Germination requirements of *Oenothera biennis* seeds during burial under natural seasonal temperature cycles

CAROL C. BASKIN AND JERRY M. BASKIN
School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225, U.S.A.

Received September 16, 1993

BASKIN, C.C., and BASKIN, J.M. 1994. Germination requirements of *Oenothera biennis* seeds during burial under natural seasonal temperature cycles. Can. J. Bot. **72**: 779–782.

Buried seeds of *Oenothera biennis*, which have the potential to form long-lived seed banks, were investigated to determine whether or not they (*i*) undergo seasonal changes in their dormancy states and (*ii*) require light for germination. Seeds were buried in soil and exposed to natural seasonal temperature changes. Samples of seeds were exhumed at monthly intervals for 31 months and tested for germination in light and darkness at 12-h daily thermoperiods of 15:6, 20:10, 25:15, 30:15, and 35:20°C. At maturity in autumn, seeds germinated to 84–95% in light at 30:15 and 35:20°C, but to 0–69% at other test conditions. By late winter, seeds germinated to 95–100% at the five thermoperiods in light and in darkness. In summer and autumn, germination in light decreased at 15:6°C, and in darkness it dropped to 0% at 15:6°C and decreased at 20:10, 25:15, 30:15, and 35:20°C. Following the second winter of burial, seeds germinated to near 100% at all thermoperiods in light and darkness. Thus, seeds exhibited an annual nondormancy – conditional dormancy cycle, being nondormant from midwinter to late spring and conditionally dormant in summer and autumn. *Oenothera biennis* is 1 of 10 species whose seeds live for 39–40 years or longer in soil and also have an annual conditional dormancy – nondormancy cycle. Seeds of six of these species, including *O. biennis*, can germinate in darkness in spring or summer at simulated habitat temperatures. Therefore, a light requirement for germination is not necessarily a prerequisite for long-term survival of buried seeds, and something other than darkness prevents germination of seeds of some species buried in soil.

Key words: seed banks, buried seeds, germination, dormancy cycles, light requirement, *Oenothera*.

BASKIN, C.C., et BASKIN, J.M. 1994. Germination requirements of *Oenothera biennis* seeds during burial under natural seasonal temperature cycles. Can. J. Bot. **72** : 779–782.

Les auteurs ont suivi les graines enterrées de l'*Oenothera biennis*, capables de former des banques de graines à longue durée de vie, afin de déterminer dans quelle mesure (*i*) ces graines connaissent des changements saisonniers dans leurs états de dormance, et (*ii*) ces graines ont besoin de lumière pour germer. Ils ont enterré des graines dans le sol, lesquelles ont été exposées aux changements saisonniers de température. Des échantillons de graines ont été prélevés à intervalles mensuelles pendant 31 mois, et elles ont été mises à germer à la lumière et à l'obscurité 12:12 h, avec des thermopériodes de 15:6, 20:10, 25:15, 30:15 et 35:20°C. À maturité à l'automne, les graines ont germé à 84–95% à la lumière et 30:15 et 35:20°C. Vers la fin de l'hiver, les graines germent à 95–100% aux cinq thermopériodes à la lumière aussi bien qu'à l'obscurité. À l'été et à l'automne la germination diminue à la lumière à 15:6°C, et à l'obscurité elle tombe à 0% avec 15:6°C et diminue avec les températures de 20:10, 25:15, 30:15, et 35:20°C. Après un deuxième hiver passé sous terre, les graines germent à près de 100% quelles que soient les conditions de lumière et de température. Ainsi, les graines montrent un cycle annuel de non-dormance–dormance conditionnelle, étant non-dormantes du milieu de l'hiver à la fin du printemps, et conditionnellement dormante à l'été et à l'automne. L'*O. biennis* fait partie d'un groupe de 10 espèces dont les graines vivent dans le sol pendant 39–40 ans ou plus, et qui montrent également un cycle annuel de non-dormance–dormance conditonnelle. Les graines de six de ces espèces, incluant l'*O. biennis*, peuvent germer à l'obscurité au printemps ou été, lorsqu'on simule les températures de l'habitat. Conséquemment, le besoin de lumière pour la germination n'est pas un prérequis nécessaire pour la survie à long terme des graines enterrées, et il y aurait quelque chose d'autre que l'obscurité qui préviendrait la germination des graines de certaines espèces enterrées dans le sol.

Mots clés : banques de graines, graines enterrées, germination, cycles de dormance, besoin de lumière, *Oenothera*.
[Traduit par la rédaction]

Introduction

If seeds of a plant species fail to germinate in the first germination season following maturation but germinate during the second or some subsequent germination season, the species has a persistent seed bank (sensu Grime 1981). Many species in a variety of plant communities form persistent seed banks, and in many of these species the seeds eventually become covered with soil (Roberts 1981). An understanding of the germination ecology of species that form persistent buried seed banks requires information about the seeds while they are buried. If seed coats are permeable to water, buried seeds may undergo cyclic changes in their dormancy state in response to seasonal temperature changes (Baskin and Baskin 1981*a*; Bouwmeester and Karssen 1992).

If seeds are dormant, they do not germinate at any temperature in light or darkness, while nondormant ones germinate over the widest range of temperatures possible for the species or populations; light and dark requirements for germination vary with the species (Baskin and Baskin 1985*a*). If seeds germinate at some but not at all the temperatures possible for the species, they are in conditional dormancy (sensu Vegis 1964). Buried seeds can lose their dormancy and remain nondormant (Baskin and Baskin 1985*b*), or they may cycle between dormancy and nondormancy (Baskin and Baskin 1980) or between conditional dormancy and nondormancy (Baskin and Baskin 1981*a*).

Oenothera biennis L. is an example of a species with long-lived buried seeds. Seeds of this species remained viable for 80 years in the Beal buried seeds experiment (Kivilaan and Bandurski 1981) and for 39 years in the Duvel buried seed experiment (Toole and Brown 1946). In the Duvel study, 17 and 18% of the seeds buried at 55 and 105 cm, respectively, germinated when exhumed after 39 years (Toole and Brown 1946). Seeds of *O. biennis* have been found in seed banks in natural plant communities in Denmark (Odum 1978), Poland (Symonides 1986), and in the United States in Illinois (Johnson and Anderson 1986), Massachusetts (Livingston and Allessio 1968), Maryland (Bigwood and Inouye 1988), North Carolina (Oosting and Humphreys 1940), and New Hampshire (Prince and Hodgdon 1946).

The purpose of this study was to determine (*i*) whether or not buried seeds of *O. biennis* undergo seasonal changes in their dormancy state and (*ii*) if light is required for germination.

Materials and methods

Ripe seeds were collected from plants of *O. biennis* growing in Fayette County, Ky., on 2 October 1985 and from plants in Robertson County, Ky., on 29 October 1989. In both years, studies were initiated the day after seed collection.

Freshly matured seeds and those exhumed after 1−31 months of burial in soil were tested for germination; seeds were exhumed on the 1st day of each month. For burial, seeds were placed in fine-mesh (average area of openings was 0.13 mm^2) nylon bags. Each bag was buried to a depth of 7 cm in soil (3:1 v/v mixture of limestone-derived topsoil and river sand) in 15-cm diameter plastic pots with drainage holes. Pots were placed under a bench in a nonheated greenhouse in Lexington, Ky. This greenhouse received no heating or air-conditioning, and its windows were kept open throughout the year; continuous thermograph records were made inside a small weather house located in the greenhouse. Mean daily maximum and minimum temperatures for each month of the year in the nonheated greenhouse are usually within 3°C of those recorded 10 cm below bare soil on the University of Kentucky Research Farm 15 km north of the nonheated greenhouse (Baskin and Baskin 1985*b*). From 1 September to 30 April of each year, the soil in the pots was watered to field capacity daily, unless frozen, and during the remainder of the year it was watered to field capacity once a week. This watering regime was given to simulate soil moisture conditions in the field in north central Kentucky throughout the year.

For germination tests, seeds were placed in five incubators in light (14-h daily photoperiod of ca. 20 μmol · m^{-2} · s^{-1}, 400−700 nm, cool white fluorescent light) or in continuous darkness for the duration of a germination test. Incubators were set on 12-h daily thermoperiods approximating mean daily maximum and minimum air temperatures for each month during the growing season in north central Kentucky (Wallis 1977): March and November, 15:6°C; April and October, 20:10°C; May, 25:15°C; June and September, 30:15°C; and July and August, 35:20°C. For each thermoperiod, the photoperiod extended from 1 h before to 1 h after the hight temperature period.

Seeds were incubated on white quartz sand moistened with distilled water in 5.5-cm Petri dishes. All dishes were wrapped with plastic film to retard the loss of water, and those incubated in darkness also were wrapped with two layers of aluminum foil. Three replications of 50 seeds each of freshly matured seeds were incubated in light and darkness at each thermoperiod. When buried seeds were exhumed, those to be incubated in darkness were not exposed to any light. In darkness, the bag of seeds was removed from the pot of soil and cut open, and the seeds were poured into a dish. A pinch of 50−75 seeds was placed in each Petri dish to be incubated in darkness. Fluorescent room light was used to count seeds incubated in light. Thus, for exhumed seeds, three replications of 50 seeds each were incubated in light and three replications of 50−75 seeds each were incubated in darkness for each thermoperiod. Final germination percentages were determined after 15 days and protrusion of the radicle was the criterion of germination. Ungerminated seeds were pinched with forceps under a dissecting microscope to determine whether or not the embryos were firm and white; tetrazolium tests revealed that such embryos were alive.

Results

Seeds of *O. biennis* collected in different years and in different locations exhibited very similar patterns of germination responses. Freshly matured seeds of *O. biennis* germinated to high percentages (84−95%) only at high temperatures (30:15, 35:20°C) and in light (Figs. 1 and 2). However, by the end of the first winter of burial (March), 95−100% of exhumed seeds germinated at all thermoperiods in light and darkness, except only 40% of 1989 seeds germinated in darkness at 15:6°C. From the first spring until the end of the studies, 84−100% of exhumed seeds incubated in light germinated at 20:10, 25:15, 30:15, and 35:20°C, while germination at 15:6°C reached a maximum of 97−100% from January to June and a minimum of 1−54% from July to November (Figs. 1*b* and 2*b*).

Exhumed seeds incubated in darkness reached maximum germination at all thermoperiods in the winter and spring and a minimum in the summer and autumn (Figs. 1*c* and 2*c*). In late spring − early summer, germination in darkness decreased first at low and then at progressively higher temperatures. Declines in germination in darkness during summer were greater for 1989 than for 1985 seeds (Figs. 1*c* and 2*c*). The highest germination in darkness in summer and autumn was often at 30:15°C. In the first spring (March) after burial, only 40% of 1989 seeds incubated in darkness at 15:6°C germinated (Fig. 2*c*), while 97% of 1985 seeds germinated (Fig. 1*c*). In the second and third springs following burial, however, germination of 1989 seeds incubated in darkness at 15:6°C was 96 and 97%, respectively.

Discussion

Since 84−95% of freshly matured seeds of *O. biennis* germinated at 30:15 and 35:20°C, 1−69% germinated at 15:6, 20:10, and 25:15°C, and later 100% germinated at all these thermoperiods (Figs. 1 and 2), most seeds were conditionally dormant at maturity in autumn. Conditional dormancy was broken during winter and seeds were nondormant in middle to late winter and spring. Conditional dormancy was induced in seeds in late spring and summer and broken during winter. Therefore, seeds of *O. biennis* have an annual conditional dormancy − nondormancy cycle, with the dormancy break occurring during the season with low temperatures and induction into conditional dormancy occurring during the season with high temperatures. Buried seeds of *Verbascum thapsus* and *Verbascum blattaria* also exhibit an annual conditional dormancy − nondormancy cycle, with seeds coming out of dormancy in winter and entering conditional dormancy in spring or summer (Baskin and Baskin 1981*b*). Vanlerberghe and van Assche (1986) demonstrated that cold stratification at 4°C broke dormancy in seeds of *V. thapsus*. On the other hand, placing nondormant seeds of *V. thapsus* at 10°C for 1 month and then at 20°C for 1 month caused decreases in germination percentages of seeds tested at 15:6 and 20:10°C but not at 35:20°C. Thus, changes in dormancy state were controlled by changes in temperature.

One ecological implication of the annual conditional dormancy − nondormancy cycle in buried seeds of *O. biennis* is that any seeds exposed to light in the field in late autumn when temperatures are low (15:6°C) would not germinate. Thus, germination would be prevented at the end of the growing season, when chances for seedling survival may be low. However, seeds could germinate in light at low (15:6°C) tempera-

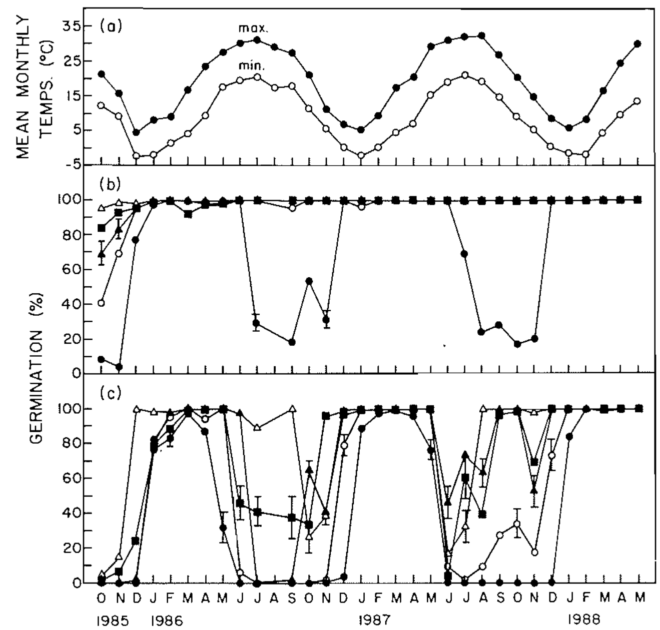

FIG. 1. (*a*) Mean monthly air temperatures in the nonheated greenhouse for the duration of the study. (*b*) Germination percentages (means ± SE, if ≥5%) of 1985 *Oenothera biennis* seeds incubated in light for 15 days following 0–31 months of burial in soil. (*c*) Germination percentages (means ± SE, if ≥5%) of 1985 *Oenothera biennis* seeds incubated in darkness for 15 days following 0–31 months of burial in soil. (*b* and *c*) ●, 15:6°C; ○, 20:10°C; ▲, 25:15°C; △, 30:15°C; ■, 35:20°C.

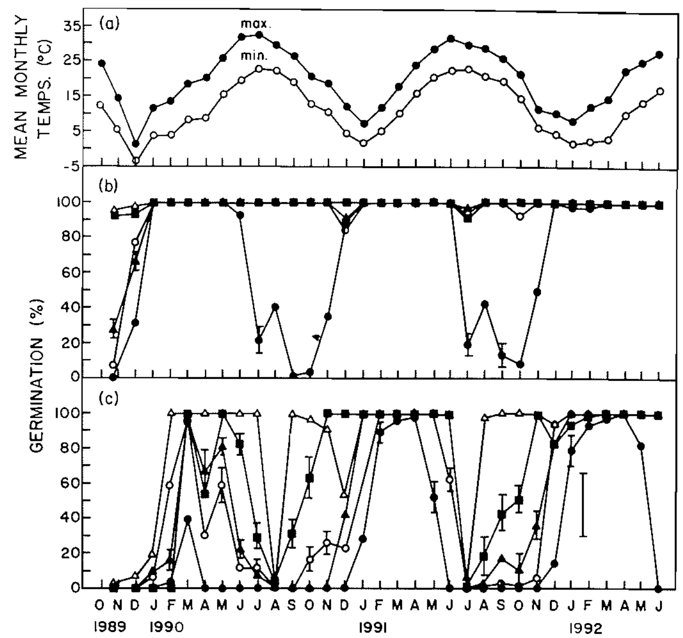

FIG. 2. (*a*) Mean monthly air temperatures in the nonheated greenhouse for the duration of the study. (*b*) Germination percentages (means ± SE, if ≥5%) of 1989 *Oenothera biennis* seeds incubated in light following 0–31 months of burial in soil. (*c*) Germination percentages (means ± SE, if ≥5%) of 1989 *Oenothera biennis* seeds incubated in darkness following 0–31 months of burial in soil. (*b* and *c*) ●, 15:6°C; ○, 20:10°C; ▲, 25:15°C; △, 30:15°C; ■, 35:20°C.

tures in early spring, permitting seedling establishment at the beginning of the growing season.

Although we obtained 100% germination in darkness at 25:15°C in many germination tests of exhumed 1985 and 1989 seeds (Figs. 1*c* and 2*c*), Toole et al. (1957) failed to obtain germination of *O. biennis* seeds in darkness at 15, 20, 25, 30, 35, 25:15, and 30:20°C. Further, in an investigation of the effects of irradiance and spectral quality on germination of *O. biennis*, Gross (1985) did not obtain any germination of seeds incubated in darkness at 25:15°C. Both Toole et al. (1957) and Gross (1985) used seeds that had not been given a cold stratification treatment, whereas our seeds received cold stratification in the nonheated greenhouse during winter (Figs. 1*a* and 2*a*). In some species, e.g., *Solanum nigrum* L. (Roberts and Lockett 1978), cold stratification overcomes the light requirement for germination. We suggest that Toole et al. (1957) and Gross (1985) did not obtain germination in darkness because their seeds were not cold stratified and thus were partly dormant.

In the Beal (Kivilaan and Bandurski 1981) and Duvel (Toole and Brown 1946) buried seed experiments, seeds of 41 species, including *O. biennis*, were alive after 39–40 years. Studies were done on buried seeds of 13 of these species to determine whether or not they undergo seasonal changes in their germination responses. In addition to *O. biennis*, buried seeds of *Amaranthus retroflexus* L. (Baskin and Baskin 1977; C.C. Baskin and J.M. Baskin, unpublished data), *Capsella bursa-pastoris* (L.) Medic (Baskin and Baskin 1989), *Chenopodium album* L. (Baskin and Baskin 1977; C.C. Baskin and J.M. Baskin, unpublished data), *Phytolacca americana* L. (C.C. Baskin and J.M. Baskin, unpublished data), *Rumex obtusifolius* L. (van Assche and Vanlerberghe 1989), *Setaria glauca* (L.) Beauv. (C.C. Baskin and J.M. Baskin, unpublished data), *Solanum nigrum* (Roberts and Lockett 1978), and *V. blattaria* and *V. thapsus* (Baskin and Baskin 1981*b*) exhibit annual conditional dormancy – nondormancy cycles. Buried seeds of *Ambrosia artemisiifolia* L. (Baskin and Baskin 1980) undergo annual dormancy–nondormancy cycles, while those of *Lepidium virginicum* L. (C.C. Baskin and J.M. Baskin, unpublished data), and *Rumex crispus* L. (Baskin and Baskin 1985*b*) come out of dormancy shortly after burial and remain nondormant. Thus, available data indicate that long-lived buried seeds undergo cyclic changes in their dormancy states and that seeds of most species cycle between nondormancy and conditional dormancy, like those of *O. biennis*.

Wesson and Wareing (1969) concluded that "... under natural conditions in the field, the germination of buried seeds, following a disturbance of the soil, is completely dependent upon exposure to light." Further, Thompson and Grime (1979) noted that germination in many species that form persistent seed banks is inhibited by darkness. Of the 13 species listed above, only seeds of *Amaranthus retroflexus*, *Chenopodium album* (Baskin and Baskin 1977; C.C. Baskin and J.M. Baskin, unpublished data), and *Rumex crispus* (Baskin and Baskin 1985*b*) required light for germination after they were exhumed. Not only did exhumed seeds of *O. biennis* (Figs. 1*c* and 2*c*), *Phytolacca americana* (C.C. Baskin and J.M. Baskin, unpublished data), *Rumex obtusifolius* (van Assche and Vanlerberghe 1989), *Setaria glauca* (C.C. Baskin and J.M. Baskin, unpublished data), and *V. thapsus* and *V. blattaria* (Baskin and Baskin 1981*b*) germinate in darkness, but they germinated in darkness in spring or summer at simulated habitat temperatures. Thus, a strict light requirement for germination is not a prerequisite for the formation of a long-lived seed bank in all species.

Although exhumed seeds of *O. biennis* germinated to high percentages in darkness in spring or summer at simulated habitat temperatures, few seeds germinated in spring or summer while they were buried in the soil in the nonheated greenhouse. Two hundred (7%) 1985 seeds germinated in April 1986, 2 (0.001%) 1989 seeds germinated in February 1990, and 30 (0.01%) 1989 seeds germinated in March 1990 while buried in the soil in the nonheated greenhouse. Similar results were obtained for buried seeds of *V. thapsus* and *V. blattaria* (Baskin and Baskin 1981*b*). Apparently, some factor(s) associated with the burial environment other than darkness prevented germination. Inhibition of germination of seeds in the soil has been attributed to improper aeration (Bibbey 1948) and the presence of inhibitory volatile products of anaerobic respiration (Holm 1972). Much remains to be learned about the factors that prevent germination of seeds during burial in the soil.

Baskin, J.M., and Baskin, C.C. 1977. Role of temperature in the germination ecology of three summer annual weeds. Oecologia, **30**: 377–382.

Baskin, J.M., and Baskin, C.C. 1980. Ecophysiology of secondary dormancy in seeds of *Ambrosia artemisiifolia*. Ecology, **61**: 475–480.

Baskin, J.M., and Baskin, C.C. 1981*a*. Seasonal changes in the germination responses of buried *Lamium amplexicaule* seeds. Weed Res. **21**: 299–306.

Baskin, J.M., and Baskin, C.C. 1981*b*. Seasonal changes in germination responses of buried seeds of *Verbascum thapsus* and *V. blattaria* and ecological implications. Can. J. Bot. **59**: 1769–1775.

Baskin, J.M., and Baskin, C.C. 1985*a*. The annual dormancy cycle in buried weed seeds: a continuum. BioScience, **35**: 492–498.

Baskin, J.M., and Baskin, C.C. 1985*b*. Does seed dormancy play a role in the germination ecology of *Rumex crispus*? Weed Sci. **33**: 340–343.

Baskin, J.M., and Baskin, C.C. 1989. Germination responses of buried seeds of *Capsella bursa-pastoris* exposed to seasonal temperature changes. Weed Res. **29**: 205–212.

Bibbey, R.O. 1948. Physiological studies on weed seed germination. Plant Physiol. **23**: 467–484.

Bigwood, D.W., and Inouye, D.W. 1988. Spatial pattern analysis of seed banks: an improved method and optimized sampling. Ecology, **69**: 497–507.

Bouwmeester, H.J., and Karssen, C.M. 1992. The dual role of temperature in the regulation of the seasonal changes in dormancy and germination of seeds of *Polygonum persicaria* L. Oecologia, **90**: 88–94.

Grime, J.P. 1981. The role of seed dormancy in vegetation dynamics. Ann. Appl. Biol. **98**: 555–558.

Gross, K.L. 1985. Effects of irradiance and spectral quality on the germination of *Verbascum thapsus* L. and *Oenothera biennis* L. seeds. New Phytol. **101**: 531–541.

Holm, R.E. 1972. Volatile metabolites controlling germination in buried weed seeds. Plant Physiol. **50**: 293–297.

Johnson, R.G., and Anderson, R.C. 1986. The seed bank of a tallgrass prairie in Illinois. Am. Midl. Nat. **115**: 123–130.

Kivilaan, A., and Bandurski, R.S. 1981. The one hundred-year period for Dr. Beal's seed viability experiment. Am. J. Bot. **68**: 1290–1292.

Livingston, R.B., and Allessio, M.L. 1968. Buried viable seeds in successional field and forest stands, Harvard Forest, Massachusetts. Bull. Torrey Bot. Club, **95**: 58–69.

Odum, S. 1978. Dormant seeds in Danish ruderal soils. Royal Veterinary and Agricultural University, Horsholm Arboretum, Horsholm, Denmark.

Oosting, H.J., and Humphreys, M.E. 1940. Buried viable seeds in a successional series of old field and forest soils. Bull. Torrey Bot. Club, **67**: 253–273.

Prince, F.S., and Hodgdon, A.R. 1946. Viable seeds in old pasture soils. Univ. N. Hamps. Agric. Exp. Stn. Tech. Bull. No. 89.

Roberts, H.A. 1981. Seed banks in soils. Adv. Appl. Biol. **6**: 1–55.

Roberts, H.A., and Lockett, P.M. 1978. Seed dormancy and field emergence in *Solanum nigrum* L. Weed Res. **18**: 231–241.

Symonides, E. 1986. Seed bank in old-field successional ecosystems. Ekol. Pol. **34**: 3–29.

Thompson, K., and Grime, J.P. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. J. Ecol. **67**: 893–921.

Toole, E.H., and Brown, E. 1946. Final results of the Duvel buried seed experiment. J. Agric. Res. (Washington, D.C.), **72**: 201–210.

Toole, E.H., Toole, V.K., Hendricks, S.B., and Borthwick, H.A. 1957. Effect of temperature on germination of light-sensitive seeds. Proc. Int. Seed Test. Assoc. **22**: 196–204.

van Assche, J.A., and Vanlerberghe, K.A. 1989. The role of temperature on the dormancy cycle of *Rumex obtusifolius* L. Funct. Ecol. **3**: 107–115.

Vanlerberghe, K.A., and van Assche, J.A. 1986. Dormancy phases in seeds of *Verbascum thapsus* L. Oecologia, **68**: 479–480.

Vegis, A. 1964. Dormancy in higher plants. Annu. Rev. Plant Physiol. **15**: 185–215.

Wallis, A.L., Jr. (*Editor*). 1977. Comparative climatic data through 1976. National Climatic Center, Environmental Data Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Asheville, N.C.

Wesson, G., and Wareing, P.F. 1969. The role of light in the germination of naturally-occurring populations of buried weed seeds. J. Exp. Bot. **20**: 402–413.