Echinacea — The Big Chill?

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INTRODUCTION

World demand for the medicinal properties of *Echinacea* has raised its profile above that of simply an attractive American wildflower.

Echinacea, or purple coneflower, as it is commonly known, is a genus of herbaceous perennials native to the prairies of central North America. Of the nine species, *E. purpurea* and *E. angustifolia* are the two that have been actively commercialised and upon which this report will focus.

Echinacea belongs to the daisy family Asteraceae. They produce a cluster of leaves from a short (20 to 30 mm) rhizome. *Echinacea angustifolia* has a vertical taproot whereas *E. purpurea* has fibrous roots. *Echinacea angustifolia* has narrow, entire leaves covered in stiff bristly hairs while *E. purpurea* has larger, rounded leaves that are coarsely toothed.

Single flower heads are produced at the end of simple or branched stems. The spiny raised receptacle or "cone" in the centre of the flower is a characteristic of the genus. The ray flowers of *E. purpurea* and *E. angustifolia* are an attractive pink colour and the "petals" sometimes reflex downwards.

USES

The main use of *E. purpurea* and *E. angustifolia* is medicinally as a stimulant of the immune system. Hobbs (1994) notes that of all the indigenous medicines introduced by Native Americans, *Echinacea* may be one of the most important.

Studies on the effects of *Echinacea*, mostly published in Germany, have established that *Echinacea* improves nonspecific immunity and stimulates new tissue growth. It is regarded as effective in treating certain viral and bacterial infections, healing wounds, and controlling inflammations (Foster, 1991).

Herbalists commonly believe that quality *Echinacea* products should contain at least one part of, or a combination of, the roots, leaves, and flowers of *E. purpurea* or this plus the roots of *E. angustifolia* (Hobbs, 1994). Preparations are available as liquid extracts, dried or powdered extracts in capsules and tablets, or fresh juice preparations of *E. purpurea* tops stabilised with ethanol.

In addition to medicinal uses, *E. purpurea* is also popular as an ornamental garden plant and several cultivars have been selected, e.g. 'White Swan' a white-flowered cultivar and 'The King' with crimson rays.

The resurgence in popularity in the United States of using native wildflowers in landscape plantings, and interest in prairie preservation, has stimulated research on the germination and cultivation of *Echinacea* species.

MARKET DEMAND

The largest market for herbal medicines using *Echinacea* is in Europe, where in 1990 phytomedicine counter sales were US$2.4 billion. Sales in Germany made up 65% of this trade (Douglas and Parmenter, 1993). The popularity of *Echinacea* preparations is increasing worldwide and it is possibly the most popular herb in the United States (Hobbs, 1994).

Hobbs (1994) comments that due to the harvesting pressure on dwindling wild supplies of *Echinacea*, the focus is shifting to organically grown commercially cultivated supplies of *Echinacea*.

ECHINACEA RESEARCH

Research trials of *Echinacea* by The New Zealand Institute for Crop & Food Research Ltd, began in 1988. Examples of this research includes trials on germination, field production, and chemical analysis of root product. Two species in particular, *E. angustifolia* and *E. purpurea*, have received most attention and these have been the subject of production trials at several sites around New Zealand (Parmenter et al., 1992).

The following is a summary of one aspect of that research — a trial to investigate the chilling requirement of commercial *Echinacea* seed. Full details of that trial are published in the N. Z. J. Crop and Hort. Sci. 1996. 24:109-114.

ECHINACEA — THE BIG CHILL?

Poor germination of commercial *E. angustifolia* seed and conflicting evidence in the literature about the benefits of a period of cold-moist treatment (stratification) of *Echinacea* seed indicated the need for an examination of methods of reducing seed dormancy in *Echinacea*.

Foster (1991) has reviewed some of the information available on stratification requirements in *Echinacea*, including studies by Hemmerly (1976) and Ottoson (1978), which appear to indicate the need for long periods (up to 10 to 15 weeks) in cold, moist conditions to produce maximum germination of *E. purpurea* and *E. angustifolia*. Other studies indicate germination improvements after much shorter periods of stratification. For example, maximum germination rates have been reached after 8 weeks of stratification for *E. angustifolia* (Baskin et al., 1992) and after only 4 weeks of stratification at 5C for *E. purpurea* (Bratcher et al., 1993). In this latter study the increase in germination over controls was only from 89% to 99%. Wartidiningsih et al. (1994) recently showed that stratification improved germination in five out of six commercial *E. purpurea* seed lots, with the greatest improvement occurring at 10C for 10 days. In the five lots that responded to stratification, the mean improvement in germination was from 45% to 80%. One study has shown no benefit to *E. angustifolia* or *E. purpurea* of chilling at 0C for 1 or 2 months (Smith-Jochum and Albrecht, 1987). In the same study, *E. pallida* germination was improved from 15% to 65% after 1 month of chilling.

Removal or cutting of the seed coat may improve germination. Foster (1991) quotes studies which suggest that trimming the seed coat may have some effect on the

dormancy of nonstratified seed, either by allowing more rapid hydration of the seed or leaching of germination inhibitors. When the seed coat of *E. angustifolia* was removed, germination was improved from 13% to 92% (Sorenson and Holden, 1974).

METHOD

Experiment 1. Seed of *E. angustifolia* and *E. purpurea* was sown in Sept 1992 in cell trays filled with a steam-sterilised seed-raising mix of peat and sand (8.5 : 1.5, v/v). In each tray, five replicates of 10 cells of each species were sown, each cell containing a single seed. Half of the *E. angustifolia* seed in each replicate was left whole, whereas the other half had the pappus trimmed from the distal end of the seed coat with a scalpel, exposing, but not cutting, the seed. Trimmed and untrimmed seeds were randomly assigned to cells within each of the five replicates.

A total of 10 trays were sown. Given evidence that germination of *Echinacea* is enhanced by light (Foster, 1991), seed was pressed horizontally into the surface of the mix without being buried.

Each tray was watered and allowed to drain before being placed in a plastic bag to retain moisture. All but one of these trays were placed in a dark coolstore (3-5C). A single tray was placed immediately in a glasshouse, without a plastic bag covering it. Every 1 or 2 weeks thereafter, a further tray was removed from the coolstore and placed in the glasshouse. Trays in the glasshouse were watered lightly each day to keep the seed moist. Glasshouse temperatures were regulated and ranged between 10C at night (8 h) and 20C during the day.

Each week for 4 weeks after placement in the glasshouse, the number of seeds that had germinated (cotyledons visible) in each tray were counted. The last tray was removed from the coolstore 11 weeks after the first.

The effect of storage time on percentage germination and the time to maximum percentage germination was determined for each species, as well as the effect of seed coat trimming on *E. angustifolia*.

Experiment 2. Experiment 2 was similar to Experiment 1, except that *E. purpurea* was not tested. Both experiments began on the same day in mid September 1992.

In each of 10 seed trays, five replicates of 10 seeds of *E. angustifolia* were sown with half the seeds trimmed and each seed pressed horizontally into the surface of each cell. The trays were watered. One tray was immediately placed in a glasshouse and the rest were covered in plastic and placed in a dark coolstore (0 to 1C). Trays were removed from the coolstore at 2 weekly intervals, with the last tray removed 18 weeks after the first. Weekly assessments of the number of germinated seeds continued for 4 weeks after removal from the coolstore. In this experiment, glasshouse temperatures were unregulated, but maximum and minimum temperatures were recorded. Maximum and minimum temperatures averaged 24 and 6C for October, 26 and 10C for November and 27 and 11C for December. Because of the warmer conditions in Experiment 2, trays were sometimes watered twice each day.

RESULTS

Maximum Germination Percentage. *Echinacea purpurea* appeared to have no stratification requirement, with a maximum germination rate of 84% averaged over all stratification periods (Fig. 1A). *Echinacea angustifolia*, in contrast, showed a marked improvement in germination percentage once stratification periods exceeded

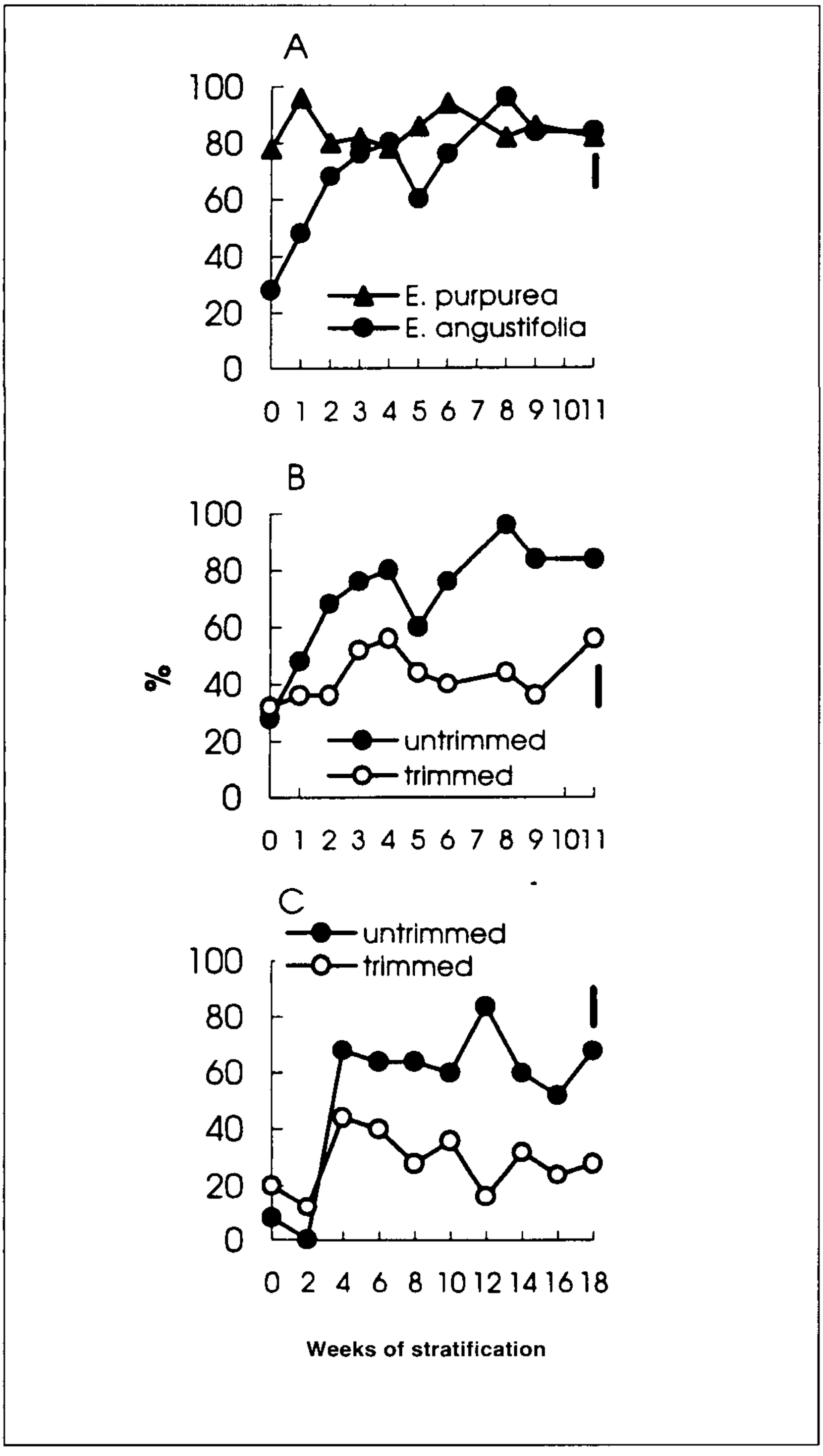

Fig. 1. Effect of stratification at 3 to 5C (Experiment 1) or 0 to 1C (Experiment 2) for periods ranging from 0 to 18 weeks on the maximum germination percentage of *Echinacea*. **A,** Comparison of *E. purpurea* and *E. angustifolia* in Experiment 1; **B,** Comparison of trimmed and untrimmed seed of *E. angustifolia* in Experiment 1; and **C,** Comparison of trimmed and untrimmed seed of *E. angustifolia* in Experiment 2.

2 weeks, from an average of 48% germination to 80% in Experiment 1 (Fig. 1B) and from 10 to 48% in Experiment 2 (Fig. 1C).

Trimming the seed coat approximately halved the maximum germination percentage in *E. angustifolia* seed stratified for more than 2 weeks from a mean of 80% to 47% in Experiment 1 (Fig. 1B) and from c. 65% to 31% in Experiment 2 (Fig. 1C). However, in nonstratified seed (0 weeks stratification), there was no significant difference in the germination percentage of trimmed and untrimmed seed — 32% trimmed compared to 28% untrimmed in Experiment 1 (Fig. 1B) and 20% trimmed compared with 8% untrimmed in Experiment 2 (Fig. 1C).

Maximum germination rates of untrimmed *E. angustifolia* seed were greater in Experiment 1 than in Experiment 2 (80% to 100% compared with 60% to 70%) (Fig. 1B,C).

Time to Maximum Germination. The time taken to reach maximum germination decreased as the length of the stratification period increased. The time taken decreased by 1.02 days per week of stratification for the species comparison in Experiment 1 (Fig. 2A), by 0.76 days per week of stratification for the trimming comparison in Experiment 1 (Fig. 2B), and by 0.73 days per week of stratification for the trimming treatment comparison in Experiment 2 (Fig. 2C). No significant evidence was found that *E. purpurea* and *E. angustifolia* differed in time to maximum germination after the same length of stratification. However, untrimmed seed took longer than trimmed seed to reach maximum germination, by 1.7 days per week of stratification.

DISCUSSION

Echinacea angustifolia required only 2 to 3 weeks of stratification to raise germination rates to maximum levels of around 80% or more, close to the best germination rates reported elsewhere for this species. This is a relatively short period of stratification compared with outdoor winter chilling which has been shown to be effective in breaking dormancy, or the longer periods of time (8 to 16 weeks) apparently required to reach maximum germination in some other experiments. However, other recent results also indicate high germination of this species after 2-weeks stratification, although only in combination with other treatments such as ethylene and light (Feghahati and Reese, 1994).

The lack of response of *E. purpurea* to stratification confirms the results of Smith-Jochum and Albrecht (1987), who had germination rates of 70% to 80% with or without stratification. Wartidiningsih and Geneve (1994) also found two of six seed sources had germination rates of 80% to 90% without stratification, indicating little dormancy. Bratcher et al. (1993) did show an apparent improvement in germination as a result of stratification, although nonstratified seed still had a high (89%) germination rate. Wartidiningsih et al. (1994) showed a clear improvement in germination as a result of stratification in five out of six seed lots. However, seed primed in salt solutions or polyethylene glycol, without chilling, showed a similar improvement in germination rates. Samfield et al. (1991) also improved germination rates from 60% to 90% without chilling by priming seed, although distilled water was as effective as salt solutions.

These results indicated that chilling is not a prerequisite for good germination of *E. purpurea*. Where initial tests of germination percentage of *E. purpurea* indicate that some improvement in germination appears necessary, priming for 3 to 9 days

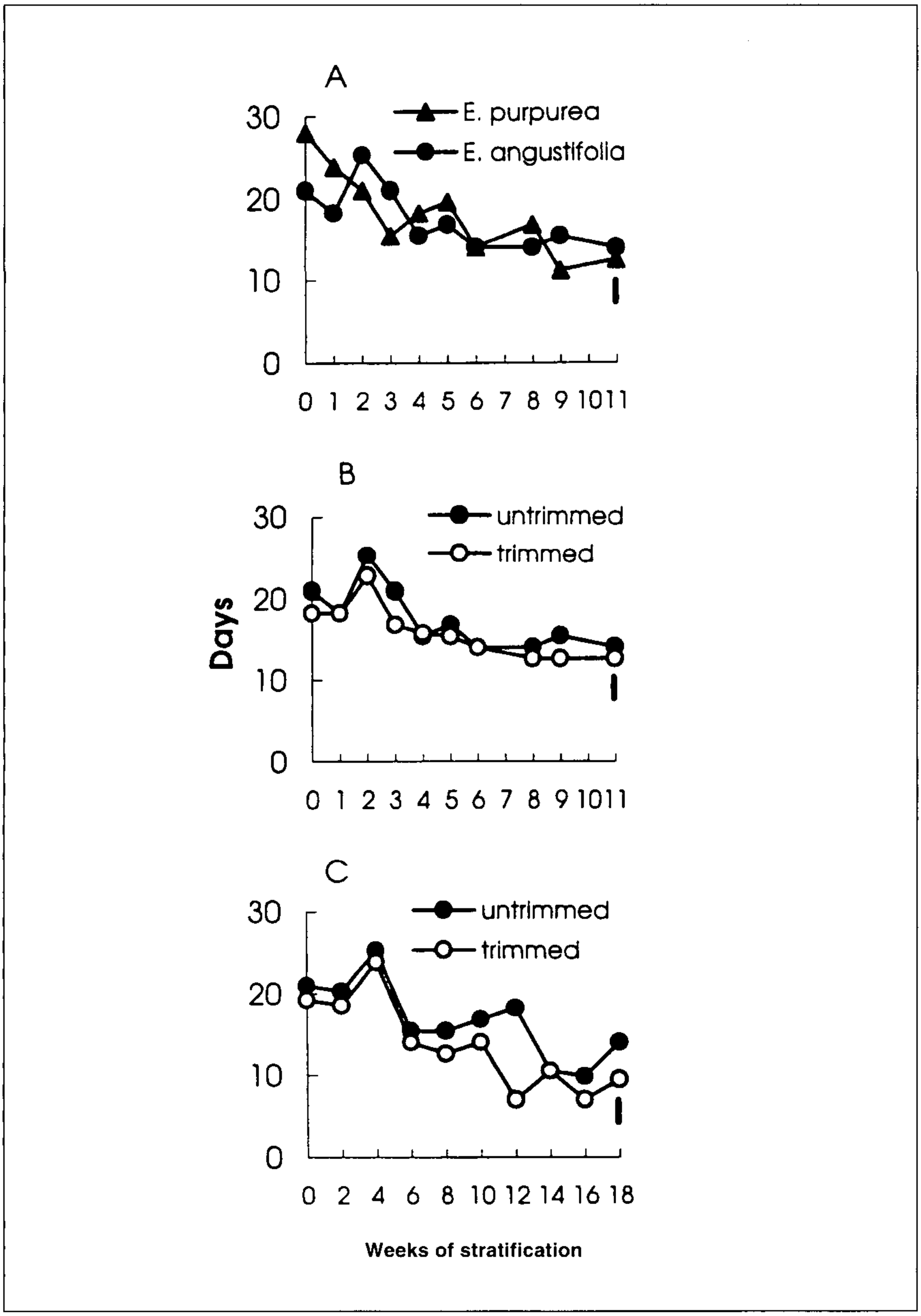

Fig. 2. Effect of stratification at 3 to 5C (Experiment 1) or 0 to 1C (Experiment 2) for periods ranging from 0 to 18 weeks on the time taken (in days after removal from the chiller) for *Echinacea* to reach maximum germination percentage. **A,** Comparison of *E. purpurea* and *E. angustifolia* in Experiment 1; **B,** Comparison of trimmed and untrimmed seed of *E. angustifolia* in Experiment 1; and **C,** Comparison of trimmed and untrimmed seed of *E. angustifolia* in Experiment 2. Vertical bars are SEDs.

in aerated distilled water (Samfield et al., 1991) is likely to be as effective as 10 to 20 days of moist chilling.

Echinacea purpurea and *E. angustifolia* had similar maximum germination percentages in Experiment 1, but *E. angustifolia* germination percentage in Experiment 2 was lower. The 3 to 4C cooler stratification temperature in Experiment 2 may have been a factor. There is some evidence that stratification of *E. purpurea* is more effective at 10C than at 5C (Wartidiningsih et al., 1994). Perhaps a more likely cause of the poorer germination in Experiment 2 is the hotter, and possibly drier conditions in the glasshouse. Even though watered twice daily, the soil surface, and the seeds pressed into it, are likely to have experienced considerable drying and heating on some days. High incubation temperatures (35C day/20C night) following stratification have been shown to reduce germination percentage in *E. angustifolia* (Baskin et al., 1992). Although air temperatures in Experiment 2 were not typically as high as 35C, soil surface temperatures are quite likely to have been so. In environments where high soil temperatures are likely, the benefits of exposing seed to light may be outweighed by the reduction in germination caused by drying, making shallow burial a better sowing method.

The reduction in percentage germination of *E. angustifolia* caused by trimming the seed coat was unexpected. Damage to the seed does not seem a likely explanation for this effect as the seed coat was trimmed carefully and any seed trimmed in the process was not used. That the reduction only occurred in seed stratified for more than 2 weeks appears to confirm this. Seeds were not treated with fungicide before stratification, so seed death as a result of fungal infection is a possible explanation, although the absence of progressive reduction in germination with increasing stratification period appears to make this unlikely. In a recent study scarification did not increase germination and the authors concluded that germination of *E. angustifolia* is probably not inhibited by physical limitations to imbibition imposed by the seed coat or by water soluble inhibitors carried in the seedcoat (Feghahati and Reese, 1994).

SUMMARY

Stratification did not improve maximum germination percentages of *E. purpurea*, but did improve the maximum germination of *E. angustifolia* when applied for more than 2 weeks. The time taken to reach maximum germination was reduced for both species as the stratification period was increased. Trimming the seed coat of *E. angustifolia* had no significant effect on nonstratified seed, but halved the maximum germination of seed stratified for more than 2 weeks.

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