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Commercial Seed Lots Exhibit Reduced Seed Dormancy in Comparison to Wild Seed Lots of *Echinacea purpurea*

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Abstract. **Seed germination patterns were studied in *Echinacea purpurea* (L.) Moench grouped by seed source, one group of seven lots from commercially cultivated populations and a second group of nine lots regenerated from *ex situ* conserved wild populations. Germination tests were conducted in a growth chamber in light (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or darkness at 25 °C for 20 days after soaking the seeds in water for 10 minutes. Except for two seed lots from wild populations, better germination was observed for commercially cultivated populations in light (90% mean among seed lots, ranging from 82% to 95%) and in darkness (88% mean among seed lots, ranging from 82% to 97%) than for wild populations in light (56% mean among seed lots, ranging from 9% to 92%) or in darkness (37% mean among seed lots, ranging from 4% to 78%). No germination difference was measured between treatments in light and darkness in the commercially cultivated populations, but significant differences were noted for treatments among wild populations. These results suggest that repeated cycles of sowing seeds during cultivation without treatments for dormancy release resulted in reduced seed dormancy in *E. purpurea*.**

Echinacea, commonly known as purple coneflower, is a perennial, herbaceous plant native to eastern North America. A detailed morphological classification of *Echinacea* was provided by McGregor (1968) and recently revised by Binns et al. (2002) using morphological as well as numerical and statistical methods. According to McGregor (1968), there are 11 taxa in *Echinacea* including nine species and two botanical varieties.

Echinacea has long been used by Native Americans for treating many conditions, including venomous bites, rabies, cold, headaches and stomach cramps (Foster, 1991; Kindscher, 1989; Li, 1998). In recent years, research results have demonstrated that *Echinacea* has immunostimulatory, antiviral, and antibacterial activities (Bauer and Wagner, 1991; Bodinet and Beuscher, 1991; Bodinet et al., 1993; Parnham, 1996).

Herbal products, made from the roots of *E. angustifolia* DC. and *E. pallida* (Nutt.) Nutt. and the roots and aerial parts of *E. purpurea* (L.) Moench, are among the most widely used herbal remedies in Europe and North America (Li, 1998). Increased market demand for *Echinacea* products has led to a rapid expansion of *Echinacea* cultivation worldwide (Li, 1998). *Echinacea purpurea* is the most used and cultivated species, accounting for 80% of *Echinacea* production (Li, 1998).

Seed propagation is the primary system for the establishment of new production fields of *Echinacea*. Wartidiningsih and Geneve (1994) reported erratic seed germination patterns in *E. purpurea*, with germination of non-treated seeds varying from <40% to >90% depending on seed lot. Methods used to overcome seed dormancy in *E. purpurea* have included cold-moist stratification, osmotic priming, and growth-regulator treatments (Parmenter et al., 1996; Pill et al., 1994; Pill and Haynes, 1996; Wartidiningsih et al., 1994).

Seed source, quality, and physiological maturity status may affect seed dormancy in *E. purpurea* (Wartidiningsih and Geneve, 1994). Among these factors, seed source could serve as a convenient and unambiguous indicator to help growers choose samples with minimal dormancy.

We found no dormancy in five lots of *E. purpurea* seeds from commercially cultivated sources (Qu et al., 2004). This suggested that repeated cycles of regeneration may have reduced or even eliminated seed dormancy in *E. purpurea*, as has been the case during the course of domestication of many other plant species (Copeland and McDonald, 1995; Harlan et al., 1973). However, because the seeds in that experiment (Qu et al., 2004) were stored dry under various temperature and humidity conditions for more than one year before testing, clear conclusions could not be drawn on the cause of this lack of dormancy. In a recent evaluation of untreated *E. purpurea* seeds harvested from commercially cultivated plants only a few months before testing, Qu et al. (unpublished data) also found no dormancy requirement.

Data on the variation of dormancy of seed lots from commercially cultivated populations or from wild stands have not been provided in previous reports on *E. purpurea* seed germination. Understanding differences in seed dormancy among various *E. purpurea* populations and determining the cause of dormancy reduction would be valuable, both in terms of the basic science (the genetics and evolution of this species) and in helping growers be more effective in selecting *E. purpurea* seeds. The objective of this investigation was to determine dormancy differences between *E. purpurea* seeds from commercially cultivated populations and from wild populations.

Materials and Methods

Echinacea purpurea seeds were grouped into two categories, those from commercially cultivated populations, presumably many generations removed from wild populations, and those from ex situ conserved wild populations. Commercially cultivated samples were purchased from seven seed companies (Ion Exchange, Harpers Ferry, Iowa; Johnny's Selected Seeds, Albion, Maine; Prairie Nursery, Westfield, Wis.; Prairie Moon Nursery, Winona, Minn.; Richters, Goodwood, Ontario, Canada; Stock Seed Farms, Murdock, Neb.; Wind River Seed, Manderson, Wyo.). Seeds from wild populations were provided by the U.S. Dept. of Agriculture–Agricultural Research Service, North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. Additional information for each seed lot is given in Table 1.

Germination tests of the seed lots were conducted in December 2003 under two treatment conditions, constant light and constant darkness. Each treatment consisted of three

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replications, 50 seeds per treatment for each lot from commercial sources and 16 seeds for each of the NCRPIS lots. The seed lots from the NCRPIS were retested in February 2004 with the same seed and replication numbers and treatment conditions. First, seeds were soaked in 50-mL beakers with about 20 mL deionized water for 10 min. After soaking, seeds were blotted dry on paper towels and placed in transparent plastic germination boxes (11 × 11 × 3.5 cm) on two layers of filter paper saturated with deionized water. The lids on the boxes then were sealed with Parafilm M (Pechiney Plastic Packaging, Menasha, Wis.). For the treatment in darkness, the germination boxes were wrapped with aluminum foil. Germination tests were conducted in a growth chamber (model 818; Precision, Winchester, Va.) at constant 25 °C. Cool white fluorescent lamps mounted in the chamber door provided photosynthetically active radiation at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Germination (presence of radicles >1 mm long) was recorded at four-day intervals for the treatment in light and two times (on days 12 and 20) for the treatment in darkness. The day-12 count for the treatment in darkness was conducted along with checking moisture level in the boxes, and it has been found that short exposure (<1 min) to light during germination evaluation had little effect on final germination (Qu et al, 2004). Germinated seeds were removed when counted, and the tests lasted for 20 d after initiation.

Each experiment was a completely randomized design. Since there were no significant differences between the two germination test results for the NCRPIS lots, those data were combined for final analysis. Germination percentages were normalized by transformation (arcsin$\sqrt{}$%) before being subjected to analysis of variance, following the methods of Wartidiningsih and Geneve (1994). Duncan's multiple range test was conducted on the transformed data after two-way ANOVA (seed lot × light vs. dark) to distinguish among individual lots.

Results and Discussion

Differences in seed germination were evident in the two groups (Table 1). Higher germination percentages were observed in commercial seed lots (90% mean among seed lots in light, ranging from 82% to 95%; 88% mean among seed lots in darkness, ranging from 82% to 97%) than from the wild populations (56% mean among seed lots in light, ranging from 9% to 92%; 37% mean among seed lots in darkness, ranging from 4% to 78%). For all but two populations (PI 633667 and PI 633669), seeds of the wild populations germinated significantly less frequently than did those of the commercially cultivated populations either in light or darkness.

Light vs. darkness during germination had no effect on germination percentage of commercially cultivated populations, but light increased the germination percentage of wild population (Table 1). Unpublished data from the NCRPIS (Widrlechner, personal communication) indicate that the differences observed in this experiment are, at least in part, if not entirely, due to the presence of dormancy in the wild populations. Seven of these nine seed lots from wild populations were tested for germination in February 2003 at the NCRPIS after using dormancy-breaking techniques involving ethephon and moist prechilling (modified from Sari et al., 2001), and all samples germinated within 21 d at levels between 70% and 95%.

All commercial seed lots had similarly high germination percentages (Table 1) in darkness and light, indicating that dormancy was either absent or minimal. Previously, we reported that ethephon had little effect in promoting seed germination of *E. purpurea* seeds from other commercial sources (Qu et al., 2004), and we recently determined that seeds freshly harvested from plants grown from commercial seeds had no dormancy requirements (92% to 98% germination) (Qu, unpublished data).

The history of cultivation of *E. purpurea* can help us understand the cause of the differences in germination behavior between these two groups of seeds. To learn more about the commercially cultivated seed samples, we communicated with technical personnel at the seed companies supplying the samples. Although it could not be confirmed with written records, the *Echinacea* plants used for commercial seed production were likely cultivated for more than ten generations, without dormancy-release treatments to increase germination. Available literature on the history of *E. purpurea* cultivation (Galambosi, 2004) indicates that much of the cultivated material was brought to Europe from wild populations in North America many years ago. We are aware

Table 1. Seed germination results of *Echinacea purpurea* from different seed lots.

Seed lot	Treatment		Mean	Harvest year	Origin in U.S.
	Light	Dark			
Commercial populations[z]					
C-purp 1	95[y]	97[y]	96.0 (1.374) a[x]	2002	Colorado
C-purp 2	95	88	91.5 (1.355) ab	1998	Oregon
C-purp 3	93	90	91.5 (1.283) abc	2002	Colorado
C-purp 4	90	88	89.0 (1.241) bc	2002	Colorado
C-purp 5	89	82	85.5 (1.192) c	1998	Colorado
C-purp 6	84	83	83.5 (1.161) c	1997	Colorado
C-purp 7	82	86	84.0 (1.161) c	1997	Oregon
Mean	90	88			
ANOVA (two-way)					
Seed lot	*				
Treatment	NS				
S × T	NS				
USDA/NCRPIS accessions[w]					
PI 633669	92	73	82.0 (1.153) c	2002	Louisiana
PI 633667	88	78	83.0 (1.161) c	2002	Arkansas
PI 631307	76	52	64.0 (0.933) d	2000	Missouri
PI 633668	68	47	57.5 (0.861) de	2002	Louisiana
PI 631313	67	34	50.5 (0.790) e	2002	North Carolina
PI 633670	64	31	47.5 (0.759) e	2002	Mississippi
PI 633671	27	5	16.0 (0.386) f	2002	Ohio
PI 633666	16	4	10.0 (0.298) f	2002	Arkansas
PI 633665	9	5	7.0 (0.267) f	2002	Arkansas
Mean	56	37			
ANOVA (two-way)					
Seed lot	***		***		
Treatment	***		***		
S × T	NS		*		

[z]n = 50 seeds, three replications.

[y]Percentage.

[x]Mean separation within columns by Duncan's multiple range test after a combined analysis of transformed data representing all seed lots; transformed means (in parentheses) follow the untransformed data.

[w]n = 32 seeds, three replications.

[NS,*,***]Nonsignificant or significant at $P < 0.05$ or 0.0001, respectively.

of no publications or other types of information indicating that intentional selection to reduce seed dormancy has ever been conducted in *Echinacea*. However, our results indicate that a reduction or lack of dormancy in commercial *E. purpurea* populations is most likely due to unintentional selection during repeated cycles of cultivation.

In nature, *Echinacea* seeds fall to the ground in the autumn and overwinter in the soil. Before seeds germinate the following season, they may receive a moist chilling treatment, giving dormant and non-dormant seeds similar growth opportunities. But when seeds are harvested in the fall and stored in a dry state until sowing, nondormant seeds would germinate to a greater extent, and dormant seeds put at a selective disadvantage, since they would germinate more slowly if at all (Wartidiningsih et al., 1994).

The wild populations of *E. purpurea* conserved ex situ by the NCRPIS are maintained by regenerating them in screened field cages with pollinating insects (Widrlechner et al., 1997; Widrlechner and McKeown, 2002), in a manner designed to preserve the genetic integrity of individual populations (Wilson, 1989). Populations are established in the greenhouse by treating seeds with ethephon (Sari et al., 2001) so that selection for non-dormant types would be avoided. Given the geographic diversity of wild populations represented in our experiment (Table 1), dormancy may be a common phenomenon of *E. purpurea* in nature, although the levels may vary.

Germination percentage of the NCRPIS seed lots was higher in light than in dark (Table 1). A similar phenomenon has been noted for *E. angustifolia* (Feghahati and Reese, 1994; Qu et al., 2004). Previous authors (Qu et al., 2004; Smith-Jochum and Albrecht, 1987; Wartidiningsih and Geneve, 1994) have reported variable effects of light on seed germination in *E. purpurea*. Qu et al. (2004) suggested that while fully dormant *Echinacea* seeds could not germinate either in light or darkness, germination in light becomes possible with a partial release of dormancy. Differences in seed source and dormancy status may be responsible for observed variation in germination. In addition, since the seeds used in this study have all been stored in a dry state for more than one year, germination differences between the commercial and wild seed lots suggest that dry storage had minimal effect on seed dormancy.

Our results are the first to indicate that selection during cultivation may have unintentionally reduced seed dormancy in *E. purpurea*. However, we recognize that, by testing seed lots produced under various environmental conditions and by using commercial seed lots of unknown original parentage, our results must be considered preliminary. However, we believe the effects of these confounding factors to be relatively minor for the following reasons:

1) Seed dormancy is commonly a well-defined genetic characteristic that interacts with environmental variation (reviewed by Baskin and Baskin, 1998). In our judgment, the differences among environmental conditions at seed-regeneration sites or among seed-storage conditions used are small relative to genetic effects.

2) It is very difficult, if not impossible, to trace the original population(s) from which the commercially cultivated seed lots were produced. In the worst-case scenario, the commercial seeds may have been supplied from only two production fields, one in Oregon and the other in Colorado. However, these seed lots represent a substantial proportion of the *E. purpurea* seed used for commercial field production, since we obtained seeds from firms that are among the major suppliers. We expect that commercial seed lots do not trace back to only two production fields. We recently tested *E. purpurea* seed lots produced in California and Illinois, with germination results not significantly different (data not shown) from the commercial seed lots analyzed in this study.

Our results provide useful information for *E. purpurea* growers who wish to select populations for reduced seed dormancy. Since *Echinacea* seed production has not been standardized as it has for many other more established crops, we suggest germination tests to evaluate *E. purpurea* seed dormancy before sowing, even when seed source information has been provided.

The germination of two other species, *E. angustifolia* and *E. pallida*, widely cultivated as medicinal plants (Galambosi, 2004), has been reported to be more erratic than that of *E. purpurea* (Feghahati and Reese, 1994; Macchia et al., 2001; Qu et al., 2004; Shalaby et al., 1997; Sari et al., 2001), requiring the use of special dormancy-breaking protocols for consistent germination. It may be possible to duplicate the selection process for dormancy reduction in *E. angustifolia* and *E. pallida* by using multiple cycles of cultivation through untreated seed propagation, as has occurred in *E. purpurea*.

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