Inoculation with Vesicular–Arbuscular Mycorrhizal Fungi and Rhizobacteria Alters Nutrient Allocation and Flowering of Harlequin Flower

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Additional index words. *Glomus intraradices*, *Sparaxis tricolor*, VA mycorrhizae

Summary. **We assessed whether addition of arbuscular mycorrhizal fungus (AMF) inoculum or rhizosphere organisms from AMF inoculum alters aspects of flowering, corm production, or corm quality of harlequin flower (*Sparaxis tricolor*) for two growth cycles after inoculation. Using pasteurized and nonpasteurized growth medium, plants were inoculated with either inoculum of the AMF, *Glomus intraradices*, or washings of the inoculum containing rhizobacteria. Shoots of plants inoculated with AMF emerged 2 days earlier than shoots on noninoculated plants or plants inoculated with inoculum washings. Flowers on AMF-inoculated plants opened 7-8 days earlier and plants produced more flowers per plant and per inflorescence than noninoculated plants. AMF-inoculated plants partitioned a higher proportion of biomass to cormel production than to daughter corms and had higher concentration and contents of zinc, sulfur, nitrogen, amino acids, and carbohydrates than corms from noninoculated plants. The rhizosphere organisms associated with the AMF inoculum influenced several measures of plant development, growth, and corm production suggesting that there are organisms associated with our AMF inoculum that have beneficial effects on the growth and productivity of harlequin flower. While inoculation with AMF can promote shoot emergence, leaf production, and flower production of harlequin flower, inoculation also alters aspects of biomass partitioning and corm composition that play an important role in the production of this crop for corms and cormels.**

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Plants with roots colonized by mycorrhizal fungi are more effective at nutrient and water acquisition, less susceptible to disease, and can be more productive under certain environmental growing conditions than plants without mycorrhizae (Bethlenfalvay and Linderman, 1992; Smith and Read, 1997). There is little available information describing the benefits of inoculation with mycorrhizal fungi on different aspects of productivity and flowering of lilaceous bulb crops (Scagel and Linderman, 2002) except onion (*Allium* spp.) (Giovannetti and Riess, 1980; Tawaraya, et al., 1999), and easter lily (*Lilium longiflorum*) (Ames et al., 1976; Ames and Linderman, 1977, 1978; Linderman et al., 1975; Mora, 1990). Only the presence or absence of mycorrhizae has been reported on most other liliaceous crops (Chilvers and Daft, 1981). Field-grown easter lily form mycorrhizae with species of AMF that are also capable of forming associations with onion (Ames and Linderman, 1977) and other crops. Inoculation of Easter lily with AMF was shown to increase root and stem weight (Ames and Linderman, 1978) and responses to inoculation can vary with soil fertility (Ames et al., 1976) and growing season (Mora, 1990). Recent studies by Scagel (2003a, 2003b) indicate that inoculation with AMF can alter carbon partitioning and flowering over two production cycles for 'Queen Fabiola' wild hyacinth (*Brodiaea laxa*) and three rain lily species (*Zephyranthes* spp.).

Soil fumigation practices commonly used during bulb crop production can decrease the levels of AMF in soil, resulting in decreased colonization. Scagel (2003a, 2003b) reported that soil pasteurization can influence bulb composition and plant development of wild hyacinth and *Zephyranthes* species. These studies used inoculum that consisted of spores and root fragments from AMF pot cultures and did not separate out the potential influence of other rhizosphere organisms that could be present in the inoculum.

The influence of AMF inoculation on productivity of different crops has been well documented (Aryal and Xu, 2000); however few reports detail how differences in carbon and nutrient allocation patterns between mycorrhizal and nonmycorrhizal plants can influence flowering and bulb production (Scagel, 2003a, 2003b). Flowering is the result of a complex relationship between carbon and nutrient demands within the plant, however, there is little information describing the effects of mycorrhizal fungi on flowering (Bryla and Koide, 1998). Carbon and nutrient allocation patterns can influence bulb quality of liliaceous ornamentals. Bulb quality can, in turn, affect new bulb formation and flower production and is influenced by several factors during storage (temperature, moisture) and during the growth cycle (light, nutrients, temperature) (Han et al., 1991; Hanks et al., 1986; Miller and Langhans, 1989; Suh, 1997).

The physiology of growth and flowering in geophytes is based on extensive studies of a handful of species [e.g. tulip (*Tulipa* spp.), iris (*Iris* spp.), gladiolus (*Gladiolus* spp.), easter lilies] (Halvey, 1990), yet little is known about the environmental factors influencing growth and flowering of other commercially important genera. The genus *Sparaxis* in the Iridaceae family contains several species of flowering bulbs used in landscape and cut flower production. *Sparaxis* species grow from corms with their active period of growth and flowering in the spring and a rest period in the summer. Natural vegetative multiplication rate of *Sparaxis* species is low and limited to annual corm replacement (daughter corm) and production of cormels at leaf bases. The objectives of this study were to 1) determine whether addition of AMF inoculum into the growing medium of harlequin flower alters aspects of flower production, bulb production, and bulb quality, and 2) assess whether other rhizosphere organisms present in the AMF inoculum play a role in plant response to inoculation.

Materials and methods

Plant material and treatments. Corms of harlequin flower were planted

into cylindrical 3.8-L (1-gal) pots (Lerio, El Campo, Texas) 19.4 × 18.1 cm ((7 5/8 × 7 1/8 inches) containing either a pasteurized [60 °C (140.0 °F) for 30 min] or nonpasteurized 1:1 mixture of a Willamette Valley alluvial silt loam and river sand [11 mg·kg^{-1} (ppm) available (Bray) phosphorus (P), pH of 6.3]. For the AMF treatment (M), inoculum of the AM fungus (*Glomus intraradices*) containing spores, colonized root fragments, and other propagules in a sand-based carrier at a rate of 1:166 (by volume) was placed beneath the base of each bulb at planting. For controls (C), sterilized inoculum was added at the base of each bulb at the same rate. A third inoculation treatment (W) was used to assess the influence of rhizosphere soil microflora from the AMF inoculum. For the W treatment, sterilized inoculum was added at the base of each bulb at the same rate used in the M and C treatments and 50 mL (1.7 fl oz) of washings from nonsterilized inoculum were applied to pots after being passed through a 28 μm sieve (Tyler equivalent 400-mesh) and Whatman 1 filter paper to remove AMF propagules.

MYCORRHIZAL INOCULUM. *Glomus intraradices* 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 'White Lisbon' bunching onion (*Allium cepa*) 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 (0.08 inch) in length]. Population estimates of the inoculum used in this study by the MPN method (Woomer, 1994) were on average 10 propagules/g (283.5 propagules/oz) of soil medium.

CULTURAL CONDITIONS. Plants were maintained in a glasshouse with supplemental light (16 h/8h, light/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 16/12 °C (61.0/54.0 °F). Plants were fertilized once a week with 50 mL of a liquid fertilizer (about 10% potassium (K), 10% P, 40% nitrogen (N), 20% calcium (Ca), 7% magnesium (Mg), 8% sulfur (S), 4% sodium (Na), and <0.05% manganese (Mn), copper (Cu), zinc (Zn), boron (B), and molybdenum (Mo) and watered as needed. Periodic pest and pathogen control measures were performed as needed. At the end of the growth cycle, when shoots had died-back, corms and cormels were removed from the soil, dried at 20 °C (68.0 °F) for 2 weeks, stored at 25 °C (77.0 °F) for 10 weeks, than placed at 15 °C (59.0 °F) for 2 weeks prior to planting. Corms were planted as previously described. Plants were grown under the same growing conditions for both the first and second growth cycles. To assess carry-over effects from the first growth cycle, no AMF inoculation was done for the second growth cycle and all soil was pasteurized before planting.

FIRST GROWTH CYCLE MEASUREMENTS. For each corm, the number of days after planting until shoot emergence and flower emergence was recorded as well as the number of flower buds and the total number of flowers on each plant. At the end of the growth cycle, when shoots had died-back, bulbs were removed from the soil, counted, air dried, and weighed. AM colonization was assessed on 1-cm sections of fresh roots after clearing and staining by modified procedures of Phillips and Hayman (1970), replacing lacto-phenol with lacto-glycerin. Percentage of root length with signs of AM colonization (e.g., vesicles, arbuscules, AM hyphae) was estimated by the method of Biermann and Linderman (1980). Six daughter bulbs per treatment were placed in storage. Another subsample (six bulbs per treatment) were analyzed for P, K, Ca, Mg, Mn, iron (Fe), Cu, B, Zn, carbon (C), N, and S content using standard methods (Gavlak et al., 1994). N and S were determined after automated combustion and concentrations of the remainder of the elements determined after dry ash oxidation by ICP-AES. Total soluble protein was determined colorimetrically using a BIO-RAD (Coomassie Brilliant blue) (BIO-RAD, Hercules, Calif.) (Bradford, 1976) after extraction of ground corm tissue (<50 mesh) in buffer [20 mM triethanolamine (TRIS), 10 mM sodium chloride (NaCl), 10 mM potassium chloride (KCl), 2 mM magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_20$)] with Nonidet P-40 (Roche Applied Science, Indianapolis). Total amino acid content of corms was determined colorimetrically with ninhydrin (Yemm and Cocking, 1955) after extraction with acetic acid prior to analysis. Total reducing and nonreducing sugar content of corms were determined colorimetrically using a modification of the Somolgyi-Nelson Alkaline Copper method (Dische, 1962; Nelson, 1944). Supernatant from ground corm tissue (<50 mesh) extracted with warm 80% ethanol was used to determine total reducing sugar content. The residual pellet from extraction was hydrolyzed in 0.2 N potassium hydroxide (KOH) prior to analyses for nonreducing sugars.

SECOND GROWTH CYCLE MEASUREMENTS. For each corm, the number of days after planting until shoot emergence and flower emergence was recorded as well as the number of flower buds and the total number of flowers on each plant. At the end of the growth cycle, when shoots had died-back, bulbs were removed from the soil, counted, air dried, and weighed. AM colonization was assessed as described for the first growth cycle.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES. The experiment was set up in a randomized design with each treatment unit (pot) replicated 20 times during the first growth cycle and 10 times during the second growth cycle. Morphological data were subjected to analysis of variance (ANOVA) with soil pasteurization treatment, AMF inoculation, and growth cycle after inoculation as main effects. Single degree of freedom contrasts were used to address specific questions related to interactions between main effects. Data with unequal variances between treatment groups were log-transformed to equalize variances. Back transformed data is presented in tables. Corm composition data were subjected to two-way ANOVA with soil pasteurization treatment and AMF inoculation as main effects. Bonferroni test was used to separate treatment means. Relationships between bulb composition data and plant morphological data were analyzed using Pearsons' correlation coefficient (r). All data analyses were performed using the Statistica statistical package (Statsoft, Inc., Tulsa, Okla.).

Results and discussion

PLANT DEVELOPMENT. In floral crop production, decreasing the time required to reach marketable size can decrease production costs. We found that shoots of harlequin flower inoculated with AMF emerged about 2 d

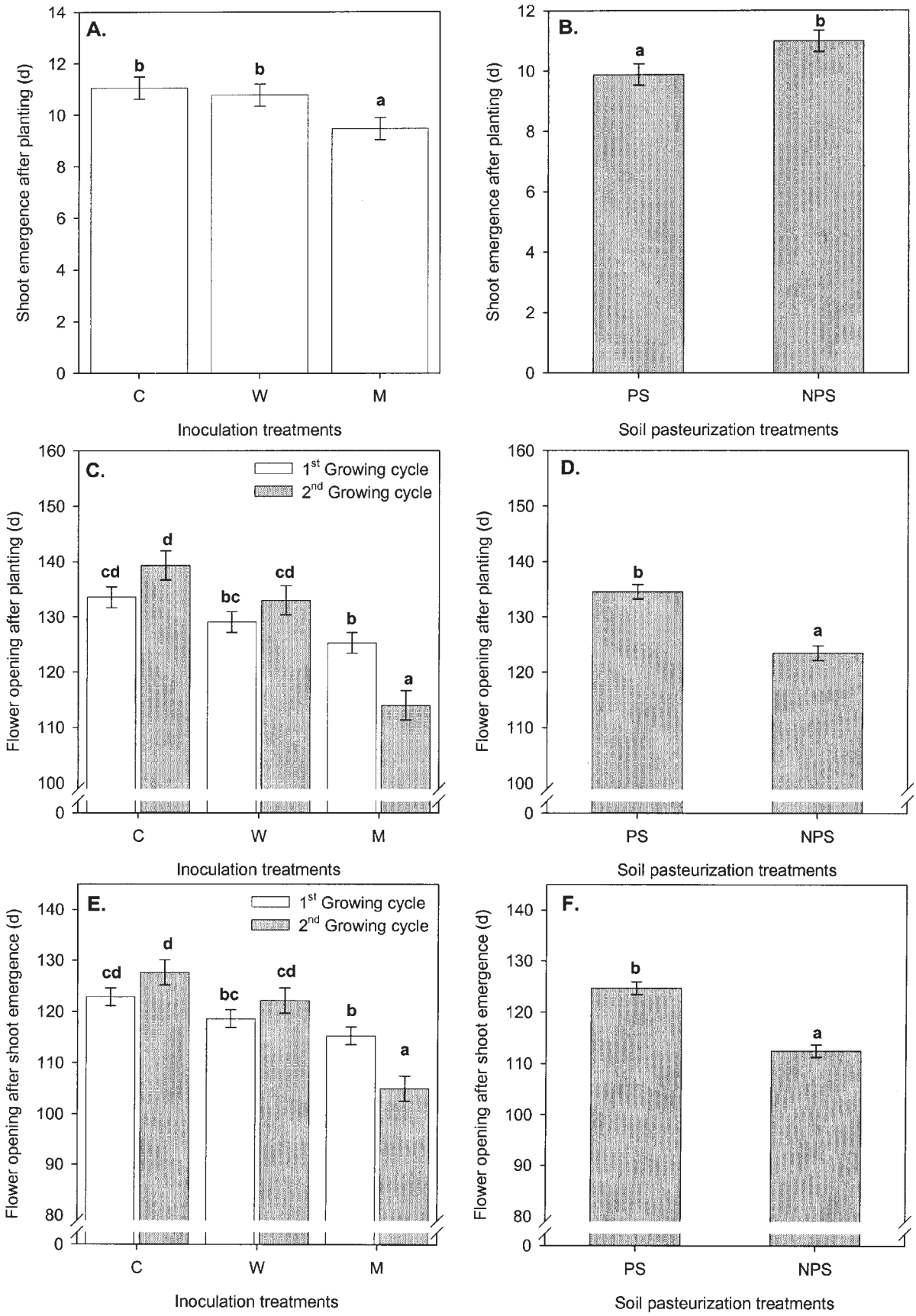

Fig. 1. Time of shoot emergence after planting (A) and flower opening after planting (B) and shoot emergence (C) of harlequin flower for two growth cycles after inoculation with arbuscular mycorrhizal fungi (AMF) and soil pasteurization treatments. Error bars are standard error of the least squares means. C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from non-sterilized inoculum; M = AMF inoculum; NPS = nonpasteurized soil; PS = pasteurized soil.

earlier than shoots on noninoculated plants (C) or plants inoculated with washings from inoculum (W) (Fig. 1A). Shoots of plants growing in pasteurized soil emerging about 2 d earlier than shoots of plants growing in nonpasteurized soil (Fig. 1B). Earlier shoot emergence of AMF-inoculated harlequin flower occurred in both years of the study, indicating that AMF may have also altered aspects of bulb quality that influenced shoot emergence for the second growth cycle after inoculation. Inoculation with AMF has also been reported to hasten shoot emergence of *Zephyranthes* species (Scagel, 2003b) but has been shown to delay shoot emergence of wild hyacinth (Scagel 2003a) and easter lily (Mora, 1990) when grown in pasteurized soil.

Root initiation in *Sparaxis* species demonstrates a high metabolic demand for macronutrients and carbohydrates (Ruiters and McKenzie, 1994). AMF promotion of shoot emergence may play a role in carbohydrate status of the plants by extending the period of photosynthetic activity in the geophytic life cycle. In other plants, AMF colonization has been shown to increase photosynthetic activity and hasten leaf appearance (Eissenstat et al., 1990; Lynch et al., 1991) and may be an adaptive response of the plant to the increased carbohydrate demands resulting from AMF colonization. In our study, even though shoots of inoculated harlequin flower emerged earlier than those of noninoculated plants, the total number of leaves produced per plant was only increased by AMF inoculation when plants were grown in pasteurized soil (Fig. 2A and B). With *Zephyranthes* species shoots of inoculated plants emerged earlier than those of noninoculated plants and the total number of leaves on plants was increased by AMF inoculation, but the dry weight of leaves was decreased by inoculation. This suggests that the photosynthetic surface area of AMF inoculated plants may be different than noninoculated plants. Further analysis of the photosynthetic efficiency and leaf area development in response to AMF inoculation in harlequin flower would allow for a better understanding of the role of these fungi in carbon partitioning and efficiency of vegetative growth during production.

In *Sparaxis* species, flower initiation and growth occurs 6 to 8 weeks after planting (De Hertogh and Le Nard, 1993). Differences in resource allocation during flower initiation resulting from AMF colonization have the potential to cause delayed flowering. In our study, the number of days between shoot emergence and flower opening was influenced by both soil pasteurization and inoculation treatments, but the response to inoculation varied with growth cycle after inoculation. Flowers on plants growing in pasteurized soil opened about 12 d later after planting (Fig. 1D) and shoot emergence (Fig. 1F) than flowers on plants growing in nonpasteurized soil. In the first growth cycle, flowers on plants inoculated with AMF opened 7 to 8 d earlier after planting (Fig. 1C) and shoot emergence (Fig. 1E) than flowers on noninoculated plants, and flowers on plants inoculated with inoculum washings opened at about the same time as flowers on plants inoculated with AMF. The effects AMF inoculation on the time of flower opening was more pronounced in the second growth cycle than the first growth cycle (Fig. 1C and E). In most geophytes, the major factor controlling flowering is seasonal thermoperiodicity (Han et al., 1991; Rees, 1985). Mora (1990) found that inoculation of easter lily with *G. intraradices* did not affect flower emergence, although flower emergence varied with nitrogen type used in the fertilizer. AMF inoculation of pink fawn lily (*Zephyranthes robusta*) and white rain lily (*Z. candida*) has been reported to delay flower emergence but not the time of flower opening (Scagel, 2003b). With yellow zephyr lily [*Z. sulphurea* (syn. *Z. citrina*)] and wild hyacinth, AMF inoculation hastened flower emergence (Scagel, 2003a, 2003b).

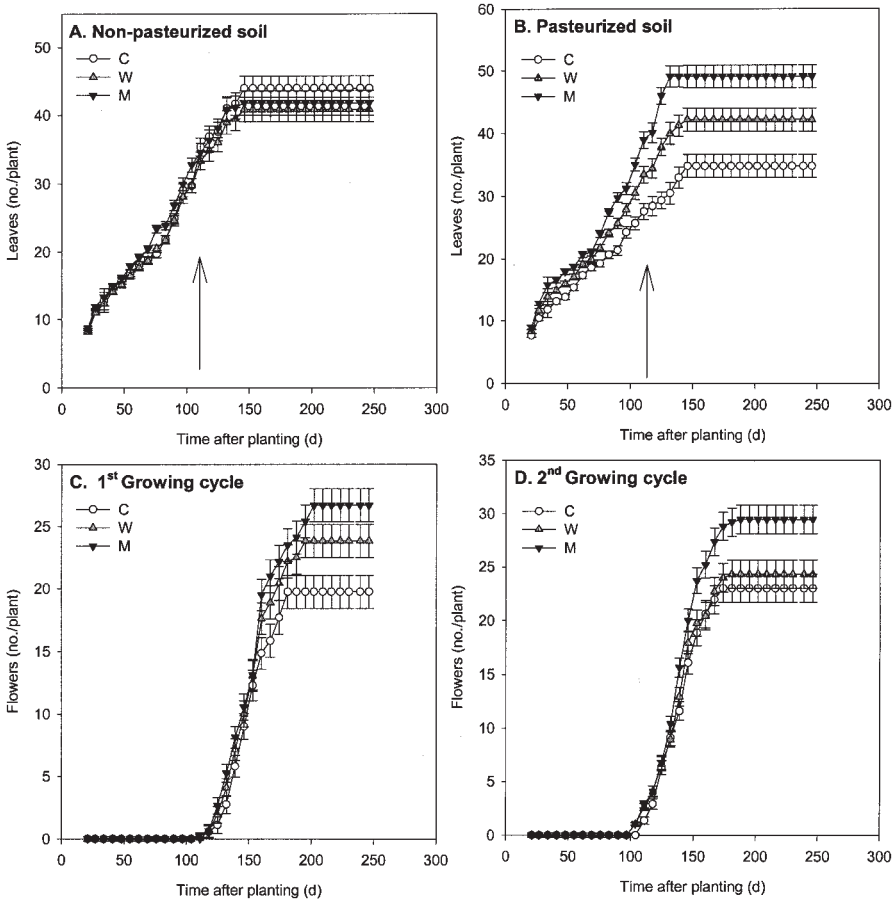

Fig. 2. Total number (cumulative) of fully expanded leaves (A and B), and flowers (C and D) of harlequin flower in response to inoculation with arbuscular mycorrhizal fungi (AMF) and soil pasteurization treatments. Error bars are standard error of the least squares means within measurement dates. Arrows (A and B) represent the average number of days until flower emergence. Different letters above bars represent treatment means significantly different from each other ($P < 0.05$, Bonferroni test). C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from nonsterilized inoculum; M = AMF inoculum.

FLOWER PRODUCTION. The total number of flowers per plant was influenced by inoculation treatments,

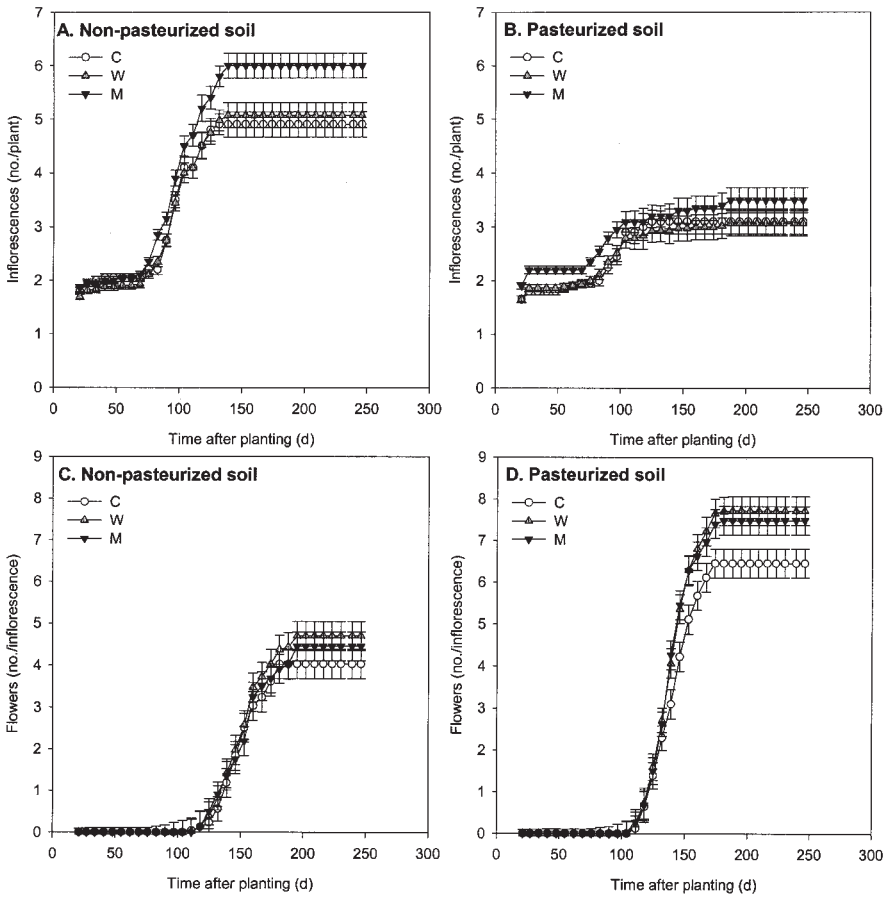

Fig. 3. Total number (cumulative) of inflorescences (A and B), and flowers per inflorescence (C and D) of harlequin flower in response to inoculation with arbuscular mycorrhizal fungi (AMF) and soil pasteurization treatments. Error bars are standard error of the least squares means within measurement dates. Different letters above bars represent treatment means significantly different from each other ($P < 0.05$, Bonferroni test). C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from nonsterilized inoculum; M = AMF inoculum.

and response to inoculation varied with growth cycle after inoculation. Plants inoculated with AMF produced about eight more flowers per plant than noninoculated plants during both growth cycles (Fig. 2C and D). Differences in flower production between AMF-inoculated and noninoculated plants were detectable about 150 d after planting. By about 180 d after planting, noninoculated plants stopped producing new flowers, while AMF-inoculated plants continued to produce newly expanded flowers until about 200 d after planting. Plants that received inoculum washings produced more flowers than noninoculated plants during the first growth cycle, but produced a similar number of flowers as noninoculated plants during the second growth cycle (Fig. 2C and D).

The number of inflorescences and the number of flowers per inflorescence were influenced by inoculation treatments, and response to inoculation varied with soil pasteurization treatment. When grown in nonpasteurized soil plants inoculated with AMF produced more inflorescences than noninoculated plants or plants inoculated with inoculum washings (Fig. 3A) resulting in slightly more flowers per inflorescence on inoculated plants (both M and W treatments) (Fig. 3C). In pasteurized soil, plants produced the same number of inflorescences regardless of inoculation treatment (Fig. 3B). Inflorescences on inoculated plants (both M and W treatments) had about two more flowers per inflorescence than noninoculated plants (C treatment) (Fig. 3D).

Corm weight and biomass partitioning. Differences in resource allocation during growth of harlequin flower could differentially influence aspects of production for corms or cut flowers. Colonization of roots by AMF has been shown to cause differences in plant biomass partitioning (Lynch et al., 1991; Smith and Read, 1997). With harlequin flower, inoculation with AMF increased the number of flowers per inflorescence and increased the combined weight of corms and cormels produced. In the first growth cycle, plants inoculated with AMF produced a greater combined weight of corms and cormels than noninoculated plants, irrespective of soil pasteurization treatment (Fig. 4A). In the second growth cycle after inoculation, AMF inoculation only increased the combined weight of corms and cormels on plants growing in nonpasteurized soil. Noninoculated plants produced daughter corms that were similar in weight or heavier than daughter corms produced by plants inoculated with AMF or washings from AMF inoculum (Fig. 4B). Plants growing in nonpasteurized soil generally produced larger daughter corms than plants growing in pasteurized soil (Fig. 4B).

Daughter corm biomass of AMF-inoculated plants was lower than that of noninoculated plants, and AMF-inoculated plants partitioned a higher proportion of biomass to cormel production than to daughter corm production (Fig. 4C). Although cormel biomass was higher in AMF-inoculated plants, these plants also produced more cormels than noninoculated plants (Fig. 4D), resulting in a smaller average cormel size in AMF-inoculated plants compared to noninoculated plants (Fig. 4E). In our experimental system, the effects of AMF inoculation on daughter corm biomass and cormel size of harlequin flower were not conducive for increasing corm or cormel size.

Earlier flowering of AMF-inoculated plants suggests that any changes in resource allocation, resulting from the establishment of the symbiosis, do not negatively affect flower development. When plants exhibit a delayed emergence of flowers in response to AMF inoculation, there may be different resource allocation patterns in AMF-inoculated plants during early stages of colonization (Koide, 1991) that influence flower development. The different flowering responses of bulbs and corms to AMF inoculation may be a result of genetic differences in carbon partitioning and demands of the different species during flower development. For example, pink fawn lily produces much larger flowers than those of yellow zephyr lily. AMF in-

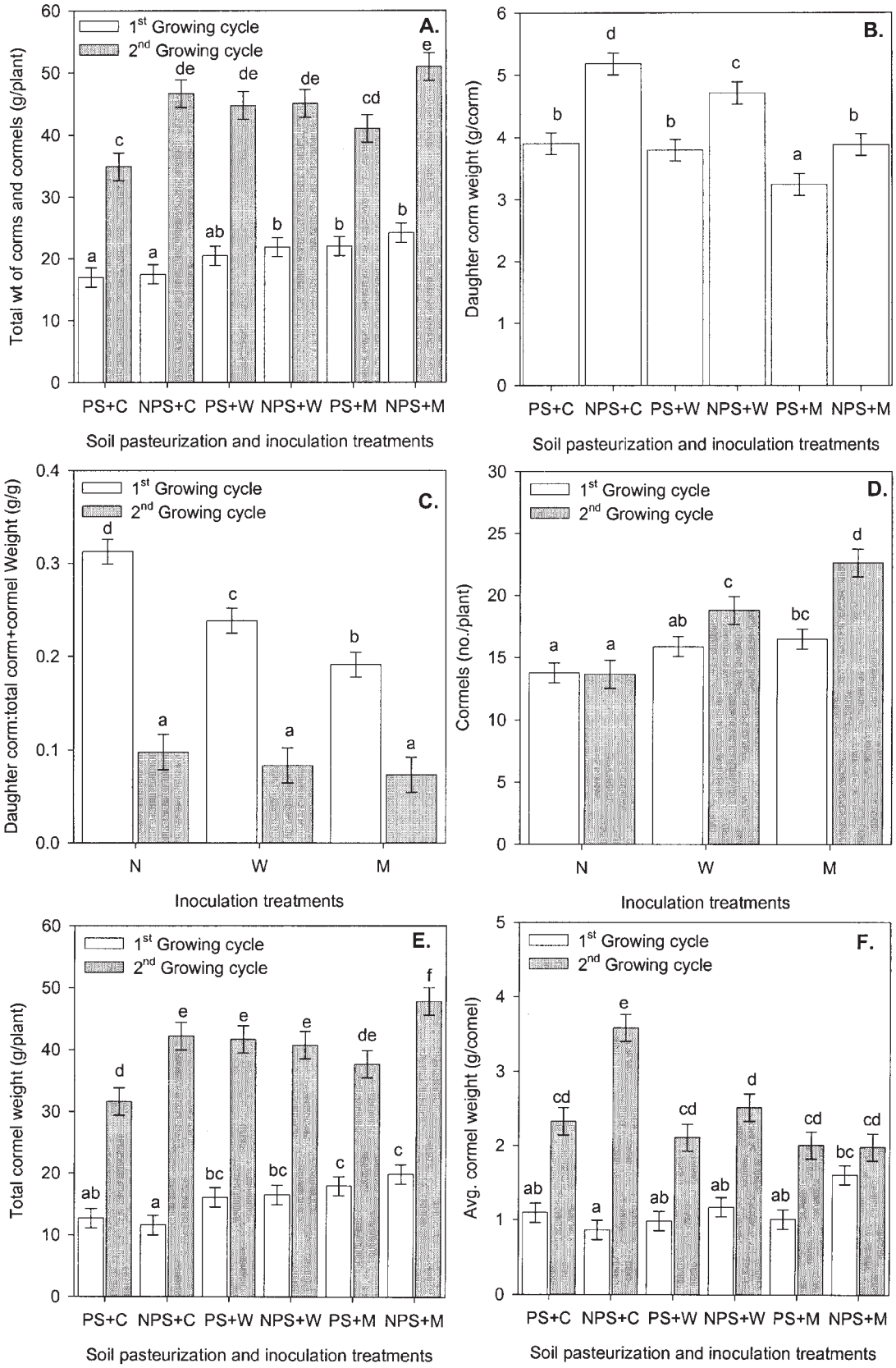

Fig. 4. Combined weight of corms and cormels (A), weight of daughter corms (B), ratio of daughter corm weight to combined weight of corms and cormels (C), cormel production (D), and average weight per cormel (E) of harlequin flower for two growth cycles after inoculation with arbuscular mycorrhizal fungi (AMF) and soil pasteurization treatments. Error bars are standard error of the least squares means. Different letters above bars represent treatment means significantly different from each other ($P < 0.05$, Bonferroni test). C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from non-sterilized inoculum; M= AMF inoculum; NPS = nonpasteurized soil; PS = pasteurized soil. 28.35 g = 1.0 oz.

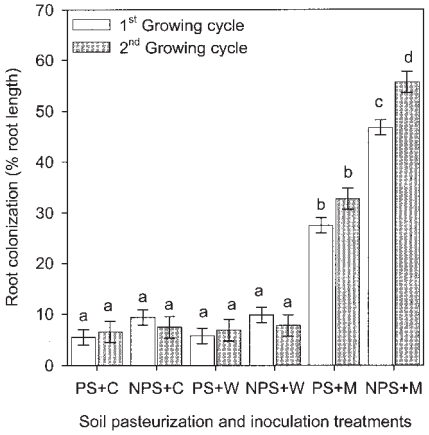

Fig. 5. Root colonization by AMF of harlequin flower for two growth cycles after inoculation with arbuscular mycorrhizal fungi (AMF) and soil pasteurization treatments. Error bars are standard error of the least squares means. Different letters above bars represent treatment means significantly different from each other ($P < 0.05$, Bonferroni test). C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from nonsterilized inoculum; M = AMF inoculum; NPS = nonpasteurized soil; PS = pasteurized soil.

oculation may have more influence on altering carbon partitioning during flower development of pink fawn lily than yellow zephyr lily as a result of a higher demand for carbon based on flower size (Scagel, 2003b). During the reproductive period of *Sparaxis* species., Ruiters and McKenzie (1994) found a higher demand for starch and other carbohydrates in the daughter corms than in the flowers. In our study plants of harlequin flower inoculated with AMF produced smaller daughter corms than noninoculated plants but more flowers, suggesting that AMF inoculation may have altered carbohydrate partitioning to corms during flower development.

COMPOSITION OF CORMS. The size of corms is commonly used for grading purposes in corm production systems. In general, corm size is thought to be related to aspects of corm quality. In *Sparaxis* species, there is a large reuse of K, N, P, and carbohydrates from vegetative and reproductive structures during senescence when new daughter corms are the major sink for allocation of dry matter and resources (Ruiters and McKenzie, 1994). Minerals and organic carbon and nitrogen stored in corms at the end of a growth cycle are important for growth during the following growth cycle when storage reserves in corms are depleted by new growth. Even though AMF-inoculated plants of harlequin flower produced daughter corms with a lower biomass than noninoculated plants, the concentrations and contents of Zn, S, N, amino acids, and carbohydrates in corms of AMF-inoculated plants were higher than that in noninoculated plants (Table 1, Table 2). Also, plants inoculated with washings had the highest concentrations of P and K in corms and higher concentrations and contents of amino acids and carbohydrates than corms from noninoculated plants. Increased content and concentration of these elements and storage compounds suggests uptake or availability of elements and storage of amino acids and carbohydrates is enhanced by AMF inoculation. We also found that plants having increased concentrations of Zn, S, and N were correlated to a decrease in the number of days until flower opening (Zn: $r = 0.44$, $P < 0.0001$; S: $r = 0.58$, $P < 0.0001$; N: $r = 0.55$, $P < 0.0001$). Increased S and N concentrations in corms were correlated to the number of leaves produced per plant (S: $r = 0.31$, $P < 0.016$; N: $r = 0.33$; $P < 0.009$), and increased concentrations of carbohydrates were correlated with the total number of flowers produced (reducing sugars: $r = 0.48$; $P < 0.0001$; nonreducing sugars: $r = 0.51$; $P < 0.0001$).

Increased uptake of elements in response to AMF inoculation frequently has been related to plant growth responses to inoculation (Smith and Read, 1997). Increased uptake of

Table 1. Analysis of variance (ANOVA) results of the influence of fertilizer amendment and inoculation with the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* on phosphorus (P), potassium (K), calcium (Ca), zinc (Zn), sulfur (S), and nitrogen (N) concentration and content of harlequin flower corms at the end of the first growth cycle.

Treatment[z]	P	K	Ca	Zn	S	N
Concn [g·kg^{-1} (except Zn mg·kg^{-1})][y]						
PS	10.6 b[x]	17.3 a	2.30 a	72.6 b	4.04 b	66.9 b
NPS	8.9 a	17.5 a	2.49 a	61.1 a	2.99 a	55.3 a
Soil SE[w]	(0.20)	(0.19)	(0.076)	(1.41)	(0.082)	(1.44)
C	9.2 a	17.3 ab	2.38 a	63.8 a	2.97 a	57.6 a
W	10.1 b	18.0 b	2.47 a	69.0 b	3.53 b	63.2 b
M	9.9 ab	16.9 a	2.34 a	67.7 ab	3.93 c	65.7 b
Inoculation SE[v]	(0.24)	(0.23)	(0.093)	(1.53)	(0.100)	(1.76)
ANOVA effects[u]	M, S	M	NS	M, S	M, S, M*S	M, S
Treatment content (mg/bulb)						
PS	207 b	342 a	4.5 a	1.42 b	78.5 b	1306 b
NPS	184 a	356 a	5.1 a	1.26 a	61.7 a	1143 a
Soil SE	(6.5)	(11.4)	(2.1)	(0.044)	(2.5)	(41.6)
C	153 a	290 a	39.7 a	1.06 a	55.9 a	951 a
W	208 b	373 b	51.4 b	1.42 b	74.6 b	1302 b
M	225 b	385 b	53.6 b	1.53 b	79.7 b	1490 c
Inoculation SE	(8.0)	(13.9)	(2.5)	(0.054)	(2.9)	(50.9)
ANOVA effects	M, S	M	M	M, S	M, S	M, S

[z]C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from nonsterilized inoculum; M = AMF inoculum; NPS = nonpasteurized soil; PS = pasteurized soil.
[y]1 g·kg^{-1} = 1000 ppm, 1.0 mg·kg^{-1} = 1.0 ppm, 28,350 mg = 1 oz.
[x]Means followed by the same letter or letters within inoculation or soil pasteurization treatments are not significantly different ($P < 0.05$, Bonferroni test).
[w]Soil SE = standard error of least square means for soil pasteurization treatments, n = 30.
[v]Inoculation SE = standard error of least square means for inoculation treatments, n = 20.
[u]ANOVA main effects and interactions significant at $P < 0.05$. S = soil pasteurization treatment; M = AMF inoculation treatment, Y = growth cycle from time of inoculation.

Table 2. Analysis of variance (ANOVA) results of the influence of fertilizer amendment and inoculation with the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* on protein, amino acid, and sugar concentration and content of harlequin flower corms and cormels at the end of the first growth cycle.

Treatment[z]	Proteins	Amino acids	Reducing sugars	Nonreducing sugars
Concn (g·kg^{-1})[y]				
PS	3.93 a[x]	125.3 a	8.89 b	8.20 b
NPS	3.85 a	130.6 a	7.67 a	7.98 a
Soil SE[w]	(0.174)	(3.51)	(0.21)	(0.31)
C	3.89 a	104.9 a	5.93 a	7.20 a
W	3.85 a	132.8 b	8.55 b	8.71 ab
M	3.93 a	146.2 b	10.36 c	9.39 b
Inoculation SE[v]	(0.213)	(3.02)	(0.26)	(0.38)
ANOVA effects[u]	ns	M	M, S, M*S	M, S
Content (mg/bulb)				
PS	76.5 a	2504 a	180.5 a	176.1 a
NPS	79.4 a	2708 a	161.0 a	166.3 a
Soil SE	(4.06)	(107)	(7.17)	(6.42)
C	64.7 a	1758 a	100.4 a	121.7 a
W	79.8 b	2749 b	177.2 b	180.1 b
M	89.3 b	3311 c	234.7 c	211.7 c
Inoculation SE	(4.98)	(131)	(8.78)	(10.1)
ANOVA effects	M	M	M	M

[z]C = sterilized AMF inoculum; W = sterilized AMF inoculum and washings from nonsterilized inoculum; M = AMF inoculum; NPS = nonpasteurized soil; PS = pasteurized soil.
[y]1 g·kg^{-1} = 1000 ppm, 1.0 mg·kg^{-1} = 1.0 ppm, 28350 mg = 1 oz.
[x]Means followed by the same letter or letters within inoculation or soil pasteurization treatments are not significantly different ($P < 0.05$, Bonferroni test).
[w]Soil SE= standard error of least square means for soil pasteurization treatments, n = 30.
[v]Inoculation SE= standard error of least square means for inoculation treatments, n = 20.
[u]ANOVA main effects and interactions significant at $P < 0.05$. S = soil pasteurization treatment; M = AMF inoculation treatment, Y = growth cycle from time of inoculation.

certain elements by geophytes has been associated with disease tolerance and dormancy (De Hertogh and Le Nard, 1993). With *Zephyranthes* species we found that AMF inoculation increased the uptake, availability, or storage of P, K, Zn, and carbohydrates in bulbs (Scagel, 2003b) and AMF inoculation of wild hyacinth increased uptake and storage of K, Zn, N, and nonreducing sugars in corms (Scagel, 2003a). Our results with harlequin flower suggest that even though the biomass of daughter corms of AMF-inoculated plants was lower than that of corms from noninoculated plants, differences in corm composition resulting from AMF inoculation may increase corm quality.

ROOT COLONIZATION. The extent of root colonization by mycorrhizal fungi has been related to several plant growth responses to inoculation (Smith and Read, 1997). In our study, mycorrhizal colonization of noninoculated harlequin flower was less than 10% of total root length at the time of harvest, irrespective of soil pasteurization treatment (Fig. 5). AMF-inoculated plants growing in nonpasteurized soil had higher root colonization than AMF-inoculated plants growing in pasteurized soil, and root colonization by AMF increased slightly between the first and second growth cycles. Inoculation increased colonization over background levels of colonization found in noninoculated plants in both pasteurized and nonpasteurized soil. Under the cultural conditions of our experiment, 30-60% of the total root length of the inoculated harlequin flower plants showed signs of colonization by the end of the growth cycle. Others have reported that AMF colonization peaks at mid-bulb-filling stage in garlic (*Allium sativum*)(Al-Karaki, 2002). It is possible that levels of root colonization measured at the end of the growth cycle in our experiment are not representative of the highest levels of colonization present during the rest of the growth cycle.

In our study, soil pasteurization decreased colonization of AMF inoculated plants but had no effect on the low level of colonization on noninoculated plants or plants inoculated with washings from inoculum. This suggests that soil pasteurization created soil characteristics that slightly inhibited AMF colonization and that differences in plant responses to AMF inoculation between pasteurized and nonpasteurized soil (e.g., number of inflorescences, number of flowers per inflorescence, daughter corm weight, weight of cormels) could be related to the level of AMF colonization.

RHIZOSPHERE ORGANISMS. Soil contains populations of organisms that can have beneficial or detrimental effects on plant growth and productivity. Cultural treatments used to control detrimental organisms may not only influence the presence of natural population of mycorrhizal fungi in soil, but also influence the effects of plant inoculation with AMF. Vosátka (1995) found that the growth responses of onion to inoculation were higher when indigenous fungi in the soil were eliminated by steam sterilization. In our experiment, soil pasteurization promoted shoot emergence and increased the number of flowers per inflorescence but delayed flower opening, decreased leaf production and decreased daughter corm biomass, total cormel biomass, and average cormel weight. This suggests that soil pasteurization either removed certain soil organisms present in our experimental system which influence these plant parameters or altered the physical or chemical attributes of the soil which influenced these plant parameters. Similar results were also seen with *Zephyranthes* species, where soil pasteurization generally decreased biomass of leaves, bulbs, and offsets and increased flower production (Scagel 2003b).

Soil fumigation practices commonly used during bulb crop production (De Hertog and Le Nard, 1993) can decrease the levels of AMF in soil, resulting in decreased root colonization. Scagel (2003a, 2003b) reported that soil pasteurization can influence bulb composition and plant development of wild hyacinth and *Zephyranthes* species. These studies used inoculum that consisted of spores and root fragments from AMF pot cultures and did not separate out the potential influence of other rhizosphere organisms that could be present in the inoculum. When we inoculated harlequin flower with washings from AMF inoculum, we found that the rhizosphere organisms associated with the *Glomus intraradices* inoculum significantly influenced several measures of plant development, growth, and corm composition. For instance, flowers on plants inoculated with washings from inoculum opened slightly earlier after planting than flowers on noninoculated plants. When growing in pasteurized soil, plants inoculated with washings from AMF inoculum produced more leaves than noninoculated plants and more flowers per inflorescence. Plants inoculated with washings from AMF inoculum generally produced smaller daughter corms and more cormels than noninoculated plants, and daughter corms had higher concentrations and contents of P, K, Zn, S, N, amino acids, and carbohydrates than corms from noninoculated plants. Since root colonization by AMF on plants inoculated with washings from AMF inoculum was equal to that of noninoculated plants, our results suggest that there are organisms associated with our *Glomus intraradices* inoculum that have beneficial effects on the growth and productivity of harlequin flower, similar to the responses described by Linderman (1988).

Conclusions

Inoculation of harlequin flower with the AMF, *Glomus intraradices*, can influence several aspects of plant development and growth via changes in mineral uptake, resource storage, and biomass partitioning. There are also other organisms associated with our *Glomus intraradices* inoculum that have beneficial effects on the growth and productivity of harlequin flower. While inoculation with AMF can promote shoot emergence, leaf production, and flower production of harlequin flower, inoculation also alters aspects of biomass partitioning and corm composition and quality that play an important role in the production of this crop for corms and cormels.

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