# Influence of Temperature Gradients on Pale and Purple Coneflower, Feverfew, and Valerian Germination

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SUMMARY. **Seeds of pale coneflower (*Echinacea pallida*), purple coneflower (*Echinacea purpurea*), feverfew (*Tanacetum parthenium*), and valerian (*Valeriana officinalis*), classified as "old" (1-year-old seed) or "fresh" (seed crop produced in the current year), were germinated at 62, 65, 69, 72, 75, 78, 82, 85, 89, and 92 °F, (16.7 , 18.3, 20.6, 22.2, 23.9, 25.6, 27.8, 29.4, 31.6, and 33.3 °C). The optimum germination temperature, defined in this study as the temperature range within which the percent germination is greatest in the shortest period of time, was determined. Old and fresh pale coneflower seed germinated optimally after 5 days at 69 °F. Old purple coneflower seed required 5 d at 78 to 82 °F, but fresh seed germinated optimally after 3 days at 75 °F. Old feverfew germinated optimally after 5 days at 65 °F, but fresh seed germinated to its optimum after 5 days at 69 °F. Old and fresh valerian seed germinated to its optimum after 3 days at 75 °F.**

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Medicinal plants traditionally are collected from the wild, but the quantities needed in the dietary supplement industry exceed available raw product supply (Li, 1998). With increased harvest pressure and plant destruction, species that were once plentiful are becoming endangered in certain regions of the world. To meet the increasing raw product demand of the dietary supplement industry, medicinal plants must be cultivated (Li, 2000). The first step in commercial production of medicinal plants is to identify germination protocols that insure the production of usable, uniform seedlings for field planting. Four environmental factors are involved in seed germination: water, air, temperature, and sometimes light (Copeland and McDonald, 2001). In our study, we examined only temperature effects on germination. Determination of the cardinal temperatures, defined as the minimum, optimum, and maximum temperatures at which germination will occur (Copeland and McDonald, 2001), is critical to the development of recommendations for commercial transplant producers. Of greatest importance to transplant growers is the optimum germination temperature that will provide the highest level of emergence and viable seedling production. The optimum germination temperature is defined as the temperature range giving the greatest percentage of germination within the shortest period of time (Copeland and McDonald, 2001).

A limitation for commercial field production of *Echinacea* sp. is poor and erratic seed germination (Li, 1998; Smith-Jochum and Albercht, 1987). Of the four species evaluated in our study, only purple coneflower is defined in Association of Official Seed Analysis (AOSA) (1998) official rules for testing seed and requires a range of 68 to 86 °F (20.0 to 30.0 °C) in the presence of light and pre-chilled for optimum germination with first counts to be taken between 4 and 6 d with final counts taken at 12 d. Wartidiningsih et al. (1994) indicated that chilling stratification increased germination of purple coneflower in the field. Parmenter et al. (1996) reported that the length of stratification did not increase germination. Seed treatments before planting can improve germination. Samfield et al. (1991) reported that priming purple coneflower seeds resulted in a faster, more uniform germination. Wartidiningsih and Geneve (1994) reported that seed size and inflorescence did not affect purple coneflower seed germination; however, seed source accounted for the most variation in germination, ranging from 91% to 30%. Li (1998) recommended that seeds be placed in flats held at 64 to 68 °F (17.8 to 20.0 °C) for 7 to 10 d with supplemental light for best germination. Researchers have reported that coneflowers [black-eyed susan (*Rudbeckia fulgida*)] required a range of 68 to 86 °F for optimum germination (Fay et al., 1993, 1994). Despite this research, erratic and poor germination continues to be a problem in the seed industry, using all recommended practices (J. Eckert, personal communication).

Feverfew has been reported to germinate at only 70 °F (21.1 °C) after 5 d without light (Buhler and Hoffman, 1999); however, it is suggested the addition of up to 3 h light exposure per day may be necessary for maximum germination (Li, 2000).

Recently harvested valerian seeds exposed to low temperatures germinated immediately, but those not exposed to low temperatures did not germinate until the following spring (Dorph-Peterson, 1924). Valerian's germination increased when seeds were exposed to light at 59 to 68 °F (15.0 to 20.0 °C) (Grime et al., 1981).

High quality, vigorous, medicinal plant seed true to its genotype is not always available since most seed is heterogeneous, not field rogued, and bulk mixed after harvest. The only guidelines available for medicinal plant seed germination are from seed companies and producers, but these are based on sparse research or from trade publications (J. Eckert, personal communication). For greenhouse producers to grow uniform seedlings, information concerning germination and emergence processes mediated by temperature is critical. Additionally, a test that rapidly determines the optimum germination temperature range would be helpful to expedite this process. Therefore, the objectives of this research were to determine: 1) the optimum temperature ranges for maximum germination to occur in a minimum amount of time for feverfew, pale and purple coneflower, and valerian; and 2) if optimal germination ranges differ by lot age and condition.

## Materials and methods

All seed lots of the four species were obtained from Johnny's Selected Seeds, Albion, Maine (Table 1). "Fresh seed" in this study was defined as seed produced in the current year and was used immediately upon arrival in 1999. Seed not used immediately in experiments was stored at 57 ± 3.6 °F (13.9 ± 2 °C) and 50% relative humidity (RH). Seed was defined as "old" in this study if the seed crop was produced in the previous year. "Old" seeds (lots 14911–pale coneflower, 14877–purple coneflower, 14576–feverfew, and 16053–valerian) in our study were purchased in 1998 and stored at room temperature in paper bags for ~8 months and then transferred to 57 ± 3.6 °F, 50% RH for ~2 months until used in the first replication. Another group of seed lots (10696–pale coneflower, 16362–purple coneflower) was purchased in 1998 and stored immediately upon delivery at 57 ± 3.6 °F, 50% RH. The history of all these lots was unknown since the seed was not contract grown by the company.

Five hundred seed of each lot was divided into 10 groups of 50 seed each, then equally spaced in individual polystyrene petri dishes [3.9 inches (10 cm) in diameter] containing a blue germination blotter (Hoffman Manufacturing Co., Albany, Ore.). Blotters were moistened with 0.27 fl oz (8 mL) of distilled water and covered with polystyrene lids to prevent moisture loss. These containers were placed along a temperature gradient on a Type 5001 thermogradient table (Seed Processing Holland, Enkhuizen, The Netherlands) and allowed to germinate over a 7-d period. The temperature treatments ([±2 °F (1.1 °C)] were: 62, 65, 69, 72, 75, 78, 82, 85, 89, and 92 °F. Temperatures were measured within the container using thermocouples (Barnant Co., Barrington, Ill.). Containers representing each lot within a species were arranged randomly within each temperature treatment location on the thermogradient table. The experiment was a factorial combination of seed lot, temperature, and day of germination counts. The experiment was replicated three times with a replicate being a repetition of the entire experiment over a 6-week period in Spring 1999. Germination counts were taken at the same time each day in the presence of light. A seed was considered germinated when a 0.1–0.2 inch (3–5 mm) long radicle protruded through the seedcoat (Copeland and McDonald, 2001). All experiments were conducted over a 6-week period. We did not find any differences in germination response because we ran each replication at a different time, not simultaneously. Therefore, storage of fresh seeds for all species for that period of time did not affect vigor and germination in our experiments.

**Table 1. Source of variation[z] in the analysis of variance (ANOVA) for fresh and old seed lots of four medicinal species germinated at a range of temperatures on a thermogradient table.**

| Source of variation | Percentage of total sums of squares[z] | | | |
|---|---|---|---|---|
| | Pale coneflower | Purple coneflower | Feverfew | Valerian |
| Replication | 0 | 0 | 0 | 1 |
| Temp (T) | 21** | 10** | 27** | 16** |
| Lot (L) | 2 | 12** | 0 | 0 |
| Day (D) | 23** | 7** | 20** | 17** |
| T × L | 2 | 2** | 4** | 1 |
| T × D | 10** | 18** | 16** | 19** |
| L × D | 2 | 8** | 1** | 1 |
| T × D × L | 8** | 13** | 6** | 5 |
| Error | 32 | 30 | 26 | 40 |

[z]The sum of the squares for each factor in the ANOVA converted to a percentage of the total sum of squares.
**F values significant at $P = 0.01$.

All germination data were tested for normality and required arcsin transformation before conducting analysis of variance (ANOVA). Data analysis was performed on transformed data, including mean separation; then transformed data mean separation results were posted to untransformed means. Since we were not interested in germination differences among species, each ANOVA was done separately by species. Our first statistical goal was to determine the first day of maximum germination rate for the fresh and old seed lots. Since the exact day of optimal germination was unknown, the first step in analysis was performed with all temperature treatments pooled in each species. Then, we specifically examined the lot × day interaction effect to determine if lots behaved similarly over time. Then, if the interaction between seed lot and day was significant at $P = 0.05$, the least significant difference (LSD) at $P = 0.05$ was derived to detect the first day that germination was maximum by each seed lot. However, if seed lot did not interact with day, the day main effect means were separated using LSD at $P = 0.05$ if the F test for this main effect was significant at $P = 0.05$. Once the identity of the first day of optimal germination rate was identified, we then determined any differences in germination response of the lots to the various temperature ranges (lot × temperature interaction) on the single day that germination was optimal. If the F-test was significant at $P = 0.05$ for this interaction, the LSD at $P = 0.05$ was derived to delineate the specific range of temperatures at which germination percentage was maximum for individual seed lots within each species. In cases where the interaction was nonsignificant, but the main effect of temperature was significant, the LSD at $P = 0.05$ was determined.

## Results and discussion

**Pale coneflower.** All three factors of temperature, day of germination counts, and lot affected germination (Table 1). Of the three factors, day of germination count accounted for most of the variation in germination. Lot did not interact with day, and we concluded that old as well as fresh seed lots responded similarly with regard to the time required to germinate optimally (Table 2). Seeds of all three lots germinated similarly to their highest level by 5 d after initiation of thermogradient testing. After 6 d, the number of seeds that germinated declined significantly. Germination percentages in Table 2 are low because all temperatures were pooled and sub-optimal germination temperatures diluted the optimal germination percentages given in Table 3. Prior to 3 d, no visible signs of minimally acceptable radicle lengths were observed. Therefore, first counts for pale coneflower can be taken on day 5 and would provide vigor information as well as a standard germination test.

Germination data from thermo-

**Table 2. Influence of seed age, seed lot, and days from initiating thermogradient testing on daily seed germination percentage of four medicinal plant species.**

| Seed lot | Seed age + condition | Days from initiation of thermogradient testing 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| | | ---------- Daily germination (%) ---------- | | | | |
| | | *Pale coneflower*[z] | | | | |
| 14911 | 1998[y] | 2 | 11 | 12 | 14 | 8 |
| 16696 | 1998[x] | 2 | 11 | 16 | 13 | 9 |
| 16481 | 1999[x] | 3 | 10 | 14 | 15 | 7 |
| Mean | | 2 d | 11 b | 14 a | 14 a | 8 c |
| | | *Purple coneflower*[w] | | | | |
| 14877 | 1998[y] | 7 fg | 19 ab | 19 ab | 13 de | 5 g |
| 16362 | 1998[x] | 19 ab | 20 ab | 15 cd | 14 cd | 6 g |
| 16544 | 1999[x] | 22 a | 17 bc | 17 bc | 10 ef | 5 g |
| Mean | | 16 | 17 | 17 | 13 | 5 |
| | | *Feverfew*[w] | | | | |
| 14576 | 1998[y] | 3 ef | 11 c | 34 a | 8 cde | 4 def |
| 18153 | 1999[x] | 2 f | 12 c | 25 b | 9 cd | 4 def |
| Mean | | 2 | 12 | 30 | 8 | 4 |
| | | *Valerian*[z] | | | | |
| 16053 | 1998[y] | 23 | 9 | 8 | 6 | 7 |
| 18803 | 1999[x] | 23 | 5 | 10 | 4 | 4 |
| Mean | | 22 a | 6 cd | 10 b | 5 d | 7 c |

[z]F test for the interaction between lot and days nonsignificant at $P = 0.05$; therefore, main effect of time separated by least significant difference (LSD) at $P = 0.05$.
[y]Seed aged by storage at room temperatures in a paper bag for 8 months.
[x]Seed properly stored after harvest in cold room.
[w]F test for the interaction between lot and days significant at $P = 0.05$ and means separated by LSD at $P = 0.05$.

gradient testing for pale coneflower on only 5 d was isolated from all other data and appropriate mean separation applied (Table 3). Since seed lot did not interact with temperature (Table 1), fresh and aged seed lots reacted similarly to temperature variation. All seed lots germinated to the highest germination percentage after 5 d at 69 °F, with significantly lower germination at temperatures above and below this optimum. Germination counts in Table 3 are low, but they are only for the optimal germination day and the entire period. Table 3 indicates the final germination percentage of all seed lots. These germination percentages are characteristic of these species. Total germination after 7 d at 69 °F indicated that all three lots were of similar viability and that old seed germinated as well as fresh seed. Based on these results, the temperature range suggested for purple coneflower (AOSA, 1998) of 68 to 86 °F is inappropriate and too broad for pale coneflower, and that 86 °F germination is very adverse.

**PURPLE CONEFLOWER.** Temperature, day of germination count, and seed lot affected purple coneflower

**Table 3. Influence of temperature and seed lot on germination (germ.) percentage on the day of optimal germination for four medicinal species.**

| Seed lot No. | Age | Days to max. germ. | Temp (°F)[z] 62 | 65 | 69 | 72 | 75 | 78 | 82 | 85 | 89 | 92 | Final germ. (%)[y] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ---------- Germination (%) ---------- | | | | | | | | | | |
| | | | *Pale coneflower*[x] | | | | | | | | | | |
| 14911 | 1998[w] | 5 | 8 | 14 | 23 | 14 | 14 | 10 | 12 | 18 | 7 | 2 | 66 a |
| 16696 | 1998[v] | 5 | 10 | 22 | 25 | 27 | 21 | 18 | 15 | 18 | 1 | 2 | 76 a |
| 16481 | 1999[v] | 5 | 12 | 21 | 25 | 23 | 13 | 13 | 7 | 20 | 6 | 1 | 68 a |
| Mean | | | 10 e | 19 b | 24 a | 21 b | 16 c | 14 cd | 12 de | 19 b | 5 e | 1 f | 70 |
| | | | *Purple coneflower*[u] | | | | | | | | | | |
| 14877 | 1998[w] | 4 | 1 lm | 8 ijk | 17 fg | 27 e | 33 d | 34 cd | 37 cd | 25 e | 4 klm | 4 klm | 67 b |
| 16362 | 1998[v] | 3 | 1 lm | 9 ij | 28 e | 33 d | 42 ab | 36 cd | 27 e | 14 gh | 5 jkl | 0 m | 91 a |
| 16544 | 1999[v] | 3 | 3 lm | 11 hi | 36 cd | 35 cd | 46 a | 38 bc | 28 e | 20 f | 2 lm | 0 m | 84 a |
| Mean | | 2 | 4 | 27 | 32 | 40 | 36 | 31 | 20 | 4 | 1 | 81 | |
| | | | *Feverfew*[u] | | | | | | | | | | |
| 14576 | 1998[w] | 5 | 60 c | 67 a | 62 bc | 52 d | 43 e | 35 f | 18 i | 6 k0 m | 0 m | 90 a | |
| 18153 | 1999[v] | 5 | 37 f | 60 c | 64 b | 22 h | 26 g | 25 g | 13 j | 3 k | 0 m | 0 m | 93 a |
| Mean | | | 48 | 63 | 63 | 37 | 35 | 30 | 16 | 5 | 0 | 0 | 92 |
| | | | *Valerian*[x] | | | | | | | | | | |
| 16053 | 1998[w] | 3 | 14 | 24 | 35 | 36 | 48 | 33 | 34 | 7 | 0 | 0 | 67 a |
| 18803 | 1999[v] | 3 | 13 | 27 | 32 | 34 | 44 | 34 | 28 | 12 | 5 | 0 | 62 a |
| Mean | | | 14 d | 26 c | 34 b | 35 b | 46 a | 34 b | 27 c | 10 d | 3 e | 0 e | 65 |

[z]5/9(°F – 32) = °C; temperature varied on the thermogradient table as a standard deviation of approximately + 2 °F.
[y]Total germination after 7 d at the optimum temperature. Means with different letters within columns within medicinal plant species separated by least significant difference (LSD) at $P = 0.05$.
[x]F test for the interaction between lot and days nonsignificant at $P = 0.05$; therefore, main effect of time separated by LSD at $P = 0.05$.
[w]Seed aged by storage at room temperatures in a paper bag for 8 months.
[v]Seed properly stored after harvest in cold room.
[u]F test for the interaction between lot and days significant at $P = 0.05$ and means separated by LSD at $P = 0.05$.

germination, similar to pale coneflower (Table 1), yet purple coneflower responded differently to temperature than pale coneflower, necessitating separate recommendations for each species. Seed lot interacted with day, indicating a difference in viability between fresh and old seed lots and the manner in which older seed was stored. Of the three lots, the old seed lot stored at room temperature (14877) required 4 d for maximum germination, but the other two seed lots (16362 and 16544) germinated optimally on 3 d (Table 2). The old seed lot (14877), acquired in 1998, was stored at room temperature for 8 months before thermogradient testing. Prior to 3 d, no signs of minimally acceptable radical lengths were visible with any lot. In contrast, lot 16362 was acquired in 1998 also, but stored properly upon delivery from the seed company until experimentation at 57± 3.6 °F, 50% RH. Both the fresh seed lot (lot 16544) and older seed lot (16362), refrigerated upon delivery, required only 3 d to germinate optimally. These results suggested that seed storage conditions are more critical for purple coneflower than for pale coneflower, with seed of purple coneflower losing vigor quickly if not properly stored.

Evaluation of thermogradient testing data on only the day of maximum germination (as derived in Table 2) indicated that purple coneflower seed lots interacted with temperature and that ideal temperature requirements differed by seed lot (Table 3). The old seed lot (14877) germinated best (after 4 d) from 78 to 82 °F. Lots 16362 and 16544 germinated similarly and optimally (after only 3 d) at the single temperature of 75 °F. Further, these temperatures are warmer than those we proposed for pale coneflower. If 69 °F, as recommended for pale coneflower, is used to germinate purple coneflower, germination would be hindered. Storage at room temperature for 8 months is detrimental and reduced final germination percentage, in contrast to refrigerated older seeds, which suggested that all purple coneflower seeds should be stored in a proper seed storage facility immediately upon delivery to maintain high viability.

**Feverfew**. The interaction between seed lot and day was significantly different, indicating that fresh and aged seed react differently to the germination factors studied (Table 1). Even though lot and day interacted, after mean separation, both lots germinated to their greatest level within 5 d, although the aged seed lot germinated to a higher percentage by 5 d compared to fresh seed (Table 2). On the 6 and 7 d after initiation of thermogradient testing, only a small percentage of the seeds germinated. Prior to 3 d, we saw no visible signs of minimally acceptable radicle lengths. DeBaggio (1999) also stated feverfew required 5 d for acceptable germination, which agrees with our results. Our results suggested that storage environment for feverfew seeds is not as critical as it is for purple coneflower.

Analysis of only thermogradient testing data on 5 d indicated that feverfew germinated best at 65 to 69 °F (Table 3), which is only slightly lower than 70 °F, as suggested by DeBaggio (1999). Thermogradient testing indicated that seed lots interacted with temperature with subtle differences between fresh and old seed. The old lot (14576) germinated best after 5 d at 65 °F, but the fresh seed lot (18153) germinated best after 5 d at 69 °F. Total germination after 7 d at 65 and 69 °F, respectively by lot, indicated that both lots were of superior quality, with excellent viability even if aged.

**Valerian**. Seed lot did not interact with day of germination count, indicating that old seed germinated similarly to fresh seed (Table 1). Seeds of both lots uniformly germinated to their highest level within 3 d after initiation of the thermogradient testing (Table 2). After 3 d, seed germination rate declined significantly. We saw no visible signs of minimally acceptable radicle lengths prior to 3 d.

Thermogradient testing at temperatures ranging from 62 to 92 °F on the third day indicated that valerian seed lots did not interact with temperature and that both seed lots reacted similarly to temperature variations (Table 3). All seed lots germinated optimally at 75 °F, with significantly lower germination at temperatures above and below this optimum. It should be noted that the vigor of both seed lots was poor since only 65% of the seed germinated after 7 d at 75 °F. Our results differ from those of Grime (1981), who reported 59 to 68 °F to be ideal temperature regime for valerian germination.

The differences between our results and those of other researchers may be attributed to differences in germplasm that are known to exist within any given species of medicinal plants.

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