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JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE

Volume 46, Number 3, July, 1971

SEED DORMANCY AND GERMINATION IN *LEAVENWORTHIA STYLOSA* (CRUCIFERAE)

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ABSTRACT

Seeds of *Leavenworthia stylosa* are shed in late may or early June, but fail to germinate until September. Investigations of possible reasons for this summer quiescence gave the following results. Dormacy appeared to be due to mechanical resistance of seed coats to expansion of the embryos. Germination occurred with equal magnitude in darkness and in light in both leached and unleached seeds. Germination was not enhanced by treatment with KNO_3, 3-IAA or H_2O_2.

Although initiation of germination in the field coincides with an average daily temperature of 18 to 23C, germination of freshly harvested seeds at such temperatures is very low (1-4%). Laboratory-stored seeds at ages from 1 to 8 months germinated only to 20 to 45% under various temperature regimes although 4-month-old field-stored seeds germinated from 80 to 90% when brought into the laboratory. Five-month-old laboratory-stored seeds reached germination percentages comparable to those of field-stored seeds after an initial moist period of 1-5 days followed by a 20- to 25-day dry period at 24C. After this treatment germination occurred promptly upon re-wetting of seeds.

Complete termination of quiescence and initiation of germination in the field depend on the combined effects of at least 3 interdependent factors, alternate wetting and drying, lapse of time, and occurrence of moderate automnal tempartures

INTRODUCTION

The herbaceous winter annual, *Leavenworthia stylosa* A. Gray, is an endemic member of the cedar glade flora that occurs in the Central Basin of middle Tennessee. Rollins (1963) assumes it to have originated in the Basin and considers it to be the basic member of the n=15 group of *Leavenworthia* special. The objective of the present study was to investigate conditions influencing field germination of its seeds.

Seeds are shed in late May and early June, a process that scatters them only a few inches from the parent plant. They lie on the surface or slightly buried in thin soil through the summer, when soil surface temperatures may range from 31 to 50C (Freeman 1933). In September, profuse germination follows the simultaneous occurrence of an average daily temperature of 19 to 23C (Fig. 1) and a heavy rain shower. Seedlings develop into rosettes having 4-7 leaves and, during the winter months, are often exposed to frost-heaving, ice

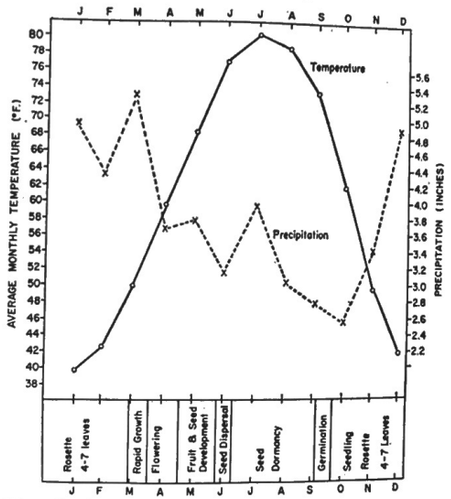

Figure 1. A summary of average monthly temperatures, average monthly precipitation and life cycle of the winter annual *Leavenworthia stylosa* in the cedar glades of Middle Tennessee. Climatological data, based on a 29-year period (1921-1950), were obtained from the United States Weather Bureau Station located near the study area.

and snow. Rapid growth occurs in March followed by flowering in late March and early April. Pollination of the highly self-service species may be effected by wasps, bees, and flies, all of which have been observed visiting the flowers. Seeds mature by late May or early June.

Middle Tennessee lies in an area with a cool season maximum precipitation pattern (Quarterman 1949; Trewartha 1954). Cedar glade soil is saturated, therefore, during the winter and early spring months after which rapid drying results in desiccation during August, September and October. The winter annual habit of *Leavenworthia* is well adapted to utilize the wet season advantageously and to persist through the summer drouth as quiescent seed (Fig. 1).

Large populations of *L. stylosa* are frequent in open cedar glades on soil one-fourth to three inches in depth (Gattinger 1901; Harper 1926; Freeman 1933; Quarterman 1950 a,b). *Leavenworthia* plants usually occur in low places or small ditches along which water flows freely. Dense populations occurring where flowing water is blocked by clumps of grass roots or other obstacles indicate that water probably serves as the chief agent of seed dispersal.

EXPERIMENTAL PROCEDURES AND RESULTS

Seeds were collected from a large population in Rutherford County, Tennessee, and stored at laboratory temperatures (24-39C). Prior to sowing, each lot of seeds was dusted lightly with Semesan to control fungal contaminants. Demineralized or distilled water was used in all experiments except where otherwise noted.

SUMMER DORMANCY AND GERMINATION TEMPERATURES

To investigate the effects of after-ripening on germination, two series of successive plantings were made on filter paper and place, respectivey, (1) in a laboratory with naturally fluctuating temperatures that closely resermbled readings at a U.S.

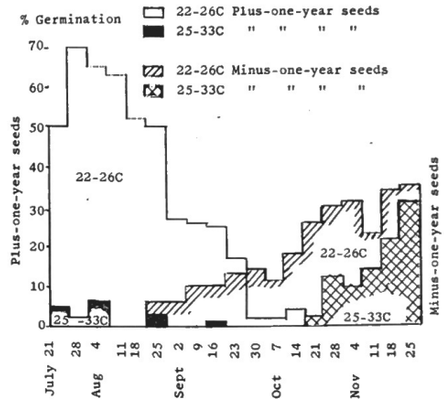

Figure 2. Effect of temperature on germination of plus- and minus-one-year seeds. (Plus-one-year after 45 days of incubation; minus-one-year, after 75 days.)

Weather Bureau Station in a glade area (25 to 33C, with occasional extremes of 17C and 33C), and (2) in an air-conditioned room whose temperatures were controlled within a narrower range (22 to 25C).

Seeds were from harvests of two consecutive years and ranged in age from one month (minus-one-year) to a year and one month (plus-one-year) at time of planting. Each planting consisted of 100 seeds of each age group under each temperature regime.

Minus-one-year seeds

Under naturally fluctuating temperatures, there was no germination of minus-one-year seeds until they had after-ripened for nearly three months. Germination was meager (31%) even after 75 days (Fig. 2). Under controlled temperatures there was sparse germination (1 to 4 %) of minus-one-year seeds in all plantings after two months of after-ripening; germinability increased with age during the summer and peaked at 35% in November.

Plus-one-year seeds

Under both temperatures regimes, all germination of plus-one-year seeds occurred within 45 days of planting. In the fluctuating natural temperatures germination was negligible (1 to 6%); in controlled temperatures germination was good in seed planted during the early part of the summer, maximum germination (70%) occurring in the lot sown on June 14 (Fig. 2). After that peak there was a continuing decrease in germinability until all germination ceased in September.

One may assume from these experiments that *Leavenworthia stylosa* seeds undergo a summer dormant period of about three months and that they do not remain viable under laboratory storage conditions for much more than a year (Fig. 3). Dry

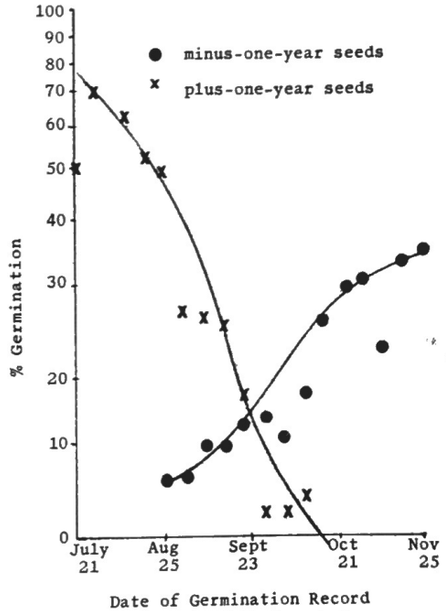

Figure 3. Effect of age of seed: Total germination percentage of minus-one-year and plus-one-year seeds through summer and fall months. Plantings were made every 2 weeks begnning June 7. Total germination of plus-one-year seeds was attained after 45 days; minus-one-year seeds after 75 days. Temperature range 22-25C.

laboratory storage may break dormancy in plus-one-year seeds up to 70% (Fig. 2).

Germination temperature requirements were more nearly met by temperatures ranging from 22 to 25C than by naturally fluctuating summer temperatures (25-33C), sugesting that high summer temperatures inhibit germination of both minus- and plus-one-year seeds.

BREAKING DORMANCY

(1) Seed coats. *Leavenworthia* seed coats are brittle but not thick and hard. Seeds swell quickly when wet, indicating a high degree of permeability and elasticity of the coats. Scarification of seed coats failed to increase germination percentage. Flemion's (1938) excised embryo viability test was applied to freshly harvested and to 3-month-old laboratory-stored seeds. Growth occurred after four days at 22-30C in 50% of the embryos from freshly harvested seeds and in 75% of those from 3-month-old seeds, as compared with no germination after 45 days from intact seeds. Although apparently permeable, seed coats appear to be contributing agents in the quiescence of freshly matured seeds..

(2) Inhibitory substances.

(a) Leaching. Two-month-old *Leavenworthia stylosa* seeds were leached in the dark in running tap water (25C) for 48 hours, then removed under a safe-light (McDonough 1963) into darkened germination plates, 100 seed per plate. Unleached controls were placed in dark and light (12 hour photoperiod), respectively. All seeds were placed under a 7-25C thermoperiod, a temperature regime that approximates field conditions in middle Tennessee during September when *Leavenworthia* seeds germinate in nature.

After 7 days, no germination had occurred among the leached seeds and 2% had germinated in both dark and light controls, indicating that summer dormancy or quiescence in *Leavenworthia stylosa* is not caused by the presence of water-soluble chemical inhibitors in the seeds.

(3) Chemical agents. Three chemical agents were used in efforts to break the dormancy of *Leavenworthia stylosa* seeds. Two 100-seed lots were used in each treatment, one darkened, one lighted. Treatments consisted of: KNO_3 (0.1%; 0.2%); 3 IAA (100, 500, and 1000 ppm); H_2O_2 (3.0%); and demineralized water, all at a 7-25C thermoperiod. After ten days there was no appreciable germination in any of the treatments although KNO_3, 3-IAA (100 ppm) and 3% H_2O_2 did increase germination percentages over those of the controls (Table 1). No significant differences occurred between light and dark treatments.

TABLE 1. Effect of KNO_3, 3-IAA, and H_2O_2 on germination of 2-month-old seeds of *Leavenworthia stylosa* in light (24C) and dark (7C) after 10 days.

Treatment	Percentage germination	
	Light : 12 hrs.	Dark : 12 hrs.
KNO_3 (0.1%)	25	15
KNO_3 (0.2%)	22	26
3-IAA (100ppm)	18	13
3-IAA (500ppm)	9	7
3-IAA (1000ppm)	4	6
H_2O_2 (3.0%)	23	8
Water	3	0

GERMINATION CONDITIONS

(1) *Light vs. dark.* In the leaching and chemical agents experiments yith 2-month-old seeds, germination did not appear to be influenced by presence or absence of light. To determine if after-ripened seeds were light sensitive, two lots of 8-month-old seeds were tested for a light/dark response. After ten days, 48% of the seeds in the dark and 54% of those in alternating light and dark had germinated. Seeds were still insensitive to light, although after-ripening allowed an expected increase in germination over that of 2-month-old seeds.

(2) *Amount of moisture.* Lots of 100 seeds each were placed on saturated filter paper, saturated filter paper with one edge in a reservoir of water, and saturated filter paper with an excess of water covering the seeds. After ten days germination percentages under all conditions ranged between 37 and 41%.

(3) *Temperature.* Eight-month-old laboratory-stored seeds were tested under various diurnally fluctuating temperature regimes.

Low. At low temperatures germination was poor (4C, 0% germination; 4-8C, 8%; 0-9C, 36%; 6-10C, 48%).

Low followed by continuous high temperature. Seeds were placed for 1, 2, 4, 6, and 8 hours, respectively, at 15C, then moved to 26.6C for 10 days; two sets of controls were kept, one at 15C and one at 26.6C. Under all conditions, germination ranged from 40 to 50%.

Alternating low and high temperature.

After 1, 2, 4, 6, and 8 hours respectively at 15C, seeds moved daily for ten days into 26.6C for the remainder of each day germinated from 20 to 45%, best germination being in the lots kept for 8 hours each day at 15C.

Field-storage. Since plants in the field are so dense as to suggest higher germination percentages under natural conditions than occurred in any of the germination experiments, seeds were collected from the surface of the soil three months after shedding, but prior to field germination. These field-stored seeds were sown in lots of 100 each on glade soil in clay pots. Pots were divided into three groups, one of which was placed at each of the following locations: out-of-doors (12-29C), cool room (22-26C), and greenhouse (18.9-32.2C). Water was added as needed to maintain a saturated substrate. Within eight days, 82% of the seeds outside, 80% of those in the cool room, and 90% of those in the greenhouse had germinated.

Germination percentages should be much higher, therefore, than those so far obtained from laboratory-stored seeds. The field-stored seeds had been germinated on soil and, during the after-ripening period, had been wet and dried with each rain shower. These factors were the basis of the next experiments.

Wetting and drying. Four-moth-old laboratory-stored seeds were sown in groups of 100 each on filter paper in petri dishes. One-half of these were watered throughout the experiment with demineralized water, the remaining with a dilute glade soil solution prepared by soaking 100 ml of glade soil in one liter of

TABLE 2. Effects of alternate short-wet and short-dry periods, and of glade soil extract, upon the germination of laboratory-stored seeds under naturally fluctuating and controlled temperatures.

Initial Wet Period (Days)	Dry Period (Days)	PER CENT GERMINATION: Naturally Fluctuating Temperatures 13 - 29C		Controlled Temperatures 22 - 26C	
		Demineralized Water	Glade Soil Extract	Demineralized Water	Glade Soil Extract
Control 20					
	0	8	10	2	30
	1	40	30	52	40
1	2	40	42	40	38
	3	18	38	40	24
	4	28	62*	0	40
	1	32	30	8	40
	2	0	68*	38	26
2	3	0	12	18	48
	4	0	58*	70*	48
	1	32	38	28	20
	2	8	50	50	38
3	3	34	48	46	80*
	4	42	16	41	48
	1	0	62*	56	16
	2	30	54	38	56
4	3	64*	76*	64*	40
	4	44	76*	54	44

*Germination percentages above 57%.

dimineralized water for 48 hours, then filtering. The test was conducted under controlled temperatures (22-26C) and under naturally fluctuating laboratory temperatures (13-29C). Four experimental groups were distinguished from each other on the basis of initial wet periods of 1, 2, 3, and 4 days, respectively (Table 2). Subdivisions of each group were subjected to dry periods of from 1 to 4 days. After dry treatment seeds were rewet and kept saturated for 20 days. Control groups were kept moist for 20 consecutive days.

In ten of the experimental groups 58-80% of the seed germinated (Table 2), results that are noticeably higher than those in preceding experiments (Table 1) with seeds of comparable age (3 to 8 months). The comparison involving demineralized water and soil extract was significant, but not highly so (Table 2). Since neither control group (demineralized water or glade soil extract) reached germination percentages of 80-90%, it seems doubtful that the wetting solutions used were responsible for the increase in germination.

Analysis of variance indicated that differences in length of wet and dry treatments were not significant; however, eight of the high germination percentages appear in the groups that were subjected to the longer dry periods (3-4 days).

The average temperature in both locations was approximately the same (24C), but the range of the naturally fluctuating temperatures encompassed those of the controlled, so there was no reliable evidence that either of the two temperature conditions was more favorable to germination than the other.

To single out the effect of alternate wet and dry treatments on germination, groups of 5-month-old laboratory-stored seeds were exposed to short initial wet periods (1-5 days) followed by relatively long dry periods (5-25 days) under temperatures of 22-26C (Table 3). Oemineralized water was used on all groups of treated seeds and on the control group, which remained wet for 10 successive days.

Maximum germination (Table 3) was reached 10 days after each group was rewet. After dry periods of 20 or 25 days, regardless of the duration of the initial wet periods, germination in all groups exceeded 69%, and in one half the groups was 80% or higher. Thus, short wet periods, followed by relatively long dry periods, effectively broke dormancy of laboratory-stored seeds, and dry periods of 20-25 days brought the percentage of germination to within the expected range of 80-90%. Analysis of variance (Table 3) showed the length of the dry period to be significant at the 1% level, and the length of the initial wet period to be insignificant.

TABLE 3. Effects of alternate short-wet and long-dry periods upon the germination of laboratory-stored seeds at 22 - 26C.

Initial Wet Period (Days)	DRY PERIOD (DAYS)					
	0	5	10	15	20	25
1	-	58	72	64	70	70
2	-	68	60	70	72	76
3	-	20	58	72	82	88
4	-	64	72	72	82	80
5	-	66	60	68	72	84
10 (Control)	42	-	-	-	-	-

Wetting and drying of freshly-harvested seeds. The seeds used in the preceding alternate wetting and drying experiment had been laboratory-stored for 4-5 months; therefore, this test could not indicate whether the effect of alternate wet and dry treatments during dormancy was immediate or delayed. Field conditions provide alternate wetting and drying throughout the entire period. To test the effectiveness of alternate wetting and drying before after-ripening and to determine if one such treatment would overcome dormancy during the summer, 5 groups of freshly-harvested seeds were wet for one day and dried for 5, 10, 15, 20, and 25 days, respectively, at 22-25C. The procedure was duplicated using an initial wet period of 5 days. Following the dry treatment, each group was rewet for 10 successive days.

No germination occurred after a single wet and dry cycle in any of the groups of freshly-harvested seeds, indicating that either a series of wet and dry periods is necessary during the summer to break dormancy, or a time lag occurs between the treatment and the effect, or other factors are influential in overcoming dormancy.

DISCUSSION

The dormancy of certain genera in the family Cruciferae, *Brassica, Amaranthus, Alisma, Capsella* and *Lepidium,* (Kidd, 1914; Griswold, 1936) is due to mechanically resistant seed coats, which, although completely permeable to water and oxygen, are not ruptured by expanding embryos. A constant wet period promotes no change; however, a wet period followed by a long-dry period alters the cells of the seed coats, so that, upon rewetting, the embryos develop sufficient pressure to burst the coats, and germination occurs (Meyer, Anderson, and Bohning, 1960). *Leavenworthia stylosa* follows the same pattern. In the glades during the summer, *Leavenworthia* seeds are repeatedly wet and dried, since most of the precipitation occurs as showers of short duration after which the area dries out quickly and thoroughly. In germination tests, *Leavenworthia* seeds that had been laboratory-stored for 5 months reached maximum germination (70%-80%) after exposure to a short wet period (1-5 days) followed by a long dry treatment (20-25 days) under temperatures of 22-25C. (Table 3). This same cycle under the same temperatures, when applied to freshly-harvested seeds, failed to promote any germination, indicating that one such treatment does not break dormancy and that time also may be an essential factor. That time is involved is indicated by the fact that seeds, which had been laboratory-stored for 4-5 months responded to one alternate wet and dry treatment and reached germination percentages comparable to those occurring in field-stored seeds (Tables 2 and 3). Germination of laboratory-stored seeds increased with the length of the storage period up to 13 months, but the only occurrence of germination within the expected range (80%-90%) was in groups of seeds that had been alternately wet and dried. No apparent differences resulted from storage under continuously low temperatures (22-25C) or under naturally fluctuating temperatures (25-40C).

Toole (1941) states that under low temperatures germination of seeds having coat restrictions may actually occur in spite of the resistance of the seed coat. In the case of *Leavenworthia stylosa,* although low temperature alone breaks dormancy in only a small percentage of the seeds (1%-4%), it undoubtedly is a contributing factor in the initiation of germination. In the field, seedlings were observed in abundance 5 days after a heavy rainfall combined with an average daily temperature of 19-21C. In the laboratory, seeds whose dormancy had been largely overcome by 12 months of dry storage, reached maximum germination (50%-70%) under continuously moderate temperatures (22-26C), and only 0-6% germination under natural sum-