The effect of 13 ectomycorrhizal fungi on the enhancement of rooting of stem cuttings of bearberry (Arctostaphylos uva-ursi 'Oregon Hybrid') was evaluated in preliminary studies. Pisolithus tinctorius, Thelephora terrestris and Rhizopogon vinicolor were selected for further 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 evaluate 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 T. terrestris, enhanced rooting occurred in the absence of any mycorrhizal association, i.e. actual infection. Cell-free culture filtrates of P. tinctorius stimulated rooting, but not as effectively as did the P. tinctorius mycelium inoculum. Non-mycorrhizal fungi, Fusarium oxysporum and Sclerotium rolfsii, stimulated rooting during the optimum rooting period, September-October, when mycorrhizal
fungi did not. Root systems formed in the presence of mycorrhizal inocula 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. tinctorius* 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 *Arctostaphylos uva-ursi* 'Oregon Hybrid' with Ectomycorrhizal Fungi

by

Christopher Alan Call

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

Completed May 20, 1977

Commencement June 1978
APPROVED:

Professor of Horticulture

in charge of major

Head of Department of Horticulture

Dean of Graduate School

Date thesis is presented _______ May 20, 1977 _______

Typed by Christopher A. Call
ACKNOWLEDGMENTS

I wish to express my appreciation to A. N. Roberts and James L. Green for their assistance in the preparation of this manuscript. I especially wish to thank Bob Linderman and the folks at the USDA Ornamentals Research Laboratory for their guidance and friendship during my research. Also, I would like to thank my wife, Noelle, for her understanding, encouragement, and baking ability.
# TABLE OF CONTENTS

I. Introduction ......................................................... 1

II. Review of Literature .............................................. 3
   Classification of Mycorrhizae ................................... 3
   Growth Regulators Produced and Released by
   Ectomycorrhizal Fungi ........................................... 6
   Stimulation of Rooting by Free Living Ectomycorrhizal
   Fungi ................................................................. 13
   Stimulation of Rooting by Cell-Free Culture Filtrates
   of Ectomycorrhizal Fungi ......................................... 15
   Seasonal Effects of Ectomycorrhizal Fungi on
   Rooting and Growth of Cuttings ................................. 15

III. Materials and Methods ........................................... 19
   Preliminary Rooting Study ....................................... 19
   Seasonal Rooting Studies .......................................... 22
   Statistical Interpretation .......................................... 26

IV. Results and Discussion .......................................... 27
   Stimulation of Rooting by Free Living Ectomycorrhizal
   Fungi ................................................................. 27
   Stimulation of Rooting by Cell-Free Culture Filtrates
   of Ectomycorrhizal Fungi ......................................... 33
   Seasonal Effects of Ectomycorrhizal Fungi on
   Rooting and Growth of Cuttings ................................. 35

V. Conclusions ......................................................... 47

VI. Nursery Application .............................................. 48

VII. Literature Cited .................................................. 51

Appendix ................................................................. 57
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Preliminary study showing rooting response of cuttings to: A) absence of mycorrhizal inoculum, B) presence of <em>Pisolithus tinctorius</em> inoculum, C) presence of <em>Thelephora terrestris</em> inoculum, D) presence of <em>Rhizopogon vinicolor</em> inoculum in rooting medium.</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Comparison of top growth of rooted cuttings from non-inoculated rooting medium and rooting medium inoculated with <em>Pisolithus tinctorius</em> and <em>Thelephora terrestris</em>.</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>Comparison of top growth of rooted cuttings from non-inoculated rooting medium and rooting medium inoculated with <em>Rhizopogon vinicolor</em>.</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Comparison of top growth of rooted cuttings from non-inoculated rooting medium and rooting medium inoculated with <em>Pisolithus tinctorius</em> and <em>Thelephora terrestris</em>. Inoculated and control cuttings treated with 10% Jiffy Grow.</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>Comparison of top growth of rooted cuttings from non-inoculated rooting medium and rooting medium inoculated with <em>Rhizopogon vinicolor</em>. Inoculated and control cuttings treated with 10% Jiffy Grow.</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>Ectendomycorrhizae observed on rooted cuttings four months after transplanting from April-August rooting trial: A) trilobate mycorrhiza with <em>Pisolithus tinctorius</em> showing associated mycelia, B) trilobate mycorrhiza with <em>Thelephora terrestris</em>, C) simple and trilobate mycorrhizae with <em>Rhizopogon vinicolor</em>.</td>
<td>44</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Preliminary study showing rooting response of cuttings to the presence of mycorrhizal fungal inocula in the rooting media.</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>Seasonal sampling times showing rooting response of cuttings to different types of mycorrhizal fungal inocula in the rooting medium.</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>Rooting response of cuttings to a dilution series of mycorrhizal fungal inocula in the rooting medium at two seasonal sampling times.</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>Rooting response of cuttings to the presence of non-mycorrhizal fungal inocula in the rooting medium.</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>Rooting response of cuttings to the presence of four-month-old mycorrhizal fungal inocula in the rooting medium.</td>
<td>46</td>
</tr>
</tbody>
</table>
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 desirable 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 arbuscules within cortical cells (13,37,38). Thin-walled, spherical to ovate vessels may also be produced in the cortex by the infecting hyphae (13,37,38). The term vesicular-arbuscular (VA) mycorrhiza 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 intracellular hyphae, and both tend to disappear in the basal portion of older roots when the cortex senesces (18,72). The identity of many ectendomycorrhizal 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 ectendomycorrhizae 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 fungal 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
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 ect-endomycorrhizal 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, cytochrome oxidase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and starch hydrolyzing 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 redistribution of amide and amino nitrogen compounds in the cutting (22,58); and 4) promotion of cell wall extensibility and subsequent cell elongation via non-enzymatic reactions, or via the induction of hydrogen ion secretion and cell wall acidification which enhance the activity of cell-wall loosening enzymes (23).

IAA is apparently produced by some ectomycorrhizal fungi. Thimann (62), in 1935, reported a growth hormone produced by Rhizopus suinus. MacDougal and Dufrenchy (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 pericycle 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 conjugates, synthesized by the action of IAA oxidase, polyphenol oxidase (PPO), and possibly other enzymes (16,24). The lack of root primordium 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 separation of enzymes and reactants on account of cellular compartmentalization (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 oxidizing 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 (cellulases, 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, kinetin, and 6-benzyl adenine have not stimulated or prevented root initiation in stem cuttings (19). The influence of cytokinins in root initiation may depend upon the particular stage of initiation and the concentration (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) demonstrated 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*) *subtomentosus* and *Cenococcum graminiforme* produced pantothenic acid (51). Vitamins released by microorganisms 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-mycorrhizal 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 identification, 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). Tomazewski and Wojciechowska (65) showed that the culture filtrate of a mycorrhizal fungus, Suillus variegatus, may contain up to $2 \times 10^{-8}$ M IAA equivalent while growing in 0.05 percent NH$_4$NO$_3$ 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 concentration some 100 times higher. The mycelial filtrates must contain other substances besides auxin which facilitate the mycorrhizal association (65). Acetic 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, Shemakhanova (51) found that culture filtrates of four Boletus species promoted 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 physiological condition of the stock plant and treatment of the cuttings with
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 seasonal changes in plant development (19,22). The propagation of plants by cuttings should not be guided by calendar dates alone since the beginning 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 conversion to soluble sugars can cause poor rooting in early winter (22,46). Generally, after the first part of January, increasing levels of endogenous 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$_2$PO$_4$, 0.25 g (NH$_4$)$_2$PO$_4$, 0.15 g MgSO$_4$ • 7H$_2$O, 0.02 g sequestrine (NaFe-EDTA), 100 mg thiamine • HCl, 3.0 g malt extract, 10.0 g glucose, 20.0 g Bacto-agar, distilled H$_2$O 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 • HCl, 2.5 g glucose, no addition of malt extract or Bacto-agar); flasks containing the medium were sterilized by autoclaving at 121°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$_2$O, 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°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 following treatments on rooting were evaluated: 1) control (1:5 ratio of non-inoculated culture medium to standard rooting medium); 2) single mycelial 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 treatment); 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 H₂O). 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 MMW 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 H₂O 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 Juice¹, 0.60 g CaCO₃, 1.0 g sucrose, distilled H₂O 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 rooting 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 growth in this species occurs in May, cuttings taken during April were from the previous year's growth; cuttings for subsequent sampling periods (July, October, and December) were from growth produced in the spring of 1976.

¹Campbell 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 (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 opaque 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

2Jiffy 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 \text{ g } \text{KH}_2\text{PO}_4, 1.0 \text{ g } (\text{NH}_2)_2\text{SO}_4, 0.5 \text{ g } \text{MgSO}_4, 1.5 \text{ g peptone, } 100 \text{ ag thiamine} \cdot \text{HCl, } 15.0 \text{ g Bacto-agar, plus distilled } \text{H}_2\text{O to } 1000 \text{ ml, was autoclaved at } 121^\circ\text{C for } 15 \text{ minutes; after autoclaving, } 5.0 \text{ ag Benlate, } 50.0 \text{ ag Neomycin } \text{SO}_4 \text{ and } 50.0 \text{ ag Streptomycin } \text{SO}_4 \text{ 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 mycorrhizal inocula were added to the rooting medium. Also, a greater number of short lateral roots were observed on root systems of cuttings in inoculated 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. terrestris* 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 concentration 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 medium. Cuttings taken December 18, 1975, harvested April 9, 1976.

<table>
<thead>
<tr>
<th>Trtmnt. No.</th>
<th>Treatment</th>
<th>Rooting Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Untreated Control</td>
<td>% Rooting Root Vol.</td>
</tr>
<tr>
<td>2.</td>
<td><em>Pisolithus tinctorius</em></td>
<td>75</td>
</tr>
<tr>
<td>3.</td>
<td><em>Thelephora terrestris</em></td>
<td>75</td>
</tr>
<tr>
<td>4.</td>
<td><em>Corticium bicolor</em></td>
<td>45</td>
</tr>
<tr>
<td>5.</td>
<td><em>Laccaria laccata</em></td>
<td>70</td>
</tr>
<tr>
<td>6.</td>
<td><em>Rhizopogon vinicolor</em></td>
<td>65</td>
</tr>
<tr>
<td>7.</td>
<td><em>Lactarius sanguifluus</em></td>
<td>65</td>
</tr>
<tr>
<td>8.</td>
<td><em>Cenococcum graniforme</em></td>
<td>90</td>
</tr>
<tr>
<td>9.</td>
<td><em>Lactarius deliciosus</em></td>
<td>70</td>
</tr>
<tr>
<td>10.</td>
<td><em>Hebeloma crustuliniforme</em></td>
<td>70</td>
</tr>
<tr>
<td>11.</td>
<td><em>Amanita muscaria</em></td>
<td>55</td>
</tr>
<tr>
<td>12.</td>
<td><em>Tricholoma flavovirens</em></td>
<td>80</td>
</tr>
<tr>
<td>13.</td>
<td><em>Poria terrestris var. cyaneus</em></td>
<td>55</td>
</tr>
<tr>
<td>14.</td>
<td><em>Poria terrestris var. subluteus</em></td>
<td>95</td>
</tr>
</tbody>
</table>

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. vinicolor did not form mycorrhizal associations (i.e. actual infection) in the propagation bed supports the hypothesis that free living mycorrhizal fungi can stimulate
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>38.30a</td>
<td>18.75a</td>
<td>61.66a</td>
<td>22.33a</td>
<td>10.00ab</td>
<td>2.50a</td>
<td>0a</td>
<td>0a</td>
</tr>
<tr>
<td>Auxin-Control (10% JG)</td>
<td>23.30a</td>
<td>13.00a</td>
<td>76.66a</td>
<td>26.16a</td>
<td>45.00ab</td>
<td>15.33a</td>
<td>20.00ab</td>
<td>7.16ab</td>
</tr>
<tr>
<td>P. tinctorius (1:5 + JG)</td>
<td>83.30a</td>
<td>42.50a</td>
<td>93.33a</td>
<td>20.33a</td>
<td>20.00ab</td>
<td>8.33a</td>
<td>71.66fg</td>
<td>21.83bc</td>
</tr>
<tr>
<td>P. tinctorius (1:10)</td>
<td>51.60a</td>
<td>44.50a</td>
<td>93.33a</td>
<td>29.00a</td>
<td>38.33ab</td>
<td>14.16a</td>
<td>48.33cdef</td>
<td>15.50abc</td>
</tr>
<tr>
<td>T. terrestris (1:5 + JG)</td>
<td>58.30a</td>
<td>35.25a</td>
<td>95.00a</td>
<td>31.33a</td>
<td>20.00ab</td>
<td>7.83a</td>
<td>70.00fg</td>
<td>20.16bc</td>
</tr>
<tr>
<td>T. terrestris (1:10 + JG)</td>
<td>41.65a</td>
<td>36.75a</td>
<td>90.00a</td>
<td>33.33a</td>
<td>55.00ab</td>
<td>20.66a</td>
<td>51.66defg</td>
<td>13.33abc</td>
</tr>
<tr>
<td>T. terrestris (1:5)</td>
<td>100.00a</td>
<td>57.75a</td>
<td>93.33a</td>
<td>28.33a</td>
<td>40.00ab</td>
<td>13.66a</td>
<td>73.33fg</td>
<td>24.83c</td>
</tr>
<tr>
<td>P. tinc. (1:5) + T. terr. (1:5)</td>
<td>76.95a</td>
<td>45.50a</td>
<td>95.00a</td>
<td>29.83a</td>
<td>23.33ab</td>
<td>9.33a</td>
<td>71.66fg</td>
<td>19.83bc</td>
</tr>
<tr>
<td>R. vinicolor (1:5 + JG)</td>
<td>50.00a</td>
<td>29.75a</td>
<td>93.33a</td>
<td>30.50a</td>
<td>60.00ab</td>
<td>22.66a</td>
<td>63.33efg</td>
<td>21.16bc</td>
</tr>
<tr>
<td>R. vinicolor (1:10)</td>
<td>81.65a</td>
<td>48.75a</td>
<td>75.00a</td>
<td>25.33a</td>
<td>52.50ab</td>
<td>17.16a</td>
<td>58.33efg</td>
<td>15.16abc</td>
</tr>
<tr>
<td>R. vinicolor (1:10 + JG)</td>
<td>61.65a</td>
<td>37.25a</td>
<td>85.00a</td>
<td>27.83a</td>
<td>33.33ab</td>
<td>13.33a</td>
<td>40.00bcde</td>
<td>13.00abc</td>
</tr>
<tr>
<td>P. tinc. (1:5) + R. vin (1:5)</td>
<td>91.60a</td>
<td>53.50a</td>
<td>93.33a</td>
<td>29.50a</td>
<td>21.66ab</td>
<td>7.66a</td>
<td>78.33g</td>
<td>24.83c</td>
</tr>
<tr>
<td>T. terr. (1:5) + R. vin (1:5)</td>
<td>63.50a</td>
<td>36.50a</td>
<td>96.66a</td>
<td>27.83a</td>
<td>50.00ab</td>
<td>19.83a</td>
<td>46.66cdef</td>
<td>13.00abc</td>
</tr>
<tr>
<td>P. tinc. (1:5) + T. terr. (1:5) + R. vin. (1:5)</td>
<td>73.30a</td>
<td>39.25a</td>
<td>85.00a</td>
<td>23.83a</td>
<td>76.66b</td>
<td>25.66a</td>
<td>55.00efg</td>
<td>16.50bc</td>
</tr>
<tr>
<td>P. tinctorius (filtrate soak)</td>
<td>86.66a</td>
<td>33.50a</td>
<td>36.66ab</td>
<td>14.16a</td>
<td>23.33bc</td>
<td>7.83ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. tinctorius (filtrate drench)</td>
<td>80.00a</td>
<td>24.83a</td>
<td>46.66ab</td>
<td>15.83a</td>
<td>43.33cde</td>
<td>11.16abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. tinctorius (leachate soak)</td>
<td>86.66a</td>
<td>33.50a</td>
<td>100.00ab</td>
<td>26.16a</td>
<td>45.00ab</td>
<td>15.33a</td>
<td>20.00ab</td>
<td>7.16ab</td>
</tr>
<tr>
<td>P. tinctorius (leachate drench)</td>
<td>80.00a</td>
<td>24.83a</td>
<td>100.00ab</td>
<td>26.16a</td>
<td>45.00ab</td>
<td>15.33a</td>
<td>20.00ab</td>
<td>7.16ab</td>
</tr>
</tbody>
</table>

Comparison of treatment means by Student-Newman-Keuls's multiple range test: any means followed by a common letter are not significantly different at the .05 level.
Comparison of treatment means by Student-Newman-Keul's multiple range test: any values followed by the same 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>July</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>66.66ab</td>
<td>22.33a</td>
</tr>
<tr>
<td>Auxin-Control (10% JG)</td>
<td>76.66ab</td>
<td>26.16a</td>
</tr>
<tr>
<td>Pisolithus tinctorius (1:5)</td>
<td>93.33b</td>
<td>20.33a</td>
</tr>
<tr>
<td>P. tinctorius (1:10)</td>
<td>95.00b</td>
<td>31.33a</td>
</tr>
<tr>
<td>P. tinctorius (1:25)</td>
<td>68.33ab</td>
<td>19.83a</td>
</tr>
<tr>
<td>P. tinctorius (1:50)</td>
<td>56.66a</td>
<td>24.66a</td>
</tr>
<tr>
<td>P. tinctorius (1:100)</td>
<td>56.66a</td>
<td>21.83a</td>
</tr>
<tr>
<td>Thelephora terrestris (1:5)</td>
<td>93.33b</td>
<td>28.33a</td>
</tr>
<tr>
<td>T. terrestris (1:10)</td>
<td>91.66b</td>
<td>27.50a</td>
</tr>
<tr>
<td>T. terrestris (1:25)</td>
<td>81.66ab</td>
<td>27.50a</td>
</tr>
<tr>
<td>T. terrestris (1:50)</td>
<td>76.66ab</td>
<td>28.83a</td>
</tr>
<tr>
<td>T. terrestris (1:100)</td>
<td>71.66ab</td>
<td>28.33a</td>
</tr>
</tbody>
</table>
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 (*Sclerotium rolfsii* and *Fusarium oxysporum*) in the rooting medium. Cuttings taken October 19, 1976, harvested February 19, 1977.

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

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 treatments (except treatment numbers 10, 14 and 19) rooted significantly better than the auxin-control. The majority of the mycorrhizal treatments (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 limiting 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 apparently 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 sampling 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 sampling 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 sampling 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 = *Rhizopogon vinicolor* (1:5 + JG), B = *R. vinicolor* (1:10 + JG), C = Auxin-Control (10% JG).
and reduced aeration in the rooting medium; and, a build-up of saprophytic fungal populations (Fusarium spp., Penicillium 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 trilobate 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 transplanting 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 uninfected.

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 mycorrhizal 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Rooting</th>
<th>Root Vol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>25</td>
<td>0.38a</td>
</tr>
<tr>
<td>Auxin-Control (10% JG)</td>
<td>20</td>
<td>0.23a</td>
</tr>
<tr>
<td><em>Pisolithus tinctorius</em></td>
<td>60</td>
<td>1.08b</td>
</tr>
<tr>
<td><em>Thelephora terrestris</em></td>
<td>70</td>
<td>1.35b</td>
</tr>
<tr>
<td><em>Rhizopogon vinicolor</em></td>
<td>95</td>
<td>1.55b</td>
</tr>
<tr>
<td><em>Cenococcum graniforme</em></td>
<td>100</td>
<td>1.63b</td>
</tr>
<tr>
<td><em>Hebeloma crustuliniforme</em></td>
<td>50</td>
<td>1.10b</td>
</tr>
<tr>
<td><em>Poria terrestris var. subluteus</em></td>
<td>95</td>
<td>1.73b</td>
</tr>
</tbody>
</table>

* Rooting response based on 20 cuttings per treatment.

* All mycorrhizal treatments at a 1:5 inoculum dilution.

* 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 polyphonol, 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 beneficial 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 natural conditions, approximately three percent of the higher plants (including many woody ornamental species) are in association with ectomycorrhizal fungi (40). These fungi are beneficial 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 forming mycorrhizae with transplants having established root systems. Once the plants become mycorrhizal they should be more efficient in the nursery production system and better adapted to outplanting stresses.

Bearberry cuttings were harvested and propagated according to established 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 materials (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 government 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°C to 29°C, the optimum being approximately 26°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.
VII. LITERATURE CITED


29. Linderman, R.G. USDA Ornamental Plants Research Laboratory, Corvallis, Oregon 97330 (personal communication).


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°C. 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): NH₄NO₃, 1.65; H₂BO₃, 6.20; KH₂PO₄, 170.0; KI, 0.83; NaMoO₄ • 2 H₂O, 0.25; CoCl₂ • 6 H₂O, 0.025; CaCl₂ • 2 H₂O, 440.0; MgSO₄ • 7 H₂O, 370.0; MnSO₄ • H₂O, 22.30; ZnSO₄ • 7 H₂O, 8.60; CuSO₄ • 5 H₂O, 0.025; Na₂EDTA, 37.30; FeSO₄ • 7 H₂O, 27.80; thiamine • 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 omitted. The actively growing perimeter of the mycelial mat was sectioned into small pieces (approximately 75 mm³) 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. vinicoloi, 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 cytokinins. 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 observed 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.