Evaluation of Ericoid Mycorrhizae and Media on Establishment of Micropropagated *Rhododendron chapmanii*, Gray[1]

Lee R. Barnes and Charles R. Johnson[2]
Ornamental Horticulture Department
University of Florida
Gainesville, FL 32611

Abstract

Rooted microcuttings of Chapman's rhododendron (*Rhododendron chapmanii* Gray) were transplanted into MetroMix-500 (M500) and fired montmorillonite clay: Canadian peat [(2FMC:1CP) by vol] and half the plantlets were inoculated with the ericoid mycorrhizal fungus, *Pezizella ericae* Read. Plantlet survival was improved with mycorrhizal colonization after 16 weeks in 2FMC:1CP medium, but no differences were observed for plants grown in the M500 medium. Plant growth parameters after 16 weeks were greater for all plants grown in the M500 medium and there were no beneficial mycorrhizal growth responses in either media. Mycorrhizal colonization appeared to be of no benefit to growth of rooted *R. chapmanii* microcuttings except improved survival in high pH 2FMC:1CP medium.

Index words: tissue culture, *in vitro* propagation, *Pezizella ericae*, Chapman's rhododendron

Introduction

Micropropagation systems have been developed for several *Rhododendron* species (1, 2, 11). Recently, a micropropagation system with potential for commercial use has been developed for the endangered Chapman's rhododendron (*Rhododendron chapmanii* Gray), an evergreen Florida endemic (3).

Several micropropagated woody species, including apple (7), cherry (14), and raspberry (13), have been inoculated with mycorrhizal fungi, often resulting in more uniform and vigorous plants. Moore-Parkhurst and Englander (12) were successful in colonizing *Rhododendron maximum* with *Pezizella ericae in vitro* although this system would be impractible for commercial use. Read (15) observed that the symbiosis of *Rhododendron* with *Pezizella* resulted in enhanced N and P supply to the plant as well as increased resistance to heavy metal toxicity, especially Cu and Al. The purpose of this study was to determine if rooted microcuttings of *Rhododendron chapmanii* can be colonized with the ericoid mycorrhizal fungus *Pezizella ericae* and determine if there are benefits from the symbiosis.

Materials and Methods

Softwood shoot-tips with 5–8 nodes were taken from stock plants grown in growth chambers maintained at 25°C +/− 2°C (77°F +/− 4°F), 16 hr light/8 hr dark and with

[1]Received for publication April 21, 1986; in revised form July 1, 1986. University of Florida Journal Series No. 7264. This research was supported, in part, by the nursery industry through contributions to the Horticultural Research Institute, Inc., a non-profit organization devoted to progress through research.

[2]Graduate Assistant and Professor, resp. Present address of senior author: P.O. Box 2705, Durham, NC 27705; present address of second author: Department of Horticulture, University of Georgia, Experiment, GA 30212.

The *Journal of Environmental Horticulture* (USPS Publication No. 698-330) is published quarterly in March, June, September, and December by the Horticultural Research Institute. Subscription rate is $30.00 per year in USA; $45.00 per year for others.Second-class postage paid at Washington, D.C. and at additional mailing office. Send address changes to HRI, 1250 I Street, N.W., Suite 500, Washington, D.C. 20005.

an irradiance of 150 μM m^{-2} s^{-1} measured at the top of the plant canopy. Hand pinching of shoot-tips resulted in additional axillary shoots which were used as explants after 3 or 4 weeks. Explants were washed in soapy water and held under running water for 1 hr, lower leaves were removed leaving 0.5 cm (0.2 in) petiole stubs and were then surface disinfested with 20% Clorox for 15–20 minutes. Base and apex of the explants were further trimmed resulting in a 5 node stem section. Explants were placed horizonally on solidified Woody Plant Medium (WPM)(10) amended with 10 mg/l (10 ppm) 2-i-P [2-iso-(-isopentyl adenine)], 30 g/l sucrose, 100 mg/l myo-inositol, 100 mg/l NaH_2PO_4, 80 mg/l adenine sulfate and 0.8% Bacto-agar. Clumps of shoots which developed at each node were divided and subcultured to yield an average 7.6 fold increase in number of additional shoots per cycle. Shoots (microcuttings) larger than 15 mm (0.6 in) were excised and placed into a polyethylene rooting chamber receiving intermittant mist. Medium temperature was maintained at approximately 26.6°C (80°F) and light levels of 300 μM m^{-2} s^{-1}. Rooting medium was a finely screened mixture of Canadian peat: vermiculite (1:1 by vol). Microcuttings were rooted after 4 weeks and were transferred to 7.6 cm^2 (1.2 in^2) plastic cell packs containing one of the 2 soilless mixes, (1) MetroMix-500 (M500) or (2) fired montmorillonite clay: Canadian peat [(2FMC:1CP) by vol]. Each medium was amended with superphosphate (8.7% P) and Perk (a micronutrient formulation of Estech, Inc., Chicago, IL) at rates of 0.25 g/m^3 (6.7 oz/yd^3). The pH values for M500 and 2FMC:1CP were 4.3 and 6.5, respectively. Half of the rooted plantlets were inoculated with the ericoid mycorrhizal fungus *Pezizella ericae* as described by Marx & Kenny (9). Pure cultures of *Pezizella ericae* (obtained from the American Type Culture Collection) were cultured in a modified Melin-Norkrans medium (9) with a mixture of 28:1 (by vol) vermiculite:Canadian peat substrate. After 4 weeks of hyphal growth, the fungal inoculum was wrapped in several layers of cheese cloth and rinsed for 3 to 4 minutes under cool tapwater to remove excess glucose and nutrients which might support intrusive saprophytes. The vermiculite inoculum was then dried at 25°C (77°F) for a 2 to 3 day period. Approximately 1 g (1 tsp) of inoculum was placed immediately under half of the rooted plantlets in each soilless medium at transplanting into 7.6 cm^2 (1.2 in^2) cell-pack. Plantlets were placed into a greenhouse maintained at approximately 28°C day/24°C night (85°F/75°F) and maximum daylight irradiance of 450 μM m^{-2} s^{-1} for 16 weeks. Each treatment was replicated 12 times, with 4 plantlets per replication as an experimental unit. After 16 weeks, 40 plants grown in M500, half of which were mycorrhizal, were transplanted into 4-liter (#1) pots for 24 additional weeks.

Survival and growth measurements (height, number of stems and caliper, at 2 cm (0.75 in) above medium level, number of leaves and top dry weight) were recorded after 16 and 40 weeks following inoculation. Root colonization was determined following a 10 minute treatment with Lactophenol-Trypan blue stain at 90°C (195°F) (4). Clearing with KOH was unnecessary due to the fine 'hair root' structure. Percent colonization was estimated by use of the grid:plate method of Giovanetti and Mosse (5).

Results and Discussion

Plantlets grown in M500 were greater in all growth measurements after 16 weeks compared to plantlets grown in FMC:CP, regardless of colonization (Table 1). Plantlets grown in FMP:CP were smaller and did not survive establishment as well as those in M500. Colonization improved survival in the FMC:CP medium, but there were no beneficial mycorrhizal responses for plantlets grown in the M500 medium. This suggests that highly organic matter media may minimize or negate ericoid mycorrhizal responses.

Table 1. Influence of mycorrhizal colonization with *Pezizella ericae* and 2 soilless media on *in vitro* propagated *Rhododendron chapmanii* plantlets after 16 wks growth.

Media[y]	Treatment	Plantlet survival %	Height main stem (cm)	Number leaves	Caliper (mm)	Colon. %
M500	+M	91.7 a[z]	6.7 a	23.6 a	0.98 a	61.4 a
M500	−M	95.8 a	6.1 a	26.7 a	1.00 a	0
FMP:CP	+M	83.9 b	2.8 b	14.9 b	0.45 b	41.1 a
FMP:CP	−M	62.0 c	2.3 b	12.8 b	0.23 b	0

[y]Media M500 = MetroMix-500; FMC:CP = 2:1 (v:v) fired montmorillonite clay:Canadian peat

[z]Means in a column followed by the same letter are not significantly different at the 1% level by Duncan's Multiple Range Test.

Table 2. Influence of mycorrhizal colonization with *Pezizella ericae* on *in vitro* propagated *Rhododendron chapmanii* plantlets after 40 wks growth.

Mycorrhizal treatment	Main stem caliper (mm)	Number stems/plant	Height main stem (cm)	Number leaves/plant	Dry wt. (g)	Colon. %
+m[y]	4.2 a[z]	5.0 a	28.0 a	96.8 a	7.1 a	53.2
−m	3.9 a	7.0 b	25.6 a	114.0 b	7.3 a	0

[y]Means based on 20 plants per treatment except dry weights which were based on 5 plants per treatment.

[z]Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Johnson and Hummel (8) similarly noted that vesicular-arbuscular (VA) mycorrhizal fungi conferred greater beneficial response in media containing fired montmorillonite clay compared to organic media. The authors attributed this lack of mycorrhizal growth response in organic media to higher levels of P and lower pH.

After 24 additional weeks of growth in 4-liter (#1) pots, non-inoculated plants had a greater number of stems and leaves (Table 2). These differences were primarily due to the larger number of short basal shoots which developed on non-inoculated plants. There were no differences in main stem caliper, height and whole plant dry weight between treatments.

Other inoculated micropropagated species, including apple, cherry and raspberry varied in their response to VA mycorrhizal inoculation. Morandi *et al.* (13) reported significant differences in shoot dry weight of raspberry after inoculation with *Glomus mosseae* after 9 weeks. Granger *et al.* (7) reported significant differences in height, leaf dry weight and total dry weights between inoculated apple clones after 15 weeks. Pons *et al.* (14) observed no differences in growth of cherry plants inoculated with *Gigaspora margarita* after 9 weeks. It is possible that inoculation with VA mycorrhizae results in more distinctive growth responses than with ericoid mycorrhizae or that colonization is only observably beneficial for *Rhododendron* species growing in less than optimal conditions. Gordon (6) suggested that ericoid mycorrhizae were not obligate since normal root and shoot development can occur in axenic culture in sterilized sand:peat medium. Stribley and Read (16) suggested that colonization with ericoid mycorrhizae may be primarily advantangeous to plants growing in nutrient poor soils of the natural environment.

Significance to the Nursery Industry

These data indicate that inoculation of Chapman's rhododendron with an ericoid mycorrhizal fungus is of no benefit in organic container media. However, results from the FMC:CP medium suggest this fungus might improve survival in high pH media or soils. This could possibly be important in the establishment and survival of Chapman's rhododendron in high pH-calcareous landscape soils.

Procedures for inoculation of the rooted microcuttings resulted in high levels of colonization with an ericoid mycorrhizal symbiont. The techniques used should be applicable for other mycorrhizal symbionts and micropropagated plant species.

Literature Cited

1. Anderson, W.C. 1975. Propagation of rhododendrons by tissue culture: Part 1. Development of a culture medium for multiplication of shoots. Proc. Intern. Plant. Prop. Soc. 25:129–135.

2. Anderson, W.C. 1978. Rooting of tissue cultured rhododendrons. Proc. Intern. Plant Prop. Soc. 28:135–139.

3. Barnes, L.R. 1984. Micropropagation of endangered native *Rhododendron chapmanii* Gray. p. 303. In: R.D. Henke, K.W. Hughes (eds.), M. Forestry and Agriculture. Plenum Press, New York.

4. Englander, L. 1982. Endomycorrhizae by septate fungi. p. 11. In: N.C. Schenck, (ed.), Methods and principles of mycorrhizal research. Amer. Phytopath. Soc., St. Paul, Minn.

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

6. Gordon, H.D. 1937. Mycorrhiza in rhododendrons. Ann. Bot. 1:593–613.

7. Granger, R.L., C. Plenchette and J.A. Fortin. 1983. Effect of a vesicular-arbuscular (VA) endomycorrhizal fungus (*Glomus epigaeum*) on the growth and leaf mineral content of two apple clones propagated *in vitro*. Can. J. Plant Sci. 63:551–555.

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

9. Marx, D.H. and D.S. Kenny. 1982. Production of ectomycorrhizal fungus inoculum. p. 131–146. In: N.C. Schenck, (ed.), Methods and principles of mycorrhizal research. Amer. Phytopath. Soc., St. Paul, Minn.

10. McCown, B.H. and G. Lloyd. 1981. Woody plant media—a mineral nutrient formulation for microculture of woody plant species. HortScience 16:453. (Abstr.).

11. McCown, B.H. and G. Lloyd. 1983. A survey of the response of *Rhododendron* to *in vitro* culture. Plant Cell, Tissue and Organ Culture 2:77–85.

12. Moore-Parkhurst, S. and L. Englander. 1981. A method for the synthesis of a mycorrhizal association between *Pezizella ericae* and *Rhododendron maximum* seedlings growing in a defined medium. Mycologia 73:994–997.

13. Morandi, D., S. Gianinazzi and V. Gianinazzi-Pearson. 1979. Interet de l'endomycorhization dans la reprise et la croissance du Framboisier issu de multiplication vegetative *in vitro*. Ann. Amelior. Plantes 29:623–630.

14. Pons. F., V. Gianinazzi-Pearson, S. Gianinazzi and J.C. Navatel. 1983. Studies of VA mycorrhizae *in vitro*: mycorrhizal synthesis of axenically propagated wild cherry (*Prunus avium* L.) plants. Plant and Soil 79:217–221.

15. Read, D.J. 1982. The biology of mycorrhiza in the Ericales. Can. J. Bot. 61:985–1004.

16. Stribley, D.P. and D.J. Read. 1975. Some nutritional aspects of the biology of ericaceous mycorrhizas. New Phytol. 73:1149–1163.