Plant Species Biol. **6** : 111-115, 1991

PLANT SPECIES BIOLOGY

Temperature Response Pattern during Afterripening of Achenes of the Winter Annual *Krigia oppositifolia* (Asteraceae)

Carol C. Baskin[1,2], Jerry M. Baskin[1,2] and Edward W. Chester[2]

[1] *School of Biological Sciences, University of Kentucky, Lexington, KY 40506, U.S.A.*
[2] *Center for Field Biology, Austin Peay State University, Clarksville, TN 37044, U.S.A.*

Abstract Freshly-matured achenes of *Krigia oppositifolia* Raf. were buried in soil at near-natural temperatures for 0–35 months and then exhumed and tested in light and darkness at (12/12 hr) daily thermoperiods of 15/6, 20/10, 25/15, 30/15 and 35/20°C. Achenes required light for germination and exhibited an annual dormancy/nondormancy cycle, being dormant in spring and nondormant in autumn. High summer temperatures (30/15, 35/20°C) fully promoted afterripening, whereas low temperatures (5, 15/6°C) prevented it. As buried seeds came out of dormancy in summer, they first germinated at medium temperatures (20/10, 25/15°C), but with additional afterripening the maximum and minimum temperatures for germination increased and decreased, respectively. Thus, during afterripening, achenes exhibit type 3 temperature responses, which otherwise are known only in two perennial Asteraceae and one perennial Liliaceae. The physiological responses of achenes of *K. oppositifolia* are unlike those of most winter annuals, which have type 1 responses—i.e., the maximum temperature for germination increases during afterripening. Also, they are unlike the majority of Asteraceae, which have type 2 responses—i.e., the minimum temperature for germination decreases during afterripening. Type 1 responses, typical of most winter annuals, have yet to be reported in the Asteraceae.

Key words: *Krigia*, Asteraceae, winter annual, seed afterripening, temperature response.

The question addressed in this study is: what are the temperature requirements for germination as achenes of winter annual Asteraceae come out of dormancy? Is the pattern of changes in temperature responses like that of other Asteraceae in temperate regions? Or is it like that of winter annuals in general? Vegis (1963) described three patterns (types) of changes in temperature responses as seeds come out of dormancy (afterripen): 1) the maximum temperature for germination increases; 2) the minimum temperature for germination decreases; and 3) both 1) and 2) occur. In a survey of the dormancy breaking and germination requirements of herbaceous plant species in a temperate region (Baskin and Baskin, 1988), 13 of 14 species of Asteraceae with dormant achenes (not including *Heterotheca subaxillaris*) had a type 2 response. The other composite (*Aster ptarmicoides* (Nees) T. and G.) had a type 3 response, as does *Echinacea angustifolia* DC. var. *angustifolia* (Baskin, Baskin and Hoffman, ms.).

In the 1988 survey, 44 of 51 species of winter annuals had a type 1 temperature response, but three species had a type 2 response. Only four species of winter annual Asteraceae were included in the survey, and three of them had nondormant achenes. The fourth, *Heterotheca subaxillaris* (Lam.) Britt. and Rusby had nondormant disc and dormant ray achenes (Baskin and Baskin, 1988), which exhibited type 2 temperature responses as they afterripened (Baskin and Baskin, 1976a).

On May 7, 1988, we found thousands of plants of the winter annual *Krigia oppositifolia* Raf. with ripe achenes in a first-year oldfield on the floodplain of the Cumberland River in Montgomery County, Tennessee, U.S.A. Spring maturation of achenes of a winter annual composite was of great interest because the achenes of many winter annual members of this family in the region do not mature their achenes until mid-summer or autumn. Further, achenes of these late-maturing species are nondormant (Baskin and Baskin, 1988). By contrast, freshly-matured achenes of *K. oppositifolia* proved to be dormant, so that we could use this species to investigate the temperature responses in achenes of a winter annual in the Asteraceae as they come out of dormancy. In this paper, we report the type of temperature responses in achenes of *K. oppositifolia* and their light requirements for germination.

K. oppositifolia is a native North American species that occurs from southern Virginia to Florida and westward to southern Illinois, Kansas and Texas (Gleason, 1952). It grows in a variety of habitats, including moist, low places (Gleason and Cronquist, 1991; Steyermark, 1963), fields, roadsides, pastures (Radford, Ahles and Bell, 1968), sandy alluvial ground, meadows, glades and prairies (Steyermark, 1963).

Materials and Methods

On May 7, 1988, heads containing dark brown (ripe) achenes were individually picked by hand from plants of *K. oppositifolia* growing in a first-year oldfield in Montgomery County, Tennessee. Achenes were allowed to dry for five days in the laboratory, and then germination studies were initiated.

Germination tests were performed in light- and temperature-controlled incubators either at a 14-hr daily photoperiod (ca. 20 μmol m^{-2} s^{-1}, 400–700 nm of cool white fluorescent light) or in constant darkness, and at alternating temperature regimes (12/12 hr) of 15/6, 20/10, 25/15, 30/15 and 35/20°C. At each thermoperiod, the photoperiod extended from 1 hr before the high temperature period began to 1 hr after it ended. Achenes were incubated in 5.5 cm Petri dishes on white quartz sand moistened with distilled water. Three replications were used for each treatment, and unless otherwise stated each dish contained 50 achenes. Dishes containing achenes to be incubated in light were wrapped with plastic film to retard water loss. Those incubated in darkness were wrapped with plastic film and then with two layers of aluminum foil. Final germination percentages were determined after 15 days of incubation, and protrusion of the radicle was the criterion of germination.

1. *Germination Requirements of Exhumed Achenes*

On May 12, 1988, about 3,000 achenes were placed in each of 14 fine-mesh nylon bags. Each bag was buried to a depth of 7 cm in soil in 15-cm-diameter clay pots with drainage holes. These pots were placed under a bench in a nontemperature-controlled greenhouse, where windows were kept open all year. Mean daily maximum and minimum monthly temperatures in the greenhouse for the duration of the study were calculated from continuous thermograph records (Table 1). Soil in the pots was watered to field capacity once each week from May 1 to August 31 and daily from September 1 to April 30, unless it was frozen. These watering regimes were given to simulate soil moisture conditions that could occur in the field.

Freshly-matured achenes and those exhumed on the first day of each month from June 1988 through April 1989 and on October 1, 1989, April 1 and October 1, 1990 and April 1, 1991 were tested in light and darkness at the five thermoperiods. Achenes in-

Table 1. Mean maximum and minimum monthly temperatures (°C) in the nontemperature-controlled greenhouse in Lexington, Kentucky, U.S.A., during the period when seeds of *Krigia oppositifolia* were buried in soil.

Month	1988		1989		1990		1991	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
January	—	—	11.3	3.1	11.7	3.7	7.3	1.8
February	—	—	8.1	−0.1	13.5	3.8	12.1	5.0
March	—	—	17.8	6.2	18.9	8.0	18.1	10.2
April	—	—	22.8	10.5	20.2	8.6		
May	29.9	13.7	25.5	14.3	26.6	15.6		
June	32.9	18.9	30.6	20.0	32.0	19.8		
July	34.1	22.3	34.1	22.5	32.5	22.9		
August	33.3	21.9	32.9	20.9	29.9	22.5		
September	27.9	17.7	27.6	17.1	26.4	19.0		
October	18.5	8.7	24.2	12.1	20.4	12.8		
November	14.5	5.3	14.2	5.4	18.9	10.7		
December	9.2	0.9	1.2	−4.8	12.1	4.7		

cubated in darkness were not exposed to any light from the time they were buried until the end of the germination tests. In darkness, the bag of achenes was retrieved from the soil, cut open and the achenes poured into a dish. A "pinch" of about 50 achenes was placed in each Petri dish, and then the dishes were wrapped with plastic film and aluminum foil. Achenes to be incubated in light were counted into three replications of 50 achenes each for each thermoperiod using fluorescent room light. Germination percentages were arcsin transformed, and the least significant difference for all germination percentages in light was computed using Tukey's Honestly Significant Difference Test (HSD) at the 5% level of significance. The HSD value was reverse transformed to a percentage.

2. *Temperature Requirements for Afterripening*

On May 12, 1988, about 1,500 achenes were placed in each of six fine-mesh nylon bags and buried to a depth of 7 cm in soil in 15-cm-diameter plastic pots with drainage holes. One pot was placed at a constant temperature of 5°C and at each of the five thermoperiods. Soil in the pots was kept at or near field capacity. The bags of achenes were exhumed on September 15, 1988, and achenes from each temperature were tested in light over the range of thermoperiods.

Results

1. *Germination Requirements of Exhumed Achenes*

Achenes were dormant at maturity in May, and they afterripened during summer (Fig. 1a). Regardless of achene age, light was required for germination; only an occasional achene germinated in darkness (Fig. 1b). As achenes came out of dormancy, the highest germination percentage in light in June and July was at 20/10°C, and in August it was at 25/15°C. By October, achenes germinated to 90% or more at 20/10, 25/15 and 30/15°C, but to only 79 and 71% at 15/6 and 35/20°C, respectively. Buried achenes exhibited an annual dormancy/nondormancy cycle. During the course of the study, achenes were dormant (May 1988), nondormant (October 1988), dormant (April 1989), nondormant (October 1989), dormant (April 1990), nondormant (October 1990) and dormant (April 1991) (Fig. 1a).

2. *Temperature Requirements for Afterripening*

Achenes of *K. oppositifolia* buried at 5 and 15/6°C did not come out of dormancy (Table 2). Although achenes buried for 4 months at these two temperatures were viable (embryos were firm and white), 4% or less of them germinated when incubated over the range of thermoperiods in light. The degree of afterripening, as indicated by germination percent-

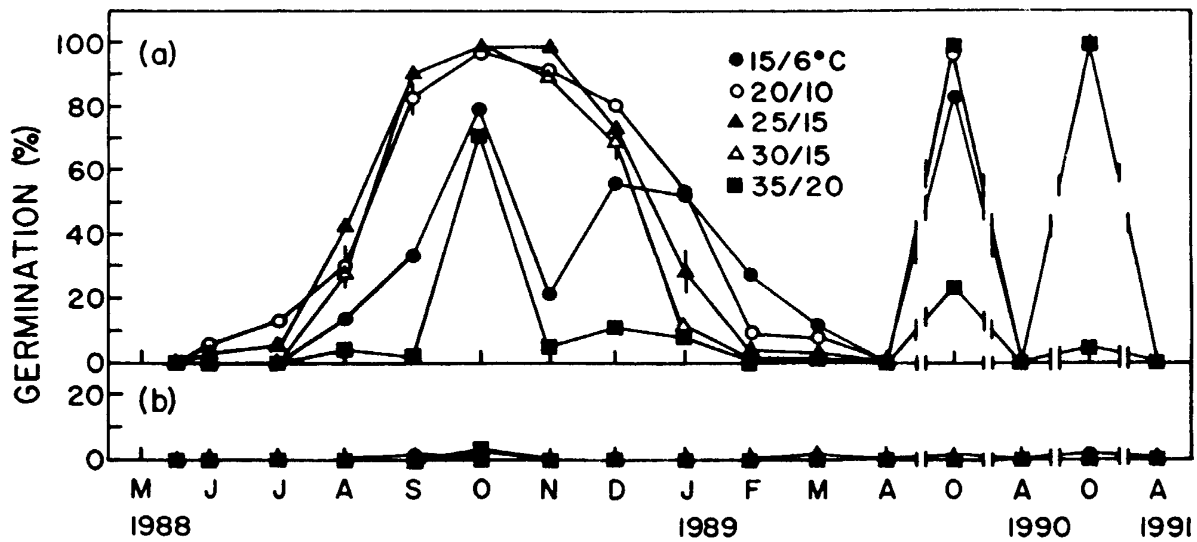

Fig. 1. (a). Germination percentages (mean ± SE) of *Krigia oppositifolia* seeds incubated at a 14-hr daily photoperiod at 15/6 (●), 20/10 (○), 25/15 (▲), 30/15 (△) and 35/20°C (■) for 15 days following various periods of burial in soil in the nontemperature-controlled greenhouse. Tukey's HSD = 19% (P < 0.05). (b). Germination percentages (mean ± SE) of *Krigia oppositifolia* seeds incubated in darkness at 15/6 (●), 20/10 (○), 25/15 (▲), 30/15 (△) and 35/20°C (■) for 15 days following various periods of burial in soil in the nontemperature-controlled greenhouse.

Table 2. Germination percentages (mean±SE) of *Krigia oppositifolia* seeds buried in moist soil at various temperatures from May 12 until September 15, 1988, when they were exhumed and incubated in light (14 h photoperiod) at the five thermoperiods for 15 days.

Burial Temps. (°C)	Incubation temperatures (°C)				
	15/6	20/10	25/15	30/15	35/20
5	0	0	0	0	0
15/6	0	1±1	4±1	0	0
20/10	72±1	79±3	56±7	13±2	0
25/15	74±4	100	100	89±5	15±1
30/15	93±0	96±3	100	84±6	83±6
35/20	91±1	99±1	100	100	79±3

ages, increased with each increase in burial temperatures above 15/6°C, and achenes buried at 30/15 and 35/20°C afterripened completely.

Discussion

Achenes of *K. oppositifolia* are like seeds of other winter annuals in two important ways. One, dormant achenes required high temperatures for the breaking of dormancy (Table 2). Afterripening in seeds of a number of winter annuals, including *Viola rafinesquii* Greene (Baskin and Baskin, 1972), *Stellaria media* (L.) Cyrillo, *Valerianella umbilicata* (Sulliv.) Wood, *Phacelia purshii* Buckl. (Baskin and Baskin, 1976b), *Lamium amplexicaule* (Baskin and Baskin, 1984a), *L. purpureum* L. (Baskin and Baskin, 1984b), *Thlaspi perfoliata* L., *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa-pastoris* (L.) Medic, *Arenaria serpyllifolia* L., *Cardamine hirsuta* L., *Holosteum umbellatum* L., *Cerastium viscosum* L. (Baskin and Baskin, 1986), *Veronica hederifolia* L. (Roberts and Neilson, 1982a) and *Aphanes arvensis* L. (Roberts and Neilson, 1982b), is fully promoted by high summer temperatures and wholly or partially inhibited by low winter temperatures. Two, achenes of *K. oppositifolia* buried in soil in the nontemperature-controlled greenhouse exhibited an annual dormancy/nondormancy cycle, with the achenes being dormant in spring and nondormant in autumn. This type of annual dormancy/nondormancy cycle has been documented in the winter annuals *Lamium purpureum* (Baskin and Baskin, 1984b), *Arabidopsis thaliana* (Baskin and Baskin, 1983a), *Phacelia dubia* (L.) Trel. (Baskin and Baskin, 1973) and *Chaerophyllum tainturieri* Hook. (Baskin and Baskin, 1990). In these winter annuals as well as in *K. oppositifolia*, germination occurs only in autumn, and the species behave as strict winter annuals. In contrast, seeds of facultative winter annuals lose their ability to germinate at high but not at low temperatures during winter. Thus, seeds can germinate in autumn or spring, and plants behave as winter annuals or short-lived spring emphemerals (Baskin and Baskin, 1985).

As achenes of *K. oppositifolia* came out of dormancy, they germinated first at medium temperatures (20/10, 25/15°C), but with additional afterripening the maximum and minimum temperatures for germination increased. Therefore, the achenes exhibited type 3 temperature responses (*sensu* Vegis, 1963), as they afterripened. Type 3 temperature responses are not known in other winter annuals; however, they occur in three perennials: *Allium cernuum* Roth. (Liliaceae) and *Aster ptarmicoides* (Baskin and Baskin, 1988) and *Echinacea angustifolia* (Baskin, Baskin and Hoffman, ms.) in the Asteraceae.

Type 1 temperature responses are very common in winter annuals, and they occur in the Apiaceae (Baskin and Baskin, 1974), Boraginaceae (Baskin and Baskin, 1988), Brassicaceae (Baskin and Baskin, 1971a), Campanulaceae (Baskin and Baskin, 1988), Caryophyllaceae (Baskin and Baskin, 1982), Crassulaceae (Baskin and Baskin, 1977), Fumariaceae (Baskin and Baskin, unpubl.), Hydophyllaceae (Baskin and Baskin, 1971b), Labiatae (Baskin and Baskin, 1981), Plantaginacaeae (Baskin and Baskin, unpubl.), Rosaceae (Roberts and Neilson, 1982b), Scrophulariaceae (Baskin and Baskin, 1983b), Valerianaceae (Baskin and Baskin, 1976c) and Violaceae (Baskin and Baskin, 1972). A few winter annuals, namely *Barbarea vulgaris* R. Br., *Lesquerella filiformis* Rollins (Brassicaceae) *Lobelia gattingeri* A. Gray (Campanulaceae) and *Heterotheca subaxillaris* (Astereaceae), have type 2 temperature responses (Baskin and Baskin, 1988). It should be noted, however, that although *B. vulgaris* (Baskin and Baskin, 1989) and *L. gattingeri* (Baskin and Baskin, 1979) can behave as winter annuals, they also exhibit other types of life cycles.

Type 1 temperature responses have not been found in the Asteraceae. Achenes of winter annual members of this family are either nondormant, or they have type 2 or 3 temperature responses. Thus, it appears that composites may be phylogenetically constrained from having type 1 temperature responses. However, we can not draw such a general conclusion until more species of winter annual Asteraceae with dormant achenes have been studied. Knowledge of the type of temperature responses as achenes of Asteraceae with various kinds of life cycles and geographical distributions afterripen may shed some light on the evolutionary history of the family.

References

Baskin, C. C. and Baskin, J. M. 1988. Germination ecophysiology of herbaceous plant species in a temperate region. Am. J. Bot. **75**: 286–305.

Baskin, C. C., Baskin, J. M. and Hoffman, G. R. Seed dormancy in the prairie forb *Echinacea angustifolia* var. *angustifolia* (Asteraceae): Afterripening pattern during cold stratification. ms. (submitted).

Baskin, J. M. and Baskin, C. C. 1971a. Germination ecology and adaptation to habitat in *Leavenworthia* spp. (Cruciferae). Am. Midl. Nat. **85**: 22–35.

——— and ———. 1971b. Germination ecology of *Phacelia dubia* var. *dubia* in Tennessee glades. Am. J. Bot. **58**: 98–104.

——— and ———. 1972. Physiological ecology of germination of *Viola rafinesquii*. Am. J. Bot. **59**: 981–988.

——— and ———. 1973. Delay of germination in seeds of *Phacelia dubia* var. *dubia*. Can. J. Bot. **51**: 2481–2486.

——— and ———. 1974. Some aspects of the autecology of *Bupleurum rotundifolium* in Tennessee cedar glades. J. Tenn. Acad. Sci. **49**: 21–24.

——— and ———. 1976a. Germination dimorphism in *Heterotheca subaxillaris* var. *subaxillaris*. Bull. Torrey Bot. Club **103**: 201–206.

——— and ———. 1976b. High temperature requirement for afterripening in seeds of winter annuals. New Phytol. **77**: 619–624.

——— and ———. 1976c. Germination ecology of winter annuals: *Valerianella umbilicata* f. *pattellaria* and f. *intermedia*. J. Tenn. Acad. Sci. **51**: 138–141.

——— and ———. 1977. Germination ecology of *Sedum pulchellum* Michx. (Crassulaceae). Am. J. Bot. **64**: 1242–1247.

——— and ———. 1979. The ecological life cycle of the cedar glade endemic *Lobelia gattingeri*. Bull. Torrey Bot. Club **106**: 176–181.

——— and ———. 1981. Seasonal changes in the germination responses of buried *Lamium amplexicaule* seeds. Weed Res. **21**: 299–306.

——— and ———. 1982. Germination ecophysiology of *Arenaria glabra*, a winter annual of sandstone and granite outcrops of southeastern United States. Am. J. Bot. **69**: 973–978.

——— and ———. 1983a. Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological interpretation. Bot. Gaz. **144**: 540–543.

——— and ———. 1983b. The germination ecology of *Veronica arvensis*. J. Ecol. **71**: 57–68.

——— and ———. 1984a. Effect of temperature during burial on dormant and nondormant seeds of *Lamium amplexicaule* and ecological implications. Weed Res. **24**: 333–339.

——— and ———. 1984b. Role of temperature in regulating timing of germination in soil seed reserves of *Lamium purpureum*. Weed Res. **24**: 341–349.

——— and ———. 1985. The annual dormancy cycle in buried weed seeds: A continuum. BioScience **35**: 492–498.

——— and ———. 1986. Temperature requirements for afterripening in seeds of nine winter annuals. Weed Res. **26**: 375–380.

——— and ———. 1989. Seasonal changes in the germination responses of buried seeds of *Barbarea vulgaris*. Can. J. Bot. **67**: 2131–2134.

——— and ———. 1990. Germination ecophysiology of seeds of the winter annual *Chaerophyllum tainturieri*: A new type of morphophysiological dormancy. J. Ecol. **78**: 993–1004.

Gleason, H. A. and Cronquist, A. 1991. Manual of Vascular Plants of the Northeastern United States and Adjacent Canada, 2nd ed. 910 pp. The New York Botanical Garden, New York.

Radford, A. E., Ahles, H. E. and Bell, C. R. 1968. Manual of the Vascular Flora of the Carolinas, 1183 pp. University of North Carolina Press, Chapel Hill.

Roberts, H. A. and Neilson, J. E. 1982a. Role of temperature in the seasonal dormancy of seeds of *Veronica hederifolia* L. New Phytol. **90**: 745–749.

Roberts, H. A. and Neilson, J. E. 1982b. Seasonal changes in the temperature requirements for germination of buried seeds of *Aphanes arvensis* L. New Phytol. **92**: 159–166.

Steyermark, J. A. 1963. Flora of Missouri, 1725 pp. Iowa State University Press, Ames.

Vegis, A. 1963. Climatic control of germination, bud break, and dormancy. *In*: Evans, L. T. (ed.), Environmental Control of Plant Growth, 265–287. Academic Press, New York.

Received November 10, 1991. Accepted December 20, 1991.