds. We need to develop better techniques for collecting, extracting, and storing pollen, as well as better bagging procedures. A logical first step is to learn more about collecting and extracting pollen. In a recent study I found that extracting pollen of jack pine (*Pinus banksiana* Lamb.) and white pine (*P. strobus* L.) at a high relative humidity favors the development and maintenance of pollen vigor.

In the literature there are several guides to the collection and storage of pollen. The recommendations are designed to obtain high germination from extracted pollen. But high germination does not necessarily indicate that the pollen is also high in its capacity to complete fertilization. If freshly collected catkins are placed in an environment of dry air until the pollen is shed, it is conceivable that moisture within the catkin will be reduced. It is also conceivable that a moisture deficiency at this stage of pollen maturation could cause physiological disturbance and result in reduced pollen vigor and low fertilizing ability.

My objective was to determine how different relative humidities during artificial extraction affect pollen vigor. The length of tubes produced by pollen grains germinating in distilled water was used as a judge of vigor.

Pollen came from four jack pines and four eastern white pines. Catkins were collected from the trees just after the formation of the generative and tube cells of individual pollen grains. This was during the fourth week of April for jack pine and the third week of May for white pine. The catkins were kept in chambers maintained at relative humidities of 15 to 20, 30 to 40, 60 to 70, and 100 percent until the pollen was released.

Pollen shedding was completed in less than 48 hours from the time the catkins were collected from each tree. Pollen permitted to shed normally from catkins left on each tree was collected also and compared with the artificially extracted pollen.

All pollen was stored over calcium chloride at 4° C. for approximately 24 hours before germination tests began. This should have served to minimize moisture differences of pollen samples. Any differences in vigor could then be attributed to differences in humidity during extraction.

The germination technique used was a modification of the method described by

Righter in 1939 1/. Petri dishes were used as germination chambers. A mixture of pollen and distilled water was put into cavities cut in paraffin. Enough pollen was mixed with the water so that clumping occurred in each cavity. The effects of clumping are therefore uniform for all trials. The lids of the dishes were sealed to prevent moisture loss, and the dishes were set in an upright position during an incubation period of 65 hours at approximately 25° C. At this time the percent of pollen germinating was calculated and the length of pollen tubes measured. Tube length was considered to indicate pollen vigor more accurately than percent of germination.

Germination of different samples was so variable that these data were not subjected to statistical analysis. However, there did seem to be a tendency for higher humidity to favor germination (Figure 1).

On the other hand, pollen tube lengths were consistent, and an analysis of variance revealed statistically significant differences between humidities as well as between trees. At a given humidity, both samples from a tree had about the same tube lengths.

Jack pine pollen extracted at 60-70% or at 100% relative humidities produced tubes nearly as long as unextracted pollen collected from the tree (Table 1). White pine pollen extracted at these humidities actually produced longer tubes than unextracted pollen collected from the tree. The data thus indicate that vigor, as measured by tube elongation, is reduced by extracting pollen at low humidity.

In this study, I was unable to use the extracted pollen to actually pollinate strobili for the determination of actual fertilizing ability. But my choice of pollen tube length as a measure of pollen vigor has recently received some support from Ehrenberg 2/. He found that pollen stored at -18° C. was more likely to produce long tubes than pollen stored at higher temperatures. Moreover, more seeds developed in cones fertilized with the pollen stored at -18° C.

This study indicates that jack pine and white pine pollen should be extracted at high relative humidity to favor the development and maintenance of pollen vigor. This should not be construed, however, to mean that pollen should be stored at high humidity.

Though pollen is easier to handle at lower humidities, the increased vigor obtained should make it worthwhile to work out techniques for extracting it at high humidity.

1/ Righter, F. I. 1939. Simple method of making germination tests of pine pollen. Jour. Forestry 37:574-576.

2/ Ehrenberg, C. E. 1960. Studies on the longevity of stored pine pollen (*Pinus sylvestris* L. and *Pinus contorta* var. *murryana* Engelm). Meddelanden Fran Statens Skogsforsknings Institut 49(7):1-31.

Table 1.--Mean length of pollen tubes after 65-hour germination period

Treatment	Jack pine	Eastern white pine
	<u>Microns</u>	
Shed on tree	132	136
15-20% rel.hum.	100	113
30-40% rel.hum.	126	125
60-70% rel.hum.	128	166
100% rel.hum.	128	195

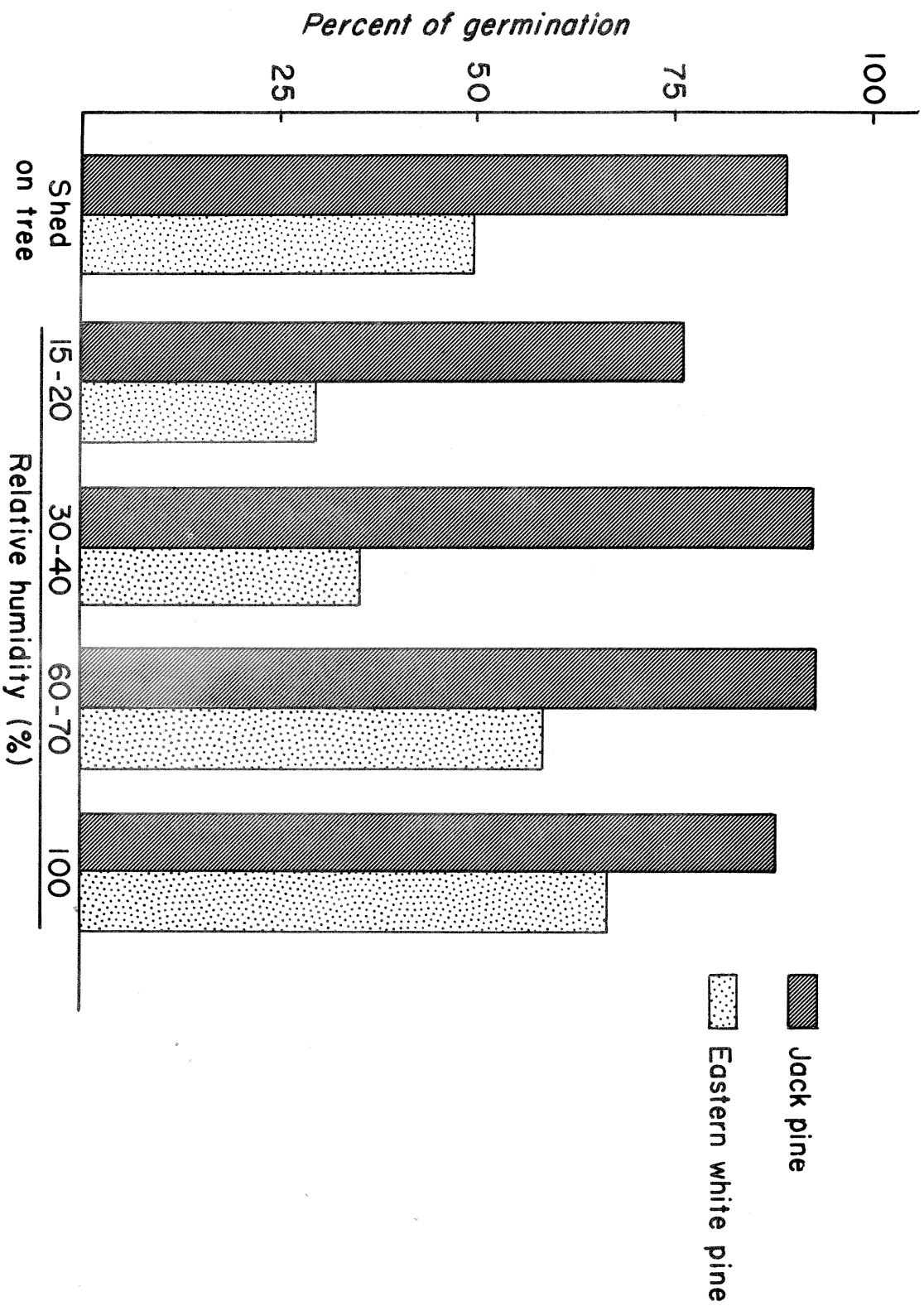


Figure 1.--Percent of pollen germinating when extracted under different relative humidities.