

Differences in light and temperature responses determine autumn versus spring germination for seeds of *Schoenolirion croceum*

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Abstract: Seeds of the southeastern North American *Schoenolirion croceum* (Michx.) Wood are dormant when dispersed in late spring to early summer. Fresh seeds buried in soil after dispersal germinate in autumn, whereas those sown on the soil surface do so the following late winter – early spring. To understand this difference in germination phenology, we examined the light and temperature requirements for dormancy break and germination. Seeds germinated to high percentages in darkness over 12:12 h thermoperiods ranging from 15:6 to 35:20 °C following warm stratification (25:15 °C) in darkness, whereas no seeds germinated in light following stratification in light. On the other hand, seeds germinated to high percentages in light and in darkness following cold stratification (5 °C) in light or darkness. Seeds exposed to light during autumn germinated in winter–spring regardless of the light regime in summer or winter–spring, whereas those in darkness during autumn germinated in autumn regardless of the light regime in summer. Thus, light conditions during autumn are critical for determining whether seeds will germinate in autumn versus early spring. In contrast with many other species in which germination phenology is mostly temperature controlled, timing of germination for *S. croceum* depends on the light conditions in relation to temperatures experienced during dormancy release.

Key words: dark, germination phenology, Hyacinthaceae, negative photoblastic seeds, photoecology, seed dormancy.

Résumé : Les graines du *Schoenolirion croceum* (Michx.) Wood, une plante du sud-est de l'Amérique du Nord, sont dormantes lorsqu'elles sont dispersées à la fin du printemps ou au début de l'été. Des graines fraîches, enterrées dans le sol après leur dispersion, germent à l'automne, alors que celles qui sont placées en surface du sol ne germent qu'à la fin de l'hiver ou au début du printemps de l'année suivante. Afin de comprendre cette différence phénologique de la germination, les auteurs ont examiné les besoins en lumière et en température pour briser la dormance et obtenir la germination. Un fort pourcentage de graines germent à l'obscurité sous un ensemble de thermopériodes (12:12 h), allant de 15:6 à 35:20 °C, suite à une stratification au chaud (25:15 °C) à l'obscurité, alors qu'aucune graine ne germe à la lumière suivant une stratification à la lumière. D'autre part, les graines germent à de fort pourcentages à la lumière et à l'obscurité, suite à une stratification au froid (5°), à la lumière aussi bien qu'à l'obscurité. Les graines exposées à la lumière au cours de l'automne germent à la fin de l'hiver début du printemps, indépendamment du régime lumineux en été ou en fin d'hiver début printemps, alors que celle maintenues à l'obscurité au cours de l'automne germent à l'automne indépendamment du régime lumineux au cours de l'été. Ainsi, les conditions lumineuses au cours de l'automne sont critiques pour déterminer si les graines germeront à l'automne ou au début du printemps. Contrairement à plusieurs autres espèces où la phénologie de la germination est surtout contrôlée par la température, la chronologie de la germination chez le *S. croceum* dépend des conditions lumineuses en relation avec les températures vécues au cours du bris de la dormance.

Mots clés : obscur, phénologie de la germination, Hyacinthaceae, graines négativement photoblastiques, photoécologie, dormance des graines.

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Introduction

Many environmental factors influence the germination of seeds. Temperature, light, water, gases such as oxygen, car-

bon dioxide, and ethylene, and inorganic and organic chemicals determine whether germination takes places and the rate at which it does so. Moreover, these environmental factors can interact, either antagonistically or synergistically, with each other (Mayer and Poljakoff-Mayber 1989; Bewley and Black 1994). Once dormancy is broken, seed germination can be controlled and influenced by manipulating external factors either in the laboratory or in the field, for example, application of nitrate into Petri dishes or as fertilizer (Fawcett and Slife 1978). On the other hand, other factors vary seasonally and have an additional governing influence on the timing of germination in nature. In temperate zones

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of the world, temperature has a major role in determining the periodicity of seed germination if soil moisture is favorable (Stoller and Wax 1973; Baskin and Baskin 1988).

Temperature plays a dual role of overcoming dormancy and permitting embryo growth with subsequent radicle emergence. Cold (0–10 °C), moist temperature conditions break dormancy in seeds of some species (e.g., Courtney 1968), whereas warm (≤ 15 °C), dry or moist temperature conditions do so for others (e.g., Baskin and Baskin 1971, 1986). On the other hand, seeds of a few species require a warm-temperature period followed by a cold-temperature period for loss of dormancy (e.g., Baskin and Baskin 1995). Once dormancy is broken, germination takes place at temperatures that are conducive for survivorship and growth of the seedlings. During the dormancy-breaking period, biochemical and physiological changes in the seeds enable them to become nondormant and germinate at temperatures that were prohibitive when they had just attained maturity and dispersed, at which time they were dormant or conditionally dormant (Walck et al. 1997a).

Light also is extremely important in regulating germination, but not necessarily the timing of it per se. In particular, the germination response to the quantity of light is largely responsible for enabling seeds to persist in a soil seed bank. Nondormant seeds that do not germinate in darkness, i.e., while buried in soil, have greater longevity in a soil seed bank than those that do germinate (Walck et al. 1998; Milberg et al. 2000). Germination responses in light versus darkness vary among species, but species that germinate to a greater or equal percentage in light than darkness are more abundant than those that complete germination to a greater percentage in darkness than light (Baskin and Baskin 1998). However, germination in darkness also is dependent upon whether the light requirement can be fulfilled prior to burial allowing seeds to germinate in darkness when normally seeds buried immediately would not do so (Walck et al. 1997b). On the other hand, quality of light can regulate the timing of germination, if it is linked to some type of disturbance regime, especially one that forms gaps in a plant canopy. The high far-red/red ratio of leaf-filtered light generally inhibits seed germination ensuring that seeds of species, particularly shade-intolerant ones, do not germinate when the amount of light is inadequate for growth and survival (Silvertown 1980; Horvitz and Schemske 1994).

Regulation for the timing of germination in nature can be viewed, and has been theoretically modeled, as an adaptive strategy optimized under natural selection (Venable 1989; Masuda and Washitani 1992). Correlating germination with the best time for successful establishment and reproduction is influenced more so by the predictability of seasonal temperature regimes rather than by irregular disturbances with concomitant changes in light quality or quantity (Levins 1969; Schaal and Leverich 1981). As such, seed germination of many plant species in temperate regions occurs in spring and (or) autumn (Baskin and Baskin 1988). Seed germination in spring is regarded as advantageous for evading winter mortality, while that in autumn provides a competitive advantage to seedlings that survive winter (Masuda and Washitani 1992). Although seed germination during only one season is generally common, germination in two seasons is known for some species of annuals (e.g., Baskin and

Baskin 1981; Masuda and Washitani 1992) and perennials (e.g., Walck et al. 1997c). However, germination responses to the light environment are similar between the two periods of emergence, and temperature regulates the timing of this germination (e.g., Walck et al. 1997d).

Herein, we describe a situation in which the light environment during dormancy break determines whether seed germination takes place during autumn versus spring in the plant species, *Schoenolirion croceum* (Michx.) Wood. We investigated the light and temperature requirements for dormancy break and germination under laboratory conditions, and link this information to understanding the phenology of germination in the field. To our knowledge, this is the first study to report that light interacts with temperature influencing the timing of germination in nature.

Materials and methods

Study species

Schoenolirion croceum (Liliaceae sensu lato, Hyacinthaceae, or Agavaceae; for a discussion on the familial placement of this taxon, see Reveal and Pires 2002) occurs from middle Tennessee to North Carolina and south to Florida and Alabama (USA), with a disjunct range being found in western Louisiana and eastern Texas (Sherman 1969, 2002). The states of Tennessee, South Carolina, and Alabama consider the plant to be imperiled (NatureServe 2003). The species grows in a variety of habitats throughout its distribution: cedar (limestone) glades; sandstone, grit, chalk, and granite outcrops; open pinelands; edges of cypress bogs; and open pine barrens or wet grasslands. These habitats are fully exposed to sunlight, very poorly drained, and subjected to a winter-wet, summer-dry cycle (Sherman 1969). The species is a herbaceous polycarpic (perennial) cryptophyte with a bulb on top of a vertical rhizome. It flowers from mid-March to mid-April in the southern and western portions of its range, and from April to mid-May in the northern portion (Sherman 1969, 2002; Baskin et al. 1995).

Plant material and sizes of seeds and embryos

The glossy, black (mature, ripe) seeds of *S. croceum* were collected in Rutherford County, Tennessee, on 3 June 2000, and from the same population on 10 June 2001. At the time of collection, some seeds already had dispersed from the capsules. Seeds collected in 2000 and 2001 were stored dry for 1 and 7 d, respectively, between collection and the initiation of laboratory studies and for 36 and 24–35 d, respectively, between collection and the initiation of phenological studies. Twenty seeds collected in 2000 were cut longitudinally with a razor blade, and the lengths of seeds and their embryos were measured under a dissecting microscope equipped with a micrometer.

Phenology of seed germination

Three replications of 300 seeds each collected in 2000 were sown on soil in plastic pots (15 cm (width) \times 11 cm (depth)) on 9 July 2000. In addition, three replications of 200 seeds each collected in 2001 were sown on soil in plastic pots on 7 July 2001. Seeds collected and sown in 2000 were placed directly on the surface of a garden topsoil mix (mostly humus and peat). In contrast, seeds collected and

sown in 2001 were placed on top of a 15 cm × 15 cm piece of fine mesh nylon positioned on a limestone-derived topsoil to prevent them from being covered with soil. The pots were placed out-of-doors, covered with metal screen, and protected from direct precipitation. The soil in the pots was watered once each week from the time of sowing to 31 August, and it was watered daily, except when frozen, during the remainder of the year. This watering regime simulates soil moisture that might occur in the field, i.e., soil wet in autumn, winter, and early spring, and alternately wet and dry in late spring and summer. At weekly intervals all germinated seeds were counted and removed from the pots until 7 April 2002 or 1 June 2002 for seeds collected and sown in 2000 and 2001, respectively.

Five hundred seeds collected in 2001 were placed in each of five fine mesh nylon bags, and on 7 July 2001 they were buried out-of-doors 8 cm deep in soil at 30-cm intervals. One bag of seeds each was chosen randomly and exhumed on 4 August 2001, 29 September 2001, 3 November 2001, 2 February 2002, and 30 March 2002. For each exhumation date, the bag of seeds was cut open, and germinated (seedlings) and nongerminated seeds were counted. Nongerminated seeds were divided equally among three dishes, incubated in light over a range of thermoperiods (see below) for 2 weeks, and then examined for germination.

Mean maximum and minimum daily air temperatures for each week of the study were calculated from data collected at the Murfreesboro, Tennessee weather station, approximately 5.5 km from the study site, (9 July 2000 – 4 August 2001) or from continuous thermograph records made inside a weather house near the pots and buried bags (5 August 2001 – 31 May 2002).

Germination tests

Germination studies were done in temperature- and light-controlled incubators and in a refrigerator equipped with a light and a time clock. The five incubators were set at 12:12 h daily thermoperiods of 15:6, 20:10, 25:15, 30:15, and 35:20 °C. The alternating thermoperiods approximate mean daily maximum and minimum monthly air temperatures in Tennessee and adjacent states during March and November, 15:6; April and October, 20:10; May, 25:15; June and September, 30:15; and July and August, 35:20 °C (National Oceanic and Atmospheric Administration 2003). The daily photoperiod in the incubators was 14 h, extending from 1 h before the beginning of the high-temperature period to 1 h after the beginning of the low-temperature period. The refrigerator was set at a constant 5 °C and a 14-h photoperiod. The 25:15 °C thermoperiod was used for warm stratification and 5 °C was used for cold stratification, since they are near-optimal for seeds of many species that require warm or cold temperatures, respectively, to come out of dormancy (Stokes 1965; Nikolaeva 1969). Cool white, 20-W fluorescent tubes, which produced a photon flux density (400–700 nm) at seed level of ca. 50–70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, were used as the light source in the incubators; 15-W tubes producing ca. 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were used in the refrigerator.

Seeds were placed on white quartz (play) sand, moistened with distilled water, in 6.0-cm (diameter) plastic Petri dishes. Water was added to the dishes as needed to keep the substrate moist. Three replications of 25 seeds per dish were

used in each treatment. Dishes containing seeds undergoing light treatments were wrapped with plastic film, and those in dark treatments also were wrapped in aluminum foil.

Protrusion of the radicle was the criterion for germination. Ungerminated seeds were checked under a dissecting microscope to see if they contained firm, white (viable) embryos or soft, brown (nonviable) ones. A tetrazolium test (Grabe 1970) confirmed that white embryos were alive and that brown ones were not.

Germination of freshly matured seeds

Freshly matured seeds collected in 2000 or 2001 were incubated in light or darkness at the five thermoperiods. Seeds incubated in light were checked at 2-week intervals for a total of 14 weeks, during which time seedlings were counted and removed from the dishes. Seeds incubated in darkness were checked only at the end of 2 weeks (2000 seeds) or 2, 8, and 14 weeks (2001 seeds). Seeds incubated in light or darkness for 14 weeks served as the controls for the warm or cold stratification treatments (see below).

Effects of warm or cold stratification on dormancy break and germination

Seeds collected in 2000 were warm (or cold) stratified for 12 weeks in light and incubated at the five thermoperiods for 2 weeks in light, or stratified for 12 weeks in darkness and incubated for 2 weeks in darkness. Seeds collected in 2001 were warm (or cold) stratified for 4, 8, or 12 weeks in light and incubated over the range of temperature regimes for 2 weeks in light, or stratified for 4, 8, or 12 weeks in darkness and incubated for 2 weeks in darkness. Seeds both stratified and incubated in darkness were not checked until the end of the incubation period.

Effect of simulated sequence of seasonal temperature and light regimes on dormancy break and germination

Seeds collected in 2001 were exposed to various light regimes during a simulated sequence of natural seasonal temperature periods (Table 1). The sequence began at summer temperature regimes, and the seeds were moved to autumn regimes followed by winter – early spring regimes. The summer regimes were monthly intervals at 30:15 °C (June) → 35:20 °C (July and August) → 30:15 °C (September), autumn regimes were at 20:10 °C (October) → 15:6 °C (November), and winter – early spring regimes were at 5 °C (December–February). One set of controls was kept in light, and a second set was kept in darkness, during summer, autumn, and winter – early spring. Dishes incubated in light were monitored for moisture at 2-week intervals. Seeds incubated in darkness were not checked for germination until the end of the dark-treatment period. Germination was recorded at the end of each seasonal regime.

Statistical analyses

Germination data were transformed to percentages based on the number of viable seeds, and means and standard errors were calculated. Means of germination percentages were compared by analyses of variances (ANOVAs) followed by protected least significant difference tests or *t* tests (SPSS 2000). A three-way ANOVA was used to examine the effects and interactions of condition (nonstratified, warm

Table 1. Outline of the procedure used to study the effect of a simulated sequence of seasonal temperature and light regimes on the dormancy break and germination of *Schoenolirion croceum* seeds.

Seasonal temperature regime	Light regime treatments						
Summer	Light	Dark	Dark	Dark	Light	Light	Light
Autumn	Light	Light	Dark	Dark	Dark	Light	Dark
Winter – early spring	Light	Light	Light	Dark	Dark	Dark	Light

Note: Seeds were exposed to various light regimes during a sequence of monthly 12:12 h thermoperiods for summer (30:15 °C (June) → 35:20 °C (July and August) → 30:15 °C (September)), followed by autumn (20:10 °C (October) → 15:6 °C (November)), and ending with winter – early spring (5 °C (December–February)).

stratified, cold stratified), light regime, and thermoperiod for the stratification experiments conducted on the 2000-collected seeds, and a four-way ANOVA examining the effects and interactions of condition, light regime, thermoperiod, and length of incubation or stratification for the 2001 seeds. In addition, a two-way ANOVA analyzed the effects and interactions of seasonal temperature and light regimes on germination percentages. Type III sums of squares were examined for the ANOVAs involving the stratification experiments, and Type IV sums of squares were examined for the ANOVA involving the seasonal temperature–light experiment because it was an unbalanced design. Percentages of germination data were arcsine square-root transformed for statistical tests.

Results

Seed and embryo size

The mean (\pm SE) lengths of seeds and embryos at the time of dispersal were 3.16 ± 0.05 and 2.12 ± 0.03 mm, respectively. The embryos were approximately 67% of the length of seeds. An empty space (0.02 mm wide) was present between the seed coat and the endosperm at the end of the embryo in 12 of the 20 seeds measured.

Phenology of seed germination

Seeds sown on top of the soil surface on 9 July 2000 began germinating between 28 January and 3 February 2001, when mean weekly maximum and minimum temperatures were 8.8 and -2.0 °C, respectively (Fig. 1). Peak germination occurred between 18 and 24 February 2001, when mean maximum and minimum temperatures were 11.0 and -1.3 °C, respectively. Of the 900 seeds sown, 35% of them germinated in late winter or early spring 2001. No additional seeds germinated after 28 April 2001 until 7 April 2002, when the study was terminated. Many seeds were destroyed by an unknown seed predator during the course of the study, accounting for the low percentages of germination.

Seeds sown on top of nylon placed on the soil surface on 7 July 2001 began germinating between 7 and 13 October 2001, when mean weekly maximum and minimum temperatures were 23.4 and 11.4 °C, respectively (Fig. 2). After 27 October 2001, no seeds germinated until the week of 20–26 January 2002, when mean weekly maximum and minimum temperatures were 14.5 and -0.1 °C, respectively. Peak germination occurred between 17 and 30 March 2002, when mean maximum and minimum temperatures were 16.9 and

Fig. 1. Cumulative germination percentages (mean \pm SE; SE shown only if $\geq 5\%$) of *Schoenolirion croceum* seeds collected on 3 June 2000 and sown on top (\bullet) of the soil surface on 9 July 2000 (arrow) in pots placed outdoors. Mean weekly maximum (\blacktriangledown) and minimum (\blacktriangle) temperatures are shown from 9 July 2000 to 26 May 2001. Letters on the x-axis represent the months between July 2000 and May 2001.

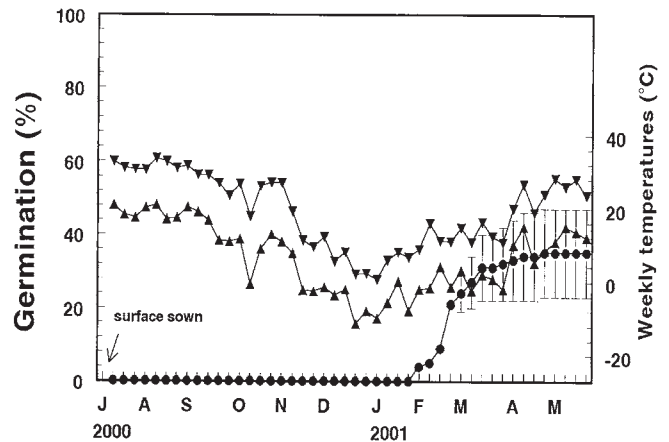


Fig. 2. Germination percentages (mean \pm SE; SE shown only if $\geq 5\%$) of *Schoenolirion croceum* seeds collected on 10 June 2001 and either sown on top (\bullet) of nylon placed on the soil surface on 7 July 2001 in pots placed outdoors or buried (\blacksquare) 8 cm deep in soil on 7 July 2001 outdoors (arrow). Mean weekly maximum (\blacktriangledown) and minimum (\blacktriangle) temperatures are shown from 7 July 2001 to 25 May 2002. Letters on the x-axis represent the months between July 2001 and May 2002. Percentages are cumulative for surface-sown seeds.

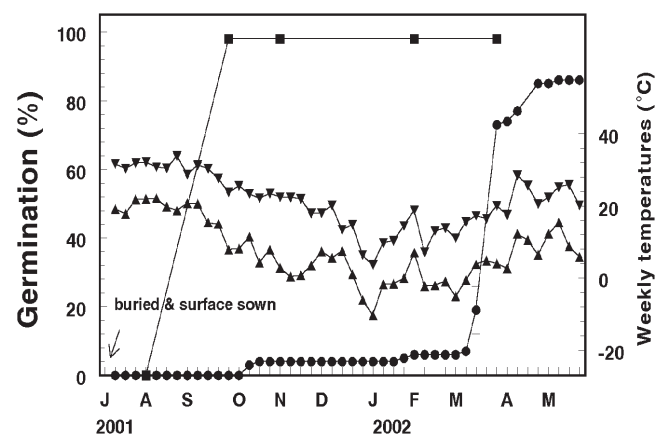


Table 2. Germination percentages (mean \pm SE) of nonstratified, warm-stratified, and cold-stratified seeds of *Schoenolirion croceum* collected in 2000.

Condition	Light regime		Incubation thermoperiod ($^{\circ}$ C)				
	Stratification	Incubation	15:6	20:10	25:15	30:15	35:20
Nonstratified							
Fresh		Light	0Aa	0Aa	0Aa	0Aa	0Aa
		Dark	0Aa	0Aa	0Aa	0Aa	0Aa
Control		Light	9 \pm 5Ba	0Ab	0Ab	0Ab	0Ab
Warm-stratified	Light	Light	0Aa	0Aa	0Aa	0Aa	0Aa
	Dark	Dark	37 \pm 11Ca	88 \pm 7Bb	79 \pm 5Bbcd	63 \pm 10Bab	55 \pm 11Bad
Cold-stratified	Light	Light	40 \pm 8Ca	67 \pm 3Cb	29 \pm 13Ca	7 \pm 1Cc	1 \pm 1Ac
	Dark	Dark	93 \pm 3Da	100Db	91 \pm 4Dad	55 \pm 4Bc	81 \pm 7Cd

Note: Fresh seeds were incubated in light or in darkness for 2 weeks, or in light for 14 weeks (control). Seeds were stratified and incubated in light or were stratified and incubated in darkness. Stratification occurred at 25:15 $^{\circ}$ C (warm) or at 5 $^{\circ}$ C (cold) for 12 weeks, and incubation followed for 2 additional weeks. Values with different uppercase letters within columns or lowercase letters within rows are significantly different (protected least significant difference test, $P = 0.05$).

4.3 $^{\circ}$ C, respectively. Of the 600 seeds sown on top of the soil surface, 4% and 82% of them germinated in autumn 2001 and late winter – early spring 2002, respectively. No additional seeds germinated after 25 May 2002 until 14 June 2002, when the study was terminated.

None of the seeds buried at an 8-cm depth in soil on 7 July 2001 and exhumed on 4 August 2001 had germinated (Fig. 2). Moreover, none of these seeds germinated when they were incubated in light over the range of thermoperiods for 2 weeks (data not shown). However, 98% of the seeds had germinated in the bags exhumed between 29 September 2001 and 30 March 2002. The mean weekly maximum and minimum temperatures between 7 July and 4 August 2001 were 31.2 and 20.1 $^{\circ}$ C, and those between 5 August and 29 September 2001 were 29.7 and 17.6 $^{\circ}$ C, respectively.

Germination of freshly matured, warm-stratified, and cold-stratified seeds

Seed condition, light regime, and thermoperiod had significant effects on germination ($P < 0.0001$) of 2000-collected seeds. Moreover, germination responses within thermoperiods and light regimes were highly dependent on the condition of the seed (two- and three-way interactions, $P \leq 0.017$). None of the freshly matured seeds germinated over the range of thermoperiods during 2 weeks of incubation in light or darkness, and 0%–9% germinated during 14 weeks of incubation in light (Table 2). No germination occurred when seeds were warm stratified for 12 weeks in light and incubated for 2 weeks in light, but up to 88% germinated when seeds were warm stratified and incubated in darkness. On the other hand, up to 67% germination occurred when seeds were cold stratified for 12 weeks in light and incubated for 2 weeks in light, and up to 100% germination occurred when seeds were cold stratified and incubated in darkness.

Seed condition, light regime, thermoperiod, and length of incubation or stratification had significant effects on germination ($P < 0.0001$) of 2001-collected seeds. Moreover, germination responses within thermoperiods and light regimes were highly dependent on the condition of the seed and on the length of incubation or stratification (two-, three-, and four-way interactions, $P < 0.0001$). None of the freshly matured seeds germinated over the range of thermoperiods dur-

ing 2 weeks of incubation in light or darkness (Figs. 3a, 3b). During 14 weeks of incubation, nonstratified seeds germinated to 0%–37% over the range of thermoperiods in light and to 84%–95% at thermoperiods from 15:6 to 25:15 $^{\circ}$ C and 0% at those from 30:15 to 35:20 $^{\circ}$ C in darkness. On the other hand, seeds that were warm stratified for 12 weeks in light and incubated for 2 weeks in light did not germinate at any thermoperiod, but those that were warm stratified and incubated in darkness germinated to 80%–95% over the range of thermoperiods (Figs. 3c, 3d). In contrast, seeds that were cold stratified for 12 weeks in light and incubated for 2 weeks in light germinated to 51%–69% at 15:6–25:15 $^{\circ}$ C and 1%–21% at 30:15–35:20 $^{\circ}$ C, and those that were cold stratified and incubated in darkness germinated to 72%–93% at 15:6–25:15 $^{\circ}$ C and 17%–53% at 30:15–35:20 $^{\circ}$ C (Figs. 3e, 3f).

Effect of simulated sequence of seasonal temperature and light regimes on dormancy break and germination

Light treatment, seasonal temperature regime, and their interaction had significant effects on germination ($P < 0.0001$). During summer, seeds that were in light and darkness germinated to 0%–1% (Table 3). Seeds that were in darkness during autumn germinated to 76%–97%, regardless of whether they were in light or darkness during the preceding summer period. On the other hand, seeds that received light during autumn germinated to 10%–20% during autumn, irrespective of the light regime during summer, but germinated to 85%–96% in light or darkness during winter–spring. If seeds were in light or darkness during summer and darkness during both autumn and winter–spring, they had germinated to 91%–98% when they were checked at the end of the winter–spring period with most of this germination probably taking place during the autumn period.

Discussion

Seeds of *S. croceum* are dormant when dispersed in late spring to early summer. Warm stratification allows seeds to germinate in darkness over a range of thermoperiods from 15:6 to 35:20 $^{\circ}$ C, whereas cold stratification permits them to germinate in light and darkness (Table 2; Fig. 3). The light environment experienced during the autumn determines

Fig. 3. Germination percentages (mean \pm SE; SE shown only if $\geq 5\%$) of freshly matured seeds of *Schoenolirion croceum* that remained in light (a) or darkness (b) at the five 12:12 h thermoperiods (15:6 °C (●), 20:10 °C (▽), 25:15 °C (■), 30:15 °C (◇), 35/20 °C (▲)) for 14 weeks; warm-stratified seeds placed at 25:15 °C for 0–12 weeks in light and incubated over the range of thermoperiods for 2 weeks in light (c), or warm-stratified seeds placed in darkness and incubated in darkness (d); and cold-stratified seeds placed at 5 °C for 0–12 weeks in light and incubated over the range of thermoperiods for 2 weeks in light (e), or cold-stratified seeds placed in darkness and incubated in darkness (f). Percentages are cumulative in Fig. 3a. Protected least significant difference is 10% for all pairwise comparisons ($P = 0.05$).

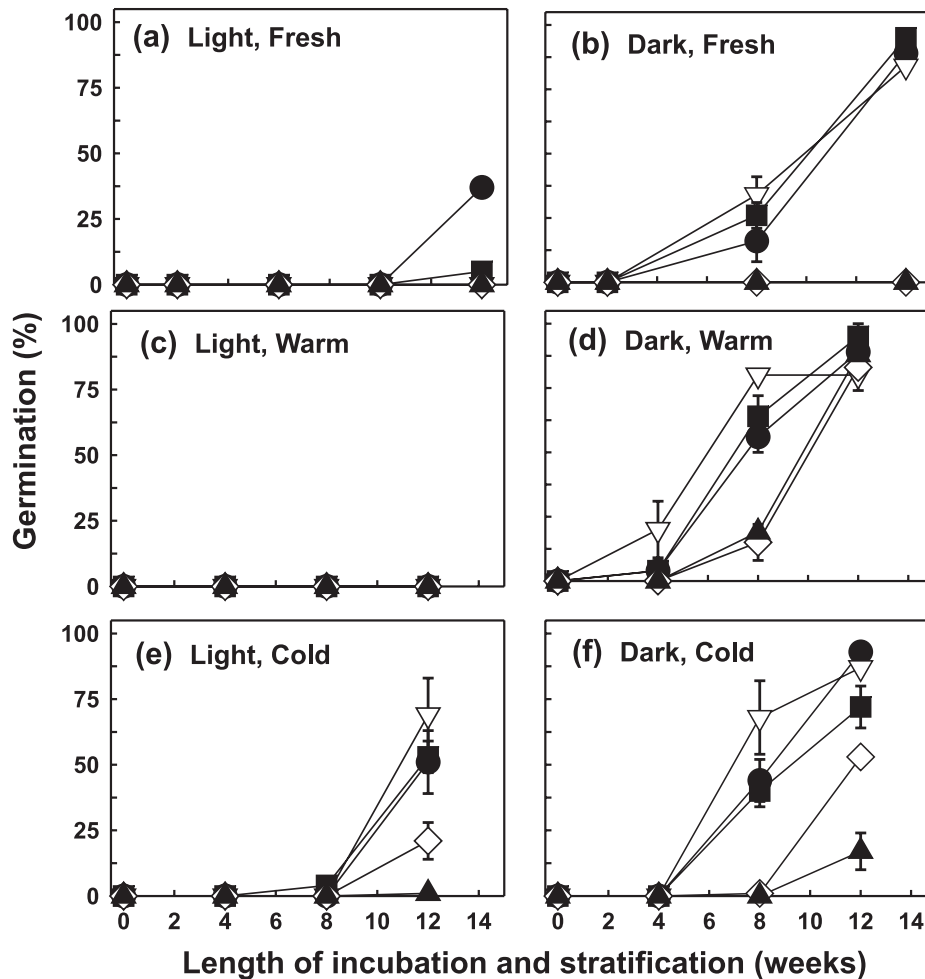


Table 3. Cumulative germination percentages (mean \pm SE) of *Schoenolirion croceum* seeds exposed to a sequence of seasonal temperature and light regimes (see Table 1 for details on treatments).

Seasonal temperature and light regimes					
Summer	Autumn	Winter–spring	Summer	Autumn	Winter–spring
Light	Light	Light	0Aa	14 \pm 4Ab	96 \pm 2Ac
Dark	Light	Light	1 \pm 1Aa	10 \pm 3Ab	85 \pm 4ABc
Dark	Dark	Light	—*	76 \pm 7Ba	81 \pm 10Ba
Dark	Dark	Dark	—*	—*	91 \pm 5AB
Light	Light	Dark	1 \pm 1Aa	20 \pm 5Ab	96 \pm 2Ac
Light	Dark	Light	0Aa	97 \pm 3Cb	97 \pm 3Ab
Light	Dark	Dark	0Aa	—*	98 \pm 2Ab

Note: Values with different upper-case letters within columns or lower-case letters within rows are significantly different (protected least significant difference test or *t* test, $P \leq 0.05$).

*These dishes were not examined because they were incubated in darkness during the specific season.

whether seeds germinate during the autumn or early spring. Seeds exposed to light during autumn germinate in winter–spring regardless of the light regime in summer or winter–spring, whereas those in darkness during autumn germinate in autumn regardless of the light regime in summer (Table 3). Thus, a biseasonal seed germination pattern is set up that is dependent on the light environment and temperatures experienced during dormancy release: autumn germination occurs only in darkness versus early spring germination in light or darkness (Figs. 1, 2).

In contrast, temperatures are responsible for determining the germination phenologies in other species in which ecotypes or life forms have seedlings emerging in autumn and spring. Masuda and Washitani (1992) investigated two ecotypes of the annual *Galium spurium* var. *echinospermon* that share common flowering and fruiting periods and disperse seeds in early summer. However, dormancy in seeds of this species germinating in spring was removed by cold stratification, whereas that in seeds germinating in autumn was released by warm stratification. On the other hand, most seeds of facultative winter annuals germinate in autumn and behave as obligate winter annuals, but some also germinate in spring and behave as short-lived summer annuals or ephemerals (e.g., Roberts and Lockett 1978; Baskin and Baskin 1981, 1989a). Seeds of many facultative winter annuals in the soil seed bank that do not germinate during autumn enter (secondary) conditional dormancy, remaining capable of germinating at prevailing habitat temperatures during spring (Baskin and Baskin 1998).

Seeds of winter annuals and perennials that are dispersed in late spring – early summer and germinate in autumn require warm stratification during summer to overcome dormancy (or conditional dormancy). Low winter temperatures induce secondary dormancy (or conditional dormancy) in seeds of winter annuals remaining in the soil past the autumn germination period (Baskin and Baskin 1998). Dormancy cycling may occur in both light and darkness (e.g., Baskin and Baskin 1989b) or it may take place only in light (i.e., seeds incubated in darkness never germinate) (e.g., Baskin and Baskin 1983). Little is known about what happens to seeds of perennials that, once their dormancy is alleviated during summer, nevertheless fail to complete germination in autumn (Baskin and Baskin 1998). Seeds of *S. croceum* were similar to winter annuals in that warm stratification overcame dormancy but only in darkness (Table 2; Fig. 3). However, *S. croceum* seeds were unlike winter annual seeds, since cold stratification did not induce dormancy but allowed them to germinate in light and darkness.

Only a relatively few species have been reported to germinate to higher percentages in darkness than in light (Baskin and Baskin 1998; Bell et al. 1999). White light is known to inhibit germination when exposure to it is prolonged, especially if the fluence rate is high. However, inhibition also is brought about in some species by intermittent light of a few hours each day resembling a photoperiodic effect, and it may be temperature dependent. Photoinhibition can occur when the embryo is under constraint either from the enclosing tissues or from application of osmotica. Once the embryo is isolated from enclosing tissues (even just radicle tip freed) or removed from the osmotica, light ceases to inhibit germination (Bewley and Black 1994; Baskin and Baskin

1998). This would indicate that the embryos in negatively photoblastic seeds lack sufficient growth potential. Usually if seeds are placed under appropriate dormancy-breaking treatments and (or) germination conditions, the growth potential of the embryo increases and germination occurs. In the case of *S. croceum* seeds, they germinated over a broad range of thermoperiods once dormancy was overcome, and following cold stratification, they germinated in light (Table 2; Fig. 3). Thus, it does not seem that the negative photoblastic response of *S. croceum* seeds was due to a lack of growth potential at any particular temperature.

High percentages of *S. croceum* seeds germinated in darkness at autumn temperature regimes under laboratory conditions, regardless of whether they were exposed to light during summer or not (Table 3). Indeed, nearly all of the seeds in bags buried in the field and exhumed in autumn had germinated, but all were ungerminated when exhumed in late summer (Fig. 2). In contrast, seeds that remained on the soil surface during summer, autumn, and winter germinated in late winter – early spring, e.g., like those on the nylon material in the 2001–2002 germination phenology study. During the 2000–2001 phenology study, seeds were placed directly on the soil surface (Fig. 1). Many of the 2000–2001 seeds remained on the soil surface exposed to light during the summer–autumn period, but by late winter and early spring we observed germinated seeds on the soil surface and beneath the soil surface (J. Walck and S. Hidayati, personal observation). Seedlings from seeds that germinated beneath the soil surface had the upper portion of their stem exposed above ground, but the attached seed remained 1–2 cm beneath the soil surface in some instances. Thus, probably most seeds during the 2000–2001 study germinated in spring in both light and dark conditions.

Germination in *S. croceum* is hypogeal, with a portion of the cotyledon (scutellum) remaining with the seed coat and acting as an absorbing organ and a prominent basal sheath from which the primary root and first leaf emerge (Sherman 1969). Seeds that germinate in autumn remain primarily underground, except for a shoot that emerges slightly from the soil surface and remains relatively small and green until spring when further elongation occurs. In fact, some of the seeds that germinated during autumn were placed 2 cm below the soil surface, and they readily emerged the following spring (J. Walck and S. Hidayati, personal observation). Seedlings observed in buried bags from November to March (Fig. 2) probably came from seeds that germinated primarily in September, and the seedlings survived over winter. When a bag was exhumed in April, seedlings were growing out of it and were up to 4 cm tall nearly reaching the soil surface; the upper 1 cm of a shoot was green and the rest of it was white (J. Walck and S. Hidayati, personal observation). Therefore, a substantial seedling bank establishes during autumn and remains until spring when shoots elongate. It appears that the capacity of *S. croceum* to form a persistent soil seed bank (sensu Thompson et al. 1997) is severely limited given the high percentages of seeds that germinated while buried in soil and while in darkness during laboratory experiments.

Schoenolirion croceum grows with another liliaceous cryptophyte, *Nothoscordum bivalve*, (NatureServe 2003) that has a similar germination response to light and darkness.

Flowering and seed production of *N. bivalve* occurs primarily in spring with seed dispersal in May–June; some plants also flower in autumn with seed dispersal in October. Both spring- and autumn-dispersed seeds germinate from late March to mid-April (Baskin and Baskin 1979). Cold-stratified seeds of *N. bivalve*, like those of *S. croceum*, germinated to higher percentages in darkness than in light. However, the effects of warm stratification were not tested on *N. bivalve* seeds. Dry laboratory storage did not overcome dormancy in seeds of *N. bivalve*, and neither did it do so in seeds of *S. croceum* (J. Walck and S. Hidayati, unpublished data).

From an evolutionary ecology perspective, seed germination of *S. croceum* underground during autumn could be advantageous for a species that grows in a habitat for which the driest soil conditions typically occur in autumn. Although the southeastern United States is classified as temperate climate with no distinct dry season (Trewartha 1968), the months of August to October have the lowest monthly precipitation during the year and the fewest number of days with precipitation of >0.025 cm (National Oceanic and Atmospheric Administration 2003). Soil moisture would be higher a few centimetres beneath the soil surface than directly on top of it, especially during autumn. The ability to germinate in darkness coupled with adequate soil moisture underground would ensure seedling survival and growth as opposed to seed germination in light, i.e., on the soil surface, where frequent dry periods could occur and severely damage seedling establishment. On the other hand, soil moisture would be sufficient in late winter and early spring allowing seedling establishment on top of the soil surface, as well as below it.

A similar idea has been postulated for species growing in sandy habitats (Thanos et al. 1991; Bell et al. 1999). Seed germination in many of these species is photoinhibited. This response to light has been suggested as a mechanism to prevent seedling establishment on the soil surface in a harsh environment, particularly with regards to soil moisture. However, the habitat in which *S. croceum* grows is not constantly harsh throughout the year. Light and temperature interact to regulate the timing of germination for this species: seeds germinate only in darkness following warm stratification but in light and darkness after cold stratification. Thus, seed germination of *S. croceum* is photoinhibited in autumn but not in spring.

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