This is to certify that the

thesis entitled

VESICULAR-ARBUSCULAR MYCORRHIZAE IN ASPARAGUS (ASPARAGUS OFFICINALIS L.): ASSESSMENT OF A COMMERCIAL PEAT-BASED INOCULUM AND EFFECTS OF PHOSPHORUS AND PHENOLIC ACIDS

presented by

CHRISTIAN THOMAS PEDERSEN

has been accepted towards fulfillment
of the requirements for

M.S. degree in BOTANY AND PLANT PATHOLOGY

Gene R. Safir
Major professor

Date AUGUST 7, 1990

VESICULAR-ARBUSCULAR MYCORRHIZAE IN ASPARAGUS (*ASPARAGUS OFFICINALIS* L.): ASSESSMENT OF A COMMERCIAL PEAT-BASED INOCULUM AND EFFECTS OF PHOSPHORUS AND PHENOLIC ACIDS

By

Christian Thomas Pedersen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1990

ABSTRACT

VESICULAR-ARBUSCULAR MYCORRHIZAE IN ASPARAGUS (*ASPARAGUS OFFICINALIS* L.): ASSESSMENT OF A COMMERCIAL PEAT-BASED INOCULUM AND EFFECTS OF PHOSPHORUS AND PHENOLIC ACIDS

By

Christian Thomas Pedersen

Tissue-cultured and seed-started asparagus (*Asparagus officinalis* L.) were grown in five commercially prepared, peat mixes containing five different *Glomus* spp. under greenhouse and field conditions. *G. intraradix* increased dry weight both in the greenhouse and the field. Mycorrhizal plants had a 70 % higher survival rate than non-mycorrhizal plants in the field after two seasons.

Asparagus seedlings were grown in mycorrhizal peat-mixes at different levels of applied phosphorus (P). Dry weight was not affected by applied P, but was increased by *G. intraradix* and *G. versiforme* treatments. Results suggest the growth increase was not related to tissue P concentrations.

The effects of the asparagus allelochemicals ferulic, caffeic and methylenedioxycinnamic acid were tested on asparagus. VAM colonization was reduced with increasing concentrations of all phenolics applied. $Ca(OH_2)$ extracts from a non-asparagus and a twenty year-old asparagus field soil were assayed on asparagus seedlings. VAM formation and asparagus fresh weight were inhibited by the asparagus soil extract, but not by the soil extract from non-asparagus soil.

This thesis is dedicated to my loving parents, Arne and Margrit Pedersen, who as European immigrants were able to experience the "American Dream" and instilled in me the importance of an open mind and a good work ethic.

ACKNOWLEDGEMENTS

There are many people who deserve thanks for their support and friendship. Most of all I thank my wife, Karen, for her love, patience and sense of humor, which was there when I needed it most. I also would like to thank my major professor Dr. Safir and my other committee members, Dr. Sink and Dr. Stephens for their support and assistance through all the trials and tribulations that are part of this experience. Additionally, I owe much gratitude to Dr. Siqueira who acted as a special mentor and friend, as well as Tracy Wacker, whose insights were always refreshing. I am grateful to John Bakker and Dr. Nair for their technical help and to Wendy Whitford for her cheerful wordprocessing assistance.

TABLE OF CONTENTS

PAGE

LIST OF TABLES v

LIST OF FIGURES. vi

LITERATURE REVIEW. 1

SECTION I: GROWTH OF ASPARAGUS IN A COMMERCIAL PEAT MIX CONTAINING VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGI AND THE EFFECTS OF APPLIED PHOSPHORUS

INTRODUCTION 12

MATERIALS AND METHODS. 13
Effects of Different VAM Fungi 13
Effects of Applied Phosphorus. 16

RESULTS. 17
Effects of Different VAM Fungi 17
Effects of Applied Phosphorus. 23

DISCUSSION 25

LITERATURE CITED 31

SECTION II: EFFECT OF PHENOLIC COMPOUNDS ON ASPARAGUS MYCORRHIZA

INTRODUCTION 35

MATERIALS AND METHODS. 36

RESULTS AND DISCUSSION 39

LITERATURE CITED 48

APPENDIX A 51

APPENDIX B 52

LIST OF TABLES

TABLE		PAGE
1.1	Growth and VAM rating of tissue-cultured plants precolonized with VAM fungi grown in the greenhouse and the field for 16 and 12 weeks respectively	18
1.2	Mean fresh weight, percent roots colonized and percent plants colonized at 5, 8 and 11 weeks of growth in individual peat mixes	22
1.3.	Effect of VAM fungal species and applied P on root colonization, percent P in cladophylls and dry weight of asparagus grown for 13 or 17 weeks in peat planting mix in the greenhouse	24

LIST OF FIGURES

FIGURE PAGE

1.1 Survival of tissue-cultured and seed-produced asparagus after two seasons of growth in the field. 21

1.2 Relationship between total plant dry weight (g) and VAM colonization (%) at 13 weeks and 17 weeks of growth in the phosphorus experiment 27

2.1 Effects of phenolic acids on VAM root colonization of asparagus grown in sand infested with G. fasciculatum after 7 weeks 41

2.2 Effects of phenolic acids on growth of asparagus in the presence and absence of the VAM fungus G. fasciculatum after 7 weeks 43

2.3 Effects of extracts from asparagus (ASP) and non-asparagus (NON-ASP) soils on fresh weight of asparagus and VAM root colonization by G. fasciculatum 46

LITERATURE REVIEW

Vesicular-arbuscular mycorrhizal (VAM) associations with plants are a common occurrence in nature. A wide range of host plants form VAM, however members from plant families such as Brassicae, Chenopodiaceae and Polygonaceae are less apt to become mycorrhizal (Harley and Smith, 1983). The mutualistic association between the VAM fungus (mycobiont) and the host plant (autobiont) benefits both organisms involved. The VAM fungus obtains carbohydrates from the plant and in return assists the plant primarily with the uptake of less mobile nutrients from outside of the root's nutrient depletion zone. Subsequent plant growth increases have been predominantly related to increased phosphorus (P) uptake and have been duplicated by the addition of P (Pacovsky et al., 1986). Thus, the optimal environment for a VAM-derived growth increase is where the VAM fungus can alleviate nutrient-related stress by assisting with nutrient uptake. (Cooper, 1984). On the other hand, high levels of soil P can frequently inhibit VAM colonization (Mosse, 1973).

Enhanced nutrient uptake not only increases plant growth but also drought resistance by increasing plant P uptake during drought (Nelsen and Safir, 1982) and possibly by increasing leaf water potential and transpiration rates (Cooper, 1984). Reduction of disease severity by VAM fungi has been reported in several studies (Harley and Smith, 1983). In Glomus intraradices-colonized tomato plants Caron et al. (1986) found a decrease in root rot of tomatoes caused by Fusarium

oxysporum f.sp. radicis-lycopersici. VAM infection compensated for disease-related biomass loss. Safir (1968) found a reduction in disease severity of onion infected by Pyrenochaeta terrestris. Similarly, Davis and Menge (1981) reported on different levels of resistance of VAM colonized citrus to Phytophthora parasitica. These effects, however, appear to be less related to direct effects of the VAM fungus on the pathogen as opposed to VAM-mediated effects in the root (Roncadori and Hussey, 1982). Although no host specificity has been observed (Harley and Smith, 1983), different degrees of mycorrhizal dependency of the host plant have been found, which depend on the plant's nutrient requirement, root distribution and the prevailing soil fertility (Plenchette et al., 1983). Different growth responses have been obtained in different hosts infected with the same VAM fungus (Plenchette et al., 1982) and it has also been suggested that particular host-fungus combinations may be more symbiotically effective, if physiology and growth rates of the symbionts are better matched (Smith and Gianinazzi-Pearson, 1988).

It would appear that an appropriately selected VAM fungus could have beneficial commercial applications. The primary obstacles for commercial application are the inability to grow VAM fungi axenically and the mass production of inoculum. Currently, VAM inoculum is primarily produced in pot cultures. Drawbacks to this system are the maintenance of a relatively disease-free environment as well as the time and space requirements for large-scale production.

Previous studies indicate the importance of selecting effective VAM fungi for specific growth conditions and crops (Mosse, 1973; Powell,

1984). The introduced VAM fungus should a) have the ability to compete with indigenous fungi b) be well adapted to the prevailing edaphic conditions and c) enhance growth of a wide range of host plants.

To deliver fungal inoculum to the plant a suitable inoculum carrier is required. The carrier should be easily incorporated into current plant production systems and maintain an appropriate environment for the survival of fungal propagules during long-term storage prior to use. Soilless media such as shredded bark, perlite, vermiculite and peat are typical planting media used in commercial production (Bunt, 1988). Some studies have found peat to be less effective for mycorrhizal growth enhancement, relating in part to the equilibrium P solution (Biermann and Linderman, 1983); thus to the lack of P absorption by peat (Graham and Timmer, 1984 and 1985). Other studies have been more successful (Caron and Parent, 1988; Nemec, 1987), indicating that a properly formulated mycorrhizal peat mix could be successful.

At present, asparagus fields are typically planted with 1 year-old seed-started crowns. Crowns are commonly grown in a field nursery for one year prior to transplanting to the field. Tissue-culture techniques are currently being developed for the propagation and genetic improvement of asparagus rather than growing plants from seed (Reuther, 1984). Tissue-cultured asparagus are multiplied <u>in vitro</u> (Chin, 1982) and then transferred into flats in the greenhouse, prior to moving them to the field. The advantage of greenhouse-produced transplants is the reduced risk of disease and lower requirements of labor and time for

planting (Ombrello and Garrison, 1978; Fisher, 1982). Some field studies, however, have observed a lower survival of transplants compared to crown-produced plants which may be related to low moisture conditions (Sterrett, 1990).

Asparagus has been found to benefit from VAM colonization (Burrows et al., 1990; Chang, 1985, Hussey et al., 1984; Powell et al., 1985; Wacker et al., 1990a). In fact, asparagus growth is significantly reduced when planted in fumigated soil compared to non-fumigated soil (Lacy, 1979). Roots of asparagus grown in fumigated soil were deficient in P compared to plants grown in non-fumigated soil (Evans et al., 1985), suggesting that young asparagus plants may be dependent on VAM-mediated P uptake. With increasing use of tissue-cultured asparagus, drought- and nutrient-related stress when transplanting to the field may be reduced by preinoculating asparagus with VAM fungi in the greenhouse.

It has been reported that asparagus suffers from a general decline (Grogan and Kimble, 1959), similar to other perennial cropping systems such as apple (Börner, 1959), peach (Patrick et al., 1964), citrus (Burger and Small, 1983) and alfalfa (Read and Jensen, 1989). Asparagus decline results in reduced field longevity and yield due to a decrease in plant vigor and lower plant densities (Cassini et al., 1985; Tu, 1985). Replanting of asparagus fields suffering from decline does not generally meet with success (Hanna, 1947; Grogan and Kimble, 1959). Stresses caused by environment and cultural practices are major factors predisposing asparagus to infection by pathogens (Hoddup, 1983; Takatori et al. 1970, Takatori et al. 1974; VanBakel et al., 1970). The

unsuccessful replanting of old asparagus fields is generally attributed to a high incidence of asparagus wilt and crown rot, caused by Fusarium oxysporum (Schlect.) Snyd. & Hans. f.sp. asparagi Cohen (Cohen & Heald) and Fusarium moniliforme (Sheld.) Snyd. & Hans. respectively (Johnston, 1979; Damicone and Manning, 1985). Field fumigation only partly remedies the problem (Lacy, 1979).

Several studies have suggested the involvement of autotoxic asparagus allelochemicals (Hartung, 1987; Yang, 1982; Young, 1984). Allelochemicals - often phenolic compounds - are substances produced by one organism that effect the growth and metabolism of another (Rice, 1984). These compounds have been found to not only affect interactions between plants, but between plants and microbes (Hartley and Whitehead, 1985; Rice, 1984). In fact the continuous input of these chemicals may over time change the microbial composition of the soil (Blum and Shafer, 1988) and indirectly affect plant growth. In asparagus (Wacker et al., 1990c) and coffee (Schenck et al., 1987) shifts in VAM populations have been observed with increasing field age. In asparagus, differences did not appear to be related to nutritional causes, suggesting that some other factor(s) was involved in the shift (Wacker, 1990c). Ferulic acid, one allelochemical isolated from asparagus tissue (Hartung, 1987), has been observed to reduce asparagus VAM colonization and plant growth with increasing concentrations (Wacker, 1990b). Many of the commonly found early-successional VAM fungal species are known to be effective symbionts of asparagus. If VAM populations shift in the direction of less effective species, VAM nutritional contribution to

asparagus growth, particularly P, may be reduced resulting in a subsequent decline in plant vigor.

Low P levels have been shown to increase root exudation (Ratnayake, 1978) as well as have phenolics (Tousson and Patrick, 1963). Increased root exudation may stimulate growth of pathogens such as Fusarium species which may be phenolic-tolerant. Although root exudates from white clover and citrus roots stimulate hyphal elongation of VAM fungi (Elias and Safir, 1987, Graham, 1982) allelochemicals may overcome this stimulatory effect (Wacker et al., 1990b) in addition to inhibiting plant growth and simultaneously predisposing the plant to infection (Linderman, 1970). Studies with asparagus root filtrates have found increased infection of asparagus by Fusarium oxysporum f.sp. asparagi (Pierce and Colby, 1987). Also, environmental factors such as high temperatures (Einhellig, 1987) and low nutritional regimes (Stowe and Osborn, 1980) have been found to increase the inhibitory effects of phenolics. In summary, allelochemicals have previously been shown to affect mycorrhizae and consequently asparagus decline may be related to interactions of asparagus-produced allelochemicals, VAM fungi and other soil organisms and enhanced by environmental stresses.

LITERATURE CITED

Biermann, B. and Linderman, R. G. 1983. Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular-arbuscular mycorrhizae. J. Amer. Soc. Hort. Sci. 108: 962-971.

Blum, U. and Shafer, S. 1988. Microbial populations and phenolic acids in soil. Soil Biol. Biochem. 20: 793-800.

Börner, H. 1959. Liberation of organic substances from higher plants and their role in the soil sickness problem. Bot. Rev., 26: 395-424.

Bunt, A. C. 1988. Media and mixes for container grown plants. Unwin Hyman Ltd., London.

Burger, W. P. and Small, J. G. C. 1983. Allelopathy in citrus orchards. Sci. Hort. 20: 361-375.

Burrows, R., Pfleger, F. L. and Waters, L. Jr. 1990. Growth of seedling asparagus inoculated with Glomus fasciculatum and phosphorus supplementation. HortScience 25: 519-521.

Caron, M. and Parent, S. 1988. Definition of a peat-lite medium for the use of vesicular-arbuscular mycorrhizae (VAM) in horticulture. Acta Horticultureae 221: 289-294.

Cassini, R. C., El Medawar, S. and Cassini, R. P. 1985. A biological control technique to prevent Fusarium decline in the fields. Proc. Sixth International Asparagus Symposium. Eds. Lougheed, E. C. and Tiessen, H., University of Guelph. pp. 228-237.

Chang, D. C. N. 1985. Asparagus mycorrhizae: Growth effect and structural changes. Proc. Sixth International Asparagus Symposium. pp. 307-316.

Chin, C. 1982. Promotion of shoot and root formation in asparagus in vitro by ancymidol. Hort Sci. 17: 590-591.

Cooper, K. M. 1984. Physiology of VA mycorrhizal associations. *In*: VA Mycorrhiza. Eds. Powell, C. L. and Bagyaraj, D. J. CRC Press, Boca Raton, Florida. pp. 155-186.

Damicone, J. P. and Manning, W. J. 1985. Frequency and pathogenicity of Fusarium species isolates from first year asparagus grown from transplants. Pl. Dis. 69: 413-416.

Davis, R. M. and Menge, J. A. 1980. Influence of Glomus fasciculatus and soil phosphorus on Phytophthora root rot of citrus. Phytopath. 70: 447-452.

Einhellig, F. A. 1985. Interactions among allelochemicals and other stress factors of the plant environment. *In*: Allelochemicals: Role in Agriculture and Forestry. Ed. G. R. Waller. ACS Symposium series 330. Washington, DC. Chap. 32.

Elias, K. S. and Safir, G. R. 1987. Hyphal elongation of Glomus fasciculatus in response to root exudates. Appl. Envir. Microb. 53: 1928-1933.

Evans, T. A., Safir, G. R. and Stephens, C. T. 1985. Vesicular-arbuscular mycorrhizal fungi and their importance in asparagus decline. Proc. Sixth International Asparagus Symposium. pg. 399 (Abstr.).

Fisher, K. J. 1982. Comparison of the growth and development of young asparagus plants established from seedling transplants and by direct seeding. New Zealand J. Expt. Agr. 10: 405-408.

Graham, J. H. 1982. Effect of citrus root exudates on germination of chlamydospores of the vesicular-arbuscular mycorrhizal fungus, Glomus epigaeum. Mycologia 74: 831-835.

Graham, J. H. and Timmer, L. W. 1984. Vesicular-arbuscular mycorrhizal development and growth response of rough lemon in soil and soilless media: Effect of phosphorus source. J. Amer. Soc. Hort. Sci. 109: 118-121.

Graham, J. H. and Timmer, L. W. 1985. Rock phosphate as a source of phosphorus for vesicular-arbuscular mycorrhizal development and growth of citrus in a soilless medium. J. Amer. Soc. Hort. Sci. 110: 489-492.

Grogan, R. G. and Kimble K. A. 1959. The association of Fusarium wilt with the asparagus decline and replant problem in California. Phytopath. 49: 122-125.

Hanna, G. C. 1947. Asparagus production in California. Calif. Agr. Ext. Serv. Circ. 91. pp. 23.

Harley, J. L. and Smith, S. E. 1983. Mycorrhizal Symbiosis. Academic Press, New York, pp. 483.

Hartley, R. D. and Whitehead, D. C. 1985. Phenolic acids in soils and their influence on plant growth and soil microbial processes. *In*: Soil Organic Matter and Biological Activity. Eds. D. Vaughan, and R. E. Malcolm, Martinus Nijhoff/Dr. W. Junk Publishers, Boston. Chap. 3.

Hartung, A.C. 1987. Allelopathic potential of asparagus (Asparagus officinalis L.). Ph.D. dissertation. Michigan State University, 142 pp.

Hoddup, R. M. 1983. Investigation of factors which contribute to asparagus (Asparagus officinalis L.) decline in Michigan. M.S. thesis. Michigan State University. 53 pp.

Hussey, R. B., Peterson, R. L. and Tiessen, H. 1984. Interactions between vesicular-arbuscular mycorrhizal fungi and asparagus. Plant and Soil 79: 403-416.

Johnston, S. A., Springer, J. K. and Lewis, G. D. 1979. Fusarium moniliforme as a cause of stem and crown rot of asparagus and its association with asparagus decline. Phytopath. 69: 778-780.

Lacy, M. L. 1979. Effects of chemicals on stand establishment and yields of asparagus. Pl. Dis. Rep. 63: 612-616.

Linderman, R. G. 1970. Plant residue decomposition products and their effects on host roots and fungi pathogenic to roots. Phytopath. 60: 19-22.

Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Ann. Rev. Plant Path. 11: 171-196.

Nelsen, C. E. and Safir, G. R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154: 407-413.

Nemec, S. 1987. Glomus intraradices infection and citrus growth in soil-free potting media. *In*: Mycorrhizae in the next decade. Proc. 7th NACOM, Gainesville, Florida. pg. 282.

Ombrello, T. M. and Garrison, S. A. 1978. Establishing asparagus from seedling transplants. HortScience 13: 663-664.

Pacovsky, R. S., Bethlenfalvay, G. J. and E. A. Paul. 1986. Comparisons between P-fertilized and mycorrhizal plants. Crop Sci. 26: 151-156.

Patrick, Z. A., Toussoun, T. A. and Koch, L. W. 1964. Effect of crop-residue decomposition products on plant roots. Ann. Rev. Phytopath. 2: 267-292.

Pierce, L. C. and Colby, L. W. 1987. Interaction of asparagus root filtrate with Fusarium oxysporum f. sp. asparagi. J. Am. Soc. Hort. Sci. 112: 35-40.

Plenchette, C., Fortin, J. A. and Furlan, V. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant and Soil 70: 199-209.

Plenchette, C. Furlan, V. and Fortin, J. A. 1982. Effects of different endomycorrhizal fungi on five host plants grown on calcined montmorillonite clay. J. Am. Soc. Hort. Sci. 107: 535-538.

Powell C. L., Bagyaraj, D. J., Clark, G. E. and Caldwell, K. I. 1985. Inoculation with vesicular-arbuscular mycorrhizal fungi in the greenhouse production of asparagus seedlings. N. Z. J. of Agr. Res. 28: 293-297.

Ratanayke, M., Leonard, R. T. and Menge, J. A. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytol. 81: 543-552.

Read, J.J. and Jensen, E.H. 1989. Phytotoxicity of water-soluble substances from alfalfa and barley soil extracts on four crop species. J. Chem. Ecol. 15: 619-628.

Reuther, G. 1984. Asparagus. *In*: Handbook of plant cell culture. Eds. W. R. Sharp, D. A. Evans, P. V. Ammirato and Y. Yamada. Vol. 2, pp. 211-242.

Rice, E. L. 1984. Allelopathy. 2nd edition. Academic Press, Inc. New York. pp. 422.

Roncadori, R. W. and R. S. Hussey. 1982. Mycorrhizae in interactions with other microorganisms. *In*: Methods and Principles in Mycorrhizal Research. Ed.: N. C. Schenck. The American Phytopath. Soc., St. Paul Minnesota. pp. 219-223.

Safir, G. R. 1968. The influence of vesicular-arbuscular mycorrhizae on the resistance of onion to Pyrenochaeta terrestris. M.S. Thesis, University of Illinois, Urbana. pp. 36.

Schenck, N. C., Siqueira, J. O. and Oliviera, E. 1987. Incidence of VA mycorrhizal fungi in native and cultivated Brazilian soil. Phytopath. 77: 1736 (Abstr.).

Smith, S. E. and Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Ann. Rev. Plant Physio. Plant Mol. Biol. 39: 221-244.

Sterrett, S. B., Ross, B. B. and Savage, C. P. Jr. 1990. Establishment and yield of asparagus as influenced by planting and irrigation method. J. Amer. Soc. Hort. Sci. 115: 29-33.

Stowe, L. G. and Osborn, A. 1980. The influence of nitrogen and phosphorus levels on the phytotoxicity of phenolic compounds. Can. J. Bot. 58: 1149-1153.

Takatori, F., Stillman, J. I. and Souther, F. D. 1970. Asparagus yields and plant vigor as influenced by time and duration of cutting. Calif. Agri. 24: 8-9.

Takatori, F., Stillman, J. I. and Souther, F. D. 1974. Influence of planting depth on production of green asparagus. Calif. Agri. 28: 4-5.

Tousson, T. A., and Z. A. Patrick. 1963. Effect of phytotoxic substances from decomposing plant residues on root rot of bean. Phytopath. 53: 265-270.

Tu, C. C. 1985. Major diseases of asparagus and their control in Taiwan. Proc. Sixth International Asparagus Symposium. Eds. E. C. Lougheed, and H. Tiessen. University of Guelph. pp. 168-180.

Wacker, T. L., Safir, G. R. and Stephens, C. T. 1990a. Effect of Glomus fasciculatum on the growth of asparagus and the incidence of Fusarium root rot. J. Amer. Soc. Hort. Sci. 115: 550-554.

Wacker, T. L., Safir, G. R. and Stephens, C. T. 1990b. Effects of ferulic acid on Glomus fasciculatum and associated effects on phosphorus uptake and growth of asparagus (Asparagus officinalis). J. Chem. Ecol. 16: 901-909.

Wacker, T. L., Safir, G. R. and Stephenson, S. N. 1990c. Evidence for succession of mycorrhizal fungi in Michigan asparagus fields. Acta Hort. 271: 273-279.

Yang, H.-J. 1982. Autotoxicity of Asparagus officinalis L. J. Am. Soc. Hort. Sci. 107: 860-862.

Young, C. C. 1984. Autointoxication in root exudates of Asparagus officinalis L. Plant and Soil 82: 247-253.

SECTION I

GROWTH OF ASPARAGUS IN A COMMERCIAL PEAT MIX CONTAINING VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGI AND THE EFFECTS OF PHOSPHORUS

INTRODUCTION

Asparagus can benefit from vesicular-arbuscular mycorrhizal (VAM) infection in the greenhouse (Burrows et al., 1990; Hussey et al., 1984; Powell et al., 1985) and in the field (Powell, 1983; Wacker et al., 1990). The primary advantage of VAM formation is derived from enhanced nutrient uptake (Cooper, 1984) resulting in, for example, optimal plant growth in Boston fern (Ponton et al., 1990), increased drought tolerance in soybean (Nelsen and Safir, 1982) and disease resistance in tomato (Caron et al., 1986). With the advances in tissue culture aimed at genetic improvement and mass production of asparagus and other crops such as strawberries (Kiernan et al., 1984), development of techniques to produce vigorous transplants with efficient root systems is needed. Attempts to preinoculate plants in vitro have been made (Ravolanirina et al., 1989), however, since soilless substrates are commonly used in greenhouse transplant production, preinoculation with VAM fungi in the greenhouse prior to transplanting to the field would be less laborious and fit into current commercial production practices. Although attempts have been made to use soilless media, especially peat-based mixes

(Caron and Parent, 1988; Ponton et al., 1990 and Powell et al., 1985), very few commercial VAM inoculants have been developed.

The objective of this study was to examine the asparagus growth response and VAM formation of asparagus in a commercial peat planting medium infested with different VAM fungi under greenhouse and field conditions and and at different levels of applied P.

MATERIALS AND METHODS

Effects of different VAM fungi. For the field and greenhouse experiment, asparagus (clone 362-M, Nourse Farms, South Deerfield, MA) was propagated by tissue culture (modified from Chin, 1982). Basal crown segments were first cultured on medium A (Appendix A) for 4-6 weeks to promote shoot growth, and then on medium B for 4-5 weeks to induce root production. The agar-based medium was washed from the roots and plants were transferred to plastic cell-packs (48 cells, 72 cm^3/cell) into a peat mix (mean: pH = 5.5, NO_3 = 5 ppm, P = 6.8 ppm, K = 5 ppm, Ca = 20 ppm and Mg = 4 ppm) infested with one of the following VAM fungi : none (= control), Glomus clarum Nicolson & Schenck (GC), G. intraradix Schenck & Smith (GI) renamed by Almeida (1989), G. monosporum Gerdemann & Trappe (GM), G. versiforme Berch & Fortin (= G. epigaeum Daniels and Trappe) (GVR), or G. vesiculiferum (Thaxter) Gerdemann & Trappe (GVS). Peat-based inocula were produced by Premier Peat Moss, Ltd., Rivière-du Loup, Québec. Transplants were covered with a plastic lid for 4 days under reduced lighting (9 $umol \cdot s^{-1} \cdot m^{-2}$) at 23°C. Subsequently, plants were transferred to a greenhouse bench

covered with heavy shade cloth (65 % light transmission) with mean temperatures at approximately 24°C. Eight days later the plastic lid and shade cloth were removed.

Also for the greenhouse and field experiment, asparagus seeds (cv. 'Mary Washington') were sterilized for 25 min. in a 20 % commercial bleach solution, rinsed with sterile distilled water several times and then sown in a soil mix (2 parts sand and 1 part loamy soil) pasteurized for 2 hours at 100°C (pH = 7.2, P = 28 ppm - Bray I, K = 27 ppm, Ca = 659 ppm and Mg = 107 ppm). Three week-old seedlings were transplanted into cell-packs containing the peat-based VAM inoculum at the same time as the tissue-cultured plants. Three weeks later, all plants were transplanted to plastic pots (400 cm^3) containing the corresponding VAM peat mix that had been used in the cell-packs. Each liter of air-dry peat was amended with 4 g dolomitic lime, 0.8 g $Ca(NO)_3$ and 1.3 g Peter's Fritted Trace Elements (W.R. Grace & Co., Fogelsville, PA). Plants in the plastic pots were fertilized weekly with 250 $mg \cdot l^{-1}$ N and K (25-0-25, W.R. Grace & Co.).

Ten week-old tissue-cultured plants, including the VAM-infested peat from the 400 cm^3 pots, were transplanted into larger pots (1500 cm^3) containing the pasteurized sand/soil mix described before. Plants were set up in a completely randomized design with fungal treatments replicated 8 times and were maintained in the greenhouse at 23°/28°C (day/night). Plants received 250 $mg \cdot l^{-1}$ N and K (25-0-25) and 20 mg S.T.E.M./l of soil (W.R. Grace & Co.) weekly and a monthly application of 95 $mg \cdot l^{-1}$ P as a phosphoric acid (85 % H_3PO_4) solution. After the fourth week each pot received 500 ml of tapwater containing 0.27 ml

nitric acid (71 % HNO_3) weekly to compensate for alkaline water conditions. Plants were harvested after 16 weeks and plant dry weight, fern and bud numbers were measured. A random sample of roots was taken from each treatment, cleared (Phillips and Hayman, 1970) and stained with 0.5 % aniline blue in lacto-glycerol (875 ml lactic acid, 63 ml glycerol and 63 ml tap-distilled water). VAM root colonization was assessed at 160X using 30 one cm root segments per slide and treatment. VAM colonization was rated as: 1 = 0 % infection, 2 = 1-25 %, 3 = 26-50 %, 4 = 51-75 % and 5 = over 75 % of each segment colonized.

In July 1988, 24 tissue-cultured (8 weeks old) and 32 seed-started plants (10 weeks old) grown as previously described, were transplanted to a fumigated (96 kg methylbromide/ha) field soil (Spinks sandy loam, pH = 6.5, 219 ppm P, 92 ppm K, 421 ppm Ca, 109 ppm Mg) located at the Asparagus Research Farm in Hart, MI. VAM treatments were randomly arranged in rows. Surface trickle irrigation was used when plants showed signs of drought stress. After the first light frost (October 1988) and 12 weeks in the field, a minimum of 5 randomly chosen plants from the tissue-culture treatment were harvested. Root samples were taken for VAM colonization assessment and other parameters were measured as previously described.

The seed-started and remaining tissue-cultured plants were left in the field for a second season of growth. Plant survival was determined 14 months after transplanting (September 1989).

VAM infectivity of the peat planting mixes infested with different VAM fungi was studied in a separate experiment. 24 plastic cell-packs

(185 cm^3/cell) were planted with surface-sterilized (Elmer and Stephens, 1988), pre-germinated asparagus seedlings (cv. 'Mary Washington'). Plants were harvested at 5, 8 and 11 weeks. The whole root system from each plant was washed free of soil, cleared and stained as previously described. VAM colonization was assessed by the line-intersect method (Kormanik and McGraw, 1982). A root system was counted as colonized if there was a minimum of one hyphal penetration point.

Effects of applied phosphorus. The effects of a weekly application of P, as phosphoric acid, on asparagus growth and VAM colonization in the VAM infested peat planting mixes (mean: pH = 6.1, NO_3 = 3 ppm, P = 6 ppm, K = 11 ppm, Ca = 60 ppm and Mg = 18 ppm) was evaluated. The four treatments included: control, GF (VAM fungus resembling Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske), G. intraradix (GI) or G. versiforme (GVR). Asparagus seeds (cv. 'Mary Washington') were surface-sterilized (Elmer et al., 1988) and pregerminated in sand at 30°C for one week after which seedlings of uniform size were transferred to 24 cell-packs (185 cm^3/cell) containing one of the peat mixes. Plants were fertilized with a solution containing 100 $mg \cdot l^{-1}$ N and K (25-0-25, W.R. Grace & Co.) and 19 $mg \cdot l^{-1}$ Fe from ferric sodium EDTA at the rate of 1 l/flat weekly. They were grown under high-pressure sodium vapor lights (14 h photoperiod; 200 $umol \cdot s^{-1} \cdot m^{-2}$ at plant level) in the greenhouse (26°/23°C, day/night) for three weeks. At this time they were transplanted to plastic pots (400 cm^3/pot). Plants were fertilized weekly with N and K (100 $mg \cdot l^{-1}$ each from 25-0-25, 100 ml/pot) and Fe

(19 $mg \cdot l^{-1}$) was added during the first four weeks. After three weeks in the pots, 100 ml of a P solution (0, 50, 100 or 150 $mg \cdot l^{-1}$ from 85 % H_3PO_4, pH ranging from 7.6 to 6.4) was applied weekly to each pot. The experiment was conducted as a 4 x 4 factorial (4 VAM x 4 P levels), randomized complete block design with 18 replications per treatment. Half of the plants were harvested after 13 weeks and the remainder after 17 weeks. Root samples for VAM colonization were cleared and stained as before and assessed using the line intersect method (Kormanik and McGraw, 1982). Plants were dried at 80°C for two days and the cladophyll P content determined. Samples were analyzed at the M.S.U. Plant and Soil testing facility. Data from each harvest were subjected to an analysis of variance using MSTAT-C (Michigan State University, 1989) and the means were separated using Duncan's multiple range test.

RESULTS

Effects of different VAM fungi. The effect of the different VAM inocula on asparagus growth both in the greenhouse and in the field are presented in Table 1.1. The dry weight of plants inoculated with GI was significantly larger ($P \leq 0.05$) than the control plants in the field (300 % increase) and in the greenhouse (43 % increase). Control, GC, GM, GVR and GVS were not significantly different in dry weight. Fern number of the GI plants was twofold higher ($P \leq 0.01$) than the control in the field, however there were no significant differences among the treatments in the greenhouse. The number of buds in field plants was

TABLE 1.1. Growth and VAM rating of tissue-cultured plants precolonized with VAM fungi grown in the greenhouse and the field for 16 and 12 weeks respectively.

VAM species	Field				Greenhouse			
	Total dwt. (g)	Fern (#)	Bud (#)	VAM rating[a]	Total dwt. (g)	Fern (#)	Bud (#)	VAM rating[a]
Control	8.5 b	6 c	11 bc	0 c	43.3 bc	10 a	17 ab	0 c
GC	23.0 ab	7 bc	15 ab	2.2 b	54.4 ab	10 a	15 b	1.2 b
GI	27.1 a	13 ab	15 ab	3.4 a	61.8 a	9 a	20 ab	2.4 a
GM	19.6 ab	10 abc	16 ab	3.3 a	42.1 b	10 a	22 ab	2.3 a
GVR	8.1 b	6 c	7 c	2.6 b	39.1 c	9 a	17 ab	1.6 b
GVS	22.4 ab	15 a	20 a	2.4 b	50.1 abc	9 a	22 a	1.4 b

Means in columns followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

[a]VAM rating of asparagus root segments with values ranging from 0 (no infection) to 4 (>75 % of segment colonized).

significantly higher ($P \leq 0.01$) in the plants inoculated with GVS (20 buds per plant) compared to the control plants (11 buds per plant). There were no differences for number of buds in the greenhouse. Root colonization was highest in the plants grown in the GI and GVR mycorrhizal inocula, whereas control plants remained non-mycorrhizal both in the field and greenhouse. No attempt was made to isolate VAM fungi from roots in the field.

Survival data after 14 months in the field are shown in Figure 1.1. The mean survival rate of all seed-started, mycorrhizal plants was 35 %, whereas only 6 % of the control plants survived. The mean survival of mycorrhizal tissue-cultured plants was 66 %, while only 38 % of the control plants survived. In the tissue-cultured plants GC and GVS showed increased survival over the control. Overall the mycorrhizal tissue-cultured plants had a survivability almost twice that of the seed-started plants.

In the VAM colonization experiment, whole plant fresh weight and number of plants colonized after 5, 8 and 11 weeks is shown in Table 1.2. At 5 weeks significant ($P \leq 0.05$) increases in fresh weight were found in GC-, GI- and GVR-inoculated plants compared to the the control. Fresh weight was significantly ($P \leq 0.05$) depressed in the GF and GM treatments in the eighth week compared to the control. At 11 weeks GC, GI and GVR had significantly ($P \leq 0.05$) larger fresh weights than the control. The mean percent plants colonized was highest in the GC and GI treatments at 11 weeks and only GI had infected 100 % of the plants at this point in time. The percent root colonization increased at each harvest only for these two fungi; whereas, colonization in the

Figure 1.1. Survival of tissue-cultured and seed-produced asparagus after two seasons of growth in the field (percent survival ± SE).

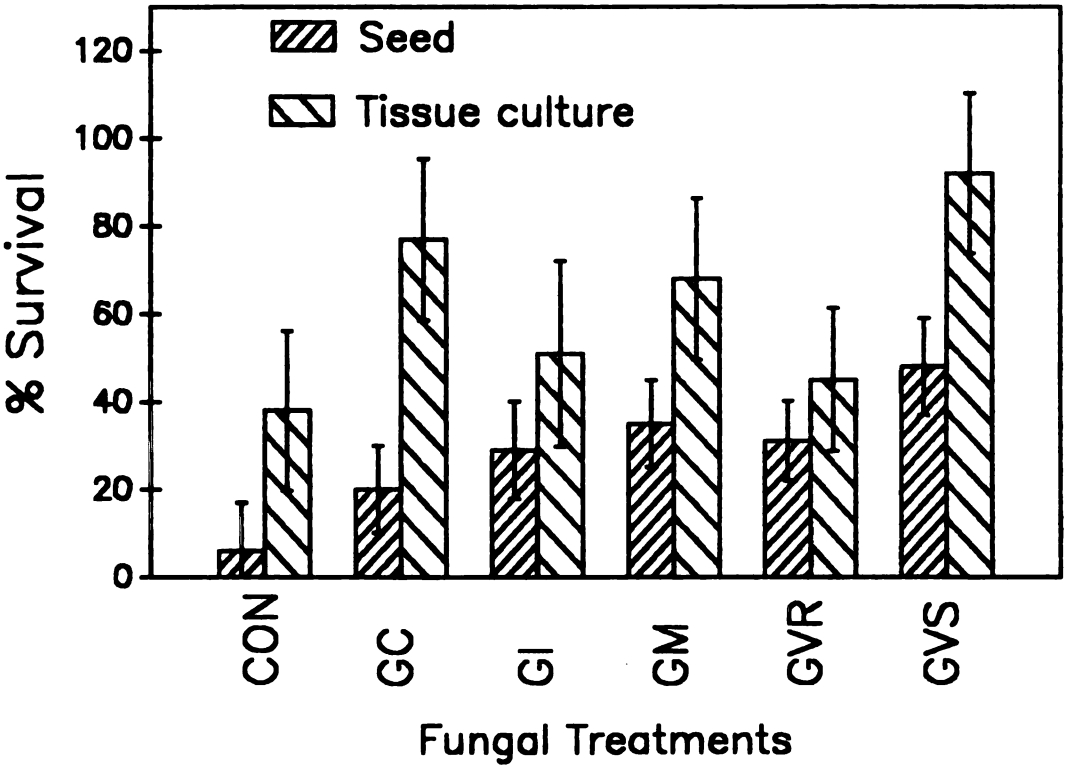

Seed
Tissue culture
% Survival
120
100
80
60
40
20
0
CON
GC
GI
GM
GVR
GVS
Fungal Treatments

TABLE 1.2. Mean fresh weight, percent roots colonized and percent plants colonized at 5, 8 and 11 weeks of growth in individual peat mixes.

VAM species	5 weeks			8 weeks			11 weeks		
	Fresh weight (g)	[ab]Roots colonized (%)	Plants colonized (%)	Fresh weight (g)	[ab]Roots colonized (%)	Plants colonized (%)	Fresh weight (g)	[ab]Roots colonized (%)	Plants colonized (%)
Control	.67 c	-	0	1.78 a	-	0	2.35 b	-	0
GC	.87 b	8 a	63	1.58 a	21 a	63	4.13 a	38 a	63
GF	.51 c	4 a	38	.67 c	10 a	63	2.46 b	11 a	50
GI	1.09 a	2 a	88	1.25 ab	16 a	50	4.15 a	39 a	100
GM	.63 c	-	0	.78 bc	-	0	1.48 b	-	17
GVR	.92 ab	-	13	1.39 a	17 a	13	4.55 a	18 a	17
GVS	.57 c	-	0	1.69 a	9 a	76	2.25 b	-	0

Means in columns followed by the same letter are not significantly different at $P \leq 0.05$ (Duncan's multiple range test).

[a]Mean based on number of colonized plants only.

[b]"-" = no colonization

other four species had leveled off at 8 weeks. At 11 weeks no VAM colonization was detected in the asparagus plants sampled from the GVS mix. Although the production of each peat-based mycorrhizal mix was similar, colonization of individual plants in a single treatment varied considerably and subsequently treatments were not significantly different.

Effects of applied phosphorus. Mean dry weights of plants inoculated with GI and GVR were significantly higher than either the control or plants grown in the GF infested peat mix across all applied P levels at weeks 13 and 17 (Table 1.3). There was no significant VAM x P interaction ($P \leq 0.05$) for plant dry weight. The GI- and GVR-treated plants developed normal shoots, whereas the control and GF-treated plants had stunted growth. GF plants were colonized at much lower levels than GI or GVR. The initial P level in the peat mixes was in the range of 6 ug/g (Bray I). Cladophyll P concentrations at both harvests increased significantly with increasing levels of applied P (Table 1.3). However, P application had no significant effect on dry weight at either week 13 or 17. GI and GVR plants had significantly lower tissue P concentrations than the control or GF plants (Table 1.3). Tissue concentrations of tested nutrients other than P were significantly lower in the GI and GVR treatments at week 13, with the exception of K which was higher in these treatments (Appendix B).

P application had a significant inhibitory effect ($P \leq 0.05$) on root colonization (Table 1.3). Root colonization decreased with increasing levels of P ($r = -0.98$, $P \leq 0.01$) at 13 weeks, but not at 17 weeks ($P = 0.21$). Increases in plant dry weight were correlated with

TABLE 1.3. Effect of VAM fungal species and applied P on root colonization, percent P in cladophylls and dry weight of asparagus grown for 13 or 17 weeks in peat planting mix in the greenhouse.

	Roots colonized (%)		Cladophyll P (%)		Total dry weight (g)	
	13 weeks	17 weeks	13 weeks	17 weeks	13 weeks	17 weeks
VAM:						
Control	0 -	0 -	0.47 a	0.57 a	0.59 c	0.89 b
GF	13 b	15 b	0.43 b	0.55 a	0.47 c	0.69 b
GI	47 a	89 a	0.36 c	0.43 b	1.38 b	3.93 a
GVR	59 a	84 a	0.38 c	0.44 b	1.88 a	4.45 a
Applied P (ppm):						
0	48 a	69 a	0.35 c	0.46 b	1.13 a	2.50 a
50	43 a	64 ab	0.41 b	0.48 b	1.04 a	2.48 a
100	38 ab	56 b	0.41 b	0.53 a	1.04 a	2.44 a
150	30 b	62 ab	0.46 a	0.53 a	1.11 a	2.54 a

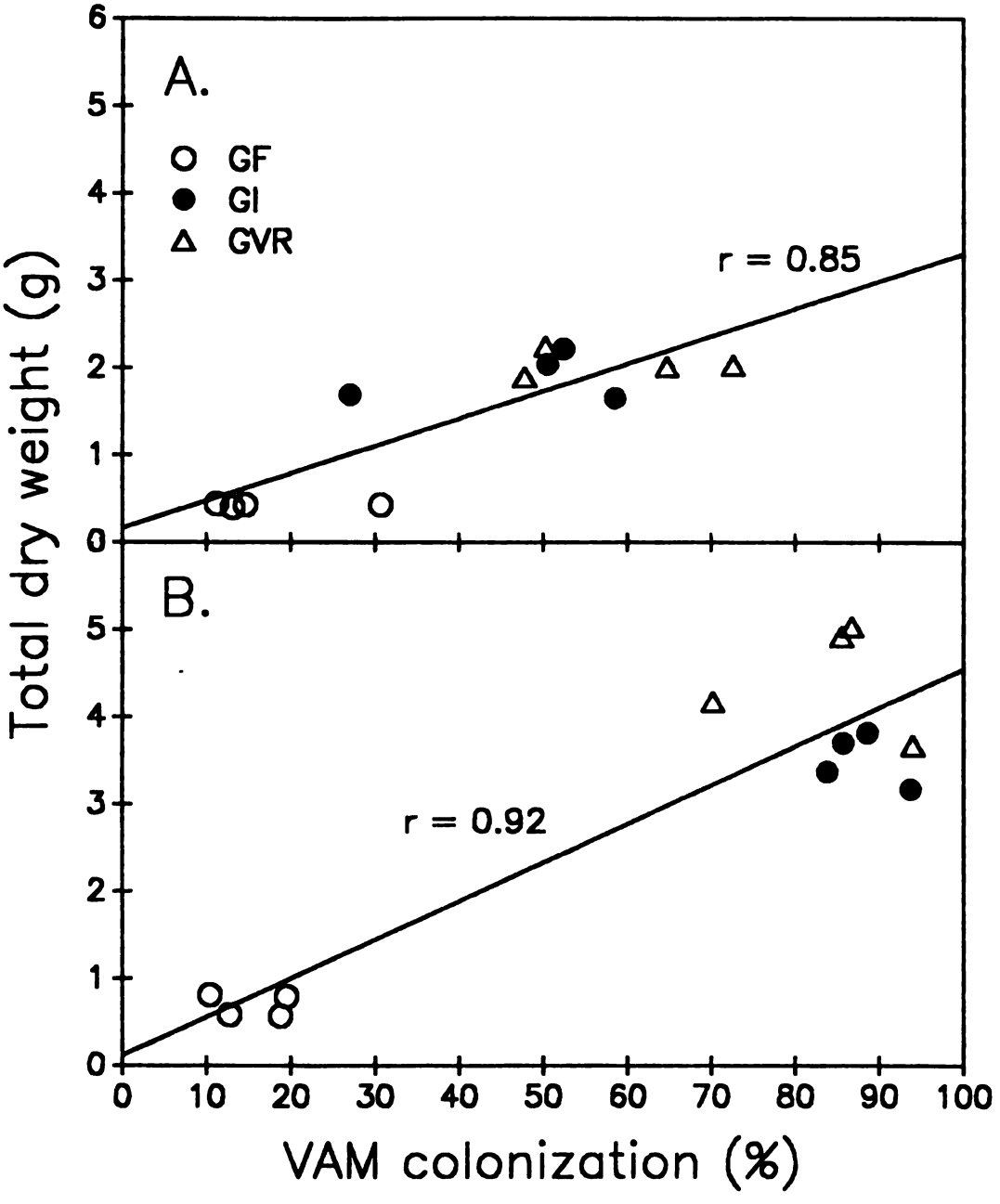
Means in columns within treatment group followed by the same letter are not significantly different at $P \leq 0.05$ (Duncan's multiple range test).

VAM root colonization rate (Figure 1.2). This experiment was repeated with a different shipment of mycorrhizal peat and a similar response to applied P was obtained.

DISCUSSION

VAM formation on plants in the greenhouse and in the field was well developed at the end of the experiments. GI increased growth substantially under both conditions; however, the response was more pronounced in the field irrespective of pre-existing high soil P levels. The drought conditions prevailing in 1988 may have reduced mobility of soil P, thus reducing any inhibitory effect of high soil P levels on VAM colonization. The number of shoots, which previously was found to correlate well with yield (Ellison and Sheer, 1959), was significantly increased by the GI and GVS treatments. In spite of the irrigation, the physiologically stressing field conditions, especially the drought, may have enhanced the VAM response. Precipitation during transplant establishment in the field was below the monthly mean for July (9.5 cm deficit) and August (7.1 cm deficit). In fact, field surface trickle irrigation in the field does not appear to improve survival or growth of asparagus transplants as does subsurface trickle irrigation (Sterrett et al., 1990). The increased survival of the preinoculated plants compared to the control after 14 months in the field for both seed-started and tissue-cultured plants may be associated with the drought conditions in the first year of growth. Increased survival of mycorrhizal plants compared to non-mycorrhizal

Figure 1.2. Relationship between total plant dry weight (g) and VAM colonization (%) in phosphorus experiment at A. 13 weeks and B. 17 weeks of growth. Significant at $P \leq 0.001$).


A.
GF
GI
GVR
r = 0.85
B.
r = 0.92
Total dry weight (g)
VAM colonization (%)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
90
100

plants has also been found in other transplanted crops (Cooper, 1981; Menge et al., 1978; J.O. Siqueira, personal communication). The increased survival of the tissue-cultured plants over the seed-started plants may prove to be of economic significance in the future as increasing numbers of growers use micropropagated plants.

Although in the colonization experiment no VAM formation was observed in the plants grown in the GVS-infested peat, it did occur after a similar time span in other replications. Previous studies have indicated that a substrate high in organic matter, such as peat, may not be suitable for VAM formation (Biermann and Linderman, 1983; Johnson and Hummel, 1986). The given conditions (e.g. type of peat, pH, nutrient availability) may exhibit differential effects on the VAM fungal species tested, which are known to differ in sensitivity to edaphic factors (Mosse, 1975, Sylvia and Schenck, 1983). The effectiveness of the VAM species tested may also depend on the characteristics of the soil from which they were originally isolated. Louis and Lim (1988) observed different responses in VAM development of two isolates of G. clarum obtained from sites with different P levels.

As shown in the colonization assay, VAM colonization was low in asparagus grown in GVR-containing peat, however plant fresh weight was increased over the control in that same treatment. Although all fungi appeared to stain satisfactorily, GVR may not stain equally well (Daniels et al., 1981). In addition root colonization is not always as good an indicator of VAM effectiveness as is the amount of external mycelium, which is the site of nutrient absorption (Graham et al. 1982). The beneficial effect of GVR for asparagus growth has also been

demonstrated in other studies (Plenchette et al., 1982; Hussey et al. 1984). This fungus showed growth-promoting effects in the phosphorus and colonization experiments, but not in the experiment comparing different fungal species in the field. The experiments were carried out with batches of peat prepared at different times and the responses may be related to this.

In the P experiment, the GI- and GVR-colonized plants had higher dry weights and lower tissue P concentrations than did the control at all levels of applied P. This suggests that P was not the limiting factor for growth in the medium and that the increased growth in the GI and GVR treatments may involve other growth response mechanisms. This supports previous field results (Wacker et al., 1990), which suggest that the increased growth of asparagus preinoculated with VAM after one season in the field was unrelated to increased tissue P concentration. Other physiological effects of VAM, such as increased transpiration of mycorrhizal plants, are also unrelated to P (Augé, 1989). Development of mycorrhizal plants was normal compared to the non-mycorrhizal control, suggesting that adequate levels of nutrients were present for growth. VAM fungi have been found to moderate the effects of unbalanced nutrients in soil (Siqueira et al., 1990) and in peat (Graham and Fardelmann, 1986). The observed growth differences in this study may be related to the ability of VA mycorrhizal fungi to compensate for such a nutrient imbalance.

The decrease in asparagus root colonization found at higher P application rates has been reported in asparagus (Burrows et al., 1990)

and in other plants (Graham and Timmer, 1984; Plenchette et al., 1983; Schubert et al., 1986). The reason for the decrease in root colonization found at 13 weeks, but not at 17 weeks is not readily clear; however, the results may be related to the dilution of P in plants with increasing plant age (Jarrell and Beverly, 1981). In contrast to other work with GF (Wacker et al., 1990; Burrows et al., 1990), in the present study, colonization and symbiotic effectiveness by GF were substantially lower than GI or GVR. This indicates that these two fungi may be better adapted to the growing conditions in the peat.

The data presented here supports previous studies on the benefits of VAM fungi to asparagus growth in the field (Powell, 1983; Wacker et al., 1990) and in the greenhouse (Burrows et al., 1990; Hussey et al, 1984; Plenchette et al., 1982; Powell et al., 1985). The use of a peat planting mix infested with VAM fungal propagules proved to be an effective way to introduce VAM fungi into commercial transplant production systems in the greenhouse and may be of importance for field growth and survival. These studies indicate that G. intraradix, and possibly G. versiforme, may be better suited for preinoculation of asparagus under the biological, physical and chemical growth conditions in this particular peat mix.

LITERATURE CITED

Almeida, R. T. 1989. Scientific Names in the Endogonales, Zygomycotina. Mycotaxon 1: 147-159.

Augé, R. M. 1989. Do VA mycorrhizae enhance transpiration by affecting host phosphorus content? J. Plant Nutr. 12: 743-753.

Biermann, B. and Linderman, R. G. 1983. Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular-arbuscular mycorrhizae. J. Amer. Soc. Hort. Sci. 108: 962-971.

Burrows, R., Pfleger, F. L. and Waters, L. Jr. 1990. Growth of seedling asparagus inoculated with Glomus fasciculatum and phosphorus supplementation. HortScience 25: 519-521.

Caron, M., Fortin, J. A. and Richard, C. 1986. Effect of inoculation sequence on the interaction between Glomus intraradices and Fusarium oxysporum f. sp. radicis-lycopersici in tomatoes. Can. J. Plant Path. 8: 12-16.

Caron, M. and Parent, S. 1988. Definition of a peat-lite medium for the use of vesicular-arbuscular mycorrhizae (VAM) in horticulture. Acta Hort. 221: 289-294.

Chang, D. C. N. 1985. Responses of asparagus seedlings to three Glomus endomycorrhizal fungi. Proc. 6th NACOM, pg. 369.

Chin, C. 1982. Promotion of shoot and root formation in asparagus in vitro by ancymidol. Hort Sci. 17: 590-591.

Cooper, K. M. 1984. Physiology of VA mycorrhizal associations. *In*: VA Mycorrhiza. Eds. C. L. Powell, and D. J. Bagyaraj, CRC Press, Boca Raton, Florida. pp. 155-186.

Cooper, K. M. 1981. The role of VA mycorrhizas in the development of a new commercial crop - tamarillo - in New Zealand. Proc. 5th N. Amer. Conf. on Mycorrhizae, Univ. Laval. Quebec. pg. 54. (Abstr.).

Daniels, B. A., McCool, P. M. and Menge, J. A. 1981. Comparative inoculum potential of spores of six vesicular-arbuscular mycorrhizal fungi. New Phytol. 89: 385-391.

Dufault, R. J. and Waters, L. Jr. 1984. Propagation methods influence asparagus transplant quality and seedling growth. HortScience 19: 866-868.

Ellison, J. H. and Scheer, D. F. 1959. Yield related to brush vigor in asparagus. Proc. Amer. Soc. Hort. Sci. 73: 339-344.

Elmer, W. H. and Stephens, C. T. 1988. Comparison of technique for eliminating contaminants from asparagus seeds. HortScience 23: 1031-1032.

Graham, J. H. and Fardelmann, D. 1986. Inoculation of citrus with root fragments containing chlamydospores of the mycorrhizal fungus Glomus intraradices. Can. J. Bot. 64: 1739-1744.

Graham, J. H., Linderman, R. G. and Menge, J. A. 1982. Development of external hyphae by different isolates of mycorrhizal Glomus spp. in relation to root colonization and growth of Troyer citrange. New Phytol. 91: 183-189.

Graham, J. H. and Timmer, L. W. 1984. Vesicular-arbuscular mycorrhizal development and growth response of rough lemon in soil and soilless media: Effect of phosphorus source. J. Amer. Soc. Hort. Sci. 109: 118-121.

Hussey, R. B., Peterson, R. L. and Tiessen, H. 1984. Interactions between vesicular-arbuscular mycorrhizal fungi and asparagus. Plant and Soil 79: 403-416.

Jarrell, W. M. and Beverly, R. B. 1981. The dilution effect in plant nutrition studies. Adv. Agron. 34: 197-224.

Johnson, C. R. and Hummel, R. L. 1986. Influence of media on endomycorrhizal infection and growth response of Severinia buxifolia. Plant and Soil 93: 35-42.

Kiernan, J. M., Hendrix, J. W., Stoltz, L. P. and Maronek, D. M. 1984. Characterization of strawberry plants produced by tissue culture and infected with specific mycorrhizal fungi. HortScience 19: 883-885.

Kormanik, P. P. and McGraw, A.-C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In* Methods and Principles of Mycorrhizal Research. Ed. N. C. Schenck, APS Press, St. Paul, Minnesota. pp. 37-45.

Louis, I. and Lim, G. 1988. Differential response in growth and mycorrhizal colonization of soybean to inoculation with two isolates of Glomus clarum in soils of different P availability. Plant and Soil 112: 37-43.

Menge, J. A., Davis, R. M., Johnson, E. L. V. and Zentmyer, G. A. 1978. Mycorrhizal fungi increase growth and reduce transplant injury in avocado. Cal. Agric. 32: 6-7.

Michigan State University 1989. User's Guide to MSTAT-C. Michigan State University, East Lansing, Michigan.

Mosse, B. 1975. Specificity of VA mycorrhizas. *In*: Endomycorrhizas. Eds. F. E. Sanders, B. Mosse, and P. B. Tinker. Academic Press, New York. pp. 469-484.

Nelsen, C. E. and Safir, G. R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154: 407-413.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Myc. Soc. 55: 158-161.

Plenchette, C., Furlan, V. and Fortin, J. A. 1983. Responses of endomycorrhizal plants grown in calcined montmorillonite clay to different levels of soluble phosphorus. I. Effect on growth and mycorrhizal development. Can. J. Bot. 61: 1377-1383.

Plenchette, C., Furlan, V. and Fortin, J. A. 1982. Effects of different endomycorrhizal fungi on five host plants grown on calcined montmorillonite clay. J. Amer. Soc. Hort. Sci. 107: 535-538.

Ponton, F. and Piché, Y., Parent, S. and Caron, M. 1990. Use of vesicular-arbuscular mycorrhizae in Boston fern production: II. Evaluation of four inocula. HortScience 25: 416-419.

Powell, C. L. 1983. Vesicular-arbuscular mycorrhizae and asparagus. Proc. Agron. Soc. N. Z. pp. 107-109.

Powell, C. L., Bagyaraj, D. J., Clark, G. E. and Caldwell, K. I. 1985. Inoculation with vesicular-arbuscular mycorrhizal fungi in the greenhouse production of asparagus seedlings. N. Z. J. of Agr. Res. 28: 293-297.

Ravolanirina, F., Gianinazzi, S., Trouvelot, A. and Carre, M. 1989. Production of endomycorrhizal explants of micropropagated grapevine rootstocks. Agric., Ecosys. and Environ. 29: 323-327.

Schubert, A. and Hayman, D. S. 1986. Plant growth responses to vesicular-arbuscular mycorrhiza XVI. Effectiveness of different endophytes at different levels of soil phosphate. New Phyto. 103: 79-90.

Siqueira, J. O., Rocha, W. F. Jr., Oliviera, E. and Colozzi, A. 1990. VA mycorrhiza-lime relationship and its associated effects on growth and nutrient concentration of brachiaria grass (Brachiaria decumbens). Biol. Fert. Soils (In press).

Sterrett, S. B., Ross, B. B. and Savage, C. P. Jr. 1990. Establishment and yield of asparagus as influenced by planting and irrigation method. J. Amer. Soc. Hort. Sci. 115: 29-33.

Sylvia, D. M. and Schenck, N. C. 1983. Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. New Phytol. 95: 655-661.

Wacker, T. L., Safir, G. R. and Stephens, C. T. 1990. Effect of Glomus fasciculatum on the growth of asparagus and the incidence of Fusarium root rot. J. Amer. Soc. Hort. Sci. 115: 550-554.

SECTION II

EFFECT OF PHENOLIC COMPOUNDS ON ASPARAGUS MYCORRHIZA

INTRODUCTION

Asparagus produces several cinnamic acid derivatives which have been shown to be allelochemicals (Hartung et al., 1990, Young, 1984, Young and Chou, 1985). It has been suggested that these allelochemicals may either be directly involved in asparagus decline by affecting plant vigor (Young, 1984; Young and Chou, 1985) or indirectly through chemical-mediated changes in the microbial community (Hartung and Stephens, 1983), which in turn affect plant growth. Relatively little work has been done on the influence of allelochemicals on VAM fungi. One study has shown that ferulic acid, a well known allelochemical also found in asparagus roots, decreases root colonization by VAM and also growth of mycorrhizal asparagus plants (Wacker et. al., 1990b).

Previous work from this laboratory has shown a shift in VAM species composition in asparagus fields with increasing age up to 20 years (Wacker et al., 1990b). Root colonization and spore density in the soil also decreased with increasing field age. Since VAM fungi are known to be important for asparagus growth (Hussey et al., 1984; Wacker et al., 1988) plant health may be adversely affected if the nutritional contribution to asparagus by native VAM fungal species decreases with

plant age. Because available phosphorus (P) decreased with field age, soil P may not be the factor responsible for reduction in root colonization and VAM fungal shift in Wacker's study (1990b). Accumulation of asparagus-produced phenolic allelochemicals could be a mechanism by which asparagus monocropping alters VAM species composition. We report here on the effects of ferulic, caffeic and methylenedioxycinnamic (MDC) acids and the effects of soil extracts from an asparagus field soil and from an adjacent non-cultivated soil on VAM formation and plant growth.

MATERIALS AND METHODS

To examine the effects of three phenolic acids on VAM formation in asparagus, asparagus seeds (Asparagus officinalis L. 'Mary Washington') were surface-sterilized (Elmer and Stephens, 1988), pregerminated and sown into plastic cell-pack units (2 x 2) with 80 g of washed sand per cell. A 9.0 cm diameter Petri dish bottom was used under each four-cell unit to avoid chemical cross-contamination. A VAM fungus resembling Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske was multiplied in pot cultures with sorghum (Sorghum vulgare L.) and used as inoculum. Soil inoculum was diluted with washed sand in order to obtain approximately 3 spores$\cdot g^{-1}$. To equilibrate microbial populations between mycorrhizal and non-mycorrhizal plants an inoculum filtrate passed through a 38 um screen was added to the non-mycorrhizal seedlings. After transplanting, plants were watered weekly with 10 ml of 0.1 strength Hoagland's solution (Hoagland and Arnon, 1950) without

P prepared with tap-distilled water, and P (34 $mg \cdot l^{-1}$ from $NH_4H_2PO_4$) was added once in the fourth week. Plants were kept in a growth chamber at 21°C/30°C (night/day) and under cool-white light bulbs providing 300 $umol \cdot m^{-2} \cdot s^{-1}$ for 14 hours each day. After the first week of growth, solutions containing the phenolic acids were applied weekly below the soil surface next to the plant roots. Ten mls per cell of ferulic and caffeic acids were applied at concentrations of 0, 25, 250 and 2000 uM and MDC acid at concentrations of 0, 25, 250 and 500 uM. Compounds were dissolved in methanol (1.5 % of the final volume) and then brought up to volume with distilled water. After seven weeks of growth plants were harvested and VAM colonization and plant growth were measured. Roots were cleared (Phillips and Hayman, 1970), stained with 0.5 % aniline blue in lactoglycerol and then assessed for VAM colonization by a line-intersect method (Kormanik and McGraw, 1982). This experiment was set up in a completely randomized design (3 phenolic acids x 4 concentrations) with 12 replications per treatment and repeated once with similar results.

To determine the effects of extracts from asparagus soils on VAM formation in asparagus, random soil samples were taken from the top 20 cm layer of two soils with different cropping histories selected from Ramey Farms in Oceana County, MI. Samples were taken in May and August 1989 from a replanted asparagus field, previously grown in asparagus for twenty years (asparagus soil with pH = 6.4, P = 170 ppm, K = 208 ppm, Ca = 506 ppm, Mg = 124 ppm, Cu = 2 ppm and Mn = 9 ppm) and from an adjacent strip of non-cultivated soil (non-asparagus soil with pH = 7.5, P = 208 ppm, K = 89 ppm, Ca = 547 ppm, Mg = 124 ppm, Cu = 3 ppm

and Mn = 13 ppm). Samples were stored at 4°C until used. Soil extracts were prepared by adding 500 ml of a 40.5 mM $Ca(OH)_2$ solution to 500 g of soil and then mixed on a rotary shaker for 48 h. The suspension was allowed to settle, and filtered through glass fiber. The solutions were centrifuged at 2500 rpm for 10 min. and the supernatant passed through a series of successively finer filter papers, the pH adjusted to 6.5 and then filter-sterilized through a 0.22 um filter. The extracts were assayed for total phenolic concentration using a colorimetric test and *p*-hydroxybenzoic acid as a standard (Bray and Thorpe, 1954).

Soil extracts from the soils collected in May 1989 were further assayed for their effects on VAM formation and growth as previously described using washed sand infested with G. fasciculatum and planted with pregerminated asparagus seeds. Plants were watered weekly with ten mls of 0.1 strength Hoagland's nutrient solution without P (= control) and asparagus and non-asparagus soil extracts, which were amended with the same nutrients as the control. Plants were grown for four weeks in the growth chamber under similar light and temperature conditions and assayed as before. In both experiments twelve plants were used per treatment. The data from both experiments were subjected to an analysis of variance and a correlation analysis using MSTAT-C (Michigan State University, 1989) and the means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

The effects of three phenolic acids on VAM formation and plant growth are presented in Figures 2.1 and 2.2, respectively. The inhibitory effects of the phenolic acids on VAM root colonization increased linearly with increasing concentration of all three compounds (Figure 2.1). MDC acid showed the highest inhibitory effects on root colonization. Reduction of mycorrhizal plant growth, shown in Figure 2.2, was significantly ($P \leq 0.05$) correlated with increasing concentration of caffeic and MDC acids, but not with ferulic acid ($P = 0.12$). In the absence of VAM, plant growth was inhibited by ferulic and MDC acids ($P \leq 0.05$), but not by caffeic acid ($P = 0.21$). Ferulic acid, a well known allelochemical (Rice, 1984), showed significant inhibitory effects on growth of non-mycorrhizal plants, but not on mycorrhizal plants. This indicates that VAM fungi, may alleviate the phytotoxic effects of ferulic acid and may act as detoxifiers as suggested in studies with ericoid mycorrhizae (Leake et al., 1989).

Extracts from asparagus soil showed higher total phenolic concentrations in May (92 $ug \cdot g^{-1}$) than in August (34 $ug \cdot g^{-1}$). A similar trend was found for the non-asparagus soil extracts which showed 60 $ug \cdot g^{-1}$ in May and 14 $ug \cdot g^{-1}$ in August. The asparagus soil extract had 53 % more total phenolics in May than the non-asparagus soil extract and 142 % more in August. The reduction in total soil phenolics later in the season has also been found in other ecosystems (Lodhi, 1975). Soil phenolics are subjected to various biological and environmental processes that may lead to the degradation, immobilization and leaching

Figure 2.1. Effects of phenolic acids on VAM root colonization in asparagus grown in sand infested with G. fasciculatum after 7 weeks. ($P \leq 0.05$). Data points represent means of two experiments.

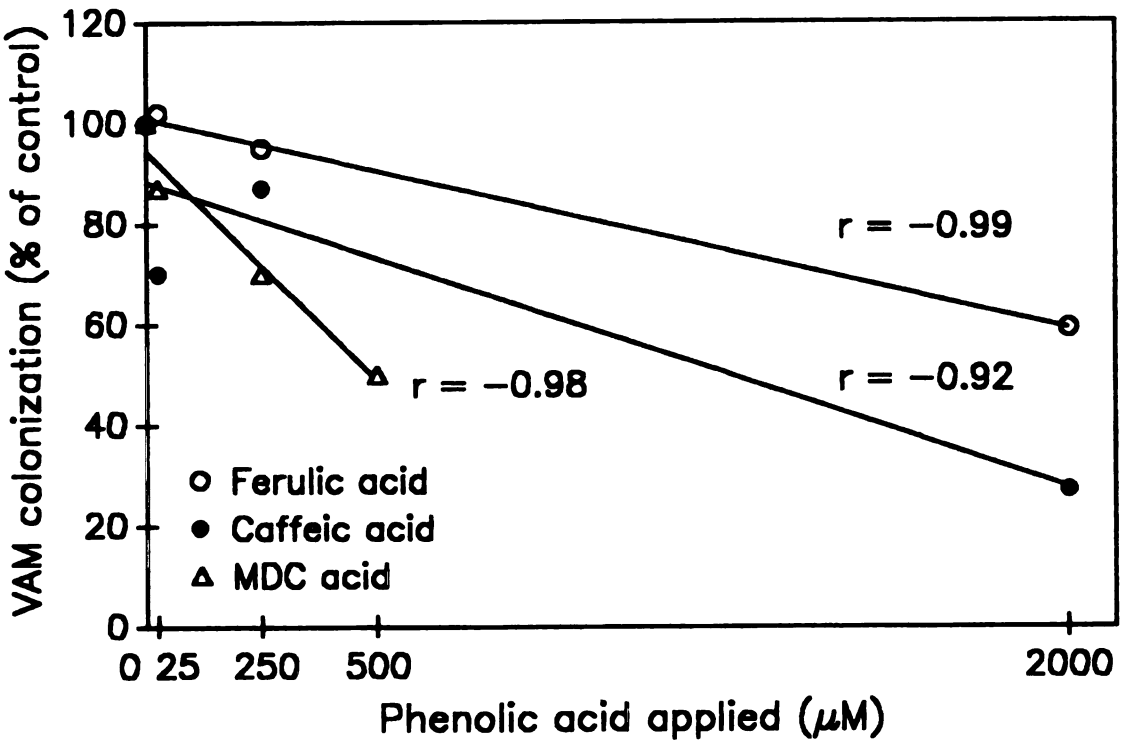
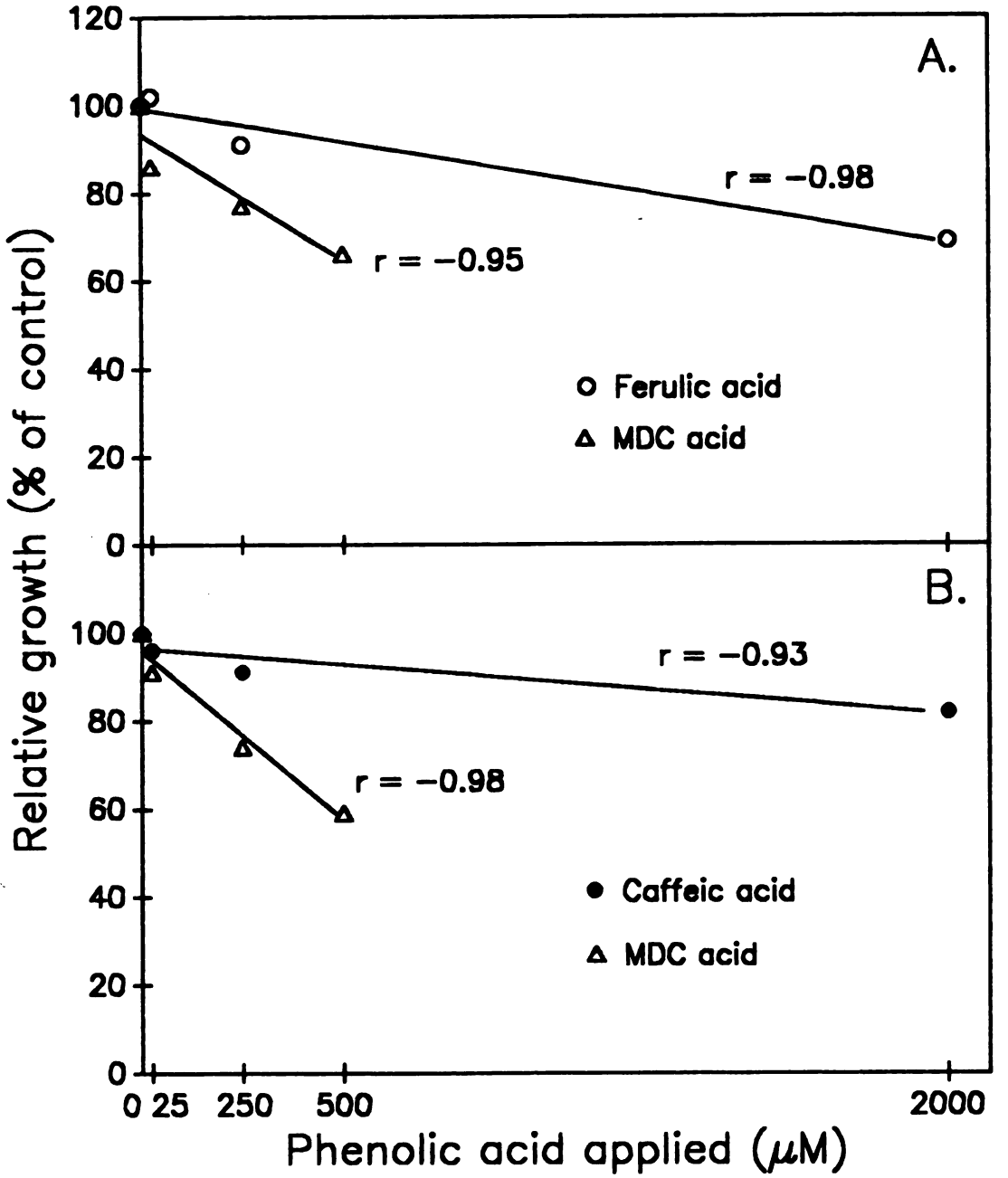

VAM colonization (% of control)
120
100
80
60
40
20
0
0 25 250 500 2000
Phenolic acid applied (μM)
r = −0.99
r = −0.92
r = −0.98
Ferulic acid
Caffeic acid
MDC acid

Figure 2.2. Effects of phenolic acids on growth of asparagus in the presence (A) and absence (B) of the VAM fungus G. fasciculatum after 7 weeks ($P \leq 0.05$). Data points represent means of two experiments.

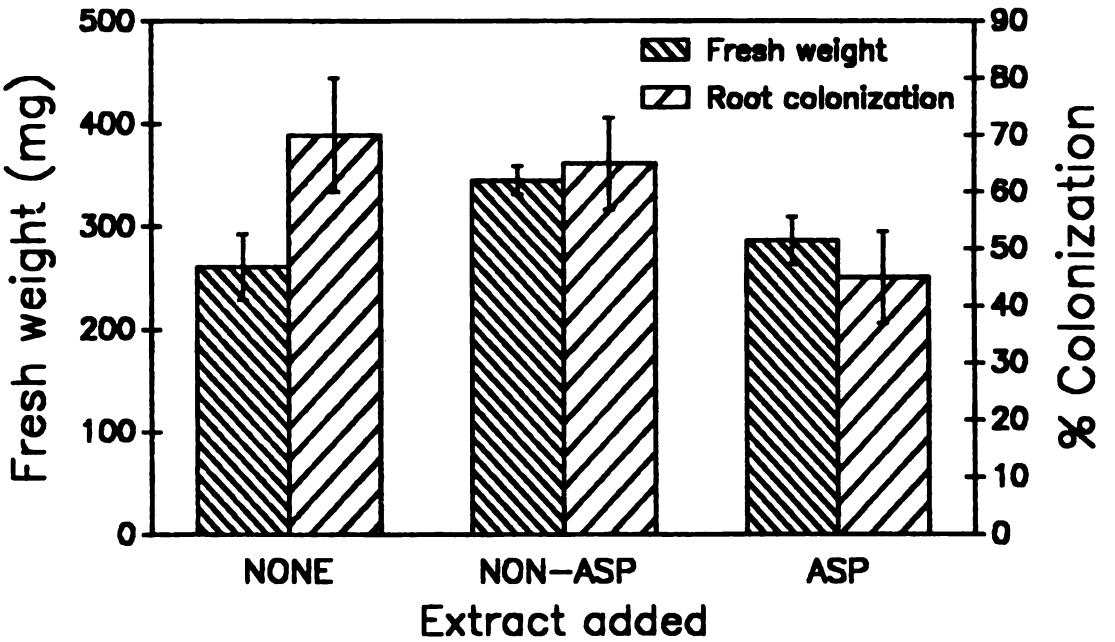

A.
B.
r = −0.98
r = −0.95
r = −0.93
r = −0.98
Ferulic acid
MDC acid
Caffeic acid
MDC acid
Relative growth (% of control)
Phenolic acid applied (μM)
0
20
40
60
80
100
120
0 25 250 500 2000

of these compounds during the growing season, thus reducing their phytotoxicity.

Application of extracts from non-asparagus soil stimulated growth of asparagus plants, when compared to the mycorrhizal control (Figure 2.3). This may be due to the additional nutrients present in the soil extracts. Plants grown with extracts from the asparagus soil did not differ from the control receiving no soil extract. Nutrient applications have been shown to reduce the phytotoxic effects of phenolics (Stowe and Osborn, 1980; Blum and Shafer, 1988). In our experiment, application of soil extracts containing nutrients in excess of the control, which contained Hoagland's solution only, may have improved plant growth and counteracted the inhibitory effects of soil phenolics. VAM root colonization was inhibited in the plants treated with asparagus soil extracts, but not in those treated with the non-asparagus soil extract or in the control. Since there was no major difference in soil nutrient concentrations between asparagus and non-asparagus soils, the reduced root colonization and plant growth in the presence of extracts from the former compared to the latter may be due to a higher phenolic concentration in the asparagus soil extract. More detailed studies should be carried out on possible synergistic interactions of phenolic compounds as indicated by Einhellig (1985), and possibly using adsorption resins to remove phenolics from the extracts and using more accurate extraction and quantification techniques in order to test the above hypothesis.

Besides asparagus, several perennial cropping systems, i.e. apple (Börner, 1959), citrus (Burger and Small, 1983) and alfalfa (Read and

Figure 2.3. Effects of extracts from asparagus (ASP) and non-asparagus (NON-ASP) soils on fresh weight of asparagus and VAM root colonization by G. fasciculatum. Bars represent mean of 12 replicates ± SE.


Fresh weight
Root colonization
Fresh weight (mg)
% Colonization
500
400
300
200
100
0
90
80
70
60
50
40
30
20
10
0
NONE
NON-ASP
ASP
Extract added

Jensen, 1989) experience replant and decline problems and phenolic compounds have been associated with these problems (Rice, 1984). Phenolic acids are ubiquitous in soil systems because of the constant input by growing plants and their residues (Patrick, 1971). They may build up over time and selectively influence soil microbial populations (Hartley and Whitehead, 1985). Possible effects of plant-produced allelochemicals on soil microorganisms (Blum and Shafer, 1988; Hartung and Stephens, 1983), including mycorrhizal fungi (Chu-Chou, 1978; Wacker et al., 1990a; Perry and Choquette, 1987), have been suggested. Shifts in VAM fungal populations in long-term monocropping systems (Schenk et al., 1987; Wacker et al.; 1990b) may be related to accumulation of allelochemicals. Asparagus cinnamic acids used in the present study, including the highly phytotoxic MDC acid (Hartung et al., 1990), were found to inhibit VAM formation and asparagus growth at biologically relevant concentrations and could be important components in the asparagus decline problem.

LITERATURE CITED

Blum, U. and Shafer, S. 1988. Microbial populations and phenolic acids in soil. Soil Biol. Biochem 20: 793-800.

Bray, H. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. 1: 27-52.

Börner, H. 1959. Liberation of organic substances from higher plants and their role in the soil sickness problem. Bot. Rev., 26: 395-424.

Burger, W. P. and Small, J. G. C. 1983. Allelopathy in citrus orchards. Sci. Hort. 20: 361-375.

Chu-Chou, M. 1978. Effects of root residues on growth of Pinus radiata seedlings and a mycorrhizal fungus. Ann. Appl. Biol. 90: 407-416.

Einhellig, F. A. 1985. Interactions amoung allelochemicals and other stress factors of the plant environment. *In*: Allelochemicals: Role in Agriculture and Forestry. Ed. G. R. Waller. ACS Symposium series 330. Washington, DC. Chap. 32.

Elmer, W. H. and Stephens, C. T. 1988. Comparison of technique for eliminating contaminants from asparagus seeds. HortScience 23: 1031-1032.

Hartley, R. D. and Whitehead, D. C. 1985. Phenolic acids in soils and their influence on plant growth and soil microbial processes. *In*: Soil organic matter and biological activity. Eds. D. Vaughan and R.E. Malcolm. Martinus Nijhoff/Dr. W. Junk Publishers, Boston. Chap. 3.

Hartung, A. C. 1987. Allelopathic potential of asparagus (Asparagus officinalis L.). Ph.D. dissertation. Michigan State University, East Lansing, 142 pp.

Hartung, A. C. Nair, M. G. and Putnam, A. R. 1990. Isolation and characterization of phytotoxic compounds from aspargus (Asparagus officinalis L.) roots. J. Chem Ecol. 16: 1707-1718.

Hartung, A. C. and Stephens, C. T. 1983. Effects of allelopathic substances produced by asparagus on incidence and severity of asparagus decline due to Fusarium crown rot. J. Chem. Ecol. 9: 1163-1174.

Hoagland, D. R. and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Cal. Agric. Expt. Sta. Circ. 347.

Kormanik, P. P. and McGraw A.-C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In* Methods and Principles of Mycorrhizal Research. Ed. N.C. Schenck. APS Press, St. Paul, Minnesota. pp. 37-45.

Leake, J. R., Shaw, G. and Read D. J. 1989. The role of ericoid mycorrhizas in the ecology of ericaceous plants. Agric., Ecosys. Envir. 29: 237-250.

Lodhi, M. A. K. 1975. Soil-plant phytotoxicity and its possible significance in patterning of herbaceous vegetation in a bottomland forest. Amer. J. Bot. 62: 618-622.

Patrick, Z. A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. 111: 13-18.

Patrick, Z. A., Toussoun, T.A. and Koch, L.W. 1964. Effect of crop-residue decomposition products on plant roots. Ann. Rev. Phytopath. 2: 267-292.

Perry D. A. and Choquette C. 1987. Allelopathic effects on mycorrhizae, influence on structure and dynamics of forest ecosystems. *In*: Allelochemicals: Role in Agriculture and Forestry. Ed. G. R. Waller. ACS Symposium series 330. Washington, DC. Chap. 17

Phillips, J. M. and D. S. Hayman 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhial fungi for rapid assessment of infestion. Trans. Brit. Myc. Soc. 55: 158-161.

Read, J. J. and Jensen, E. H. 1989. Phytotoxicity of water-soluble substances from alfalfa and barley soil extracts on four crop species. J. Chem. Ecol. 15: 619-628.

Rice, E. L. 1984. Allelopathy. 2nd edition. Academic Press, Inc. New York. pp. 422.

Schenck, N. C., Siqueira, J.O. and Olviera, E. 1987. Incidence of VA mycorrhizal fungi in native and cultivated Brazilian soil. Phytopath. 77: 1736 (Abstr.).

Stowe, L. G. and Osborn, A. 1980. The influence of nitrogen and phosphorus levels on the phytotoxicity of phenolic compounds. Can. J. Bot. 58: 1149-1153.

Wacker, T. L. 1988. The role of vesicular-arbuscular mycorrhizal fungi in the asparagus (Asparagus officinalis L.) agroecosystem. MS Thesis, Michigan State University, East Lansing, 101 pp.

Wacker, T. L., Safir, G. R. and Stephens, C. T. 1990a. Effects of ferulic acid on Glomus fasciculatum and associated effects on phosphorus uptake and grwoth of asparagus (Asparagus officinalis). J. Chem. Ecol. 16: 901-909.

Wacker, T. L., Safir, G. R. and Stephenson, S. N. 1990b. Evidence for succession of mycorrhizal fungi in Michigan asparagus fields. Acta Hort. 271: 273-279.

Young, C. C. and Chou, T. C. 1985. Autointoxication in residues of Asparagus officinalis L. Plant and soil 85: 385-393.

Young, C. C. 1984. Autointoxication in root exudates of Asparagus officinalis L. Plant and Soil 82: 247-253.

APPENDIX A: ASPARAGUS TISSUE-CULTURE MEDIA

Ingredients used in addition to Murashige and Skoog salts:

	Media A:	Media B:
A-Rest	8.0 ml	5.0 ml
Nicotinic acid	3.0 mg	0.5 mg
Pyridoxine HCl	3.0 mg	0.5 mg
Thiamine	0.5 mg	0.5 mg
Glycine	2.0 mg	2.0 mg
Myo-inositol	100.0 mg	100.0 mg
Sucrose	40.0 g	40.0 g
1-Napthaleneacetic acid (NAA)	0.3 mg	0.3 mg
(2-Isopentenyl)adenine	-	0.1 mg
Kinetin	0.7 mg	-

APPENDIX B: PLANT TISSUE ANALYSIS

Cladophyll nutrient analysis of asparagus after 13 weeks of growth at 0, 50, 100 and 150 ppm applied phosphorus.

	applied P	% P	% K	% Ca	% Mg	ppm Al	ppm Fe	ppm Cu	ppm Mn
Control	0	.40	1.42	2.13	.73	6.4	13.1	3.0	4.5
	50	.48	1.71	1.85	.62	5.5	13.0	1.7	4.9
	100	.51	1.69	2.11	.71	5.7	14.5	1.4	4.9
	150	.51	1.69	1.81	.61	5.3	12.9	1.3	4.7
		*.47	1.63	1.98	.68	5.7	13.4	1.9	4.8
GF	0	.35	1.30	2.02	.78	5.1	10.0	1.5	6.5
	50	.46	1.35	2.27	.79	5.2	11.8	1.3	7.7
	100	.41	1.42	2.26	.79	5.2	13.0	1.3	8.6
	150	.48	1.46	2.15	.79	5.4	12.2	1.2	7.9
		*.43	1.38	2.18	.79	5.2	11.8	1.3	7.7
GI	0	.32	2.26	1.23	.45	5.9	10.1	1.1	2.6
	50	.35	2.13	1.36	.48	6.4	11.4	1.1	3.0
	100	.33	1.91	1.31	.45	7.4	10.8	1.1	2.6
	150	.43	2.11	1.36	.49	4.5	11.5	1.1	3.8
		*.36	2.10	1.32	.46	6.0	11.0	1.1	3.0
GVR	0	.32	2.33	1.24	.48	5.7	8.3	1.0	2.6
	50	.36	2.22	1.37	.52	5.8	8.6	0.9	3.2
	100	.39	2.17	1.28	.49	5.7	8.1	1.2	2.6
	150	.44	2.14	1.29	.47	6.7	9.2	1.1	3.3
		*.38	2.21	1.30	.49	6.0	8.6	1.1	2.9

*Overall mean for each peat-mix across all applied P levels

DRY ASHING AND DIGESTING PROCEDURE:

1) 0.25 g of dried asparagus cladophylls placed in covered ceramic crucibles were dry ashed in a muffle furnace at 500°C for 6 hours. One standard reference material was included for every 25 samples.

2) Ashed samples were dissolved for one hour in 25 ml of digesting solution (3 normal HNO_3 containing 1000 ppm LiCl).

3) Solution was poured into labelled vials until assayed in a direct-current plasma analysis optical emission spectrophotometer.