AN ABSTRACT OF THE THESIS OF

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Title: ENHANCED ROOTING OF <u>ARCTOSTAPHYLOS UVA-URSI</u> 'OREGON HYBRID' WITH
ECTOMYCORRHIZAL FUNGI

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                        A. N. Roberts

The effect of 13 ectomycorrhizal fungi on the enhancement of root-
ing of stem cuttings of bearberry (<u>Arctostaphylos uva-ursi</u> 'Oregon
Hybrid') was evaluated in preliminary studies. <u>Pisolithus tinctorius</u>,
<u>Thelephora terrestris</u> and <u>Rhizopogon vinicolor</u> were selected for fur-
ther study on cuttings taken at different stages of development during
the year. Cuttings were taken for treatment on April 21, July 15,
October 19, and December 12, 1976, based on traditional optimum rooting
periods (September-October, March-April) and a non-optimum period
(December-January). Percent rooting and root volume were used to eval-
uate fungal enhancement of rooting. Inoculation with mycorrhizal fungi
was most beneficial during the non-optimum period for rooting, however,
during the optimum period, September-October, no enhancement occurred.
With the exception of <u>T</u>. <u>terrestris</u>, enhanced rooting occurred in the
absence of any mycorrhizal association, i.e. actual infection. Cell-
free culture filtrates of <u>P</u>. <u>tinctorius</u> stimulated rooting, but not as
effectively as did the <u>P</u>. <u>tinctorius</u> mycelium inoculum. Non-mycorrhi-
zal fungi, <u>Fusarium oxysporum</u> and <u>Sclerotium rolfsii</u>, stimulated rooting
during the optimum rooting period, September-October, when mycorrhizal

fungi did not. Root systems formed in the presence of mycorrhizal in-
ocula had greater numbers of short laterals than those on cuttings in
non-mycorrhizal inocula or non-inoculated media. Only T. terrestris
formed ectendomycorrhizae in the propagation bench. However, P. tinc-
torius and R. vinicolor formed mycorrhizae after transplanting rooted
cuttings including attached rooting medium. Possible mechanisms for
enhancement of rooting by mycorrhizal fungi during non-optimum periods
include the production and excretion of growth substances, i.e. auxins,
auxin synergists, cytokinins, gibberellins and B-vitamins, into the
rooting medium that interact with endogenous growth substances in the
cutting to activate metabolic processes required for root initiation.

Enhanced Rooting of <u>Arctostaphylos</u> <u>uva-ursi</u>
'Oregon Hybrid' with Ectomycorrhizal Fungi

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Christopher Alan Call

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_____
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in charge of major

_____
Head of Department of Horticulture

_____
Dean of Graduate School

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ENHANCED ROOTING OF ARCTOSTAPHYLOS UVA-URSI 'OREGON HYBRID' WITH ECTOMYCORRHIZAL FUNGI

I. INTRODUCTION

Bearberry (Arctostaphylos uva-ursi (L.) Spreng.) is considered one of the best ground-cover ornamentals for covering sandy banks, rocky slopes and dry exposed areas in the Pacific Northwest (21,59). It also has a potential use for erosion control and revegetation of disturbed areas (3). Bearberry and other plants native to the region require less care than non-natives, because they have evolved and adapted to the native climate and soil (60). However, difficulties encountered in commercial propagation and establishment of bearberry have limited its wider use in landscaping and revegetation.

Large, uniform bearberry plants can be obtained more readily from cuttings than from seeds. Seeds of Arctostaphylos have a double dormancy, hard seed coats and dormant embryos, which must be overcome by scarification and warm-cold stratification (3). Seed treatments are time consuming, and germination may only reach 50 percent (3). Bearberry also shows a high degree of hybridization in some localities, so it is desireable to propagate it from cuttings to maintain genetic uniformity (3).

Limitations are also encountered in the vegetative propagation of A. uva-ursi. Bearberry cuttings are difficult to root during most of the year. Commercial propagators suggest that bearberry has two optimum periods for root initiation, September 15 to October 15, and March 1 to April 1 (21). Following this practice, cuttings are stuck in the

propagation bench from September 15 to October 15, and rooted cuttings are transplanted from March 1 to April 1.

When properly transplanted from cuttings beds to containers at the optimum time (March 1 to April), propagators have attained 85 percent survival. However, losses of 50 percent or greater can result from improper timing (21).

Considering the above problems, it was hypothesized that ectomycorrhizal fungi might enhance root regeneration and transplant survival of bearberry cuttings by:

1) Producing and excreting growth regulators into the rooting medium in the absence of any association between vegetative mycelium and the host plant (cutting).

2) Producing and excreting growth regulators into liquid culture medium; cell-free extracts of which may be as effective as vegetative mycelial inoculum in promoting rooting.

3) Overcoming certain physiological conditions within the stock plants at certain stages of development during the year and thereby predisposing the cuttings to root.

II. REVIEW OF LITERATURE

Classification of Mycorrhizae

Mycorrhizae are symbiotic associations between non-pathogenic fungi and the roots of higher plants (18). The gross morphology of various mycorrhizae are sufficiently different for them to be grouped into three classes: the endomycorrhizae, ectomycorrhizae, and ectendomycorrhizae (32). Plants with mycorrhizae have: 1) a large physiologically active root-fungus surface area for nutrient and water absorption (38); 2) an increased nutrient uptake from soil low in one or several nutrients (5,9); 3) the ability to absorb and accumulate nitrogen, phosphorus, potassium, and calcium rapidly and for long periods of time (38); 4) the ability to degrade certain complex minerals and organic substances in soil thereby increasing the availability of these to the plant (38); 5) an increased tolerance to drought, high soil temperatures, soil toxins (organic and inorganic), and extremes of soil pH (15,38,61); 6) an increased resistance to root pathogens (27,35,36,40, 73).

Endomycorrhizae

Endomycorrhizal fungi form a loose network of hyphae on feeder root surfaces, and do not develop the dense fungal mantle found on ectomycorrhizae (37,38). Most often, these fungi form large, conspicuous, thick-walled spores on the root surfaces, in the rhizosphere, and sometimes in feeder root tissue (37,38). The fungi colonize epidermal and cortical cells but never invade the endodermis, stele or root meristem (13).

Soon after infection the fungi form specialized haustoria (absorbing or nutrient-exchanging structures) called arbuscles within cortical cells (13,37,38). Thin-walled, spherical to ovate vessicles may also be produced in the cortex by the infecting hyphae (13,37,38). The term vesicular-arbuscular (VA) mycorrhizae is used to describe this type of endomycorrhiza. Most of the endomycorrhizal fungi are Phycomycetes (38).

Endomycorrhizae are found in Bryophytes, Pteridophytes, Gymnosperms and Angiosperms (13). They occur on species in most families with the following exceptions: 1) families that are ectomycorrhizal, primarily Pinaceae, Betulaceae, and Fagaceae; 2) families that are endomycorrhizal with separate endophytes, primarily Orchidaceae, and Ericaceae; 3) certain plant families that are non-mycorrhizal, primarily Crucifereae, Fumariaceae, Cyperaceae, Commelinaceae, Utricaceae, and Polygonaceae (12,13). There are also some plant groups in which both endomycorrhizae and ectomycorrhizae occur; Salicaceae, Juglandaceae, Tiliaceae, Myrtaceae, Fagaceae, Juniperus, and Chamaecyparis (13).

Ectomycorrhizae

Approximately three percent of higher plants form ectomycorrhizal associations. Ectomycorrhizal fungi form a mantle of hyphae covering the slow growing, unsuberized parts of the roots, and it penetrates, intercellulary, to varying depths, into the host cortex (32). The mycelium between the cortical cells forms a network known as the Hartig net, which never penetrates the endodermal cells and the stele (40).

Most ectomycorrhizal fungi are Basidiomycetes, which produce mushrooms
and puffballs (40). Ectomycorrhizae are most common in the families
Pinaceae, Salicaceae, Betulaceae, and Fagaceae; in other families, they
exist in only a few genera (4,18,32,38,39).

Ectendomycorrhizae

Ectendomycorrhizae exhibit a fungal mantle which in some cases is
well developed and closely resembles typical ectomycorrhizae of forest
trees (72,74). A Hartig net of thick hyphae surrounds the cortical
cells, and coarse intracellular hyphae occupy the cortical cells (72).
The intercellular hyphae grow toward the root tip in advance of the in-
tracellular hyphae, and both tend to disappear in the basal portion of
older roots when the cortex senesces (18,72). The identity of many ect-
endomycorrhizal fungal symbionts remains largely unknown. However, most
ectendomycorrhizal fungi are Basidiomycetes and are very likely the same
species that form ectomycorrhizae of forest trees (74). Mikola (41)
has suggested that the same fungal species may produce ectendomycorrhi-
zae one time and ectomycorrhizae another. Meyer (40) states that under
certain circumstances (aging ectomycorrhizae, unfavorable conditions
for the host plant, and/or diminishing amounts of available glucose in
host roots), hyphae of the ectomycorrhizal Hartig net will penetrate
into the cortical cells forming an ectendomycorrhizal association.
Wilcox (72) states that ectomycorrhizae are caused by a variety of fung-
al species rather than by a single species as in the ectendomycorrhizal
type. As a result, ectomycorrhizae show a much greater variation in
anatomical features than ectendomycorrhizae. Ectendomycorrhizal fungi

are found mainly in the Ericaceae (49).

Ectendomycorrhizae of A. uva-ursi are "arbutoid"; they possess a well developed mantle and closely resemble typical ectomycorrhizae of associated forest trees in the Pacific Northwest (49,74). Both ectendomycorrhizal and ectomycorrhizal associations are formed by the fungi used in this study, and the physiology and ecology of both types are similar.

Growth Regulators Produced and Released
by Ectomycorrhizal Fungi

Growth regulators produced and released by ectomycorrhizal fungi may affect the metabolism, growth and development of the host plant (54). There is no direct evidence that ectomycorrhizal fungi release growth regulators in vivo. However, indirect evidence supports the hypothesis: ectomycorrhizal fungi are known to produce auxins, auxin synergists, cytokinins, gibberellins and/or B-vitamins in vitro (54,65); and anatomical and physiological changes in roots infected with mycorrhizal fungi can be mimicked by applications of pure preparations of synthetic growth regulators and/or culture filtrates from the fungi (54).

Auxins

Naturally occurring auxin, indole-3-acetic acid (IAA), and synthetic auxins, indolebutyric acid (IBA) and naphthaleneacetic acid (NAA), affect the rooting of woody stem cuttings in the following manner: 1) enhancement of root primordium initiation and cell division by stimulating

messenger RNA synthesis through the derepression of genes which could
lead to the de novo synthesis of specific enzymes (peroxidase, cyto-
chrome oxidase, succinate dehydrogenase, glucose-6-phosphate dehydrog-
enase, glyceraldehyde-3-phosphate dehydrogenase, and starch hydrolyz-
ing enzymes) and their increase at the base of the cutting or in root
primordia (17); 2) enhancement of the hydrolysis of starch into sugars
and stimulation of the translocation of sugars to the basal end of the
cutting where they supply energy and the necessary carbon skeletons for
root primordia (17); 3) enhancement of the amount and rate of redistri-
bution of amide and amino nitrogen compounds in the cutting (22,58);
and 4) promotion of cell wall extensibility and subsequent cell elonga-
tion via non-enzymatic reactions, or via the induction of hydrogen ion
secretion and cell wall acidification which enhance the activity of
cell-wall looseneng enzymes (23).

IAA is apparently produced by some ectomycorrhizal fungi. Thimann
(62), in 1935, reported a growth hormone produced by Rhizopus suinus.
MacDougal and Dufrenoy (31), in 1944, discovered abundant auxin in the
fungal hyphae and, particularly, in the fungus mantle. Auxin was also
present in the hyphae intermeshed with cortical cells and in the peri-
cycle of the roots. Moser (45) tested 23 species of ectomycorrhizal
Basidiomycetes, isolated from mycorrhizae of Pinus, Larix, Picea,
Betula, and Fagus spp., for auxin production. As a precursor for auxin
production he added tryptophan to the nutrient solution. The majority
of the fungi tested produced IAA. Some mycorrhizal fungi are able to
synthesize IAA and other indole compounds in tryptophan-deficient media
(45,70). Ulrich (70) has also shown that mycorrhizae-forming fungi of

Boletus and Amanita spp. produce extracellular auxins in pure culture. Slankis (52,53) induced ectomycorrhizae-like root structures in excised and attached root systems of Pinus sylvestris by adding synthetic auxins such as IAA, indolepropionic acid (IPA), IBA, and NAA to the nutrient solution. The roots exhibited a radial elongation of outer cells, reduced growth in length along with an increase in diameter of the roots, and the absence of root hairs on the swollen root parts (40).

The amounts of auxins produced, their composition, and time necessary for their production in detectable amounts vary between different species and even between strains (54). The differences in auxin production between symbiotic and saprophytic fungi are more of a quantitative nature, and it seems that this capability increases in the course of symbiotic life (40). Moser (45) has found that auxin production by ectomycorrhizal fungi is greatly influenced by the source of nitrogen and its concentration. Nitrogen compounds, such as alanine, asparagine, aspartic acid, glycine, glutamine, and inorganic ammonium salts, which can by more readily assimilated than tryptophan will inhibit auxin production (54).

Auxin Synergists

Auxin synergists (polyphenols) have been postulated to enhance predisposition and primordium initiation during adventitious root formation of cuttings by: 1) acting as auxin protectors which inhibit the activity of IAA oxidizing enzymes (11,16,50); 2) stimulating auxin synthesis (14,16); 3) freeing IAA to act at sites that only IAA can satisfy

in non-auxin treated cuttings (16); and 4) forming auxin-phenolic con-
jugates, synthesized by the action of IAA oxidase, polyphenol oxidase
(PPO), and possibly other enzymes (16,24). The lack of root primord-
ium initiation in response to applied or endogenous auxin may result
from: 1) lack of necessary enzymes to synthesize the root-inducing
auxin-phenol conjugates; 2) lack of enzyme activators; 3) presence of
enzyme inhibitors; 4) lack of substrate phenolics; or 5) physical sep-
aration of enzymes and reactants on account of cellular compartment-
alization (16).

IAA biosynthesis has been positively correlated with the production
of polyphenolic pigments, e.g. boviquinones and variegatic acid, in
mycelial cultures of mycorrhiza-forming fungi (65). The polyphenolic
pigments proved to act as auxin protectors both in mycelial cultures
and on pine root surfaces (65), by inhibiting the activity of IAA ox-
idizing enzymes (64,65). Both the fungus and the root may form auxin
protectors, but the fungus does so at relatively low nitrogen supply
(65).

It appears that polyphenols might maintain the high auxin level
required for mycorrhizae formation. Auxin is known to induce hydrolase
activity which affects cell wall components and carbohydrate reserves,
and since mycorrhizal fungi are devoid of hydrolytic enzymes (cell-
ulases, gluconases and pectinases) they may induce the host's hydrolases
through auxin excretion (38,40,65).

Cytokinins

Generally, natural and synthetic cytokinins such as zeatin, kin-
etin, and 6-benzyl adenine have not stimulated or prevented root initia-
tion in stem cuttings (19). The influence of cytokinins in root initia-
tion may depend upon the particular stage of initiation and the concen-
tration (19). Cytokinin actions are linked to protein and RNA synthesis,
carbohydrate mobilization, and cell wall extension (15). Cytokinins
also influence synthesis of phenolic compounds and related pigments,
apparently protect against certain deleterious effects of both high and
low temperatures and of wilting, and protect against invading organisms
(44).

The production and release of cytokinins by fungi and bacteria which
associate with higher plants is common (54). Miller (7,43,44) demon-
strated that mycorrhizal fungi also produced and liberated cytokinins.
Using cytokinin-dependent soybean tissue cultures he obtained growth
stimulation when callus tissue pieces were placed on agar away from the
mycelial inoculum of Rhizopogon roseolus and R. ochraceorugens (7,44).
From 200 liters of fungus culture solution, Miller (43) isolated the
cytokinins zeatin and zeatin riboside. However, not all ectomycorrhizal
fungi produce and liberate cytokinins, i.e. Cenococcum graniforme,
Thelephora terrestris, and Hebeloma crustuliniforme which were used in
this study (7,44).

Gibberellins

Gibberellins affect the rooting of stem cuttings by regulating nucleic acid and protein synthesis associated with early cell divisions involved in the transformation of mature stem tissue to a meristematic condition (19). Gibberellins at relatively high concentrations (up to 10^{-3} M) consistently inhibit adventitious root formation, whereas lower concentrations (10^{-11} to 10^{-7} M) promote rooting when applied at a time coincident with the first observable stage of root initiation (19,55). Promotion of adventitious root formation in cuttings has been obtained experimentally by applying chemical substances (Alar, abscisic acid) that interfere with gibberellin activity (19).

The fruiting bodies, mycelium, and culture medium of Boletus edulis var. pinicolus contained three gibberellin-related compounds: gibberellic acid, gibberellic esters, and an unidentified compound (54). At this time, the role of fungal gibberellins in mycorrhizae formation has not been thoroughly studied.

B-Vitamins

The literature concerning the effects of B-vitamins on root formation in cuttings is contradictory. Some investigators (71) consider that B-vitamins stimulate root formation, while others (63) have not observed any positive effect of B-vitamins on the rooting of pine cuttings. In entire plants (rooted cuttings), vitamins enhance the growth of both the root system and aerial parts (51).

Ectomycorrhizal fungi synthesize and liberate vitamins (51).
Culture solutions of these fungi, assayed with different species of
yeast, showed that nicotinic acid, biotin, and pantothenic acid are
produced by Suillus (Boletus) luteus, Boletus scaber, S. (B.) edulis,
and Paxillus involutus. Amanita muscaria and B. luridus synthesized
only biotin, and Xerocomus (Bolitus) subtomemtosus and Cenococcum
graniforme produced pantothenic acid (51). Vitamins released by micro-
organisms in the rhizosphere may influence the conversion of nitrate
nitrogen in the roots. This is of significance when nitrogen metabolism
in the plants is inadequate to completely utilize the available nitrogen
in the medium (51).

Ethylene

The effect of ethylene on root formation on stem cuttings is a
controversial subject. Ethylene itself may have no direct effect on
rooting of cuttings, but it is possible that ethylene may be indirectly
increasing the sensitivity of cuttings to endogenous and exogenous
auxins (1). Some investigators reported increases in auxin levels after
ethylene treatment while others reported decreases in auxin levels (1).
Ethylene has been shown to regulate auxin levels by controlling the
activity of auxin biosynthesis, and it has also been shown to inhibit
auxin transport from the site of production to the site of action. It
is important to distinguish between a direct effect on the machinery
which moves auxin in the plant tissue and an indirect effect on the
general metabolism resulting in reduced transport (1). The non-mycor-
rhizal fungi used in this study, Sclerotium rolfsii and Fusarium

oxysporum, are known to produce ethylene (29). At present, detailed ethylene studies have not been performed with ectomycorrhizal fungi.

Stimulation of Rooting by Free Living Ectomycorrhizal Fungi

Levisohn (26,28) demonstrated that free living ectomycorrhizal fungi in soil could stimulate the development of tree seedlings prior to infection. Mycelia of ectomycorrhizal fungi also stimulated the growth of endomycorrhizal tree species even though they could not form mycorrhizae (26). The fact that certain ectomycorrhizal fungi produce and liberate growth regulators, and stimulate the development of tree seedlings prior to infection suggests the free living fungus could stimulate initiation and development of rooting of many woody plant cuttings.

Mycorrhization of Rooting Medium

Theoretically, the best method for introducing ectomycorrhizal fungi into a nursery system is by the use of pure cultures as they require little space for culture and storage, are easy to handle, involve no risk of introducing pests, and allow inoculation of nursery plants with specific fungi (42). The use of pure cultures at the present time has limitations: 1) lack of knowledge and technology regarding indentification, isolation and in vitro culture for production of potential mycorrhizal fungi (67); 2) slow growth and specific growth substances required for many fungi now in culture (42). However, a number of studies have shown that mycelial forms of inoculum can survive successfully after inoculation into sterile soils (6).

The majority of mycorrhizal fungi require: 1) an adequate oxygen supply (sensitive to poor aeration); 2) a weakly acid substrate (approximately pH 5.0); 3) sufficient water supply; 4) a simple carbohydrate substrate (sucrose, glucose); and 5) an adequate nutrient supply (ammonium and organic nitrogen compounds) (18,39). Marx et al. (34) demonstrated growth optima of 26°C in pure culture for isolates of Pisolithus tinctorius and mycorrhizal formation with same fungi at pH 4.0 (33).

The non-mycorrhizal fungi used in this study (S. rolfsii and F. oxysporum) have requirements similar to those of ectomycorrhizal fungi with the exception of having the ability to degrade a greater variety of organic substances (i.e. more complex carbohydrates); F. oxysporum is also a facultative anaerobe (47).

Before host infection occurs, free living mycorrhizal fungi are known to be poor competitors with soil saprophytes for complex substrates (18). However, while in the mist bench, the fungi may be acquiring simple carbohydrates from: 1) exudates from the basal end of cuttings; 2) exudates from newly formed adventitious roots; and 3) metabolites leached from cuttings into the rooting medium. Tukey (69), found that depending on the plant, large amounts of carbohydrates (including glucose, fructose and sucrose), inorganic nutrients, amino acids, and organic acids are leached from cuttings under mist. In regard to mist propagation, wetting and rewetting the foliage of cuttings makes them more susceptible to leaching than would the same amount of water applied as a continuous drench (69). Also, Tukey hypothesized

that increased solar radiation stimulates the photosynthetic activity
of the leaf, elaborating carbohydrates which are readily water soluble
and easily leached after manufacture (68).

Stimulation of Rooting by Cell-Free
Culture Filtrates of Ectomycorrhizal Fungi

As mentioned previously, ectomycorrhizal fungi are known to produce
and release growth regulators into the culture medium (54,65). Tomazew-
ski and Wojciechowska (65) showed that the culture filtrate of a mycor-
rhizal fungus, Suillus variegatus, may contain up to 2 X 10^{-8} M IAA
equivalent while growing in 0.05 percent NH_4NO_3 as the sole nitrogen
source. They found that these filtrates could induce mycorrhiza-like
swellings, whereas pure IAA solution would do the same, but at a conc-
entration some 100 times higher. The mycelial filtrates must contain
other substances besides auxin which facilitate the mycorrhizal assoc-
iation (65). Acidic ether extracts of filtrates analyzed by thin-layer
chromatography confirmed the presence of polyphenols, e.g. variegatic
acid and boviquinones, in the following species: Suillus variegatus,
S. bovinus, S. grevillei, and S. granulatus (65). In addition, Shemak-
hanova (51) found that culture filtrates of four Boletus species pro-
moted root formation in bean cuttings.

Seasonal Effects of Ectomycorrhizal Fungi on
Rooting and Growth of Cuttings

The rooting potential of woody stem cuttings at certain stages of
development during the year is dependent, in part, upon the physiolog-
ical condition of the stock plant and treatment of the cuttings with

growth regulators (19,22).

Physiological Condition of Stock Plant

The internal factors of the stock plant (e.g. growth regulator
levels, enzyme activity, carbohydrate storage, and inorganic nutrient
status), affecting root initiation of cuttings, are influenced by seas-
onal changes in plant development (19,22). The propagation of plants
by cuttings should not be guided by calendar dates alone since the be-
ginning of the growing season as well as the rate of shoot growth and
development in the same plant may vary considerably in different years,
depending on the weather (22). Broadleaved evergreens usually root most
readily if the cuttings are taken after a growth flush and the wood is
partially matured (19,22). This period in late summer to autumn
(September-October for bearberry) is characterized by carbohydrate
accumulation and a gradual decrease in endogenous auxin concentration
and activity of hydrolytic enzymes (22). In several woody species,
shorter photoperiods induce the formation of some inhibitor, such as
abscisic acid, which accumulates in the buds and stem apices with the
onset of rest (25). The presence of an inhibitor, low endogenous auxin
levels and low activity of hydrolyzing enzymes facilitating starch con-
version to soluble sugars can cause poor rooting in early winter (22,46).
Generally, after the first part of January, increasing levels of endog-
enous auxin give rise to increased hydrolytic enzyme activity and starch
conversion in most woody species (22). DeFrance (8) demonstrated that
the rooting percentage of bearberry cuttings, taken in early February,
was increased when cuttings were soaked in 40 ppm IBA for 24 hours.

During flowering (March-May for bearberry) and the vegetative growth period (April-June for bearberry) auxin levels are high, but rooting potential is usually low as a result of competition for food reserves (22).

Treatment of Cuttings with Growth Regulators

The influence of growth regulators on the rooting of cuttings depends on the concentration of solution and duration of treatment, and on the physiological factors mentioned above (22). For the majority of woody species IBA and NAA are recommended, and in many cases mixtures of equal parts of IBA and NAA have been found to induce better rooting than either material alone (19,22). A mixture of 500 ppm IBA and 500 ppm NAA is recommended for bearberry cuttings during the optimum rooting period (September-October) (21). Cuttings of some species which are difficult to root will still root poorly after treatment with auxin due to limiting amounts of rooting cofactors (auxin synergists) (19). Also, application of synthetic auxins to stem cuttings at high concentrations, or when endogenous auxin levels are high, can inhibit bud development even though root formation has been adequate (19).

Auxin treatment apparently accelerates the processes that normally occur in untreated tissues (22,57). A decrease in the respiration rates at basal ends of treated and untreated cuttings usually occurs during the first few days after removal from the stock plant (57). This decrease could be due to a lack of synthetic processes which, in turn would cause a lack of phosphate acceptors (57). After the first few

days the major energy-requiring reactions start at the basal end,

changes occur in phosphate equilibrium, and respiration and nitrogen

metabolism increase sharply (57). The starch content of most cuttings

declines rapidly during root primordium initiation and may then increase

(17).

III. MATERIALS AND METHODS

Preliminary Rooting Study

Culture of Fungi

Ectomycorrhizal fungi were initially isolated from sporophores or rhizomorphs, or from mycorrhizae of Douglas-fir or Pinus spp.; Pisolithus tinctorius (Pers.) Coker & Couch; Thelephora terrestris Ehrh. ex Fr.; 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.) Quel.; Amanita muscaria (L. ex Fr.) Hooker; Tricholoma flavovirens (Pers. ex Fr.) Lund.; Poria terrestris D.C. ex Fries; var. cyaneus and var. subluteus, grown in plates on modified Melin-Norkrans (MMN) agar medium [0.05 g $CaCl_2$, 0.025 g NaCl, 0.5 g KH_2PO_4, 0.25 g $(NH_4)_2PO_4$, 0.15 g $MgSO_4 \cdot 7H_2O$, 0.02 g sequestrine (NaFe-EDTA), 100 mg thiamine $\cdot$ HCl, 3.0 g malt extract, 10.0 g glucose, 20.0 g Bacto-agar, distilled H_2O to 1000 ml, pH adjusted to 5.5 with HCl] and then transferred to vermiculite-peat culture according to the method of Marx and Zak (33). To one liter flasks was added 420 cc vermiculite, 30 cc fine peat moss, and 300 ml MMN nutrient solution (identical to MMN agar medium with following exceptions: 25 mg thizmin $\cdot$ HCl, 2.5 g glucose, no addition of malt extract or Bacto-agar); flasks containing the medium were sterilized by autoclaving at $121^{\circ}C$ for 15 minutes. Flasks were then inoculated with ectomycorrhizal fungi by adding ten ml of agar culture homogenate (two

actively growing agar plate cultures in 100 ml distilled H_2O, blended

for 30 seconds in an osterizer). Non-inoculated control flasks were

prepared in the same manner except for the addition of fungal inoculum.

Flasks were maintained at $21^{\circ}C$ in the laboratory, and after two months,

inoculated and non-inoculated culture media were washed out of the

flasks onto cheese cloth, rinsed with one liter of tap water, and the

solid substrate was added to a standard rooting medium (peat-perlite

1:1. v/v).

Source of Plant Material and Type of Cutting

Plant materials were obtained from eight-year-old bearberry plants

in roadside plantings along Highway 34 in west Corvallis, Oregon.

Single stem tip cuttings, from lateral branches, of the 'Oregon Hybrid'

cultivar of A. uva-ursi were taken December 18, 1975.

Propagation Techniques, Treatments and Facilities

Tip cuttings were cut (square basal cut 3-5 mm below node) to

7.5-10.0 cm lengths and the lower one-third leaves were removed before

treatment and placement in the propagation bench. Effect of the follow-

ing treatments on rooting were evaluated: 1) control (1:5 ratio of non-

inoculated culture medium to standard rooting medium); 2) single mycel-

ial inocula of 13 ectomycorrhizal fungi (1:5 ratio of inoculated culture

medium to standard rooting medium). Rooting mixtures (treatments) were

added to 11 X 11 X 6 cm fiber flats placed on a mist bench. Each

treatment consisted of 20 cuttings per flat (one replication per treat-

ment); four rows of five cuttings equally spaced within and between rows.

The propagation bench was located in a greenhouse equipped with "automatic leaf" controlled misting and maintained at 16°C minimum and 21°C maximum ambient temperatures. Evaporative coolers were used during the summer months to maintain moderate daytime maximums. Cuttings were provided with supplemental light for 16 hours daily (6 am to 10 pm) at approximately 12,000 lux by high pressure sodium vapor lamps (Lucalox lamps). The standard rooting medium consisted of one part fine grade sphagnum moss peat and one part horticultural grade perlite (both media used directly from bag without sterilization). After addition of inoculated and non-inoculated culture media the average rooting mixture had a pH of 4.0 before sticking and 4.3 after harvesting cuttings, and a soluble salt concentration of 45 ppm before sticking and 33 ppm after harvesting cuttings (pH meter and solubridge readings taken from suspension of 75 cc rooting mixture in 50 ml distilled H_2O). Rooting mixture temperatures were controlled thermostatically at approximately 23-25°C.

Rooting Response

After a period of four months in the propagation bench, cuttings were harvested and evaluated for rooting on a percentage basis, and root volume was measured by root ball size. Root volume ratings, based on root ball diameter with adhering medium, were as follows: 0 = none; 0.5 = root ball less than 1.0"; 1.0 = root ball 1.0-1.5"; 1.5 = root ball 1.5-2.0"; 2.0 = root ball 2.0-2.5"; 2.5 = root ball 2.5-3.0"; 3.0 = root ball 3.0-3.5"; and 3.5 = root ball greater than 3.5". This rating system was adopted due to the fibrous nature of the roots and the difficulty encountered in removing the medium from rooted cuttings.

Evaluation of Infection

Root systems of ten rooted cuttings from each treatment were washed to remove rooting medium particles, excised from the cuttings, and examined for mycorrhizal formation by clearing in 10% KOH and staining with trypan blue according to the method of Phillips and Hayman (48) as modified by Ames and Linderman (2). Mycorrhizal formation was not quantified by any rating system, only presence or absence of mycorrhizae was noted.

Seasonal Rooting Studies

After reviewing the rooting response data of the 13 fungi, three fungi were selected for studies during 1976 and early 1977. P. tinctorius, T. terrestris and R. vinicolor were selected for the following reasons: 1) growth on MMN agar plates and throughout peat-vermiculite culture flasks after transfer; 2) enhancement of rooting of bearberry cuttings; and 3) development of ectendomycorrhizae with bearberry as demonstrated by Zak (74).

Culture of Fungi

Three types of inocula were prepared with the three ectomycorrhizal fungi: 1) peat-vermiculite, mycelial-containing inocula were prepared and added to standard rooting medium in the same manner as in the preliminary trial; 2) cell-free liquid inoculum was obtained by leaching and filtering the peat-vermiculite culture medium of P. tinctorius (culture medium was leached with 700 ml distilled water, the leachate

was then filtered through Whatman No. 1 Qualitative Filters twice to
remove mycelium) liquid cultures of P. tinctorius and T. terrestris
(grown in 100 ml MMN nutrient solution in 250 ml Erlenmeyer flasks at
20°C for two months).

Non-mycorrhizal fungi (Fusarium oxysporum Schlecht. and Sclerotium
rolfsii Sacc.) were grown in plates on potato-dextrose agar medium (25.0
g dehydrated potatoes, 20.0 g dextrose, 20.0 g Bacto-agar, distilled H_2O
to 1000 ml; pH adjusted to 5.5 with HCl) and then transferred to peat-
vermiculite flask cultures moistened with V-8 nutrient solution (40.0
ml V-8 Juice[1], 0.60 g $CaCO_3$, 1.0 g sucrose, distilled H_2O to 1000 ml).
Culture flasks were sterilized and inoculated in the same manner as with
ectomycorrhizal fungi. After one month, inoculated and non-inoculated
culture media were washed out of flasks and added to the standard root-
ing medium in the same manner as with ectomycorrhizal fungi.

Source of Plant Material and Type of Cutting

Single stem tip cuttings, from lateral branches, were taken at four
different times during 1976 (Spring-April 21, Summer-July 15, Autumn-
October 19, and Winter-December 12) from the same block of stock plants.
Since extension frowth in this species occurs in May, cuttings taken
during April were from the previous year's growth; cuttings for subse-
quent sampling periods (July, October, and December) were from growth
produced in the spring of 1976.

[1]Campbell Soup Co., Camden, N.J.

Propagation Techniques, Treatments and Facilities

Tip cuttings were cut and prepared for placement in the propagation bench as in the preliminary study. In the first sampling period (April), two replications of 30 cuttings were used for each treatment; the remaining sampling periods (July, October, and December) had three replications of 20 cuttings per treatment.

Inoculation of Rooting Medium

Effect of the following inoculation treatments on rooting were evaluated (see Table 2 except where noted otherwise):

1) To determine the optimum mycelial inocula concentration for stimulation of rooting, a dilution series of single mycelial inocula was prepared by mixing ratios of 1:5 and 1:10 concentrations (treatments 3, 5, 7, 9, 11 and 13), and 1:25, 1:50 and 1:100 concentrations (Table 3); combinations of mycelial inocula were prepared by mixing 1:5 concentrations of single mycelial inocula together (treatments 15-18); and single mycelial inocula of two non-mycorrhizal fungi (to compare with effectiveness of mycorrhizal inocula) were prepared in 1:5 and 1:10 concentrations (Table 4) for the October sampling period only.

2) To compare the effectiveness of P. tinctorius cell-free culture filtrate and leachate with mycelial inocula of the same fungus, cuttings were soaked (one cm of basal end submersed) for 24 hours in filtrate (treatment 19); or, rooting medium around cuttings was drenched with filtrate (treatment 20) each week for the first six weeks of the October and December sampling periods (75 ml filtrate applied to 700 cc rooting

medium); or, cuttings were both soaked (treatment 21) and drenched (treatment 22) with leachate (July sampling period only).

3) To determine the interaction of mycorrhizal fungi with applied auxin and endogenous auxin in the cutting, cuttings were dipped for five seconds in 10% Jiffy Grow[2](JG) prior to insertion into 1:5 and 1:10 concentrations of single mycelial inocula (treatments 4, 6, 8, 10, 12 and 14).

4) To determine the survivability of free living mycorrhizal fungi in propagation media, 1:5 inoculated rooting media was stored for four months in opague bags under a greenhouse bench before use in the April sampling period only (Table 5).

All inoculated treatments were compared to an untreated control treatment (treatment 1) and an auxin-control [five second dip in 10% Jiffy Grow prior to insertion in 1:5 non-inoculated rooting medium (treatment 2)]. Rooting mixtures were added to 11 X 11 X 6 cm fiber flats and placed on the mist bench in a completely randomized design. Environmental conditions were the same as those in the preliminary study except the misting interval which was changed to 15 seconds mist every 30 minutes.

Rooting Response

After a four-months rooting period the cutting sample for each date

[2]Jiffy Grow Tonic No. 2 (5000 ppm IBA, 5000 ppm NAA, 100 ppm phenylmercuric acetate, 175 ppm boron from boric acid), G & W Products, Estacada, Oregon.

was harvested and evaluated for percent rooting and root volume as described previously.

Recovery of Free-Living Ectomycorrhizal Fungi from Rooting Medium

Selective Taylor's medium [1.0 g KH_2PO_4, 1.0 g $(NH_2)_2SO_4$, 0.5 g $MgSO_4$, 1.5 g peptone, 100 ug thiamine · HCl, 15.0 g Bacto-agar, plus distilled H_2O to 1000 ml, was autoclaved at $121^{\circ}C$ for 15 minutes; after autoclaving, 5.0 ug Benlate, 50.0 ug Neomycin SO_4 and 50.0 ug Streptomycin SO_4 was added] was used in attempts to reisolate ectomycorrhizal fungi from inoculated media before sticking and after harvesting cuttings.

Evaluation of Infection

Root systems of ten rooted cuttings from each replication of each treatment were examined for mycorrhizal formation using the same technique as in the preliminary study.

Statistical Interpretation

Statistical methods used to interpret the data were those used by Steel and Torrie (56), and Little and Hills (30). Analyses were based on the analysis of variance, least significant difference, and Student-Newman-Keul's tests. All percentage values for sampling times were transformed via arcsin $\sqrt{X}$ transformation prior to analysis to provide normal distributions for periods with percentages covering a wide range of values.

IV. RESULTS AND DISCUSSION

Stimulation of Rooting by Free Living
Ectomycorrhizal Fungi

Preliminary data (Table 1, Fig. 1) showed that both percent rooting

and root volume of cuttings taken in December were greater when mycor-

rhizal inocula were added to the rooting medium. Also, a greater number

of short lateral roots were observed on root systems of cuttings in in-

oculated rooting media. Of the 13 mycorrhizal fungi, P. tinctorius and

T. terrestris exhibited high rooting percentages and the highest root

volume ratings.

Single and combined mycelial inocula of P. tinctorius, T. terres-

tris and R. vinicolor significantly increased rooting only in December

(Table 2). The only exception was the significant increase in rooting

percentage with treatment 18 (P. tinctorius 1:5 + T. terrestris 1:5 +

R. vinicolor 1:5) in October. Single mycorrhizal inocula were more

effective in stimulating rooting than combinations at all times sampled

except October (Table 2).

In the majority of single mycorrhizal treatments, no significant

differences were noted in rooting between inoculum dilutions of 1:5 and

1:10 (inoculum to standard rooting medium, v/v). As the inoculum con-

centration was reduced from 1:25 to 1:100 there was a corresponding

decrease in effectiveness (Table 3).

The stimulation of rooting by ectomycorrhizal fungi, at certain

seasons, may possibly be attributed to fungal growth substances (auxins,

auxin synergists, cytokinins, B-vitamins) produced and excreted slowly

Table 1.   Preliminary study showing rooting response of cuttings to the
presence of ectomycorrhizal fungal inocula in the rooting med-
ium. Cuttings taken December 18, 1975, harvested April 9,
1976.

Trtmt. No.	Treatment [y]	Rooting Response [w] [x]	
		% Rooting	Root Vol.
1.	Untreated Control	15	0.15a
2.	Pisolithus tinctorius	75	1.10bc
3.	Thelephora terrestris	75	1.18bc
4.	Corticium bicolor	45	1.03bc
5.	Laccaria laccata	70	0.73abc
6.	Rhizopogon vinicolor	65	0.85bc
7.	Lactarius sanguifluus	65	0.68ab
8.	Cenococcum graniforme	90	1.00bc
9.	Lactarius deliciosus	70	0.88bc
10.	Hebeloma crustuliniforme	70	1.40c
11.	Amanita muscaria	55	0.48ab
12.	Tricholoma flavovirens	80	0.98bc
13.	Poria terrestris var. cyaneus	55	0.55ab
14.	Poria terrestris var. subluteus	95	1.00bc

w
 Rooting response based on 20 cuttings per treatment.
x
 Explanation of rooting response ratings in Materials and Methods, p. 21.
y
 All mycorrhizal treatments at a 1:5 dilution with rooting mix.
z
 Comparison of root volume means by Student-Newman-Kuel's multiple range
test; any values followed by the same letter are not significantly
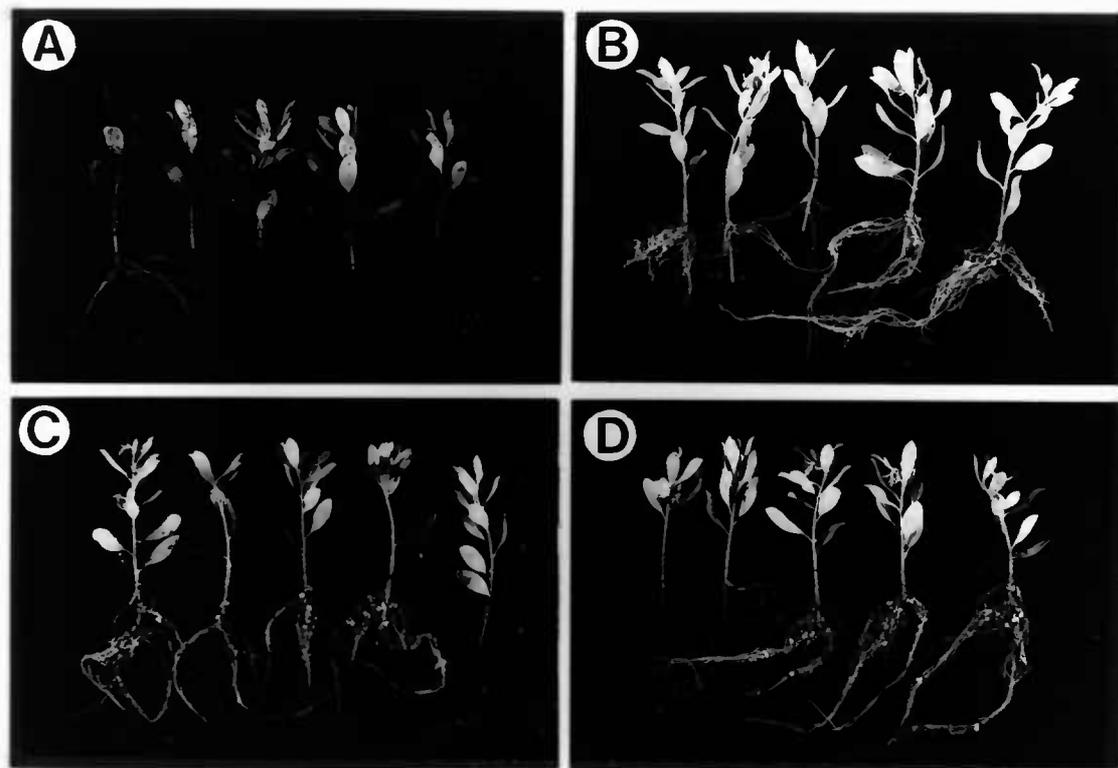different at the .05 level.

Figure 1. Representative cuttings showing the rooting of cuttings:
A) without mycorrhizal fungal inoculum in the rooting medium;
B) with P. tinctorius (1:5) added to the rooting medium;
C) with T. terrestris (1:5) added to the rooting medium;
D) with R. vinicolor (1:5) added to the rooting medium.

into the rooting medium as they would also in liquid culture medium. These substances, excreted in close proximity to the basal end of the cutting, could interact with endogenous growth substances in the cutting. 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 (45); this may explain differences in rooting responses to individual and combined mycorrhizal fungi. In addition, enhancement of rooting by fungal growth regulators would depend upon: populations of mycorrhizal fungi in the rooting medium (as noted in the dilution series); susceptibility of these substances to leaching by repeated misting; and the competitive use of these substances by other microorganisms present in the rooting medium.

In inoculated treatments and auxin-control treatments, root elongation occurred after approximately five weeks in the preliminary study and at all times sampled except October when root elongation occurred after approximately seven weeks. In untreated controls, with the exception of December, root elongation occurred after eight weeks in April and July, and after ten weeks in October.

As mentioned previously, root systems produced in mycorrhizal inocula in both the preliminary and year round studies had a greater number of short lateral roots, but after clearing and staining no infections were observed except in roots inoculated with T. terrestris at inoculum dilutions of 1:5 and 1:10 (inoculum to standard rooting medium, v/v). The fact that P. tinctorius and R. vinvcolor did not form mycorrhizal associations (i.e. actual infection) in the propagation bed supports the hypothesis that free living mycorrhizal fungi can stimulate

Table 2. Effect of ectomycorrhizal fungal mycelial, filtrate, and leachate treatments on cutting rooting percentage and root volume at different sampling times.

| Trtmt. No. | Treatment [z] | Sampling Time | | | | | | | |
| | | April | | July | | October | | December | |
		% Rooting	Root Vol.	% Rooting	Root Vol.	% Rooting	Root Vol.	% Rooting	Root Vol.
1.	Untreated Control	38.30a	18.75a	66.66a	22.33a	10.00ab	2.50a	0a	0a
2.	Auxin-Control (10% JG)	23.30a	13.00a	76.66a	26.16a	45.00ab	15.33a	20.00ab	7.16ab
3.	Pisolithus tinctorius (1:5)	83.30a	42.50a	93.33a	20.33a	20.00ab	8.33a	71.66fg	21.83bc
4.	P. tinctorius (1:5 + JG)	51.60a	44.50a	93.33a	29.00a	38.33ab	14.16a	48.33cdef	15.50abc
5.	P. tinctorius (1:10)	58.30a	35.25a	95.00a	31.33a	20.00ab	7.83a	70.00fg	20.16bc
6.	P. tinctorius (1:10 + JG)	41.65a	36.75a	90.00a	33.33a	55.00ab	20.66a	51.66defg	13.33abc
7.	Thelephora terrestris (1:5)	100.00a	57.75a	93.33a	28.33a	40.00ab	13.66a	73.33fg	24.83c
8.	T. terrestris (1:5 + JG)	93.30a	49.25a	93.33a	28.66a	71.66ab	27.00a	75.00g	24.83c
9.	T. terrestris (1:10)	55.00a	44.50a	91.66a	27.50a	3.33a	1.33a	65.00efg	18.00bc
10.	T. terrestris (1:10 + JG)	64.95a	38.75a	93.33a	31.50a	21.66ab	8.16a	30.00bcd	7.83ab
11.	Rhizopogon vinicolor (1:5)	76.95a	45.50a	95.00a	29.83a	23.33ab	9.33a	71.66fg	19.83bc
12.	R. vinicolor (1:5 + JG)	50.00a	29.75a	93.33a	30.50a	60.00ab	22.66a	63.33efg	21.16bc
13.	R. vinvcolor (1:10)	81.65a	48.75a	75.00a	25.33a	52.50ab	17.16a	58.33efg	15.16abc
14.	R. vinicolor (1:10 + JG)	61.65a	37.25a	85.00a	27.83a	33.33ab	13.33a	40.00bcde	13.00abc
15.	P. tinc. (1:5) + T. terr. (1:5)	91.60a	53.50a	93.33a	29.50a	21.66ab	7.66a	78.33g	24.83c
16.	P. tinc. (1:5) + R. vin. (1:5)	91.60a	50.00a	83.33a	30.83a	31.66ab	13.00a	43.33cde	14.33abc
17.	T. terr. (1:5) + R. vin. (1:5)	63.50a	36.50a	96.66a	27.83a	50.00ab	19.83a	46.66cdef	13.00abc
18.	P. tinc. (1:5) + T. terr. (1:5) + R. vin. (1:5)	73.30a	39.25a	85.00a	23.83a	76.66b	25.66a	55.00efg	16.50bc
19.	P. tinctorius (filtrate soak)					36.66ab	14.16a	23.33bc	7.83ab
20.	P. tinctorius (filtrate drench)					46.66ab	15.83a	43.33cde	11.16abc
21.	P. tinctorius (leachate soak)			86.66a	33.50a				
22.	P. tinctorius (leachate drench)			80.00a	24.83a				

[z] Comparison of treatment means by Student-Newman-Kuel's multiple range test; any means followed by a common letter are not significantly different at the .05 level.

Table 3. Rooting response of cuttings to a dilution series of mycorrhizal fungal inocula in the rooting medium in July and December, 1976.

Treatment [z]	Rooting Response			
	July		December	
	% Rooting	Root Vol.	% Rooting	Root Vol.
Untreated Control	66.66ab	22.33a	0a	0a
Auxin-Control (10% JG)	76.66ab	26.16a	20.00bc	7.16ab
Pisolithus tinctorius (1:5)	93.33b	20.33a	71.66d	21.83c
P. tinctorius (1:10)	95.00b	31.33a	70.00d	20.16c
P. tinctorius (1:25)	68.33ab	19.83a	58.33d	21.00c
P. tinctorius (1:50)	56.66a	24.66a	33.33c	6.33ab
P. tinctorius (1:100)	56.66a	21.83a	36.66c	7.00ab
Thelephora terrestris (1:5)	93.33b	28.33a	73.33d	24.83c
T. terrestris (1:10)	91.66b	27.50a	65.00d	18.00bc
T. terrestris (1:25)	81.66ab	27.50a	18.33abc	4.33ab
T. terrestris (1:50)	76.66ab	28.83a	5.00ab	1.00a
T. terrestris (1:100)	71.66ab	28.33a	1.66a	0.50a

z
Comparison of treatment means by Student-Newman-Kuel's multiple range test; any values followed by the same letter are not significantly different at the .05 level.

rooting of bearberry cuttings.

Stimulation of Rooting by Non-Mycorrhizal Fungi

Non-mycorrhizal fungi (F. oxysporum and S. rolfsii) significantly increased percent rooting when diluted into the rooting medium at an inoculum dilution of 1:5 (inoculum to standard rooting medium, v/v) during October (Table 4). Also, rooting response in non-mycorrhizal inocula was greater than that in mycorrhizal inocula, indicating that F. oxysporum and S. rolfsii may be more active in the rooting medium. However, it was noted that root systems of cuttings harvested from non-mycorrhizal inocula were extremely "string-like" and possessed few lateral roots characteristic of root systems developed in mycorrhizal inocula.

Stimulation of Rooting by Cell-Free
Culture Filtrates of Ectomycorrhizal Fungi

Treatment responses of cuttings taken in October and December (Table 2) support Shemakhanova's (51) observations that cell-free culture filtrates of certain ectomycorrhizal fungi stimulate the rooting of cuttings. Filtrates from liquid cultures of P. tinctorius significantly increased percent rooting of bearberry cuttings taken in December. However, the response of December cuttings to treatment with filtrate inocula was less than with vegetative mycelial inocula, suggesting that slow continuous release of growth substances by fungal mycelia was more effective in stimulating rooting than drenching or soaking with a limited amount of growth regulators in liquid culture. The October

Table 4. Rooting response of cuttings to the presence of non-mycorrhizal fungal inocula (<u>Sclerotium rolfsii</u> and <u>Fusarium oxysporum</u>) in the rooting medium. Cuttings taken October 19, 1976, harvested February 19, 1977.

	Rooting Response	
Treatment[z]	% Rooting	Root Vol.
Untreated Control	10.00a	2.50a
Auxin-Control (10% JG)	45.00abc	15.33a
<u>Pisolithus</u> <u>tinctorius</u> (1:5)	20.00ab	8.33a
<u>Thelephora</u> <u>terrestris</u> (1:5)	40.00abc	13.66a
<u>Rhizopogon</u> <u>vinicolor</u> (1:5)	23.33ab	9.33a
<u>Sclerotium</u> <u>rolfsii</u> (1:5)	80.00bc	22.33a
<u>S</u>. <u>rolfsii</u> (1:10)	60.00abc	20.50a
<u>Fusarium</u> <u>oxysporum</u> (1:5)	86.66c	25.66a
<u>F</u>. <u>oxysporum</u> (1:10)	46.66abc	15.16a

[z] Comparison of controls, mycorrhizal, and non-mycorrhizal treatment means by Student-Newman-Kuel's multiple range test; any values followed by the same letter are not significantly different at the .05 level.

cuttings showed the opposite response to mycelial inocula and culture filtrate inocula. The culture filtrate drench treatment (treatment number 20) had a greater rooting response than with mycelial inocula treatments (treatment numbers 3, 5, 7, 9 and 11) and equal to the auxin-control treatment. The rooting medium temperature was lower with this set of cuttings and may have affected the activity of the fungal mycelium, possibly reducing their ability to produce and release auxins.

Addition of leachates, obtained from washing and filtering peat-vermiculite culture flasks of P. tinctorius, slightly enhanced the rooting of cuttings taken in July (Table 2, treatment numbers 21 and 22). There were no significant differences between drenching and soaking treatments for leachates or culture filtrates of P. tinctorius.

Seasonal Effects of Ectomycorrhizal Fungi
on Rooting and Growth of Cuttings

Rooting of untreated cuttings was maximum with cuttings taken in July, and minimum in December (Table 2). Cutting response to auxin treatment was also greatest in July and poorest in December. The poor rooting of cuttings taken in October was attributed to a malfunction in the bottom heat system which lowered the rooting medium temperature below the optimum (23-25°C) for rooting (8,21). However, the rooting values denote a trend among the inoculated and non-inoculated treatments during that period.

Inoculation with ectomycorrhizal fungi had different effects on rooting and growth of rooted cuttings at different times of the year (Table 2). The most striking rooting increase to mycorrhizal inocula

occurred in cuttings taken in December. In December, all inoculated
treatments significantly increased rooting, and most inoculation treat-
ments (except treatment numbers 10, 14 and 19) rooted significantly
better than the auxin-control. The majority of the mycorrhizal treat-
ments (treatment numbers 3, 5, 7, 8, 9, 11, 12, 15 and 18) significantly
increased root volume, but only a few (treatment numbers 7, 8 and 15)
significantly increased rooting over that of the auxin treatment alone.
Enhancement of rooting at other times of the year, however, cannot be
substantiated statistically.

While exogenous auxin alone (Jiffy Grow 10%) increased rooting in
December, increases were obtained by the addition of mycorrhizal fungi,
indicating that endogenous rooting factors other than auxin were limit-
ing rooting. Perhaps the mycorrhizal fungi excrete rooting factors
(auxins, cytokinins, polyphenols, polyphenol oxidase complex, vitamins)
into the rooting medium at concentrations adequate to have an additive
effect with endogenous growth substances in the cutting at non-optimum
times, and activate the metabolic processes required for root initiation
(i.e. derepression of nucleic acids, synthesis of hydrolyzing enzymes,
degradation of starch and mobilization of sugars).

Jiffy Grow (10%), alone and in combination with mycorrhizal inocula,
had different effects on rooting at different times of the year (Table
2). Jiffy Grow (10%) alone significantly increased rooting in December.
At other times of the year, Jiffy Grow (10%) alone was either inhibitory
(April), beneficial (July), or very beneficial (October). Propagators
claim September-October to be the optimum time period for rooting when
auxin treatment is used (10,21). With few exceptions, Jiffy Grow (10%)

in combination with mycorrhizal inocula was inhibitory in April and December, very beneficial in October, and apparently had little or no effect in July.

Fungal growth regulators (IAA and auxin synergists) may be interacting with endogenous (IAA) and applied (10% Jiffy Grow containing 500 ppm IBA + 500 ppm NAA) auxins to alter the intensity or balance of growth regulator substances in the cutting at different seasons. In December, where Jiffy Grow (10%) in combination with fungal inocula appeared to be inhibitory, greater inhibition occurred in the 1:10 inoculum dilutions than at 1:5. Greater amounts of IAA could have been added in the 1:5 inoculum dilution than at the 1:10 dilution. IAA excreted by the fungi may have affected the ratio of IAA:IBA:NAA in the cutting. More than likely, the ratio of IAA + IBA:NAA was affected, i.e. greater IAA + IBA:NAA when greater IAA was added as inoculum. There is evidence that when using NAA in equal proportion to IBA or IAA in auxin mixtures for rooting, the proportion of NAA should be reduced below 50 percent due to its high activity in several evergreens (20). Auxin synergists (polyphenols) excreted by the mycorrhizal fungi may also be increasing the endogenous IAA level in the IAA + IBA:NAA ratio in the cutting via the inhibition of IAA oxidase activity.

The interaction of applied auxins with specific mycorrhizal fungi, as indicated by rooting response, was contradictory at different seasons. Jiffy Grow (10%) in combination with T. terrestris treatments, i.e. enhancement at 1:10, inhibition at 1:5. In October, Jiffy Grow (10%) increased rooting in combination with 1:5 and 1:10 inocula dilutions of mycorrhizal fungi except for a decrease when using the 1:10

dilution of R. vinicolor (treatment number 14). Again, this may be the result of varied amounts of auxin or other growth regulators being excreted by the fungi, thus affecting the ratio of IAA + IBA:NAA in the cutting.

Bearberry cuttings rooted in mycorrhizal inocula also exhibited enhanced top growth in both the preliminary study and seasonal studies (Figs. 2-5). During the preliminary study (December, 1975-April, 1976) and the December sampling period (December, 1976-March, 1977), cuttings in inoculated rooting media exhibited earlier flower and vegetative bud break, and greater subsequent shoot growth than cuttings in non-inoculated rooting media. Cuttings taken from flowering stock plants in April also showed earlier vegetative bud break and greater extension in inoculated treatments. Earlier rooting in inoculated treatments apparantly accounted for this earlier bud break and greater top growth. Adventitious roots formed on the cuttings would allow for greater water and nutrient uptake, and would be producing hormones (cytokinins, gibberellins) affecting the growth and development of the shoot (66).

Survival of Ectomycorrhizal Fungi in Propagation Media

Using selective Taylor's medium, it was possible to reisolate the three ectomycorrhizal fungi used in these rooting tests from freshly inoculated rooting medium, but not from inoculated rooting medium exposed to the greenhouse environment for several weeks. Mycorrhizal activity in the propagation bed may have been suppressed by excess moisture in certain areas of the mist bench, resulting in waterlogging

Figure 2. Comparison of top growth of rooted cuttings from April samp-
           ling period. A = Pisolithus tinctorius (1:5), B = P.
           tinctorius (1:10), C = Thelephora terrestris (1:5), D = T.
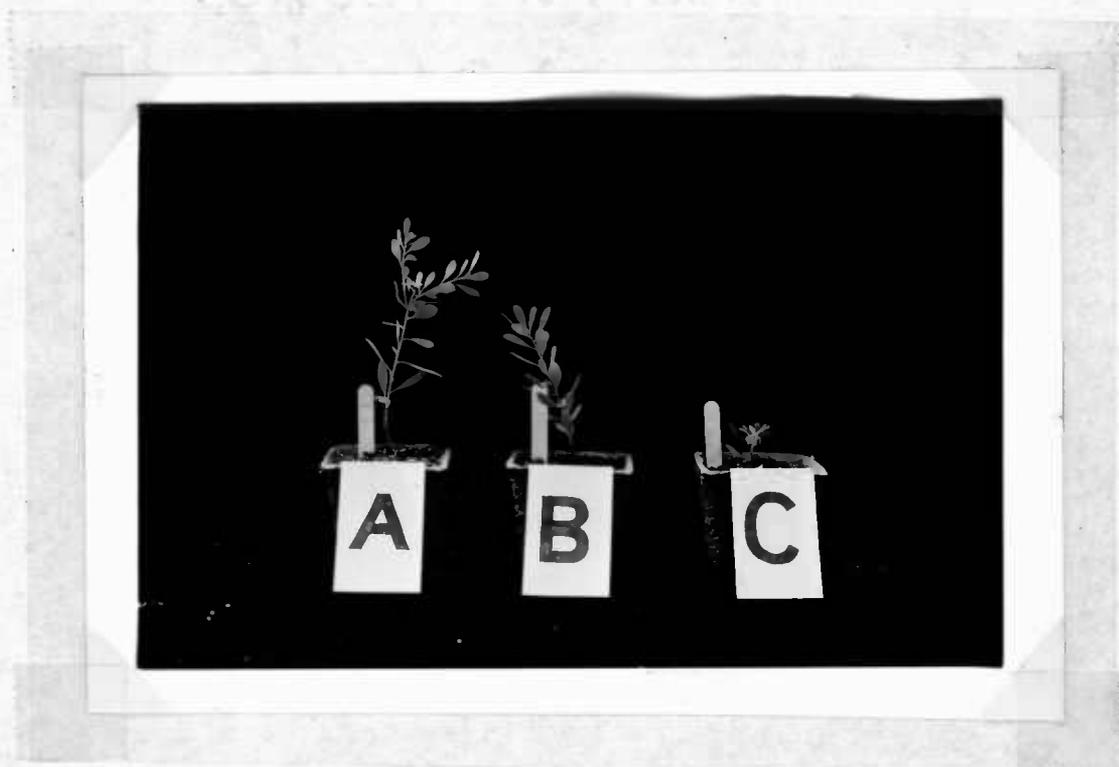   terrestris (1:10), E = Untreated Control.

Figure 3.   Comparison of top growth of rooted cuttings from April samp-
            ling period.   A = Rhizopogon vinicolor (1:5), B = R.
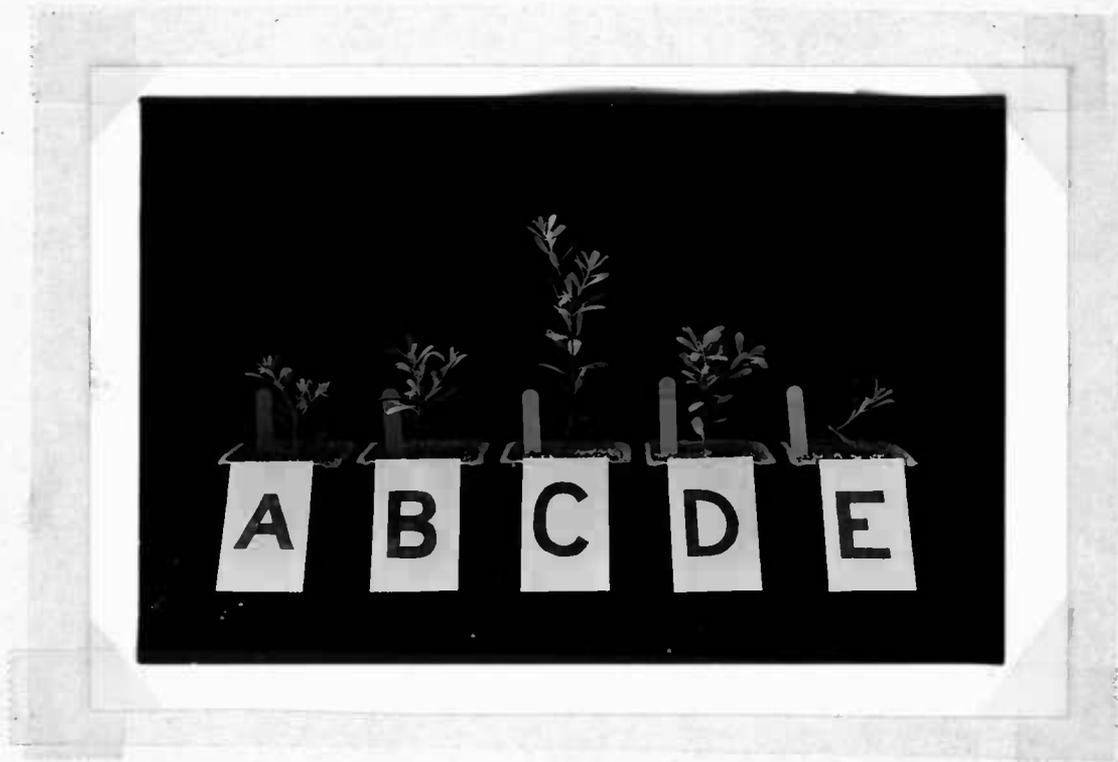   vinicolor (1:10), C = Untreated Control.

Figure 4. Comparison of top growth of rooted cuttings from April samp-
ling period. A = Pisolithus tinctorius (1:5 + JG), B = P.
tinctorius (1:10 + JG), C = Thelephora terrestris (1:5 + JG),
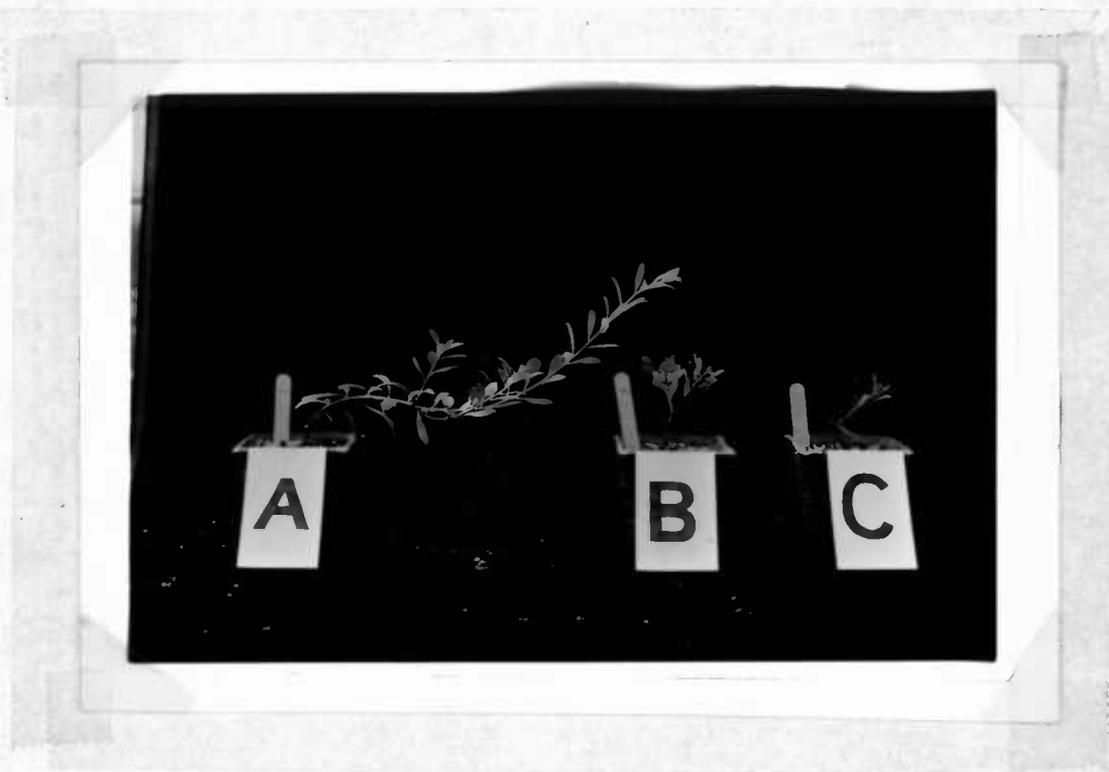D = T. terrestris (1:10 + JG), E = Auxin-Control (10% JG).

Figure 5. Comparison of top growth of rooted cuttings from April sampling period. A = <u>Rhizopogon</u> <u>vinicolor</u> (1:5 + JG), B = <u>R</u>. <u>vinicolor</u> (1:10 + JG), C = Auxin-Control (10% JG).

and reduced aeration in the rooting medium; and, a build-up of sapro-
phytic fungal populations (Fusarium spp., Penecillium spp., Trichoderma
spp.) in the rooting medium may have suppressed the mycorrhizal fungi
or interfered with the reisolation process. However, T. terrestris did
form mycorrhizae in the rooting medium in all rooting tests and all three
fungi formed mycorrhizae four months after rooted cuttings and adhering
rooting medium were transplanted into a pasteurized soil mix at the end
of the April sampling trial (August 20, 1976). Mycorrhizae were formed
on only one auxin-control transplant, and this was apparently due to
contamination from a neighboring inoculated transplant. The mycorrhizae
formed on bearberry by P. tinctorius, T. terrestris and R. vinicolor
were easily visible with the naked eye and very conspicuous when viewed
under a stereoscope. All three mycorrhizae had characteristic, swollen,
short, lateral roots, light to dark brown in color, with simple and tri-
lobate tips (Fig. 6). Smooth to fibrous mantles surrounded by loose
wefts of mycelium were observed after clearing and staining. These
characteristics were similar to those of bearberry ectendomycorrhizae
described by Zak (74).

The results suggest that mycorrhizal fungi remained viable in the
rooting medium and formed mycorrhizae on rooted cuttings after trans-
planting into a soil mixture with a more favorable oxygen and moisture
status. Contamination of transplanted cuttings by spores blown into the
greenhouse was discounted since other susceptible hosts remained unin-
fected.

Rooting medium, inoculated with mycorrhizal fungi stored for four
months after harvesting from flasks, was comparable to freshly inoculated
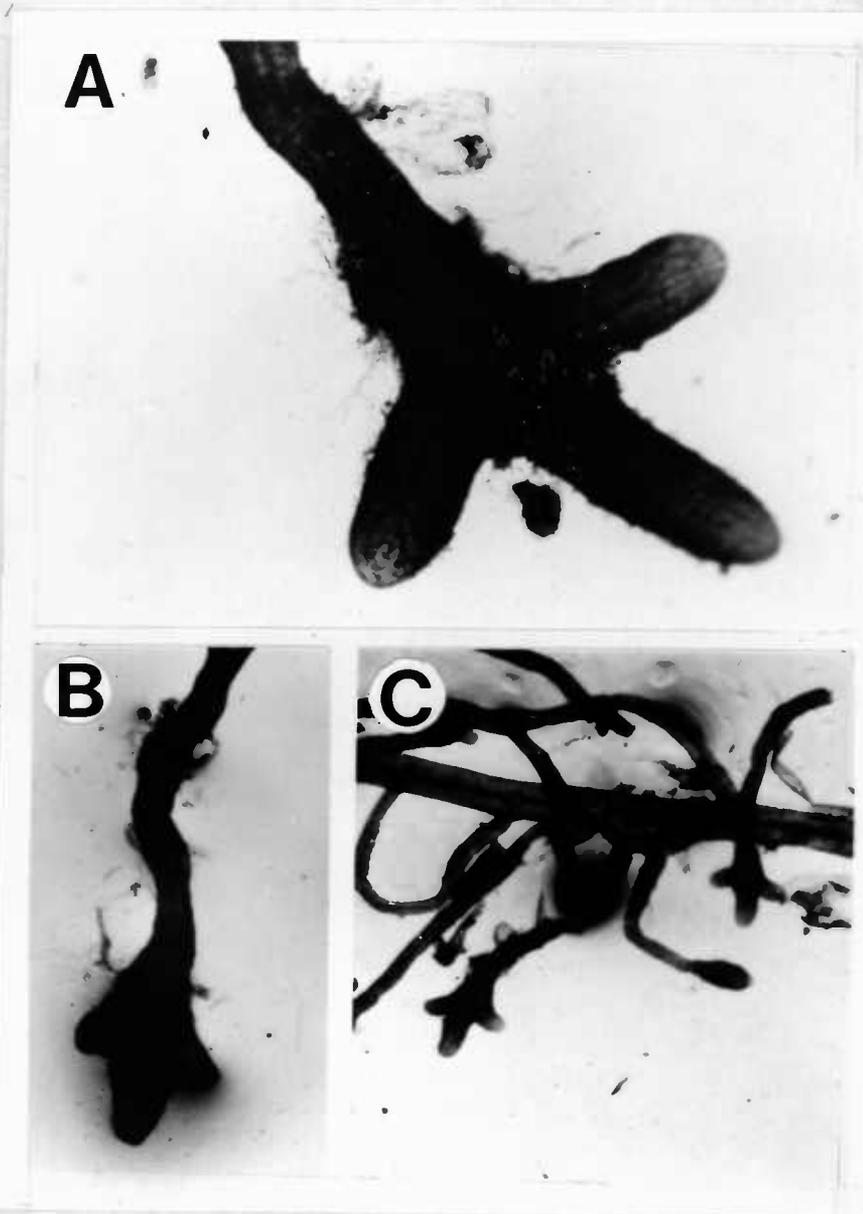
Figure 6. Ectendomycorrhizae observed on rooted cuttings four months
           after transplanting from April-August, 1976 rooting trial.
           A) Trilobate mycorrhiza with Pisolithus tinctorius showing
              associated mycelia.
           B) Trilobate mycorrhiza with Thelephora terrestris.
           C) Simple and trilobate mycorrhizae with Rhizopogon vinicolor.

rooting medium in the enhancement of rooting in April cuttings (Table 5).
This also supports the hypothesis that free living mycelia of mycorrhi-
zal fungi can survive successfully for a period of time (several weeks)
in the absence of a mycorrhizal association.

Table 5. Rooting response of cuttings to the presence of four-month-old mycorrhizal fungal inocula in the rooting medium. Cuttings taken April 21, 1976, harvested August 20, 1976.

	Rooting Response[x]	
Treatment[y]	% Rooting	Root Vol.[z]
Untreated Control	25	0.38a
Auxin-Control (10% JG)	20	0.23a
Pisolithus tinctorius	60	1.08b
Thelephora terrestris	70	1.35b
Rhizopogon vinicolor	95	1.55b
Cenococcum graniforme	100	1.63b
Hebeloma crustuliniforme	50	1.10b
Poria terrestris var. subluteus	95	1.73b

[x] Rooting response based on 20 cuttings per treatment.

[y] All mycorrhizal treatments at a 1:5 inoculum dilution.

[z] Comparison of root volume means by Student-Newman-Kuel's multiple range test; any values followed by the same letter are not significantly different at the .05 level.

V. CONCLUSIONS

The necessity for further research on the mechanisms involved in the enhancement of rooting of woody cuttings by ectomycorrhizal and non-mycorrhizal fungi is evident. This study suggests a possible mechanism for enhancement of rooting by ectomycorrhizal fungi at non-optimum rooting periods, and non-mycorrhizal fungi at optimum periods, may be the production and excretion of growth substances, i.e. auxins, auxin synergists, cytokinins, gibberellins, B-vitamins and ethylene, into the rooting medium that interact with endogenous growth substances in the cutting to activate the metabolic processes required for root initiation. These ectomycorrhizal fungi vary in the type and amount of growth regulators produced and excreted, yet the majority stimulate rooting, suggesting the production of some common denominator such as a polyphenol, which may control the high auxin level required for mycorrhizae initiation, i.e. infection of the host plant.

Other mechanisms may be involved in this enhancement of rooting. Mycorrhizal and non-mycorrhizal fungi may be altering the structural nature of the rooting medium providing more optimum aeration and moisture conditions for rooting, and/or altering the nutrient status of the rooting medium by degrading the organic matter. Mycorrhizal fungi may also be releasing metabolites that are inhibitory to other microorganisms that cause the deterioration of cuttings in the rooting medium. Further research regarding these mechanisms will provide more insight into the stimulation of rooting by various fungi.

VI. NURSERY APPLICATION

In a typical nursery operation producing woody plant species, plant material is sexually or asexually propagated, transplanted into containers for growing-on in the nursery, and ultimately lined out. Production practices stress the maintenance of aseptic conditions in the nursery to reduce pest problems. Seeds and cuttings are quite often propagated in sterile media, transplanted to sterile soil mix, and when necessary, plant materials are protected by the use of pesticides. Plants in the nursery scheme usually receive a surplus of nutrients, resulting in lush top and root growth. Some of these practices may reduce populations of harmful organisms, but they may also reduce the populations of benefic-ial organisms, e.g. mycorrhizal fungi.

When these sterile nursery plants are lined out in the field they will encounter a completely different environment which will influence their survival. Natural outplanting stresses include temperature extremes, water availability, nutrient deficiencies and imbalances, extremes in soil reaction, and pathogens. Under narural conditions, approximately three percent of the higher plants (including many woody ornamental species) are in association with ectomycorrhizal fungi (40). These fungi are benificial to the host plant in several ways. Plants with abundant ectomycorrhizae have a larger root surface for nutrient and water absorption, the ability to breakdown complex minerals and organic matter, an increased tolerance to high soil temperatures and toxic levels of aluminum, sulfur and other elements, and they function as biological deterrents to infection of feeder roots by pathogens (38).

Ectomycorrhizal fungi may be incorporated into the nursery using soil from natural forest sites, mycorrhizal seedlings, or pure cultures of mycorrhizal fungi (42). The use of pure cultures of ectomycorrhizal fungi remains the best method of inoculation since they require little space, are easy to handle, and provide no risk of introducing pests. Pure culture inoculum may be introduced during two stages of the nursery system: 1) in the propagation medium before seeding or sticking cuttings; and 2) in containers or beds along with seedling or rooted cutting transplants. Ectomycorrhizal fungi were shown to enhance the rooting of bearberry cuttings in this study, but they are more effective in form-ing mycorrhizae with transplants having established root systems. Once the plants become mycorrhizal they should be more efficient in the nur-sery production system and better adapted to outplanting stresses.

Bearberry cuttings were harvested and propagated according to est-ablished practices used by commercial propagators. These experiments were conducted in a manner such that the practices and procedures could be easily incorporated into a nursery production system. A large, diversified nursery operation could easily afford the equipment and mat-erials (small transfer hood, glassware, nutrient media, miscellaneous lab equipment) for the pure culture synthesis of ectomycorrhizal fungi. Specific fungal isolates may be obtained from universities and govern-ment research institutions, and once in culture, these fungi are easy to maintain. The fungi can be grown on different media (Melin-Norkrans medium, potato dextrose medium, tissue culture medium), in the presence or absence of light, and at temperatures ranging from 14^{0} to $29^{0}C$, the optimum being approximately $26^{0}C$ (34). Relatively small quantities of

inoculum would be required to inoculate large volumes of rooting medium or soil mix. Fungal inoculum and inoculated rooting or soil medium can be stored for periods of several months. In the near future, a small nursery operation may obtain mycorrhizal inoculum from commercial laboratories specializing in pure culture synthesis.

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APPENDIX

Bioassay for Fungal Cytokinin Production

The most simple and direct method for detecting cytokinin production by fungi is the placement of the fungi on a cytokinin-free agar surface surrounded by pieces of cytokinin-dependent soybean, carrot, or tobacco callus tissue (25). The increase in fresh weight of the callus tissue in culture is taken as a measure of cytokinin stimulated cell division (25).

The following ectomycorrhizal fungi were screened for cytokinin production: P. tinctorius; T. terrestris; R. vinicolor; Cenococcum graniforme; and Poria terrestris var. subluteus. The fungi were grown on MMN agar plates for two weeks at 20^oC. Cytokinin-dependent tobacco callus (Nicotiana tabacum var. Wisconsin No. 38) was obtained from Dr. D. Armstrong. Tobacco callus was maintained on Revised Tobacco Tissue Culture Medium (RM-1965) containing (mg/l): NH_4NO_3, 1.65; H_3BO_4, 6.20; KH_2PO_4, 170.0; KI, 0.83; $NaMoO_4 \cdot 2 H_2O$, 0.25; $CoCl_2 \cdot 6 H_2O$, 0.025; $CaCl_2 \cdot 2 H_2O$, 440.0; $MgSO_4 \cdot 7 H_2O$, 370.0; $MnSO_4 \cdot H_2O$, 22.30; $ZnSO_4 \cdot 7 H_2O$, 8.60; $CuSO_4 \cdot 5 H_2O$, 0.025; Na_2EDTA, 37.30; $FeSO_4 \cdot 7 H_2O$, 27.80; thiamine $\cdot$ HCl, 0.40; sucrose, 30,000; myo-inositol, 100; IAA, 2.0; kinetin, 0.020; and Bacto-agar, 10,000. The pH was adjusted to 5.6 before adding agar. Small pieces of callus (approximately 60 mg each) from five week old cultures were transferred to 250 ml Erlenmeyer flasks containing 100 ml of the same medium but with the kinetin ommitted. The actively growing perimeter of the mycelial mat was sectioned into small pieces (approximately 75 mm^3) which were placed in the center of three tobacco tissue pieces with approximately 20 mm between the

tobacco and fungus. In another treatment the inoculum of fungus was placed at the end of a row of tobacco tissue pieces with approximately 20 mm and 40 mm between the tobacco pieces and the fungus. Control treatments consisted of tobacco tissue pieces on tissue culture medium minus kinetin and on tissue culture medium with kinetin (0.020 mg/l). Three replications of each treatment were incubated at 20°C in darkness for 25 days.

Negative results were obtained for all mycorrhizal fungi in each experiment with the exception of one replicate of R. vinicolor, which stimulated growth of tobacco tissue (final mean fresh weight 1.05 g) comparable to that of the positive cytokinin control (final mean fresh weight 1.16 g). This suggests that these species do not produce cyto- kinins. However, these negative results must be viewed with caution since cytokinins might occur but at levels too low to be detected with this bioassay system, or the fungus might produce some substance which inhibits the growth and response of tobacco tissue (7). Also, as ob- served with P. tinctorius and R. vinicolor in this series of experiments, the fungi may grow too fast and overgrow the tissue before stimulation occurs. In some instances, Miller (44) has noticed that callus tissue inhibits the growth of the fungus. Poria terrestris var. subluteus may fall into this category. Modification of the culture medium and/or environmental conditions may produce more positive results.