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Isolate-specific rooting responses of *Leucothoe fontanesiana* cuttings to inoculation with ericoid mycorrhizal fungi

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SUMMARY
We assessed whether adding ericoid mycorrhizal fungi (EMF) to the rooting substrate during cutting propagation altered rooting and root growth of *Leucothoe fontanesiana* 'Rainbow'. Hardwood cuttings, treated or untreated with rooting hormone prior to sticking into rooting substrate, were grown with one of three isolates of EMF as inoculum or with no inoculum (control). Cuttings were placed under mist in a greenhouse, with no bottom heat, and harvested 63, 84 and 109 d after sticking. Cuttings in substrate inoculated with two of the three EMF isolates showed better rooting than in non-inoculated substrate 63 d after sticking. However, by the end of the experiment, there were no differences in rooting between cuttings in the different inoculation treatments. Only cuttings in pots inoculated with one of the EMF isolates had consistently higher root initiation and weight than non-inoculated cuttings at all harvest dates. Root initiation and weight of cuttings in pots inoculated with either of the other two EMF isolates were greater than non-inoculated cuttings only when the cuttings had been treated with rooting hormone. In general, roots on EMF-inoculated cuttings were less branched and longer than roots on non-inoculated cuttings. Root colonisation was positively correlated with root initiation, length and weight 63 d after sticking; while, at later harvest dates, root colonisation was positively correlated only with root weight. The ability of EMF to increase rooting during cutting propagation of easy-to-root species, may decrease root production time. EMF-induced increases in root initiation during cutting propagation appear to be related to specific plant-fungus interactions and to interactions with rooting hormone. Although there were isolate-specific differences in rooting response, EMF-induced changes in root size and anatomy appear to be time-dependent and may influence the function of the new root system relative to water use and nutrient uptake, as well as survival, during transplanting.

Most ericaceous plants are calcifuges that grow naturally in acid soils with low to moderate fertility. Roots of many wild ericaceous species are heavily colonised by ericoid mycorrhizal fungi (EMF), which form extensive hyphal networks in the soil and potentially enhance plant nutrient uptake (Read, 1996). Mycorrhizal benefits are thought to be particularly high when plants are colonised early during development (Chang, 1994). Thus, when plants are propagated from cuttings, maximum benefit should occur when mycorrhizal inoculum is present during adventitious root formation. Mycorrhizal inoculum added during cutting propagation has been found to increase root production in several woody plant species, including *Sciadopitys verticillata* (Douds *et al*., 1995), *Arctostaphylos uva-ursi* (Linderman and Call, 1977), *Rosa* spp. (Scagel, 2000; 2001); *Viburnum dentatum* (Verkade and Hamilton, 1987) and *Cornus sericea* (Verkade *et al*., 1988).

Rooting of woody stem cuttings is dependent, in part, upon the physiological condition of the stock plant and treatment of the cuttings with rooting hormone. Rooting many ericaceous species from cuttings is sometimes difficult and attempts to root cuttings outside specific optimal times during the growing season can result in low percentages of rooted cuttings and poor survival after transplantation (Górecka, 1979). During commercial nursery production, however, it is not always possible to stick cuttings at optimal times for rooting. Thus, the time it takes for cuttings to grow adequate roots may increase production time. Therefore, propagators are looking for methods to increase successful propagation and decrease the time required for rooting, particularly when large quantities of rooted cuttings are required quickly.

Leucothoe fontanesiana (Steud.) Sleumer is an ericaceous plant native to the South-Eastern United States that can easily be propagated vegetatively by rooting stem cuttings (Dirr and Heuser, 1987). It roots readily, without exogenous auxin application, from cuttings taken between June–December (Dirr and Heuser, 1987). The objectives of this study were to determine whether an EMF inoculum, added to the rooting substrate during cutting propagation, can alter root production by *L. fontanesiana*, and whether responses to inoculation depend on the EMF isolate used as inoculum. Although commercially-produced inocula of certain types of mycorrhizal fungi (arbuscular and ectomycorrhizal fungi) are readily available to horticulturists, little is known about the benefits, if any, of inoculation with EMF on cutting propagation in woody ericaceous plants, and whether the response of cuttings to EMF depends on the fungal isolate.

MATERIALS AND METHODS

Plant culture

In late February 1999, rooted tissue culture plantlets of *L. fontanesiana* (Steud.) Sleumer 'Rainbow' ('Girard's Rainbow') were obtained from Briggs Nursery

(Olympia, WA, USA) and transplanted into 0.64 l pots (10 cm Gage Dura Pot #GDP400) filled with a mix of 80% composted Douglas-fir bark (Whitney Farms, Independence, OR, USA) and 20% sphagnum peat (Sunshine Grower Grade White, SunGrow, Hubbard, OR, USA). The mix was amended with 5.5 g l^{-1} of slow release fertiliser (SLR; Osmocote Plus 15-9-12, Scotts Company, Marysville, OH, USA). Fertiliser was mixed into media on a per-pot basis to ensure uniform distribution. Plants were maintained in a glasshouse with supplemental light (16 h photoperiod), average day/night temperatures of 21°/16°C and watered as needed. Pest control included diflubenzuron as needed for fungus gnats (*Bradysia* spp.) and *Neoseiulus cucumeris* predators for thrips (*Frankiniella* spp.). After 16 weeks, plants were transplanted into cylindrical 3.8 l pots (19 cm × 18 cm) containing a mix of 30% composted Douglas-fir bark, 60% peat and 10% perlite amended with 9.2 g l^{-1} of SLR per pot and grown outside under ambient conditions in Corvallis, OR (lat. 44°59'041" N, long. 123°27'217" W) and watered as needed.

Mycorrhizal inoculum

Isolates of three species of EMF (*Oidiodendron griseum* Robak; *Hymenoscyphus ericae* (Read) Korf and Kernan; and *Pezizella ericae* Pearson & Read) were grown as separate isolates in sterile cultures of liquid Modified Melins-Norkrans (MMN) media (Molina and Palmer, 1982). Single isolates of each species were used: *O. griseum* (OG) (ATCC#60377, originally from *Vaccinium corybosum*), *H. ericae* (HE) (HMER-100, USDA-FSL, Corvallis, OR, USA originally from an ascocarp), and *P. ericae* (PE) (ATCC#32985, originally from ericaceous roots). The day before inoculation, hyphae from 16 week-old cultures of each isolate were harvested by filtration and re-suspended in 1 l sterile water. The number of colony forming fragments (c.f.u.) was 21.2, 15.1 and 6.9 cfu ml^{-1} for OG, HE and PE, respectively. Inocula were used within 24 h.

Experimental design

On 15 November 2000, *L. fontanesiana* stems were cut from new growth on 2 year-old plants grown in pots (see above). The cuttings were sorted for uniformity (based on fresh weight and length), disinfected by dipping one end into 10% NaOCl solution for 20 min and rinsed with water. Cuttings were then left untreated, or treated with rooting hormone by dipping the disinfected end into 3 g l^{-1} Woods Hormone Solution (1.03% indole-3-butyric acid, 0.66% 1-naphthalene acetic acid; Earth Science Products Corp., Wilsonville, OR, USA) for 5 s. Treated and untreated cuttings were stuck into 10 cm pots (1 cutting per pot) filled with a 4:1 perlite:peat mix. Pots were inoculated with one of the three EMF isolates immediately after sticking, or left un-inoculated. Inoculum was applied by drenching the rooting substrate at the cutting base with 10 ml of inoculum mixture (see above).

Treatments were arranged in a completely randomised design with two hormone (treated and untreated) and four inoculation (*O. griseum*, *H. ericae*, *P. ericae* and non-inoculated) treatments and 15 single cutting replications (120 cuttings total). Pots were placed on an expanded metal bench top in a glasshouse and cuttings were rooted under intermittent mist with supplemental light (16 h photoperiod) and average day/night temperatures of 21°/16°C. No bottom heat was used during the experiment. Five cuttings per treatment were selected at random and harvested at 63, 84 and 109 d after sticking. Gaps created by sub-sampling were filled with 10 cm pots containing only the same perlite-peat mix.

Measurements

At each harvest, both primary and secondary roots on each cutting were counted, removed, measured for length and weighed. Cuttings with no roots were recorded as having zero roots. The above-ground portion (i.e., the region above the rooting zone) was also weighed. Approximately 50% of the fresh roots on cuttings were used to determine EMF colonisation. The remaining roots were dried at 60°C and weighed. Fresh root samples were cleared with 10% (w/v) KOH and stained following a modification of the procedure outlined by Phillips and Hayman (1970), with lacto-phenol replaced by lacto-glycerin. The percentage of root length with signs of EMF colonisation was estimated on 1 cm root segments following Biermann and Linderman (1980). Inoculated cuttings with no roots at the time sampling were recorded as having no colonisation.

Experimental design and statistical analyses

All data, except EMF colonisation and percentage rooting data, were subjected to a 3-factor analysis of variance (ANOVA) with two rooting hormone treatments, four inoculation treatments and three harvest dates. As plants in non-inoculated treatments showed no evidence of mycorrhizal colonisation, EMF colonisation data were subjected to a three-factor ANOVA with two rooting hormone treatments, three inoculation treatments and three harvest dates. Dry weight and root colonisation, number and size data were square-root transformed prior to analysis to correct for unequal variance and to achieve best model fit. Actual data and standard errors are reported in the Tables and Figures. The Bonferroni test was used to separate treatment means of data analysed by ANOVA at $P < 0.05$. Linear contrasts were used to compare treatment means averaged over time. Data are presented in Figures only when significant main effects or interactions ($P < 0.05$) were indicated by ANOVA. Because the percentage of cuttings with roots was determined for each treatment at each harvest, without replication, the Kolmogorov-Smirnov test ($P < 0.05$) was used to assess the effects of inoculation on percentage rooting over time (data pooled across rooting hormone treatments; n = 10), and of rooting hormone and inoculation on percentage rooting (data pooled across harvest dates; n = 15). Relationships between EMF colonisation and root growth characteristics were assessed using Pearson's Correlation Coefficient (r). All data were analysed using the Statistica® statistical package (Statsoft Inc., Tulsa, OK, USA).

RESULTS

Inoculated cuttings had 4–17%, 12–43% and 24–49% of their root length colonised by EMF at 63, 84 and 109 d

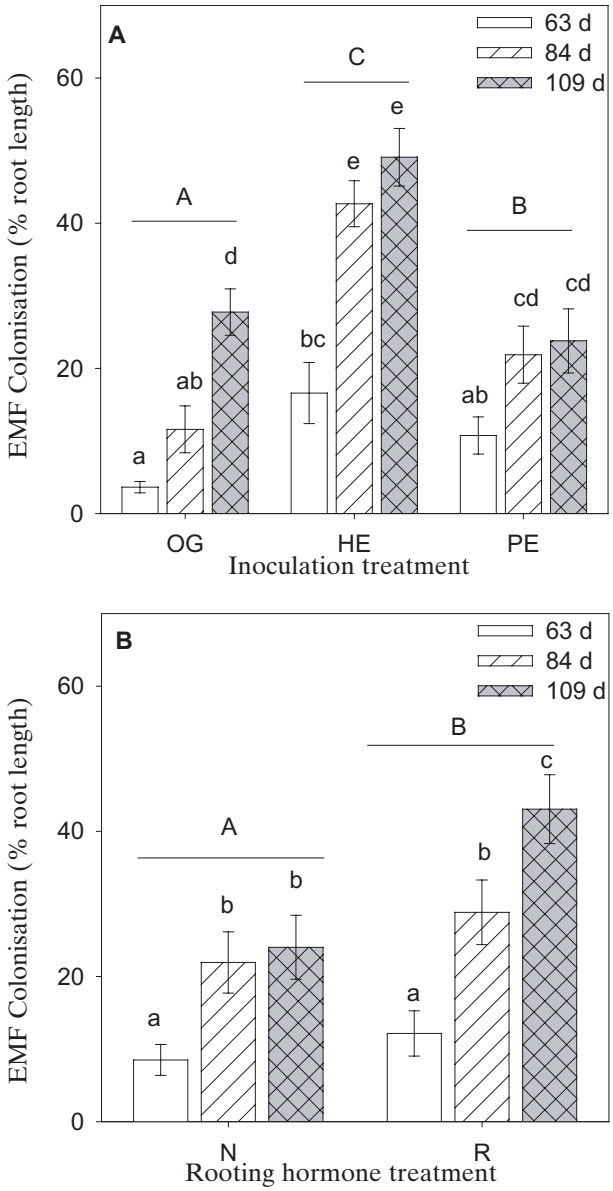

FIG. 1
Root colonisation of *Lecothoe racemosa* 'Rainbow' cuttings by ericoid mycorrhizal fungi (EMF), 63, 84 and 109 d after sticking and inoculation with mycorrhizal fungi (Panel A); or treatment with rooting hormone (Panel B). Inoculation treatments: OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n = 20). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$, Bonferroni test). Groups of columns with the same upper case letter above them within one panel denote no significant difference in treatment response over time ($P < 0.05$).

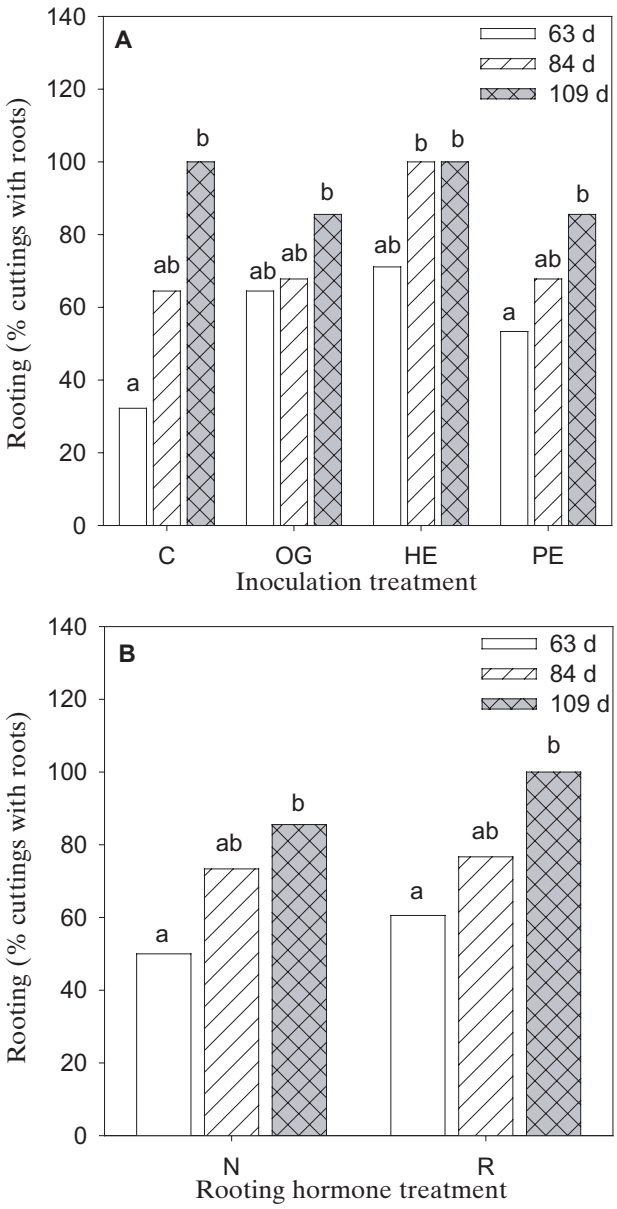

FIG. 2
Percentage of *Lecothoe racemosa* 'Rainbow' cuttings with roots 63, 84 and 109 d after sticking and inoculation with ericoid mycorrhizal fungi (EMF) (Panel A); or treatment with rooting hormone (Panel B). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Columns with the same letter above them are not significantly different from each other ($P < 0.05$, Kolmogorov-Smirnov test).

after sticking, respectively (Figure 1A). Non-inoculated cuttings showed no signs of root colonisation by EMF (data not shown). Percentage colonisation was higher when cuttings were inoculated with HE than with OG or PE. Cuttings in pots inoculated with HE became colonised more rapidly than cuttings in pots inoculated with the other EMF isolates. Colonisation was also higher, regardless of EMF isolate, when rooting hormone was applied, but only at the last harvest (109 d; Figure 1B).

Sixty-three d after sticking, less than 40% of non-inoculated cuttings showed any signs of visible roots, regardless of rooting hormone treatment, while more than 60% of cuttings inoculated with the OG or HE isolates had roots (Figure 2A). However, 109 d after sticking, the percentage of cuttings with roots was similar among all inoculation treatments. Treatment of cuttings with rooting hormone had no influence on the percentage of cuttings with roots, regardless of inoculation treatment (Figure 2B).

In general, inoculation with EMF increased the number of primary roots produced at each harvest

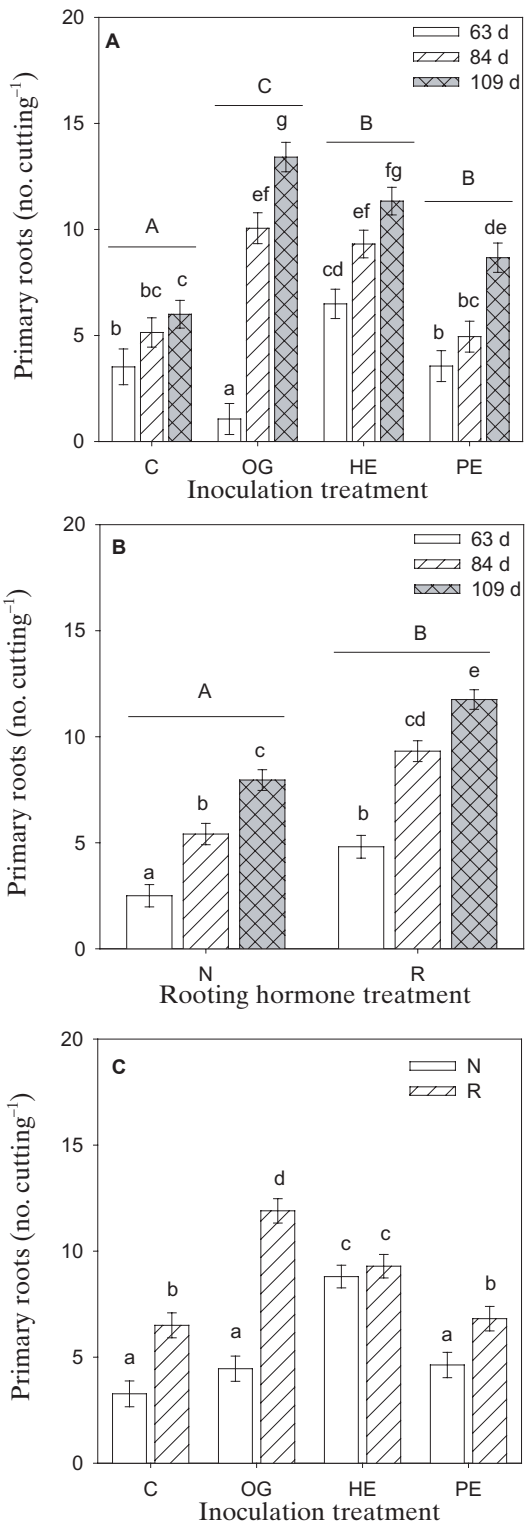

FIG. 3
Number of primary roots on *Lecothoe racemosa* 'Rainbow' cuttings 63, 84, and 109 d after sticking and inoculation with ericoid mycorrhizal fungi (EMF; Panels A, C); or treatment with rooting hormone (Panels B, C). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n = 20; Panel C, n = 15). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$; Bonferroni test). Groups of columns with the same upper case letter above them within a panel denote no significant difference in treatment response over time ($P < 0.05$).

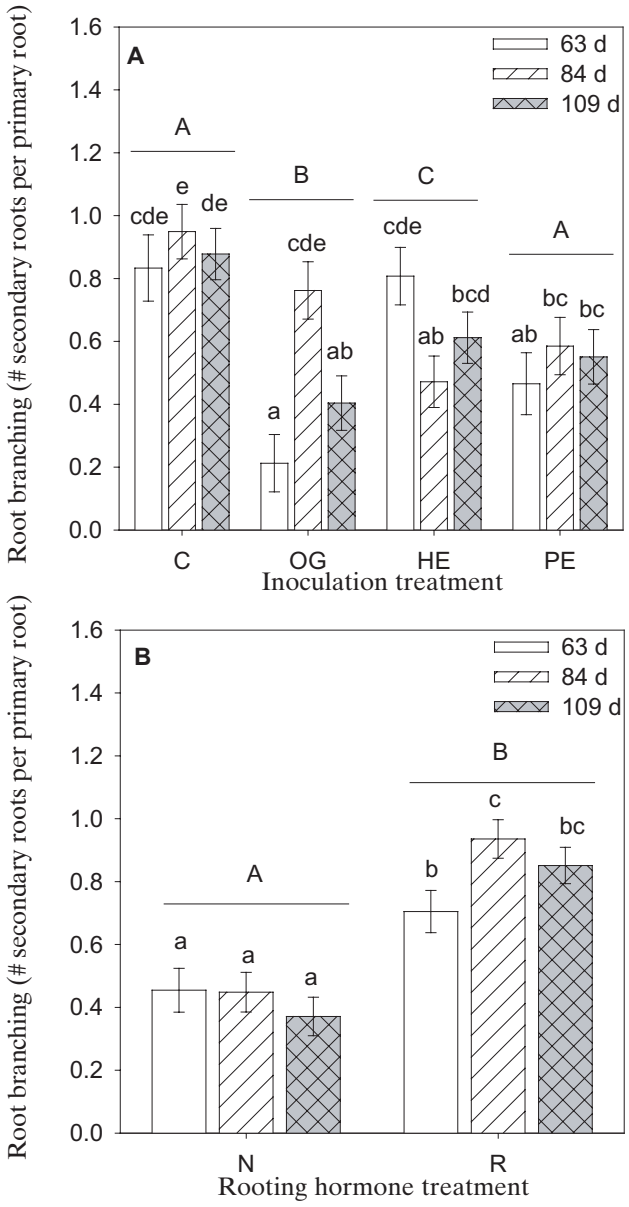

FIG. 4
Root branching on *Lecothoe racemosa* 'Rainbow' cuttings 63, 84, and 109 d after sticking and inoculation with ericoid mycorrhizal fungi (EMF; Panel A); or treatment with rooting hormone (Panel B). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n =20). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$; Bonferroni test). Groups of columns with the same upper case letter above them within a panel denote no significant difference in treatment response over time ($P < 0.05$).

(Figure 3A), but reduced root branching, as indicated by the number of secondary roots located on primary roots (Figure 4A). Rooting hormone, on the other hand, increased the production of primary roots (Figure 3B) and increased root branching (Figure 4B). However, rooting hormone effects on primary root production varied with EMF isolate (Figure 3C). For instance, when no rooting hormone was applied to cuttings, only cuttings inoculated with the HE isolate had more primary roots than non-inoculated cuttings; but, when rooting hormone was applied to cuttings, cuttings inoculated with either

isolate OG or HE had more primary roots than non-inoculated cuttings. The timing of increased primary root production also varied with the EMF isolate used for inoculation. For instance, cuttings inoculated with HE had more primary roots than non-inoculated cuttings at all harvest dates, while cuttings inoculated with isolate PE had more primary roots than non-inoculated cuttings only at the final harvest, 109 d after sticking (Figure 3A).

Cutting root weights and lengths varied with EMF isolate and harvest date; and the response of cuttings to EMF also varied with rooting hormone treatment. Cuttings in pots inoculated with OG or HE had greater total root lengths than non-inoculated cuttings, 109 d after sticking (Figure 5A); while cuttings inoculated with the HE or PE isolate had greater root weights than non-inoculated cuttings 84 and 109 d after sticking (Figure 6A). Cuttings treated with rooting hormone had greater total root lengths than non-hormone treated cuttings, 84 and 109 d after sticking (Figure 5B; Figure 6B). Application of rooting hormone to non-inoculated cuttings and cuttings inoculated with either the OG or the PE isolate increased the total root length on cuttings, while the application of rooting hormone to cuttings inoculated with the HE isolate had no influence on total root length (Figure 5C). Specific root length (SRL; cm mg^{-1} root) was used to assess root morphology. Lower values denote coarser or denser roots compared to higher values which represent thinner or less-dense roots. Differences in root length and weight resulted in a more rapid decrease in SLR in EMF-inoculated cuttings than in non-inoculated cuttings (Figure 7A). Conversely cuttings treated with rooting hormone showed a slower decline in SLR than non-hormone treated cuttings (Figure 7B).

The amount of water per weight of stem (g H_2O g^{-1} stem dry weight) was used to assess stem moisture content. In general, stem moisture contents were similar in cuttings inoculated with isolates of EMF species OG and HE at each harvest date, while stem moisture contents of non-inoculated cuttings and cuttings inoculated with the PE isolate varied between harvest dates (Figure 8A). Stem moisture contents of cuttings that received no rooting hormone were similar at all harvest dates, while the stem moisture contents of cuttings treated with rooting hormone varied between harvest dates (Figure 8B).

Root colonisation was positively correlated with primary root production ($r^2 = 0.748$, $P < 0.05$), total root length ($r^2 = 0.755$, $P < 0.05$) and root dry weight ($r^2 = 0.831$, $P < 0.05$) at 63 d after sticking. Colonisation at 84 and 109 d after sticking was also positively correlated with root dry weight ($r^2 = 0.750$, $P < 0.05$) and negatively correlated with specific root length ($r^2 = 0.664$, $P < 0.05$).

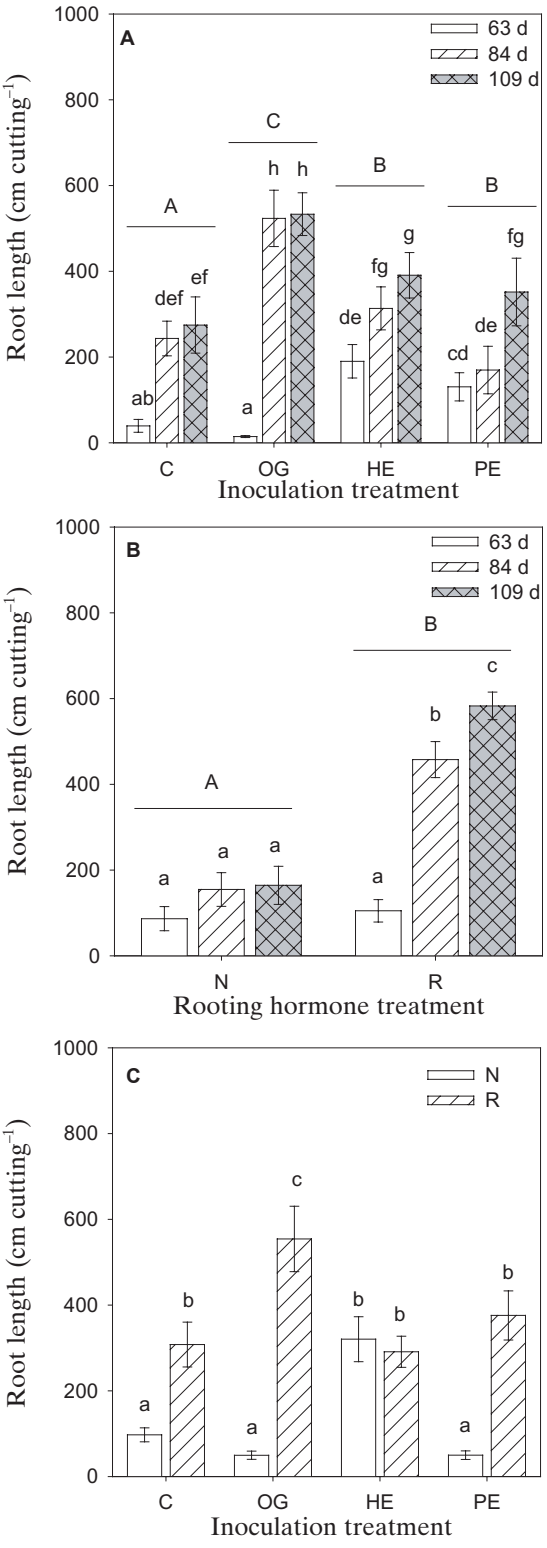

FIG. 5
Total length of primary roots on *Lecothoe racemosa* 'Rainbow' cuttings 63, 84 and 109 d after sticking and inoculation with ericoid mycorrhizal fungi (EMF; Panels A, C); or treatment with rooting hormone (Panels B, C). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = Pezizella ericae. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n = 20; Panel C, n = 15).). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$; Bonferroni test). Groups of columns with the same upper case letter above them within a panel denote no significant difference in treatment response over time ($P < 0.05$).

DISCUSSION

In general, we found that EMF inoculation of the substrate increased early root production on *L. fontanesiana* cuttings. When the propagation cycle for *L. fontansiana* from cuttings was less than 84 d, we found that inoculation with certain EMF species isolates increased the percentage of cuttings with roots. However, in longer propagation cycles, inoculation with EMF may not increase rooting success. Douds *et al.*

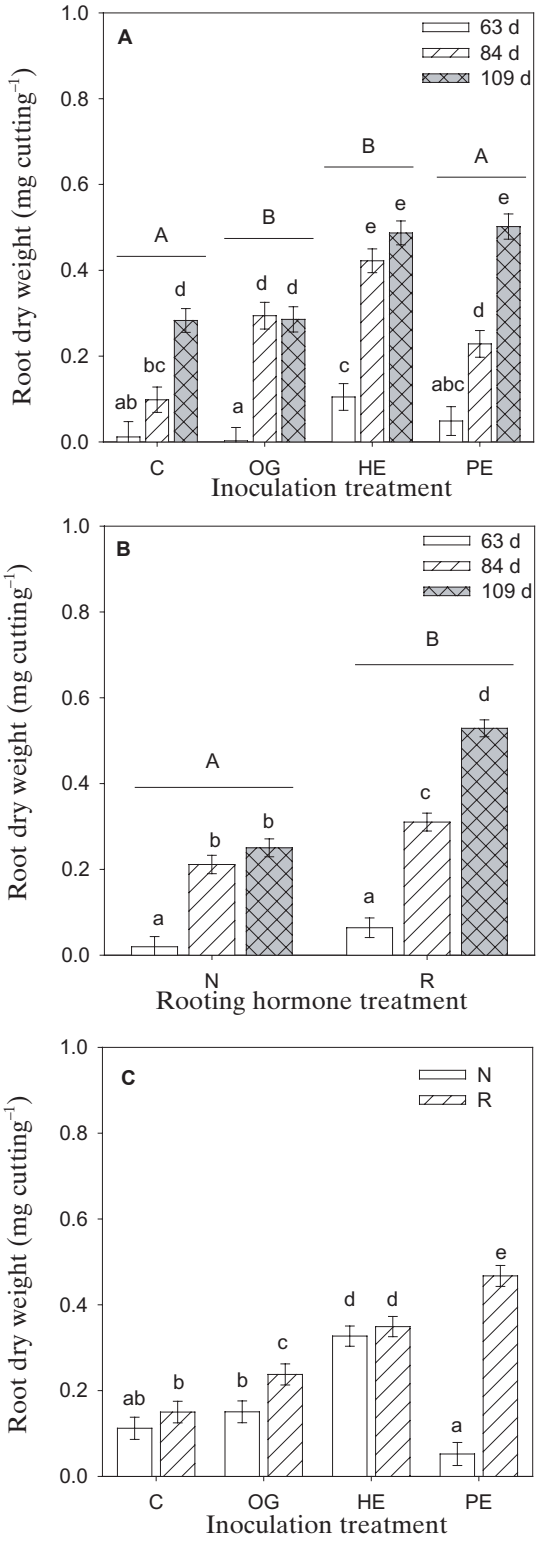

FIG. 6
Total dry weight of roots on *Lecothoe racemosa* 'Rainbow' cuttings 63, 84 and 109 d after sticking and inoculation with ericooid mycorrhizal fungi (EMF; Panels A, C); or treatment with rooting hormone (Panels B, C). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n = 20; Panel C, n = 15).). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$; Bonferroni test). Groups of columns with the same upper case letter above them within a panel denote no significant difference in treatment response over time ($P < 0.05$).

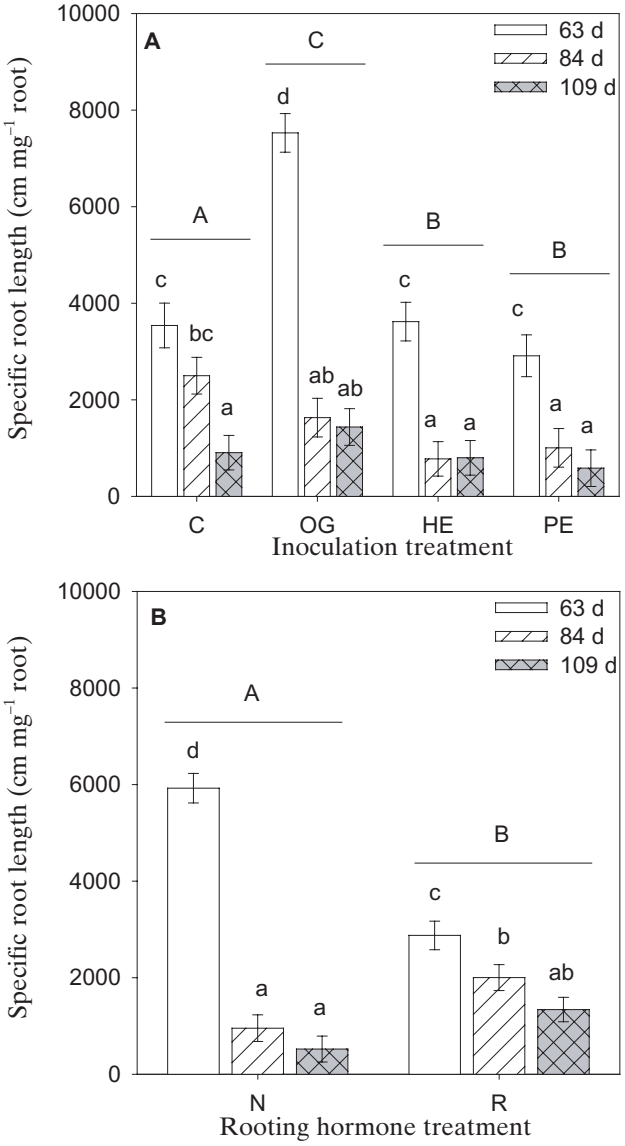

FIG. 7
Specific root length of *Lecothoe racemosa* 'Rainbow' cuttings 63, 84 and 109 d after sticking and inoculation with ericoid mycorrhizal fungi (EMF; Panel A); or treatment with rooting hormone (Panel B). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n = 20). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$; Bonferroni test). Groups of columns with the same upper case letter above them within a panel denote no significant difference in treatment response over time ($P < 0.05$).

(1995) increased rooting of *S. verticillata* cuttings by adding an inoculum of an arbuscular mycorrhizal fungus (AMF) to the rooting substrate. They also reported that AMF increased cutting survival and callus development. Increased rooting was also obtained when a mycorrhizal inoculum was added to the rooting substrate of miniature rose (Scagel, 2001) and kinnikinnick (*Arctostphylos uva-ursi*) cuttings (Scagel, 2004b). The ability of mycorrhizal fungi to increase percentage rooting during propagation of cuttings of easy-to-root species, may be of benefit to growers by decreasing production times for rooting. In cuttings of easy-to-root

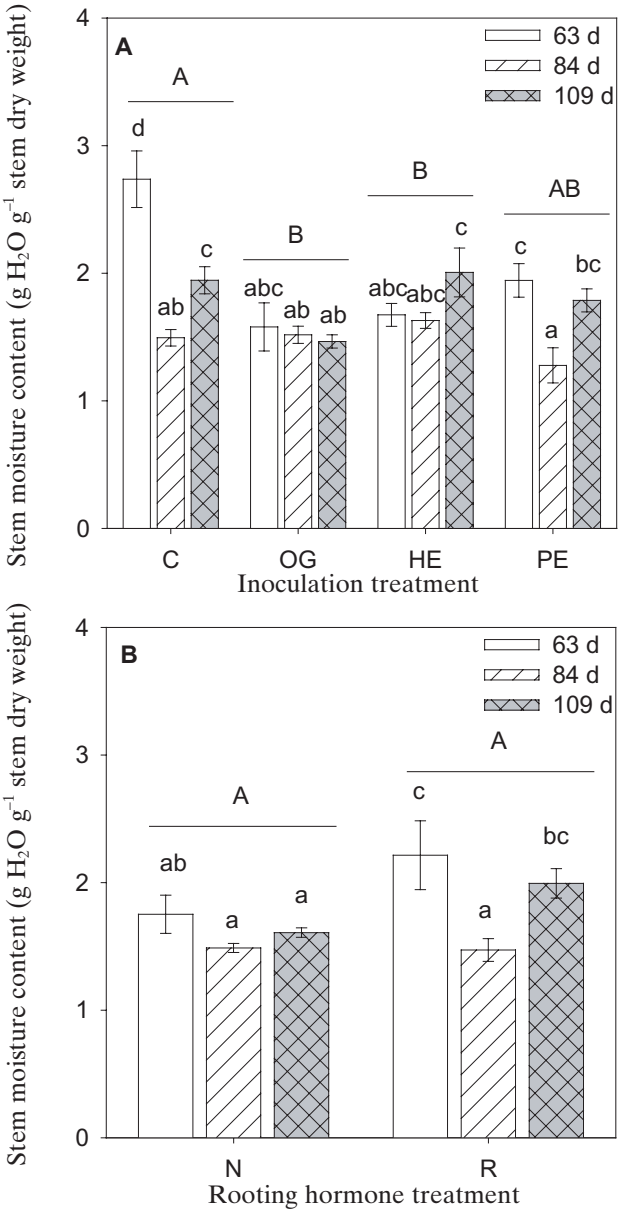

FIG. 8
Stem moisture content *Lecothoe racemosa* 'Rainbow' cuttings 63, 84 and 109 d after sticking and inoculation with ericoid mycorrhizal fungi (EMF; Panel A); or treatment with rooting hormone (Panel B). Inoculation treatments: C = control, no EMF inoculum; OG = inoculated with *Oidiodendron griseum*; HE = *Hymenoscyphus ericae*; or PE = *Pezizella ericae*. Rooting hormone treatments: N = no rooting hormone, and R = root hormone applied to cutting. Bars on data points represent standard errors (Panel A, n = 10; Panel B, n = 20). Columns with the same lower case letter above them are not significantly different from each other ($P < 0.05$; Bonferroni test). Groups of columns with the same upper case letter above them within a panel denote no significant difference in treatment response over time ($P < 0.05$).

plant species, such as *L. fontanesiana*, the percentage of cuttings that produce roots is generally not a limiting factor for production; but the time it takes for cuttings to grow adequate roots for transplanting may increase production time, and utilise valuable space and energy resources.

During vegetative propagation, the number of roots initiated influences the length of the production cycle, and the final quality of the rooted cutting produced. When the propagation cycle for *L. fontansiana* from cuttings was 109 d, we found that inoculation with any of the three EMF species isolates used in our study increased the number of primary roots per cutting. However, only one of the three EMF isolates used in our study (*O. griseum*) consistently increased the number of primary roots on *L. fontanesiana* cuttings regardless of the length of the propagation cycle or any rooting hormone application.

Verkade and Hamilton (1987) found that the presence of AMF in the rooting substrate increased root development and growth of *V. dentatum,* but not root initiation; while Scagel (2004b) found that inoculating kinnikinnick cuttings with an arbutoid mycorrhizal fungus increased root initiation, particularly when cuttings were treated with rooting hormone. With miniature rose, Scagel (2001) found that although adding AMF inoculum to the rooting substrate did not always increase root initiation, in some cultivars a combination of AMF inoculum and rooting hormone did increase root initiation. The influence of mycorrhizal fungi on root initiation during cutting propagation appears to be related not only to specific plant-fungus interactions, but also to interactions with the application of rooting hormone.

We found that application of rooting hormone to cuttings of *L. fontanesiana* increased root branching, while roots on EMF-inoculated cuttings were generally less branched and longer than roots on non-inoculated cuttings. This suggests that rooting hormone application and inoculation with EMF do not have the same effect on second-order root development. Some researchers have reported that cytokinins play a dominant role in root branching (Blonstein *et al*., 1991), while others report that cytokinins do not act as a root-derived signal for the regulation of shoot branching (Schmulling, 2002). Auxins are reported to induce branching in roots (Biondi *et al*., 1997). Ectomycorrhizal fungi have been found to produce auxins and cytokinins and to induce changes in root hormone levels (Niemi *et al*., 2002; 2004); however, little is known about hormone production by ericoid mycorrhizal fungi or the changes they may cause in plant hormone levels (Gay and Debaud, 1986). EMF may produce hormones or induce plants to produce hormones that stimulate root development and growth by different mechanisms to those caused by rooting hormone.

Although we found that EMF did not have a substantial influence on the morphology of individual roots, roots on EMF-inoculated cuttings generally became thicker, or attained greater mass or density more rapidly than roots on non-inoculated cuttings. Comas *et al*. (2002) suggested that plants with higher specific root length have higher maintenance costs due to respiratory losses. During cutting propagation, high respiration rates without adequate replenishment of energy from photosynthesis can decrease cutting quality (Friend *et al*., 1994). Scagel (2001) reported that addition of AMF to the rooting substrate of miniature rose had little influence on root size, 28 d after sticking, compared to cuttings from untreated controls. However, roots on cuttings treated with rooting hormones were smaller (shorter or thinner) than cuttings from other treatments. Scagel (2004a) found that roots on miniature rose, 21 d after sticking, were generally thicker or longer after

treatment with hormones or inoculation with AMF. When AMF inoculum was added to the rooting substrate of Hick's yew cuttings, roots were similar in size to untreated controls 108 d after sticking, but were significantly smaller after 156 d (Scagel *et al*., 2003). The alteration in root size, induced by inoculation of cuttings with mycorrhizal fungi, appears to be time-dependent and may influence water use, nutrient uptake and respiratory losses of the new root system, as well as cutting survival during transplanting.

Moisture retention during adventitious rooting is important for cutting survival (Hartmann *et al*., 1997). We found that when cuttings of *L. fontanesiana* were inoculated with EMF, stem moisture contents were lower 63 d after sticking than in non-inoculated cuttings. By the end of the experiment, however, the stem moisture contents of inoculated and non-inoculated cuttings were similar. Hormone and AMF treatments resulted in increased moisture retention by miniature rose roots (Scagel, 2004a), and AMF-inoculated cuttings of Hick's yew (*Taxus x media* 'Hicksii') have also been reported to have a higher moisture content than non-inoculated cuttings (Scagel *et al*., 2003). The lower moisture content observed in inoculated cuttings of *L. fontanesiana* in the current experiments may be due to decreased callus induction (data not shown). Mycorrhizal fungi may alter root or stem exudation patterns, allowing increased metabolite flow from roots or stems during the first stages of fungus-plant interaction (Larose *et al*., 2002; Buee *et al*., 2000). As rooting hormone did not cause similar moisture losses 63 d after sticking, this response in EMF-inoculated cuttings may not be related to hormonally-mediated mechanisms of root formation.

In soilless substrates that lack indigenous mycorrhizal fungi, mycorrhizal inoculation and colonisation have been found to increase crop uniformity, reduce transplant mortality and increase productivity in several plant species (Biermann and Linderman, 1983; Chavez and Cerrato, 1990; Powell and Bagyaraj, 1984; Vosátka *et al*., 1999). When cuttings of *L. fontanesiana* were inoculated with different species isolates of EMF, the intensity of root colonisation varied with isolate and time after sticking, and was correlated with root initiation, root growth and root morphology. When cuttings of kinnikinnick were inoculated with an arbutoid mycorrhizal fungus, root colonisation was correlated with root initiation and root length (Scagel, 2004b). Scagel (2001) reported that increases in root initiation and root growth of miniature rose cuttings rooted in substrate containing the AMF, *Glomus intraradices*, were not always associated with increased levels of colonisation. Verkade *et al*. (1988) found that increased colonisation of *C. sericea* cuttings by *G. fasciculatum*, substantially increased plant growth during the later stages of root development on cuttings. In our experiments, although EMF inoculum did not consistently increase rooting, it is possible that root colonisation could result in higher quality cuttings that are better able to withstand the stress of transplanting and increase growth during later stages of plant development.

The degree and type of response of cuttings to inoculation with mycorrhizal fungi appears to vary with plant species and cultivar. Cultivar-specific responses to mycorrhizal fungi have been documented for several factors, including nutrition and fungal-plant recognition (Clark and Zeto, 2000; Graham and Eissenstat, 1994). Scagel (2001; 2004a) reported cultivar-specific responses to AMF during the propagation of miniature rose cuttings. Plant responses to mycorrhizal fungi during propagation of cuttings could result from genotype-specific interactions between the fungus and traits specific to each plant genotype which influence responses to factors such as environment, nutrition, or hormonal requirements for optimal rooting. Our current study suggests that cutting responses may also be a function of the isolate of the mycorrhizal fungus used for inoculation. Moreover, specific application rates of mycorrhizal inoculum may be required for optimal rooting responses as described for Hick's yew (Scagel *et al*., 2003).

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