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Growth Response of Purple Coneflower (*Echinacea purpurea*) to Five Single Species Arbuscular Mycorrhizal Treatments

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Abstract

This study aimed to investigate the growth-promoting effects of five different arbuscular mycorrhizal fungi (AMF) single species treatments on *Echinacea purpurea*. Forty-two echinacea seedlings were inoculated with one each of five different AMF species and grown outside for 12 weeks. Photosynthetic rates were measured at the eight-week period. Samples were harvested and divided into above- and belowground biomass after 12-weeks. Total leaves were counted, leaf area was calculated, above- and belowground biomass was weighted, and root to shoot ratios were determined. One-way analyses of variance (ANOVA) were conducted on all data collected. Three of the five AMF species were shown to have significant (P= .0025, ANOVA) growth-promoting effects on the belowground biomass only. The responses of echinacea to AMF single species treatments suggests that there may be potential horticultural applicability.

1. Introduction

Echinacea purpurea is a popular perennial herb of the Asteraceae family, commonly found in prairies of central and southeastern United States[1]. There are nine species in the *Echinacea* genus that are becoming increasingly valuable due to a wide range of bioactive metabolites produced by the plants, including phenolics, alkylamides, and polysaccharide / glycoproteins[2]. *Echinacea* extracts have been shown in experimentally tested pharmaceutical studies to exhibit antiviral and anticancer activity, as well as immunomodulatory properties. On the continents of Africa, North and South America, and Asia, traditional medicine continues to perform an important function. Products containing *Echinacea* were reported to have a global market value of over one billion dollars[3].

Arbuscular mycorrhizal fungi (AMF) form symbiotic relations with the roots of approximately 80% of known plant species, serving to extend the area of nutrient uptake, particularly for phosphorous[6] (van der Heijden et at. 1998). AMF are an important part of the carbon cycle since they exchange nutrients for photosynthates, and their presence and diversity can affect plant productivity[4]. AMF have been shown to increase the survivability and lateral root growth of *Echinacea pallida* during micropropagation by stimulating auxin synthesis[5]. In a study by Gualandi Jr. et al. (2014), phytochemical content was increased up to 30-fold in *Echinacea purpurea* by the inoculation with two species of AMF, *Rhizophagus intraradices* and *Gigaspora margarita*[7].

Because many people depend on traditional herbal medicine there is a growing demand for products containing medicinally active compounds such as those found in *E. purpurea*. This study examined the relationships between AMF single species inoculation and *E. purpurea* performance and productivity. Plant performance was examined by measuring photosynthetic rates, stomatal conductance, and transpirations rates across five single species treatments and a control. Plant productivity was also measured by analyzing above- and belowground biomass, root to shoot ratios, leaf counts, and total leaf areas. Plant performance and productivity were hypothesized to increase with AMF single species inoculations.

2. Methodology

2.1 Sterilization

Approximately 3000 mL of Sungro Horticulture Professional Growing Mix soil were placed inside 35, 731.5-cm^2 aluminum pans. Wood chips larger than 0.25 cm were removed and soil clods larger than 0.5 cm were pulverized. A graduated cylinder was used to add 150 mL of deionized (DI) H_2O to each pan and mixtures were stirred to an even moisture consistency. Soil was evenly spread into a 5.08 cm layer in each pan. All pans were covered and sealed with aluminum foil and placed in a forced convection oven at 100ºC for one hour. Pans were removed and allowed to cool for 24 hours.

All planting materials were sterilized by soaking in 7.4% sodium hypochlorite (Clorox bleach) for five minutes. Materials were then thoroughly rinsed with water and allowed to completely dry on sterile plastic. Sterile lab technique was followed throughout the entire planting procedure.

2.2 Planting

Echinacea purpurea seeds were purchased from Sow True Seed (Asheville, N.C.) and cold stratified for eight weeks inside a refrigerator at 4ºC. Sterilized soil was placed inside 2, 72-cell starter trays and lightly pressed to fill each cubicle to the top of the tray. A sterilized 0.25 cm diameter metal rod was used to create 1 cm deep seed holes in each tray cubicle. One *Echinacea purpurea* seed was placed inside each hole and soil was lightly pressed to cover each seed. A spray bottle was used to dampen the tops of each tray evenly. Both trays were initially bottom watered with 750 mL of DI H_2O. Seed trays were placed inside a sealed growth chamber under 8 hours of florescent light for four weeks.

After four weeks 42 healthy seedlings were transferred to 3.8-L pots filled with premoistened inoculated soil. Before transplanting each pot was filled with 2500 mL of moist sterile soil and a 5.08-cm x 5.08-cm cavity was created and lined with one of five different mycorrhizal inoculates. Each cavity received 50 g of mycorrhizal / sand inoculate from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM). A control treatment containing no inoculate was created. Each treatment was replicated seven times. After 24 hours samples were moved outside into full sun and placed on an elevated bench. Samples received filtered water until saturated every two days as needed for 12 weeks. After eight weeks photosynthetic rate was measured for each sample on an intact leaf using a LI-6400 (LI-COR Biosciences Lincoln, NE).

Once the 12-week growth period was completed pots were transferred to a refrigerator to stop growth while they were processed. Plants were removed from their pots and root masses were cleaned in a sink over a sieve with a spray hose. Once root masses were cleaned plant samples were separated into above- and belowground biomass at the root / shoot junction. Total leaf numbers were recorded. Total leaf area for each sample was also recorded using an LI-3100C area meter (LI-COR Biosciences Lincoln, NE). Above- and belowground biomasses were placed in separate paper bags and labeled. All samples were then placed inside a drying oven for 48 hours at 65ºC. Samples were removed and above- and belowground biomass was weighted.

2.3 Analysis

Statistical analyses were performed using SAS analytical software (SAS Institute Cary, NC). Aboveground and belowground biomass dry weights were used to calculate the root to shoot ratio. One-way analysis of variance (ANOVA) tests were performed on root to shoot ratios, total leaf count, photosynthetic rates, stomatal conductance, transpiration rates, and above- and below ground biomasses separately. Tukey's Studentized Range (HST) tests were also conducted on root to shoot ratios, photosynthetic rates, stomatal conductance, transpiration rates, and above- and belowground biomasses separately.

3. Results

Results from the initial measurements of photosynthetic rates, stomatal conductance, and transpiration rates showed that single species mycorrhizal treatments had no significant ($P = 0.73, 0.81, 0.96$) effect on *Echinacea purpurea* (see

Table 1.). Results of leaf counts, root to shoot ratios, and aboveground biomasses showed that single species mycorrhizal treatments also had no significant (P= 0.52, 0.81, 0.40) effect on *Echinacea purpurea* growth (see Table 2.) Belowground biomass was indicated to be significantly affected by three of five single species mycorrhizal treatments (see Figure 1.) Treatments SD, GR, and DH resulted in significant (ANOVA, Df = 5, F = 4.57, P = 0.0025) increases in belowground biomass growth compared to the control. Treatments X and RI (Group AB) were shown to not be significantly different from Group A (SD, GR, DH) or group B (control) (see Figure 1.). The mean belowground biomass for Group A was 7.16 g. The mean belowground biomass for Group B was 4.95 g. Group AB had a belowground biomass mean of 6.14 g. The belowground biomass sample mean for all groups was 6.49 g.

Table 1. Mean photosynthetic rates, stomatal conductance, and transpiration rates for *Echineacea purpura* with five different mycorrhizal species.

Species	Photosynthetic Rate (µmol)	Stomatal Conductance (mmol)	Transpiration Rate (mol)
X	11.37	0.33	3.8
RI	14.62	0.38	4.16
SD	14.01	0.25	3.14
GR	13.38	0.27	3.31
DH	13.31	0.28	3.52
C	13.35	0.29	3.58

Table 2. Mean leaf number, root / shoot ratio, and above- and belowground biomass of *Echinacea purpurea* with five different mycorrhizal fungi species.

Species	Total Leaf No.	Root / Shoot Ratio (g)	Aboveground Biomass (g)	Belowground Biomass (g)
X	16.86	1.81	3.58	6.12
RI	22.57	1.6	3.9	6.16
SD	23.57	1.65	4.39	6.99
GR	22	1.8	4.02	6.96
DH	20.71	1.82	4.22	7.53
C	24.86	1.61	3.54	4.95

Table 3. Five mycorrhizal species

Species	Origin
(RI) Rhisophagus intraradices	WV
(X) Claroideoglomus etunicatum	WV
(GR) Gigaspora rosea	UT
(DH) Dentiscutata heterogama	FL
(SD) Septoglomus deserticola	SP (Spain)

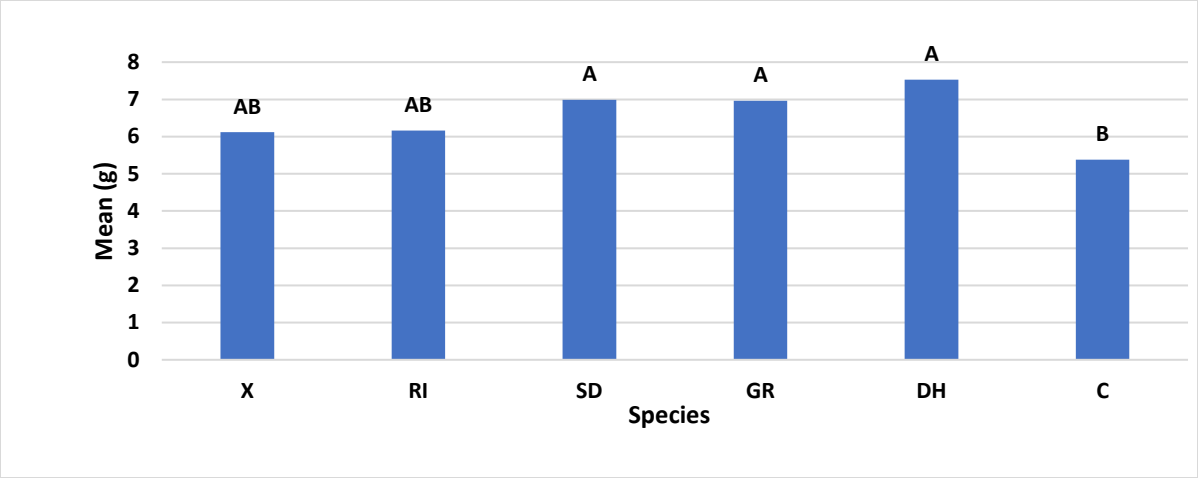

Figure 1. Mean belowground biomass growth response of Purple Coneflower (*Echinacea purpurea)* to five different mycorrhizal fungi species treatments over a 12-week period. Means with the same letter (A,B) are not significantly different.

4. Discussion

Observations showed that results for belowground biomass growth response of *Echinacea purpurea* to single species mycorrhizal fungi treatments were significant only for three species. Results support the hypothesis that symbiotic associations between plants and fungi would increase *Echinacea purpurea* growth. Similar reports from Kapoor et al. (2017) indicate that mycorrhizal symbioses free nutrients within plants that would otherwise be allocated to root exudates, for nutrient cycling, which can contribute to increased biomass growth in host plants[8]. Increased belowground biomass growth in *Echinacea purpurea* examined in this study might have been from an increased availability of nutrients within sample plants due to less exudates being lost to soil microbe exchange for nutrient cycling.

Physiological responses were not found to have significant changes due to mycorrhizal symbioses. Photosynthetic rates, stomatal conductance, and transpiration rates did not increase with inoculation treatments. This is counter to findings from Zhu et al. (2011) who reported that colonization by arbuscular mycorrhizal fungi lead to significant increases in host plant photosynthesis rates, stomatal conductance, and transpiration rates[9]. The lack of physiological responses in this study might indicate that the specific mycorrhizal species tested do not stimulate enhanced metabolism, although three of the species did facilitate enhanced belowground biomass growth.

Growth parameters that were shown to not be significant include root to shoot rations, leaf counts, and aboveground biomass growth. There is evidence that all three of these factors can be significantly increased by mycorrhizal symbioses. A review by Kapoor et al. (2017) noted that leaf mass, shoot weight, and number of nodes were significantly increased in different studies involving mycorrhizal inoculation treatments[8]. The lack of these growth responses in *Echinacea purpurea* may indicate that the symbiotic combinations used in this study do not result in significant increased growth of any of the aboveground organs for this host plant. Since three of the five species tested did have significant increased belowground biomass response it is worth noting that there may be potential application for using these species in horticultural practices where belowground biomass is the target harvest organ. By using mycorrhizae to enhance host plant root growth and nutrient utilization ability, scientists and cultivators may be able to reduce soil nutrient input and more efficiently manage sustainable medicinal plant production.

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