

Some Eco-Physiological Aspects of Seed Dormancy in *Geranium carolinianum* L. from Central Tennessee

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Summary. In central Tennessee *Geranium carolinianum* L. behaves as a winter annual. Seed germination occurs in autumn, and seed ripening and dispersal are completed in May. Freshly-matured seeds have hard coats and will not imbibe water unless scarified. Embryos of freshly-matured seeds are conditionally dormant; scarified seeds germinate better in darkness than in light at high temperatures. After a short after-ripening period the embryo is essentially nondormant, and scarified seeds germinate to high percentages over a wide range of temperatures in both light and darkness. About 50% of the nonscarified seeds become germinable after 4.5 months of dry-laboratory storage. In order for the seed coat to become permeable (without scarification), seeds must be kept either dry or alternately wet and dry at relatively high temperatures during the summer dormancy period. The ecological significance of seed dormancy in *G. carolinianum* in central Tennessee is that it allows this non-drought tolerant species to avoid droughts that frequently occur in its habitat between late spring and early autumn. Conditional dormancy of the embryo of freshly-matured seeds, hard seed coats and the inability of seeds (non-scarified but permeable) to germinate in summer at high summer temperatures all seem to play a role in delaying germination until autumn.

Introduction

Geranium carolinianum L. is an annual or biennial that occurs in disturbed habitats throughout much of the United States (Fernald, 1950; Gleason and Cronquist, 1963). In central Tennessee and Kentucky where we have studied the species, plants of *G. carolinianum* behave strictly as winter annuals and are always found growing in disturbed habitats such as roadsides, lawns, pastures, disturbed rock outcrops and railroad embankments. Most of the germination occurs from mid September to mid October after which an over-wintering rosette is formed. Flower buds are initiated in late February and early March, and flowering occurs from mid April to mid May. Seeds are dispersed during May and lie dormant until at least through the following summer. Preliminary studies in the laboratory indicated that unlike most of the other species of winter annuals that inhabit these disturbed areas, seed dormancy in *G. carolinianum* is caused by a "hard" (impermeable to water) seed coat.

The purposes of the present investigation were 1. to further document the causes of dormancy and 2. to ascertain the ecological factor(s) that render the seeds nondormant.

General Methods

On May 2, 1971, and May 15, 1973, seeds of *G. carolinianum* were collected from several thousand plants growing in a disturbed field-roadside habitat in Davidson County, Tennessee. All germination tests were carried out in temperature- and light-controlled incubators either at a 14-h photoperiod or in constant darkness and at constant and alternating temperature regimes. All temperatures used were $\pm 1^\circ\text{C}$. At the alternating temperatures the thermoperiod was 12 h, and the light period extended from 1 h before the high temperature period began to 1 h after it ended. Light was supplied by "cool white" fluorescent tubes, and light intensity at seed level was ca. 2100 lux. Constant darkness was maintained by wrapping the Petri dishes first with Saran wrap to retard water loss and then with aluminum foil. The scarified seeds used in this study were made permeable by cutting a small hole in the radicle end of the seed with a razor blade. Radicle emergence was the criterion used for germination.

Experimental Procedures and Results

Effect of Scarification on Imbibition

Imbibition rates of freshly-matured scarified and nonscarified seeds (collected May 2, 1971) were compared. Two replications of 100 seeds each of scarified and nonscarified seeds were placed in Petri dishes on filter paper moistened with distilled water and allowed to imbibe at 25°C for 24.5 h. Immediately after the initial wetting and at irregular intervals throughout the 24.5 h period, scarified and nonscarified seeds were blotted, weighted to the nearest 0.1 mg and then returned to wet filter paper at 25°C .

Scarified seeds imbibed water rapidly, and after 2.5 h their weight had increased by 88.9% (Fig. 1). At this point they were essentially fully imbibed as seeds imbibed for 24.5 h increased by only 91.9%. On the other hand, nonscarified seeds imbibed very little water, and at the end of the 24.5-h imbibition period their weight had increased by only 4.1% (Fig. 1).

Germination of Scarified and Nonscarified Laboratory-Stored Seeds

The purpose of this experiment was to compare germination of scarified and nonscarified seeds after various periods of storage in the laboratory at $25 \pm 2^\circ\text{C}$. Seeds used in this study were collected May 2, 1971, and germination tests were performed on freshly-matured and 0.5-, 1.5-, 2.5-, 3.5- and 4.5-month-old seeds. Each germination experiment consisted of comparing scarified and nonscarified seeds over a range of constant (5, 10, 15, 20 and 25°C) and alternating temperatures

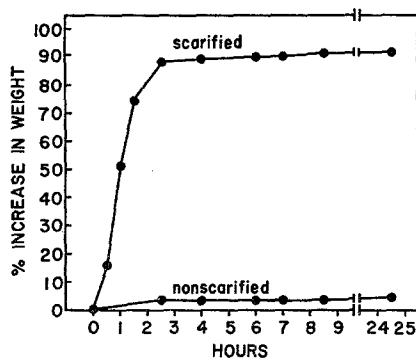


Fig. 1. Imbibition curves for scarified and nonscarified seeds of *G. carolinianum*

(15/6, 20/10, 30/15 and 35/20° C) in light and constant darkness. Three replications of 50 seeds each were used for each treatment, and seeds were placed in 9-cm Petri dishes on a 1-cm layer of clean, white sand moistened with distilled water. Germination counts were made at 5 day intervals for 30 days, and seeds germinated in darkness were examined in a darkened room with the aid of a green safe light. Although germination counts were made at 5 day intervals only the final germination percentages (after 30 days) are presented in Fig. 2.

In 0.0-month-old, scarified seeds some germination occurred in both light and darkness at all temperatures; however, at all temperatures except 10° C germination was better in darkness than in light (Fig. 2). At 5, 20, 25, 20/10 and 35/20° C germination was considerably higher (40% or more) in darkness than in light. Very little or no germination occurred in the freshly-matured, nonscarified seeds. After the seeds had aged for 0.5 months, there was very little difference in scarified seeds between germination in light and darkness, but nonscarified seeds still germinated to very low percentages. By the time the seeds had aged for 1.5 months, scarified seeds germinated to near 100% at all temperatures in both light and darkness except at 35/20° C in light where only 62% of the seeds germinated. Scarified seeds never did gain the ability to germinate to 100% in light at 35/20° C, and 3.5- and 4.5-month-old seeds germinated to a lower percentage at 30/15° C in light than did 1.5- and 2.5-month-old seeds. Germination of nonscarified seeds increased slowly throughout the experiment, but even after 4.5 months of storage the highest percentages of germination in darkness and light were 56.7 and 53.3, respectively, at 25° C. In 4.5-month-old, nonscarified seeds some germination occurred at all temperatures tested, and except at 30/15° C there was very little difference (16% or less) between per-

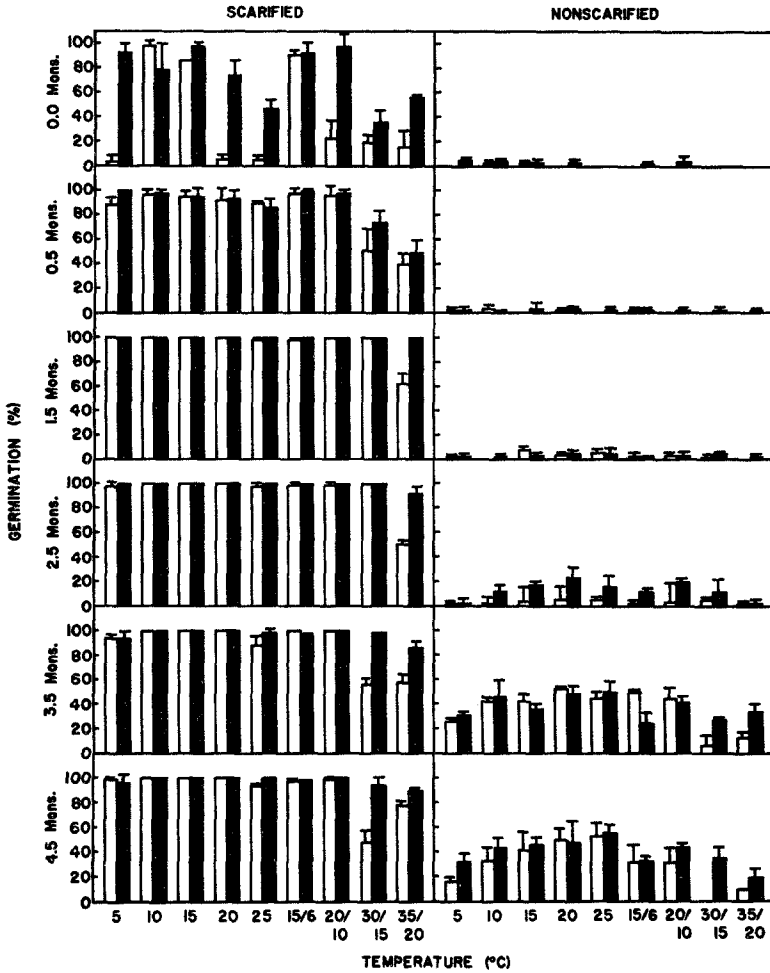


Fig. 2. Germination of scarified and nonscarified seeds of *G. carolinianum* in light (unshaded bars) and darkness (shaded bars). Lines at tops of bars indicate one standard deviation

centages of germination of seeds in light and darkness. At 30/15° C none of the seeds in light germinated while 36% of those in darkness did.

Effect of Temperature and Soil Moisture Regime on Seed Coat Breakdown

In this experiment the effect of various storage temperatures and soil moisture regimes upon breakdown of the hard seed coat and subsequent germination at the storage temperatures and at the simulated

October temperature was investigated. On May 21, 1973, freshly-matured seeds were placed on a 1-cm layer of dry greenhouse potting soil in 9-cm Petri dishes, and then they were subjected to three soil moisture regimes (wet, wet-dry and dry) at each of five constant (5, 10, 15, 20 and 25° C) and four alternating temperature regimes (15/6, 20/10, 30/15 and 35/20° C) until October 2, 1973. Six replications of 100 seeds each for each of the three soil moisture regimes were placed at each of the nine temperatures. Soil receiving the wet moisture treatment was watered to field capacity on the first day of the experiment and was kept at or near field capacity until the experiment was terminated on November 1, 1973. From May 21 to October 2, soil receiving the dry treatment was not watered, and soil receiving the wet-dry treatment was alternately kept wet for 5 days and then allowed to dry for 10. In the latter treatment the soil was watered to field capacity on the first day of the wet period, then after 5 days the lids were removed from the Petri dishes. Ten days after removal, the soil was rewet and the lids were replaced, and so forth. Depending on the temperature, it took 1–4 days for the soil to dry. On October 2 soil receiving the wet-dry and dry treatments was watered and then kept at or near field capacity until the end of the experiment. On this same date half of the seeds (3 replications of 100 seeds each) from each temperature and soil moisture regime were moved to 20/10° C which simulates the mean daily maximum and minimum monthly temperatures for October in middle Tennessee (U.S.D.C., 1965). The other half of the seeds from each temperature and soil moisture regime remained at their respective storage temperatures. Germination was checked at 15-day intervals throughout the experiment.

Seeds kept on wet soil throughout the experiment germinated to only low percentages at the temperatures at which they were stored as well as the 20/10° C October regime (Fig. 3A and D). Seeds kept on soil that was alternately wet and dry during the summer at 25, 30/15 and 35/20° C and then were moved to the 20/10° C regime in October germinated to 90% or greater (Fig. 3E). In those seeds not moved the best germination (63.7%) occurred at 30/15° C and at no other temperature did the seeds germinate to as much as 50% (Fig. 3B). Only a very small percentage of the seed coats became permeable under the wet-dry soil moisture regime at the lower temperatures (*i.e.*, 5, 10, 15, 15/6 and 20/10° C), and only a very small percentage or none of the seeds germinated either at these storage temperatures or after removal to 20/10° C (Fig. 3B and E). As in the wet-dry soil moisture regime, seed coats of seeds stored on dry soil all summer became permeable when stored at 25, 30/15 and 35/20° C (Fig. 3C and F). Seeds stored dry at 25, 30/15 and 35/20° C germinated to 86% or better at the re-

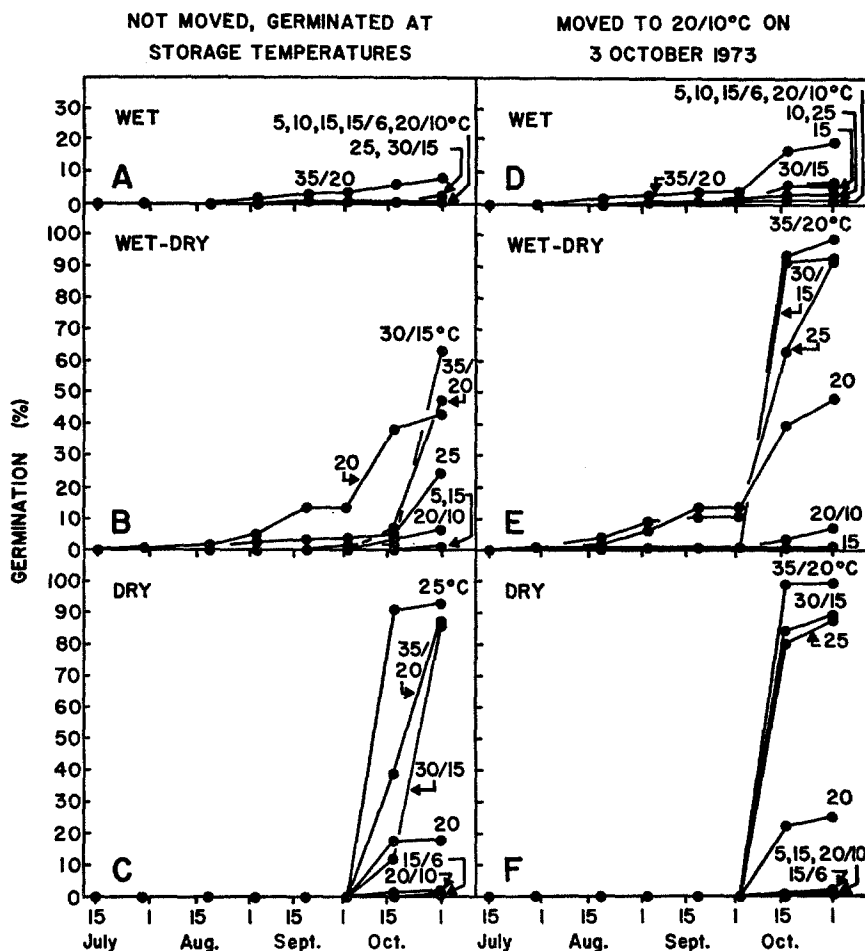


Fig. 3 A—F. Effect of storage temperatures and soil moisture regime on germination of nonscarified seeds of *G. carolinianum*

spective storage temperatures or when moved to 20/10° C. Again, very little germination occurred when the seeds were stored at the lower temperatures.

Effect of Soil Moisture Regime and Simulated Summer Temperatures on Seed Coat Breakdown

In this experiment the progress of seed coat breakdown of seeds stored at three soil moisture regimes (wet, wet-dry and dry) and at simulated summer temperatures was followed. Three replications of

Table 1. Percent (mean \pm SE) germination of seeds of *G. carolinianum* stored at three soil moisture regimes at simulated summer temperatures and germinated on moist soil at 20/10° C

Date seeds were moved to 20/10° C	Treatment while at simulated summer temperatures		
	Wet	Wet-dry	Dry
June 1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
July 1	0.3 \pm 0.3	0.7 \pm 0.7	0.3 \pm 0.3
August 1	0.7 \pm 0.3	54.0 \pm 4.3	26.7 \pm 2.0
September 1	5.7 \pm 2.3	98.7 \pm 0.3	86.3 \pm 2.2
October 1	12.0 \pm 2.0	96.7 \pm 1.9	81.7 \pm 1.8

100 seeds each were used for each treatment, and seeds were placed on a 1-cm layer of dry greenhouse potting soil in 9-cm Petri dishes. The three moisture regimes were applied to the soil as previously described. At the beginning of the experiment (May 21, 1973) all seeds were placed in an incubator set on the May temperature regime. On the first day of each month from June to October the temperature setting on this incubator was changed to correspond to the approximate mean daily maximum and minimum monthly temperatures for that particular month in middle Tennessee: May 20/10, June 30/15, July 35/20, August 35/20, September 30/15 and October 20/10° C (U.S.D.C., 1965). Also, on the first day of each month from June to October one set (3 replications of 100 seeds each) of seeds from each soil moisture regime was removed to a second incubator set at the October regime, which is optimal for germination, and a 14-h photoperiod. Soil receiving dry or wet-dry regimes while at the summer temperatures was watered to field capacity at the time of removal to 20/10° C and kept moist. Germination percentages were determined at 15-day intervals throughout the experiment.

With the exception of two seeds (stored on the wet-dry soil moisture regime) that germinated at the September temperature regime in September, all germination occurred after the seeds were placed at the 20/10° C October regime. Only a small percentage of the seed coats of seeds stored on continuously wet soil became permeable, and the highest percentage of germination (12.0) was in those seeds kept at simulated summer temperatures from May 21 to October 1 and then at 20/10° C during October (Table 1). On the other hand, a high percentage of the seeds stored on the wet-dry and dry soil moisture regimes became permeable by September 1, and they germinated to 98.7 and 86.3%, respectively, after removal to the 20/10° C regime.

Discussion

There are a number of winter annuals in which the embryo is non-dormant and in which dormancy is caused by a hard seed coat. Most winter annuals with impermeable coats belong to the Leguminosae, and all the studies that we are aware of on the germination ecology of hard seeded winter annuals have been done on economically important pasture legumes. A number of these studies have emphasized the role of daily fluctuating temperatures in causing the breakdown of the seed coat. Williams and Elliott (1960) studied the ecological aspects of seed coat impermeability of three winter annual legumes (*Trifolium incarnatum* L., *T. subterraneum* L. and *T. hirtum* All.) in California. In general, they found that high temperatures at the soil surface during the Mediterranean-type summer promoted seed coat breakdown but that the mild winter environment in this area was not conducive to softening of the seed coat. Quinlivan (1961) working in southwestern Australia found that a high percentage of the seed coats of *Lupinus digitatus* Forsk., *L. lutens* L., *Medicago tribuloides* Desr. and *Trifolium subterraneum* became permeable when seeds were stored dry during the summer rest period at temperatures simulating those at the soil surface in pastures during the summer. Seeds stored at 15.6/60° C fluctuating temperatures gave a higher percentage of germination than seeds stored at constant temperatures of 15.6 or 60° C. Quinlivan and Millington (1962) compared germination of seeds of *T. subterraneum* stored dry in the laboratory at simulated soil surface temperatures (15.6/60° C) with germination of seeds collected from the field at various intervals during the summer. The decline in impermeability of laboratory stored seeds was found to be comparable to that of seeds collected from the field. In a later study Quinlivan (1966) found that the fluctuations in temperature must be of a certain magnitude to promote maximum rate of breakdown. Subjecting the seeds of *T. subterraneum* to diurnal fluctuations of 15/30° C for 210 days was almost totally ineffective in causing seed coat breakdown, but 15/45, 15/60 and 15/75° C for the same interval caused most of the seed coats to become permeable.

In addition to daily fluctuating temperatures, relative humidity and seed moisture content also are important factors in causing hard seed coats to become permeable. In 1954 Hyde reported that during ripening of *Trifolium repens* L., *T. pratense* L. and *Lupinus arboreus* L. seeds the moisture content decreased from over 150% of dry weight to approximately 25% via water loss through the epidermis. At this point the hilum began to function as a hygroscopic valve; opening when external relative humidity was low so that water vapor escaped from the seed by diffusion and closing when relative humidity was high. Seeds attained a moisture content in equilibrium with the lowest relative

humidity to which they were exposed. At 14% moisture content the seed coats became impermeable. Gladstones (1958) found that *Lupinus digitatus* seeds could be prevented from developing hard coats if they were stored at high relative humidity so that the moisture content did not drop below 14%. Below 14% moisture content, permeability decreased with decreasing moisture content and at 11% almost all of the seeds had impermeable coats. In *Lupinus varius* L. seeds with 10% moisture content were conditionally hard and would soften after several months when placed on a moist substrate; whereas, seeds with 8.5% moisture content were absolutely hard and would not soften when exposed to moist conditions (Quinlivan, 1968). The seeds with 8.5% moisture content became permeable after exposure to daily fluctuating temperatures of 15 and 65° C which fractured the coat at the strophiole. Fluctuating temperatures were not effective in softening the coats of seeds with high (10%) moisture content. Quinlivan (1968) concluded that any beneficial effect of fluctuating temperatures on seeds with high moisture content would be to indirectly cause drying of the seeds. After the seeds dried to 8.5% moisture content then fluctuating temperatures would cause the coat to become permeable.

In our study storage conditions greatly influenced the number of *G. carolinianum* seeds that became permeable (Fig. 3). Storage at high temperatures (25, 30/15 and 35/20° C) and at either wet-dry or dry soil moisture conditions was the most favorable for breakdown of the seed coat. It is believed that drying and not fluctuating temperatures *per se* was responsible for softening the seed coat because a high percentage of the seeds stored dry at 30/15 and 35/20° C as well as 25° C became permeable. Also, freshly-matured seeds dried in an oven at a constant temperature of 80° C for 15 days gave 100% germination after 7 days when they were placed on moist sand at 20/10° C (Baskin and Baskin, unpubl.). It is not known if the hilum functions as a hygroscopic valve in seeds of *G. carolinianum*.

In freshly-matured seeds of *G. carolinianum* the seed coat is impermeable, and the embryos are conditionally dormant. Only a low percentage of the scarified seeds germinated in light at 20/10° C and in either light or darkness at 30/15° C; 20/10 and 30/15° C are the mean daily maximum and minimum monthly temperatures for middle Tennessee in May and June, respectively (U.S.D.C., 1965). Thus, even if the seed coat was permeable, freshly-matured seeds probably would not germinate in the field during late May and early June. However, the embryo soon loses its dormancy, and by the time the seeds are 1.5 months old (July) scarified seeds germinate to a high percentage over a wide range of temperatures in both light and darkness (Fig. 2). From the data presented in Fig. 2, it could be concluded that seeds under

natural conditions remain dormant during the summer due to an impermeable seed coat. However, over 50% of the seeds stored at simulated summer temperatures and soil moisture regime (wet-dry) became permeable and germinated in August when placed at 20/10° C (Table 1), but they did not germinate if left at the August temperature (35/20° C). Thus, even though the seed coat was permeable it still exerted enough mechanical resistance to prevent germination at normal summer temperatures. Since field temperatures in summer are above those required for germination, germination of naturally-softened seeds is delayed until autumn when temperatures are lower. One reason why laboratory-stored, scarified seeds germinate at 35/20° C in August and the seeds stored at simulated summer temperatures and soil moisture did not may be that the scarified seed coat is weaker than the nonscarified seed coat which has become permeable naturally. Thus, the embryo may have enough growth potential to penetrate the scarified coat but not enough to penetrate the naturally softened coat. Differences in growth potential of the embryo have been proposed as an explanation for dormancy in seeds imposed by darkness (Scheibe and Lang, 1965), light (Chen, 1969, 1970), high temperature (Chen and Thimann, 1966), low temperature (Junttila, 1973) and the need for low temperature stratification (Baskin and Baskin, 1971a).

Seedlings of *G. carolinianum* are non-drought tolerant and the few that germinate during occasional cool, moist periods in summer do not survive (Baskin and Baskin, 1971b). Thus, in order to survive in its disturbed, summer-dry habitat, *G. carolinianum* must remain in the dormant seed stage during the summer. In the field dormancy in freshly-matured seeds is due to a partially dormant embryo and to a hard seed coat. Later, in July and August after the seed coat on many of the seeds becomes permeable, high temperature in combination with the mechanical restraint of the seed coat prevents germination. With the arrival of lowered autumn temperatures the growth potential of the embryo apparently is increased, and the seeds germinate.

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