

Inoculation with Arbuscular Mycorrhizal Fungi Alters Nutrient Allocation and Flowering of *Freesia x hybrida*¹

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Abstract

The influence of inoculation with arbuscular mycorrhizal fungi (AMF) on flower and corm production of three *Freesia x hybrida* cultivars grown in pasteurized or non-pasteurized soil was assessed during two growth cycles after inoculation. Shoots on AMF-inoculated plants emerged approximately 2 to 3 days earlier than shoots on non-inoculated plants. AMF had no influence on flower opening in the first growth cycle, but inoculated plants produced flowers approximately 20 days earlier than non-inoculated plants in the second growth cycle. When grown in non-pasteurized soil, inoculated plants produced more leaves, flowers, inflorescences, and flowers per inflorescence than non-inoculated plants. AMF-inoculated plants produced heavier daughter corms than non-inoculated plants. Inoculation with AMF increased the number of cormlets produced by two of the three cultivars, however only one cultivar produced larger cormlets when inoculated. AMF-inoculated plants produced corms with zinc, sulfur, protein, amino acid, and sugar concentrations and contents equal to or higher than corms from non-inoculated plants. Inoculation altered aspects of plant morphology and biomass partitioning important to flower and corm production of *Freesia*. Other soil organisms associated with non-pasteurized soil, and washings from AMF inoculum also play a role in the response of *Freesia* to AMF inoculation.

Index words: arbuscular mycorrhizal fungi, AM, *Glomus intraradices*.

Species used in this study: *Freesia* (*Freesia x hybrida*).

Significance to the Nursery Industry

A balance between resources allocated to flowering and corm production is necessary for maximum productivity and quality of geophytes, such as *Freesia*, used for both flower and corm production. The symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and their plant symbiont alters several aspects of plant development and growth via changes in mineral uptake and biomass partitioning that can differentially influence productivity and quality of corm crops. Addition of AMF to the growing medium of different *Freesia x hybrida* cultivars alters aspects of flower production, corm production, and corm composition during two growth cycles after inoculation. There may also be other organisms associated with AMF inoculum that have beneficial effects on the growth and productivity of *Freesia*. While inoculation can promote shoot and flower emergence and increase the weight of daughter corms produced at the end of the growing cycle, other responses to AMF vary with *Freesia* cultivar and whether the growth medium is pasteurized.

Introduction

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 (30). Several benefits of inoculation with mycorrhizal

fungi on different aspects of productivity and flowering of lilaceous bulb crops have been described for Easter lily (*Lilium longiflorum* L.) (2, 23), however only the presence or absence of mycorrhizae has been reported on most other lilaceous crops (7) and little is known about the influence of AMF inoculation on the production of geophytes (perennial plants with underground storage organs) used in floral crop production. Inoculation of Easter lily with AMF can increase root and stem weight, and responses to inoculation can vary with soil fertility and growth cycle (3, 23). Recent studies by Scagel (27, 28, 29) indicate that inoculation with AMF can alter partitioning of carbon and nutrients that influence flowering and vegetative propagation of Wild Hyacinth (*Brodiaea laxa* Benth. 'Queen Fabiola'), Harlequin Flower (*Sparaxis tricolor* (Schneev.) Ker Gawl.), and species of *Zephyranthes*.

Soil fumigation practices commonly used during bulb crop production can decrease the levels of AMF in soil, resulting in decreased colonization. Scagel (27, 28) reported that soil pasteurization can influence bulb composition and plant development of Wild Hyacinth and *Zephyranthes* spp. These studies involved inoculum that consisted of spores and root fragments from AMF-inoculated plants and did not discern out the potential influence of other rhizosphere organisms that were present in the inoculum. In another study (29), the influence of AMF and other organisms in inoculum was separated by comparing plant growth and productivity of Harlequin Flower by inoculating corms with either inoculum of the AMF, *Glomus intraradices*, or washings from the inoculum containing other organisms present in the inoculum but not AMF. This study showed that other organisms associated with inoculum fostered the growth and productivity of Harlequin Flower.

The genus *Freesia* is in the Iridaceae family, the same family as Harlequin Flower. There are several species and hybrids of *Freesia* used in cut flower production. *Freesia* spp. grow from corms with their active period of growth and flowering in the spring and a rest period in the summer. The objectives

¹Received for publication April 10, 2003; in revised form October 1, 2003. The author gratefully acknowledges the technical assistance of Kathleen Eggemeyer, Jesse Mitchell, Lisa Tribbet, and Benjamin Jackson. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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of this study were (1) to determine whether addition of AMF inoculum into the growing medium of different *Freesia x hybrida* cultivars alters flower production, corm production, and corm chemical composition, and (2) to assess whether the 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 three *Freesia* single-flowering cultivars (*Freesia x hybrida* 'Blue', 'White', and 'Yellow') were obtained from the Netherland Bulb Co., Inc. (Easton, PA) and were planted into cylindrical (1 gal) pots (Lerio 7 5/8 × 7 1/8 in) containing either a air-steam pasteurized [60C (140F) for 30 min] or non-pasteurized 1:1 (by vol) mixture of a Willamette Valley alluvial silt loam and river sand [11 mg/kg available (Bray) phosphorus (P), pH of 6.3]. For the AMF treatment (AMF), inoculum of the AM fungus (*Glomus intraradices* Schenck & Smith) containing spores, colonized root fragments, and other propagules in a sand-based carrier at a rate of 1:166 (by vol; inoculum:soil) was placed beneath the base of each corm at planting. For controls (None), sterilized inoculum was added at the base of each corm at the same rate. A third inoculation treatment (WASH) was used to assess the influence of rhizosphere soil microflora from the AMF inoculum. For the WASH treatment, sterilized inoculum was added at the base of each corm at the same rate used in the AMF and None treatments, and 50 ml of washings from non-sterilized inoculum were applied to pots as a soil drench after being passed through a 38- μ m sieve (Tyler equivalent 400-mesh) and Whatman 1 filter paper.

Mycorrhizal inoculum. *Glomus intraradices* Schenck & Smith was originally obtained from Native Plants Incorporated, (Salt Lake City, UT) and maintained in pot cultures at the USDA-ARS, Horticultural Crops Research Laboratory in Corvallis, OR. The fungus was propagated in pot cultures on roots of bunching onion (*Allium cepa* L. 'White Lisbon') grown in 1:1 (by vol) loam:sand for 5 months. Inoculum consisted of a mixture of the soil medium, extraradical hyphae and spores, and colonized root segments [<2 mm (0.079 in) in length]. Population estimate of the inoculum by the MPN method (35) averaged 10 propagules/g of soil medium.

Cultural conditions. Plants were maintained in a glasshouse with supplemental light (16/8 h, light/dark) provided by high pressure multi-vapor lamps with an average of 700 μ mol/sq m/s at canopy level, and average day/night temperatures of 16/12C for 6 weeks, then 20/15C for the remainder of the growth cycle. Plants were fertilized once a week with 50 ml of a liquid fertilizer [approximately 10% potassium (K), 10% P, 40% nitrogen (N), 20% calcium (Ca), 7% magnesium (Mg), 8% sulfur (S), 4% sodium (Na), and less than 0.05% of 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 in the greenhouse and included Diflubenzuron for fungus gnats (*Bradysia* spp.), *Neoseiulus fallacis* predators for spider mites (*Tetranychus* spp.), and *Neoseiulus cucumeris* predators for thrips (*Frankiniella* spp). At the end of the first growth cycle, when stems had died-back, corms were removed from the soil, dried at 25C (77F) for 2 weeks, stored at 30C (85F) for six weeks, then stored at 20C (68F) for 2

weeks until planting. Corms were planted into cylindrical (1 gal) pots (Lerio 7 5/8 × 7 1/8 in) containing a 1:1 (by vol) 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 growth cycles. To assess carry-over effects from the first growth cycle, no inoculation treatments were done for the second growth cycle and all soil was pasteurized.

First growth cycle measurements. For each corm the number of days after planting until shoot emergence and flower emergence was recorded. Flower emergence was indicated by at least one fully open floret with little to no chlorophyll in the tepals (31). The number of inflorescences and the total number of flowers on each plant was recorded. At the end of the growth cycle, when shoots had died back, plants were harvested and corms and cormlets were counted, air dried, and weighed. AM colonization of fresh roots was assessed on 1-cm sections after clearing and staining by modified procedures of Phillips and Hayman (26), replacing lacto-phenol with lacto-glycerin. Percentage of root length with signs of AM colonization was estimated by the method of Biermann and Linderman (4). Ten daughter corms per treatment were placed in storage. Another 10 daughter corms per treatment were analyzed for P, K, Ca, Mg, Mn, iron (Fe), Cu, B, Zn, carbon (C), N, and S content using standard methods (12). N and S were determined after automated combustion and concentrations of the remainder of the elements determined after dry-ash oxidation by optical emission spectrometry with inductively coupled plasma (ICP-AES). Total soluble protein was determined colorimetrically using BIO-RAD (Coomassie Brilliant blue) (6) after extraction of ground corm tissue (<50 mesh) in buffer (20mM TRIS, 10mM NaCl, 10mM KCl, 2 mM MgCl₂·6H₂O) with Nonidet P-40. Total amino acid content of corms was determined colorimetrically with ninhydrin (37) after extraction with acetic acid prior to analysis. Total reducing and non-reducing sugar content of corms were determined colorimetrically using a modification of the Somogyi-Nelson Alkaline Copper method (9, 25). 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 KOH prior to analyses for non-reducing sugars.

Second growth cycle measurements. For each corm the number of days after planting until shoot emergence and flower emergence was recorded. The number of inflorescences and total number of flowers on each plant were also recorded. At the end of the growth cycle, when shoots had died back, plants were harvested and corms and cormlets were counted, air-dried, and weighed. Daughter corms were analyzed for mineral composition and storage compounds as described for samples from 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. Data were subjected to analysis of variance (ANOVA) for with cultivar, soil pasteurization treatment, AMF inoculation, and growth cycle from inoculation as main effects. Single-degree-of-freedom contrasts were used to address specific questions re-

Table 1. Influence of soil pasteurization on the time of shoot emergence and flower opening after planting and number of flowers and inflorescences of three *Freesia x hybrida* cultivars.

Soil pasteurization treatment ^a	Cultivar ^b	Time after planting (d) ^a		Flowers and inflorescences (no./plant)	
		Shoot emergence	Flower opening	Flowers	Inflorescences
PS	B	10.6a*	180c	30.8a	3.0a
	W	11.3ab	182c	35.3a	6.4d
	Y	10.9ab	151a	37.2ab	5.2bc
NPS	B	11.4b	166b	48.9bc	4.6b
	W	14.0c	165b	56.7c	6.1cd
	Y	11.0ab	154a	40.8ab	4.9bc

^aPS = pasteurized soil; NPS = non-pasteurized soil.

^bB = Blue, W = White, Y = Yellow *Freesia x hybrida* cultivars.

^aMeans averaged over two growth cycles and inoculation treatments.

^aMeans followed by the same letter or letters within a variable are not significantly different from each other ($p < 0.05$, Bonferroni's Test).

lated to interactions between main effects. Data with unequal variances between treatment groups were log-transformed to equalize variances. Back-transformed data are presented in tables. Relationships between corm composition data and plant morphological data were analyzed using Pearson's Correlation Coefficient (r). Bonferroni test was used to separate treatment means. All data analyses were performed using the Statistica® statistical package (Statsoft, Inc., Tulsa, OK).

Results and Discussion

Leaf development. Shoot emergence of *Freesia* can occur 9–20 d after planting depending on temperature (15). In our study shoot emergence of the different *Freesia* cultivars varied between 10–15 d after planting, and inoculation with AMF decreased the time until shoot emergence. We found that inoculation with AMF altered leaf development of *Freesia x hybrida*, and responses to inoculation depended on the *Freesia* cultivar and whether the growing medium had been pasteurized. Soil pasteurization decreased the number of days until stem emergence for the blue and white cultivars but not the yellow cultivar (Table 1). Shoots on plants inoculated with AMF emerged approximately 2 to 3 days earlier than shoots on non-inoculated plants during both growth cycles and response to AMF inoculation was greater in the second than the first growth cycle (Table 2).

AMF inoculation has also been reported to hasten shoot emergence of Harlequin Flower (29), and *Zephyranthes* spp. (28) but can delay emergence of Wild Hyacinth (27), and Easter lily (23). AMF promotion of shoot emergence may play a role in carbohydrate status of the plant by extending the period of photosynthetic activity in the geophyte life cycle. In other plants, AMF colonization increases photosynthetic activity and hastens leaf appearance (11, 19) and may be an adaptive response of the plant to the increased carbohydrate demands resulting from AMF colonization.

Flower development. Many factors can influence the time of flowering of *Freesia x hybrida* including greenhouse temperatures, corm storage temperatures, and growth regulators (15, 36). Continuous lighting or lighting during flower development can also hastened flowering but reduce the number of flowering stems per corm, as well as stem length and weight (5). We found that both soil pasteurization and inocu-

lation treatments influenced flower opening of *Freesia*, and response to inoculation treatments was independent of cultivar. Flowers on blue and white cultivars opened later when grown in pasteurized soil than non-pasteurized soil; the yellow cultivar was not influenced by soil pasteurization (Table 1C). Plants inoculated with AMF opened slightly earlier (~10 d) than flowers on non-inoculated plants during the first growth and approximately 20 d earlier than flowers on non-inoculated plants during the second growth cycle. (Table 2D).

Differences in resource allocation during flower initiation resulting from AMF colonization have the potential to delay flowering. Mora (23) reported that inoculation of Easter lily with AMF did not affect flower emergence, although flower emergence varied with nitrogen type used in the fertilizer. AMF inoculation of Pink Fairy Lily (*Z. robusta* (Herb. Ex Sweet) baker syn *Habaranthus robustus* Sweet) and White Rain Lily (*Z. candida* (Lindl.) Herb.) has been reported to delay flower emergence but not the time of flower opening (28), while inoculation of Harlequin Flower (29), Yellow Zephyr Lily (*Z. sulphurea* syn. *Z. citrina* Baker) (28) and Wild Hyacinth (27) with AMF hastened flower emergence. Earlier flowering of AMF-inoculated plants suggests that any changes in resource allocation resulting from the establish-

Table 2. Influence of inoculation treatments on the time of shoot emergence and flower opening after planting of three *Freesia x hybrida* cultivars for two growth cycles after inoculation.

Growth cycle ^a	Inoculation treatment ^b	Time after planting (d) ^a	
		Shoot emergence	Flower opening
1	AMF	10.9b*	162ab
	WASH	11.4bc	168b
	NONE	11.9c	165b
2	AMF	9.9a	156a
	WASH	12.0c	185b
	NONE	13.1d	180b

^a1 = first growth cycle, 2 = second growth cycle after inoculation treatments.

^bNONE = sterilized inoculum of arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*, WASH = sterilized AMF inoculum and washings from non-sterilized AMF inoculum, AMF = AMF inoculum.

^aMeans averaged over soil pasteurization treatments and cultivars.

^aMeans followed by the same letter or letters within a variable are not significantly different from each other ($p < 0.05$, Bonferroni's Test).

ment 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 that influence flower development (18).

Altered plant development in response to AMF inoculation suggests that some sort of signaling occurs between the fungus or microorganisms in the inoculum and the plant. It is known that endogenous hormones are involved in growth and dormancy control of *Freesia* corms and cormlets. Increases in cytokinins (21), indoleacetic acid (IAA) (13), and ethylene (33) are related to dormancy release and hastened plant development. Mycorrhizal fungi produce or induce production of cytokinins, IAA, and ethylene (30, 32). It is possible that AMF may alter aspects of *Freesia* development including shoot emergence and flowering through hormonal signals between the fungus and the plant.

Leaf production. In *Freesia*, leaf differentiation in new corms for the following growth cycle ceases when mother plants finish flowering, and resumes when dormancy is broken. Leaf number of *Freesia* can vary from 15 to 50 leaves per plant depending on temperature (15). In our study plants produced between 25 to 40 leaves per plant depending on soil pasteurization and inoculation treatment, and response to inoculation was similar in both production cycles. Plants growing in pasteurized soil produced a similar number of leaves regardless of inoculation treatment, while in non-pasteurized soil, plants that were inoculated with either AMF or washings from AMF inoculum produced more leaves than non-inoculated plants (Table 3). Differences in the number of leaves on plants from the different inoculation treatments were detectable approximately 120 d after planting. Leaf production stopped prior to flower emergence for all treatments.

Increased leaf production in AMF-inoculated plants during both production cycles indicates that inoculation with AMF not only increased photosynthetic leaf surface during the year of inoculation but had carry-over effects on leaf production in the following growth cycle. A similar response was reported for *Zephyranthes* spp. Shoots of inoculated plants emerged earlier than those of non-inoculated plants and the total number of leaves on plants was increased by AMF inoculation (28). In contrast, even though shoots of AMF-inoculated Harlequin Flower emerged earlier than those of non-inoculated plants, the total number of leaves produced per plant was only increased by AMF inoculation when plants were grown in pasteurized soil (29). These differences in response of plants when grown in pasteurized and non-pasteurized soil suggests that soil pasteurization alters the soil environment in such a way that AMF inoculation does not promote leaf production. This means that there are several factors regulating changes in the photosynthetic surface area of AMF-inoculated plants. Further analysis of the photosynthetic efficiency and leaf area development in response to AMF inoculation would allow for a better understanding of the role of these fungi in carbon partitioning and efficiency of vegetative growth during production of *Freesia*.

Flower production. The size and number of inflorescences on plants is important for *Freesia* cut flower production (15). We found that the total number of flowers and inflorescences per plant and flowers per inflorescence were influenced by

Table 3. Influence of soil pasteurization inoculation treatments on the number of flowers, inflorescences, and leaves of three *Freesia x hybrida* cultivars.

Soil pasteurization treatment ^a	Inoculation treatment ^b	Flowers	Inflorescences	Leaves
		(no./plant)		
PS	AMF	42.0bc*	5.2ab	30.3bc
	WASH	34.9ab	4.9a	30.5abc
	NONE	26.3a	4.4a	28.8ab
NPS	AMF	63.6d	6.2b	34.8c
	WASH	49.5c	5.3ab	30.9bc
	NONE	33.2ab	4.1a	24.7a

^aPS = pasteurized soil; NPS = non-pasteurized soil.

^bNONE = sterilized inoculum of arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*, WASH = sterilized AMF inoculum and washings from non-sterilized AMF inoculum, AMF = AMF inoculum.

^cMeans averaged over two growth cycles and cultivars.

^dMeans followed by the same letter or letters within a variable are not significantly different from each other ($p < 0.05$, Bonferroni's Test).

both soil pasteurization and inoculation treatments, and responses to inoculation were independent of cultivars. Soil pasteurization decreased the number of flowers and inflorescences on the blue *Freesia* cultivar, had no effect on the number of flowers or inflorescences on the yellow *Freesia* cultivar, and decreased the number of flowers but had no effect on the number of inflorescences on the white cultivar (Table 1). Plants inoculated with AMF produced more flowers than non-inoculated plants, regardless of soil pasteurization treatment, while inoculation only increased the number of inflorescences on plants growing in non-pasteurized soil (Table 3). Differences in the number of flowers and inflorescences between inoculated and non-inoculated plants in the different soil pasteurization treatments resulted in plants inoculated with AMF having more flowers per inflorescence than non-inoculated plants when growing in pasteurized soil. Plants with earlier flower emergence produced more flowers ($r = 0.664$, $p < 0.001$) and plants with more leaves produced more inflorescences ($r = 0.884$, $p < 0.0001$).

The different flowering responses of bulbs and corms to AMF inoculation may be a result of genetic difference 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 and leaves of a similar size (28). The influence of AMF inoculation on carbon partitioning during flower development of Pink Fawn Lily may be greater than the influence of AMF inoculation on Yellow Zephyr Lily as a result of a higher demand for carbon based on flower size. Although flowers of *Freesia* are much larger than those of *Zephyranthes* spp., leaf production is also much greater and lasts for a longer period of time before flowering. It is possible that the increase in leaf production resulting from AMF inoculation may help compensate for the increased carbohydrate demand of the symbiosis without negatively influencing flower production.

Differences in resource allocation during growth of *Freesia* could differentially influence production for corms or cut flowers. Colonization of roots by AMF causes differences in plant biomass partitioning (18). *Freesia* flower initiation occurs after shoot emergence at the same time as new corms are developing. One important characteristic for commer-

Table 4. Influence of soil pasteurization and inoculation treatments on cormlet production and weight of three *Freesia x hybrida* cultivars.

Cultivar ^a	Inoculation treatment ^b	Soil pasteurization treatment ^c	Weight (g/plant) ^d		Ratio of Corm:Corm + Cormlet (g/g) ^e
			Corms + Cormlets	Corms	
B	AMF	PS	12.5a [*]	4.5b	0.41cd
		NPS	23.8b	5.6c	0.28a
	WASH	PS	12.7a	4.4b	0.43de
		NPS	25.3b	5.6c	0.36b
	NONE	PS	12.1a	3.8a	0.47e
		NPS	26.8b	5.5c	0.37bc
W	AMF	PS	85.1d	4.8b	0.06a
		NPS	52.7b	5.3b	0.10a
	WASH	PS	68.3c	4.5ab	0.07a
		NPS	49.2ab	4.7ab	0.09ab
	NONE	PS	47.9ab	3.9a	0.12b
		NPS	43.2a	3.8a	0.17c
Y	AMF	PS	56.3b	4.8a	0.11a
		NPS	56.9b	4.9a	0.11a
	WASH	PS	52.5ab	4.4a	0.09a
		NPS	52.1ab	4.8a	0.10a
	NONE	PS	45.9a	4.2a	0.20b
		NPS	44.6a	4.8a	0.18b

^aB = Blue, W = White, Y = Yellow *Freesia x hybrida* cultivars.

^bNONE = sterilized inoculum of arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*, WASH = sterilized AMF inoculum and washings from non-sterilized AMF inoculum, AMF = AMF inoculum.

^cPS = pasteurized soil; NPS = non-pasteurized soil.

^dMeans averaged over two growth cycles.

^eMeans followed by the same letter or letters within a variable and cultivar are not significantly different from each other ($p < 0.05$, Bonferroni's Test).

cial flower production of *Freesia* includes lateral stem number and the number of flowers per inflorescence (15). Reports of low light intensity decreasing flower initiation (15) and elevated CO₂ concentrations increasing flower production (10) suggest that photosynthetic efficiency plays an important role in flower production of this crop. In our study, inoculation with AMF increased the numbers of leaves on plants and but increased flower production only when plants were growing in non-pasteurized soil. Similar results were reported for Harlequin Flower (29) and Wild Hyacinth (27); AMF increased the number of flowers per inflorescence and the number of leaves per plant, in both pasteurized and non-pasteurized media.

Corm and cormlet production. Daughter corm size and vegetative production via cormlets is important to corm production of *Freesia* (15). We found that differences in daughter corm and cormlet weight and partitioning of biomass between daughter corms and cormlets was influenced by inoculation with AMF, however responses to inoculation were generally dependant on both soil pasteurization treatment and cultivar. Soil pasteurization decreased the total weight of daughter corms and cormlets and inoculation treatments had no effect on the total weight of daughter corms and cormlets of the blue *Freesia* cultivar (Table 4). Inoculation with AMF increased the total weight of corms and cormlets of the white and yellow *Freesia* cultivar regardless of soil pasteurization treatment. The weight of daughter corms of the white *Freesia* cultivar was increased by inoculation with AMF in regardless of soil pasteurization treatment while inoculation with AMF had no effect on the weight of daughter corms of the yellow *Freesia* cultivar (Table 4). Daughter corms of the

Table 5. Influence of soil pasteurization and inoculation treatments on biomass partitioning to corms (daughter corms) and cormlets of three *Freesia x hybrida* cultivars.

Cultivar ^a	Inoculation treatment ^b	Soil pasteurization treatment ^c	Cormlets ^d	
			Number (#/plant)	Weight (g/cormlet)
B	AMF	PS	2.2a [*]	3.6a
		NPS	5.5c	3.5a
	WASH	PS	2.0a	3.9b
		NPS	4.7bc	4.1b
	NONE	PS	2.1a	3.4a
		NPS	3.9ab	4.2b
W	AMF	PS	17.3d	4.6a
		NPS	11.2b	4.4a
	WASH	PS	14.9c	4.3a
		NPS	9.6ab	4.5a
	NONE	PS	12.1b	4.6a
		NPS	7.6a	4.4a
Y	AMF	PS	7.2a	7.0b
		NPS	8.1a	6.8b
	WASH	PS	7.1a	6.6b
		NPS	7.3a	6.7b
	NONE	PS	6.8a	6.2a
		NPS	6.6a	6.1a

^aB = Blue, W = White, Y = Yellow *Freesia x hybrida* cultivars.

^bNONE = sterilized inoculum of arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*, WASH = sterilized AMF inoculum and washings from non-sterilized AMF inoculum, AMF = AMF inoculum.

^cPS = pasteurized soil; NPS = non-pasteurized soil.

^dMeans averaged over two growth cycles.

^eMeans followed by the same letter or letters within a variable and cultivar are not significantly different from each other ($p < 0.05$, Bonferroni's Test).

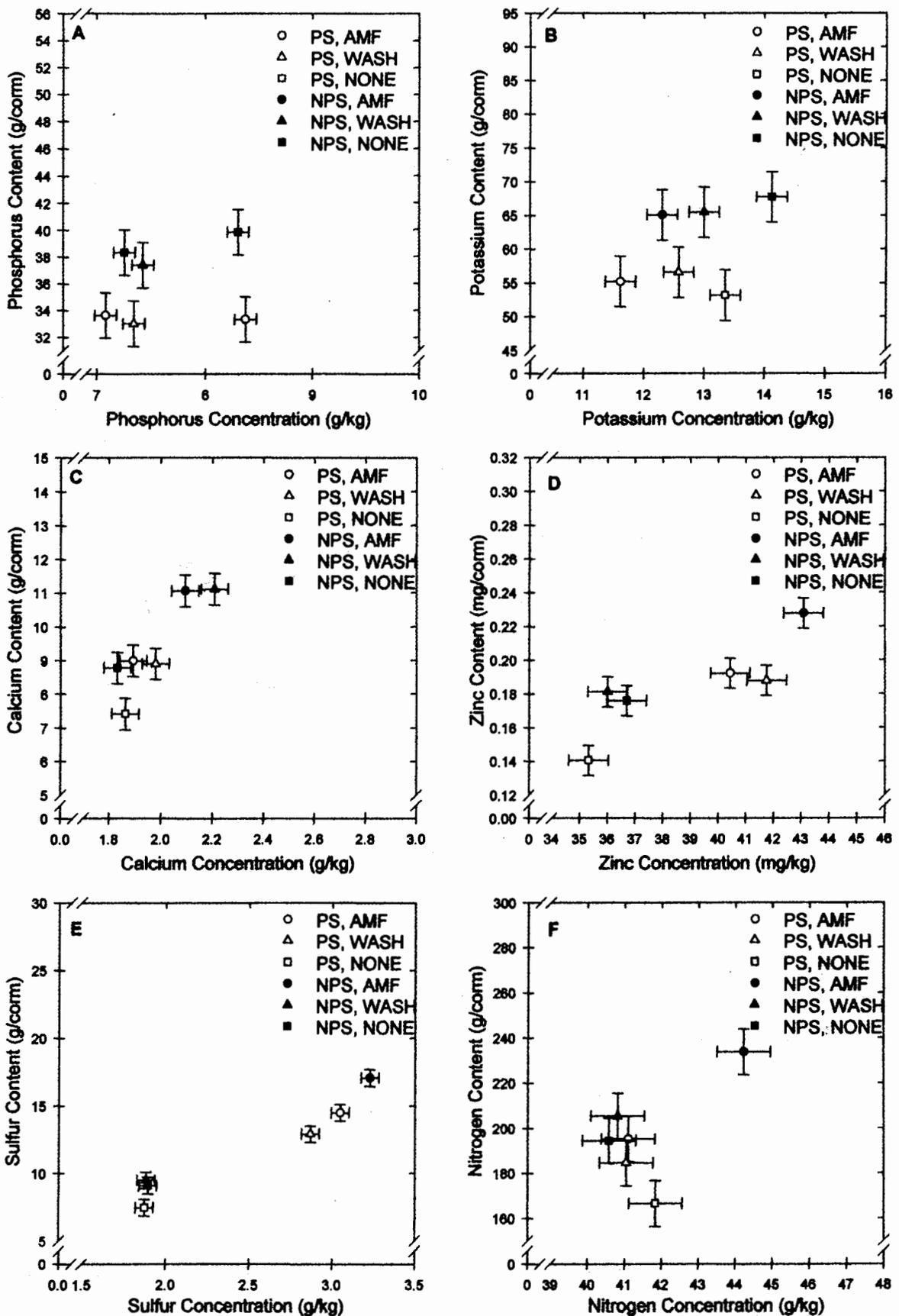


Fig. 1. Phosphorus, potassium, calcium, zinc, sulfur, and nitrogen concentration and content of *Freesia x hybrida* corms in response to inoculation and soil pasteurization treatments. Error bars are standard error of the least squares means across cultivars. NONE = sterilized AMF inoculum; WASH = sterilized AMF inoculum and washings from non-sterilized inoculum; AMF = AMF inoculum (*Glomus intraradices*); NPS = Non-pasteurized soil; PS = Pasteurized soil.

blue *Freesia* cultivar were heavier when inoculated with AMF, but only when plants were grown in pasteurized soil. The ratio of daughter corm weight to the combined weight of daughter corms and cormlets was used to compare biomass partitioning between corms and cormlets. In general, non-inoculated plants partitioned more biomass towards corms than cormlets in comparison to plants inoculated with AMF or washings from AMF inoculum (Table 4).

AMF-inoculated plants of the blue *Freesia* cultivar produced more cormlets when grown in non-pasteurized soil than in pasteurized soil (Table 5), however, AMF-inoculated plants generally produced smaller cormlets than non-inoculated plants (Table 5). The white *Freesia* cultivar produced more cormlets when inoculated with AMF, regardless of soil pasteurization treatment but inoculation had no influence on cormlet size. The number of cormlets produced by the yellow *Freesia* cultivar were not influenced by soil pasteurization or inoculation treatment, however, plants inoculated with AMF generally produced larger cormlets. Cormlet production was positively correlated with leaf number ($r = 0.671$, $p < 0.0001$).

The yield of *Freesia* corms and cormlets can also be influenced by supplemental lighting (5) and elevated CO₂ concentrations (10) during plant growth. *Freesia* stocks are usually increased asexually from cormlets (15). Propagation of *Freesia* occurs by cormlets that develop from lower axillary buds of the developing daughter corm. Cormlets start to develop after flowering and reach maturity at the same time as new daughter corms. Anatomically, cormlets consist of swollen stem tissues. In our study, we found that for two of the three cultivars, daughter corm biomass was slightly increased by inoculation with AMF (Table 4), however AMF only increased total cormlet weight and the number of cormlets produced for only one of the three cultivars tested (Table 5). Plants inoculated with AMF tended to partition a higher proportion of biomass to the cormlets than to the main daughter corm (Table 4), however because AMF inoculated plants produced more cormlets than non-inoculated plants, the average size of cormlets on inoculated plants was similar or smaller than that on non-inoculated plants (Table 5). Inoculation of Wild Hyacinth with AMF was also found to increase daughter corm weight, and inoculated plants produced more but smaller cormlets than non-inoculated plants (27). In contrast, daughter corm biomass of AMF-inoculated Harlequin Flower was lower than that of non-inoculated plants, and AMF-inoculated plants partitioned a higher proportion of biomass to cormlets than daughter corms; however inoculated plants also produced more cormlets than non-inoculated plants, resulting in a smaller average cormlet size in AMF-inoculated plants compared to non-inoculated plants (29). It appears with *Freesia*, Harlequin Flower, and Wild Hyacinth that the effects of AMF inoculation are conducive for increasing the number of vegetative propagules but not propagule size.

Corm chemical composition. In general, corms from plants grown in pasteurized soil had lower concentrations of K and Ca, and lower total content of most elements than plants grown in non-pasteurized soil (Fig. 1A–F). Corms from plants inoculated with AMF or washings from AMF inoculum had lower P, K concentrations than corms from non-inoculated plants, but similar total P and K content (Fig. 1A, B). This suggests that P and K were not limiting to growth in our

experimental system and differences in P and K concentrations resulting from inoculation treatments were primarily a result of increased corm weight of AMF inoculated plants. Both the concentrations and total content of Zn and S were higher in corms of AMF-inoculated plants than non-inoculated plants (Fig. 1D, E). Increased concentrations and content suggests that inoculation with AMF increased uptake of Zn and S. Washings from AMF inoculum increased uptake of Zn and S, but only when plants were grown in pasteurized soil. The concentration and total content of Ca were higher in corms inoculated with AMF or washings from AMF inoculum than in non-inoculated corms, but only when plants were grown in non-pasteurized soil (Fig. 1C). Corms of AMF-inoculated plants contained higher concentrations and total content of N, but only when grown in non-pasteurized soil (Fig. 1F). The influence of soil pasteurization on the availability of Ca and N in our experimental system influenced the influence of inoculation treatments on uptake and storage of these elements. P concentration of corms was positively correlated with Zn ($r = 0.872$, $p < 0.0001$), S ($r = 0.793$, $p < 0.0001$), and N ($r = 0.625$, $p < 0.0001$) concentration of corms. Soil pasteurization had no influence on the ratio of P/N, K/N, or S/N in corms while corms from AMF-inoculated plants had lower P/N and K/N and higher S/N ratios than corms from non-inoculated plants.

Increased uptake of elements from soil in response to AMF inoculation frequently has been related to plant growth responses to inoculation (30). Increased uptake of certain elements by geophytes has been associated with disease tolerance and dormancy (8). Corms from *Freesia* inoculated with AMF generally had higher concentrations and content of S and Zn, indicating increased uptake of these elements. Inoculated corms also had lower concentrations of other minerals than corms from non-inoculated plants, suggesting that these minerals were not limiting to plant growth under our cultural conditions, and that increased content in corms from AMF inoculated plants was primarily a function of increased corm weight in response to inoculation. In contrast, with other geophytes inoculation with AMF has been reported to increase uptake of P (28, 29), K, Zn (27, 28, 29), S (29), and N (27, 29).

Soil pasteurization generally increased the concentration of proteins, amino acids, reducing and non-reducing sugars in corms (Fig. 2A–D). Corms from plants growing in pasteurized soil generally had total protein, amino acid, and sugar contents similar to or less than corms from plants grown in non-pasteurized soil. Plants inoculated with AMF or inoculum washings produced corms with protein, amino acid, and sugar concentrations and contents similar to or higher than corms from non-inoculated plants (Fig. 2A, B, C, D). Plants that produced larger corms or more cormlets had lower concentrations of proteins and carbohydrates in corms than plants that produced smaller corms or fewer cormlets. Amino acid concentrations were highest in plants that produced more cormlets.

The size of corms is commonly used for grading purposes in corm production systems. In general, corm size is thought to be related to corm quality. 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. In developing storage organs such as corms, translocated photosynthates are converted into carbon and nitrogen reserves

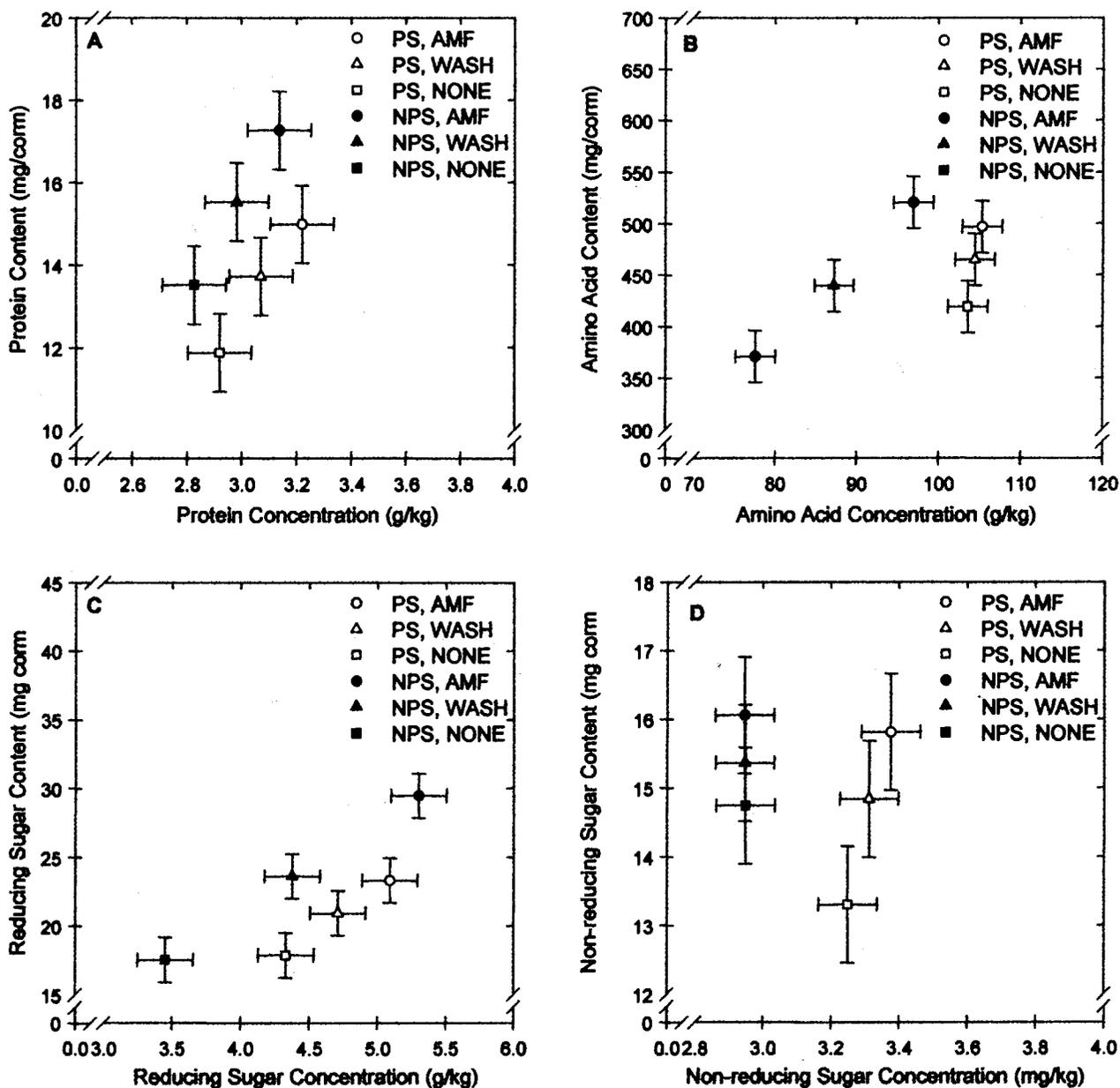


Fig. 2. Protein, amino acid, reducing sugar and non-reducing sugar concentration and content of *Freesia x hybrida* corms in response to inoculation and soil pasteurization treatments. Error bars are standard error of the least squares means across cultivars. NONE = sterilized AMF inoculum; WASH = sterilized AMF inoculum and washings from non-sterilized inoculum; AMF = AMF inoculum (*Glomus intraradices*); NPS = Non-pasteurized soil; PS = Pasteurized soil.

such as starch, fructans, oils, and storage proteins (17). Kaneko and Imanishi (16) reported that the total sugar content of *Freesia* corms was lowest at harvest (~2%) and around 8% at the end of dormancy. At harvest, we found that the total sugar content was highest in 'Blue' *Freesia* (1.6–2.1%) and much lower in the 'White' and 'Yellow' cultivars (0.8–1.2%) and that inoculation with AMF generally increased concentrations and contents of proteins, amino acids, and reducing sugars in corms. Inoculation also increases storage of sugars, proteins, and amino acids in Harlequin flower (29) and increased storage of reducing sugars in *Zephyranthes* spp. (28) and non-reducing sugars in Wild Hyacinth (27).

Root colonization. Non-inoculated plants and plants inoculated with washing from AMF inoculum all showed some

signs of colonization by mycorrhizal fungi, irrespective of soil pasteurization treatment (Fig. 3). Plants inoculated with AMF had between 30 and 80% of their roots colonized by mycorrhizal fungi, and soil pasteurization decreased root colonization (Fig. 3). The extent of root colonization by mycorrhizal fungi has been related to several plant growth responses to inoculation (30). In our study, AMF colonization of non-inoculated *Freesia* or plants inoculated with washings from AMF inoculum was less than 10% of total root length at the time of harvest, irrespective of soil pasteurization treatment. Inoculation increased colonization over background levels of colonization found in non-inoculated plants in both pasteurized and non-pasteurized soil. Under the cultural conditions of our experiment, 25–80% of the total root length of the inoculated *Freesia* plants was colonized

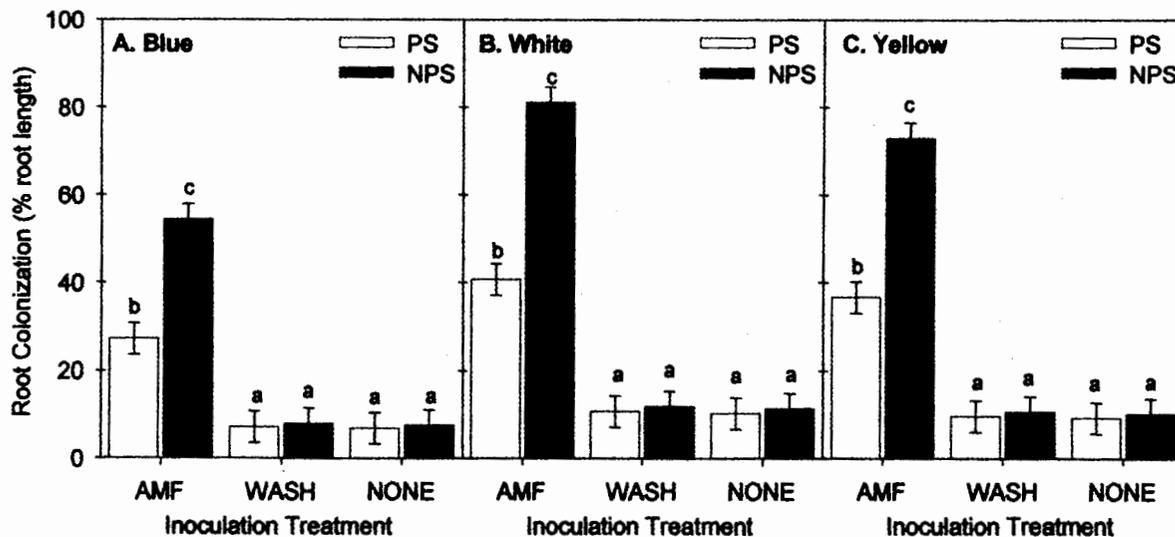


Fig. 3. Root colonization of three *Freesia x hybrida* cultivars (Blue, White, and Yellow) corms by AMF in response to inoculation and soil pasteurization treatments. Error bars are standard error of the least squares means. NONE = sterilized AMF inoculum; WASH = sterilized AMF inoculum and washings from non-sterilized inoculum; AMF = AMF inoculum (*Glomus intraradices*); NPS = Non-pasteurized soil; PS = Pasteurized soil. Different letters above bars within each cultivar represent treatment means significantly different from each other ($p < 0.05$, Bonferroni's Test).

by the end of the growth cycle. Others have reported that AMF colonization peaks at mid-bulb-filling stage in garlic (*Allium sativum* L.) (1). It is possible that levels of root colonization measured at the end of the growing 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 non-inoculated plants or plants inoculated with washings from AMF inoculum. This suggests that soil pasteurization created soil characteristics that slightly inhibited VAMF colonization and differences in plant responses to AMF inoculum between pasteurized and non-pasteurized soil could be related to the level of AMF colonization.

Rhizosphere organisms. *Freesia* are produced in greenhouses both for corm and cut flowers and greenhouse beds are generally steam sterilized before planting (8). Soil contains populations of organisms that can have beneficial or detrimental effects of plant growth and productivity. Cultural treatments used to control detrimental organisms may not only influence the presence of natural populations of mycorrhizal fungi in soil, but also influence the effects of plant inoculation with AMF. Others (34) have reported that the growth responses of onion (*Allium cepa* L.) to inoculation were higher when indigenous fungi in the soil were eliminated by steam sterilization. In our experiments, soil pasteurization promoted shoot emergence and flower emergence but decreased the numbers of flowers and inflorescences, and corm size. This suggests that soil pasteurization either removed certain soil organisms present in our experimental system that 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 Harlequin Flower and *Zephyranthes* spp.; soil pasteurization generally decreased leaf and bulb biomass and increased flower production (28, 29).

Soil fumigation practices commonly used during bulb crop production (8) can decrease the population levels of AMF in soil, resulting in decreased root colonization. Soil pasteurization influences bulb composition and plant development of Wild Hyacinth and *Zephyranthes* spp. (27, 28). AMF inoculum used in these studies consisted of spores and root fragments from AMF pot cultures and did not separate out the potential influence of other rhizosphere organisms present in the inoculum. When we inoculated *Freesia* with washings from AMF inoculum, we found that the rhizosphere organisms associated with the *Glomus intraradices* inoculum influenced several measures of plant development, growth, and corm composition. For instance, shoots on plants inoculated with washings from inoculum emerged slightly earlier after planting than shoots on non-inoculated plants during the second growing cycle. When growing in non-pasteurized soil, plants inoculated with washings from AMF inoculum produced more flowers and leaves than non-inoculated plants in the second growing cycle. Large bacterial populations are associated with the mycelium from AM fungi (20) and some rhizosphere bacteria can synergistically effect AMF colonization and resultant plant growth and mineral nutrition (14, 24). Since AMF colonization on roots of plants inoculated with washings from AMF inoculum was similar to that of non-inoculated plants, our results suggest that there are organisms associated with our *G. intraradices* inoculum that have beneficial effects on the growth and productivity of *Freesia* similar to the responses of Harlequin Flower to rhizosphere organisms (29).

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