Ruter, J.M., M.P. Garber, and **D.J. Moorhead.** 1994. Early lifting and transplanting of flowering dogwood seedlings increases survival in the southern United States. J. Environ. Hort. 12:164-166.

SAS Institute Inc. 1999. SAS user's guide. Version 8.2. SAS Institute, Inc. Cary, North Carolina.

Sibley, J.L., B.C. Wilson, and **J.C. Wilson.** 2001. Chilling affects foliar budbreak of ornamental trees. Comb. Proc. Intl. Plant Prop. Soc. 51:66-71.

Wilson, B.C., J.L. Sibley, and **J.E. Altland.** 2002. Chilling duration affects foliar budbreak of linden cultivars. HortTechnology 12:660-662.

Initial Shoot Growth and Development of Micropropagated Blueberry Plants Following Inoculation with an Ericoid Mycorrhizal Isolate©

Mark C. Starrett

University of Vermont, Department of Plant and Soil Science, Hills Science Bldg., Burlington, Vermont 05405-0082 U.S.A.

INTRODUCTION

Hymenoscyphus ericae (Read) Korf and Kernan is a widespread ericoid mycorrhizal fungus found on North American and European continents. *Hymenoscyphus ericae* has demonstrated mycorrhizal associations with many ericaceous species including *Vaccinium angustifolium* Ait. (lowbush blueberry) and *V. corymbosum* L. (highbush blueberry).

Inoculation of species of *Vaccinium* with isolates of *H. ericae* have resulted in mixed responses. In several studies, in vitro inoculation of *V. corymbosum,* with *H. ericae* resulted in positive effects on shoot growth. However, in some instances, mycorrhizal colonization has had a negative impact on shoot growth.

In the field, seasonal conditions can dramatically effect the intensity of root colonization. To avoid the influence of seasonal fluctuations on mycorrhizal activity in the host plant, this study used controlled environmental conditions in a greenhouse to investigate the effects of isolates of *H. ericae* on shoot growth of *V. corymbosum* 'Bluecrop' grown in a commercially available growing medium. Although several field studies involving ericoid mycorrhizal inoculation have used the highbush blueberry cultivar 'Bluecrop' as the host, there have been no apparent studies conducted using this selection in greenhouse conditions.

MATERIALS AND METHODS

Microshoots of blueberry (*V. corymbosum* 'Bluecrop') were rooted directly in autoclaved Jiffy-7® Peat Pellets containing established cultures of one of five different isolates of the ericoid mycorrhizal fungus, *H. ericae* or remained non-inoculated. Microshoots were grown for 8 weeks under aseptic conditions in a growth chamber and then transferred to a greenhouse for 16 weeks. In the greenhouse, light was provided by filtered natural sunlight and supplemented by 400-W sodium-vapor high-intensity discharge (HID) lamps which provided an average daily maximum PPF of 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a 16-h day. The greenhouse was air conditioned and therefore

temperature was maintained at 24/21 ± 2°C [75/70 ± 4°F (day/night)]. While in the greenhouse, all plants were watered daily with deionized water and once weekly with 1/8-strength Woody Plant Medium (WPM) (mineral nutrients only) until the medium was saturated. The experimental design was a randomized complete block with three replications and 1 month between replications for manageability at harvest. At harvest the following were recorded: shoot length, shoot number, leaf number, leaf area, shoot dry weight, and extent of root colonization. Statistical analyses were performed using general linear modeling in SAS. Comparisons of the percentage of the root system colonized by each isolate (i.e., mean percent colonization) are based on analysis of variance limited to the inoculated plants. In order to satisfy the homogeneity of variance assumption associated with the analysis of variance, data corresponding to percent colonization was arcsine square-root transformed. Multiple comparisons are based on the Student-Newman-Keuls test. Measures of association are based on Pearson's Correlation Coefficient. Statistical significance was determined using $p<0.05$.

RESULTS AND DISCUSSION

Regardless of isolate, all shoot measurements of inoculated plants were similar to the non-inoculated plants. While some studies have demonstrated an increase in shoot growth of ericaceous plants when grown in vitro, others have demonstrated that once the plants are placed in a growth chamber or greenhouse, these growth benefits are not sustained. The lack of response in plants colonized by *H. ericae* may be attributed to the fact that all plants in this study were supplied with sufficient mineral nutrients in the 1/8-strength WPM. Research has shown that if adequate nutrients are present, the benefits of mycorrhizae may not be evident. Woody Plant Medium used without dilution is the industry standard for proper growth and development of ericaceous plants during micropropagation. Providing the blueberry plants with a very dilute nutrient solution on a weekly basis should not have masked the effects of inoculation with and colonization by *H. ericae* based on previous studies (data not presented). Plants in the current study exhibited symptoms of both nitrogen and phosphorus deficiencies although quantitative foliar nutrient analysis of the resultant plants was not performed.

Associations were examined across all treatments (excluding non-inoculated plants) and across all blocks however, there was no correlation between percentage of roots colonized and shoot growth response. Although there was no overall correlation, there was a positive correlation between extent of root colonization by the isolate LPA and shoot length ($r = 0.70$, $p<0.01$), leaf area ($r = 0.68$, $p<0.01$), and shoot dry weight ($r = 0.69$, $p<0.01$). None of the other isolates demonstrated either a positive or negative correlation between extent of root colonization and any shoot growth measurements.

There were, however, significant differences between extent of root colonization among the isolates investigated (Fig. 1). Roots inoculated with HE were colonized to a much greater extent than any other isolate tested (94%). Both isolates, DA and LFE colonized roots similarly (77% and 72%, respectively). Although the isolate, LPA had significantly lower root colonization (55%), this isolate did result in a direct and positive correlation between extent of colonization and several shoot growth measurements. This result was somewhat unexpected as the LPA isolate originated from roots on rhododendron and not blueberry. In addition, the BMA isolate, which

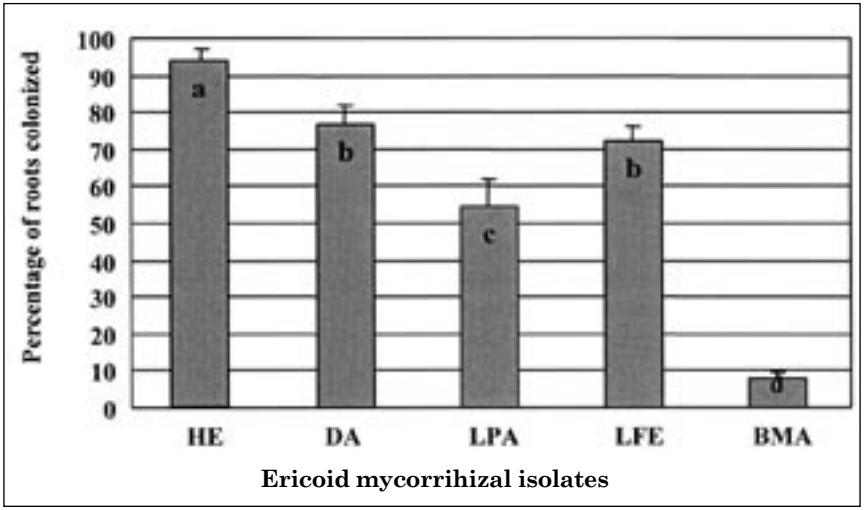

Fig. 1. Extent of roots of *Vaccinium corymbosum* 'Bluecrop' colonized by select isolates of *Hymenoscyphus ericae.* Columns with similar letters are not significantly different at $p<0.05$.

was the only ericoid mycorrhizal isolate originally collected from the same species of blueberry as used in the present study, had the least capacity to colonize the roots of this host (8%).

CONCLUSION

Insertion of microshoots of *V. corymbosum* 'Bluecrop' into peat pellets previously inoculated with *H. ericae* provides a useful technique for successful colonization of these plants. This protocol may have applications to other ericaceous plants produced using micropropagation procedures and should be investigated further. There was no shoot growth response to inoculation by any of the isolates used in this study when compared to non-inoculated plants. Despite the fact that all isolates used were purportedly isolates of *H. ericae*, there was considerable variability in the extent of colonization of the roots by each isolate. Further studies need to be conducted to evaluate the plant growth response following nursery and field conditions.