Ethylene production by ectomycorrhizal fungi, *Fusarium oxysporum* f. sp. *pini*, and by aseptically synthesized ectomycorrhizae and *Fusarium*-infected Douglas-fir roots[1]

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The ectomycorrhizal fungi *Cenococcum geophilum*, *Hebeloma crustuliniforme*, and *Laccaria laccata* produced ethylene *in vitro* in modified Melin–Norkrans liquid medium only if amended with 2.5–10 m*M* methionine; *Pisolithus tinctorius* failed to produce ethylene unless the cultures were renewed with fresh methionine-amended medium before ethylene assay. An additional 19 ectomycorrhizal fungi, plus five isolates of *Fusarium oxysporum* f. sp. *pini*, all produced ethylene in renewed and (or) nonrenewed media. Although the rates varied, ethylene production by many ectomycorrhizal fungi equaled that of *Fusarium*.

Culture filtrates of *H. crustuliniforme* and *L. laccata* also evolved ethylene that was apparently of nonenzymatic origin.

Ethylene was produced by aseptically grown Douglas-fir seedlings inoculated with *C. geophilum*, *H. crustuliniforme*, and *L. laccata* and appearance of ethylene coincided with the formation of mycorrhizae; production by *P. tinctorius* inoculated seedlings was inconsistent. Lateral root formation of Douglas-fir was stimulated by inoculation with *C. geophilum*, *H. crustuliniforme*, and *L. laccata* but was inhibited by *P. tinctorius*. *Fusarium*-inoculated seedlings produced more ethylene sooner than seedlings inoculated with mycorrhizal fungi. The disparity in the levels of ethylene associated with ectomycorrhiza formation compared with *Fusarium* infection suggests a possible differential role for ethylene in symbiotic and pathogenic fungus–host interactions.

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Les champignons ectomycorhizateurs *Cenococcum geophilum*, *Hebeloma crustuliniforme* et *Laccaria laccata* ne produisent de l'éthylène *in vitro*, en milieu liquide Melin–Norkrans modifié, que si ce milieu est amendé avec 2,5 à 10 m*M* de méthionine; *Pisolithus tinctorius* ne parvient pas à produire de l'éthylène à moins que les cultures soient renouvelées avec du milieu amendé de méthionine fraîche avant l'essai d'éthylène. Un groupe additionnel de 19 champignons mycorhizateurs, plus cinq isolats de *Fusarium oxysporum* f. sp. *pini*, ont tous produit de l'éthylène dans ce milieu, qu'il soit renouvelé ou non. Bien que les taux ont varié, plusieurs champignons mycorhizateurs ont égalé les *Fusarium* dans la production d'éthylène.

De l'éthylène qui n'était apparemment pas d'origine enzymatique s'est aussi dégagé des filtrats de culture de *H. crustuliniforme* et *H. laccata*.

De l'éthylène a été produit par des plantules de sapin de Douglas croissant en milieu aseptique mais inoculées avec *C. geophilum*, *H. crustuliniforme* et *L. laccata*; l'apparition de l'éthylène coïncidait avec la formation des mycorhizes. La production d'éthylène par les plantules inoculées avec *P. tinctorius* s'est avérée inconsistante. L'inoculation des plantules de sapin de Douglas avec *C. geophilum*, *H. crustuliniforme* et *L. laccata* a favorisé la formation de racines latérales, alors que *P. tinctorius* l'a inhibée. Les plantules inoculées par *Fusarium* ont produit plus d'éthylène et plus hâtivement que celles qui furent inoculées par champignons mycorhizateurs. La disparité des niveaux d'éthylène associés à la formation d'ectomycorhizes, comparée à l'infection par *Fusarium*, suggère que l'éthylène a possiblement un rôle différentiel dans les interactions, symbiotiques et pathogéniques, hotes–champignons.

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Introduction

Ethylene, a natural plant growth regulator, is produced by a wide variety of soil-borne microorganisms including bacterial and fungal plant pathogens (Archer and Hislop 1975; Abeles 1973). Ethylene, evolved from soil and diseased plant tissues, has been implicated in a number of plant growth and development phenomena including inhibition of root elongation in waterlogged soil (Smith and Russell 1969) and development of disease symptoms such as petiolar epinasty (Dimond and Waggoner 1953), abscission (Williamson 1950), and early ripening of fruit (Freebairn and Buddenhagen 1964).

It is now well established that certain disease symptoms caused by *Fusarium* infection are due to ethylene (Gentile and Matta 1975). Bulbs infected with *Fusarium oxysporum* f. sp. *tulipae* evolve sufficiently high levels of ethylene to cause a number of physiological disorders in tulips (de Munk 1972). Swart and Kamerbeek (1976) reported that all *Fusarium* species and formae speciales they examined produced ethylene but *F. oxysporum* f. sp. *tulipae* produced the highest amounts, although isolates varied considerably.

Ethylene production by ectomycorrhizal fungi and mycorrhizae has not been examined, although a number of other plant hormones produced by ectomycorrhizal fungi, such as auxins and cytokinins, have been suggested as playing a key role in mycorrhiza formation (Slankis 1972, 1976). Apparently not all ectomycorrhizal fungi produced auxins (Shemakhanova 1962) or cytokinins (Miller 1971) *in vitro*, a situation which raises the question of the universality of their role in ectomycorrhiza formation. In view of the similarity in effects of ethylene and auxins on root growth and development (Abeles 1973; Zobel 1973) and the widespread capability of fungi for ethylene production (Ilag and Curtis 1968), the role of ethylene in ectomycorrhiza formation needs to be characterized and contrasted with its function in disease development.

We therefore conducted comparative studies on ectomycorrhizal fungi and *Fusarium oxysporum* Schlecht. f. sp. *pini* (Hartig) Synd. and Hans. to determine the ability of these fungi to produce ethylene *in vitro* and in aseptic synthesis culture with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

Materials and methods

Fungi

Cultures of ectomycorrhizal fungi (Table 1) were obtained from R. J. Molina, United States Department of Agriculture Forest Service, Corvallis, Oregon, and maintained on modified Melin–Norkrans (MMN) agar (Marx 1969). *Fusarium oxysporum* f. sp. *pini* was isolated from surface-sterilized and washed Douglas-fir seed and infected and dying Douglas-fir seedlings. Isolates were tested for pathogenicity on Douglas-fir (J. H. Graham and R. G. Linderman, unpublished data) and maintained on V-8 juice[2] agar (Toussoun and Nelson 1968).

Experiment 1: Ethylene production by ectomycorrhizal fungi and Fusarium

Culture preparation and handling

Ectomycorrhizal fungi, which had been grown in MMN liquid medium for 4–6 weeks, were prepared for experimental inoculations by aseptically filtering mycelium and washing and resuspending the mycelium in 150–200 ml of sterile-distilled water (sdw). The suspension was then homogenized in a Waring blendor for 5–20 s, the exact time period depending on the fungus treatment, and 6-mL aliquots of the mycelial slurry, equivalent to 6–10 mg dry weight of mycelium, was pipetted into replicate volume-calibrated 125-mL flasks containing 40 mL of MMN nutrient solution. *Fusarium oxysporum* f. sp. *pini*, which had been grown on MMN agar for 7–10 days, was prepared by suspending 8-mm agar plugs containing macro- and microconidia and chlamydospores in 20 mL of sdw and shaking vigorously for 1 min. One-millilitre aliquots of the spore suspension (10^4–10^5 spores/mL) plus 5 mL of sdw were pipetted into the test flasks. Uninoculated control flasks received an equivalent amount of sdw to give the same total liquid volume. All treatments were incubated in the dark at 20°C for the requisite time before ethylene was assayed.

For treatments requiring renewal of the culture medium, mycelial cultures were transferred to tubes and centrifuged at 12 000 × *g* for 15 min. The mycelial pellet was washed in sdw, recentrifuged, and transferred to a calibrated flask containing fresh medium and ethylene was assayed.

Ethylene assay

For the ethylene assay, flasks were flushed with sterile air in a laminar flow hood to remove any residual ethylene accumulated in the culture headspace, sealed with ethanol-sterilized serum stoppers, and incubated at 20°C in the dark. After 2 h, a 1-cm^3 gas sample was withdrawn and injected into a Perkin–Elmer 3920 gas chromatograph equipped with a flame-ionization detector and a 2.4-m Poropak N (80–100 mesh) column operated isothermally at 80°C. Ethylene was identified by cochromatography with an ethylene-in-air standard. The quantity of ethylene produced in cultures was calculated as the total volume of ethylene in the headspace of treatment cultures minus the volume of ethylene produced by the control medium. Fungal biomass was determined as oven-dry weight after 24 h at 70°C.

Characterization of culture ethylene production

The importance of methionine as a precursor for ethylene production was examined using ectomycorrhizal fungus species 1–4 (Table 1) by amending MMN liquid medium with 0, 2.5, 5.0, 7.5, and 10.0 m*M* DL-methionine. Five replicate samples of each fungus treatment were incubated in the dark at 20°C for 21 days before assaying for ethylene production. In subsequent experiments the MMN liquid medium was ammended with 5.0 m*M* methionine.

The time of maximum ethylene production for species 1–4 (Table 1) was determined by assaying for ethylene production at

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TABLE 1. Identification and origin of fungus isolates

Fungus species	No.	Origin	Host(s)
(1) *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quel.	S-260	Oregon	Pc*
	B-1	Oregon	Bp
	S-166	Oregon	Pm
(2) *Laccaria laccata* (Scop. ex Fr.) Berk. & Br.	S-167	Oregon	Pm
	S-282	Oregon	Pc, Pm
	S-283	Oregon	Pc, Pm
(3) *Pisolithus tinctorius* Coker & Couch	S-359	California	Pl
	S-360	California	Am, Ld, Pm
	S-210	Georgia	Pt
(4) *Cenococcum geophilum* Fr.	A-145	Oregon	Pm
	A-161	Oregon	Aa, Th
	A-150	Oregon	Pm
(5) *Rhizopogon vulgaris* (Vitt.) M. Lange	S-218	Oregon	Ag, Pm
(6) *R. villosulus* Zeller	S-249	Oregon	Pm
(7) *R. sepelibilus* Smith	S-278	Washington	Ag, Pm
(8) *R. ellenae* Smith	S-248	Oregon	Ag
(9) *R. subcaerulescens* Smith	S-268	Oregon	Ag, Pm
(10) *R. verisporus* Smith	S-280	Washington	Ag, Pm
(11) *R. abietis* Smith	S-221	Washington	Tm
(12) *Suillus brunnescens* Smith & Thiers	S-357	California	Pl
(13) *S. albidipes* (Peck) Singer	S-236	Oregon	Pc
(14) *S. tomentosus* (Kauffm.) Singer, Snell & Dick	S-313	Oregon	Ar, Ps, Pc
(15) *S. brevipes* (Peck) O. Kuntze	S-256	Oregon	Pp, Pm
(16) *S. ponderosus* Smith & Thiers	S-337	Oregon	Pm
(17) *S. subolivaceus* Smith & Thiers	S-269	Oregon	Th
(18) *S. lakei* (Murrill) Smith & Thiers	S-243	Oregon	Pm
(19) *Amanita pantherina* (D. C. ex Fr.) Schum.	A-168	Oregon	Pm, Th
(20) *A. muscaria* (Fr.) S. F. Gray	S-212	Oregon	Al, Pc, Tm
(21) *Cortinarius elegantior* (Fr. ex Fr.) Fr.	S-173	Europe	Unknown
(22) *Lycoperdon pyriforme* Persoon	S-305	Oregon	Pp
(23) *Calvatia fumosa* Zeller	S-391	Oregon	Al, Pm, Th
(24) *Fusarium oxysporum* Schlect f. sp. *pini* (Hartig) Synd. and Hans.	DF-1	Oregon	Pm
	DF-2	Oregon	Pm
	DF-4	Oregon	Pm
	DF-5	Oregon	Pm
	DF-7	Oregon	Pm

*Host abbreviations: Aa, *Abies amabilis*; Ag, *Abies grandis*; Al, *Abies lasiocarpa*; Ar, *Alnus rubra*; Am, *Arbutus menziesii*; Bp, *Betula pendula*; Ld, *Lithocarpus densiflorus*; Ps, *Picea sitchensis*; Pc, *Pinus contorta*; Pl, *Pinus lambertiana*; Pp, *Pinus ponderosa*; Pt, *Pinus taeda*; Pm, *Pseudotsuga menziesii*; Th, *Tsuga heterophylla*; Tm, *Tsuga mertensiana*.

0, 1, 2, 4, 6, 8, 14, and 21 days. Five replicate samples were assayed at each sampling time.

The effect on ethylene production of fungal metabolites accumulating in the culture medium during the incubation period was examined by comparing ethylene production in culture where the medium was renewed with the ethylene production in cultures of the same age but without culture renewal. For species 1–4 (Table 1) this comparison was made after 7, 14, and 21 days of incubation. For the remaining 27 ectomycorrhizal fungi and five isolates of *Fusarium oxysporum* f. sp. *pini* (Table 1) this comparison was made after 7 days. Assays were performed in five replicate samples.

Ethylene evolution from cell-free culture filtrates was determined for two ectomycorrhizal fungi, *Hebeloma crustiliniforme* (S-166) and *Laccaria laccata* (S-167). Ethylene production by fungus plus filtrate was assayed after 7 days of incubation. Cultures were then filtered (0.45-μm Millipore) to remove the mycelial fraction and one-half of the cell-free cultures was assayed immediately. To identify whether or not the source of ethylene was enzymic, the remaining one-half of the cell-free cultures was aseptically transferred to test tubes, incubated at 60°C for 1 h, and cooled to 20°C. The heat-treated filtrates were then transferred to flasks and assayed immediately. For further assay, cell-free culture treatments were incubated at 20°C and ethylene evolution was determined at 7 and 14 days.

Experiment 2: Ethylene production by ectomycorrhiza and Fusarium-*infected Douglas-fir*

Aseptic Douglas-fir seedlings were grown in large test tubes (200 mm × 32 mm) containing 60 cm^3 vermiculite and 5 cm^3 finely ground peat moss, mixed thoroughly, and moistened with 45 mL of MMN nutrient solution. The tubes were covered with 50-mL glass beakers and autoclaved at 121°C for 30 min.

Washed Douglas-fir seed was surface-sterilized in 30% H_2O_2 for 1 h and germinated on MMN agar. Germlings were transplanted into the culture tubes and the root portion of the tube was wrapped in foil to exclude light. To direct root growth along the side of the tube, cultures were incubated on a slant in a growth chamber under fluorescent–incandescent lights (250 $\mu E \cdot m^{-2} \cdot s^{-1}$ at 400–700 nm) set on a 16-h photoperiod and 25°C–15°C day–night temperature regime. After 1 month, healthy seedlings free of visible contamination were selected for inoculation.

To determine the production of ethylene by the fungus–host

seedling interaction, the same four ectomycorrhizal species examined in experiment 1 and one isolate of *F. oxysporum* f. sp. *pini* (DF-4) were chosen for study. Aseptic cultures with and without Douglas-fir seedlings were inoculated with 10 mL of blended mycelium of ectomycorrhizal fungi or 10 mL of *Fusarium* spore suspension prepared as described in experiment 1. Uninoculated controls received an equal volume of sdw.

For the ethylene assay, six culture tubes per treatment were sealed with ethanol-sterilized serum stoppers and incubated under growth chamber conditions. After 24 h, a 1-cm^3 gas sample was withdrawn from each tube and analyzed as described in experiment 1. The quantity of ethylene produced in the treatments was calculated as the concentration of ethylene (parts per million) in fungus–seedling cultures minus the concentrations in the respective fungus culture alone and seedling culture alone combined. Starting at time of inoculation, ethylene was assayed at 2-week intervals during a 14-week period using the same cultures at each assay time.

Mycorrhizal and control tree cultures were harvested at 16 weeks and total number of lateral roots less than 2 cm in length, number of mycorrhiza-mantled lateral roots, and seedling oven-dry weight (70°C for 24 h) were detemined. As a check, samples of culture medium were plated on MMN medium to confirm the absence of contaminating microorganisms. For confirmation of ectomycorrhiza formation, mycorrhizal tips were collected, fixed in 50% formalin – acetic acid – alcohol, embedded and sectioned in paraffin, and stained with safranin followed by fast green (Johansen 1940).

Results

Experiment 1: Ethylene production by ectomycorrhizal fungi and Fusarium

Cenococcum geophilum, *H. crustuliniforme*, and *L. laccata* produced ethylene in MMN liquid medium only if ammended with DL-methionine (Fig. 1). The levels of ethylene produced by *L. laccata* and *H. crustuliniforme* were similar and reached a maximum at 7.5 m*M* methionine; production by *C. geophilum* was 100 times lower, with a maximum at 2.5 m*M* methionine; *P. tinctorius* failed to produce ethylene at any methionine concentration tested.

Ethylene production levels peaked within 2–4 days after inoculation and by 21 days decreased to about 50% of the maximum level (Fig. 2). *Pisolithus tinctorius* only produced ethylene at the time of inoculation, suggesting that fungal metabolites accumulating in the medium over time might be inhibitory to ethylene production by this fungus.

When cultures were renewed with fresh medium before the ethylene assay, *P. tinctorius* was stimulated to produce ethylene at 7, 14, and 21 days after inoculation (Fig. 3). Culture renewal also significantly increased ethylene production by *C. geophilum* but decreased production by *H. crustuliniforme* and *L. laccata*.

All of the additional 27 ectomycorrhizal fungi tested in renewed and nonrenewed cultures produced ethylene (Figs. 4–6). Amounts of ethylene produced by three isolates each of *H. crustuliniforme* and *L. laccata* varied only slightly. All isolates of *P. tinctorius* required culture renewal to produce ethylene whereas isolates of *C. geophilum* differed in their requirement for culture renewal (Fig. 4).

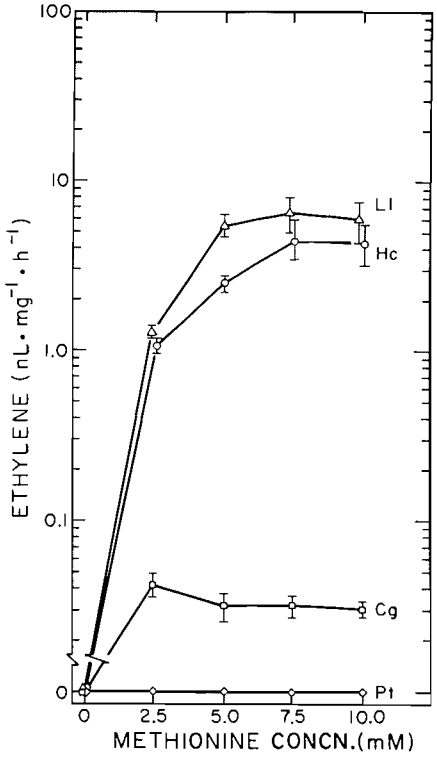

FIG. 1. Effect of methionine on ethylene production by ectomycorrhizal fungi grown in MMN liquid medium for 21 days. *Cenococcum geophilum* (Cg), *Hebeloma crustuliniforme* (Hc), *Laccaria laccata* (Ll), *Pisolithus tinctorius* (Pt). Error bars represent the standard deviation of the mean of five replications.

Suillus species also varied in their requirements for culture renewal for ethylene production (Fig. 5). *Rhizopogon* species all produced ethylene in nonrenewed media but varied in that two species (*R. versisporus*, *R. abietis*) showed increased production on culture renewal whereas the opposite effect was noted with the other five species listed.

Amanita pantherina and *Cortinarius elegantior* behaved like *C. geophilum* isolate A-145 in that culture renewal increased ethylene production. *Lycoperdon pyriforme*, *Calvatia fumosa*, and *Amanita muscaria* all required culture renewal for ethylene production to occur (Fig. 6).

The levels of ethylene production by isolates of *F. oxysporum* f. sp. *pini* were similar in both renewed and nonrenewed culture. In renewed culture, which is the more comparable estimate of ethylene production, nearly all ectomycorrhizal fungi produced more ethylene than *Fusarium*.

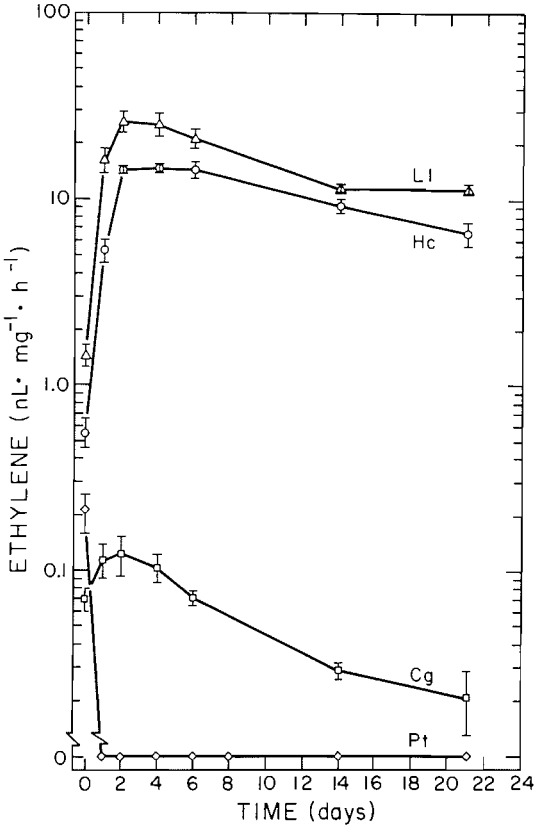

FIG. 2. Production of ethylene by ectomycorrhizal fungi during a 21-day period. Fungi were grown in 5 m*M* methionine amended MMN liquid medium. *Cenococcum geophilum* (Cg), *Hebeloma crustuliniforme* (Hc), *Laccaria laccata* (Ll), *Pisolithus tinctorius* (Pt). Error bars represent the standard deviation of the mean of five replications.

In many cases culture renewal decreased ethylene production, suggesting that the filtrate contributed to production. Ethylene evolved by cell-free culture filtrates of *H. crustuliniforme* and *L. laccata* represented a substantial proportion (>50%) of the total ethylene evolved by the fungal cultures (Table 2). Heating the filtrate at 60°C to nullify extracellular enzyme activity did not lower ethylene production.

Experiment 2: Ethylene production by ectomycorrhizae and Fusarium*-infected Douglas-fir*

The amount of ethylene produced by cultures of either Douglas-fir seedlings or the test fungi was minimal and ranged from 0 to 0.002 ppm within each treatment. Seedling cultures inoculated with ectomycorrhizal fungi or *F. oxysporum* f. sp. *pini* produced significantly more ethylene than the total of fungus alone and seedling alone cultures (Fig. 7). Time of ethylene production by seedlings inoculated with *C. geophilum*, *H. crustuliniforme*, and *L.*

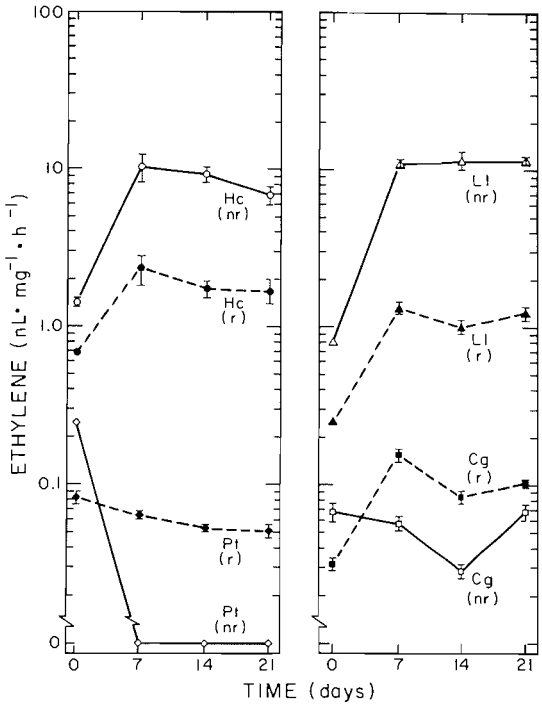

FIG. 3. Effect of renewal of the culture medium on ethylene production by ectomycorrhizal fungi during a 21-day period. Fungi were grown in 5 m*M* methionine amended MMN liquid medium. For renewed treatments the culture medium was replaced with fresh medium prior to ethylene assay. Nonrenewed (*nr*), renewed (*r*), *Cenococcum geophilum* (Cg), *Hebeloma crustuliniforme* (Hc), *Laccaria laccata* (Ll), *Pisolithus tinctorius* (Pt). Error bars represent the standard deviation of the mean of five replications.

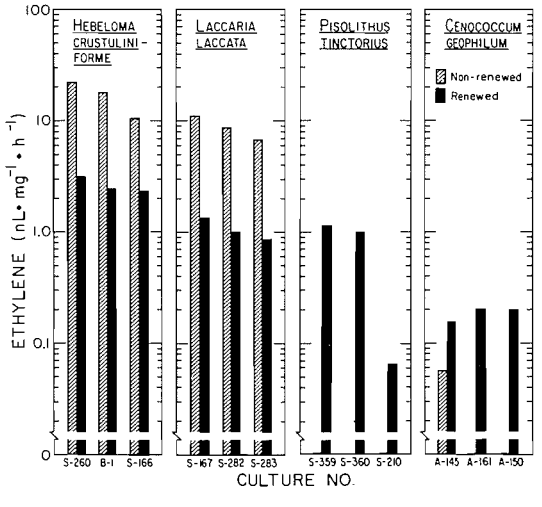

FIG. 4. Ethylene production by selected isolates of *Hebeloma crustuliniforme*, *Laccaria laccata*, *Pisolithus tinctorius*, and *Cenococcum geophilum*. Fungi were grown in 5 m*M* methionine amended MMN liquid medium for 7 days and ethylene assayed in renewed and nonrenewed cultures. Each ethylene level is the mean of five replications.

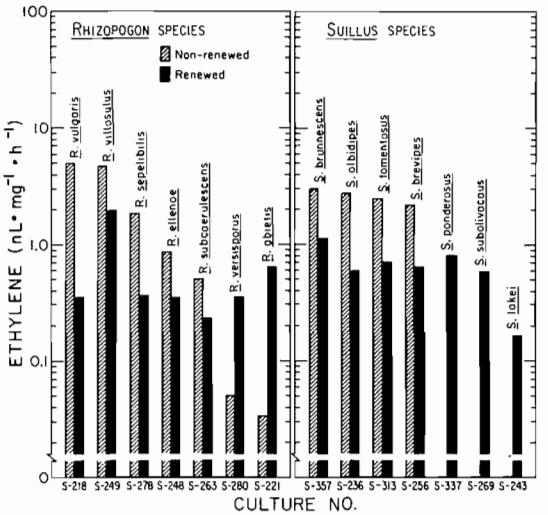

FIG. 5. Ethylene production by selected species of *Rhizopogon* and *Suillus*. Fungi were grown in 5 m*M* methionine amended MMN liquid medium for 7 days and ethylene assayed in renewed and nonrenewed cultures. Each ethylene level is the mean of five replications.

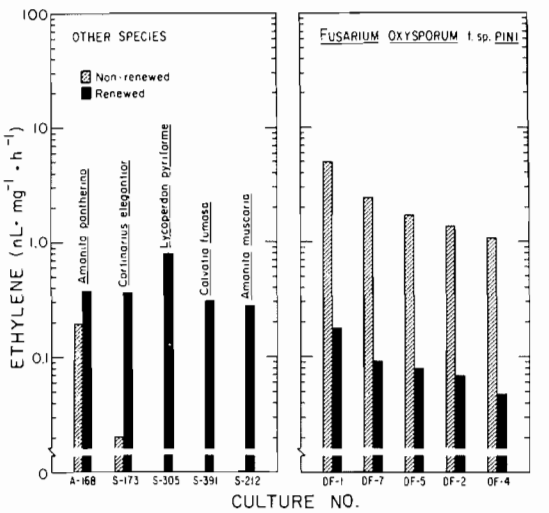

FIG. 6. Ethylene production by selected ectomycorrhizal fungi and isolates of *Fusarium oxysporum* f. sp. *pini*. Fungi were grown in 5 m*M* methionine amended MMN liquid medium for 7 days and ethylene assayed in renewed and nonrenewed cultures. Each ethylene level is the mean of five replications.

laccata coincided with the formation of mycorrhizal short roots as noted by visual observation through the glass tube. Production by *P. tinctorius* inoculated seedlings was inconsistent. *Fusarium*-inoculated seedlings produced more ethylene 1 week after inoculation than mycorrhizal fungus inoculated seedlings did at any time during the 14-week assay period. Ethylene production continued to increase up to 4 weeks after inoculation and was correlated with the colonization of the root cortex and development of stunting, chlorosis, and wilting of the top. After 4 weeks, ethylene production decreased as a result of seedling mortality.

Seedlings inoculated with the four different mycorrhizal fungi showed no differences in dry weight or percentage mycorrhizal formation but *C. geophilum*, *H. crustuliniforme*, and *L. laccata* stimulated, whereas *P. tinctorius* inhibited, lateral root formation compared with uninoculated controls (Table 3). Transverse sections of *Pisolithus*-mantled roots revealed the absence of a Hartig net whereas the other three fungi formed a Hartig net between one to three layers of cortical cells.

Discussion

Ectomycorrhizal fungi and *F. oxysporum* f. sp. *pini* required methionine for ethylene production in MMN liquid medium containing mineral salts and glucose. Glucose and methionine are the precursors for ethylene production by a number of fungi and bacteria (Lynch and Harper 1974; Chalutz *et al.* 1977; Primrose 1976*a*).

Several ectomycorrhizal fungi also required renewal of the culture medium to induce or enhance ethylene production, suggesting that fungal metabolites accumulating in the medium during incubation may be inhibitory. Problems in assessing microbial ethylene production in liquid cultures over extended incubation periods have been noted by other workers (Lynch and Harper 1974; Bonn *et al.* 1975). In our experiments the effect of fungal metabolites was minimized by assaying for ethylene production in fresh culture medium.

In many cases, culture renewal decreased ethylene production, an indication that the culture filtrate was a source of ethylene. Cell-free culture filtrates of *H. crustuliniforme* and *L. laccata* evolved substantial quantities of ethylene and contributed to over half of the ethylene produced by fungus plus filtrate. Ethylene from filtrates appeared to be of nonenzymatic origin because ethylene evolution was unaffected by a heat treatment that would nullify enzymatic activity.

Nonenzymatic release of ethylene from cell-free filtrates has been reported for both fungi and bacteria. Lynch (1974) suggested that *Mucor hiemalis* released flavinoid compounds into the culture medium which act as a cofactor in the photochemical conversion of methionine to ethylene (Yang *et al.* 1967). Primrose (1976*b*) identified several isolates of bacteria that exuded pigments with spectral properties of flavinoids into the culture medium. Cell-free filtrates from these cultures released ethylene more rapidly than filtrates from bacterial

TABLE 2. Comparison of ethylene evolution from cultures and cell-free culture filtrates of *Hebeloma crustuliniforme* (S-166) and *Laccaria laccata* (S-167)

Treatment	7 days*		14 days*		21 days*	
	S-166	S-167	S-166	S-167	S-166	S-167
Fungus and filtrate	43.4†	49.9	—	—	—	—
Filtrate	28.9	37.5	5.4	8.8	0	0
Heated filtrate	29.4	36.9	5.0	9.1	0	0
% contribution from filtrate‡	66.4	75.2	—	—	—	—

*Days after time of inoculation.
†Ethylene evolved in nanolitres per hour. Values are the mean of five replications.
‡Percentage contributions of filtrate to total ethylene evolved from fungus plus filtrate.

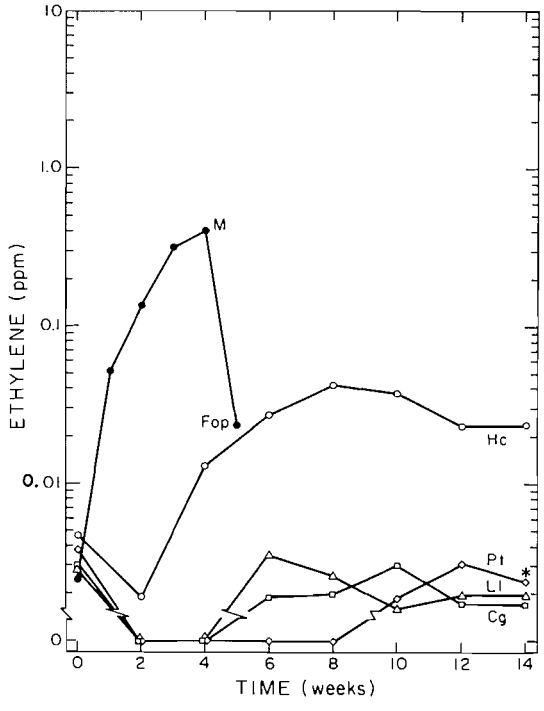

FIG. 7. Ethylene production by aseptically synthesized ectomycorrhizae and *Fusarium*-infected roots of Douglas-fir. *Cenococcum geophilum* (Cg), *Hebeloma crustuliniforme* (Hc), *Laccaria laccata* (Ll), *Pisolithus tinctorius* (Pt), *Fusarium oxysporum* f. sp. *pini* (Fop). M indicates time of seedling mortality in *Fusarium*-inoculated cultures. All ethylene levels greater than zero are significantly different from the combined levels in the respective fungus alone and seedling alone cultures at the 0.01 probability level except (*).

isolates not exuding pigment. Chalutz *et al.* (1977) reported that filtrates of *Penicillium digitatum* evolved ethylene by both enzymatic and nonenzymatic reactions. Boiling the filtrate or treating with protein-degrading enzyme reduced ethylene formation by over 50%. Tracer experiments indicated that the fungus took up methionine and released a metabolite which was then converted to ethylene. Chalutz *et al.* (1977) suggested that the ethylene-evolving systems in cell-free filtrates of fungi and bacteria may be similar but require further investigation.

Ethylene was also produced by aseptically synthesized ectomycorrhizae of Douglas-fir. Inoculation with *C. geophilum*, *H. crustuliniforme*, and *L. laccata* stimulated lateral root development on Douglas-fir but *P. tinctorius* inhibited formation. The absence of a Hartig net in *P. tinctorius* mantled short roots indicated that a functional mycorrhizal association was not formed (Marks and Foster 1972). Thus, the lack of a clear ethylene response may relate to the failure of *P. tinctorius* to form a true mycorrhizal association.

Our studies have shown that ethylene is produced by ectomycorrhizal fungi in culture and in the mycorrhizal association. Methionine, the precursor for ethylene production by ectomycorrhizal fungi and their culture filtrates, is in root exudate of ectomycorrhizal hosts such as *Pinus radiata* (Rovira 1965) and *Eucalyptus calophylla* (Malajczuk and McComb 1977). Thus, ethylene formation by mycorrhizal fungi in the rhizosphere or in mycorrhizal association may depend on a supply of methionine from plant roots.

Culture filtrates of mycorrhizal fungi and synthetic auxins are known to stimulate root initiation (Slankis 1972). However, ethylene has root growth promoting properties similar to auxins (Abeles 1973; Zobel 1973). We found that when a functional mycorrhizal association was formed, low levels of ethylene were produced and lateral root formation increased. These observations suggest that there is a relationship among ethylene, lateral root formation, and establishment of ectomycorrhizae.

Compared with mycorrhiza formation, the role of ethylene in *Fusarium* disease development has been well characterized (Gentile and Matta 1975; de Munk 1972). Douglas-fir seedlings infected with *Fusarium* produced much more ethylene than mycorrhizal seedlings. High levels of ethylene associated with *Fusarium* disease may function in

TABLE 3. Effect of ectomycorrhizal fungi on growth and development of Douglas-fir in aseptic culture

Fungal treatment	Seedling dry weight (g)	% mycorrhiza-mantled lateral roots	Lateral root count
Uninoculated control	0.33*a**	—	210*a**
Cenococcum geophilum	0.31*a*	47*a**	293*b*
Hebeloma crustuliniforme	0.33*a*	55*a*	358*b*
Laccaria laccata	0.32*a*	41*a*	287*b*
Pisolithus tinctorius	0.30*a*	65*a*	121*c*

*Comparison of means of seven replications by Duncan's multiple range test; column values followed by the same letter are not significantly different at the 0.01 probability level.

lowering the disease resistance of the host as shown with *Fusarium* wilt of tomato (Collins and Scheffer 1958).

The disparity in the levels of ethylene associated with mycorrhiza formation compared with *Fusarium* infection suggests a differential role for ethylene in symbiotic and pathogenic fungus–host interactions. Further comparative studies will examine the effect of ethylene on ectomycorrhizae and *Fusarium* disease of Douglas-fir in more detail.

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