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Enhanced Rooting of Woody Plant Cuttings by Mycorrhizal Fungi[1]

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Abstract. When inoculum of ectomycorrhizal fungi was added to the rooting medium, the percentage of rooted cuttings and the root volumes on cuttings of bearberry (*Arctostaphylos uva-ursi* L. Spreng.) and huckleberry (*Vaccinium ovatum* Parsh) were significantly greater than those of the uninoculated controls. This enhanced rooting occurred before or in the absence of any mycorrhizal association. In some tests, inoculum of one fungus enhanced rooting of one cultivar of bearberry, but not another, suggesting a specific interaction between the cultivar and the fungus. Of the 13 fungi tested, only *Thelephora terrestris* Ehrh. ex Fr. formed ectendomycorrhizae in the propagation bed, although several others did so under other conditions.

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Mycorrhizal fungi are known to enhance the growth and development of their host plants. The evidence is strong that the fungi increase nutrient uptake in the host plant and produce growth promoting or regulatory compounds, such as auxins, cytokinins, gibberellins, and/or growth-regulating B vitamins (9). Evidence that the fungi actually release these materials in the host root comes indirectly from anatomical and physiological changes in mycorrhizae that can be induced or mimicked by pure preparations of these compounds and/or by culture filtrates from the fungi. Because these fungi do exude such compounds into the culture growth medium, they could, therefore, be capable of releasing the same materials into the root zone before penetration. Several workers, in fact, have reported stimulation of shoot and root growth on seedlings by these fungi before, or in the absence of, any mycorrhizal association (3, 8).

We hypothesized that ectomycorrhizal fungi might produce growth-promoting substances that could enhance the rooting of cuttings of vegetatively-propagated woody ornamental plants. Of particular interest were some native and cultivated species known to be difficult to propagate by normal rooting procedures, namely bearberry [*Arctostaphylos uva-ursi*], and huckleberry (*Vaccinium ovatum*). The purpose of our study, therefore, was to determine whether addition of ectomycorrhizal inoculum to the rooting medium would influence rooting of cuttings from these plants.

Materials and Methods

The fungi used in these studies supplied by Dr. B. Zak (11), had been isolated either directly from sporophores or rhizomorphs, or from mycorrhizae of Douglas fir or *Pinus* sp.: *Pisolithus tinctorius* (Pers.) Coker & Couch; *Thelephora terrestris; Corticium bicolor* Peck; *Laccaria laccata* (Scop. ex Fr.) Cke.; *Rhizopogon vinicolor* A. H. Smith; *Cenococcum graniforme* (Sow.) Ferd. & Winge; *Lactarius sanguifluus* Fr.; *Lactarius deliciosus* (L. ex Fr.), S. F. Gray; *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quél: *Amanita muscaria* (L. ex Fr.) Hooker; *Tricholoma flavovirens* (Pers. ex Fr.) Lund.; *Poria terrestris* DC. ex Fries, var. *cyaneus* and var. *subluteus.* The fungi were grown in vermiculite-peat flask cultures according to the method of Marx and Zak (5). After 2 months, the fungi had grown throughout the medium, and the fungal inoculum was washed out of the flasks onto cheese cloth, rinsed thoroughly, and added in various proportions to the rooting medium, usually in a 1:5 ratio of inoculum to rooting medium. The latter was a 1:peat moss:perlite mix. Control mixtures were composed of washed, non-inoculated flask medium and rooting medium. The rooting mixtures were added to fiber flats placed under mist. The bottom heat pads were at 24°C, and the bench was lighted for 16 hr daily at about 12 klx by sodium vapor lamps.

Terminal cuttings of test plant species were harvested from plants grown outside, or in one case, in the greenhouse. In the case of *A. uva-ursi,* several cultivars were tested: Pt. Reyes, Native, Alaska, Massachusetts, and Oregon Hybrid. *Vaccinium ovatum* cuttings were collected from native plants in the Coast Redwood forest area of Northern California. In our first experiment, we used 10 cuttings per treatment, and all treatments (including non-inoculated controls) were dipped in commercial rooting hormone (Jiffy Grow, G&W Products, Estacada, Oregon).[3] That experiment was terminated after 2 months. In the second experiment, we used 20 cuttings per treatment with bearberry and 10 cuttings per treatment with huckleberry; commercial hormone was used only on the non-inoculated controls, and the experiment was harvested after 4 months.

At the time experiments were harvested, cuttings were carefully removed from the medium and rated for rooting percentage and root ball size. Root ratings, based on the approximate diameter of the root ball with adhering medium, were as follows: 0 = none; 1 = root ball to 2.5 cm; 1.5 = root ball from 2.5–4 cm; 2 = root ball 4–5 cm; 2.5 = root ball 5–7 cm; 3 = root ball 7 cm or greater.

Roots were cleared in 10% KOH, stained with trypan blue according to the method of Phillips and Hayman (7) as modified by Ames and Linderman (1), and examined for mycorrhizal formation.

Results and Discussion

In these studies, we observed that addition of mycorrhizal fungi to the rooting medium usually enhanced the rooting of cuttings by increasing both percentage rooting and root ball size (Fig. 1, and Tables 1 and 2). With certain combinations, the response was very striking. In addition, we observed earlier vegetative bud break and growth and less discoloration and spotting of leaves than on control plants.

When roots of rooted cuttings were examined, we noted that more laterals were formed in the presence of mycorrhizal fungi. However, when the roots were cleared and stained, we observed no infections except in roots inoculated with *Thelephora terrestris.* In other experiments to be reported elsewhere, ectendomycorrhizae did form with some of the other fungi tested here.

Certain fungi enhanced the rooting of one cultivar of bearberry, but did not enhance rooting in another. For example, most of the fungi tested enhanced both percentage rooting and root ball size with both 'Native' and 'Oregon Hybrid', but

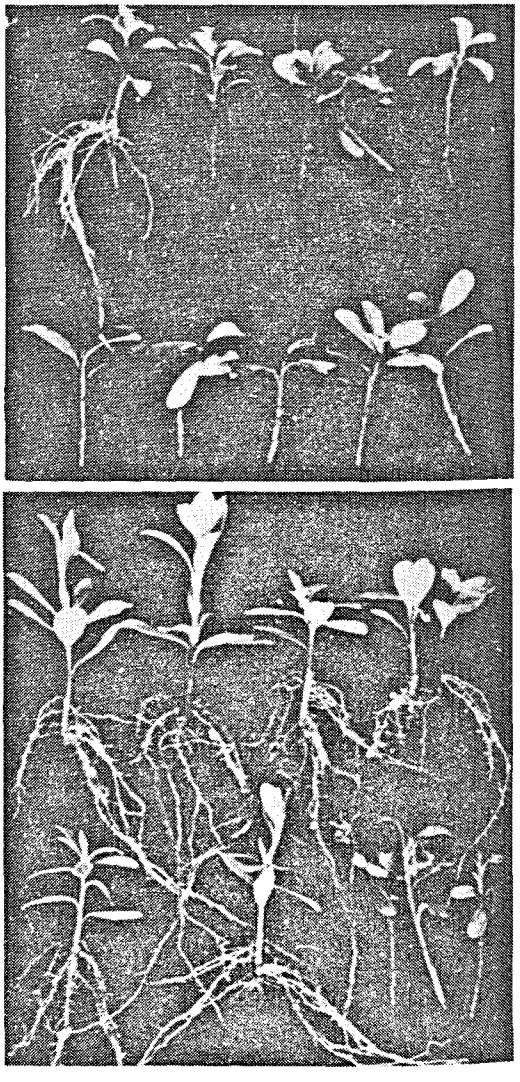

Fig. 1. Rooting response of bearberry (*Arctostaphylos uva-ursi* 'Native') to the presence (lower) or absence (upper) of inoculum of the mycorrhizae fungus, *Thelephora terrestris,* in the rooting medium.

Table 1. Rooting response of cuttings of 4 cultivars of *Arctostaphylos uva-ursi* to the presence of mycorrhizal fungus inoculum in the rooting medium. All treatments including the non-inoculated control were treated with hormone.

Mycorrhizal fungus	Rooting response[z]							
	Massachusetts		Pt. Reyes		Native		Alaska	
	% rooting	Avg root ball rating[y]	% rooting	Avg root ball rating[y]	% rooting	Avg root ball rating[y]	% rooting	Avg root ball rating[y]
Control	40	0.35	40	0.50	10	0.15	20	0.15
Thelephora terrestris	60	1.00	60	1.00	70	1.20**	40	0.40
Pisolithus tinctorius	40	0.50	70	1.20	90	1.20**	80	1.00**
Cenococcum graniforme	70	1.10*	60	1.10	40	0.60	70	0.80**
Laccaria laccata	70	0.80	90	1.50*	50	0.85*	20	0.15
Rhizopogon vinicolor	30	0.40	30	0.50	70	0.80	60	0.60*
Corticium bicolor	70	1.20*	80	1.40*	60	0.80	50	0.50
LSD 5%		0.68		0.		0.68		0.42
LSD 1%		0.90		1.01		0.90		0.56

[z]Root response based on 10 cuttings per treatment inserted Dec. 6, 1974, rated Feb. 13, 1975; all cuttings treated with 1:10 dilution of Jiffy Grow rooting hormone.

[y]Root ball size ratings: 0 = no roots; 1 = 2.5 cm root ball; 1.5 = 2.5–4 cm root ball; 2 = 4–5 cm root ball; 2.5 = 5–7 cm root ball; and 3 = 7 cm or greater root ball. Ratings were based on an estimate of the root ball including the adhering rooting medium.

*,** Statistical significance at 5% (*) and 1% (**) levels (analysis of variance).

Rhizopogon vinicolor did not enhance rooting with 'Massachusetts' and 'Pt. Reyes' (Table 1 and 2). Further, fungi that enhanced rooting in 'Oregon Hybrid' bearberry didn't always do so with huckleberry.

Commercial propagators of bearberry cuttings have indicated to us that rooting is optimum only if cuttings are taken during Sept.–Oct. or Feb.–March. When our experiments were begun in Dec. (non-optimum), we observed the most striking enhancement of rooting. When we stuck cuttings in mid-March, enhancement of rooting by mycorrhizal inoculum was much less striking because most of the cuttings, even the controls (with hormone), rooted. However, the root ball size on cuttings rooted in mycorrhizal inoculum was noticeably greater than on the controls.

To the best of our knowledge, this is the first report of enhanced rooting of cuttings of woody plant species by ecto- or ectendomycorrhizal fungi, although F. Hendrix (personal communication) has shown that the size of root balls increased

Table 2. Rooting response of cuttings of *Arctostaphylos uva-ursi* 'Oregon Hybrid' and *Vaccinium ovatum* in the presence of mycorrhizal fungus inoculum in the rooting medium. Cuttings in treatments with mycorrhizal fungi were not treated with hormone.

Mycorrhizal fungus	Rooting response[z]			
	Arctostaphylos (Oregon Hybrid)		Vaccinium	
	% rooting	Avg. root ball ratings	% rooting	Avg. root ball ratings[z]
Control with hormone	15	0.15	30	0.40
Pisolithus tinctorius	75	1.10**	30	0.40
Thelephora terrestris	75	1.18**	60	0.95**
Corticium bicolor	45	1.03**	10	0.10
Laccaria laccata	70	0.73**	50	0.50
Rhizopogon vinicolor	65	0.85**	50	0.85
Cenococcum graniforme	90	1.00**	80	1.05*
Lactarius sanguifluus	65	0.68*	30	0.35
Lactarius deliciosus	70	0.88**	60	0.75
Hebeloma crustuliniforme	70	1.40**	80	1.30**
Amanita muscaria	55	0.48	80	1.45**
Tricholoma flavovirens	80	0.98**	90	1.45**
Poria terrestris var. *cyaneus*	55	0.55	70	1.40**
Poria terrestris var. *subluteus*	95	1.00**	40	0.65
LSD 5%		0.43		0.53
LSD 1%		0.57		0.75

[z]Rooting response based on 20 cuttings per treatment of 'Oregon Hybrid', and 10 cuttings per treatment of *Vaccinium;* cuttings inserted Dec. 18, 1975 and rated April 9, 1975; Jiffy Grow rooting hormone used only on non-inoculated control cuttings.

[y]Root ball size ratings: 0 = no roots; 1 = 2.5 cm root ball; 1.5 = 2.5–4 cm root ball; 2 = 4–5 cm root ball; 2.5 = 5–7 cm root ball; and 3 = 7 cm or greater root ball. Ratings were based on an estimate of the root ball including the adhering rooting medium.

*,** Statistical significance at 5% (*) and 1% (**) levels (analysis of variance).

on woody cuttings rooted in a medium to which spores of vesicular arbuscular mycorrhizal fungi were added. This enhancement apparently followed infection of initial roots. Shemakhanova (8) reported enhancement of root formation on bean hypocotyl cuttings treated with mycorrhizal fungus culture filtrate. She also enhanced seed germination and root and shoot growth on pine and oak seedlings by adding culture filtrates to the medium. This enhanced growth in the presence of mycorrhizal fungi (or culture filtrate) in the absence of a mycorrhizal association (i.e., actual root infection) was also demonstrated by Levisohn (3). Levisohn further showed that mycelium of ectomycorrhizal fungi could enhance growth of endomycorrhizal tree species where no infection would occur. Our experiments showed root enhancement before infection by most of the test fungi. Zak (11) synthesized mycorrhizae on bearberry with some of the same isolates we used, but he was unsuccessful with the isolates of *Cenococcum graniforme* and *Corticium bicolor.* We obtained rooting enhancement with these two fungi even though no mycorrhizae formed. However, *C. graniforme* was observed by Zak to form mycorrhizae on bearberry in nature (11).

Several mechanisms could be operating in the reported rooting response. Some mycorrhizal fungi produce growth substances like auxins, cytokinins, gibberellins (9), or vitamins (8) *in vitro,* but that others do not (8). Comparing the fungi we used with the fungi reported to produce certain of these substances, we find no correlation. That approach may be illogical anyway, since genera, species, varieties, and even individual isolates of these fungi can vary considerably in how fast, how much, and what kinds of growth substances are produced (6).

At present we are trying to determine if the effect on rooting can be attributed to some substance(s) released into the culture medium. At the same time we are aware that substances produced by the fungi in our tests would be produced slowly and released into the rooting medium rather than into a liquid culture medium. In addition, the rooting medium is not sterile and is repeatedly leached by the mist system.

We are also considering other possible mechanisms for the rooting phenomenon. For example, these mycorrhizal fungi may be producing substances which may act protectively or synergistically with the growth substances already in the cuttings (10). It is also possible that these fungi may be altering the nutrient status of the medium, as suggested by Levisohn (3), or they may be releasing substances that are inhibitory to other microbes that cause cuttings to deteriorate. Since such a range of fungi used in our tests enhanced rooting, it would appear that some mechanism common to them all is involved.

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