Soil Pasteurization and Mycorrhizal Inoculation Alter Flower Production and Corm Composition of *Brodiaea laxa* ‘Queen Fabiola’

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Abstract: The ornamental flowering bulb *Brodiaea laxa* Benth. ‘Queen Fabiola’ was grown with or without arbuscular mycorrhizal fungal (AMF) inoculum in pasteurized or nonpasteurized soil to determine if inoculation altered flower and corm production. The first growing cycle after planting, mycorrhizal inoculation decreased the days to anthesis and increased the number of flowers produced per inflorescence and flower longevity. It also affected the quality of the daughter corm, which influenced flowering the following year. Inoculated plants produced larger daughter corms and more cormels than uninoculated plants, and allocated more biomass to the corms than the cormels, which lowered the average weight of the corms. Corms produced by inoculated plants also had higher concentration of nitrogen, potassium, zinc, and nonreducing sugars than those produced by uninoculated plants. The beneficial effects of AMF inoculation on flowering and corm/cormel production were generally increased by soil pasteurization. The results indicate that mycorrhizal inoculation may enhance commercial cut flower and corm production of this crop.

Arbuscular mycorrhizal fungi (AMF) are known to enhance nutrient uptake and increase growth and production in many plant species (Smith and Read 1997). Field-grown Easter lily form mycorrhizae with species of arbuscular mycorrhizal fungi (AMF) that are also capable of forming associations with onion (Ames and Linderman, 1977) and soil fertility level influences colonization by AMF (Ames et al., 1976). Inoculation of Easter lily with AMF increases root and stem weight (Ames and Linderman, 1978) and plant response to inoculation can vary with soil fertility (Ames and Linderman, 1978) and growing season (Mora, 1990). Except for onion (Giovannetti and Ries, 1980; Tawaraya, et al., 1999) and Easter lily (*Lilium longiflorum* Thunb.) (Ames and Linderman, 1977; Ames et al., 1976; Linderman et al., 1975; Mora, 1990), however, little is known about the benefits of AMF on liliaceous bulb crops (Scagel, 2002).

Furthermore, few reports detail how different carbon and nutrient allocation patterns between mycorrhizal plants and nonmycorrhizal plants can influence flowering (Bryla and Koid, 1998; Johnson et al., 1982) and bulb production (Charron et al., 2001). Carbon and nutrient allocation patterns can influence bulb quality of liliaceous ornamentals. Bulb quality can, in turn, effect new bulb formation and flower production and is influenced by several factors during storage (temperature, moisture) and during the growing season (light, nutrients, temperature) (Han, 1993; Han and Halvey, 1993; Han et al., 1990; Marinangeli and Curvetto, 1997; Miller and Langhans, 1989; Suh, 1997).

The objective of this study was to determine whether addition of AMF inoculum into the growing medium of *B. laxa* ‘Queen Fabiola’ alters aspects of flower production, corm production, and corm quality. *Brodiaea laxa* is a late-spring flowering corn (bulb) in the Themidaceae family that grows from summer dormant corms (De Hertogh and Le Nard, 1993). *Brodiaea* produces an umbrellate inflorescence with many flowers on one scape that is used for cut flower production. Flower initiation in this genus occurs after planting.

The size of the apical meristem in the corm rather than the amount of reserves in the corm is thought to determine the corm’s ability to form flowers (Halvey, 1990).

Materials and Methods

**Mycorrhizal inoculum.** Glomus intraradices Schenck & Smith was originally obtained from Native Plants Inc., (Salt Lake City, Utah) and maintained in pot cultures at the USDA–ARS, Horticultural Crops Research Laboratory, in Corvallis, Ore. The fungus was propagated in pot cultures on roots of bunching onion (*Allium cepa* L. ‘White Lisbon’) grown in 1 loam : 1 sand for 5 months. Inoculum consisted of a mixture of the soil medium, extraradical hyphae and spores, and colonized root segments (<2 mm in length). Population estimates of the inoculum used in this study by the MPN method (Woomer, 1994) were on average 10 propagules/g of soil medium.

**Plant material and treatments.** Corms of wild hyacinth (B. *laxa* ‘Queen Fabiola’) were planted into cylindrical 3.78-L pots (Lerio 19.4 x 18.1 cm) filled with a steam pasteurized (60 °C for 30 min) or nonpasteurized 1:1 mixtures of Willamette Valley alluvial silt loam and river sand. The mixture had 11 mg·kg⁻¹ available phosphorus and a pH of 6.3. AMF inoculum (*G. intraradices*), which contained spores, colonized root fragments, and other propagules in a sand-based carrier at a rate of 1:166 (v/v), was placed beneath the base of each corm at planting. For controls, sterilized inoculum was added at the base of each corm at the same rate.

**Cultural conditions.** During the first growing cycle, plants were maintained in a greenhouse with supplemental light (16 h light/8 h dark) provided by high-pressure multi-vapor lamps with an average of 700 µmol·m⁻²·s⁻¹ at canopy level, and average day/night temperatures of 20/16 °C (75/65 °F). Plants were fertilized once a week with 50 mL of a liquid fertilizer (LF) (10% K, 40% N, 20% Ca, 7% Mg, 8% S, 4% Na, and <0.05% of Mn, Cu, Zn, and Mo) and watered as needed. Periodic pest and pathogen control measures were performed as needed and included Diflubenzuron for fungus gnats (*Bradysia sp.*), *Neoseiulus fallacis* Garman predators for spider mites (*Tetranychus sp.*), and *Neoseiulus cucumeris* predators for thrips (*Frankiennella sp.*). At the end of the first growing cycle, when shoots had died-back, corms were removed from the soil, dried at 20 °C for 2 weeks, then stored at 5 °C for 10 weeks. After 10 weeks of storage, corms were planted into cylindrical 3.78-L pots (Lerio 19.4 cm x 18.1 cm) containing a 1:1 mixture of a Willamette Valley alluvial silt loam and river sand. Plants were grown under the same growing conditions for both the first and second growing cycles. To assess carryover effects from the first growing cycle, no AMF inoculation was included for the second growing cycle and soil was pasteurized as previously described.

**First growing cycle measurements.** For each corm, the number of days after planting until shoot emergence and flower emergence was recorded. The number of flowers per inflorescence and the length of flower survival on each plant was recorded. The end of the growing cycle, when shoots had died-back, corms were removed from the soil, counted, air dried, and weighed. Colonization of fresh roots by AMF was assessed on 1-cm sections after clearing and staining by a modified procedure of Phillips and Hayman (1970), replacing lactophenol with lactoglycerin. Percentage of root length with colonization by AMF was estimated by the method of Biermann and Linderman (1980). Six daughter corms per treatment were dried and placed in storage. A subsample (six corms per treatment) was analyzed for P, K, Ca, Zn, C, N, and S concentrations using standard methods (Gaulak et al. 1997). Nitrogen and S were determined after automated combus-
Root colonization by AMF. AMF inoculation significantly increased the proportion of root length colonized by fungi in both pasteurized and nonpasteurized soil, although pasteurization reduced colonization in inoculated plants by 17% in the first growing cycle after inoculation and by 22% in the second growing cycle (Fig. 1).

Shoot emergence. When plants were grown in pasteurized soil, AMF inoculation significantly increased the days to shoot emergence both the first and second growing cycle after planting (Fig. 2A). However, when they were grown in nonpasteurized soil, inoculation had no effect on shoot emergence the first growing cycle after planting and reduced days to shoot emergence the second growing cycle after planting (Fig. 2A).

Flower development. Inoculation significantly decreased the days to flower bud emergence (Fig. 2B) and flower opening (Fig. 2C), flower longevity (Fig. 2D), and flowers per inflorescence (Fig. 2E), and of Brodiaea laxa ‘Queen Fabiola’ during the first (YR1) and second (YR2) growing cycle after inoculation. For both growing cycles, inoculation significantly increased the number of flowers produced per inflorescence and reduced days to flower bud emergence (Fig. 2A) and flower longevity (Fig. 2B). Inoculation also increased flower longevity in both growing cycles, but only when the soil was not pasteurized (Fig. 2D).

Results

Root colonization by AMF. AMF inoculation significantly increased the proportion of root length colonized by fungi in both pasteurized and nonpasteurized soil, although pasteurization reduced colonization in inoculated plants by 17% in the first growing cycle after inoculation and by 22% in the second growing cycle (Fig. 1).
longevity when plants were not inoculated (Fig. 2D). Plants growing in nonpasteurized soil stopped producing new flowers 8 to 12 d earlier than plants growing in pasteurized soil (data not shown).

Corm and cormel development and composition. Inoculation with AMF increased the total weight of daughter corms and cormels in both production cycles (Fig. 3A). Inoculation significantly increased the weight of daughter corms produced at the end of each growing cycle in both soils (Fig. 3C) and inoculated plants partitioned a higher proportion of biomass to daughter corms than cormels (Fig. 3E). Inoculation also increased the concentrations of nitrogen (Fig. 4C) and nonreducing sugars (Fig. 5D) in the corms produced in both soils, but lowered the concentrations of amino acids (Fig. 5B) and reducing sugars (Fig. 5C) at the end of the first growing cycle (Fig. 4C), but decreased the concentration of potassium (Fig. 4B) and reducing sugars (Fig. 5C). Pasteurization decreased the total number of cormels produced both growing cycles, but only when plants were not inoculated (Fig. 3D). The total weight of cormels produced, however, was increased by pasteurization whether plants were inoculated or not (Fig. 3B). Higher cormel production from inoculated plants growing in pasteurized soil resulted in a lower average weight per cormel than in noninoculated plants (Fig. 3F). Plants growing in nonpasteurized soil had the smallest cormels and plants growing in pasteurized soil without AMF inoculum had the largest cormels (Fig. 3F).

Discussion

Liliaceous plants are important in a wide variety of horticultural systems used for the production of food and ornamental crops. The beneficial effects of AMF on growth and nutrition of onion has been well documented in many experimental systems designed to look at the mechanisms involved in the interaction between the fungus and plant, however the influence of AMF on liliaceous plants that are used in the production of cut flowers and ornamental crops has not been investigated to a similar depth. We found that inoculation of B. laxa ‘Queen Fabiola’ bulbs with AMF can alter plant development and biomass partitioning which can, in turn influence crop productivity in terms of bulb and flower production and bulb composition.

In general, inoculation with AMF has been found to increase plant growth; however, there are many reports which describe an initial lag-phase after inoculation when noninoculated plants are larger than inoculated plants. Mora (1990) found that inoculation of L. longiflorum with the AMF G. intraradices significantly delayed shoot emergence of plants growing in pasteurized media in the growing cycle plants were inoculated. This delay in shoot emergence in pasteurized soil could be a result of an increase in carbohydrate demand during the early stages of root colonization and may potentially have a negative influence on the timing of production of B. laxa for cut flowers. In nonpasteurized soil inoculation with AMF had no effect on the time of shoot emergence during the first growing cycle, but reduced the number of days to emergence in the second growing cycle. This indicates when corms are grown in nonpasteurized soil, inoculation with AMF alter aspects of corm quality that influence the timing of shoot emergence in the growing cycle following inoculation.

In most geophytes, including B. laxa, the major factor controlling flowering is seasonal thermoperiodicity (Han et al., 1991; Rees, 1985). Although inoculation delayed shoot emergence of B. laxa, this delay did not cause a delay in the timing of flower emergence. Mora (1990) found that inoculation of L. longiflorum with G. intraradices did not significantly affect flower emergence or number of flowers produced although response varied with the type N fertilizer. Our results suggest that initial
Colonization of plants by AMF can alter indirect effects on flowering in the following growing cycle after inoculation. Error bars are standard error of the least squares means (n = 12). NPS+NONE = no soil pasteurization and sterilized AMF inoculum, NPS+AMF = no soil pasteurization and inoculated with AMF, PS+NONE = pasteurized soil, PS+AMF = pasteurized soil and inoculated with AMF.

Organic sources of carbon and nitrogen stored in bulbs at the end of a growing cycle are important for growth during the following season when storage reserves in mother corms are depleted by new growth. In developing storage organs such as corms, translocated photoassimilates are converted into carbon and nitrogen reserves such as starch, fructans, oils, and storage proteins (Kavalič et al., 2000). In B. laxa the primary area of storage is the
the corms’ ability to form flowers (Halvey, 1990). At the end of the first growing cycle, soil pasteurization also influenced storage quality of corms. Increased accumulation of amino acids and carbohydrates possibly as a result of respiratory turnover generally decreased in response to cooling, but changes in concentration were associated with proportional increases in total content and corm weight, showing that soil pasteurization increased accumulation and storage of proteins in corms. Others (Krishna and Bagyaraj, 1983; Vázquez et al., 2001) have found that AMF can increase root and shoot total protein content, however responses depended on the species and inoculation had a different effect on the concentration and total content of protein (A), amino acids (B), reducing sugars (C), and nonreducing sugars (D) in daughter corms of Brodiaea laxa ‘Queen Fabiola’ at the end of the first growing cycle after inoculation. Error bars are standard error of the least squares means (n = 12). NPS+NONE = no soil pasteurization and sterilized AMF inoculum; NPS+AMF = no soil pasteurization and inoculated with AMF; PS+NONE = pasteurized soil; PS+AMF = pasteurized soil and inoculated with AMF.

In our study, the concentration of protein in corms at the end of the first growing cycle was significantly increased by soil pasteurization and was not influenced by AMF inoculation. Soil pasteurization increased the concentration and total content of proteins in corms at the end of the first growing without increased corm weight, showing that soil pasteurization increased accumulation and storage of proteins in corms. Others (Krishna and Bagyaraj, 1983; Vázquez et al., 2001) have found that AMF can increase root and shoot total protein content, however responses depended on the species of AMF used in the study. In our study, we only used one isolate of AMF. It is possible that other AMF may alter corm total protein content.

Cultural treatments commonly used during bulb crop production to control detrimental organisms may not only influence the presence of natural populations of mycorrhizal fungi in soils, but also influence the effects of plant inoculation with AMF. Vósátká (1995) found that the growth response of onion to inoculation was higher when indigenous fungi in the soil were eliminated by steam sterilization. In our experiment colonization of inoculated plants was slightly decreased by soil pasteurization. However, flower production and biomass of corms and cormels were generally increased by pasteurization. The beneficial effects of AMF inoculation on flowering and corm/cormel production were generally increased by soil pasteurization, suggesting that although soil pasteurized decreased AMF colonization, either the efficiency of the symbiotic association was greater or pasteurization suppressed other organisms that were detrimental to plant growth.

Conclusions

Maximum productivity and quality of geophytes used for both flower and corm production requires a balance between resources allocated to flowering and corm production. The symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and their plant symbiont can alter aspects of biomass partitioning and metabolism that differentially influence productivity and quality of corm crops. Inoculation of B. laxa ‘Queen Fabiola’ can decrease the time until flower opening and increase the number of flowers per inflorescence. Flowers on inoculated plants generally lasted longer. Inoculation can also increase daughter corm size and production of cormels, but inoculated plants preferentially increase biomass partitioning to daughter corms over cormels resulting in a lower average weight per corm than in noninoculated plants. Our results indicate that adding AMF inoculum into the growing medium of B. laxa alters aspects of flowering and biomasses partitioning that are important in the commercial production of this crop for cut flowers and corms.

Literature Cited

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