# Cold Temperature Treatment As a Means of Breaking Seed Dormancy in Hemerocallis[1]

By R. A. Griesbach

Dormancy of freshly harvested daylily seeds has been demonstrated to be the consequence of an insufficient amount of oxygen reaching the embryo (1, 2). Thus, germination follows within a few days after removal of some or all of the enveloping structures from around the embryo. In order that a high percentage germination be obtained, a greater portion of the embryo must be exposed directly to air at relatively low (60°F.) than at high (70°–80°) temperatures. Such response to temperature might either be the result of a decrease in the permeability of the enveloping structures to oxygen or to an increase in the oxygen requirement at low temperatures.

Hybridizers usually sow daylily seeds in open soil in autumn or in flats which are in turn subjected to a cold treatment and then later transferred to a greenhouse in early spring for germination.

The following is a report of various aspects of stratification of daylily seeds. The experimental work described in the present paper was carried out during the autumn, winter, and spring months of 1954-1955 with the purpose of obtaining subject matter for the writer's doctoral dissertation. The investigations were carried out in the botany laboratories, greenhouses and gardens of the University of Chicago under the direction of Professor Paul D. Voth.

EFFECT OF STRATIFICATION TEMPERATURE ON GERMINATION—Of a mixture consisting of many varietal and seedling crosses, lots of 50 or 100 fully-imbibed seeds were placed on the surface of moistened filter paper in Petri dishes and maintained for two weeks at each of nine different temperatures ranging from 20° to 60°F. Following stratification the seed lots were transferred to 60°F. for germination (Table 1).

*Table 1:* Effect of Stratification Temperature on Germination. Percentage germination during a 20-day incubation period at 60°F. following two weeks of cold treatment at various temperatures. Fifty or 100 seeds/lot.

| Stratification Temperature (°F.) | 20° | 25° | 32° | 34° | 38° | 45° | 50° | 55° | 60° Control |
|---|---|---|---|---|---|---|---|---|---|
| Percentage Germination | 7 | 2 | 56 | 75 | 59 | 37 | 9 | 4 | 2 |

As seen in Table 1, the highly effective temperature range for stratification of daylily seeds lies between 32° and 45°F. with the optimum slightly above freezing.

EFFECT OF DURATION OF STRATIFICATION ON GERMINATION—In one experiment a mixture of seeds all derived from a common pollen parent ('Evelyn Claar') was brought to saturation, divided into 6 lots,

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

and then stratified in Petri dishes at 34°F. for 1, 2, 4, 8, 16 and 32 days. At the end of the specific stratification period the individual lots of 100 seeds each were incubated at 60°F. (Table 2).

*Table 2:* Effect of Length of Cold Treatment on Germination. Percentage germination during a 20-day incubation period at 60°F. following stratification at 34°F. 100 seeds/lot.

| Duration of Stratification (Days) | 0 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|
| Percentage Germination | 4 | 19 | 18 | 36 | 81 | 91 | 93 |

As indicated in Table 2, daylily seeds, when stratified under ideal temperature conditions (34°F.), after-ripened very quickly, almost all seeds of a given population having reached the capacity to germinate at 60°F. after only 16 days of treatment. It might be mentioned here that very seldom can 100% germination be realized since it has been observed in *Hemerocallis* that some seeds are without embryos (2). The frequency of such normally appearing, but germless seeds varies with the particular parents in question. In one case, V 42 (Voth seedling) selfed, 33% of the seeds were devoid of embryos. Out of 4,747 mixed seeds that were peeled, cut into, or excised during various experiments, 4.2% were found to be without embryos. The cause of this condition in daylilies, so far as is known, has not been determined.

On November 1, 1954, in another experiment dealing with the length of stratification, seeds of four different crosses were sown in soil in greenhouse flats, watered, and then placed outdoors and mulched. One flat, containing 100 seeds, acted as the control for the experiment; being incubated directly at room temperature (65°–72°F.) without a cold treatment. Of the remaining flats, one was removed from cold storage each month and incubated for two weeks at the same temperature (Table 3).

*Table 3:* Effect of Length of Cold Treatment on Germination. Percentage germination (average of 4 crosses) during a 14-day incubation period at 65°-72°F. 100 seeds/lot.

| Stratification Period (Months) | 0 Nov. 1 | 1 Dec. 1 | 2 Jan. 1 | 3 Feb. 1 | 4 Mar. 1 | 5 April 1 |
|---|---|---|---|---|---|---|
| Percentage Germination | 47 | 63 | 64 | 72 | 42 | 10 |

The slightly erratic nature of the results summarized in Table 3, obtained following natural stratification, can probably be explained on the basis of outdoor temperature fluctuations during the 5 months duration of the experiment.

The relatively low percentage emergence obtained even after 3 months of cold treatment might well reflect a certain degree of lethality of seeds arising from exposure to freezing temperatures. After the incubation period ungerminated seeds were unearthed and cut open to determine why they had not emerged. In contrast to the control lot, there were no viable seeds remaining after one or more months of cold treatment; lack of emergence being due either to the embryoless condition or loss of viability as indicated by rotting. As indicated by unearthing, the progressive decrease in percentage emergence

with increased duration of cold treatment could in part be attributed to lethality of ungerminated seeds and apparently partially to the death of partially germinated seeds. As will be seen later in the present paper, seeds receiving a prolonged cold treatment are able to begin germination at temperatures that are only slightly above freezing. Under conditions of natural stratification, then, germination might well begin during a relatively warm period in early spring; and if followed by very cold weather some of the recently germinated seeds might possibly be killed. Had the flats in the above experiment not been mulched, the percentage emergence undoubtedly would have been still lower.

EFFECT OF TEMPERATURE AND DURATION OF STRATIFICATION ON GERMINATION AT VARIOUS INCUBATION TEMPERATURES—A mixture of seeds was obtained by combining some 150 cross-pollinated lots. A control portion of the mixture was stored (dry) at 70°-72°F. while other fractions were brought to full saturation in tap water and then placed in cold storage at 25°, 38°, or 50°F. Upon completion of the desired period of stratification (1, 2, 4, or 8 weeks), one lot of 40 or 80 seeds in a Petri dish was placed at each of 5 different incubation temperatures 50°, 60°, 70°, 80° and 90°F.) for two weeks (Table 4).

*Table 4*: Effect of Temperature and Duration of Stratification on Germination at Various Incubation Temperatures. Percentage germination during a 2-week incubation period.

| Length of cold treatment (weeks) | Incubation Temperature (°F.) 50° | 60° | 70° | 80° | 90° | Temperature of cold Treatment (°F.) |
|---|---|---|---|---|---|---|
| 0 (Control) | 0 | 2 | 43 | 28 | 0 | |
| 1 | 0 | 2 | 20 | 12 | 0 | |
| 2 | 2 | 22 | 37 | 29 | 1 | 25° |
| 4 | 1 | 7 | 36 | 17 | 0 | |
| 8 | 2 | 25 | 30 | 0 | 2 | |
| 0 (Control) | 0 | 2 | 43 | 28 | 0 | |
| 1 | 0 | 10 | 70 | 40 | 0 | |
| 2 | 5 | 65 | 97 | 53 | 0 | 38° |
| 4 | 29 | 88 | 92 | 51 | 2 | |
| 8 | 86 | 95 | 90 | 69 | 16 | |
| 0 (Control) | 0 | 2 | 43 | 28 | 0 | |
| 1 | 0 | 6 | 35 | 32 | 0 | |
| 2 | 0 | 6 | 50 | 66 | 0 | 50° |
| 4 | 5 | 25 | 90 | 57 | 5 | |
| 8 | 17 | 72 | 95 | 80 | 0 | |

Increased percentage germination at the 50° and 60°F. incubation temperatures following stratification (Table 4) indicates that at least some after-ripening of daylily seeds can occur at temperatures below freezing. Decreased percentage germination at the 70° and 80°F. incubation temperatures following low-temperature stratification, on the other hand, can be explained by

the high degree of lethality following subjection of imbibed seeds to freezing temperatures. From these results it is apparent that in practice one should attempt to maintain the stratification temperature above freezing.

Stratification carried out at 38°F. (Table 4) was highly effective in increasing percentage germination over the entire range of incubation temperatures dealt with. A high percentage germination (86%) was obtained at 50°F. following two months of cold treatment; and in other experiments appreciable germination occurred even at the 38° stratification temperature when held at that temperature for one or more months.

Stratification at 50°F. likewise resulted in after-ripening of daylily seeds, however, longer periods of cold treatment were necessary than at 38°F. for equivalent responses at the various incubation temperatures dealt with.

INFLUENCE OF DEGREE OF SATURATION ON EFFECTIVENESS OF COLD TREATMENT—A mixed population of seeds was weighed and then brought to full saturation by keeping the seeds moist for a period of 2 days at 70°-72°F. and 3 days at 60°F. The seeds were then blotted until no surface moisture remained and reweighed. Since the amount of water held by saturated seeds above that of air-dry seeds could thereby be determined the weight of seeds of any desired degree of saturation could easily be calculated. Thus, by merely placing the moist seeds in an open container in the laboratory to dry, seed lots were progressively obtained that were ¾, ½, and ¼ saturated along with a control lot that was dried down to the original air-dry weight. One lot (50 seeds) of each degree of saturation was then placed at 18°, 25° and 38°F. for two weeks. During the stratification period the seed lots were maintained at approximately their original levels of saturation by placing them on slightly moistened filter paper in Petri dishes and wrapping each lot separately in sealed plastic bags. Following the cold treatment the seed lots were gradually transferred to the 60°F. incubation temperature where they remained for 20 days (Table 5).

*Table 5:* INFLUENCE OF DEGREE OF SATURATION ON EFFECTIVENESS OF COLD TREATMENT. Percentage germination following 20 days incubation at 60°F. 50 seeds/lot.

| Stratification Temperature (°F.) | Degree of Saturation | | | | |
|---|---|---|---|---|---|
| | 0 (air-dry) | ¼ | ½ | ¾ | Fully Saturated |
| 18° | 2 | 0 | 0 | 0 | 0 |
| 25° | 0 | 12 | 14 | 58 | 16 |
| 38° | 2 | 78 | 76 | 70 | 72 |

Thus, stratification at 18°F. proved fatal to all seeds (as indicated by rotting) except those which had undergone the treatment in the air-dry condition. Of the 50 seeds in the latter lot only one germinated within the 20-day incubation period.

Stratification at 25°F. likewise proved fatal to many of the moist seeds. As indicated by the results summarized in Table 5, the degree of saturation apparently was an especially important factor in determining the extent of lethality at this temperature.

Degree of saturation apparently was not too critical a factor in the after-ripening of seeds maintained at the 38°F. stratification temperature providing the seeds undergoing cold treatment had a somewhat greater moisture content than that of air-dry seeds.

Although low-temperature dry storage, as indicated in this particular experiment as well as others not herein described, does not result in the breaking of dormancy in daylily seeds; preliminary experiments carried out in conjunction with Mr. Orville Fay of Northbrook, Illinois, indicate that seeds may be kept perfectly viable in this condition for at least two years and most likely much longer.

NECESSITY OF OXYGEN DURING STRATIFICATION—In order to determine the necessity of oxygen during cold treatment, seeds were stratified for 2 weeks at 38°F. under conditions of high and low oxygen supply. Following the cold period the various seed lots were incubated for 30 days at 50°F.—a temperature that is suitable only for after-ripened seeds.

In one instance seeds were placed in an atmosphere of nitrogen; and in another case, one lot of 50 seeds was submerged under 10 cm. of previously boiled water in a sealed container. In contrast, seeds of one lot were peeled prior to stratification while another lot underwent stratification in full oxygen pressure (Table 6).

*Table 6:* EFFECT OF OXYGEN ON AFTER-RIPENING. Percentage germination during a 30-day incubation period at 50°F. 50 seeds/lot.

| Treatment | Control (Intact) | Oxygen (Intact) | Nitrogen (Intact) | Submerged (Peeled after cold treatment) | Peeled prior to cold cold treatment | Peeled after cold treatment |
|---|---|---|---|---|---|---|
| Percentage Germination | 48 | 40 | 2 | 20 | 76 | 72 |

The results obtained in this particular experiment show that aeration is necessary for effective after-ripening of daylily seeds. However, increasing the amount of oxygen in the vicinity of the embryo beyond normal either by increasing the oxygen pressure or through removal of the seed coat, apparently was without stimulatory effect. Undoubtedly the oxygen requirement for the after-ripening process is quite low and can therefore ordinarily be satisfied by very slow diffusion through the relatively impermeable enveloping structures.

EFFECT OF DRYING OUT OF SEEDS SUBSEQUENT TO STRATIFICATION—In order to determine whether the after-ripening changes would endure drying out of the seeds following stratification, seeds were given a 3-week cold treatment at 38°F. and then dried by exposing them to air in the laboratory for 2 or 4 weeks. Following the dehydration period, 100 seeds of the two lots were again moistened and incubated for 2 weeks at 60°F. along with a control lot which had not been dried subsequent to the cold treatment (Table 7).

*Table 7:* Effect of Dehydration Following Stratification. Percentage germination during a 20-day incubation period at 60°F. 100 seeds/lot.

| Dehydration Period (Weeks) | 0 | 2 | 4 |
|---|---|---|---|
| Percentage Germination | 62 | 85 | 78 |

Since 4 weeks of dehydration following stratification was not at all detrimental, it is likely that the germination capacity of after-ripened seeds would persist even over a considerably longer period. This fact might be utilized in the formulation of a procedure whereby seeds are given a controlled cold treatment in a refrigerator, then dried and stored until early spring at which time they are sowed directly in open soil.

CONCLUSION—It is hoped that results of certain of the experiments described in this paper can be of some definite practical value to the inexperienced breeder with seed germination problems. Undoubtedly many experienced daylily hybridizers have developed methods of seed germination which consistently give good results year after year. Obviously, the present paper for the most part is merely a verification of what these people already know or take for granted.

Department of Biological Sciences
DePaul University
Chicago, Illinois

*References Cited*

[1] Griesbach, R. A. Some aspects of *Hemerocallis* seed germination. Hemerocallis Yearbook, Vol. 9:26–29. 1955.

[2] Griesbach, R. A. *The Nature of Dormancy and Seed Germination in Hemerocallis.* Thesis. University of Chicago. 1955.