

emerging, stunted and had distinctive small, dark green, concavely curved cotyledons of about 1/4 normal size and having chlorotic tips (Fig. 1). Plants were spindly and growth was slow. The true leaves were puckered or cup-like with leaf margins cupped upwards, suggesting a retardation of leaf margin expansion. Although staminate and pistillate flowers were fertile, the corollas of both remained almost closed. The anthers were small and pistillate flowers had a slender, tapering neck connecting the ovary to the corolla. When grafted to normal cucumber rootstocks (2), the stunted plants grew slightly more vigorous than on their own roots. Normal plant tops grafted on the stunted rootstock grew slowly, but failed to show puckering or indications of any transmissible factor to the scion. Stunted plants produced only a few seeds when self pollinated or pollinated with normal pollen. In the field, stunted plants grew slowly and fruit failed to set during the season.

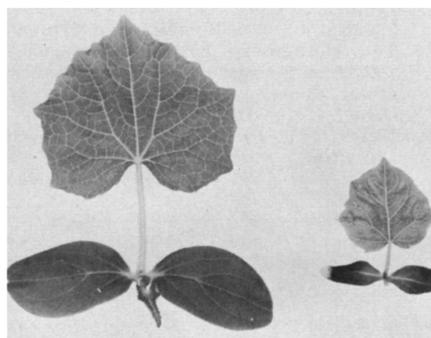


Fig. 1. Normal (left) and *sc* (right) 14-day-old cucumber seedlings.

Table 1. Inheritance of stunted cotyledon, *sc*, in cucumber.

Parents	Cotyledon		Expected ratio	X ²	P
	Normal	Stunted			
Line 9597 selfed	352	92	3:1	4.3	0.04
Line 9594 selfed	280	90	3:1	.089	0.75
Line 9597 x mutant 9597	61	56	1:1	.212	0.60
Line 9594 x mutant 9594	112	104	1:1	.296	0.65
Mutant 9594 x line 9594	54	49	1:1	.242	0.60

Because of the difficulty in producing more than a few selfed seed on the stunted plants, genetic analyses were made on the heterozygotes. From 12 normal selfed plants derived from lines 9594 and 9597, 8 progenies segregated 3 normal:1 stunted cotyledon and 4 progenies did not segregate. In progenies from 4 plants backcrossed, 2 segregated normal:1 stunted cotyledon (Table 1).

We conclude that stunted cotyledon and its accompanying syndrome is controlled by a single recessive gene which we designate *sc* for stunted cotyledon. The *sc* gene is pleiotropic for plant weakness and stunting, irregular puckering and upward cupping of the true leaves, abnormally shaped flowers, curved fruits and sickle shaped seeds. Although the mature *sc* plants may superficially resemble the "gingko leaf" mutant described by John and Wilson (3), they differ in that the gingko leaf plants are sterile and have irregularly revolute cotyledons.

The *sc* gene is maintained in the population through heterozygotes,

which cannot be distinguished from the normal plants. The seeds from the heterozygous plants either selfed or backcrossed to the mutant plants were normal whether or not they contained *sc* embryos. From 60 to 70% of the seeds obtained from mutant plants either selfed or backcrossed, were sickle shaped, and, upon germination, had sickle shaped cotyledons. The concavely curved *sc* cotyledons differ from the sickle shaped cotyledons and can be distinguished in the population.

Literature Cited

1. Aalders, L. E. 1959. Yellow cotyledon, a new cucumber mutation. *Can. J. Genet. Cytol.* 1:10-12.
2. Burnham, J., S. C. Phatak, and C. E. Peterson. 1966. Graft-aided inheritance study of a chlorophyll deficient cucumber. *Proc. Amer. Soc. Hort. Sci.* 89:386-389.
3. John, C. A., and J. D. Wilson. 1952. A "gingko leafed" mutation in cucumber. *J. Heredity* 43:47-48.
4. Shifriss, O. 1945. Male sterility and albino seedlings in cucurbits. *J. Heredity* 36:47-52.
5. Whelan, E. D. P. 1971. Golden cotyledon: a radiation-induced mutant in cucumber. *HortScience* 6:343.

Enhancement of *In Vitro* Pollen Germination of Lily with Increased Pre-inoculation Humidity¹

D. H. Simons², E. Sfakiotakis and D. R. Dilley
Michigan State University, East Lansing

Abstract. Germination of freshly dehiscid or partially dried 1-day-old pollen of lily (*Lilium longifolium* Thumb cv. Ace) was greatly enhanced by exposure to high humidity prior to inoculation in germination medium. Half the maximum response was obtained with 40% humidity or with 5 min humidifying time at saturation.

Although there have been extensive studies of storage conditions for many plant pollens as described in the reviews of Visser (6) and Pruzsinsky (4) there is little information on how to handle

pollen after storage. In practice the stored pollen is allowed to equilibrate to uncontrolled ambient conditions for unspecified periods before use. Nebel and Ruttle (2) restored the viability of apple pollen stored at laboratory temp and humidity by placing it at 2 to 8°C and 80% relative humidity for a week or more. In using stored gladiolus pollen Pfeiffer (3) improved seed set by conditioning the pollen, which had been stored at room conditions for 1 or more days, by transferring it to 10°C and 65% humidity for 3 or more days prior to inoculation. Duffield and Snow (1) increased the % germination of pine pollen, which had been stored for 413 days at 0°C and 10% humidity, by humidification at 75% humidity and 4°C for only 12 hr. Visser (6) increased

germination and tube length and decreased bursting of apple and pear pollens, which had been stored at 10% humidity and 2 to 4°C for 7 months, by humidifying at 80% for 1 day before testing. In the aforementioned studies humidification at low temp was used and in most instances the pollen had been stored dry for long periods.

Our objective was to show that exposing lily pollen to high humidity prior to inoculation is essential for maximum % germination.

'Ace' lily pollen from greenhouse grown plants was germinated in continuously ventilated hanging drop cultures (5) at 27°C. Preliminary experiments revealed that pollen taken from dehiscid anthers and kept under dry conditions before inoculation into hanging drops at 27°C germinated very poorly. Exposure to moist air for a few hours before inoculation markedly improved germination.

To analyze the effect of pre-inoculation humidity, we placed pollen in desiccators at different relative humidities at 27°C. A range of humidities was established by injecting calculated amounts of water into

¹Received for publication June 22, 1972. Michigan Agricultural Experiment Station Journal Article No. 5958.

²Present address: Laboratorium voor Plantenfysiologie, Landouwhogeschool, Arboretumlaan 4, Wageningen, The Netherlands.

desiccators previously dried with calcium sulfate and by then allowing enough time for vaporization of the water. The actual humidities were measured electronically (Model 15-4050E Hydrodynamics, Inc., Silver Springs, Md.) by suspending the sensing elements from a rubber stopper in the desiccator lid. When the humidities had stabilized, pollen (from anthers recently dehisced under room conditions) was transferred quickly into each desiccator in a small beaker suspended from the stopper. The pollen was maintained at the various humidities for 2.5 and 4.5 hr before inoculation of the hanging drops. As there were no differences in % germination for the 2 humidification times, the data were pooled. Germination was very poor at 15% pre-inoculation humidity but progressively improved to 85% as humidity was raised to 65% (Fig. 1).

We also determined the time course of humidification effect. Pollen (1-day-old) was exposed to 8% humidity for 1 hr then transferred to a desiccator at 100% humidity at 27°C. Samples were then taken during the first 60 min for inoculation into the hanging drop cultures. No pollen grains germinated when transferred directly from the dry conditions to the germination medium and % germination increased rapidly with increasing time of high humidity exposure up to 15 min (Fig. 2). There

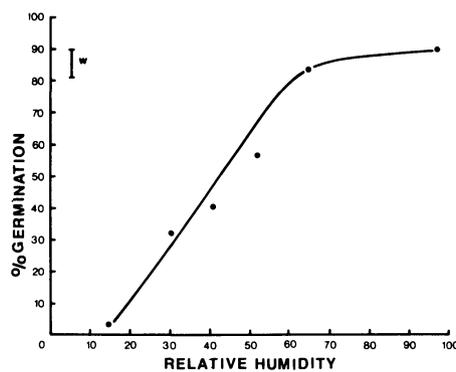


Fig. 1. Effect of pre-inoculation humidity % germination of lily pollen. (Bar indicates Tukey's ω -test at $P = 1\%$).

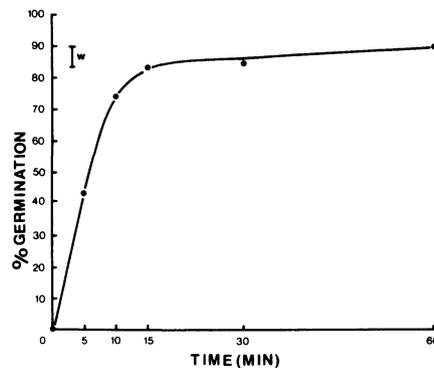


Fig. 2. Effect of exposure time at 100% humidity on % germination of lily pollen. (Bar indicates Tukey's ω -test at $P = 1\%$).

was relatively little further increase with longer exposure time.

Our experiments show that the relative humidities that are normally encountered in laboratories can seriously limit the germination of lily pollen, and that this can be quickly overcome by exposing the pollen to high humidity before inoculation. Further study is necessary to establish the consistency of this phenomenon in different species of plants and to learn if pollen germination and, therefore, seed set are limited by low relative humidities encountered in arid climates.

Literature Cited

1. Duffield, J. W., and A. G. Snow. 1941. Pollen longevity of *Pinus strobus* and *Pinus resinosa* as controlled by humidity and temp. *Amer. J. Bot.* 28:175-177.
2. Nebel, B. R., and N. L. Ruttle. 1936. Storage experiments with pollen of cultivated fruit trees. *J. Pomol.* 14:347-359.
3. Pfeiffer, N. E. 1939. Life of gladiolus pollen prolonged by controlled conditions of storage. *Contrib. Boyce Thompson Inst.* 10:429-440.
4. Pruzinsky, S. 1960. Über Trocken- und Feuchtluftresistenz des Pollens. *Akad. Wiss., Math. Naturwiss. Kl. Abt. I.* 169:43-100.
5. Sfakiotakis, E., D. H. Simons, and D. R. Dilley. 1972. Pollen germination and tube growth: Dependent on carbon dioxide and independent of ethylene. *Plant Physiol.* 49:963-967.
6. Visser, T. 1955. Germination and storage of pollen. *Meded. Landbouwhogesch., Wageningen.* 55:1-68.

Influence of Temperature on Pollen Tube Growth and Initial Fruit Development in 'd'Anjou' Pear¹

W. M. Mellenthin, C. Y. Wang and S. Y. Wang
Mid-Columbia Experiment Station, Hood River, Oregon

Abstract. Pollen tube growth in styles of 'd'Anjou' pear (*Pyrus communis* L.) is largely dependent on prevailing temp. At 21°C the process was completed within 24 hr, while at 15.5° and 10° growth was completed by 72 and 120 hr respectively. The initial fruit development 14 days following pollination was greatly influenced by day temp (ranging from 13° to 23.4°) during anthesis.

The chain of events beginning with the opening of a flower to fruit set at harvest is influenced by many factors. The pollination-fertilization processes in the chain are probably the most important links. There have been numerous investigations on the requirements of pear pollination (2, 5, 7, 9, 11, 13, 14). These relate to coincidence of bloom, pollen viability, parthenocarpy, self-fruitfulness, cross

pollination, pollinizer location, and spacing, yield, maturity and quality. Although temp is universally recognized as an important factor affecting these processes (3, 6), little data pertaining to orchard temp during anthesis have been reported. This study was initiated to determine the influence of varying temp on pollen tube growth and initial fruit development in the 'd'Anjou' pear.

'D'Anjou' pear branches were forced to the "popcorn" stage under laboratory conditions at 15.5° - 21°C, then emasculated and each cluster reduced to 5 healthy flowers prior to pollinating with fresh 'Bartlett' pollen. The pollinated branches were divided in 3 groups and placed in constant temp cabinets at 10°, 15.5° or 21°C. Air was bubbled slowly through each bucket containing water and branches to keep the pollinated flowers from wilting. Five flowers were picked at random from each cabinet at 24-hr intervals for the

first 72 hr. Subsequent sampling was done at 48-hr intervals. The flowers were infiltrated under vacuum with standard FAA fixing solution. Pollen tube growth was measured according to Martin (10). Callose fluorescence was observed with a Leitz Labolux D fluorescence microscope using a HBO 200 high pressure mercury lamp with a UV filter (200 mm UGI). Pollen tubes were measured as the no. of fields (10× objective, 10× ocular) in which the longest pollen tube was visible, divided by the total no. of fields for a given style, then expressed as %.

Table 1 shows the influence of temp on the rate of pollen tube growth in 'd'Anjou' pear styles. Pollen tubes had grown to the base of the style within 24 hr at 21.1°C, by 72 hr at 15.5°, and by 120 hr at 10°. Williams (16) reported

Table 1. Effect of temp on pollen tube growth in style of 'd'Anjou' pear flower.

Time (hr)	Pollen tube growth (tube length/style length × 100)		
	Temp (°C)		
	10.0	15.5	21.1
24	0.0	3.8	100.0
48	1.0	41.3	100.0
72	32.0	100.0	100.0
120	100.0	100.0	100.0
168	100.0	100.0	100.0

¹Received for publication July 1, 1972. Technical Paper No. 3380. Oregon Agricultural Experiment Station.