Ethephon Can Overcome Seed Dormancy and Improve Seed Germination in Purple Coneflower Species *Echinacea angustifolia* and *E. pallida*

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Summary. **Low and erratic seed germination presents a major production problem in the medicinal plants that collectively are called echinacea or purple coneflower (*Echinacea angustifolia* and *E. pallida*). In this study, nine seed lots of each *E. pallida* and *E. angustifolia* from a wide variety of commercial sources and germplasm collections were collected and treated with a solution of 1.0 mM [144.5 mg·L^{-1} (ppm)] ethephon (2-chloroethylphosphoric acid) to determine whether ethephon would sufficiently improve seed germination to be used by industry to improve the quality of echinacea seed. Application**

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of ethephon increased seed germination in both *E. pallida* and *E. angustifolia* seed lots regardless of seed sources. The increase in germination by ethephon in eight seed lots of *E. pallida* and four seed lots in *E. angustifolia* were statistically significant compared to the nontreated control seeds. The increases in germination were also significant across seed lots for both species. Average germination increases across all seed lots were 1271 and 29% for *E. pallida* and *E. angustifolia*, respectively. Average germination of ethephon treated-untreated control seed lots was 76% to 27% and 79% to 62% for *E. pallida* and *E. angustifolia*, respectively.

Echinacea is fast becoming one of the most popular medicinal herbs in the United States and demand has increased markedly. *Echinacea* used in herbal preparations comes from different species, primarily purple coneflower (*Echinacea purpurea*), narrow-leafed purple coneflower (*E. angustifolia*), and pale purple coneflower (*E. pallida*) (Schulthess et al., 1991), grown or collected from natural stands for the commercial trade. *Echinacea* was used extensively by native Americans for many purposes including treatments for venomous bites, rabies, toothaches, cough, sore mouth, throat, dyspepsia, colds, colic, headache, and stomach cramps (Kindscher, 1989; Foster, 1991). The use and demand of echinacea has been increasing because echinacea extracts have been shown to strengthen the human immune system (Bauer et al., 1990; Schulthess et al., 1991; Leung and Foster 1996; Burger et al., 1997, Robbers and Tyler, 1999). Preparations are made from the roots or aerial parts of echinacea (Blumenthal et al., 1998), and research has confirmed the product safe (McCaleb, 1998; Mengs et al., 1991), although clinical trials have not confirmed some of the uses for which the plant is acclaimed.

Cultivation of echinacea has been greatly hampered by the plants low and erratic seed germination. Shalaby et al. (1997) reported that *E. pallida* and *E. angustifolia* germinated 0% to 1%, even after 40 d of stratification at 2 °C (35.5 °F). Baskin et al. (1992) found that fresh mature seeds of *E. angustifolia* germinated only from 0% to 6% when they were placed under light and from 0% to 2% when placed in darkness. In another study, no germination was observed when *E. angustifolia* and *E. pallida* were directly field sown in the spring (Smith-Jochum and Albercht, 1988).

Ethylene has been used to break seed dormancy (Weaver, 1972). Ethephon, an ethylene-releasing compound when absorbed by plant tissues is also effective in overcoming seed dormancy imposed by the embryo, seed coat, adverse environmental conditions, or inhibitors (Weaver, 1972; Heydecker and Coolbear, 1977). The effectiveness of ethylene increases with exposure to light (Heydecker and Coolbear, 1977). Feghahati and Reese (1994) increased germination of control and weight-base-selected *E. angustifolia* seeds from 30% to 80% and 97%, respectively, by stratifying the seeds with 1.0 mM ethephon for 2 weeks at 4 °C (39.2 °F). Application of 1.0 mM ethephon alone and with 8 weeks of stratification at 0 °C (32 °F) increased seed germination of *E. angustifolia* from 65% to 94% and 99%, respectively (Sari 1998 and Sari et al. 1999).

Echinacea seed lots of the same species but from different commercial seed sources have been shown to have significantly different germination capabilities. Wartidiningsih and Geneve (1994) observed between 39 and 91% seed germination of six commercial seed lots of *E. purpurea*. Whether the high variation is due to the inherent dormancy, the genetics of the lines, the time of seed collection, harvesting method, or handling and storage methods remains unknown. The purpose of this study was to determine if the dormancy overcoming effect of ethephon observed by Feghahati and Reese (1994), Sari (1998) and Sari et al. (1999) for *E. angustifolia* seeds would be an applicable and reliable technique to 1) overcome seed dormancy and 2) improve seed germination in both *E. angustifolia* and *E. pallida* seed lots from a wide variety of sources varying in genetic and environmental backgrounds.

Materials and methods

Echinacea pallida and *E. angustifolia* seed lots obtained from seed companies and harvested from plants which originated from selected commercial sources and field-grown at the Purdue Research Farm, Lafayette, Ind., were used in this experiment to confirm the dormancy breaking effects of ethephon on different echinacea seed sources and lots (Table

Table 1. The sources and origins of *Echinacea pallida* and *E. angustifolia* seeds used in this study (x shows seed source).

Seed source	Seed origin	*Echinacea* Abbreviation	*Echinacea pallida*	*angustifolia*
Commercial Seed Companies	Johnny's Selected Seeds, Albion, Maine	JS	x	x
	Missouri Wild Flowers, Jefferson City, Mo.	MWF	x	
	Prairie Moon Nursery, Winona, Minn.	PMN	x	x
	Richters Herb, Goodwood, Ont., Canada	RH	x	x
	L.L. Olds Seed Co., Madison, Wis.	LL OS		x
Purdue Research Farm	Johnny's Selected Seeds, Albion, Maine	JS-Purdue	x	
	Companion Plants, Athens, Ohio	CP-Purdue	x	
	USDA-Ames-14445-E. pallida.	PI Ames-14445	x	
	USDA-Ames-14446-E. angustifolia.	PI Ames-14446		x
	Richters Herb, Goodwood, Ont., Canada	RH-Purdue	x	x
	Prairie Moon Nursery, Winona, Minn.	PMN-Purdue	x	x
	USDA-PI-421372-E. angustifolia	PI-421372		x
	Herb Tech-pallida sourced from Prairie Moon Nursery, Winona, Minn.	HT-Purdue		x

Table 2. Germination of control (untreated) and 1.0 mM [144.5 mg·L^{-1} (ppm)] ethephon treated *Echinacea pallida* and *E. angustifolia* seeds from different commercial sources and germplasm accessions.[z]

Seed source	Abbreviation	*Echinacea pallida* Germination (%) Control	Ethephon	*t* statistic	% Increase	*Echinacea angustifolia* Germination (%) Control	Ethephon	*t* statistic	% Increase
Johnny's Selected Seeds	JS	77	91	3.5*	18	58	81	1.6161^{NS}	40
Johnny's Selected Seeds-Purdue	JS-Purdue	75	93	2.0^{NS}	24	---	---	---	---
Missouri Wild Flowers	MWF	7	70	5.9*	950	---	---	---	---
Prairie Moon Nursery	PMN	4	85	24.4**	2025	69	79	1.3229^{NS}	14
Prairie Moon Nursery-Purdue	PMN-Purdue	1	65	11.9**	6400	72	94	3.1322*	31
Richters Herb	RH	52	90	9.1**	73	63	91	3.3077*	44
Richters Herb-Purdue	RH-Purdue	6	50	6.1*	733	60	85	2.6429^{NS}	42
Companion Plants-Purdue	CP-Purdue	7	63	16.2**	800	---	---	---	---
USDA-Ames-14445-Purdue	PI-Ames-14445	13	73	24.7**	462	---	---	---	---
HT-Purdue (Prairie Moon Nursery)	HT-Purdue	---	---	---	---	36	51	5.5*	42
L.L. Olds Seed	LL OS	---	---	---	---	82	89	0.7852^{NS}	9
USDA-14446-Purdue	PI-Ames-14446-Purdue	---	---	---	---	66	67	0.1153^{NS}	2
PI-421372-ang (USDA)	PI-421372	---	---	---	---	54	75	8**	39
Mean		27	76	1271	62	79	29		
SD		32	15	2023	13	14	16		
		df = 11			df = 16				
		t statistic=2.987** (across seed sources)			*t* statistic =2.669** (across seed sources)				

[z]Germination of each control (untreated) and ethephon treated seeds from different sources were compared with paired *t* test (df = 2, critical $t_{0.01}$ = 6.695, $t_{0.05}$ = 2.920) and germination across seed sources compared with unpooled t test (for *E. pallida*: df = 11, critical $t_{0.01}$ = 2.718, $t_{0.05}$=1.796; for *E. angustifolia*: df = 16, critical $t_{0.01}$ = 2.583, $t_{0.05}$ = 1.746).

1). Seeds from 2-year-old *E. pallida* and *E. angustifolia* plants grown at Purdue were manually harvested on 14 Aug. 1998, just before the initiation of seed shattering and stored in paper bags at 25 °C (77 °F) for around 45 d until commercial seeds were received at the beginning of Oct. 1998. The local grown seeds were threshed, cleaned and handled in an identical manner to that of the commercial seeds. A sample of 50 seeds from each species and each seed lot was placed in 100 x 15 mm round petri dishes containing two 90-mm filter papers. The filter papers were saturated with 1.0 mM ethephon solution [about 15 mL (0.5 fl oz) ethephon solution for each petri dish]. Petri dishes were immediately sealed with parafilm after ethephon application, and transferred to the germination incubator set at 25 ± 3 °C (77 ± 5 °F) (model SG-30-HR, Hoffman Refrigeration, Albany, Oregon). Germination was under constant light, 30 μmol·m^{-2}·s^{-1}. Each petri dish represented a replication and there were three replications for each seed lot. Three replications of untreated control seed from each seed lot were also placed in the germination incubator. Filter papers in the petri dishes containing control seeds were saturated with deionized water and petri dishes were sealed with parafilm and then transferred into the incubator. The experiment was conducted in the incubator of the Office of Indiana State Chemist and Seed Commissioner, Purdue University, Lafayette, Ind. The incubator was monitored on a 24-h basis for temperature. Seeds were dusted with the fungicide, captan, before starting the experiment, and were considered germinated when the radicle was visible. Germination was terminated 12 d after sowing, and data were evaluated using unpooled and paired *t* tests.

Results and discussion

Application of 1.0 mM ethephon increased seed germination of *E. angustifolia* and *E. pallida* regardless of seed source and the increase was significant for both species across seed sources (Table 2). However, the degree of germination increase was different for each species and among seed sources within species. The improvement in seed germination from ethephon was greater and broader across seed lots in *E. pallida* than in *E. angustifolia* (Table 2). Ethephon significantly increased germination of all *E. pallida* seed lots, except for JS-Purdue; and in four lots of *E. angustifolia*.

In *E. angustifolia*, the average germination increase from ethephon across seed lots was 29%, while the application of ethephon to *E. pallida* seeds resulted in 1271% average increase in seed germination across seed lots. When compared to control seeds, the increase in seed germination among seed lots ranged between 18% (JS) and 6400% (PMN-Purdue) for *E. pallida*; and 2% (PI-Ames-14446-Purdue) and 44% (RH) for *E. angustifolia* seed lots.

As we expected, there was wide germination variability among control seeds of *E. pallida* from different sources ranging from 1% to 77% with an average of 27% germination (Table 2). The germination of *E. pallida* control seed lots could be grouped into three different distinct classes exhibiting about 10%, 50%, and 75% germination (Table 2). The largest class was the group with the lowest germination percentage, around 10%. Control seeds from seed sources MWF, PMN, PMN-Purdue, RH-Purdue, CP-Purdue, and PI-Ames-14445-Purdue were in this group, their germination rates were 7, 4, 1, 6, 7, and 13%, respectively. Control seeds of JS and JS-Purdue were in the highest group, having 77 and 75% seed germination, respectively, while germination of RH was in the middle group with 52%.

Germination of *E. angustifolia* control seeds was higher than that of *E. pallida* control seeds regardless of

seed sources except for seed lots JS and JS-Purdue (Table 2). Germination of *E. angustifolia* control seeds among seed sources varied between 36 and 82% with an average of 62% (Table 2). Germination of control seeds of *E. pallida*, JS (77%) and JS-Purdue (75%) were higher than that of *E. angustifolia* from the same seed sources (69 and 72% germination, respectively) (Table 2).

Our results confirm the dormancy breaking effect of ethephon, observed in *E. angustifolia* by Feghahati and Reese (1994), Sari (1998) and Sari et al. (1999) who used 1.0 mM ethephon and increased seed germination to 99%. In this study, we show that such a technique can be a reliable tool not only in overcoming seed dormancy in *E. angustifolia*, but also in *E. pallida*. We can assume wide variations due to environment, genetics, after-ripening factors and differing response to storage between seed lots because they originated from distinct regions, and were harvested and processed by different companies. Variation in seed lots harvested from the Purdue O'Neall Research Farm should differ only genetically in germination ability since all other environmental, harvest, and storage procedures were kept relatively equal. As 1.0 mM ethephon application increased seed germination of all seed lots evaluated in this study, we conclude that ethephon has wide germination increasing effect for both *E. pallida* and *E. angustifolia* seeds regardless of variability in environmental, genetic, after-ripening and storage conditions. The dormancy breaking effect of ethephon was higher in *E. pallida* than *E. angustifolia* because *E. pallida* seed lots with 27% average germination were generally more dormant than *E. angustifolia* seed lots with 62% average germination (Table 2). The lowest germination of control seeds of *E. angustifolia* seed lots was 36% (HT-Purdue) and application of 1.0 mM ethephon increased it to 51% with a 42% increase in germination (Table 2). However, we observed up to 6400% germination increase in *E. pallida* seed lot originated from PMN-Purdue since this seed lot germinated only 1% without ethephon treatment and with 1.0 mM ethephon treatment this germination increased to 65% (Table 2). Although ethephon increased germination of all seed lots, the germination of the more dormant seed lots reached only 50% to 70% after the treatment (Table 2). Such seed lots might require higher concentrations of ethephon for an even greater germination improvement because seeds do produce ethylene during germination and nondormant seeds produce more ethylene than dormant seeds (Wareing and Saunders, 1971), though some seeds have an insufficient response mechanism to exogenous ethylene (Kepczynski and Kepczynska, 1997).

The results obtained in this study show that ethephon can stimulate germination of *E. pallida* and *E. angustifolia* seed from a wide genetic and environmental base. *Echinacea pallida* seeds appear to have stricter seed dormancy than *E. angustifolia* seeds, and untreated seeds of *E. pallida* generally germinated at {XltequalX}10%, while *E. angustifolia* seeds germinated at 50% to 60%. However, there is a wide germination variation among the untreated seed lots of both species, and germination of untreated *E. pallida* and *E. angustifolia* seed lots ranged between 1% to 77% and 36% to 82% respectively. This genetic variation can be exploited by selecting plants for high seed germination through plant breeding techniques.

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