GERMINATION REQUIREMENTS AND FIELD CULTIVATION EFFECTS ON THE FIELD ESTABLISHMENT AND OIL EXTRACTS OF THREE *ECHINACEA* SPECIES

by

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INTRODUCTION

Since its introduction to the medical profession in 1887 by Dr. John King, the therapeutic effects of *Echinacea* species has at times been disputed (Lloyd, 1924). In the past 20 to 30 years, however, scientists have successfully tested *Echinacea* extracts against common health problems. Root extracts have been found to provide resistance to viral infections (Wacker and Hilbig, 1978), show antibiotic activity (Stoll, et al., 1950), speed the wound healing process (Kuhn, 1953; Bonadeo, et al. ,1971) and effectively treat allergies (Reith, 1978). Wagner and Proksch (1981) observed that *E. purpurea* extracts provided immunostimulating properties to cells, and regarded them as a tool to further investigate the immune system. The revitalized interest has renewed testing of the plants' properties and has increased a demand for the plant. Missouri has already experienced a population decrease in the genus by wildcrafting of the roots (the uncontrolled harvesting of native/wild plant populations). Kansas, Nebraska, Oklahoma, Texas, and Arkansas are expected to experience a population decline in the future (Foster, 1984). This could be prevented by establishing commercial cultivation, of which there is little published information available to date. This investigator proposed to determine

some preliminary aspects for successful cultivation of three of the more commonly demanded species: *E. angustifolia, E. purpurea,* and *E. pallida.*

The main focus of this study will be to determine the effect of raised versus flat bed preparation and direct seeding versus transplanting three species of *Echinacea* in the spring versus the fall on the establishment and growth of the plant species and subsequent effects on the quality/quantity of the oil extracted for medicinal uses.

REVIEW OF LITERATURE

Echinacea species in this study are herbaceous perennials which can be found on dry prairies and barrens [*E. angustifolia* (D.C)]; rocky prairies, open wooded hillsides, and glades [*E. pallida* (Nutt.) Nutt.]; rocky open woods, thickets and prairies [*E. purpurea* (L.) Moench] (McGregor, 1968). The plants develop either a main taproot (*E. angustifolia* and *E. pallida*) or a fibrous root system (*E. purpurea*). Stems are erect, branched or simple, hirsute or glabrous. Leaves are mostly basal, oblong-lanceolate or ovate to ovate-lanceolate, petiolate below changing to reduced and sessile above. The ray flowers are spreading or drooping and range in color from pale white to purple to red, with *E. purpurea* exhibiting the most variation (Foster, 1984; McGregor, 1968). The three species usually begin flowering in May and June and remain blooming through the summer, with a persistent seed head.

The habitat of *E. angustifolia* ranges from Texas north to Saskatchewan and from Colorado east to Iowa. *E. pallida* is established in northeast Texas, eastern Oklahoma and Kansas north into Wisconsin and into eastern Indiana. *E. purpurea* can be found from Georgia to Louisiana, eastern Oklahoma north through Iowa, Illinois, Ohio, and

southwestern Michigan (Foster, 1984; McGregor, 1968). This species is commonly cultivated as an ornamental perennial in the United States. Some sources list it as *Rudbeckia purpurea*, an obsolete name (McGregor, 1968).

Echinacea species have been studied for reclamation and reestablishment of over-used land or for erosion control. Blake (1935) found natural seed stratification increased germination by 36% for *E. angustifolia* in the field. Sorensen and Holden (1974) observed a 79% increase in germination when the corky seed covering was removed from non-stratified seed. Artificial stratification of seed at 4°C for 15 weeks significantly increased germination without scarification with good germination at 19°C or 26°C (Hesse, 1973). Stratifying seed in an equal volume of fine moist silica or beach sand in a polyethylene bag at 0°C to 3°C for 2 months and then germinating (also without scarification) in the greenhouse at approximately 7°C has also been successful (Anonymous, 1972). Foster (1984) cited two reports [Elisabeth Kaul (Prairie Ridge Nursery, Mt. Horeb, Wis.) and R.L. McGregor (University of Kansas)] which both found light exposure helpful in germination. *Echinacea* seed tamped down onto the soil surface germinated in 5 days while seed covered with one eighth inch of soil germinated 2 weeks to one month later. Germination rates of direct seeded areas are usually much

lower than seed germinated in a greenhouse or cold frame (Salac, et al., 1982). It has been shown that plant establishment is enhanced by using transplants from cuttings or seedlings in the field (Salac, et al., 1982). Spacing transplants 45.7 cm apart with 91.4 cm between rows was recommended for field planting (Foster, 1984), which would allow 9800 plants per 0.4 hectares.

The roots reach a marketable size after 3 to 4 seasons. During this time, the tops can be harvested for sale to pharmaceutical companies and researchers. The tops reportedly have the same medicinal constituents, although lower in potency than the roots (Alstat, 1984). When roots are harvested, crowns can be divided to produce new plants. Plants should be grown in a well drained soil with a pH between 5.9 and 7.0 (Foster, 1984). Raised beds reportedly produce plants (*E. pallida* and *E. purpurea*) of greater quality than those grown in the field or wild (Alstat, 1984).

Increasing numbers of compounds are continually being isolated and identified from *Echinacea* plants. Glycoside extracts (echinoside) from the roots of *E. angustifolia* were found to have mild antibiotic activity (Stoll et al., 1950). Originally believed to be two glycosides and later found to be only one, echinoside inhibited the growth of *Staphylococcus aureus* and *Streptococcus pyogenes.* Later

tests of unidentified polysaccharide components of *E. purpurea* (termed Echinacin) exhibited direct inhibition of hyaluronidase activity which causes cellular inflamation and swelling (Busing, 1952; Koch and Haase, 1952; and Koch and Uebel, 1953). An increase in hyaluronidase affects the hyaluronic acid that surrounds living animal cells, a gel which is involved in the transfer of cell wastes, hormones, nutrients, and minerals. As hyaluronase activity increases, swelling occurs due to an enhanced intercellular diffusion rate which can influence the invasion of pathogens. Further tests showed that echinoside had an indirect influence on the increased production of fibroblasts (Koch and Uebel, 1953) and the activation of macrophages (white blood cells) (Kuhn, 1953). The causal agent of this effect was found to be a complex polysaccharide (termed echinacin B) isolated from *E. angustifolia* and *E. purpurea* (Bonadeo et al., 1971). The effect that echinacin B had on the promotion of the wound healing response was attributed to its indirect inhibition of hyaluronidase activity by forming a polysaccharide complex with the hyaluronic acid which surrounds the cells. The system was briefly stabilized, hyaluronic acid was temporarily increased and the growth of fibroblasts was stimulated. It was later found that pretreatment of cells with echinacin (an H_2O soluble extract from *E. purpurea* roots) inhibited viral infections

from 50 to 80%, and remained for 48 hours (Wacker and Hilbig, 1978). Wagner and Proksch (1981) isolated a high molecular weight polysaccharide from *E. purpurea* (above ground shoots) which blocks the virus reception sites on cells preventing the penetration of the virus, and therefore its' RNA transcription potential.

Pentane extracted oils have been found to act as an insecticide to adult houseflies, *Musca domestica* L. (Jacobson, 1954) and mimic juvenile insect hormone activity on yellow mealworm, *Tenebrio molitor* L. (Jacobson et al, 1975) which acts as a growth regulator. Voaden and Jacobson (1972) found that a compound isolated from the oil extract (from *E. angustifolia* and *E. pallida*) inhibited *in vivo* growth of Walker carcinosarcoma 256 and P-388 lymphocytic leukemia in mouse and rat cells. This compound was identified as (*Z*)-1,8-pentadecadiene and found to represent 44% of total oil extracted. The specific activity on the cellular level has not been described, and further testing is in progress.

Medicinal preparations of *Echinacea* are widely used in Europe in the practices of urology, gynecology, internal medicine, dermatology, holistic, homeopathic and allopathic practices. In the United States its use has been limited to the holistic and homeopathic professions (Moring, 1983).

There is still much research to be done with *Echinacea*

spp. Environmental conditions as well as genetic differences can influence the therapeutic characteristics. *E. angustifolia* from a specific region of Nebraska was preferred by Lloyd Brothers Pharmaceuticals (Foster, 1984), the developers of *Echinacea* products in the early twentieth century. *E. pallida* harvested from Wisconsin was found to have little activity (Alstat, 1984) when compared to *E. angustifolia*. Other researchers have found *E. pallida* to have positive therapeutic action (Voaden and Jacobsen, 1972) and contain compounds not found in *E. angustifolia* (Bauer, et al., 1987). Some species may be more suited for polysaccharide extracts, some for oils. Determining the best cultural techniques for growing *Echinacea spp.* is only one aspect of providing superior plants. Developing genetic lines is equally important. Through horticulture, *Echinacea* species can be improved to benefit those currently researching its therapeutic value and preserve the diminishing wild populations.

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GERMINATION OF THREE *ECHINACEA* SPECIES

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Abstract

Three native *Echinacea* species (coneflowers) were examined to determine germination requirements and favorable field culture techniques. In the greenhouse, *E. purpurea* exhibited significantly higher germination than *E. pallida* and *angustifolia*. No treatment (control), 1 month stratification in peatmoss or sand, and 24 hour H_2O soak showed significantly higher germination than 2 month stratification in peatmoss or sand as well as 24 hour KNO_3 soak for all species. In a second study, germination was enhanced by light exposure but unaffected by GA_3 treatments. Two types of bed preparation and planting season were studied in the field. Fall-sowing yielded higher emergence than spring-sowing. *E. pallida* had higher emergence than *E. purpurea* in the fall-sowings; however, the situation was reversed for spring-sowings.

1. Introduction

Coneflower species, native to the Great Plains and Midwest of the USA, have been used for reclamation of overused land or erosion control. Over the past few decades, biochemists and pharmacologists have been interested in 3 species

(E. angustifolia, pallida, and purpurea) due to positive clinical test of root extracts against common health problems (Reith, 1978; Wacker and Hilbig, 1978). E. laevigata and E. tennesseensis are on the Federal Rare and Endangered Species List. Population decline of native coneflower species in Missouri has occurred due to wildcrafting of roots for medicinal extractions (Foster, 1984). Commercial cultivation techniques need to be determined to prevent future losses of native populations.

Blake (1935) and Hesse (1973) found stratification without scarification increased germination. Others (Anon., 1972; Sorensen and Holden, 1974) have found scarification without stratification enhanced germination of coneflower seed. Light exposure has been shown to be necessary for faster germination (Foster, 1984). The objectives of this study were to determine 1)germination requirements of 3 coneflower species, and 2)the effect of direct sowing in the field on germination.

2. Material and methods

2.1 Greenhouse germination

Seed of E. pallida, purpurea, and angustifolia were given the following treatments before planting in the greenhouse: 1)placed in fine, pasteurized moist sand at 0°C for 1 or 2 months; 2)placed in pasteurized moist sphagnum peatmoss at 0°C for 1 or 2 months; 3)KNO_3 24 hour soak; 4)H_2O 24 hour soak; or 5)control. There were 3 replications with 10 seed/replication/ treatment. All seeds were scarified in

NaOHCl solution (Purex bleach diluted 2.5 times with water) 15 minutes prior to placement in 52 x 26 x 6 cm flats containing 1100 g of a 1 soil : 2 sphagnum peatmoss : 2 perlite (v/v/v) pasteurized mix. Seeds and flats were arranged in a completely randomized design under intermittent mist in a 25°C greenhouse. Flats were removed from the mist at 50% germination and maintained at the same temperature. The number of seed germinated was recorded weekly for 5 weeks.

Seedlings were used as spring transplants in the field. E. purpurea seeds produced from these plants were collected for use in the second experiment as non-stratified seed. E. angustifolia and pallida did not produce enough seed before the first frost, so they were not included.

2.2 Influence of GA and light on germination of E. purpurea

Stratified and non-stratified seed were surface sanitized in NaOHCl solution (Purex bleach diluted 1.5 times with water) for 5 minutes with constant agitation. Treatment groups were: 24 hours in 1000 or 2000 mg/liter GA_3, or sterile distilled H_2O. The control received the NaOHCl wash. Seeds were placed on sterile filter paper in glass Petri plates. Half of the plates for each treatment were placed in a lighted growth chamber, the other half were placed in an unlighted growth chamber (Precision Scientific, model 806 incubator). Both chambers were set at 25°C with 3 replications with 10 seeds/ replication/seed treatment in both growth chambers arranged in a split-plot design with

light conditions as the main plot. The experiment was terminated after 17 days at which time number of germinated seeds was recorded.

2.3 Field emergence

Seed of E. pallida and purpurea were planted in the field [Haynie very fine sandy loam (Mollic Udifluent; coarse-silty, mixed calcareous, mesic)] using no stratification for the spring planting. This method yielded the best results in the first experiment. Raised beds (20 cm high x 30 cm wide) and flat beds were prepared as treatments. Seeds were planted in April and October. Following sowing, plant emergence was recorded for 4 weeks. No supplemental irrigation was used due to adequate rainfall after sowing. Fall sown seed did not emerge until spring and data was recorded at that time. There were 16 replications with 100 seeds/replication for each bed type and sowing season arranged in a randomized strip plot design.

Treatment means in each experiment were compared using an LSD, P=0.05.

3. Results

3.1 Greenhouse germination

E. purpurea exhibited significantly higher germination than either E. pallida or angustifolia. Control, one month stratification in sand or sphagnum peatmoss, and 24 hour H_2O soak showed significantly higher germination than 2 months stratification in sand or sphagnum peatmoss, and 24 hour KNO_3 soak for all species (Fig. 1).

3.2 Influence of GA_3 and light on germination of E. purpurea

Light and stratification treatments were significant at the 5% level. Exposure to light significantly increased the germination rate across all chemical treatments. Stratification significantly increased germination rates with no significant difference among chemical treatments. Light X stratification interaction was not significant (Fig. 2). GA_3 treatment was ineffective.

3.3 Field emergence

For the spring sowing there was no significant difference between bed treatments with E. purpurea exhibiting highest emergence. Fall sown E. pallida showed significantly increased emergence over those sown in the spring. Within the fall sown treatment, E. purpurea planted in flat beds exhibited higher emergence than those in raised beds and E. pallida had increased emergence over E. purpurea within any treatment (Fig. 3).

4. Discussion

Germination of coneflower species for greenhouse transplant production is best achieved by using light (leaving the seeds uncovered by germination media). Soaking seeds in water or GA_3 solutions prior to sowing did not enhance germination if seeds were not exposed to light.

Stratification did not enhance germination in the greenhouse. In the field, fall-sown seed had increased emergence. This could be due to the stratifying effects of

overwintering, given an environment of less than optimal conditions compared to the greenhouse. Spring-sown seed may have desiccated prior to emergence even though adequate rainfall was received for germination.

Sowing seed in flat beds provided a better environment for germination than raised beds. This could be due to seasonal extremes in temperature and possible moisture stress experienced in the raised beds, because of an abnormally dry winter.

Germination in the greenhouse appears to occur at a higher rate than in the field. A study of transplant establishment and winter survival in the field will be discussed in a later report. Preliminary results indicate better establishment and survival of coneflowers by transplants rather than by direct-seeding to the field.

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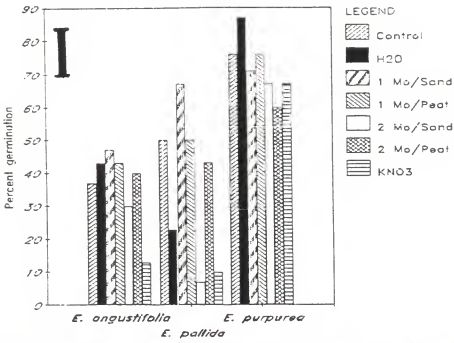

Figure 1 - Percent germination of coneflower species after exposure to various stratification treatments.

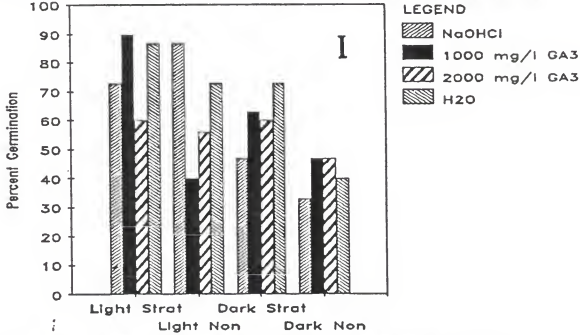

Figure 2 - Effect of GA_3 and light on germination of E. purpurea (treatments: Light Strat= light with stratification; Light + Non= light without stratification; Dark + Strat= no light with stratification; and Dark + Non= no light without stratification).

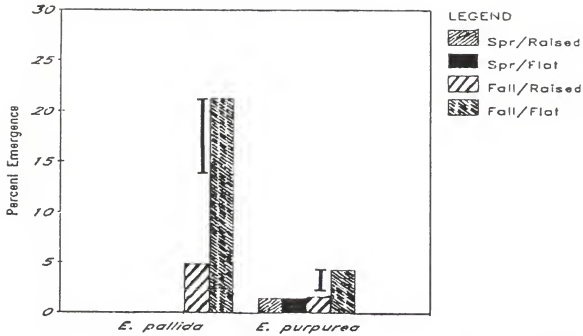

Figure 3 - Effect of sowing season and bed type on the field emergence of 2 coneflower species.

MANUSCRIPT

This manuscript is written in the style of and for publication in HortScience.

INFLUENCE OF BED PREPARATION AND PLANTING METHOD ON THE FIELD ESTABLISHMENT OF THREE *ECHINACEA* SPECIES.

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Additional index words. *Echinacea angustifolia, Echinacea pallida, Echinacea purpurea*

Abstract. Three *Echinacea* spp. were spring planted by direct seeding and transplanting in raised and flat beds. Plant height, leaf and flower number, and root and shoot dry weights were compared. Transplanting enhanced establishment for all three species with no difference between bed preparations. Direct seeded *E. angustifolia* and *E. pallida* never emerged. Raised beds enhanced the establishment of direct seeded *E. purpurea*. Transplanting in raised or flat beds and direct seeding in raised beds significantly increased root dry weight over direct seeding in flat beds of *E. purpurea*.

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Purple coneflowers are herbaceous perennials native to the Midwest and Great Plains of the U.S.A. *Echinacea* plant species have value not only as an ornamental but for therapeutic treatments as well. Root extracts have been found to have antibiotic, anti-viral, and immune stimulatory properties (4,5,6).

Plant heights range from 1 - 5 dm [*E. angustifolia* (D.C.)] to 6 - 18 dm high [*E. purpurea* (L.) Moench] (2). The plants develop either a main taproot [*E. angustifolia* and *E. pallida* (Nutt.) Nutt.] or fibrous root system (*E. purpurea*). Field establishment is enhanced by using transplants from cuttings or seedlings (3). It has been reported that field transplants should be set 46 cm apart in a well-drained soil with a pH of 5.9 - 7.0 (1). Raised beds reportedly produce plants of greater quality than those grown in the field or wild (Ed Alstat, personal communication, 1984, Eclectic Institute, Portland, OR).

This study was designed to evaluate the effect of direct seeding versus transplanting in raised and flat field beds on plant height, root and shoot dry weights, and leaf and flower number of *E. angustifolia*, *E. pallida* and *E. purpurea* (herein referred to as ANG, PAL, and PUR respectively).

The study was conducted at the Kansas State University

Horticulture Farm at Manhattan, in a Haynie, very fine, sandy loam (Mollic Udifluvent, coarse, silty, mixed calcareous, mesic). Seed was obtained from Harris Moran Seed Company, Rochester, N.Y. (PUR), Soil Conservation Service Plant Materials Center, Manhattan, KS. (PAL), and Ed Alstat Eclectic Institute, Portland, OR. (ANG, gathered in South Dakota). A randomized strip plot experimental design with planting method as main plots and plant species as subplots was used. Each treatment combination had 16 single plant replications. Raised bed sections were prepared 20 cm high by 30 cm wide with a lister planter.

Transplants were produced from seedlings planted at the 1 to 2 fully expanded true leaf stage to 165 cm^3 Supercell tubes (J.M. McConkey Co., Sumner, Wash.) each containing 150 cm^3 of 1 soil: 1 spaghnum peatmoss: 1 perlite (v/v/v) steam pasteurized mix. Plants were placed in a 24°C maximum day/18°C minimum night temperature greenhouse for 3 months. Direct seeding was performed in April 1985. Final spacing was obtained by thinning after emergence. In May 1985, plants were transplanted to the field. Plant spacing was set at 45.7 cm and row spacing was 91 cm.

Overhead irrigation was used the 4 weeks following transplanting only to supplement rainfall to provide 2.50 ± 0.5 cm of irrigation per week. Irrigation of direct seeded areas was not necessary prior to this due to

adequate rainfall. Observations on emergence were taken weekly for 4 weeks. Leaf number, plant height, and flower number were taken in late August, 1985. Samples were dug in the spring of 1986 and root and shoot dry weights were recorded.

No plants of ANG or PAL emerged from direct seeded areas, even after a second planting. This supports the results by Salac, et al. (3). Direct seeded plants of PUR in raised beds were greater in height, leaf number, root and shoot dry weights than those in flat beds (Table 1). Transplants of all species showed no significant differences between bed treatments with any plant growth parameters measured. However, PUR transplants were significantly greater in all areas but shoot dry weights when compared to direct seeded plants (Table 2). There was also a bed by planting interaction with PUR root dry weights. Transplants in raised and flat beds, and plants direct seeded in raised beds, had significantly higher root dry weights than plants direct seeded in flat beds (Table 3). Planting in raised beds seems to overcome the disadvantage of direct seeding with respect to root dry weight. It most likely aids the fibrous root development and transplants may get a head start. This may be important to those growing it for root extracts, but it may not make any ornamental differerence.

The results from this study indicate that ANG and PAL establishment is enhanced by transplanting in raised or flat beds. PUR transplants become better established than direct seeded plants in the first year. If transplants are not available, direct seeding in raised beds enhances plant establishment.

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Table 1. Bed effect on direct seeded *E. purpurea*.

Bed	Height (cm)	Flower number	Leaf number	Root dry weight(gm)	Shoot dry weight(gm)
Raised	15.9	0.1	35.8	11.3	101.1
Flat	11.2	0.0	19.2	5.6	55.3
Significance level	*	NS	*	**	**

NS,*,** Nonsignificant (NS) or significant at the 5% or 1% level, respectively, using a 2-sample t-test.

Table 2. Planting method effect on *E. purpurea* field establishment.

Planting method[Y]	Height (cm)	Flower number	Leaf number	Root dry weight(gm)	Shoot dry weight(gm)
Transplanted	48.1	10.5	152.6	12.6	74.6
Direct seeded	13.6	0.04	31.5	8.5	78.2
Significance level	***	***	***	*	NS

[Y] Raised and flat beds combined.
NS,*,*** Nonsignificant (NS) or significant at the 5% or 0.1% level, respectively, using a 2-sample t-test.

Table 3. Bed by planting interaction effect on root dry weight of *E. purpurea*.

Bed/planting	Root dry weight (gm)
Flat/transplanted	12.8 a[z]
Raised/transplanted	12.4 a
Raised/direct seeded	11.3 a
Flat/direct seeded	5.6 b

[z] Means followed by the same letter are not significantly different (P=0.05) as determined by L.S.D.

MANUSCRIPT

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FIELD CULTIVATION EFFECTS ON THE EXTRACTED OILS OF THREE *ECHINACEA* SPECIES

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ABSTRACT

Smith-Jochum, C.C., and Davis, L.C.____. Field cultivation effects on the extracted oils of three *Echinacea* species. Scientia Hortic.,_____.

Three *Echinacea* species, often used for their therapeutic properties, were direct seeded or transplanted in raised and flat beds in the spring and fall of 1985. In a preliminary comparison of extracted oils from oven and freeze-dried samples, no differences were found between the two methods in regard to extracted oil weight and total gas-liquid chromatographic peak areas. Spring seeded *E. pallida* and *E. angustifolia* never emerged. Plants were harvested one year after planting, oven dried, and the oils extracted. *E. pallida* yielded the highest amount of oil and mean peak area over *E. purpurea* and *E. angustifolia*. Bed type yielded no significant difference for all transplants or fall direct seeded *E. pallida*. The greatest mean peak area was obtained from fall transplanted/harvested *E. pallida*. No correlation existed between whole root dry weight and weight of extracted oil or GLC total peak area. The amount of extracted oil was not related to GLC total peak area. Fall transplanting

/harvesting in raised beds appears to yield the greatest quality/quantity of extracted oils.

Keywords: *Echinacea angustifolia*; *E. pallida*; *E. purpurea*; transplant/direct seeded; oven/freeze-dried.

INTRODUCTION

Echinacea spp. are perennial forbs native to the eastern, midwestern, and plains areas of the United States. Medicinal uses had beginnings with plains Indian tribes (Gilmore, 1977). The plants soon became widely used by the medical profession, which moved its use into the twentieth century (Foster, 1984). Modern research has commonly used *E. angustifolia*, *E. pallida*, and *E. purpurea* extracts to test a variety of therapeutic properties. Root extracts have been found to provide resistance to viral infections (Wacker and Hilbig, 1978). They show antibiotic activity (Stoll et al., 1950), speed the wound healing process (Kuhn, 1953; Bonadeo, et al., 1971), and effectively treat allergies (Reith, 1978). In addition, the extracts have been found to stimulate the immune system (Wagner and Proksch, 1981) and inhibit the growth of some cancerous tumors (Voaden and Jacobson, 1972).

The increasing amounts of plant material used for research alone is expected to cause a population decline in the genus by wildcrafting (uncontrolled harvesting of wild

plant populations). Often the plant material received is not even *Echinacea* but some adulterant (Bauer, et al., 1987), which can distort results. Commercial cultivation of *Echinacea* species by reputable growers is necessary for research on the species' medicinal properties and to preserve wild populations that can serve as a future source of improved genetic material. Climate and soil conditions as well as the stage of plant development at harvest can influence the concentration of plant oils (Foster, 1984). Some large differences in the chemical content among species exist (Bauer, et al., 1987). This study examined the effect that direct seeding and transplanting in the spring (spring harvested) and fall (fall harvested) in raised and flat beds had on the quantity and quality of extracted oils.

MATERIALS AND METHODS

Seeds of *Echinacea angustifolia* (D.C.) (prairie purple coneflower), *E. pallida* (Nutt.) Nutt. (pale purple coneflower), and *E. purpurea* (L.) Moench (purple coneflower), herein referred to as ANG, PAL, and PUR respectively, were field sown in April and September 1985. No supplemental irrigation was applied after the plants were established. Three month old transplants were field planted in May and October 1985. Field design was a randomized

strip plot.

In a preliminary study prior to the spring harvest, oil extracts from oven dried and freeze dried plant samples were compared. Roots and vegetative shoots of 3 of each field grown PAL and PUR plants were separated from the crown, which was discarded. Each plant was a single replication. With each replication, roots and shoots were separated, chopped, and each divided into 2 subsamples, on a fresh weight basis. These subsamples were either oven dried or freeze-dried.

Freeze-dried samples were obtained by immersing fresh tissue in liquid N_2 for 3 to 5 minutes, and drying overnight in 250 ml Erlenmeyer flasks attached to the freeze dryer (Virtis automatic freeze dryer Model No. 10-010) with a duo-seal vacuum pump (Welch Model No. 1405). Oven dried samples were obtained by overnight drying in a 70°C oven (GCA/Precision Scientific Thelco oven, Model 28) with circulating air.

All dried subsamples were weighed and then ground in a double bladed, one-quarter hp Wiley Mill through a 20 mesh screen. After grinding and stirring, one gram subsamples were removed and placed in 30 ml stoppered glass centrifuge tubes to which 10 ml of hexane was added. Each tube was vigorously shaken for 10 seconds, the sides washed down and allowed to stand for approximately 23 hours. Samples were

again shaken, then centrifuged for 20 minutes at 2000 r.p.m. at 10°C (Lourdes 30-R Clini-Fuge). Two mls of supernatant were siphoned off, placed in preweighed conical glass test tubes, and the hexane was evaporated by blowing a stream of dry N_2 gas into them. The tubes were reweighed and the difference taken as the amount of hexane extracted material (oil).

Samples were analyzed on a GLC [gas-liquid chromatograph (Hewlett-Packard 5880A with 2% diethyleneglycol succinate (DEGS) on a 100 - 120 mesh Chromosorb W packed column)]. Immediately prior to the injection of each subsample, it was resuspended in 100 ul of fresh hexane, from which one ul was used for each GLC injection. The oven temperature was 180°C, the flame ionization detector was set at 230°C, and the injection port temperature was 220°C. The carrier gas was nitrogen at a 10-15 ml/min. flow rate. Peak retention times, peak area (each peak area unit=1 picocoulomb), and total peak area were recorded. Peak area was taken as a measure of the volatile hydrocarbon production (Voaden and Jacobson, 1972).

In May and October of 1986, field grown ANG, PAL, and PUR plants were harvested. Sixteen single plant replications per treatment combination per species were planned; however, due to mortality, samples ranged from 0 (spring direct sown) to 16 single plant replications. Plant

preparation for extraction was the same as previously described for oven dried samples, with each plant as a replication. Dried sample preparation for analysis by the GLC was the same as previously described with the following modifications: The amount of hexane added to the dried material in the centrifuge tubes was increased to 20 ml per gram dried ground plant material. The amount of supernatant removed after centrifugation was increased to 12 ml. This was done to increase the amount of the oil to enhance reliability in weighing the oil fraction and to improve peak definition during GLC analysis; 1 ul represented 1.2 mg of the dried sample. Hexane evaporation was performed on a multiple-sample water aspiration apparatus with the test tubes suspended in a 40°C water bath while drawing a stream of air over the surface of the hexane vigorously. The remaining oil was weighed and then resuspended with 500 ul of fresh hexane immediately prior to the 1 ul injection into the GLC under the same conditions previously stated. Peak retention time, peak area, and total peak area per sample were recorded by the attached printer.

RESULTS AND DISCUSSION

Freeze-dried/oven dried comparison

No significant differences were found between freeze

and oven dried samples in regard to extracted oil weight and total GLC peak area for either species (Table 1). PAL roots yielded a significantly greater amount of oil than PAL shoots, but no difference was found between PAL plant parts in regard to total GLC peak area. No differences were found between PUR roots and shoots. For multiple small sample extractions, the oven dried method is faster than freeze-drying. Depending on the type of apparatus available, freeze-drying may be suitable for large batch samples. After extracting the oils, analysis should be completed as soon as possible. One week old extract (stored at 2-4°C in stoppered test tube) showed less peak definition and lower total peak area than the same samples analyzed as soon as possible after oil extraction (Table 2). Time from harvest to oil extraction and analysis may be a limiting factor in determining quantity and/or quality. Jacobson (1967) reported such instability in *Echinacea* oil.

Field culture comparisons

Spring field sown ANG and PAL never germinated, even after a second planting. Thus this portion of the original experimental design was not completed. The amounts of extracted oil and GLC peak areas from the vegetative shoots of all three species were minute to nonexistent. Extracted

oil from spring harvested ANG and PUR roots yielded half that of PAL; however, after GLC analysis, peak areas were less than 1% (PUR) or near 0% (ANG) compared to the mean peak area registered by PAL roots. Preliminary analysis of fall harvested samples gave the same results, so only PAL root results were used in the statistical analysis (general linear models procedure of analysis of variance, P=0.05, SAS Institute, Inc., 1982).

For transplants, only season was significant (Table 3). Bed type was not significant for spring and fall transplants or fall direct seeded. The greatest peak area was obtained from fall transplanted and harvested PAL roots, with no significant difference between the amount of oil extracted from one gram samples (Table 4). Significant interaction between planting and bed occured for fall with transplants in raised beds providing a higher total peak area than the other treatment combinations (Table 5). Genetic variability may be a factor in this effect, however if improved genetic lines can be developed, there is an indication of greater quality from fall transplants in raised beds harvested in the fall.

No correlation existed between whole root dry weight and weight of extracted oil or GLC total peak area. The amount of extracted oil was not related to GLC total peak area. Improved genetic lines may influence this; however,

other factors may be involved as well. High amounts of rainfall or irrigation may increase root size and weight, but could diminish the oil it produces. *Echinacea* spp. are naturally very drought tolerant (Foster, 1984) , and oil production may be enhanced in dry conditions.

Jacobson (1967) obtained 0.42% oil by pentane extraction of ANG. Several hydrocarbons constituents were later found including a pentadecadiene (Voaden and Jacobson, 1972). The extracts from this study were compared by GLC analysis by using as standards the following available hydrocarbons ; pentadecane, 1-pentadecene, heptadecane, 1-heptadecene, 1-nonadecene, 6-nonadcene (Aldrich Chemical CO., Milwaukee, WI.), 1-pentadecyne, 1-hexadecyne, 1-tetradecyne, 1-heptadecyne, 1-octadecyne, 1,13-tetradecadiene, 7-tetradecene, 1-tetradecene (Alfa Laval, Ft. Lee, NJ.) None of the peaks from the *Echianeca* oil extracted in this study appear to correspond precisely to any of these compounds. On the basis of relative retention time, the diene identified by Voaden and Jacobson (1967) would elute just after tetradecadiene. They observed only trace amounts of this constituent in PAL.

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Table 1. Comparison of freeze and oven dried *Echinacea* samples.

Species	Drying method	Extracted oil (mg/g dw)	Total GLC peak areas
E. pallida			
	Freeze	19.3[NS]	41922[NS]
	Oven	16.7	131079
E. purpurea			
	Freeze	13.9[NS]	4202[NS]
	Oven	13.3	3461

[NS] Non-significant t-test at the 5% level.

Table 2. Effect of one week storage of extracted *Echinacea* oil.

Age	Total GLC peak area
E. pallida	
fresh	41919*
1 week old	6211
E. purpurea	
fresh	4202*
1 week old	979

* Significant difference at 5% level using 2-sample t-test.

Table 3. Analysis of variance for total GLC peak area of *E. pallida* root oil extracts[z].

Source	Degrees of freedom	Sum of Squares	F value	P > F
Transplants				
Season	1	5.78×10^{11}	50.75	0.0004
error[y]	6	6.83×10^{10}	0.29	0.9375
Bed	1	1.97×10^{11}	4.76	0.0719
error[x]	6	2.48×10^{11}	1.06	0.4013
Season X Bed	1	5.38×10^{10}	1.38	0.2464
error[w]	44	1.72×10^{12}		
Corrected total	59	2.89×10^{12}		
Fall direct seeded				
Bed	1	3.38×10^{10}	3.54	0.1088
error[x]	6	5.72×10^{10}	0.57	0.7493
Corrected total	25	3.65×10^{11}		

[z] Model : Area = Season Col(Season) Bed Row(Bed) Season X Bed
[y] Col(Season) as error term
[x] Row(Bed) as error term
[w] Model error

Table 4. Total GLC peak areas of extracted oil from *E. pallida* transplanted roots.

Treatment	Extracted oil (mg/g dw)	Total GLC peak area
Season[z]		
Fall	18.9[NS]	306488*
Spring	17.9	107131
Bed[y]		
Raised	16.5[NS]	148686[NS]
Flat	20.3	264933
Season with bed		
Fall Flat	16.9[NS]	217954[NS]
Fall Raised	20.9	395023
Spring Flat	16.1	79418
Spring Raised	19.7	134843

[z] Raised and flat combined.
[y] Spring and fall combined.
[NS,*] Nonsignificant (NS) or significantly different at the 5 % level (*) using L.S.D.

TABLE 5. Planting by bed interaction effect on total GLC peak area of extracted oil from fall harvested *E. pallida* roots.

Planting/bed	Total GLC peak area
Transplanted/raised	395023 a[z]
Direct seeded/flat	240115 b
Transplanted/flat	217954 b
Direct seeded/raised	157284 b

[z] Means followed by the same letter are not significantly different (P=0.05) as determined by L.S.D.

APPENDICES

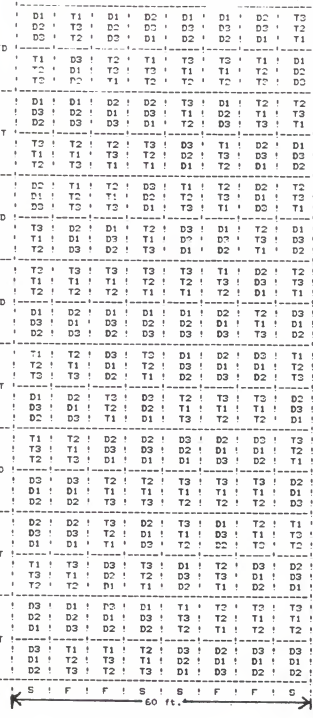


Figure 1A. Field plot plan. S=spring, F=fall, T=transplant, D=direct seeded, 1= Echinacea angustifolia, 2= E. pallida, 3= E. purpurea.

[HP] 5880A MANUAL INJECTION @ 19:35 JUL 2, 1986
AREA %

RT	AREA	TYPE	AREA %
1.50	140.15	VB	100.000

TOTAL AREA = 140.15

[HP] 5880A MANUAL INJECTION @ 18:18 JUL 2, 1986
AREA %

RT	AREA	TYPE	AREA %

TOTAL AREA = 0.00

Figure 2A. Typical GLC peak area diagrams of extracted oil from *Echinacea angustifolia* shoots (S) and roots (R) of spring transplants. Solvent peak(s) appear before one (1) minute.

S

[HP] 5890A MANUAL INJECTION @ 15:07 JUL 3, 1986
AREA %

RT	AREA	TYPE	AREA %
1.34	108.98	VV	7.118
1.47	1422.19	VB	92.882

TOTAL AREA = 1531.17

R

[HP] 5890A MANUAL INJECTION @ 22:08 JUL 3, 1986
AREA %

RT	AREA	TYPE	AREA %

TOTAL AREA = 0.00

Figure 3A.1. Typical GLC peak area diagrams of extracted oil from *Echinacea purpurea* shoots (S) and roots (R) of field spring sown plants. Solvent peak(s) appear before one (1) minute.

S

[HP] 5880A MANUAL INJECTION @ 16:04 JUL 2, 1986
AREA %

RT	AREA	TYPE	AREA %
1.47	1897.44	VB	100.000

TOTAL AREA = 1897.44

R

[HP] 5880A MANUAL INJECTION @ 21:51 JUL 2, 1986
AREA %

RT	AREA	TYPE	AREA %
3.02	985.67	BB	100.000

TOTAL AREA = 985.67

Figure 3A.2. Typical GLC peak area diagrams of extracted oil from *Echinacea purpurea* shoots (S) and roots (R) of spring transplants. Solvent peak(s) appear before one (1) minute.

S

[hp] 5880A MANUAL INJECTION @ 15:40 JUL 10, 1986
AREA %

RT	AREA	TYPE	AREA %
1.72	1302.73	VB	100.000

TOTAL AREA = 1302.73

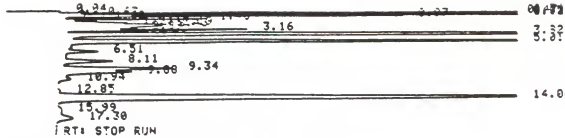
R

[hp] 5880A MANUAL INJECTION @ 15:47 JUL 10, 1986
AREA %

RT	AREA	TYPE	AREA %
1.08	20865.90	VV	16.940
1.20	826.15	VB	0.671
1.46	298.63	BB	0.242
1.66	928.14	BP	0.754
2.05	1235.27	VV	1.003
2.27	913.27	VV	0.741
2.51	1016.02	VV	0.825
2.97	1142.28	VV	0.927
3.28	3689.50	VV	2.995
3.85	73883.20	VV	59.983
4.92	15773.10	VV	12.806
6.50	1554.56	VV	1.262
8.07	1046.98	PH	0.850

TOTAL AREA = 123173.00

Figure 4A.1. Typical GLC peak area diagrams of extracted oil from *Echinacea pallida* shoots (S) and roots (R) of spring transplants. Solvent peak(s) appear before one (1) minute.

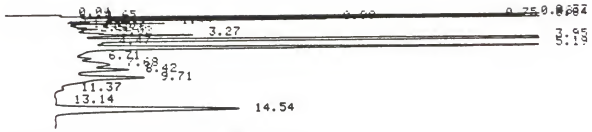


[HP] 5880A MANUAL INJECTION @ 13:03 NOV 12, 1986
AREA %

RT	AREA	TYPE	AREA %
1.18	667.00	BP	0.181
1.40	3559.61	PV	0.966
1.93	3535.71	VV	0.960
2.19	5996.45	VV	1.628
2.43	10150.70	VV	2.755
2.89	3353.65	VV	0.910
3.16	9124.25	VV	2.477
3.32	146511.30	VV	39.768
5.01	69370.80	VV	18.829
6.51	2884.06	VB	0.783
8.11	4901.13	VV	1.330
9.34	12457.20	VV	3.381
9.88	8253.95	VV	2.240
10.94	1673.50	VP	0.454
12.85	388.98	PV	0.106
14.00	80736.80	VV	21.915
15.99	1277.92	VV	0.347
17.30	3574.17	VP	0.970

TOTAL AREA = 368417.00

Figure 4A.2. Typical GLC peak area diagram of extracted oil from *Echinacea pallida* roots of fall transplants. Solvent peak(s) appear before one (1) minute.



[HP] 5880A MANUAL INJECTION @ 12:40 NOV 3, 1986
AREA %

RT	AREA	TYPE	AREA %
0.04	63.96	BV	0.001
0.37	8409290.00	SBB	97.068
0.48	1319.65	BB	0.015
0.65	37.73	BV	0.000
0.75	4859.20	VV	0.056
0.84	23838.30	VV	0.275
0.99	4270.12	VB	0.049
1.21	411.64	BP	0.005
1.44	2303.51	PV	0.027
1.82	290.57	VV	0.003
1.98	747.81	VV	0.009
2.26	1332.48	VV	0.015
2.52	1014.30	VV	0.012
3.02	2337.79	VV	0.027
3.27	6971.59	VV	0.080
3.95	90481.50	VV	1.044
4.47	2607.38	VV	0.030
5.19	43192.00	VV	0.499
6.71	3765.02	VV	0.043
7.68	9621.88	VV	0.111
8.42	8861.16	VV	0.102
9.71	16164.00	VV	0.187
11.37	1098.11	VP	0.013
13.14	478.53	PH	0.006
14.54	27973.20	HH	0.323

TOTAL AREA = 8663260.00

Figure 4A.3. Typical GLC peak area diagram of extracted oil from *Echinacea pallida* fall field sown plants. Solvent peak(s) appear before one (1) minute.

GERMINATION REQUIREMENTS AND FIELD CULTIVATION EFFECTS ON THE FIELD ESTABLISHMENT AND OIL EXTRACTS OF THREE *ECHINACEA* SPECIES

by

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B.S. University of Nebraska, Lincoln, 1981

AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

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1987

Three native Echinacea species (coneflowers) were examined to determine germination requirements and the effect that spring and fall transplanting and direct seeding in raised and flat beds had on field establishment and quality/quantity of extracted oil. In the greenhouse E. purpurea exhibited significantly higher germination than E. pallida and E. angustifolia. No treatment (control), 1 month stratification in peatmoss or sand, and 24 hour water soak showed significantly higher germination than 2 month stratification in peatmoss or sand as well as 24 hour potassium nitrate soak (a common pregermination treatement) for all species. In a second study, GA® treatments at the rates used were ineffective and germination was enhanced by light exposure or stratification. In the field, E. purpurea exhibited the highest emergence with no significance between bed treatments. Fall sown E. pallida had greater emergence than E. purpurea, both of which had higher emergence from flat beds. Overall, fall sowing gave the highest emergence for both species. Transplanting enhanced establishment for all three species in regard to height, root dry weight, and leaf and flower number, with no difference between bed treatments. Raised beds enhanced establishment of direct seeded E. purpurea. E. pallida yielded the highest amount of extracted oil and mean gas-liquid chromatographic peak areas over E. purpurea and

E. angustifolia. There was no significant differences between plants in raised and flat beds within plantings and no correlation existed between whole root dry weight and weight of extracted oil or GLC peak area. The amount of extracted oil was not related to GLC peak area. Fall seeding provides greater emergence in the field; however, transplants establish themselves better than plants from direct seeding. Fall transplanting and harvesting appears to provide the greatest quality/quantity of extracted oil.