Weed Biology and Management

Weed Biology and Management **10**, 241–248 (2010)

RESEARCH PAPER

Phenotypic selection for seed dormancy in white snakeroot (*Eupatorium rugosum*)

JOANN M. LAU and DAVID LOWELL ROBINSON*
Department of Biology, Bellarmine University, Louisville, Kentucky, USA

Eupatorium rugosum (*Ageratina altissima*), commonly known as white snakeroot, is a weedy plant that invades woodland areas in North America, Korea, and Japan. In order to examine the inheritance of seed dormancy in this species, seeds from a single population were screened for their differential germination response to stratification. After two cycles of recurrent selection, the seed from the shallow–dormant lines had 4.4 times greater germination prior to stratification than did the seed from the deep–dormant lines. The seed from the deep–dormant lines showed 3.4 times greater germination after stratification, compared to the seed from the shallow–dormant selections. This suggests that primary dormancy in the seed of white snakeroot is under some degree of genetic control. This perennial species produces overwintering rhizomes that give rise to adventitious, vegetative buds each spring. The plants selected for the production of seeds with lower levels of dormancy in the fall were observed to generate rhizomatous buds that were released from dormancy earlier in spring, compared to the plants that produced seeds with higher levels of dormancy. A statistically significant positive correlation also was observed between seed and bud dormancy in a naturally occurring population of white snakeroot. Common regulatory elements might be influencing dormancy in both the seeds and vegetative buds of this species.

Keywords: *Ageratina altissima*, bud, germination, seed dormancy, selection.

White snakeroot, *Eupatorium rugosum* Houtt. (*Ageratina altissima* [L.] King & Robinson), is a herbaceous perennial that is weedy in both its native habitat (USA and Canada) and in countries like Japan and Korea, where it has rapidly invaded forested areas since its introduction (Walck *et al.* 1997; Leckie *et al.* 2000; Kil *et al.* 2004; Mito & Uesugi 2004). Although not an overly aggressive weed in North America, white snakeroot is very invasive in Korea, covering as much as 60% of the ground in some areas (Suh *et al.* 1997). In addition to reducing native species diversity, white snakeroot produces a toxin that is harmful to the animals that graze on it or to those that drink the milk of lactating grazers (Sharma *et al.* 1998; Lee *et al.* 2003).

White snakeroot is a polycarpic hemicryptophyte, possessing a rhizome that produces vegetative buds near the soil surface each spring, which develop into new shoots annually. The rhizomes persist for at least 5 years, giving rise to adventitious buds that assume growth each spring. In North America, white snakeroot blooms in late summer and fall and disseminates its mature seeds (achenes) in fall and winter (Baskin & Baskin 1988). Each plant can produce thousands of seeds, the majority of which germinate in spring, much like summer annuals.

The fresh seeds of white snakeroot are in a conditionally dormant state at maturity, germinating only at temperatures higher than what normally occurs in their natural habitat at that time (Baskin & Baskin 1988; Walck *et al.* 1997). White snakeroot seeds exhibit a Type II response to stratification, as the minimum temperature for germination decreases as winter progresses (Walck *et al.* 1997; Baskin & Baskin 1998). By the time spring arrives, the seed can germinate at a wide range of temperatures. If germination does not occur in spring, however, the remaining seeds can re-enter a state of

Communicated by T. Yoshioka.
**Correspondence to: David Lowell Robinson, Department of Biology, Bellarmine University, 2001 Newburg Road, Louisville, KY 40205, USA.*
Email: drobinson@bellarmine.edu

The authors have no commercial interest in the findings presented.

Received 11 July 2009; accepted 5 September 2010

doi:10.1111/j.1445-6664.2010.00390.x

dormancy (secondary dormancy) that prevents them from emerging during the summer months, thus contributing to a persistent seed bank (Baskin & Baskin 1988; Walck *et al*. 1997; Donohue 2005).

The shoot development of individual white snakeroot plants also exhibits a high level of plasticity. Lee *et al*. (2003) reported many-fold differences in the number, height, and weight of the vegetative ramets that are produced by individual white snakeroot plants in Korea. They found that human activity enhanced these parameters.

Seed dormancy is a genetic trait that is empirically shown by the success at selecting against it during the process of crop domestication (Harlan *et al*. 1973). The breeding of wild relatives of domesticated *Eleusine* and *Papaver* spp. revealed extensive genetic variability in seed dormancy (Hilu & de Wet 1980; Lane & Lawrence 1995). Garbutt and Witcombe (1986) were successful in selecting for dormant and non-dormant traits in the weed, *Sinapsis arvensis*, and reported both embryonic and maternal genetic components to seed dormancy in that species. Fennimore *et al*. (1999) described a three-locus model of dormancy in *Avena fatua* seed and Staub *et al*. (1989) showed that there were from three-to-seven loci involved in the dormancy of *Cucumis sativus,* with a broad-sense heritability that ranged between 78 and 95%. Eleven quantitative trait loci for the delay of germination in *Arabidopsis thaliana* were reported by Bentsink *et al*. (2010).

The dormancy of vegetative buds is probably influenced by the same hormones (e.g. abscissic acid and gibberellin) and environmental factors (e.g. temperature) that regulate the dormancy of seeds (Baskin & Baskin 1998; Anderson *et al*. 2001; Shimizu-Sato & Mori 2001; Horvath *et al*. 2003; Kermode 2005; Cadman *et al*. 2006). Several studies have shown direct links between the physiological mechanisms of dormancy in seeds versus buds (Simmonds 1964; Dennis 1996; García-Gusano *et al*. 2004). More recently, Ruttink *et al*. (2007) reported that there were 107 genes that are associated with seed dormancy in *Arabidopsis* that also are involved in bud or cambial dormancy in *Populus* trees. A dozen of these genes were for transcription factors. Horvath *et al*. (2008) identified 42 genes that were differentially expressed in the transition to dormancy in both *Arabidopsis* seeds and the perennating underground buds of leafy spurge (*Euphorbia esula* L.). Sixteen of these were expressed in a similar manner during the dormancy transition.

In order to explore the genetic basis for dormancy in white snakeroot, three cycles of recurrent selection for seed dormancy in this species were carried out and the levels of seed and bud dormancy in the resulting germplasm were examined. The level of correlation between seed and bud dormancy also was examined in an unselected population.

MATERIALS AND METHODS

Original phenotypic selection for seed dormancy

White snakeroot seeds (achenes) were harvested from a single, naturally occurring population (~400 plants) that were growing in Louisville, Kentucky, USA, in November 1996. The seeds were after-ripened at 23°C for 18 days, manually cleaned, and stored at 4°C. Then, 2000 seeds were sown onto a single tray that was lined with germination paper (Seedburo Equipment Company, Des Plaines, IL, USA). After adding deionized water, the tray was sealed and placed into a dark growth-room that was kept at a constant 23°C. The seeds were checked for germination (defined as radicle protrusion outside the achene) daily. The first seeds to germinate (classified as "shallow–dormant") were immediately transplanted into pots of soil. After allowing sufficient time (17 days) for all the shallow–dormant seeds to germinate, the seeds were stratified at 4°C for 44 h and then were placed back into the 23°C growth-room. Again, the seeds were examined daily for germination, with new seedlings transplanted as previously described. This process was repeated for ten additional cycles until no more seeds germinated.

The plants that grew from the shallow–dormant seeds (requiring no stratification) were grown in an outdoor nursery, as were the deep–dormant plants that grew from the seeds that germinated after 1, 2, 3, 4, 5, 6, 8, or 9 cold treatments. No seed germinated after the seventh, tenth, or eleventh cold treatment. These populations of selected plants were allowed to bloom and set seed. The selections were grown in similar environments and pollination by the unselected plants was minimized. The seeds were collected as they matured between mid-October and early December, after-ripened at 23°C, and stored at 4°C for progeny testing the following year.

Progeny test 1

In June of the following year, the progeny (seeds) of the selections, described above, were evaluated for dormancy. In the first experiment, 25 seeds from each plant (24 plants in total) were sown into separate disposable Petri dishes that were lined with filter paper (no. 1 Qualitative; Whatman, Piscataway, NJ, USA). After imbibition in 3 mL of deionized water, the seeds were allowed to germinate at a constant 23°C. The seeds were examined daily for germination. Newly sprouted seeds were transplanted into soil, as previously described. The ungerminated seeds were given a 44 h cold treatment (4°C).

Following this stratification treatment, the seeds were assessed daily until germination ceased for at least 2 days. This process was repeated for a total of 10 cold-treatment cycles. As none of the seeds germinated in the tenth cycle, 1 mL of 500 μmol L^{-1} gibberellin, GA_3 (GA), was added to each dish and germination was assessed daily until seed germination ceased. A χ^2-analysis, using a contingency table, was carried out to summarize the contributions from each of the individual treatments to the overall pattern of germination.

The transplants that exhibited either of the two target phenotypes, deep–dormant or shallow–dormant, were grown to maturity, as described previously. The seeds were collected from these plants in November and December, after-ripened, and stored at 4°C until the next progeny test. The parental lines from the previous year were discarded.

Progeny test 2

In May of the next year, the seeds from the plants selected during Progeny test 1 were tested under the same conditions and procedures as described above. The phenotypic selections were made, transplanted, and grown to maturity, as previously described. To reduce inbreeding, multiple plants (five shallow–dormant and 13 deep–dormant) from the elite lines (those expressing the strongest phenotypes) were selected. The parental lines from the previous year were discarded.

The seeds from one of the selected shallow–dormant lines (that germinated before stratification in Progeny test 1) and from one of the selected deep–dormant lines (that germinated after receiving four cold treatments in Progeny test 1) were compared in a replicated trial. The progeny of each plant were assessed for germination under four conditions: (i) continuous 23°C; (ii) a 5 day cold pretreatment (4°C), followed by continuous 23°C; (iii) alternating exposure to 4°C and 23°C (12 h each); and (iv) immediate treatment with GA (500 μmol L^{-1}) at continuous 23°C. Fifty seeds per dish were planted, with four replications per treatment. Germination was assessed daily for 73 days. The Germination Speed Index (GSI) was calculated by summing each day's cumulative germination for the entire experiment and dividing by the product of the final cumulative germination × number of days (Noe & Zedler 2000). The percentage-germination data were arcsin-transformed and statistically analyzed by using one-way ANOVA (Jandel Scientific 1995).

Progeny test 3

Third-generation seeds were progeny-tested, as previously described. The experimental conditions were the same as before, except that seed germination was assessed every 3 days instead of daily. The data from all 3 years of progeny testing were compared by using contingency-table χ^2-analysis.

Assessment of perennating bud dormancy in the selections

In March of all three progeny-test years, the newly emerging buds on the perennating rhizomes of the previous year's selections were examined and the length of the longest sprout was determined. The percentage data were arcsin-transformed and statistically analyzed by using one-way ANOVA (Jandel Scientific 1995) and contingency-table χ^2-analysis.

Relationship of bud and seed dormancy in a natural population

In May 2007, 27 snakeroot plants were dug up from a horse farm in Kentucky, USA, potted, and grown for 6 months at a single location. The plants appeared to be at least 1 year-old as they had well-developed rhizomes. The seeds were collected in November and after-ripened at room temperature for 20 days. In order to examine the dormancy of the seeds that were produced by these plants, 50 seeds from each were sown onto Petri dishes, as previously described, and germination was assessed daily until germination ceased. The seeds were treated to 13 consecutive stratification treatments, as described for the progeny tests. After the last stratification, GA was added to further break the seed dormancy. The seeds that did not germinate until after a cold or GA treatment were considered to be deep–dormant.

After overwintering, the perennating bud dormancy was assessed continually in February and March 2008 by routinely examining the plants for bud growth. Bud emergence was defined as the date at which at least 2 cm of sprouting shoot tissue was seen growing from the rhizome. The relationship between the seed and bud dormancy was examined by correlation analysis (Jandel Scientific 1995).

RESULTS

Original phenotypic selection for seed dormancy

Of the 2000 seeds that were planted at the beginning of this study, 45% germinated within the first 5 days of planting. By 10 days after planting, 76% had germinated, with another 1% of the seeds sprouting 7 days after that. These seeds germinated so readily that they were categorized as shallow–dormant. The remaining 460 seeds were cold-treated 11 consecutive times, with an adequate

amount of time allotted for germination after each stratification treatment. The final seed-germination event was after nine cold treatments, 83 days after the start of the experiment.

The seeds selected for either the shallow–dormant or the deep–dormant trait were transplanted into soil and grown to reproductive maturity. Only the seedlings from the first six cold-treatment cycles were mature enough to flower and produce seed in the first season. The progeny (seeds) from these plants were examined for germinability the following year and were used to complete a second round of selection for dormancy.

Progeny tests

The χ^2-contingency test indicated that there was no statistical difference for the percentage germination of the two populations after the first year of selection for dormancy (Table 1). At least half of the seed germinated without stratification and another one-third germinated after stratification or GA treatment. In the second year, however, there were different patterns of response for germination in the progeny selected for deep dormancy versus shallow dormancy. Significantly more progeny (4.4 times) from the shallow–dormant selections germinated before stratification, compared to the progeny of the plants selected for deep dormancy, and 3.4 times more deep–dormant progeny germinated after stratification than did the shallow–dormant selections ($P < 0.001$). The χ^2-analysis of the third progeny test showed a similar pattern of response as the second test (Table 1).

In order to allow for the statistical comparison of the specific shallow–dormant and deep–dormant lines, a replicated trial was used to compare the progeny of two individual plants. The seed germination in these lines was assessed in four different experimental situations. In one treatment, the seeds from both lines were treated with GA during imbibition (Fig. 1). In this treatment, the seeds selected for shallow dormancy achieved 100% germination after planting, while the seeds selected for deep dormancy attained 99% germination (not statistically different). This shows that the seed viability in these two lines was similar. In the non-stratification treatment (constant 23°C), 99% of the shallow–dormant seed germinated, compared to only 56% in the deep–dormant line. This difference was statistically significant ($P < 0.05$). As this experiment was carried out several months after Progeny test 2, after-ripening might have resulted in additional seed germination in these lines.

The third set of conditions involved an alternating-temperature regime that was designed to simulate natural circadian variations: 12 h at 4°C and 12 h at 23°C. The final germination rate of the shallow–dormant line in this treatment was identical to that of the GA treatment (100%). The final germination rate (90%) of the deep–dormant line in the alternating-temperature treatment was not statistically different, compared to the GA treatment (99%), for that line, but it was significantly greater than that of the deep–dormant seed that was kept at a constant 23°C ($P < 0.001$).

In order to test the cumulative effect of these short, repetitive cold treatments versus the response to a one-time exposure to cold, a fourth treatment involved the exposure of the seeds to a single 4°C pretreatment for 5 days and then incubation at a constant 23°C. Whereas, 99% of the seed from the shallow–dormant line germinated under these conditions, only 71% of the seed from the deep–dormant line did so ($P < 0.001$). The final germination rate of the deep–dormant seed, following the 5 day cold treatment, was greater than when kept at a constant 23°C ($P < 0.01$), but it was less than the rate when receiving alternating cycles of cold ($P < 0.005$).

Table 1. Percentage germination (mean ± SE) of the progeny that were harvested from the plants grown from seed that was selected for shallow dormancy or deep dormancy

Progeny test	Selected phenotype	N‡	% Germination§				*P*-value
			Before stratification	After stratification¶	After GA	Ungerminated	
1	Shallow–dormant	7	60 ± 12	27	9 ± 4	4 ± 1	NS
	Deep–dormant	17	50 ± 5	18	15 ± 3	17 ± 5	
2	Shallow–dormant	3	74 ± 6	20	3 ± 1	3 ± 1	<0.001
	Deep–dormant	8	17 ± 3	67	0	16 ± 4	
3†	Shallow–dormant	5	70 ± 6	22	–	8 ± 5	<0.001
	Deep–dormant	13	20 ± 2	52	–	28 ± 5	

† GA was not used in Progeny test 3; ‡ N represents the number of parents used in each test, so is dependent on the number of selections made the year before; § the germination pattern within each year was tested by contingency χ^2-analysis; ¶ stratification involved 7–10 cycles of cold treatment (depending on the year), followed by 23°C for several days after each cycle, so the values are cumulative. GA, gibberellin; NS, not statistically significant.

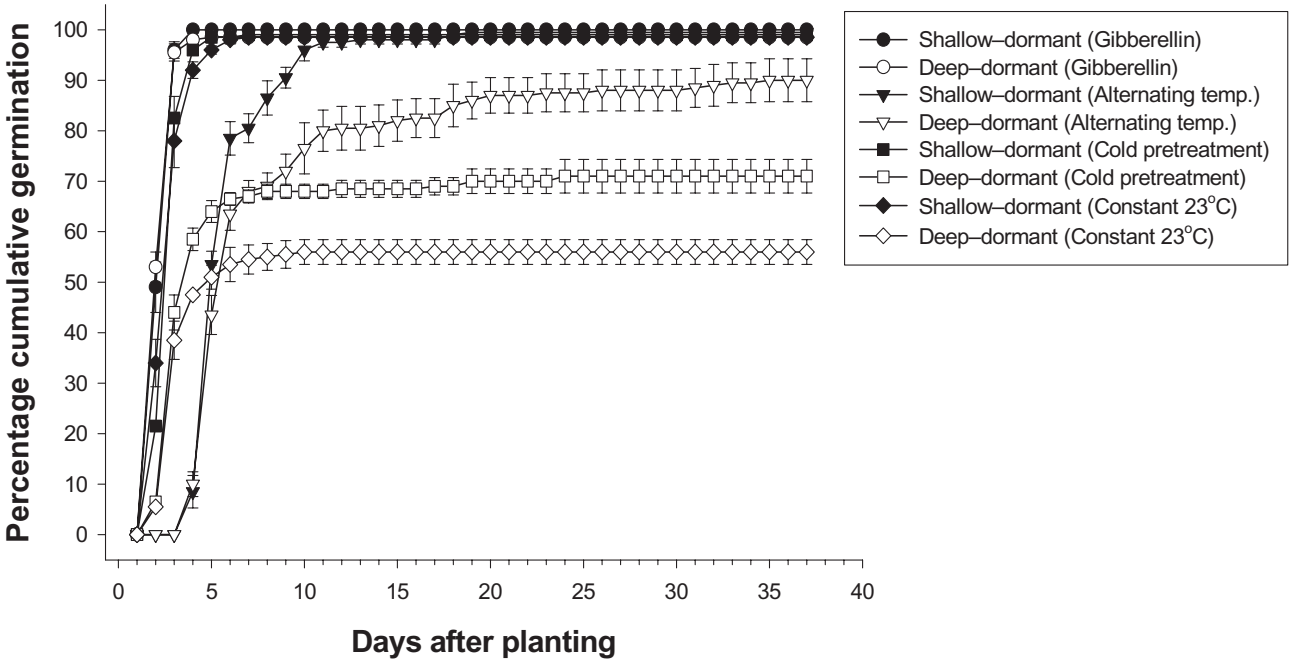

Fig. 1. Percentage cumulative germination of the seeds from a shallow–dormant line and a deep–dormant line, compared in a replicated trial in year 2. The seeds were exposed to four different treatments: (i) constant 23°C; (ii) 5 day cold pretreatment, followed by constant 23°C; (iii) alternating exposure to 4°C and 23°C (12 h each); and (iv) treatment with gibberellin at a constant 23°C. The values represent the mean ± SE.

Germination vigor was examined by calculating the Germination Speed Index (GSI), as described by Noe and Zedler (2000). The GSI varies between 0 and 1.00 (representing the lowest and highest germination vigor, respectively) and is not altered by the final amount of germination. The highest GSI was in the seed that was treated with GA (Table 2). Both the shallow–dormant and deep–dormant seed had the same vigor after the GA treatment. The two groups showed the same general pattern of vigor response across the different treatments, with the lowest vigor being in the seeds that received circadian cycles of cool and warm. Except in the GA treatment, the deep–dormant line had statistically lower germination vigor than the shallow–dormant line ($P < 0.05$).

Table 2. Germination vigor, as indicated by the Germination Speed Index, of the seeds from a shallow–dormant line and a deep–dormant line, compared in a replicated trial in year 2

Treatment	Germination Speed Index	
	Shallow–dormant	Deep–dormant
Gibberellin	0.977 a	0.978 a
Constant 23°C	0.970 b	0.962 b
Cold pretreatment	0.970 b	0.948 b
Alternating temperatures	0.923 c	0.879 c

Values followed by a different letter within a column are significantly different ($P < 0.05$), according to the Student-Newman-Keuls Test.

Assessment of perennating bud dormancy in the selections

The rhizomes of all the selections were examined for early bud elongation in March of each year. In general, the plants selected for the production of shallow–dormant seeds tended to have buds on their rhizomes that resumed growth relatively early in spring, whereas the plants selected for the production of deep–dormant seeds tended to have buds that sprouted later in the season (Table 3). These differences were seen for both the percentage of sprouted buds at a particular time-point and the height of the emerging buds at that time. The differences between the shallow–dormant and deep–dormant selections were statistically significant for all 3

Table 3. Growth of the vegetative buds on the perennating rhizomes of plants that were selected for the production of shallow–dormant or deep–dormant seed during three consecutive years of progeny testing

Phenotype	Percentage of plants with sprouts >0.5 cm			Sprout height (cm)		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Shallow–dormant	63	88	75	3.0 ± 1.05	4.5 ± 1.30	4.9 ± 2.33
Deep–dormant	36	24	0	0.6 ± 0.10	1.0 ± 0.26	0.0 ± 0.00

Values represent the mean ± SE. Each year, there was a statistically significant difference between the phenotypes for both parameters ($P < 0.001$).

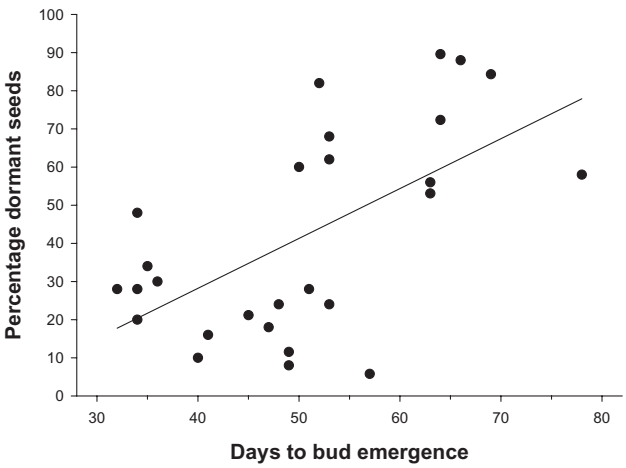

Fig. 2. Relationship between bud and seed dormancy in a randomly selected natural population of white snakeroot plants ($n = 27$). Correlation coefficient, $R = 0.60$.

years ($P < 0.001$). Differences in the plant height were not apparent by the end of the growing season.

Relationship of bud and seed dormancy in a natural population

The amount of seed dormancy in the progeny of a natural population of white snakeroot plants ranged from 6% to 90%. In the same plants, the emergence of the buds from the rhizomes after overwintering occurred between February 1 and March 18, a 47 day span. There was a positive linear correlation ($R = 0.60$) between the level of dormancy in the rhizomatous buds and in the seeds that the plants produced (Fig. 2). This correlation was statistically significant ($P < 0.001$).

DISCUSSION

A single naturally occurring population of white snakeroot plants was used in a recurrent selection program that was designed to isolate genotypes that produced seeds with varying levels of primary dormancy. Ecologically realistic temperature regimes were not used in this selection program in order to increase the likelihood of isolating variants that expressed specific physiological and morphological responses to cold that could be distinguished easily in a laboratory setting. The duration of the cold treatments (44 h) was too short to simulate natural winter stratification, as the fresh seeds of white snakeroot, which are dispersed in late fall, have been shown to require 8 weeks of stratification for maximum germination (Walck *et al.* 1997). It was hoped that exposure to shorter cold periods might reveal dormant individuals that were especially sensitive to cold stratification. Likewise, although the duration of the temperature treatments was too long to simulate the circadian changes in temperature that would occur naturally in spring, it was hoped that this time frame might make sensitive individuals more evident. Yamauchi *et al.* (2004) reported that 48 h in the cold was enough to induce the transcription of GA-inducible genes.

This approach appeared to be successful. Although there was no statistical difference between the selections following the first year of selection, they were readily apparent by the second year. It is typical for recurrent selection programs to require more than one cycle of selection to make substantial progress (Allard 1960), particularly in light of the polygenic nature of seed dormancy (Gu *et al.* 2004). For instance, Garbutt and Witcombe (1986) did not find differences between the dormant and undormant seed selections in charlock mustard (*Sinapis arvensis* L.) until the fourth cycle of selection. They reported that the line selected for dormancy actually showed enhanced germination during the first three cycles. They speculated that this was due to the "release of concealed variation during the first stages of selection" (Garbutt and Witcombe 1986: 27). The similar dormancy levels that were observed in the first progeny test also could be related to some type of maternal effect (Donohue 2009). For example, the pericarp

and seed coat (entirely maternal) and the seed endosperm (two-thirds maternal) can have considerable influence over dormancy. Likewise, the accumulation of nutrients, hormones, proteins, and mRNAs in the developing seed is largely regulated by the maternal plant. These types of effects might have interfered with the full expression of the dormancy phenotypes in the first year.

A replicated trial was used to examine the response to an alternating-temperature regime that resembled natural thermoperiods. The entire experiment was repeated a second time, with similar results (data not shown). When treated with GA, the seed from the deep–dormant line germinated as much, and as fast, as the shallow–dormant seed (Fig. 1, Table 2). This indicates that the overall seed viability and vigor were not altered by selection. Without GA, not only was the amount of germination lower in the deep–dormant line, but so was the speed of germination. The deep–dormant selections that were exposed to alternating daily temperatures showed a final germination rate that was 9% less than that of the GA treatment and 61% higher than when they were held at a continuous 23°C. Whereas, the germination rate in the GA-treated deep–dormant line reached a plateau in the first 5 days, the plateau was reached at 10 days in the 23°C treatment and at 24 days in the cold pretreatment. The deep–dormant seeds that were exposed to alternating temperatures did not reach a germination plateau until 67 days after planting. Therefore, it appears that the influence of alternating cold temperatures on germination in the deep–dormant line is cumulative. Yamauchi *et al.* (2004) reported that the levels of GA-inducible transcription in *Arabidopsis* seeds steadily increased for ≤96 h after imbibition in the cold; thus, GA could be involved.

The cumulative effect of cold temperature was also apparent when comparing germination on the tenth day of the experiment. At that time, the deep–dormant seeds that had been exposed to the circadian temperature regime had received exactly the same duration of cold as those in the single 120 h pretreatment, but had 13% more germination. Numerous researchers have shown that alternating daily temperatures, which are normal in natural habitats, typically induce higher rates of germination than constant temperatures (Vleeshouwers *et al.* 1995; Baskin & Baskin 1998; Hilhorst 1998). Alternating daily temperatures might be a way that buried seeds can detect a change in season (van Assche & Vanlerberghe 1989). It also might provide seeds with input about their depth in the soil and the amount of vegetation and litter in their proximity (Baskin & Baskin 1998).

It was observed that, once the plants in the nursery had overwintered, the buds on the rhizomes of the shallow–dormant selections sprouted earlier in spring than those on the deep–dormant selections. Earlier bud elongation in the plants selected for shallow dormancy was observed in all 3 years. Walck *et al.* (1997) reported that the peak emergence period for white snakeroot seeds that were sown outdoors in Kentucky, USA, occurred in mid-March, when the mean daily maximum and minimum temperatures were 23°C and 14°C, respectively. This is about the same time that bud dormancy appeared to break in our white snakeroot nursery in Kentucky.

A positive correlation between bud and seed dormancy also was observed in a small population of randomly selected plants that were growing in the wild. The idea that there might be common mechanisms regulating dormancy in the buds and seeds goes back a half a century (Wareing 1956; Vegis 1964; Rohde *et al.* 2000). Simmonds (1964) reported a positive correlation between seed dormancy and tuber-bud dormancy in potato (*Solanum tuberosum* L.) plants selected for either characteristic. Powell (1987) noted several common characteristics of seed and bud dormancy in woody plants. More recently, García-Gusano *et al.* (2004) reported that late-flowering almond (*Prunus dulcis* [Mill.] D.A. Webb) cultivars produced seed that required longer stratification periods in order to germinate, while the seed that was produced by early-flowering cultivars had lower levels of dormancy.

Abscissic acid, gibberellin, as well as the photoperiod and temperature, have been shown to be important factors in regulating bud and seed dormancy in many plant species (Anderson *et al.* 2001; Shimizu-Sato & Mori 2001; Foley 2002; Arora *et al.* 2003; Horvath *et al.* 2003; Donohue 2005; Gubler *et al.* 2005; Chao *et al.* 2006; Ruttink *et al.* 2007; Horvath *et al.* 2008). Although the internal and external mechanisms that regulate these two types of dormancy might be similar, there have been few publications showing a direct heritable linkage between the two. Greater levels of dormancy in the perennating buds of white snakeroot plants selected for higher levels of seed dormancy indicate that there might be a physiological or genetic link between the bud and seed dormancy of this species.

This study shows that diversity for seed and bud dormancy exists in natural populations of white snakeroot plants and that dormancy is, at least partially, inherited (Dekker 1997). This variability in dormancy might allow white snakeroot to flourish in a wide variety of climatic conditions, thus contributing to its success as a weed.

ACKNOWLEDGMENTS

We thank Dr. Carol Baskin for her advice and encouragement. The cooperation of Spring Run Farm (Prospect, Kentucky, USA) is appreciated.

REFERENCES

Allard R.W. 1960. *Principles of Plant Breeding*. John Wiley & Sons, New York.

Anderson J.V., Chao W.S. and Horvath D.P. 2001. A current review on the regulation of dormancy in vegetative buds. *Weed Sci.* **49**, 581–589.

Arora R., Rowland L.J. and Tanino K. 2003. Induction and release of bud dormancy in woody perennials: a science comes to age. *HortScience* **38**, 911–921.

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

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. and Baskin J.M. 1998. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, London.

Bentsink L., Hanson J., Hanhart C.J., Blankestijn-deVries H., Coltrane C., Keizer P. *et al.* 2010. Natural variation for seed dormancy in *Arabidopsis* is regulated by additive genetic and molecular pathways. *Proc. Natl Acad. Sci. U.S.A.* **107**, 4264–4269.

Cadman C.S.C., Toorop P.E., Hilhorst H.W.M. and Finch-Savage W.E. 2006. Gene expression profiles of *Arabidopsis cvi* seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J.* **46**, 805–822.

Chao W.S., Serpe M.D., Anderson J.V., Gesch R.W. and Horvath D.P. 2006. Sugars, hormones, and environment affect the dormancy status in underground adventitious buds of leafy spurge. *Weed Sci.* **54**, 59–68.

Dekker J. 1997. Weed diversity and weed management. *Weed Sci.* **45**, 357–363.

Dennis F.G. 1996. A physiological comparison of seed and bud dormancy. In: *Plant Dormancy: Physiology, Biochemistry and Molecular Biology* (ed. by Lang B.A.). CAB International, Wallingford, UK, 47–56.

Donohue K. 2005. Seeds and seasons: interpreting germination timing in the field. *Seed Sci. Res.* **15**, 175–187.

Donohue K. 2009. Completing the cycle: maternal effects as the missing link in plant life histories. *Philos. Trans. R. Soc. B.* **364**, 1059–1074.

Fennimore S.A., Nyquist W.E., Shaner G.E., Doerge R.W. and Foley M.E. 1999. A genetic model and molecular markers for wild oat (*Avena fatua* L.) seed dormancy. *Theor. Appl. Genet.* **99**, 711–718.

Foley M.E. 2002. Weeds, seeds, and buds – opportunities and systems for dormancy investigations. *Weed Sci.* **50**, 267–272.

Garbutt K. and Witcombe J.R. 1986. The inheritance of seed dormancy in *Sinapis arvensis* L. *Heredity* **56**, 25–31.

García-Gusano M., Martínez-Gómez P. and Dicenta F. 2004. Breaking seed dormancy in almond (*Prunus dulcis* (Mill.) D.A. Webb). *Sci. Hortic.* **99**, 363–370.

Gu X.Y., Kianian S.F. and Foley M.E. 2004. Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (*Oryza sativa*). *Genetics* **166**, 1503–1516.

Gubler F., Millar A.A. and Jacobsen J.V. 2005. Dormancy release, ABA and pre-harvest sprouting. *Curr. Opin. Plant Biol.* **8**, 183–187.

Harlan J.R., de Wet J.M.J. and Price E.G. 1973. Comparative evolution of cereals. *Evolution* **27**, 311–325.

Hilhorst H.W.M. 1998. The regulation of secondary dormancy. The membrane hypothesis revisited. *Seed Sci. Res.* **8**, 77–90.

Hilu K.W. and de Wet J.M.J. 1980. Effect of artificial selection on grain dormancy in *Eleusine* (Gramineae). *System Bot.* **5**, 54–60.

Horvath D.P., Anderson J.V., Chao W.S. and Foley M.E. 2003. Knowing when to grow: signals regulating bud dormancy. *Trends Plant Sci.* **8**, 534–540.

Horvath D.P., Chao W.S., Suttle J.C., Thimmapuram J. and Anderson J.V. 2008. Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (*Euphorbia esula* L.). *BMC Genomics* **9**, 536.

Jandel Scientific 1995. *SigmaStat User's Manual (v. 2.0)*. Jandel Scientific Corporation, San Rafael, CA.

Kermode A.R. 2005. Role of abscissic acid in seed dormancy. *J. Plant Growth Regul.* **24**, 319–344.

Kil J.H., Shim K.C., Park S.H., Koh K.S., Suh M.H., Ku Y.M. *et al.* 2004. Distributions of naturalized alien plants in South Korea. *Weed Technol.* **18**, 1493–1495.

Lane M.D. and Lawrence M.J. 1995. Genetics of seed dormancy in *Papaver rhoeas*. *Heredity* **75**, 84–91.

Leckie S., Vellend M., Bell G., Waterway M.J. and Lechowicz M.J. 2000. The seed bank in an old-growth, temperate deciduous forest. *Can. J. Bot.* **78**, 181–192.

Lee H.S., Yoo H.M. and Lee C.S. 2003. Distribution pattern of white snakeroot as an invasive alien plant and restoration strategy to inhibit its expansion in Seoripool Park, Seoul. *Korean J. Biol. Sci.* **7**, 197–205.

Mito T. and Uesugi T. 2004. Invasive alien species in Japan: the status quo and the new regulation for prevention of their adverse effects. *Global Environ. Res.* **8**, 171–191.

Noe G.B. and Zedler J.B. 2000. Differential effects of four abiotic factors on the germination of salt marsh annuals. *Am. J. Bot.* **87**, 1679–1692.

Powell L.E. 1987. Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. *HortScience* **22**, 845–850.

Rohde A., Howe G.T., Olsen J.E., Moritz T., van Montagu M., Junttila O. *et al.* 2000. Molecular aspects of bud dormancy in trees. In: *Molecular Biology of Plants*, Vol. 1 (ed. by Jain S.M. and Minocha S.C.). Kluwer Academic, Dordrecht, the Netherlands, 89–134.

Ruttink T., Arend M., Morreel K., Storme V., Rombauts S., Fromm J. *et al.* 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. *Plant Cell* **19**, 2370–2390.

Sharma O.P., Dawra R.K., Kurade N.P. and Sharma P.D. 1998. A review of the toxicosis and biological properties of the genus *Eupatorium*. *Nat. Toxins* **6**, 1–14.

Shimizu-Sato S. and Mori H. 2001. Control of outgrowth and dormancy in axillary buds. *Plant Physiol.* **127**, 1405–1413.

Simmonds N.W. 1964. The genetics of seed and tuber dormancy in the cultivated potatoes. *Heredity* **19**, 489–504.

Staub J.E., Globerson D. and Genizi A. 1989. Inheritance of seed dormancy in *Cucumis sativus* var. *hardwickii* (Royle) Alef. *Theor. Appl. Genet.* **78**, 143–151.

Suh M.H., Lee Y.M., Kil J.H. and Kim K.D. 1997. Characteristics and distribution pattern of *Eupatorium rugosum* at Mt. Namsan in Seoul. *Korean J. Ecol.* **20**, 285–291.

Vegis A. 1964. Dormancy in higher plants. In: *Annual Review of Plant Physiology* (ed. by Machlis L. and Briggs W.R.). Annual Reviews, Palo Alto, CA, 185–224.

Vleeshouwers L.M., Bouwmeester H.J. and Karssen C.M. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *J. Ecol.* **83**, 1031–1037.

Walck J.L., Baskin C.C. and Baskin J.M. 1997. Comparative achene germination requirements of the rockhouse endemic *Ageratina luciae-brauniae* and its widespread close relative *A. altissima* (Asteraceae). *Am. Midl. Nat.* **137**, 1–12.

Wareing P.F. 1956. Photoperiodism in woody plants. In: *Annual Review of Plant Physiology* (ed. by Blinks L.R., Machlis L. and Torrey J.G.). Annual Reviews, Palo Alto, CA, 191–214.

Yamauchi Y., Ogawa M., Kuwahara A., Hanada A., Kamiya Y. and Yamaguchi S. 2004. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell* **16**, 367–378.