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ARBUSCULAR MYCORRHIZAL FUNGI AND *TRICHODERMA HARZIANUM* IN RELATION TO BORDER CELL PRODUCTION AND FUSARIUM ROOT ROT OF ASPARAGUS

By

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ABSTRACT

ARBUSCULAR MYCORRHIZAL FUNGI AND *TRICHODERMA HARZIANUM* IN RELATION TO BORDER CELL PRODUCTION AND FUSARIUM ROOT ROT OF ASPARAGUS

By

Laura L. Arriola

Four species from the family Amaranthaceae were studied to determine border cell (sloughed root cap cells) production and arbuscular mycorrhizal colonization. Border cells were produced by all species studied and the number of border cells increased with increasing root length until a maximum was reached at 25 mm. Arbuscular mycorrhizal root colonization was found in all the Amaranthaceae species and was positively correlated with maximum border cell production.

In a second study, commercially available forms of the arbuscular mycorrhizal fungus *Glomus intraradices* and *Trichoderma harzianum* were investigated as biocontrol agents of *Fusarium oxysporum* f. sp. *asparagi* inoculated (at high and low concentrations) asparagus. Death rates of biocontrol treated plants were less than half those of plants inoculated only with *F. oxysporum* . Shoot height, weight and number of shoots produced was greater in biocontrol treated plants than in plants inoculated only with *F. oxysporum*.

This work is dedicated to my husband Joe and
my children Roxanna and Caleb

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INTRODUCTION

Arbuscular mycorrhizae and border cells

Arbuscular mycorrhizal (AM) fungi are ubiquitous soil organisms capable of forming symbiotic associations with many plant species (1). Taxonomically AM fungi are in the order Glomales within the Zygomycetes (4) and belong to *Glomus*, *Sclerocystis*, *Acaulospora*, *Entrophospora*, *Gigaspora*, and *Scutellospora*. Arbuscular mycorrhizal fungi are not host specific (1, 61). However, there is evidence that some fungal genera are more capable symbionts than others (1).

Arbuscular mycorrhizal symbiosis is the most common form of mycorrhizal plant association and exists in most angiosperms in a wide range of environments (27). Among the plants that form AM associations are several major crop and horticultural species, including, corn (*Zea mays*), wheat (*Triticum aestivum*), oat (*Avena sativa*), cucumber (*Cucumis sativus*), clover (*Trifolium repens*), soybean (*Glycine max*), bean (*Phaseolus vulgaris*), coffee (*Coffea arabica*) and citrus (*Citrus* spp.) (23, 39, 54). The AM fungus receives photosynthate and aids in plant growth and health. A major benefit to the plant is enhanced uptake and translocation of phosphorus (39, 51),copper, zinc, calcium and sulfur (21, 35, 62). In addition symbiosis with the AM fungus increases drought tolerance, possibly by increasing uptake of P (43). Recent research suggests that another major benefit to plants is increased disease resistance (39). Arbuscular mycorrhizae decreases

disease incidence caused by *Fusarium* spp. in asparagus (*Asparagus officinalis)* (67), tomato (*Lycopersicon esculentum*)(10,15), potato (*Solanum tuberosum*) (46) and prairie grasses (44). Decreased disease incidence also has been found in *Citrus* spp. infected with *Pythium* (56).

There are a few plant families without the capacity to form mycorrhizal symbiosis. The most notable nonmycorrhizal plants are in Brassicaceae and Chenopodiaceae (65). It has long been thought that plants in the Amaranthaceae were also nonmycorrhizal; however, recent research suggests that they are capable of forming AM symbiosis (42, 47). The factors that determine whether a particular species of plant or family of plants will be mycorrhizal are still controversial. Baylis (3) suggested that the propensity of a plant to form mycorrhizal symbiosis depends on the structure and morphology of the root system. This hypothesis proposes that those plants with very small, finely branched roots and large numbers of relatively long root hairs will not form mycorrhizal associations. Presumably, the mycorrhizal hyphae contribute no nutritional advantage. Species with thick unbranched roots lacking extensive root hairs benefit from the hyphal network and are more likely to be mycorrhizal. The endomycorrhizal Ericaceae which have extensive root systems and are highly mycorrhizal are exceptions to this hypothesis (27).

Border cells (BC) (also referred to as sloughed root cap cells) are found on the root tips of many plant species and are defined as noncontiguous root-tip cells that dislodge from the root when gently agitated in water (30, 31, 33). Hawes and Pueppke (32) found BC production in a wide range of economically important species such as cucumber (*Cucumis sativus*), soybean (*Glycine max*), bean (*Phaseolus*

vulgaris) and petunia (*Petunia* spp.). The number of BC produced within a family falls in a narrow range. However, between families there can be greater than a 100-fold difference in BC produced. For example, species within Solanaceae produce approximately 100 per root, whereas species in Fabaceae produce an average of 2000 border cells per root (32). In addition, the number of BC released from pea (*Psium sativum*) root tips increases with increasing length until a maximum of 3400 BC per root tip is reached at 25 mm (33).

The physiology of BC leads to speculation that BC may influence the rhizosphere's microbial population (7, 24, 30, 32, 47). In more than 15 plant species surveyed, the BC were found viable after separation from the root tip. Viability ranged from approximately 80% in Solanaceae to 90% in Fabaceae and Poaceae (32, 34). Viable BC of alfalfa (*Medicago sativa*) were capable of redifferentiation to form callus and roots upon culturing (34). In another study, zoospores of *Pythium* were preferentially attracted to cotton (*Gossypium hirsutum*) BC and not to cotton root tips after the BC had been removed (24). Recent research has shown that approximately 13% of proteins produced by BC are absent in root tip cells and many are excreted rapidly into the surounding media (7).

Border cells have not been observed in all plant families. Neither Brassicaceae nor Chenopodiaceae produce BC (6, 32). Interestingly, as noted above the Brassicaceae and Chenopodiaceae are the two most notable nonmycorrhizal plant families (65). Both BC production and AM colonization vary among species and between families. Niemira et al. (47) noted that species and families of plants with greater BC production tended to have a greater propensity for mycorrhizal

colonization. They hypthosized that mycorrhizal colonization is positively correlated to BC production.

Biological control with arbuscular mycorrhizal fungi and *Trichoderma* spp.

Biological control can involve the use of naturally occurring soil antagonists to control pests and disease and has received greater attention recently as disease-causing organisms become more resistant to chemical controls. There is also concern over the chemical pollution of ground water and the effects these chemicals may have on humans and organisms in the environment (11). Two organisms that have shown potential as biocontrol agents are arbuscular mycorrhizal (AM) fungi (39, 56) and the antagonistic fungus *Trichoderma* (11, 12).

Trichoderma is an anamorphic filamentous fungus of the Hyphomyces. It is ubiquitous in the soil and easily isolated from the rhizosphere of plants (11). *Trichoderma* currently is being investigated as a biocontrol agent. In several studies the addition of *Trichoderma* to the soil reduced the incidence of a variety of diseases in several agricultural crops (15, 26, 57, 60, 66, 68). Root necrosis caused by *Meloidogyne arenaria* (nematode) was significantly reduced in corn as a result of the application of either *T. harzianum* or *T. koningii* (68). Soil treatments with *T. harzianum* reduced disease incidence caused by *Sclerotium rolfsii* in sugar beets by as much as 80% (66). Hadar et al. (26) found that both *T. harzianum* and *T. Konigii* where able to significantly control disease caused by *Pythium* spp. in both cucumber (*Cucumis sativus*) and pea (*Pisum sativum).* Sivan and Chet (60) found

that disease caused by *Fusarium* spp. in cotton (*Gossypium barbardense)*, wheat (*Triticum aestivum*) and muskmelon (*Cucumis melon*) could be reduced by the addition of *T. harzianum* at the rate of 5 x 10^9 conidia/g of soil. Similar results were found in both greenhouse and field experiments. *Trichoderma harzianum* reduced root rot caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* by 50% on tomatoes (*Lycopersicon esculentum*). The reduction in disease resulted in a significant increase in yield and quality of tomato by the end of 24 weeks (57). Datnoff et al. (15) observed similar results with field tomatoes suffering from crown and root rot caused by *F. oxysporum* f. sp. *radicis lycopersici.* In this study the application of *T. harzianum* reduced disease incidence and incidence in infected tomato plants.

Recently, a commercially produced form of *T. harzianum* became available which is dispersed within a preparation of small clay granules. The concentration of *T. harzianum* is approximately 10^6 colony forming units (CFU)/g per preparation. Lo (40) found that the commercial preparation of *T. harzianum* was very effective against a variety of turfgrass diseases. Disease severity of brown patch disease caused by *Rhizoctonia solani* was reduced by 30%. Dollar spot disease produced by *Sclerotinia homoeocarp* was reduced by 40%. *Pythium* root rot was reduced by 70%.

The concentration of *T. harzianum* in the soil affects the level of disease on plants. In cucumber (*Cucumis sativus*) and pea (*Pisum sativum*), the greatest percentage of healthy seedlings was seen when the population of *T. harzianum* had reached a concentration of 4 x 10^4 CFU/g of soil (26). Sivan and Chet (60) found that disease caused by *F. oxysporum* f. sp. *vasinfectum* on cotton (*Gossypium barbardense*) was

controlled on three successive plantings of cotton in the same field when *T. harzianum* was applied. However, the percentage of diseased plants increased between the first and third plantings as the level of *T. harzianum* in the soil decreased.

Treating seeds with *T. harzianum* prior to germination can increase germination percentage and seedling survival. Tomato seedlings coated with *T. harzianum* significantly decreased the number of dead seedlings within 80 days (57). Asparagus seeds are notorious for carrying disease organisms and for being difficult to germinate (13). However, germination can increase by 60% when aparagus seeds are precoated with *Trichoderma* spp. prior to germination. (22, 48).

There are several different mechanisms by which *Trichoderma* controls pathogens depending on the pathogen and the environment. The ability to predict which mechanism may be in use is still under investigation. Competition is one mechanism by which *T. harzianum* controls pathogens on the roots of plants (60). *Trichoderma harzianum* also produces antibodies and hydrolytic enzymes that inhibit pathogen growth and spore germination (36). Finally, *T. harzianum* physically parasitizes fungal pathogens (36, 53, 66).

There is evidence to suggest that *Trichoderma* is a more effective competitor for resources than many plant pathogens (55, 59, 60). Sivan and Chet (60) noted a significant reduction of *F. oxysoporum* f.sp. *vasinfectum* population on cotton plants as the concentration of *T. harzianum* increased. In addition, as the level of *T. harzianum* increased, disease severity on the cotton plants decreased. However, the authors did not demonstrate that *Fusarium* populations were declining because they were out competed by *Trichoderma*; therefore,

the other two mechanistic models for *Trichoderma* biocontrol were still possibilities. In another study, however, Sivan and Chet (59) were able to show that there may be competition for nutrients in the rhizosphere. They demonstrated that spore germination of several *Fusarium* species (i.e. *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *vasinfectum*) increased by 45%, with carbon sources found in root exudates. They then showed that *T. harzianum* reduced disease incidence in *Fusarium*-infected plants, but that disease reduction was nullified when the soil was treated with a carbon source. They also looked at the spatial arrangement of the fungi in the rhizosphere and rhizoplane and found the highest concentration of *Trichoderma* and the lowest populations of *Fusarium* on the roots.

Trichoderma harzianum is able to control pathogens through the production of inhibitory compounds. The fungus produces a variety of hydrolytic enzymes which include endochitinases that have been shown to inhibit such plant pathogens as *Fusarium*, *Botrytis* and *Ustilago* (28). In the presence of *B. cineria*, *T. harzianum* produces several hydrolytic enzymes, including chitinases as well as antibodies (55). Neither the hydrolytic enzymes nor the antibodies inhibit *B. cineria* alone at concentrations found in vivo; however, when the two are combined there is a 50% reduction in spore germination and hyphal elongation of *B. cineria*. These observations suggest *T. harzianum* may inhibit plant pathogens by biochemically reducing the pathogen's viability and population.

Trichoderma harzianum can also exert a biocontrol effect through physical contact and subsequent mycoparasitism of fungal pathogens in the rhizosphere. Several studies have reported mycoparasitism by

T. harzianum on pathogenic fungi as well as the beneficial arbuscular mycorrhizal fungus *Glomus intraradices* (36, 53, 66). When *Sclerotium rolfsii, R. solani,* and the mycorrhizal fungus *Glomus intraradices* were grown seperately in vitro with *T. harzianum*, growth of each of the fungi ceased (36, 53, 55, 66). *Trichoderma harzianum* was observed to coil around aerial portions of *S. rolfsii* and *R. solani. Trichoderma harzianum* grew along side both pathogens and *G. intraradices* forming loops around and penetrating the hyphae. The sclerotia of *S. rolfsii* and spores of *G. intraradices* were also parasitized by *T. harzianum* (53, 66).

Trichoderma harzianum is an effective antagonist and therefore may be harmful to beneficial microorganisms in the rhizosphere. Mycorrhizal fungi are some of the most widespread soil inhabitants that form a symbiosis with many plants. The application of *T. harzianum* as a biological control agent adversely affects AM colonization (41, 63, 69). Ectomycorrhizal colonization by *Laccaria bicolor* also was reduced by *Trichoderma* spp. (63). However, the reduction in colonization does not correspond to reduced disease resistance. In fact disease resistance was enhanced when both *T. harzianum* and the AM fungus *Glomus mossaea* or *Glomus intraradices* was added to the soil (9, 15). The mechanisms involved in reducing mycorrhizal colonization may be mycoparasitism by *T. harzianum.* As mentioned previously *T. harzianum* parasitizes and lyses the AM fungus *G. intraradices* in vitro (53).

Asparagus officinalis, a member of Liliaceae, is a very important perennial crop worldwide. An estimated 140,000 ha of asparagus was produced globally in 1990 (45). In 1992, 35,000 ha worth $162 million was produced in the United States alone. The major asparagus-

producing states are California, Washington and Michigan, with smaller production sites in Illinois, Maryland, Massachusetts, New Jersey, Ohio and Oregon (20).

Asparagus benefits from inoculation and colonization with AM (5, 8, 49, 67): growth, dry weight, and disease resistance increase with inoculation both in the greenhouse and the field. Root colonization with AM fungi ranges from 20-80% depending on the fungus. Maximal colonization occurs when asparagus is inoculated with *Glomus intraradices* (49).

Asparagus usually is harvested in the spring after 1-3 years of growth. A field can remain in production anywhere from 10-20 years (20, 45). However, a condition known as asparagus decline can reduce a field's productive life by half (25). Asparagus decline is characterized by a loss of vigor, a decrease in growth, and onset of disease in the asparagus plants (14, 20, 29, 67). There are several factors that contribute to asparagus decline. Asparagus produces several phenolic chemicals that are autotoxic and possibly allelopathic (20, 50, 70). Some cultural practices such as tillage and excessive harvesting also affect the overall vigor of a field (20, 52, 64). However, the onset of disease can ultimatly cause the death and loss of entire fields (20, 50, 67). Fusarium root rot caused by *F. oxysporum* f. sp. *asparagi* (Schlect.) (FOA) is one of the major diseases that contributes to asparagus decline and loss of crops world wide (13, 18, 20, 50, 67). In Michigan, for example, FOA has been found in all fields tested. Even fields that never have been cultivated with asparagus harbor low levels of pathogenic species (29).

Chemical treatments ineffectivly control *Fusarium* root rot (18, 38). Therefore, other innovative approaches to disease control need to be found. Both AM fungi and *T. harzianum* are promising candidates as biological control agents against *F. oxysporum* f.sp. *asparagi* on asparagus. In greenhouse as well as field studies AM greatly reduces FOA on asparagus (67). *Trichoderma* also has been efficacious in reducing disease caused by a variety of plant pathogens including many *Fusarium* spp. In addition, AM fungi and *T. harzianum* are currently commercially available and therefore are more easily accessible to growers than biocontrol agents that need to be experimentally isolated in the lab and often require specialized equipment, methods and materials.

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Chapter 1

Border Cells and Arbuscular Mycorrhizae in Four Amaranthaceae Species

Abstract

Four species from the family Amaranthaceae were studied to determine border cell (sloughed root cap cells) production and arbuscular mycorrhizal colonization. It was found that border cells are produced by all species studied and that border cells increase with increasing root length until a maximum number is reached at 25 mm. However, the increase in border cells with increasing root length is not uniform between species. Arbuscular mycorrhizal root colonization was found in all the Amaranthaceae species and AM colonization was positively correlated with maximum border cell production.

Introduction

Border cells (BC) (also referred to as sloughed root cap cells) (3,7,12,25) are found on the root tips of many plant species including economically important species such as cucumber (*Cucumis sativus*), soybean (*Glycine max*), pea (*Pisum sativum*) and petunia (*Petunia* spp.) (7,12). Border cells have not been found in plants belonging to the families Brassicaceae and Chenopodiaceae (3). Border cells are

experimentally defined as cells on the root tip which are separate from each other and will disassociate from the root when gently agitated in water (9). Border cells have also been shown to be present on the root tips of 2 month old corn (*Zea mays*) plants growing in soil (25). In more than 15 plant species surveyed, the viability of BC isolated from young root tips growing in petri plates was determined to be 80 to100% (12). Viable BC of alfalfa (*Medicago sativa*) are capable of redifferentiation to form callus and roots upon culturing (13). Border cells can also synthesize and readily exude unique proteins, which are not present in the root tip proper (the root cap, root meristem, and adjacent cells) (4). These aspects of BC physiology have lead several researchers to speculate that BC may influence the rhizosphere's microbial population (7,8,9,12,20). Border cells have already been shown to influence soil fungi. Motile zoospores of *Pythium* were preferentially attracted to cotton (*Gossypium hirsutum*) BC verses root tips after the BC had been removed (8). Border cells of corn have also been shown to stimulate the growth of *Pseudomonas fluorescens*, inhibit the growth of *Rhizobium* spp. and *Escherichia coli*, and induce sporulation in *Bacillus* spp. (7).

Arbuscular mycorrhizae (AM) are ubiquitous soil fungi which are capable of forming mutualistic associations with many plant species (1). Arbuscular mycorrhizal associations are the most common form of mycorrhizae, found in most angiosperms including most major crop species such as soybean, corn, wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), clover (*Trifolium repens*), and pea (6,22). Arbuscular mycorrhizal associations are thought to benefit plants primarily through the uptake of nutrients (16,23), although increased

disease resistance resulting from AM root colonization has been suggested to be a major factor as well (19,16,18).

There are a few plant families without the capacity to form AM. The most notable nonmycorrhizal plants are in the family Brassicaceae and Chenopodiaceae (24). Although it has long been thought that plants in Amaranthaceae are also nonmycorrhizal, recent research suggests that they may be capable of forming AM associations (17). Their degree of potential AM formation, however, is a matter of ongoing discussion (20). The physiological and ecological factors that determine the mycorrhizal status of a particular plant species or family are not clear. Baylis (2) suggested that the propensity of a plant to form mycorrhizal relationships depend on the structure and morphology of the root system. An alternative hypothesis proposed by Niemira et al. (20) suggests that mycorrhizal capability may correlate with BC production. This hypothesis predicts that a relatively high degree of AM colonization will have a relatively high level of BC production. Similarly, low (or absent) mycorrhizal colonization would correspond with low (or absent) BC production.

The purposes of this study were to confirm the mycorrhizal status of species in the Amaranthaceae and to determine the strength of the correlation, if any, between BC production and AM colonization in those species.

Materials and Methods

Mycorrhizal colonization

Four species from the Amaranthaceae were studied: *Gomphrena globosa* L. cv. 'lilac', *Amaranthus tricolor* L., *Amaranthus caudatus* L.

(Stokes Seeds, Inc. Buffalo, NY), and *Celosia cristata* L. cv. 'red velvet' (W. Atlee Burpee & Co., Warminster, PA). Mycorrhizal white clover, *Trifolium repens* L. was a positive control and nonmycorrhizal field mustard, *Brassica campestris* L., a negative control. There were two treatments: 1) inoculation with the mycorrhizal fungus *Glomus intraradices* Schenck and Smith 2) noninoculated controls. Each treatment consisted of 10 plants from each of the above six species. Seeds were germinated at 25^{0}C in the dark on water agar overlaid with Whatman filter paper until the radicle emerged. The noninoculated controls were planted in a 1:1 (v/v) sterile soil and sand mix. The mycorrhizal treatments were comprised of seeds which were planted in 1:1 (v/v) sterile soil and soil from mycorrhizal pot cultures, which contained 3 spores/g of soil of *Glomus intraradices*. The pot cultures were prepared using *Sorghum bicolor* L. and *Glomus intraradices* according to the procedure outlined by Brundrett (5). The plants were grown for 8 weeks in a 25^{0}C growth chamber with 12-h/12-h, light/dark cycles and watered as needed with distilled water. Noninoculated plants received one application, at the rate of 20 ml per plant, of 1/4 strength Hoagland's solution (14) two weeks after planting. Inoculated plants received 20 ml of 1/4 strength Hoagland's solution, minus phosphorus, per plant at two weeks after planting. At 8 weeks the plants were harvested and the roots were used to determine mycorrhizal colonization using the methods of Phillips and Hayman (21), with the following modifications. The roots were washed, cleared, and stained with trypan blue. A random sample of each plant's root system was cut into 0.5 to 1 cm segments and placed in a petri plate. The root segments were then suspended in a lactoglycerol

destaining solution. Mycorrhizal root colonization percent was determined using the gridline intersect method. The root segments were placed on the stage of a dissecting microscope overlaid with a t-grid and then viewed at 250X magnification. The total number of root segments which came in contact with the grid were counted as were the number of roots with signs of mycorrhizal colonization. Presence or absence of vesicles and associated hyphae were the determining factors in establishing percent mycorrhizal colonization. Arbuscules were rarely seen in our stained roots. The ratio of mycorrhizal root segments to total root segments was used to determine the percent of mycorrhizal root colonization. Experimental procedures were repeated with similar results.

Border cell enumeration

Seeds from the same four species of Amaranthaceae (*A. tricolor, A. caudatus, G. globosa, C. cristata*) and the mycorrhizal positive (*T. repens*) and negative control (*B. campestris)* were examined for border cell production. Border cells were isolated and counted using the method of Hawes and Brigham (10), with the following modifications. Seeds were surface sterilized with 20% commercial bleach for 15 min. rinsed three times in distilled water, germinated under sterile conditions on 2% water agar overlaid with Whatman filter paper. Seeds were then incubated during germination in the dark at 25^0C until the radicles were the desired length. Border cell production was determined when the radicles were 5, 10, 15, 20, 25, and 30 mm long. Border cells were isolated by immersing seedling root tips (~2 mm) into 85µl of sterile distilled water in a microtiter plate well. The root tips

were immersed for 1 min. and the water around the roots was gently agitated with a pasteur pipette. The roots were removed and 15 µl of aqueous 0.05% Toluidine blue stain was added increasing the well volume to 100 µl. A 10 µl aliquot then was removed from each well and placed on a glass slide. The BC in the 10 µl aliquot were counted under a dissecting microscope at 500x magnification and the total number of BC per well was calculated. Ten seedlings for each species were assessed to determine average BC number per root at each length.

Regression analysis of AM colonization and maximum BC for all species was performed using SigmaStat v. 1.02 (Jandel Scientific, San Rafael, CA). The experiment was repeated with similar results.

Results

Border cells were found in all four Amaranthaceae species as well as the mycorrhizal positive control *T. repens*. Additionally, the number of border cells increased with increasing root length until a maximum number of BC per root was reached at 25 mm. This maximum ranged from an average high of 464 BC in *C. cristata* (Figure 1.1A) to a low of 72 BC per root in *A. caudatus* (Figure 1.1B). The mycorrhizal negative control *B. campestris* did not produce BC until the radicle had reached 30 mm where an average of 4 BC per root was observed, however, most roots did not produce BC. The increase in BC with increasing root length was not uniform among the species surveyed. *Celosia cristata* produced approximately 50% of its maximum number of BC at 5 mm in length (Figure 1.2A), whereas *G. globosa* (Figure 1.2A), *A. tricolor* and *A. caudatus* had produced 0 to 3% of their individual maximum (Figure 1.2B). *Trifolium repens* showed maximum BC production at 5 mm in

length (Figure 1.2B). For 10 mm roots, *C. cristata* had produced 74% of maximum and *G. globosa* 13% of maximum (Figure 1.2A). At 10 mm in length *Amaranthus tricolor* had produced 36% and *A. caudatus* had produced 7% , of their individual maximum (Figure 1.2B).

Arbuscular mycorrhizal root colonization was found in all the Amaranthaceae species as well as in the positive control, *T. repens*. *Celosia cristata* had the greatest AM colonization of the Amaranthaceae and *A. caudatus* had the lowest with 47% and 25% respectively, (Figure 1.3). The negative control *B. campestris* was not colonized. Mycorrhizal root colonization was closely and positively correlated with BC production (r^2 = 0.82 and p=0.013), (Figure 1.3) in that arbuscular mycorrhizal root colonization increased as BC production increased in the Amaranthaceae species and *T. repens*.

Figure 1.1 Average number of BC per root tip at lengths 5, 10, 15, 20, 25, and 30 mm in: A) *T. repens*, *C. cristata* and *G. globosa*; B) *B. campestris*, *A. tricolor* and *A. caudatus*.

*Note a change in scale between graph A and B
Bars represent SE and n=10 for each point.

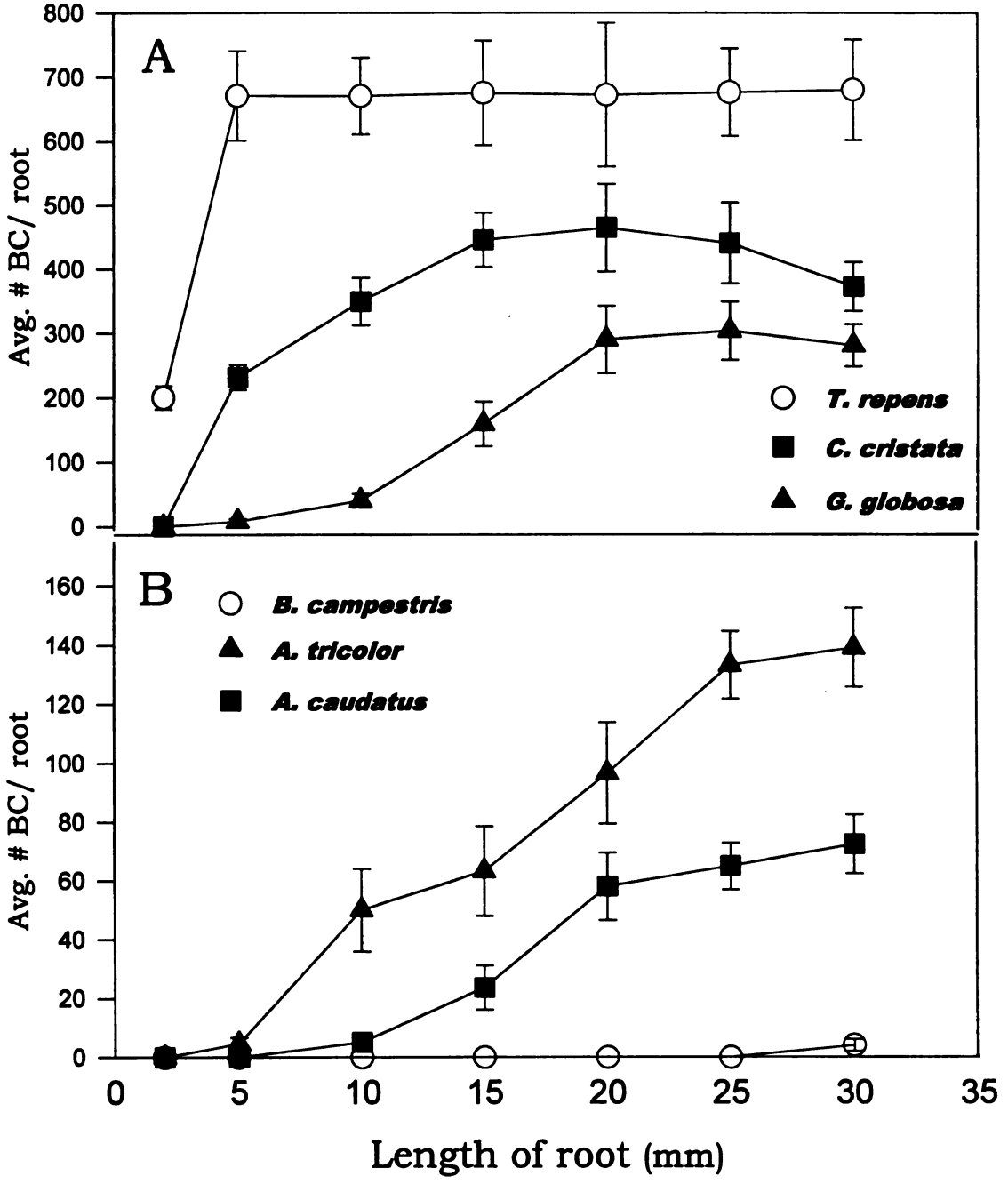
A
B
Avg. # BC/ root
Avg. # BC/ root
T. repens
C. cristata
G. globosa
B. campestris
A. tricolor
A. caudatus
800
700
600
500
400
300
200
100
0
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Length of root (mm)

Figure 1.1

Figure 1.2. Percent of maximum BC produced at lengths 5, 10, 15, 20, 25, and 30 mm in: A) *G. globosa*, *C. cristata* and *B. campestris* B) *A. caudatus*, *A. tricolor* and *T. repens*[*].

[*]Bars represent SE and n=10 for each point.

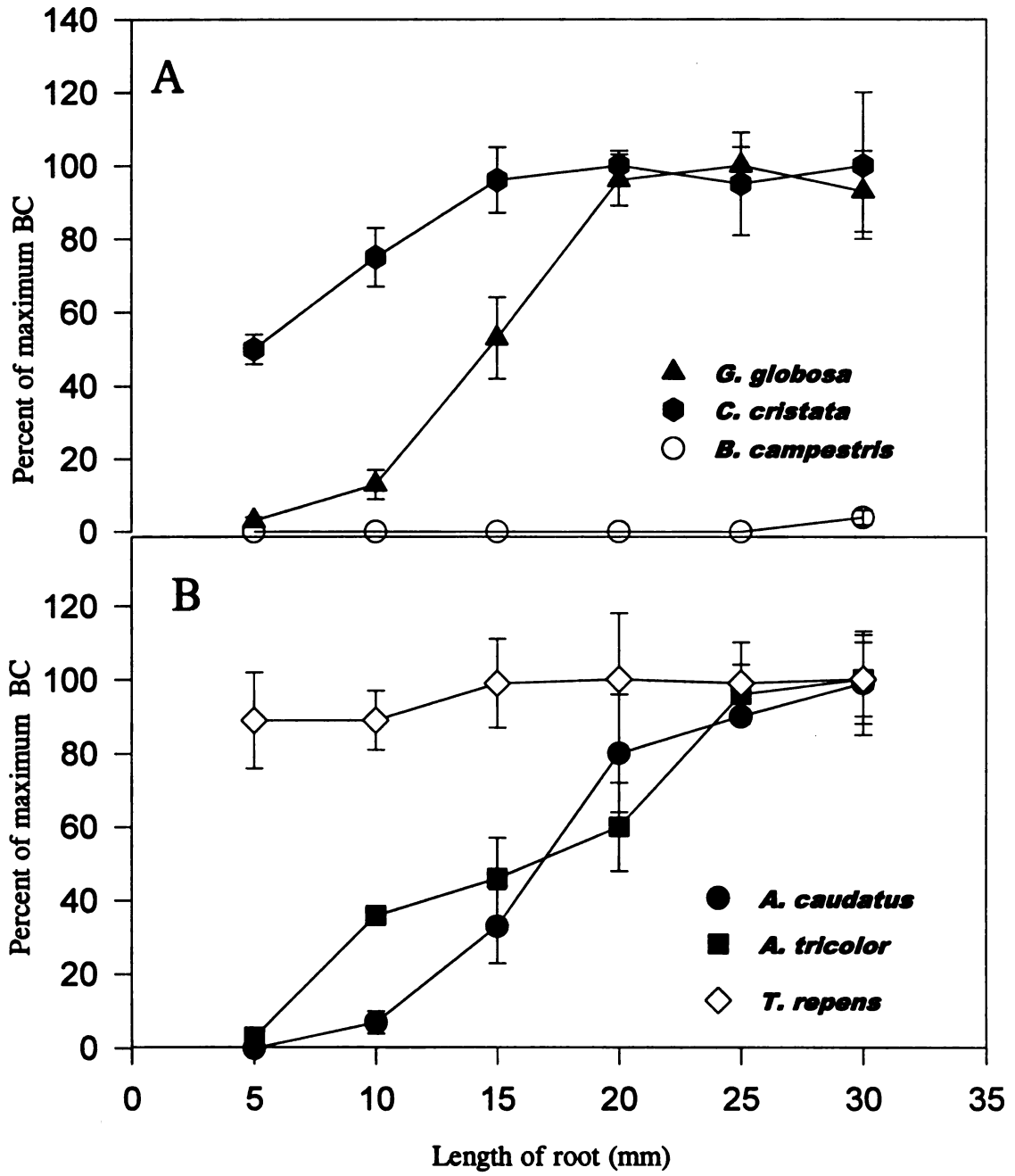
A
B
Percent of maximum BC
Percent of maximum BC
140
120
100
80
60
40
20
0
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Length of root (mm)
G. globosa
C. cristata
B. campestris
A. caudatus
A. tricolor
T. repens

Figure 1.2

Figure 1.3. AM root colonization correlated to maximum BC production[+].

[+] r^2=0.82 and p=0.013
Bars represent SE and n=10 for each point.

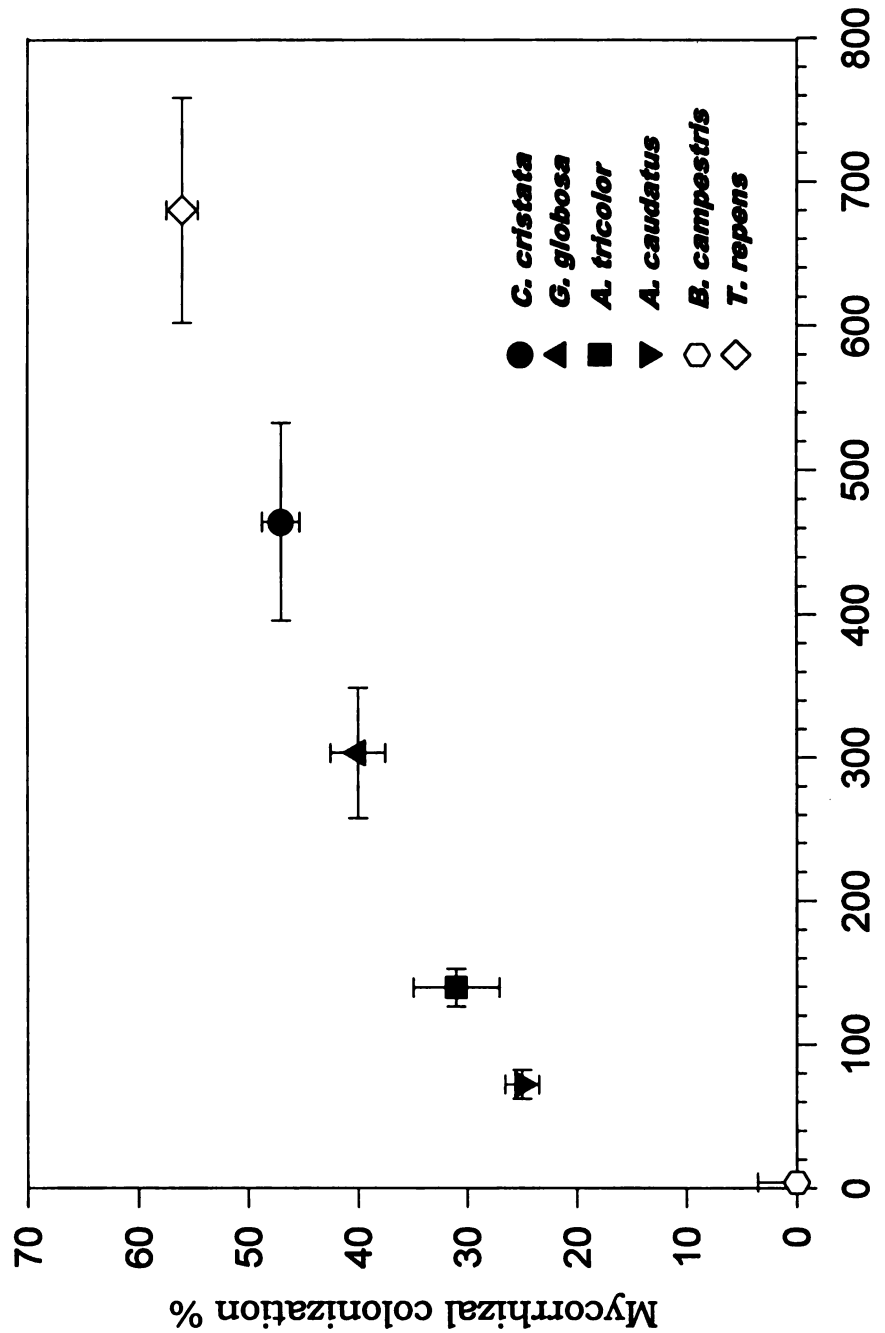
Mycorrhizal colonization %
0
10
20
30
40
50
60
70
0
100
200
300
400
500
600
700
800
Maximum BC per root
C. cristata
G. globosa
A. tricolor
A. caudatus
B. campestris
T. repens

Figure 1.3

Discussion

Border cell production in the Amaranthaceae was first reported by Hawes and Pueppke (12) in *A. tricolor* and *C. cristata.* The results in our study confirm these findings and show that two previously unstudied Amaranthaceae species, *G. globosa* and *A. caudatus,* also produce BC. Furthermore, the number of BC produced per root increases with increasing length. This was observed in all four of the Amaranthaceae species studied as well as in the positive control *T. repens.* Hawes and Lin (11) observed the same phenomena with pea seedlings. They found BC numbers increasing with increasing root length until the maximum (3400 BC per root) was reached at 25 mm. We have shown that the number of BC at a given root length as a percent of that species maximum is variable between species in the same family and between families, although less variable within a genus. Therefore, comparisons of BC production per root tip must factor in the length of the roots used. In this study, each species showed maximum BC produced per root tip in roots 25 mm in length.

We have shown that plants within the family Amaranthaceae are colonized by AM. Neeraj et al. (17) also found both *A. tricolor* and *A. caudatus* were colonized by AM. As all four species tested here were readily colonized by AM, it seems likely that mycorrhizal colonization in the Amaranthaceae is more widespread than was previously believed and this family should no longer be considered "rarely or minimally" mycorrhizal (6,20), but rather moderately mycorrhizal. The level of colonization we observed in these species is roughly comparable to plants in the mycorrhizal family Solanaceae (15).

The hypothesis suggested by Niemira et al. (20), that AM colonization propensity is correlated to BC production was supported by our results. In order to more fully test this hypothesis more research is needed to determine BC production on actively growing root tips from a wider range of mycorrhizal and nonmycorrhizal plants. Given that BC are known to influence Pythiaceous soil fungi and bacteria by the synthesis and exudation of stimulatory compounds (7,8), it seems plausable that a similar mechanism may result in the influence of BC on mycorrhizal soil fungi. Brigham et al. (4) found that 13% of proteins found in BC were not present in the root tip (root cap, root meristem and adjacent cells). It is possible that biologically active compounds, including those with specific activity towards AM fungi, may be produced by the BC. Further research into the specific nature of compounds produced exclusively by BC may further elucidate the role of BC in rhizoplane and rhizosphere ecology.

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Chapter 2

The Effect of Commercially available *Trichoderma harzianum* and Arbuscular Mycorrhizae on Fusarium Root Rot in Asparagus

Abstract

Commercially available forms of the potential biocontrol agents *Trichoderma harzianum* and the arbuscular mycorrhizal fungus *Glomus intraradices* were investigated to determine their effectiveness in controlling fusarium root rot in asparagus. High and low concentrations of *Fusarium oxysporum* f. sp. *asparagi* were combined with arbuscular mycorrhizal inoculum and / or *T. harzianum* granules. Death rates of biocontrol treatment plants were less than half of those plants inoculated only with either high or low *F. oxysporum* concentrations. At high and low *F. oxysporum* concentrations it was found that both biocontrol agents alone and in combination significantly increased the numbers of shoots produced and decreased the level of root rot over plants inoculated solely with *F. oxysporum*. In addition, shoot height and weight of biocontrol treated plants were greater than those of *F. oxysporum* treatments.

Introduction

Asparagus (*Asparagus officinalis* L.) is an important crop worldwide. An estimated 140,000 ha of asparagus were grown globally in 1990 (19). In 1992, 35,000 ha were grown in the United States alone

(19). Asparagus production has great potential for development, (19) however, asparagus fields worldwide suffer from asparagus decline, which is characterized by loss of vigor, decrease in growth and onset of disease (11, 13). Several biotic and abiotic factors are responsible for asparagus decline (11, 22,30) although, it is the onset of disease that ultimately causes the death entire crops. *Fusarium* root rot caused by *Fusarium oxysporum* (Schlectend.) f. sp. *asparagi* (S.I. Cohen) (FOA) is thought to be the major disease that contributes to asparagus decline and the loss of crops (6, 11, 22). Chemical treatments and cultural practices ineffectively control *Fusarium* root rot (10, 15) therefore, other innovative approaches to disease control need to be considered. Biological control agents such as arbuscular mycorrhizal fungi (16, 29) and the antagonistic fungus *Trichoderma harzianum* (5, 28) offer prominsing alternatives.

Arbuscular mycorrhizae (AM) are ubiquitous soil fungi capable of forming mutualistic associations with many plant species (1). Among the plants that form AM are several crop species including corn (*Zea mays*), wheat (*Triticum aestivum*), cucumber (*Cucumis sativus*), clover (*Trifolium repens*), tomato (*Lycopersicon esculentum*), and asparagus (12, 16, 25, 29). Arbuscular mycorrhizae benefit plants primarily by facilitating increased nutrient (most notably phosphorus) uptake (12), and disease resistance (3,8,16,20,29). Inoculation with AM fungi has been shown to decrease disease incidence caused by *Fusarium* spp. in asparagus (29), tomato (3, 8), and potato (*Solanum tuberosum*) (20). A commercially available peat mix containing AM propagules is an effective source of AM inoculum on asparagus (21).

Trichoderma harzianum is a natural soil inhabitant and can be isolated easily from the rhizosphere of plants (4). Application of *T. harzianum* has been shown to control *Fusarium* disease in a number of crops, including tomato, cotton (*Gossypium barbardense*), muskmelon (*Cucumis melon*) and wheat (8, 26, 27). Recently a commercially produced form of *T. harzianum* became available and is dispersed within a preparation of small clay granules. Lo (17) found that the commercial preparation effectively controlled the turf grass disease brown patch caused by *Rhizoctonia solani*, dollar spot caused by *Sclerotinia homoecarp* and root rot caused by *Pythium* spp.

The purpose of this research is to determine if the commercially available formulations of *T. harzianum* and the arbuscular mycorrhizal fungus *Glomus intraradices* effectively reduce disease incidence caused by FOA on asparagus.

Materials and Methods

An isolate of virulent FOA previously isolated from Michigan soils was obtained from Dr. Wade Elmer (Connecticut Agricultural Experiment Station). The FOA inoculum was made according to the procedure outlined by Wacker et al. (29). The FOA isolate was grown under sterile conditions on potato dextrose agar (PDA) (Difco, Detroit, Mich.) at room temperature (20° C) for 7 days. Millet seeds (200 g) in 100 ml of distilled water were autoclaved in a 1-L Erlenmeyer flask for 40 min. on two consecutive days. The flask was shaken to break up large clumps of millet seed. One 6-mm plug of FOA-infested agar was placed under sterile conditions into the flask containing millet seed.

The flask was shaken daily to distribute the fungus. After 14 days the fungus infested millet seed was allowed to dry at room temperature for 6 days. The millet seed then was ground to a powder in a Wiley intermediate mill (Thomas Scientific, Swedesboro, N.J.). The high and low FOA concentration treatments contained 1 and 0.5 g of millet inoculum per liter of peat respectively. Serial dilutions of FOA-infested peat were made and plated onto Komada's media (14) to determine FOA concentrations. The high and low FOA infested peat had approximately $1x10^6$ and $2x10^4$ colony forming units (cfu) per gram of soil, respectively .

A commercial preparation of *T. harzianum* Rifai strain KRL-AG2 (T-220) was obtained from Bioworks, Inc. (Geneva, NY). A granular clay preparation of *T. harzianum* that had approximately 10^6 colony forming units (cfu) of *T. harzianum* per g of preparation was used. The *T. harzianum* granules were applied to treatments according to the manufacturer's directions at a rate of 1 g per liter of peat.

Mycorrhizal treatments were planted in a commercial mycopeat mix from Premier Peat (Montreal, Quebec Canada). The peat contains approximately 2 propagules of *G. intraradices* (Schenck and Smith) per gram.

Seeds of *A. officinalis* L. cv. Martha Washington (Abbot and Cobb, Feasterville, Penn.) were sterilized according to the procedure outlined by Damicone and Manning (7). The seeds were surface sterilized in 20% commercial bleach for 30 min. They then were rinsed with distilled water and resuspended in 100 ml of acetone and 2.5 g of benomyl for 24 hrs on a rotatory shaker. The seeds then were rinsed several times with sterile distilled water. The seeds were germinated at

25°C under dark sterile conditions on water agar overlaid with filter paper until the radicles emerged (approximately 7 days). Two germinated seeds were sown into 250 ml pots containing amended or untreated media. Seedlings were thinned to one plant per pot 7 days after sowing.

This study was arranged in a randomized complete block design with five blocks. There were four treatments at both high and low FOA levels: 1) FOA (F), 2) FOA and *T. harzianum* (F+T), 3) FOA and mycorrhizae (F+M), and 4) FOA, *T. harzianum* and mycorrhizal inoculum (F+T+M). There was also an untreated control and an AM control treatment. There were 10 plants per treatment.

The plants were grown in a growth chamber at 25°C with 12-h/12-h light/dark cycles and watered with distilled water as needed. Numbers of shoots and shoot length were recorded every 7 days and plant death was monitored daily. The plants were harvested 38 days after planting. At harvest, plants were extracted from the soil and shaken free of peat particles. *Fusarium oxysporum* f. sp. *asparagi* root rot ratings were assessed on all plants (29). Ratings were assigned according to the percentage of roots exhibiting lesions or discoloration (reddening). The FOA ratings were as follows: 1 = 0-10%; 2 = 11-20%; 3 = 21-30%; 4 = 31-40% and 5 = more than 40%. Total weight, root and shoot fresh weights were measured on all plants. Dry weights were measured on shoots that had been dried for 5 days at 30°C.

The roots from five plants were chosen at random from each treatment with *T. harzianum* and plated onto *Trichoderma* selective media (TSM) (9) to confirm the presence of *T. harzianum*. The roots of plants from each treatment grown with mycorrhizal inoculum were

used to determine mycorrhizal root colonization using the methods of Phillips and Hayman (23), with the following modifications. The roots were washed, cleared, and stained with trypan blue. A random sample of each plant's root system was cut into 0.5 to 1 cm segments and placed in a petri plate. The root segments were then suspended in a lactoglycerol destaining solution. Mycorrhizal root colonization percent was determined using the gridline intersect method (23). The root segments were placed on the stage of a dissecting microscope overlaid with a t-grid and then viewed at 250x magnification. The total number of roots segments which came in contact with the grid were counted as were the number of roots with signs of mycorrhizal colonization. Presence or absence of vesicles and associated hyphae were the determining factors in establishing mycorrhizal colonization. The ratio of mycorrhizal root segments to total root segments was used to determine the percent of mycorrhizal root colonization. All data were subjected to an analysis of variance and Fisher's mean separation test using Minitab, release 11 for windows, 1996 (Minitab Inc., State College, PA.). The experiment was repeated.

Results

Disease pressure from FOA was severe. At harvest the plant death for the high and low FOA treatment was 50% (Figure 2.1A) and 40% (Figure 2.1B), respectively. For both high (Figure 2.1A) and low FOA (Figure 2.1B) concentrations the F+M and F+T+M treatments resulted in10% plant death 25 days after planting with no further increase in plant death. The high (Figure 2.1A) and low (Figure 2.1B)

FOA T+M treatments reached a maximum of 20% plant death at 30 days after planting with no further plant death. There was no plant death in the untreated control or the AM control (Figure 2.1)

The F+T, F+M, and F+T+M treated plants had higher average shoot heights than either the high (Figure 2.2A) or low FOA (Figure 2.2B) treatments at harvest. The high FOA treated plants reached an average maximum shoot height of only 10 cm by 15 days after planting whereas, the high F+T, F+M, and F+T+M treated plants continued to increase steadily in shoot growth throughout the course of the experiment (Figure 2.2A). The high F+T treated plants were significangly greater in height at harvest, reaching 17 cm, than those from the high FOA treatment (Figure 2.2A). The high F+M and F+T+M plants grew to an average of 16 cm which was significantly greater than the high FOA treated plants (Figure 2.2A). At low FOA concentrations plants from the low F+T treatment reached an average 18 cm which was greater than those from the low FOA which grew to 13 cm (Figure 2.2B). The low F+M and F+T+M plants were 17 cm and 17.5 cm tall respectively at harvest (Figure 2.2B).

The average number of shoots per plant at high FOA concentrations was not different between the high FOA treatment and the three biocontrol treatments (F+T, F+M, and F+T+M) at 17 days after planting. At this time the high FOA treated plants had not produced any shoots whereas shoots were present in plants from all the biocontrol treatments (Figure 2.3). At 24 days after planting the high F+T and F+M treated plants averaged 1.2 shoots per plant which was significantly more than the high FOA treatment which averaged 0.2 shoots per plant (Figure 2.3). The high F+T and F+M treatments were

not significantly different from the untreated control or the AM control, which both had an average of 1.4 shoots per plant (Figure 2.3). The high F+T+M treatment averaged 0.80 shoots per plant and was significantly greater than the high FOA treatment and significantly lower in shoot number than the untreated control (Figure 2.3). At 38 days after planting the high F+T, F+M, and F+T+M had an average range of 1.4 to 1.8 shoots per plant and were not different from the untreated controls which averaged 2 shoots per plant. The three high FOA biocontrol treated plants had significantly more shoots than those from the high FOA treatment, which averaged 0.38 shoots per plant (Figure 2.3). At 17 days after planting the average number of shoots in the three biocontrol treatments at the low FOA concentration was not significantly greater than the low FOA treatment (Figure 2.3B). At 24 days the average number of shoots in the low FOA biocontrol treatments ranged from 0.9 to 1.3 shoots per plant and was significantly greater than the low FOA treatment which averaged 0.3 shoots per plant (Figure 2.4). The low F+T, F+M and F+T+M treatments were not significantly different than the untreated controls which averaged 1.5 shoots per plant at 24 days after planting (Figure 2.4). At harvest the average number of shoots in the low FOA biocontrol treatments ranged from 1.4 to 2 shoots per plant and were not different from the control with 2 shoots per plant. These treatments, however, were greater than the low FOA treatment which averaged 0.75 shoots per plant (Figure 2.4).

At harvest the root rot levels at both high and low FOA concentrations were decreased by the biocontrol treatments (Figure 2.5). The high FOA treatment had an average root rot rating of 3.8,

which corresponds to approximately 30% of the root system with reddening and lesions (Figure 2.5A). The high F+T and F+M treatments had average root rot ratings of 2, which correspond to 11 to 20% of the root system with reddening and lesions and this was significantly less than the high FOA treatment (Figure 2.5A). The high F+T+M treatment had an average root rot rating of 1.4 (0 to 10% of the root system with reddening or lesions) which was significantly less than the high FOA treatment. The high F+T+M treatment was not significantly different than the untreated control, which had a root rot rating of 1 and had no signs of reddening or lesions (Figure 2.5A). The AM control treatment also had a root rot rating of 1 and had no signs of reddening or lesions (Figure 5A). Among the low FOA treatments the low F+T and F+M had an average root rot rating of 1.5, which is approximately 10% of the root system affected. The low F+T+M treatment had an average of rating of 1 with approximately 2% of the root system affected (Figure 2.5B). The low F+T, F+M and F+T+M treatments were not significantly different from the untreated control, but were different from the low FOA treatment, which had an average root rot rating of 3 (21 to 30% of roots affected) (Figure 2.5B).

Figure 2.1. Percent plant death of asparagus over time at A) high FOA concentrations and B) low FOA concentrations.

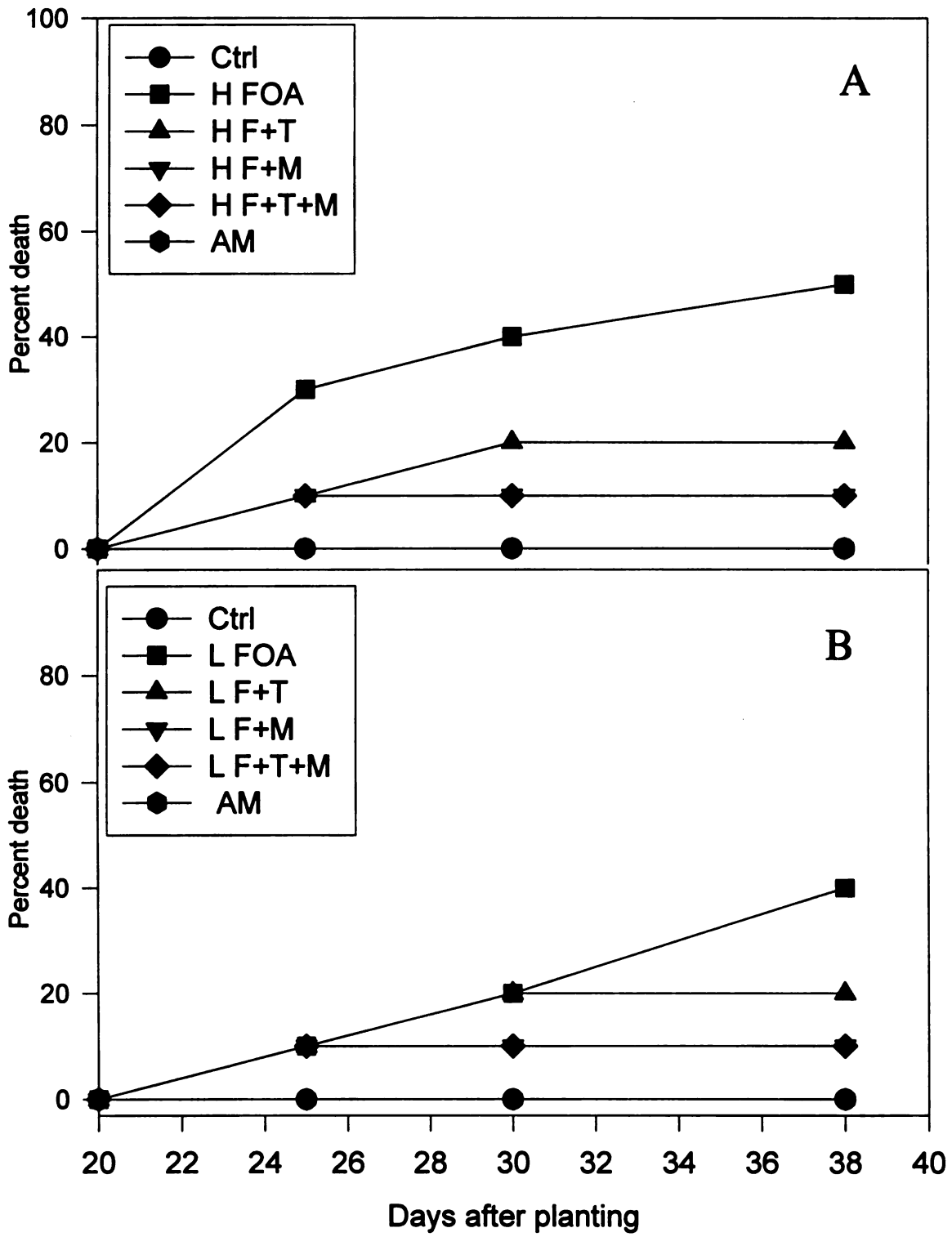
100
80
60
40
20
0
Percent death
Ctrl
H FOA
H F+T
H F+M
H F+T+M
AM
A
Ctrl
L FOA
L F+T
L F+M
L F+T+M
AM
B
20
22
24
26
28
30
32
34
36
38
40
Days after planting

Figure 2.1

Figure 2.2. Average shoot growth of asparagus over time at A) high FOA concentrations and B) low FOA concentrations*.

*Treatments followed by the same letter are not significantly different (p = 0.05), bars are SE.

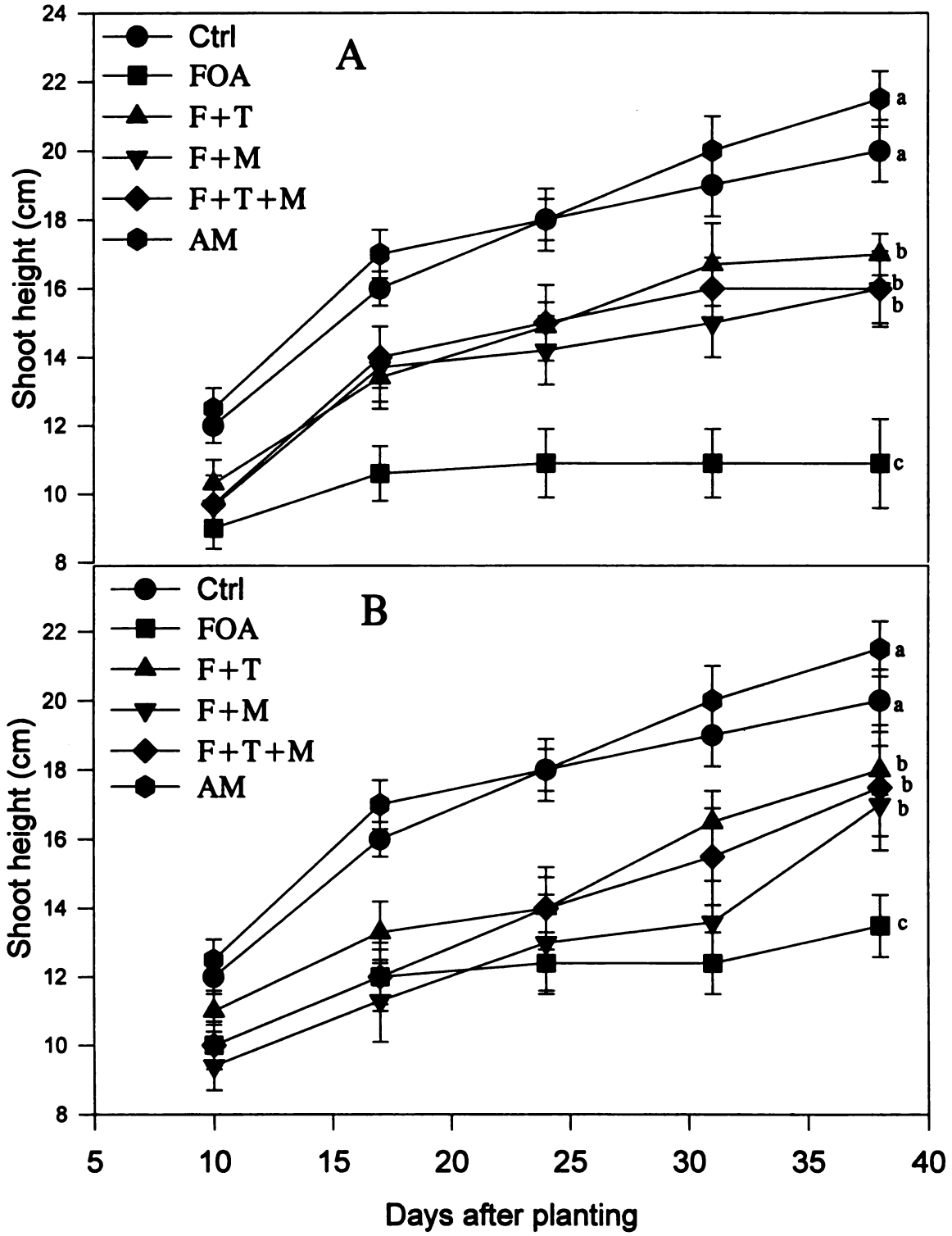
A
B
Ctrl
FOA
F+T
F+M
F+T+M
AM
Shoot height (cm)
Days after planting
a
a
b
b
b
c
24
22
20
18
16
14
12
10
8
5
10
15
20
25
30
35
40

Figure 2.2

Figure 2.3. Average number of shoots per plant at high FOA concentrations on 17, 24, 31 and 38 days after planting*.

*Treatment columns on the same day with the same letter are not significantly different (p=0.05), bars represent SE.

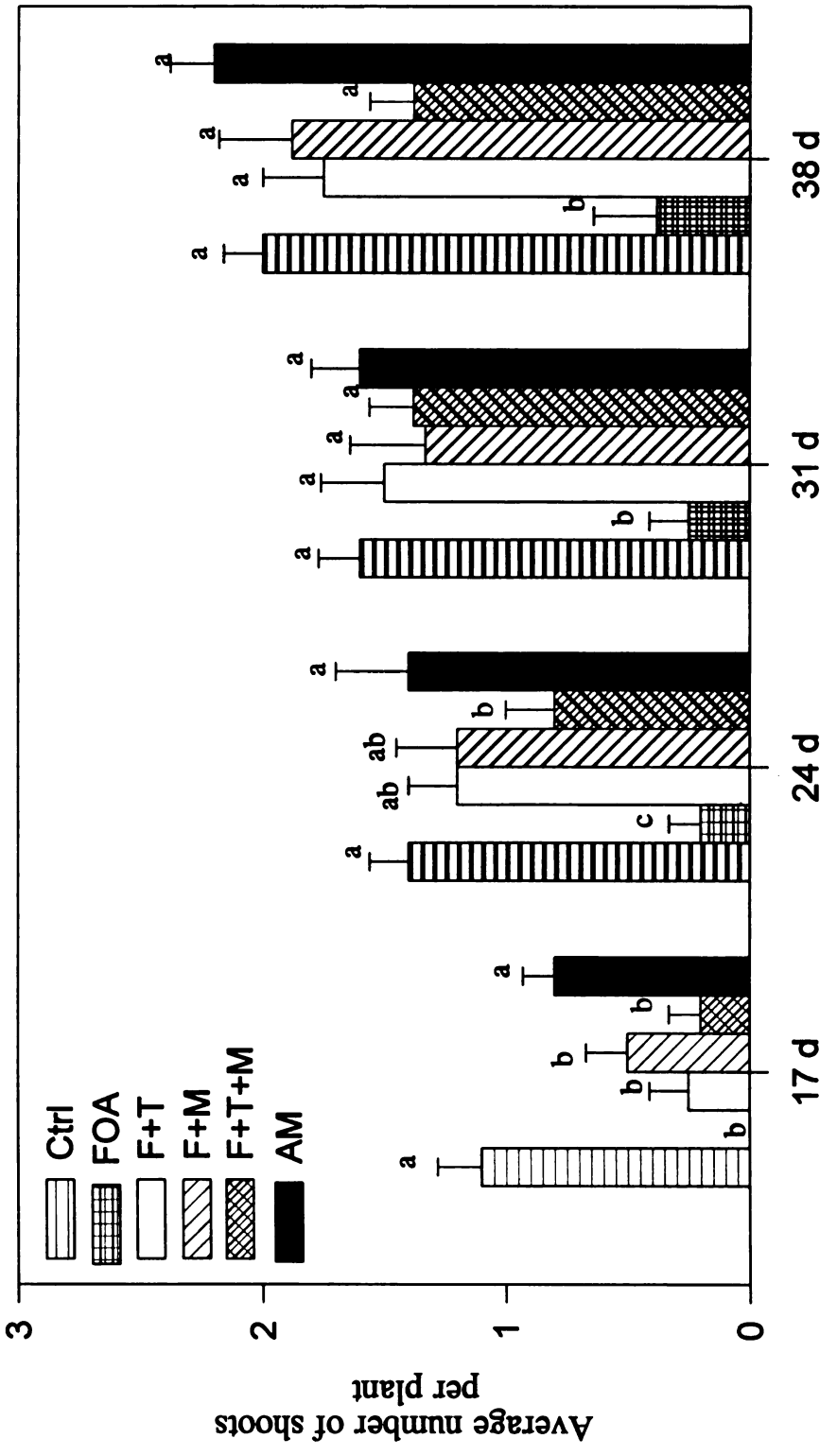
Average number of shoots per plant
3
2
1
0
Ctrl
FOA
F+T
F+M
F+T+M
AM
17 d
24 d
31 d
38 d
Days after planting

Figure 2.3

Figure 2.4. Average number of shoots per plant at low FOA concentrations on 17, 24, 31 and 38 days after planting*.

*Treatment columns on the same day with the same letter are not significantly different (p=0.05), bars represent SE.

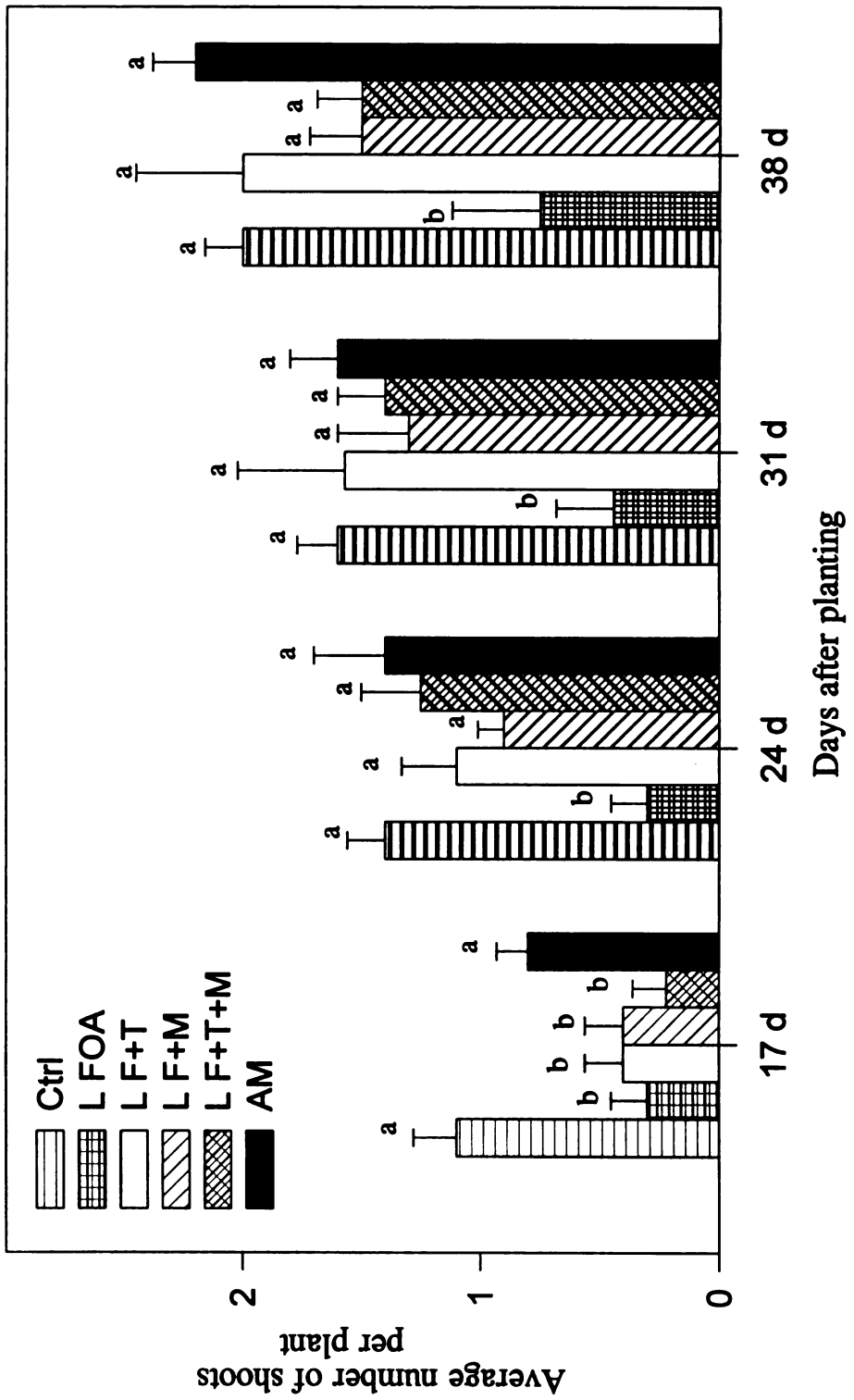
Average number of shoots per plant
0
1
2
Ctrl
L FOA
L F+T
L F+M
L F+T+M
AM
17 d
24 d
31 d
38 d
Days after planting

Figure 2.4

Figure 2.5. FOA root rot ratings[+] for A) high and B) low FOA concentrations[+].

[+]The root rot ratings are 1=0-10%; 2=11-20%;3=21-30%; 4=31-40% and 5=>40%. Bars are SE.

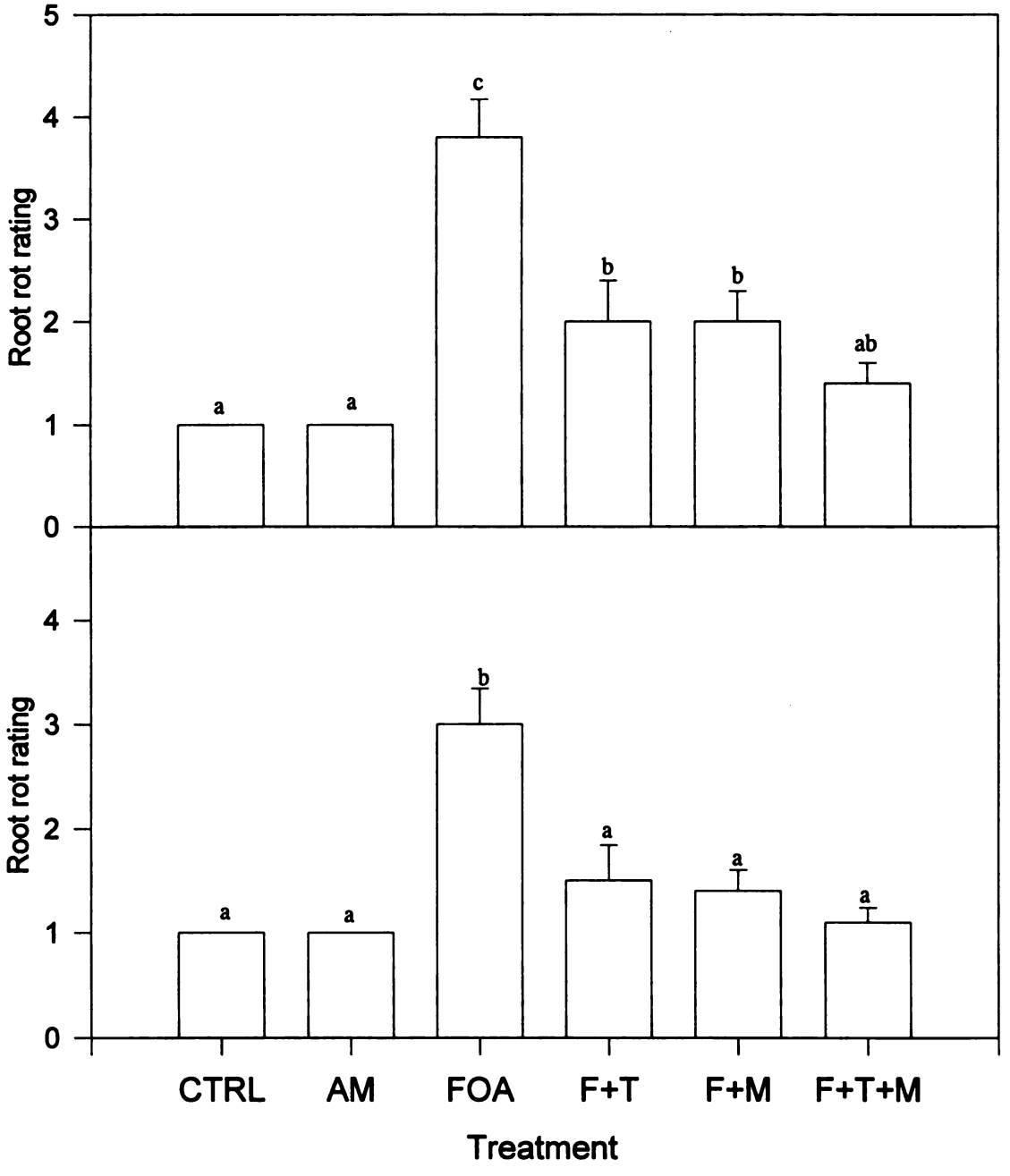
Root rot rating
5
4
3
2
1
0
a
a
c
b
b
ab
Root rot rating
4
3
2
1
0
a
a
b
a
a
a
CTRL
AM
FOA
F+T
F+M
F+T+M
Treatment

Figure 2.5

The average fresh weights of the high F+T, F+M, and F+T+M treatments were significantly greater than the high FOA treatment, Table 2.1. The fresh weight of the biocontrol treated plants ranged from an average of 0.36 g to 0.39 g and the high FOA treated plants had an average fresh weight of 0.18 g, Table 2.1. The average root, shoot and shoot dry weights in the high F+T, F+M, and F+T+M treatments were two times greater than the high FOA treatment, Table 2.1. The average fresh weight of the untreated control and AM control was 2.5 times greater than the weight of any of the high FOA biocontrol treatments, Table 2.1. At low FOA levels the low F+T treatment had a significantly greater average shoot weight and dry shoot weight than the low FOA treatment. The average fresh and root weight was not significantly different between the low FOA and the F+T, F+M, F+T+M treatments. However, the weight of the plants with the biocontrol treatments was consistently greater than the FOA treatment, Table 2.2.

Arbuscular mycorrhizal colonization was 55.3 % in the AM control, and was not significantly decreased in the high and low F+M with colonization at 49.6 and 50.2% respectively, Table 2.3. Arbuscular mycorrhizal colonization was significantly decreased in the high and low F+T+M, 39% in the latter and 35.4% in the former, Table 2.3. *Trichoderma harzianum* was found to be present on the root surface of all treated plants at both high and low FOA levels, Table 2.3.

Table 2.1. Fresh, root, shoot and shoot dry weight for treatments at high FOA concentrations.

Treat	F. wt.† (g)	Rt. wt. (g)	Sh. wt. (g)	Dry sh. wt. (g)
Ctrl	0.97 a	0.53 a	0.44 a	0.09 a
AM	1.10 a	0.56 a	0.49 a	0.10 a
FOA	0.18 b	0.07 b	0.09 b	0.02 b
F+T	0.36 c	0.15 bd	0.22 c	0.05 b
F+M	0.39 c	0.20 cd	0.18 c	0.05 b
F+T+M	0.37 c	0.18 cd	0.18 c	0.05 b

† Weights followed by the same letter are not significantly different (p=0.05).

Table 2.2. Fresh, root, shoot and shoot dry weight for treatments at low FOA concentrations.

Treat	F. wt.† (g)	Rt. wt. (g)	Sh. wt. (g)	Dry sh. wt. (g)
Ctrl	0.97 a	0.53 a	0.44 a	0.09 a
AM	1.10 a	0.56 a	0.49 a	0.10 a
FOA	0.25 b	0.09 b	0.15 b	0.04 b
F+T	0.43 b	0.18 b	0.26 cd	0.07 cd
F+M	0.30 b	0.12 b	0.18 d	0.06 bd
F+T+M	0.45 b	0.20 b	0.24 d	0.05 bd

† Weights followed by the same letter are not significantly different (p=0.05)

Table 2.3. Arbuscular mycorrhizal root colonization percent and presence of *T. harzianum* on treatments at high and low FOA concentrations.

FOA level	Treatment	AM colonization† %	*T. harzianum*‡
	Ctrl	0	-
	AM	55.3 a	-
High			
	FOA	0	-
	F+T	0	+
	F+M	49.6 a	-
	F+T+M	39.0 b	+
Low			
	FOA	0	-
	F+T	0	+
	F+M	50.2 a	-
	F+T+M	35.4 b	+

† AM treatments followed by the same letter are not significantly different (p=0.05).
‡ + or - indicates presence or absence of *T. harzianum*.

Discussion

This is the first study to our knowledge which has looked at the efficacy of *T. harzianum* as a biocontrol agent of FOA on asparagus. The results of our study suggest that the commercially available forms of *T. harzianum* and the AM fungus *G. intraradices* may be effective in reducing disease incidence in asparagus seedlings inoculated with FOA. The two biocontrol fungi alone and in combination (F+T, F+M and F+T+M) decreased plant death at both high and low FOA levels. All biocontrol treatments also increased plant shoot height, number of shoots produced and plant weight, over that of either the high or low FOA treatments. In addition, there were no significant differences between the *T. harzianum* (F+T), AM (F+M), and the *T. harzianum* plus AM combination (F+T+M) treatments. At high FOA concentrations plant mortality was at least 30% lower in all biocontrol treatments than in the high FOA treatment. The low FOA treatment had a lower death rate than the high FOA treatment. Despite the lower death rate of the low FOA treatment, the low F+T, F+M and F+T+M treatments had 20% less plant mortality than the low FOA treatment. Sivan (26) similarly found that *T. harzianum* reduced the death rate of tomatoes affected by *Fusarium* crown and root rot by 70% in the greenhouse and 25% in the field.

In our study, shoot height and weight was increased at high FOA inoculum levels in all of the biocontrol treated plants. At low levels of FOA inoculum there was also an increase in shoot height in the biocontrol treated plants. The number of shoots produced by the biocontrol treated plants at both high and low FOA levels was significantly greater than those of FOA treated plants by 24 days after

planting. The number of shoots produced by the biocontrol treated plants was not significantly different from that of the untreated control plants. The continued production of shoots could have important implications for use of these biocontrol organisms in the field since the young newly emerging shoots are the harvested product.

Root rot levels were significantly decreased by all (F+T, F+M and F+T+M) biocontrol treatments at both high and low FOA concentrations. At low FOA concentrations root rot levels of the biocontrol treated plants were not significantly different from the untreated controls. These results suggest that the progress of disease over a longer growing period may also be curtailed by the biocontrol treatments. Our results confirm observations by Wacker et al., (29) in which asparagus inoculated with AM fungi significantly reduced disease incidence in the greenhouse and in the field. Datnoff et al. (8) found that both *T. harzianum* and AM fungal inoculum, reduced disease incidence (determined by percent necrosis of the stem and root) caused by *F. oxysporum* f. sp. *radicis-lycopersici.* in tomatoes. Similarly, Sivan and Chet (27) found the application of *T. harzianum* reduced disease incidence of Fusarium spp. in cotton, wheat and muskmelon.

In our study a decrease in AM root colonization was found in plants also inoculated with *T. harzianum.* However, we did not observe an increase in disease incidence associated with inoculation of both biocontrol agents in comparison with single inoculuations. Rousseau et al. (24) observed *T. harzianum* parasitizing the AM fungus *G. intraradices* in vitro. McAllister et al. (18) also found a reduction in AM root colonization when corn was inoculated with AM fungi

simultaneously with *T. harzianum*, but not when *T. harzianum* was applied two weeks following AM fungal inoculations. More research is needed to first determine whether or not the combination of *T. harzianum* and AM is beneficial and then to determine if timing of inoculations is important.

The investigation of biocontrol treatments is particularly important to the asparagus industry because chemical controls have been found to be largely ineffective in controlling fusarium root rot (10, 15). The results of this study suggest that the commercially available forms of *T. harzianum* and the AM fungus *Glomus intraradices* may have potential as agents for the biocontrol of FOA in the field. If the decrease in root rot and increase in shoot production that we have observed is maintained in the field, the application of these biocontrol products may be effective in slowing disease development in new and existing fields.

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CONCLUSIONS

Border cells and arbuscular mycorrhizae in four Amaranthaceae

Four species of Amaranthaceae were surveyed and found to produce border cells on the root tip and form arbuscular mycorrhizal root associations. The species studied were: *Amaranthus tricolor* L. *Amaranthus caudatus* L., *Celosia cristata* L., and *Gomphrena globosa* L. Included in the study was also an arbuscular mycorrhizal positive control *Trifolium repens* L. and a negative control *Brassica campestris* L. In all the Amaranthaceae, plus the positive control *T. repens*, the number of border cells on the root increased with increasing root size from the time the radicle was 5mm until it reached 25 mm. At the length of 25 mm the number of border cells per root tip reached a maximum. The average maximum number of border cells ranged from 72 per root tip in *A. caudatus* to 464 border cells per root tip in *C. cristata*. *Amaranthus tricolor* and *G. globosa* produced a average maximum of 142 and 290 border cells per root tip respectively. The increase in border cells with increasing length of the radicle was not uniform among the species surveyed. For example, *C. cristata* produces approximately 50% of it's maximum number of border cells when the radicle was 5 mm. However, *A. tricolor*, *A. caudatus* and *G. globosa* produce between 0-3% of their individual maximum number of border cells when the radicle was 5 mm. This information on differential

development is important when comparing border cells from different species.

All four species of Amaranthaceae (*Amaranthus tricolor, Amaranthus caudatus, Celosia cristata,* and *Gomphrena globosa*) and the arbuscular mycorrhizal positive (*T. repens*) and negative (*B. campestris*) control were inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices.* Arbuscular mycorrhizal root colonization was observed in all four species of Amaranthaceae and *T. repens.* The degree of root colonization in the Amaranthaceae was however, variable. *Celosia cristata* had an average of 47% of it's roots colonized by arbuscular mycorrhizae which was the greatest of all the species surveyed. *Amaranthus caudatus* had the lowest degree of arbuscular mycorrhizal root colonization with an average of 25% of the roots colonized. *Amaranthus tricolor* and *G. Globosa* had intermediate arbuscular mycorrhizal root colonization with 34% and 40% respectively. In addition, arbuscular mycorrhizal root colonization was found to be closely and positively correlated to maximum border cell production. Among the Amaranthaceae species surveyed plus the positive and negative controls, the percent of roots colonized with arbuscular mycorrhizae increased as the maximum number of border cells produced increased. Further investigation into the nature of the relationship between border cells and arbuscular mycorrhizae is necessary.

Biological control of *Fusarium* root rot on asparagus with *Trichoderma harzianum* and arbuscular mycorrhizae

Trichoderma harzianum (Rifai strain KRL-AG2) and the arbuscular mycorrhizal fungus *Glomus intraradices* (Schenck and Smith) were found to be efficacious when commercially available forms were applied as biological control agents of *Fusarium oxysporum* f. sp. *asparagi* (Schlect.) (FOA). Both the biocontrol agents were applied alone and in combination to plants inoculated with FOA at high and low levels. There were also plants treated only with FOA at high and low concentrations and an untreated control treatment, in which plants were not treated with the biocontrol treatments or inoculated with FOA.

The mortality of plants inoculated with a high FOA concentration and treated with the biocontrol agents (*T. harzianum*, arbuscular mycorrhizae fungi or both combined) was decreased by at least 30% over plants that were inoculated with FOA only. At low FOA levels mortality of all biocontrol treatments was decreased by 20% over plants only inoculated with FOA.

Shoot height and weight were increased significantly in plants inoculated with high FOA levels and simultaneously treated with either *T. harzianum*, arbuscular mycorrhizae fungi or both. Plant height was significantly greater in plants inoculated with low FOA levels and treated with either or both of the biocontrol agents. Plant weight was not significantly greater in plants of the biocontrol treatments, however, was consistently higher.

The number of shoots produced by plants was monitored during the growing period. At 24 days after planting asparagus treated with *T. harzianum*, arbuscular mycorrhizal fungi or both and inoculated with

FOA, at both high and low levels, had significantly more shoots than plants which were solely inoculated with FOA (at high and low levels). Plants treated with the biocontrol agents (and inoculated with FOA) continued to have increased shoot production over those inoculated only with FOA until harvest, 38 days after planting. In addition the number of shoots produced at harvest in the biocontrol treatment plants were not significantly different from the untreated control plants (which were not inoculated with FOA or the biocontrol agents).

Root rot caused by FOA was significantly decreased in plants also treated with *T. harzianum*, arbuscular mycorrhizal fungi or both. Decreased root rot of biocontrol treated plants was observed at both high and low FOA levels. Root rot of plants at low FOA levels and treated with the biocontrol agents (alone or in combination) were not significantly different than the untreated controls.

Arbuscular mycorrhizal root colonization was assessed on treatments which included inoculation with the arbuscular mycorrhizal fungus *G. intraradices*. Arbuscular mycorrhizal root colonization was found to be decreased in treatments also inoculated with *T. harzianum*.

Further research is needed in the field with the commercially available *T. harzianum* and arbuscular mycorrhizal products in order to determine if similar disease reduction is observed. In addition, in a field study, the long term effects and economic feasibility could be studied.