HORTSCIENCE 33(7):1136–1137. 1998.

Mycorrhizal Infection and Plant Growth of Highbush Blueberry in Fumigated Soil Following Soil Amendment and Inoculation with Mycorrhizal Fungi

Wei Qiang Yang, Barbara L. Goulart, and K. Demchak
Horticulture Department, The Pennsylvania State University, University Park, PA 16802

Additional index words. ericoid mycorrhizae, methyl bromide, chloropicrin, soil amendment, *Vaccinium corymbosum*

Abstract. **A field trial was conducted to investigate the effectiveness of soil fumigation on maintaining nonmycorrhizal status and the effect of mycorrhizal inoculation and preplant soil amendment on the growth of tissue-cultured highbush blueberry plants. Soil fumigation using a methyl bromide/chloropicrin (67/33) mixture at the rate of 560 kg·ha^{-1} was effective in maintaining nonmycorrhizal status for one growing season. Noninoculated control plants became infected during the second growing season. Field inoculation using a native *Oidiodendron maius* was successful, but plant growth was not significantly affected by mycorrhizal inoculation in either year. Rotted sawdust amendment, however, reduced plant growth in the first year but effects were no longer measurable in the second year. Soil fumigation and field inoculation could be used to establish mycorrhizal plants and nonmycorrhizal controls for future short-term field experiments.**

Commercial highbush blueberry (*Vaccinium corymbosum* L.) plants tend to be mycorrhizal, albeit to varying degrees (Boyer et al., 1982; Goulart et al., 1993), and application of surface mulch and/or organic soil amendments are standard practices recommended for production on mineral soils (Gough et al., 1977; Goulart et al., 1996). The study of the interactions between mycorrhizal infection and organic mulch and/or soil amendment is often difficult because nonmycorrhizal control plants are often infected by indigenous fungi, which are ubiquitous and capable of living saprophytically in soils (Barron, 1962). Inoculum delivered by wind and rain makes keeping control plants uninfected even more difficult. Since the maintenance of controls is vital, seedlings have been used as control plants in most ericoid mycorrhizal studies (Stribley and Read, 1975, 1976, and 1980). Recently, plants propagated clonally using meristem tip culture have been used in such studies (Yang et al., 1996). Media sterilization techniques, such as steaming, autoclaving, and γ-irradiation, are impractical in field studies. Soil fumigation using a methyl bromide/chloropicrin (Trichloronitromethane) (67/33) mixture at the rate of 560 kg·ha^{-1} is effective in maintaining nonmycorrhizal status in field vesicular arbuscular mycorrhizae (VAM) studies with *Abutilon* (Koide et al., 1994). However, this method may not be effective under field conditions for ericoid mycorrhizae, which are in a different fungal class than VAM.

In this preliminary study we evaluated the effects of mycorrhizal inoculation and preplant soil amendment on the growth of highbush blueberry plants in fumigated soil, thus providing needed information for future field studies.

Materials and Methods

The experiment was designed as a split-plot with mycorrhizal treatments as the main plot. Sub-plots were preplant soil amendments (rotted sawdust or none) with two plants per treatment. The main plot was replicated three times. All plants were grown on silver on black polyethylene film mulch, and irrigated by trickle irrigation. Plant spacing was 1.5 × 3 m. No nitrogen fertilizer was applied during the two growing seasons of the experiment, which was conducted at the Horticulture Farm at the Russell E. Larson Agricultural Research Center, Rock Springs, Pa. The soil was a Hagerstown silt loam (Alfisol) with no previous planting history of ericaceous plant species. Prior to fumigation, the field was plowed, then tilled with a rototiller. Rotted sawdust (2 L) was then mixed manually with the soil of each planting hole (25 × 25 × 20 cm) having that treatment. When the soil temperature was above 15 °C, the entire experimental plot was fumigated with methyl bromide/chloropicrin (67/33) at the rate of 560 kg·ha^{-1} (Hendrix & Dail, Inc., Greenville, N.C.). Two weeks after fumigation, the pH of the soil in each planting hole was determined and the amount of elemental sulfur powder needed to reduce the pH to 5.0 was calculated and blended with the soil.

The ericoid mycorrhizal fungal isolate *Oidiodendron maius* (UAMH 9263) was grown in malt agar media for 20 d. Ten agar discs were cut from the leading edge of the fungal colony using a 4-mm corkborer and placed in 400 mL of MR liquid media (Mitchell and Read, 1981). After 3 weeks, the fungal slurry was harvested, washed five times with distilled water, and macerated in a blender in 100 mL of distilled water at 2000 rpm for 50 s. 'Elliot' highbush blueberry plants were propagated by rooting tissue-cultured shoots in acid-washed sand (Yang et al., 1996). For mycorrhizal inoculation, 20 mL of a suspension of the macerated fungal slurry was applied to the root system of each plant during planting; the control plants received an equal amount of sterile water.

In 1996, the new growth of each shoot and lateral was measured at the end of the growing season. In 1997, plant canopy volume and foliage density were determined and a growth index was calculated by multiplying canopy volume by foliage density (Yang et al., 1996). Root samples were taken each fall and stained using trypan blue (Phillips and Hayman, 1970). Mycorrhizal infection levels were determined by a grid-line intersect technique (Giovannetti and Mosse, 1980) modified to determine percentage of root epidermal cells infected. Mycorrhizal infection data were arcsin transformed prior to analysis. All data were analyzed by using the GLM procedure in SAS (SAS Institute, 1992).

Results and Discussion

Noninoculated controls (NM) were only minimally infected after the first growing season following soil fumigation (Table 1). After the second season, NM plants still had lower infection levels than inoculated (M) plants, but the infection percentage was similar to that in M plants the previous year. Since host plants are primarily infected by the hyphae of ericoid mycorrhizal fungi, the infection of NM plants in the second growing season suggested that the indigenous fungi were responsible. The fact that these fungi inhabit soil, wood pulp, paper mill, and even toilet tissue (Barron, 1962) illustrates their ubiquitous nature, and explains the infection of NM plants in the second growing season.

Although mycorrhizal inoculation was successful, neither growth differences between M and NM plants nor interactions between inoculation and soil amendment were observed in either growing season. This may be partially explained by the low infection rate in the first growing season and/or the infection of NM controls in the second season. In 1996, the rotted sawdust amendment suppressed plant growth. This was surprising, considering that rotted sawdust is a standard soil amendment and/or surface mulch in commercial highbush blueberry production. The decomposition of such amended material by soil microbes might have immobilized mineralized soil nitrogen, thus resulting in a more limited supply of soil nitrogen for plant growth. Studies have suggested that no net nitrogen mineralization can

Received for publication 4 May 1998. Accepted for publication 30 June 1998. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. The effects of inoculation with mycorrhizal fungi and preplant soil amendment with rotted sawdust on rate of infection and growth of field-grown 'Elliot' highbush blueberry.

	1996		1997		
Treatment	New growth[z] (cm)	Mycorrhizal infection (%)	Canopy growth index[y] (cm^3)	Adjusted canopy growth index[x] (cm^3)	Mycorrhizal infection (%)
Inoculated[w]	72.9	7.05	171,554	151,783	20.9
Noninoculated	57.3	0.17	141,414	161,185	10.6
P	0.258	0.0001	0.191	0.697	0.013
Amended	36.2	3.05	109,544	176,691	21.1
Not amended	94.1	2.61	203,424	136,227	10.4
P	0.028	0.869	<0.001	0.292	0.091
Inoc. × Amend.	NS	NS	NS	NS	NS

[z]New growth = total length of all new shoots and laterals at end of growing season.
[y]Canopy growth index = canopy volume (cm^3) × foliage density (rated on 1–5 scale).
[x]Determined by adjusting the means of canopy growth index using previous season's growth as a covariate.
[w]Inoculated plants were treated with 20 mL of the suspension of a native ericoid mycorrhizal fungal isolate at planting in 1996.
[NS]Nonsignificant.

occur when the C/N ratio of added soil organic material is above 30:1 (Tisdale et al., 1993). Since the C/N ratio in rotted sawdust used in this experiment was 59:1, the decomposition of the sawdust probably reduced the supply of nitrogen to the plants.

In summary, noninoculated blueberry plants remained free of mycorrhizae for one growing season following soil fumigation using methyl bromide/chloropicrin (67/33) at 560 $kg \cdot ha^{-1}$. Rotted sawdust amendment reduced plant growth, presumably due to its high C/N ratio which resulted in nitrogen immobilization. To compensate for the nitrogen used by the soil microbes, additional fertilizer nitrogen may be required. Experiments using organic amendments with different C/N ratios and nitrogen isotope 15 are underway to investigate the effect of M inoculation on nitrogen acquisition in highbush blueberry production systems.

Literature Cited

Barron, G.L. 1962. New species and new records of Oidiodendron. Can. J. Bot. 40:589–607.

Boyer, E.P., J.R. Ballington, and C.M. Mainland. 1982. Endomycorrhizae of *Vaccinium corymbosum* L. in North Carolina. J. Amer. Soc. Hort. Sci. 107:751–754.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.

Gough, R.E., V.G. Shutake, and D.B. Wallace. 1977. Highbush blueberry culture. Rhode Island Coop. Ext. Bul. 143.

Goulart, B.L., M. Brittingham, J. Harper, P. Heinemann, and W. Hock. 1996. Small fruit production and pest management guide, 1996–98. The Pennsylvania State Univ.

Goulart, B.L., K. Demchak, M.L. Schroeder, and J.R. Clark. 1993. Mycorrhizae in highbush blueberries: Survey results and field response to nitrogen. HortScience 28:140.

Koide, R., D.L. Shumway, and S.A. Mabon. 1994. Mycorrhizal fungi and reproduction of field populations of *Abutilon theophrasti* Medic. (Malvaceae). New Phytol. 126:123–130.

Mitchell, D.T. and D.J. Read. 1981. Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of *Vaccinium macrocarpon* and *Rhododendron ponticum*. Trans. Brit. Mycol. Soc. 76:225–260.

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

SAS Institute. 1992. SAS user's guide: Statistics. vers. 5 ed. SAS Inst., Cary, N.C.

Stribley, D.P. and D.J. Read. 1975. Some nutritional aspects of the biology of ericaceous mycorrhizas. Proc. Symp. Univ. Leeds. 105–117.

Stribley, D.P. and D.J. Read. 1976. The biology of mycorrhiza in the ericaceae VI. The effects of mycorrhizal infection and concentration of ammonium nitrogen on growth of cranberry (*Vaccinium macrocarpon* Ait.) in sand culture. New Phytol. 77:63–72.

Stribley, D.P. and D.J. Read. 1980. The biology of mycorrhiza in the ericaceae. VII. The relationship between mycorrhizal infection and the capacity to utilize simple and complex organic nitrogen sources. New Phytol. 86:365–371.

Tisdale, S.L., W.L. Nelson, J.D. Beaton, and J.L. Havlin. 1993. Soil fertility and fertilizers. Maxwell Macmillan Intl., New York.

Yang, W.Q., B.L. Goulart, and K. Demchak. 1996. The effect of aluminum and media on the growth of mycorrhizal and nonmycorrhizal highbush blueberry plantlets. Plant Soil 183:301–308.