In Vitro Colonization of Micropropagated *Pieris floribunda* by Ericoid Mycorrhizae. I. Establishment of Mycorrhizae on Microshoots

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Abstract. Selected isolates of *Hymenoscyphus ericae* (Read) Korf and Kernan, *Oidiodendron griseum* Robak, *O. maius* Barron, and a second *O. Robak* species were evaluated as inocula for in vitro establishment of micropropagated plantlets of *Pieris floribunda* (Pursh ex Sims) Benth. and Hook. Severity of shoot necrosis on microshoots differed for each isolate of *Oidiodendron*. Inoculation of micropropagated plantlets with isolates of *H. ericae* benefited initial shoot and root development on agar-solidified Woody Plant Medium (WPM) supplemented with sucrose and covered by a layer of autoclaved 1 peat : 1 vermiculite (v/v). Inoculation of microshoots with *H. ericae* or isolates of *Oidiodendron* did not stimulate production of adventitious roots.

Several species of fungi are identified as having the capacity to form mycorrhizae with roots of ericaceous plants. The most widely studied ericoid mycorrhizal fungus is *Hymenoscyphus ericae* (syn. *Pezizella ericae* Read), which forms mycorrhizae with *Calluna vulgaris* (L.) Hull (heather), (Read, 1974), *Rhododendron minus* var. *chapmanii* (A. Gray) Duncan & Pullen (syn. *R. chapmanii* A. Gray,

Chapman's rhododendron), (Barnes and Johnson, 1986), *R. maximum* L. (rosebay rhododendron), (Moore-Parkhurst and Englander, 1981), *R. ponticum* L. (Duddridge and Read, 1982), *Vaccinium angustifolium* Ait. (lowbush blueberry) (Couture et al., 1983), and *V. corymbosum* L. (highbush blueberry) (Lareau, 1985). *Oidiodendron griseum* is another species that forms mycorrhizae with *V. angustifolium* (Couture et al., 1983; Dalpé, 1986) and *V. corymbosum* (Couture et al., 1983; Lareau, 1985). A related species, *O. maius*, was isolated from roots of *Rhododendron* × 'Pink Pearl' and forms typical ericoid mycorrhizae when reassociated with rooted microshoots of this cultivar (Douglas et al., 1989). Other species of *Oidiodendron* reportedly form ericoid mycorrhizae (Currah et al., 1993; Dalpé, 1991; Stoyke and Currah, 1991; Xiao and Berch, 1992). Englander and Hull (1980) and Peterson et al. (1980) observed mycorrhizal symbiosis between *Clavaria* Fr. sp. and *Pieris japonica* (Thunb.) D. Don ex G. Don (Japanese andromeda). Starrett et al. (1996b) isolated a mycorrhizal fungus, identified tentatively as *Oidiodendron* sp., from roots of plants of *P. floribunda* collected from the wild.

Many different media are used for aseptic culture of isolates of the aforementioned mycorrhizal fungi, each with varying success. In an in vitro study with ericoid mycorrhizal fungi and seedlings of *Rhododendron* L. sp.

(rhododendron), slender roots of ericaceous seedlings did not penetrate solidified agar readily, but remained on and above the surface of the medium (Gordon, 1937). Similar results were reported by Pierik and Steegmans (1975) with aseptic cultures of rhododendrons and by Starrett et al. (1993) with adventitious roots formed in vitro on microcuttings of *P. floribunda*. Pierik and Steegmans (1975) suggested that inadequate aeration in the medium caused aerial root growth.

Various methods exist to improve in vitro root development of ericaceous species and to increase the potential for synthesis of a mycorrhizal association between fungus and host. A paper bridge support over liquid medium is used for synthesis of mycorrhizae between ericoid mycorrhizae and rhododendrons (Douglas et al., 1989; Moore-Parkhurst and Englander, 1981). This method is effective in promoting growth of both plants and fungi. However, the procedure is very labor-intensive and therefore difficult to implement commercially.

A protocol developed by Pearson and Read (1973) is widely used for synthesis of ericoid mycorrhizae in aseptic culture. This procedure employs a layer of sterilized soil placed on top of a water–agar base, and was used by Berta and Bonfante-Fasolo (1983), Berta and Gianinazzi-Pearson (1986), and Couture et al. (1983) to improve aeration and allow for root penetration. Unfortunately, the "soil" used in these studies was not characterized, making replication difficult.

Some soils used by researchers contain peat following the procedure of Pearson and Read (1973). However, autoclaved peat can release toxic substances (Harley, 1969). Read and Stribley (1973) and Stribley et al. (1975) used gamma irradiation to sterilize soil and reported fewer toxicity problems when compared with an autoclaved soil layer. Unfortunately, most laboratories are not equipped to sterilize soil with gamma irradiation. Duclos and Fortin (1983), Lareau (1985), and Dalpé (1986) added carbohydrates, mineral nutrients, or other amendments, rather than a layer of soil, to the surface of the agar-solidified medium. These additives resulted in variable growth of either host, endophyte or both.

A micropropagation protocol developed by Anderson (1978) is useful for rapid, commercial propagation of rhododendron. This procedure uses agar-solidified medium without addition of a soil layer. A soilless medium may be employed for direct rooting of microshoots, or microshoots may be induced to root in vitro (Anderson, 1978). Microshoots of rhododendron are often rooted ex vitro in a peat : vermiculite medium (Barnes and Johnson, 1986; Smagula and Litten, 1989). However, *Pieris* D. Don. sp. requires a root initiation phase in vitro (Pennell, 1990). Starrett et al. (1993) included a similar in vitro rooting step in the protocol developed for micropropagation of *P. floribunda*.

Seedlings are often used to study ericoid mycorrhizal fungi when both are cultured in vitro (Berta and Gianinazzi-Pearson, 1986; Dalpé, 1986; Pearson and Read, 1973; Starrett,

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et al. 1995). Dalpé (1986) recommended using seedlings of *Vaccinium* L. sp. (blueberry) to verify mycorrhizal potential of fungi. This suggestion applies mainly to evaluation of fungi for potential mycorrhizal capacity and effects on root morphology, but does not consider shoot growth or development as affected by mycorrhizal colonization in the roots. To evaluate mycorrhizal potential of a fungal isolate, clonally propagated host plants are desirable to eliminate seedling variability; however, micropropagated plant material is rarely utilized for evaluation of root and shoot growth because of colonization by ericoid mycorrhizal fungi (Currah et al., 1993; Douglas et al., 1989; Lareau, 1985).

No reports are known of simultaneous comparisons of media for optimum growth of several species of ericoid mycorrhizal fungi and a micropropagated host plant. Therefore, we examined six isolates of ericoid mycorrhizal fungi and their interaction with micropropagated shoots of *P. floribunda* grown on six culture media.

Materials and Methods

On 10 July 1993, terminal shoots, ≈ 3 cm in length, were taken from an actively growing, 2 1/2-year-old seedling of *P. floribunda* maintained in a greenhouse at 24 ± 3 day/ 20 ± 3 °C night with natural photoperiod. Natural irradiance was reduced by 75% by using whitewash on greenhouse glass. The plant was propagated from seed collected from a native population of open-pollinated plants growing at an elevation of 1860 m in Haywood County, N.C. Surface disinfection and establishment of aseptic cultures from actively elongating shoot tips followed the protocol of Starrett et al. (1993). Shoots were transferred to fresh medium every 4 weeks and subdivided at 8-week intervals. When an adequate supply of shoots was available, the experiment was initiated.

On 30 Nov. 1994, four unbranched microshoots of *P. floribunda* (2.5 cm in length) were selected and placed vertically to a depth of 1 cm in each of 36 jars (140 mL). Each jar contained 20 mL Woody Plant Medium (WPM) (Lloyd and McCown, 1980) solidified with 0.8% Tissue Culture (TC) agar (JRH Biosciences, Lenexa, Kans.), with $200 \text{ mg} \cdot \text{L}^{-1} \text{ NaH}_2\text{PO}_4$, $80 \text{ mg} \cdot \text{L}^{-1}$ adenine hemisulfate, 2% sucrose, and 0, 20.7, or $41.4 \mu\text{M}$ of the potassium (K) salt of 1*H*-indole-3-butyric acid (K-IBA). There were 48 shoots per concentration of K-IBA, for a total of 144 shoots. Jars were capped with Magenta B-caps (Magenta Corp., Chicago) and sealed with Parafilm® "M" (American National Can, Greenwich, Conn.). Cultures were placed in a controlled-environment chamber maintained at 23 ± 1 °C with a 16-h photoperiod provided by two, cool-white fluorescent power groove tubes (GE model F48PG17W; GE Lighting, Cleveland) and two, 25-W, soft-white incandescent bulbs suspended 60 cm above the tops of the jars. Tubes and bulbs provided a photosynthetic photon flux [PPF (400–700 nm)] of $\approx 60 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ as measured at the tops of

jars. These and all other light measurements were recorded with a LI-COR LI-185A quantum/radiometer/photometer (LI-COR, Lincoln, Nebr.). Shoots were transferred after 7 d to baby food jars (vol. = 225 mL) containing 30 mL WPM as listed above with 0%, 1%, or 2% sucrose and no K-IBA. The surface of the agar was nontreated (WPM–PV) or covered with a 3–4 mm layer of moist, autoclaved (15 min at 121 °C) 1 sifted peat : 1 fine vermiculite (WPM+PV).

In the culture vessel, each microshoot was inoculated with one of six isolates of ericoid mycorrhizal fungi or remained noninoculated (control). Three isolates obtained from the American Type Culture Collection (ATCC) (Rockville, Md.) included *H. ericae* [HE (#32985)], *O. griseum* [OG (#60377)], and *O. maius* [OM (#66504)]. Two other isolates, isolated from roots of *Rhododendron* were DA (= isolate A, a putative isolate of *H. ericae*) (Lemoine et al., 1992) and LPA (= isolate Ipae25, identified as an isolate of *H. ericae*) (Lemoine et al., 1992). Isolate C1 was obtained from roots of *P. floribunda* collected in Haywood County, N.C. and was identified tentatively as an *Oidiodendron* sp. The fungal inoculum consisted of $\approx 3 \text{ mm}^2$ of aerial hyphae collected from actively growing colonies maintained on malt agar (Difco Laboratories, Detroit). After inoculation, jars were covered with Magenta B-caps, sealed with Parafilm®, returned to the controlled-environment chamber, and incubated for 3 months.

Total shoot growth, shoot necrosis, number of primary roots, total root length, and percentage of roots colonized were recorded for each microshoot. Total shoot growth was calculated as total shoot length after 3 months in culture minus 2.5 cm (original microshoot explant length). Some shoots exhibited basal necrosis; this was quantified as the percentage of shoot length that was necrotic.

Roots were cleared and stained following a protocol adapted from Brundrett et al. (1984). The proportion of root length infected was estimated at $\times 40$ in increments of 10%.

The experimental design was a split-split-split plot with main plots in blocks and a four-factor factorial arrangement of treatments (PV, K-IBA, sucrose, and fungi). PV was assigned to main plots, K-IBA to subplots, sucrose to subsubplots, and fungi to subsubsubplots. There were four replications (blocks) in time. An experimental unit consisted of one microshoot per jar. Data were subjected to analysis of variance (ANOVA) procedures in SAS (SAS Institute, 1990).

Results

Shoot growth. The ANOVA indicated that there was a significant PV $\times$ sucrose $\times$ fungal isolate interaction for shoot growth (Table 1). Growth was not influenced by K-IBA (data not shown) nor did increasing concentrations of sucrose increase shoot growth of control microshoots or those inoculated with isolates of *Oidiodendron*. Several isolates of *Oidiodendron* significantly inhibited growth, especially at higher sucrose concentrations.

On sucrose-free WPM+PV, growth of microshoots inoculated with DA was significantly greater than that of those inoculated with OG or OM but was not significantly different from that of control shoots, or those inoculated with HE, LPA, or C1 (Table 1). Microshoots inoculated with DA were not different from controls or other inoculated plantlets when grown on sucrose-free WPM–PV. Shoots inoculated with DA or LPA on WPM+PV and 1% sucrose grew significantly more than did those inoculated with other isolates or the control on the same medium; but this was not true on 2% sucrose. DA- or LPA-inoculated microshoots grown on WPM+PV with 1% sucrose were longer than those on the medium without PV.

Shoot necrosis. PV $\times$ sucrose $\times$ fungal isolate interaction was significant for shoot necrosis (Table 1). Necrosis was not influenced by K-IBA (data not shown). In the medium without sucrose, a layer of PV suppressed basal browning by all fungal isolates. Without PV, inoculation of microshoots with any isolate caused significant necrosis. After 3 months in culture on WPM–PV without sucrose, microshoots inoculated with isolates of *H. ericae* or *Oidiodendron* were at least 80% necrotic. Control shoots were not affected by PV.

On WPM without sucrose, addition of PV reduced necrosis in all fungal treatments. Shoots treated with *H. ericae* responded better than those inoculated with *Oidiodendron* but neither was significantly different from the control. All *H. ericae* treatments on WPM–PV with sucrose had less necrosis than on the same medium without sucrose. *Oidiodendron*-treated shoots responded very differently. In general, sucrose did not reduce necrosis but PV did. Regardless of sucrose concentration, inoculation with OG killed all microshoots on WPM–PV.

Root length. Total root length was also influenced by a PV $\times$ sucrose $\times$ fungal isolate interaction (Table 1). Sucrose (2%) increased total root length in the controls in the absence of PV but not in its presence. In general, the best results for inoculated shoots were obtained on WPM+PV without sucrose.

Root number. Primary root number was influenced by a medium $\times$ fungal isolate interaction, but not by sucrose concentration (data not presented). On WPM+PV, microshoots inoculated with DA had more primary roots than did microshoots inoculated with any other fungus or the noninoculated controls. On WPM–PV, production of roots by microshoots inoculated with DA was similar to that of noninoculated microshoots on the same medium. Controls on WPM developed more primary roots than did microshoots inoculated with HE, LPA, or any isolate of *Oidiodendron*. With or without PV, *Oidiodendron*-treated microshoots had the fewest primary roots.

An auxin concentration $\times$ fungal isolate interaction also affected the number of primary roots produced after 3 months in culture (Table 2). Without root induction by K-IBA, only microshoots inoculated with isolates of *H. ericae* developed roots. Following treat-

Table 1. Effects of sucrose concentration and addition of peat/vermiculite (PV) to Woody Plant Medium (WPM) on growth (cm) of microshoots of *Pieris floribunda* during 3 months of culture in vitro with ericoid mycorrhizal fungi.

PV	Sucrose (%)	Control	Isolate ^z					
			<i>H. ericae</i>			<i>Oidiodendron</i>		
			HE	DA	LPA	OG	OM	C1
<i>Shoot growth (cm)</i>								
–	0	1.9 a/m/p ^y	1.8 a/m/p	1.7 a/m/p	1.7 a/m/p	1.3 a/m/p	1.5 a/m/p	1.1 a/m/p
	1	2.7 a/m/p	2.0 ab/m/p	1.9 ab/m/q	1.3 ab/m/q	1.4 ab/m/p	1.1 b/m/p	1.3 ab/m/p
	2	3.2 a/m/p	1.4 b/m/p	3.1 a/m/p	2.0 ab/m/p	1.2 b/m/p	0.9 b/m/p	1.3 b/m/p
+	0	1.5 abc/m/p	2.5 ab/m/p	2.7 a/m/p	2.2 abc/n/p	1.0 c/m/p	1.1 bc/m/p	2.1 abc/m/p
	1	2.0 b/m/p	1.1 b/m/p	3.9 a/m/p	4.3 a/m/p	1.7 b/m/p	1.2 b/m/p	1.0 b/m/p
	2	2.5 a/m/p	2.4 ab/m/p	2.5 a/m/p	1.5 abc/n/p	1.2 abc/m/p	1.1 bc/m/p	1.0 c/m/p
<i>Shoot necrosis (%)</i>								
–	0	24 c/m/p	82 ab/m/p	100 a/m/p	80 b/m/p	100 a/m/p	82 ab/m/p	80 b/m/p
	1	19 b/m/p	20 b/n/p	23 b/n/p	29 b/n/p	100 a/m/p	100 a/m/p	90 a/m/p
	2	20 cd/m/p	23 cd/n/p	15 d/n/p	24 cd/n/p	100 a/m/p	62 b/n/p	35 c/n/p
+	0	24 ab/m/p	18 b/m/q	25 ab/m/q	18 b/m/q	42 a/n/q	43 a/m/q	36 ab/m/q
	1	20 c/m/p	21 c/m/p	12 c/m/p	14 c/m/p	100 a/m/p	28 c/m/q	53 b/m/q
	2	18 c/m/p	16 c/m/p	15 c/m/p	21 c/m/p	88 a/m/p	23 c/n/q	45 b/m/q
<i>Total length of roots (cm)</i>								
–	0	0.0 a/n/p	2.0 a/m/q	1.5 a/n/q	1.1 a/m/q	0.0 a/m/q	0.0 a/m/q	0.0 a/m/q
	1	7.0 a/n/p	0.1 a/m/p	4.8 a/n/p	0.0 a/m/p	0.0 a/m/p	0.0 a/m/p	0.1 a/m/p
	2	30.1 a/m/p	0.4 c/m/q	16.3 b/m/p	4.6 c/m/p	0.4 c/m/p	0.0 c/m/p	0.8 c/m/p
+	0	0.0 c/m/p	18.0 b/m/p	25.0 b/m/p	18.0 b/m/p	42.0 a/m/p	43.0 a/m/p	36.0 a/m/p
	1	1.9 a/m/p	0.4 a/n/p	8.2 a/n/p	1.7 a/n/p	0.0 a/n/p	0.0 a/n/p	0.4 a/n/p
	2	2.2 b/m/q	11.3 a/m/p	4.8 ab/n/q	0.2 b/n/p	0.0 b/n/p	0.1 b/n/p	0.0 b/n/p

^zControl = noninoculated; HE = *Hymenoscyphus ericae* (ATCC #32985); American Type Culture Collection, Rockville, Md.); DA and LPA = putative isolates of *H. ericae* from Dijon, France; OG = *Oidiodendron griseum* (ATCC #60377), OM = *O. maius* (ATCC #66504), C1 = isolate from roots of *Pieris floribunda* collected from the wild in North Carolina, identified putatively as *Oidiodendron*.

^yMean separation within parameters and within rows (abc), between sucrose levels within PV and isolates (mn) and between presence and absence of PV within sucrose levels and isolates (pq) by LSD $P \leq 0.05$. LSD for no. of primary roots/shoot = 1.6; and LSD for total length of roots = 6.3. LSD's ($\alpha = 0.05$) are 1.4 for shoot growth, 19 for shoot necrosis, and 9.0 for total length of roots. In each case, the LSD should be used to compare levels of one variable when the other two variables are held fixed.

ments with 20.7 μM K-IBA, microshoots inoculated with DA produced more primary roots than did those inoculated with any other isolates or the noninoculated controls. Controls on 41.4 μM K-IBA had more primary roots than microshoots inoculated with HE, LPA, or any isolate of *Oidiodendron*, which produced few roots, regardless of K-IBA level.

Total root length also was affected by a K-IBA $\times$ fungal isolate interaction (Table 2). Microshoots induced to root with 20.7 μM K-IBA and inoculated with isolate DA had longer roots than did microshoots inoculated with any other isolate, but were not different from controls. At 41.4 μM K-IBA, DA-treated microshoots had the longest roots of any treatment but they were significantly shorter than those of the controls.

Root colonization was low and ranged from 0% to 30% (data not shown). The failure of microshoots inoculated with isolates of *Oidiodendron* to root, together with the low rooting percentages of microshoots, prevented analysis of the data.

Discussion

Shoot and root growth of microshoots inoculated with isolates of *Oidiodendron* was generally inferior to that of noninoculated microshoots or those inoculated with isolates of *H. ericae*. These results contrast with those of Dalpé (1986), who reported that growth of seedlings of *V. angustifolium* was not affected by *O. griseum*, *O. cerealis* (Thüm.) Barron, or *O. rhodogenum* Robak. These three species also did not stimulate growth of seedlings

Table 2. Influence of K-IBA on primary root number and total root length (cm) of microshoots of *Pieris floribunda* during 3 months of culture in vitro with ericoid mycorrhizal fungi.

K-IBA (μM)	Control	Isolate ^z					
		<i>H. ericae</i>			<i>Oidiodendron</i>		
		HE	DA	LPA	OG	OM	C1
<i>No. primary roots/shoot</i>							
0	0.0 aB ^y	1.0 aA	0.5 aB	0.1 aA	0.0 aA	0.0 aA	0.0 aA
20.7	1.0 bB	0.7 bA	3.4 aA	1.1 bA	0.0 bA	0.0 bA	0.4 bA
41.4	3.7 aA	1.1 bA	2.1 abA	1.6 bA	0.3 bA	0.3 bA	0.9 bA
<i>Total length of roots (cm)</i>							
0	0.0 aB	4.6 aA	1.6 aB	0.3 aA	0.0 aA	0.0 aA	0.0 aA
20.7	5.0 aB	1.7 bA	8.1 aA	0.8 bA	0.0 bA	0.0 bA	1.5 bA
41.4	16.9 aA	1.0 cA	8.1 bA	2.8 bcA	0.2 cA	0.2 cA	0.6 cA

^zControl = noninoculated; HE = *Hymenoscyphus ericae* (ATCC #32985); American Type Culture Collection, Rockville, Md.); DA and LPA = putative isolates of *H. ericae* from Dijon, France; OG = *Oidiodendron griseum* (ATCC #60377), OM = *O. maius* (ATCC #66504), C1 = isolate from roots of *Pieris floribunda* collected from the wild in North Carolina, identified putatively as *Oidiodendron*.

^yMean separation within parameters, rows (abc) and columns (AB) by LSD $P \leq 0.05$. LSD for no. of primary roots/shoot = 1.6; and LSD for total length of roots = 6.3.

(Dalpé, 1986). *Oidiodendron griseum*, *O. cerealis*, and *O. rhodogenum* form typical ericoid mycorrhizae with roots of seedlings of *V. angustifolium*. However, two other species, *O. tenuissimum* (Peck) Hughes and *O. truncatum* Barron, do not colonize roots (Dalpé, 1986). Root and shoot development of seedlings inoculated with *O. tenuissimum* are stunted despite lack of mycorrhizal association between host and fungal isolate (Dalpé, 1986). Successful mycorrhizal formation of *V. corymbosum* with *O. griseum* without a stimulatory effect on shoot development has been reported (Lareau, 1985; Starrett et al., 1995). Douglas et al. (1989) noted the first successful formation of mycorrhizae with an isolate of

O. maius and *Rhododendron* $\times$ 'Pink Pearl'. However, details were not provided on the morphological response of the host to colonization by this isolate. Currah et al. (1993) found that *O. periconioides* formed typical ericoid mycorrhizae on seedlings of *R. brachycarpum* G. Don., but the effects on growth or development were not presented.

Isolates of *Oidiodendron* caused extensive shoot necrosis after 3 months culture in vitro. The high mortality of shoots grown on WPM and inoculated with isolates of *Oidiodendron* suggests an antagonistic aspect of these isolates. Isolates OM and C1 produced hyphal growth and conidia on dead portions of inoculated microshoots, whereas necrotic portions

of microshoots inoculated with OG lacked hyphae or conidia.

Extensive basal necrosis was evident on microshoots of *P. floribunda* inoculated with *Oidiodendron* isolates, regardless of the presence or absence of sucrose in the medium. Basal necrosis was suppressed by a layer of PV on the medium, regardless of sucrose concentration, for plantlets inoculated with OG or OM. A surface-layer of PV on WPM containing 0% or 1%, but not 2% sucrose suppressed basal necrosis on microshoots inoculated with C1.

Similarly, many microshoots inoculated with *H. ericae* and cultured on WPM without sucrose were necrotic after 3 months but necrosis was not evident when such microshoots were cultured on sucrose-free WPM+PV. Lack of extensive necrosis on control plantlets grown without sucrose indicated that the fungal inoculum had adverse effects. When sucrose was provided, there was no effect of PV on necrosis of *H. ericae*-inoculated microshoots. Perhaps *H. ericae* isolates used microshoots as a carbohydrate source when sucrose was limiting, thus increasing necrosis of the lower portions of the microshoots.

Without K-IBA, roots were not produced to any significant extent regardless of the presence or absence of ericoid fungi. This result contrasts with a report by Berta and Gianinazzi-Pearson (1986) that *H. ericae*-inoculated seedlings of *C. vulgaris* produced more roots than did the controls. Given proper precursors, often found in root exudates, *H. ericae* can synthesize indole-3-acetic acid (IAA) (Gay and Debaud, 1986). In the present study, microshoots inoculated with isolates of *Oidiodendron* rooted very poorly regardless of treatment with K-IBA (a synthetic auxin). Neither isolate OG nor OM is reported in the literature as stimulating root production, nor are they described as inhibitors of rooting of microshoots. A nonmycorrhizae-forming isolate of *O. tenuissimum* retarded in vitro root development of seedlings of *V. angustifolium* (Dalpé, 1986). Similarly, during a preliminary study, in vitro inoculation with *O. griseum*, *O. maius*, or isolate C1 stunted roots on microshoots of *P. floribunda* (Starrett et al., 1996a).

A layer of PV was added to provide an organic substrate for the fungal inoculum, as well as an aerated and darkened medium for the penetration of roots. On WPM without sucrose, all isolates produced sparse hyphae; however, addition of a layer of PV to the surface, favored hyphal production, suggesting that the PV was providing nutrients to the fungus (data not presented). Unfortunately, there was no consistent beneficial effect on shoot growth.

Based on our data, isolates OG, OM, and C1 are not suitable as inocula for in vitro establishment of micropropagated plantlets of *P. floribunda*. Inoculation with isolates of *H. ericae* may be beneficial for initial shoot and root development provided the WPM is supplemented with 1% sucrose and covered by a

layer of PV. Further studies should be conducted to investigate acclimatization of micropropagated *P. floribunda* inoculated with isolates of *H. ericae*.

Literature Cited

- Anderson, W.C. 1978. Rooting of tissue-cultured rhododendrons. Proc. Intl. Plant Prop. Soc. 28:135-139.
- Barnes, L.R. and C.R. Johnson. 1986. Evaluation of ericoid mycorrhizae and media on establishment of micropropagated *Rhododendron chapmanii*, Gray. J. Environ. Hort. 4:109-111.
- Berta, G. and P. Bonfante-Fasolo. 1983. Apical meristems in mycorrhizal and uninfected roots of *Calluna vulgaris* (L.) Hull. Plant and Soil 71:285-291.
- Berta, G. and V. Gianinazzi-Pearson. 1986. Influence of mycorrhizal infection on root development in *Calluna vulgaris* (L.) Hull seedlings, p. 673-676. In: V. Gianinazzi Pearson and S. Gianinazzi (eds.). Physiological and genetical aspects of mycorrhizae. Proc. 1st European Symp. on Mycorrhizae, Dijon, France, 1-5 July 1985.
- Brundrett, M.C., Y. Piché, and R.L. Peterson. 1984. A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot. 62:2128-2134.
- Couture, M., J.A. Fortin, and Y. Dalpé. 1983. *Oidiodendron griseum* Robak: An endophyte of ericoid mycorrhiza in *Vaccinium* spp. New Phytol. 95:375-380.
- Currah, R.S., A. Tsuneda, and S. Murakami. 1993. Conidiogenesis in *Oidiodendron periconioides* and ultrastructure of ericoid mycorrhizas formed with *Rhododendron brachycarpum*. Can. J. Bot. 71:1481-1485.
- Dalpé, Y. 1986. Axenic synthesis of ericoid mycorrhiza in *Vaccinium angustifolium* by *Oidiodendron* species. New Phytol. 103:391-396.
- Dalpé, Y. 1991. Endomycorrhizal status of the genus *Oidiodendron*. Can. J. Bot. 69:1712-1714.
- Douglas, G.C., M.C. Heslin, and C. Reid. 1989. Isolation of *Oidiodendron maius* from *Rhododendron* and ultrastructural characterization of synthesized mycorrhizas. Can. J. Bot. 67:2206-2212.
- Duclos, J.L. and J.A. Fortin. 1983. Effect of glucose and active charcoal on in vitro synthesis of ericoid mycorrhiza with *Vaccinium* spp. New Phytol. 94:95-102.
- Duddridge, J. and D.J. Read. 1982. An ultrastructural analysis of the development of mycorrhizas in *Rhododendron ponticum*. Can. J. Bot. 60:2345-2356.
- Englander, L. and R.J. Hull. 1980. Reciprocal transfer of nutrients between ericaceous plants [*Rhododendron* sp., *Pieris japonica*] and a *Clavaria* [argillacea] sp. [Mycorrhizal relationship]. New Phytol. 84:661-667.
- Gay, G. and J.C. Debaud. 1986. Preliminary study of IAA synthesis by ericoid endomycorrhizal fungi, p. 677-682. In: V. Gianinazzi-Pearson and S. Gianinazzi (eds.). Physiological and genetical aspects of mycorrhizae. Proc. 1st European Symp. on Mycorrhizae, Dijon, France, 1-5 July 1985.
- Gordon, H.D. 1937. Mycorrhiza in rhododendron. Ann. Bot. (NS) 1:593-613.
- Harley, J.L. 1969. The biology of mycorrhiza. 2nd ed. Leonard Hill, London.
- Lareau, M.J. 1985. Rooting and establishment of in vitro blueberry plantlets in the presence of mycorrhizal fungi. Acta Hort. 165:197-201.
- Lemoine, M.C., S. Gianinazzi, and V. Gianinazzi-Pearson. 1992. Application of endomycorrhizae to commercial production of *Rhododendron* microplantlets. Agronomie 12:881-885.
- Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Intl. Plant Prop. Soc. 30:421-437.
- 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.
- Pearson, V. and D.J. Read. 1973. The biology of mycorrhiza in the Ericaceae. I. The isolation of the endophyte and synthesis of mycorrhizas in aseptic culture. New Phytol. 72:371-379.
- Pennell, D. 1990. Micropropagation of the Ericaceae. The Plantsman 12:120-125.
- Peterson, T.A., W.C. Mueller, and L. Englander. 1980. Anatomy and ultrastructure of a *Rhododendron* root-fungus association. Can. J. Bot. 58:2421-2433.
- Pierik, R.L.M. and H.H.M. Steegmans. 1975. Analysis of adventitious root formation in isolated stem explants of *Rhododendron*. Scientia Hort. 3:1-20.
- Read, D.J. 1974. *Pezizella ericae* sp. nov. the perfect state of a typical mycorrhizal endophyte of Ericaceae. Trans. Brit. Mycol. Soc. 63:381-419.
- Read, D.J. and D.P. Stribley. 1973. Effects of mycorrhizal infection on nitrogen and phosphorus nutrition of ericaceous plants. Nature (London) 244:81.
- SAS Institute. 1990. SAS/STAT user's guide. Vol. 2. SAS Inst., Cary, N.C.
- Smagula, J.M. and W. Litten. 1989. Effect of ericoid mycorrhizae isolates on growth and development of lowbush blueberry tissue culture plantlets. Acta Hort. 241:110-114.
- Starrett, M.C., F.A. Blazich, J.R. Acedo, and S.L. Warren. 1993. Micropropagation of *Pieris floribunda*. J. Environ. Hort. 11:191-195.
- Starrett, M.C., F.A. Blazich, L.F. Grand, and S.R. Shafer. 1995. Response of seedlings of highbush blueberry to in vitro ericoid mycorrhizal inoculation [sic]. Proc. Southern Nurserymen's Assn. Res. Conf., 40th Annu. Rpt. p. 266-268.
- Starrett, M.C., F.A. Blazich, L.F. Grand, and S.R. Shafer. 1996a. Response of microshoots of mountain andromeda to in vitro ericoid mycorrhizal inoculation. Proc. Southern Nurserymen's Assoc. Res. Conf., 40th Annu. Rpt. p. 239-242.
- Starrett, M.C., F.A. Blazich, S.R. Shafer, and L.F. Grand. 1996b. Isolation, storage, and use of ericoid mycorrhizal fungi for improved acclimatization and subsequent growth of micropropagated *Pieris floribunda*. PhD Diss., North Carolina State Univ., Raleigh.
- Stoyke, G. and R.S. Currah. 1991. Endophytic fungi from mycorrhizae of alpine ericoid plants. Can. J. Bot. 69:347-352.
- Stribley, D.P., D.J. Read, and R. Hunt. 1975. The biology of mycorrhiza in the Ericaceae. V. The effects of mycorrhizal infection, soil type and partial soil-sterilization (by gamma irradiation) on growth of cranberry (*Vaccinium macrocarpon* Ait.). New Phytol. 75:119-130.
- Xiao, G. and S.M. Berch. 1992. Ericoid mycorrhizal fungi of *Gaultheria shallon*. Mycologia 84:470-471.