Relationship among Seed Size, Source and Temperature on Germination of *Echinacea angustifolia, pallida* and *purpurea*

Richard L. Hassell, Robert Dufault and Tyron Phillips
Clemson University
Coastal Research and Education Center
2865 Savannah Highway
Charleston, SC 29414
USA

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Abstract

Three seed sources for *E. purpurea* and *pallida* (seed collected from one- and two-year-old mother plants and bought from a commercial seed company) were sized into large and small categories and then germinated on a thermogradient table ranging in temperature from 16° to 34°C. Only *E. angustifolia* had a single source, a commercial seed company. The larger seeds of *E. angustifolia* from the commercial source germinated to the highest rates after 13 d at 28 °C to 32 °C, with smaller seeds found to be non-viable. *E. pallida* seed source and seed size germinated similarly with highest germination at 22° to 30 °C. Seed from one-year-old mother plants was more viable than those from two-year-old mother plants. Large seed of *E. purpurea* from commercial source germinated best at 24° to 30 °C, with smaller seed apparently more viable that larger seed. Seed from two-year-old *E. purpurea* was less viable that seed from one-year-old mother plants. However, the increased germination received from sorted seed is a commercially significant and important operation for *E. angustifolia* but not for *E. pallida* and *purpurea*.

INTRODUCTION

Echinacea species are (*E. angustifolia*, *E. pallida*, and *E. purpurea*) native to North America and are very popular medicinal herbs, because of the medicinal properties of the roots and foliage. Native Americans used *Echinacea* extensively for the treatment of snake and other venomous bites, rabies, toothaches, sore throats, dyspepsia, colds, colic, headaches, and stomach-aches (Hobbs, 1989; Kindscher, 1989; Foster, 1991). *E. purpurea* purportedly is an effective additive to anti-cold remedies for treatment of the common cold and reduction of healing time (Scaglione and Lund, 1995). Germany leads the research efforts on the medicinal values of *Echinacea* species and has documented its immunostimulatory activity (Kindscher, 1989).

As the uses of *Echinacea* species become validated, the consumer demand for this herb will increase. To meet increasing demand for raw product by the dietary supplement industry, *Echinacea* must be commercially cultivated. The main way to propagate *Echinacea* is by seed or division (Li, 2000). In order to establish new fields of *Echinacea*, seedlings must be used; however, the major propagation problem with *Echinacea* facing the commercial transplant industry is poor and erratic seed germination (Smith-Jochum and Albrecht, 1998). The basic factors affecting seed germination are temperature, light and water (Copeland and McDonald, 1995); however, seed size is also an important factor in seed germination of many economically important crops (Singh and Rai, 1988). Previous studies have shown that the seed size within various species determines seedling germination and vigor (Singh and Rai, 1988). Generally, larger seed tend to germinate better with greater seedling vigor because of larger reserves within the seed, (Gelmond, 1978), however, other studies have shown that intermediate seed of some species have an increased advantage over large seed (Edward and Hartwig, 1971). Seed size and vigor can be directly correlated with the growth, health and vigor of the mother plants (Koller, 1972; Pollock and Roos, 1972). By altering the growth of the mother plant (fertilizer, plant age, maturity, etc.), seed germination and seedling vigor could be enhanced.

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Information on seed size and its relation to germination, as well as the influence of the mother plant on seed performance in *Echinacea* species is limited. Determining the effect of seed size and age of mother plant could make it possible to provide better cultivation recommendations to the industry and to slow the loss of native *Echinacea* populations (Smith-Jochum and Albrecht, 1987). Therefore, the objectives of this research were to determine: 1) the effect of seed size on germination of *Echinacea angustifolia* (angustifolia), *E. pallida* (pallida) and *E. purpurea* (purpurea); 2) the ideal temperature range of various size seed for optimal germination; and 3) the effect of the mother plant's age on germination.

MATERIALS AND METHODS

Seed of pallida and purpurea were grown at Clemson University's Coastal Research and Education Center, Charleston, SC. Seedlings were transplanted to the field in February 1999, and grew normally for two summers, producing seed heads by August 2000. Seed was then allowed to desiccate on mother plants before harvest in mid-September 2000. These were considered seed from 2-year-old mother plants. In another field, seedlings were transplanted in February 2000, and grew normally for one summer. One-year-old mother plants developed seed heads by August and were harvested mid September. Seed were collected by hand and then hand-cleaned, bulked and stored in a seed storage facility at 14 ^{o}C $\pm$ 2 ^{o}C with 50 % relative humidity until needed. In September 2000, commercial seed was obtained from Johnny's Selected Seeds (Albion, ME) of all three species. These seed were considered freshly harvested that summer. Upon delivery, the seed was immediately placed in a seed storage facility at 14 ^{o}C $\pm$ 2 ^{o}C with 50 % relative humidity until needed. In October 2000, all seed was then sized using a RX-86 model sieve shaker (W.S. Tyler, Mentor, OH). The three experimental sizes were defined as: 8's (>2.36 mm), 10's (2.00-2.36 mm), and >10's (<2.00 mm) using a U.S.A. standard testing sieve (ATM Testing Sieves, Inc., Milwaukee, WI). Immediately after sizing, seed was returned to the seed storage facility to await further testing.

Five-hundred seeds of each lot were divided into 10 groups of 50 seeds each, equally spaced in individual polystyrene petri dishes (10 cm in diameter) containing a blue germination blotter (Hoffman Manufacturing Co., Albany, OR) moistened with 6 ml distilled water. Polystyrene lids covered the tops to prevent moisture loss. These containers were placed along a temperature gradient on a thermogradient table, Type 5001 (Seed Processing, Holland) and allowed to germinate over a ten-day period. The gradient treatments were: ($\pm$ 1 °C) were 16°, 18°, 20°, 22°, 24°, 26°, 28°, 30°, 32°, and 34°, respectively. Temperatures were measured within the container using thermocouples (Barnant Co., Barrington, IL). Containers representing each lot within a species were randomly arranged within each temperature treatment location on the thermogradient table.

The experiment for purpurea and pallida were a factorial combinations of seed source (commercial, 1 year-old mother plants, 2 year-old mother plants), seed size (8,10, >10), temperature, and day of germination counts. Only one seed source (commercial) of angustifolia was evaluated and the experiment consisted of a factorial of seed size, temperature and day of germination counts. The experiment was replicated three times with a replicate equal to a complete repetition of the experiment over a six-week period in spring 2000. Germination counts for purpurea and angustifolia were taken on the even number days at the same time each day in the presence of light, while pallida was taken odd days. A seed was considered germinated when a 3 to 5 mm long radicle protruded through the seed coat (Copeland and McDonald, 1995). All germination data were then analyzed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Echinacea angustifolia

Angustifolia commercial seeds germinated to highest levels over 13 d period from 28 °C to 32 °C with germination lower at temperatures ≥ 34 °C and <28 °C (Table 1). Optimal germination was achieved as early as 9 d after initiating the experiment at 28 °C. By separating out the large seed (Size 8), overall germination was improved, since smaller seed (Size 10) failed to germinate and were considered non-viable.

Echinacea pallida

One-year plant, two-year plant and commercial purpurea seed germinated similarly to temperature time (Table 2). By day 4, the first day germination was counted no significant germination had occurred, but by 6 d, germination increased suggesting a preferred temperature range from 22° to 28 °C (Table 2). This range is lower than the preferred temperature for angustifolia. By 12 d, germination had reached its highest level, and by 14 d, the final preferred temperature range was 22° to 28 °C.

Echinacea purpurea

Purpurea responded differently than angustifolia and pallida. The size 10 seed from one-year mother plants germinated highest from 24° to 28 °C after 14 d with lesser germination with larger and smaller seed. The size >10 seed germinated similarly to the size 10 seed at 26 °C only. The seed from the two-year-old mother plants were not as viable as seed from the one-year-old plants and commercial seed sources. Seed from commercial source were the most vigorous, with highest germination and the preferred temperature range from 24° to 30 °C after 14 d, with all seed sources and sizes germinating similarly at 24°-28 °C.

The viability of the seed is affected by seed size, age of the mother plants, and species with these aspects being commercially important to increase production of *Echinacea*. Age of the mother plants of pallida and purpurea seemed to have some significant effect, however we considered this variation minor and negligible. Seed size does have a profound effect on germination of angustifolia, with the larger seed size germinating, while smaller seed were technically in-viable. Seed size did not matter for pallida, but smaller seed seems to perform better with purpurea. The increased germination from sorted seed size does appear to be significant enough to suggest that commercial seed companies should sort angustifolia seed. The largest factor noted in this study was that of temperature. If a single ideal temperature range is desired for the three *Echinacea* species, all three species indicated a common germination temperature for *Echinacea* 28° to 30°C.

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Tables

Table 1. Interaction[1] of seed size, days to germination and a temperature gradient on daily germination of fifty *Echinacea angustifolia* seeds.

Temperature (C)[2]

		16	18	20	22	24	26	28	30	32	34
Size[3]	Days	Germination numbers/50 seeds									
8	3	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	5	0.0	0.0	4.3	6.3	8.3	9.7	13.7	11.3	4.3	2.0
	7	2.7	3.3	6.3	7.0	11.7	12.7	20.0	16.3	8.7	5.0
	9	3.3	4.3	7.3	8.0	15.3	15.0	21.7	19.3	14.3	7.7
	11	4.0	4.3	8.0	8.7	15.7	18.7	23.3	24.0	19.7	10.7
	13	4.0	4.7	8.3	9.0	17.3	19.7	24.0	24.7	21.0	12.0
10	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[1]Interaction among seed size, temperature and day of germination count significant at $P=0.05$. LSD $(P=0.05)=4.0$.
[2]Temperature varied on thermogradient table as a standard deviation of approximately 1 C.
[3]Size 8 = seed not passing through a 2.36 sq. mm screen; Size 10 = seed not passing through a 2.0 sq. mm screen.

Table 2. Interaction[1] of days to germination and temperature gradient on daily cumulative germination of fifty *Echinacea pallida* seeds (pooled over three seed sources, and three seed sizes).

Temperature (C)[2]

	16	18	20	22	24	26	28	30	32	34
Days	Germination numbers/50 seeds									
4	0.0	0.2	0.7	1.1	1.5	1.9	2.0	2.0	0.1	0.0
6	1.0	4.6	7.8	9.3	8.7	9.4	7.4	6.1	2.5	0.9
8	3.3	10.5	14.9	17.0	21.1	19.4	16.2	12.6	7.4	2.4
10	5.5	14.2	19.0	24.4	25.4	26.7	24.9	20.3	14.0	4.7
12	7.6	17.1	23.4	28.9	31.9	31.1	29.1	25.6	16.3	6.4
14	8.7	18.1	25.3	31.8	34.5	33.9	31.9	28.7	19.2	8.3

[1]Interaction among seed size, temperature and day of germination count significant at *P=0.05*. LSD *(P=0.05)=3.0*.

[2]Temperature varied on thermogradient table as a standard deviation of approximately 1C.

Table 3. Interaction[1] of seed source, seed size and temperature gradient after 14 days on germination counts of fifty seeds of *Echinacea purpurea*.

Temperature (C)[2]

		16	18	20	22	24	26	28	30	32	34
Source[4]	Size[3]	Germination numbers/50 seeds									
One Year	8	26.7	31.0	37.7	36.3	39.7	41.0	42.0	40.3	38.3	36.3
	10	20.3	37.7	43.0	42.3	43.7	46.3	46.3	42.3	39.3	31.0
	>10	21.0	36.0	46.0	38.3	43.0	43.3	43.0	38.7	36.3	32.7
Two Year	8	13.3	28.0	36.3	36.0	41.3	40.7	41.0	42.3	40.0	31.0
	10	22.3	34.7	36.3	38.0	40.7	40.7	41.7	41.7	36.7	30.7
	>10	17.0	31.0	32.0	38.7	38.0	37.7	36.7	37.0	36.7	32.3
Commercial	8	9.0	25.3	35.0	41.0	42.7	43.7	42.3	42.0	39.0	23.0
	10	7.7	25.3	38.3	42.7	44.7	46.7	45.0	44.3	40.3	34.7
	>10	19.7	33.3	41.7	43.3	45.0	46.3	44.6	43.9	40.2	30.9

[1]Interaction among seed size, temperature and day of germination count significant at *P=0.05*. LSD *(P=0.05)=2.8*.

[2]Temperature varied on thermogradient table as a standard deviation of approximately 1 C.

[3]Size 8 = seed not passing through a 2.36 sq. mm screen; Size 10 = seed not passing through a 2.0 sq. mm screen; Size >10=seed passing through 2.0 sq. mm screen.

[4]Source of seed was one-year-old, two-year-old mother plants and fresh seed from commercial seed company.