Influence of Air Pollutant Gases on Oxygen Uptake of Pine Roots with Selected Ectomycorrhizae

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ABSTRACT

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Oxygen uptake was determined for root segments of greenhouse-grown loblolly pine seedlings, with and without ectomycorrhizae, following exposure to 50 and 500 μliters/m^3 O_3 or SO_2. Oxygen uptake was determined at 30-min intervals over a 3-hr period following a 1-hr exposure to each concentration of each gas. The root segments without ectomycorrhizae showed less O_2 uptake than those with ectomycorrhizae. The results indicate that development of ectomycorrhizal-root associations may afford some level of protection for the feeder-root system of loblolly pine trees when exposed to the concentrations of O_3 or SO_2 common in many areas subject to air pollution.

An aspect of environmental physiology and ecology which only recently has been investigated is the effects of certain environmental gases on the organisms which occupy the mycorrhizosphere. In areas with chronic high levels of atmospheric O_3 and SO_2, damage to indigenous tree species frequently is noted. This damage may result either from direct interference with normal plant metabolism or, more subtly, through upset of the critical balance existing within the rhizosphere. Such activity might include damage to the mycorrhizae system with resultant impairment of root physiological activity or decreased ability to fend-off soil pathogens.

One approach to investigate the influence of environmental gases on mycorrhizae-forming fungi was to determine any changes in O_2 uptake in root segments with and without the ectomycorrhizae association when exposed to low levels of the test gases. This paper presents the results of studies conducted to determine the influence of O_3 and SO_2 on O_2 uptake activity of loblolly pine seedling roots, with and without ectomycorrhizae, represented by selected isolates of *Thelephora terrestris* or *Pisolithus tinctorius*.

MATERIALS AND METHODS

Isolates of *Thelephora terrestris* (Erhr.) Fr. and *Pisolithus tinctorius* (Pers.) Coker and Couch were obtained from D. H. Marx, USDA-Forest Service, Southeastern Forest Experiment Station, Athens, GA 30601. Preparation of vermiculite-peat moss inocula of these isolates was followed as described by Marx and Zak (2).

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Loblolly pine (*Pinus taeda* L.) seedlings were propagated from seed which was surface-disinfested by immersion in 10% sodium hypochlorite for 5 min and washed with sterile distilled water before it was planted. The seeds were planted 1.5 cm deep in 4-liter plastic pots containing 3 liters of autoclaved potting medium (40-50 seeds per pot). The potting medium consisted of a homogenous mixture (1:1) of vermiculite and peat moss. Immediately following planting, the soil was saturated with sterile distilled water and each pot was covered with polyethylene film. Then the pots were placed in a Scientific Systems (Baton Rouge, LA 70800) walk-in environmental growth chamber at night/day temperatures of 20 and 31C, 65-75% relative humidity, and 13-hr photoperiods. Light intensity was maintained at approximately 87,000 lux.

The pots were inoculated with the ectomycorrhizae isolates 7 to 10 days after seedling emergence by first removing all of the seedlings from each of three pots and adding inoculum at a ratio of one part inoculum to nine parts medium, and replanting six seedlings in each pot. To prevent dual infections, all apparatus used in the inoculation procedure and work areas was sterilized after being used with each isolate. Ten otherwise similarly prepared noninoculated pots were used for the controls. While in the chamber, the seedlings were watered on alternate days with sterile distilled water and fertilized at 21-day intervals with 1 g/liter of sterile solution of commercial plant food (Rapid-Gro).

Ectomycorrhizae formed in the inoculated plants first were observed by stereomicroscopic examination of the root systems at 105 days after inoculation. The ectomycorrhizal root segments were excised from washed root systems exhibiting characteristic ectomycorrhizae structures at 120 days. The segments without mycorrhizae were taken from the first series of lateral roots appearing

below the root collar. Following excision from the seedling root system, the segments were washed in two changes of sterile distilled water and then placed in 20 ml of sterile Sorensen's pH 5.5 phosphate buffer until they were used in the O_2 uptake experiments. Ten 1.5-cm segments were selected and placed in single reaction vessels containing 2.8 ml of Sorensen's phosphate buffer. Oxygen uptake experiments were performed with 11 replicated vessels with or without ectomycorrhizae formed by *T. terrestris* or *P. tinctorius* and exposed to 50 and 500 μliters/m^3 O_3 or SO_2. Except for the exposure to gas, nonexposed control segments were treated identically.

Ozone produced by an Ozone Research Equipment Corp. (Phoenix, AR 85000) Model 03V92-AR ozonator was diluted with the ambient air in a growth chamber and the contaminated air was pumped from the chamber by a diaphragm-type pump (Dyna-Vac, Cole-Parmer, Chicago, IL 60600) through a single-tube flowmeter into the respirometer gassing manifold. The contaminated air was moved through the respirometer under slight positive pressure. During the exposure period, O_3 levels were monitored constantly at the gassing manifold by a McMillan Electronic Corp. (Houston, TX 77000) Model MECP 1100 Ozone Analyzer. The concentration of O_3 was held to within ± 5.0 μliters/m^3 of the desired levels. Sulfur dioxide was drawn from a pressurized cylinder of certified 99.98% SO_2 (Matheson Gas Products, Joliet, IL 60400) and dilution was accomplished by metering the gas at a flow rate of 0.12 liter/hr into a 7-liter carboy mixing chamber of dry air and used at a flow rate of 35.3 and 353.0 liter/min for 50 and 500 μliters/m^3, respectively.

All experiments were performed with a Gilson Medical Electronics (Middleton, WI 53562) Model GR14 Differential Respirometer at 26C for 4 hr. The first hour of each experiment was used for equilibration and exposure of the root segments to each test gas. Manometric readings then were made at 30-min intervals, a total of six measurements per replicate. The O_2 uptake was calculated and determinations of percent reduction in uptake activity were based on the difference between the exposed and nonexposed controls; all reduction values were calculated at the 180-min time period (3).

RESULTS

Exposure of nonmycorrhizal loblolly pine root segments to 50 and 500 μliters/m^3 demonstrated that these structures are highly susceptible to damage by that gas (Fig. 1). These segments showed a reduction of 75% in O_2 uptake activity following exposure to O_3 at 50 μliters/m^3 and 74% at 500 μliters/m^3. A tenfold increase in O_3 failed to cause appreciably more reduction in O_2

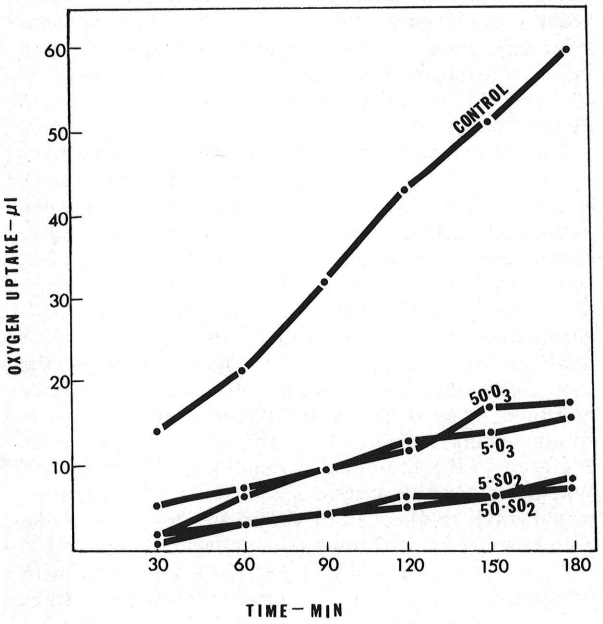

Fig. 1. Oxygen uptake in nonmycorrhizal loblolly pine root segments exposed to two concentrations of O_3 and SO_2 in pphm (1 pphm = 10 μliters/m^3).

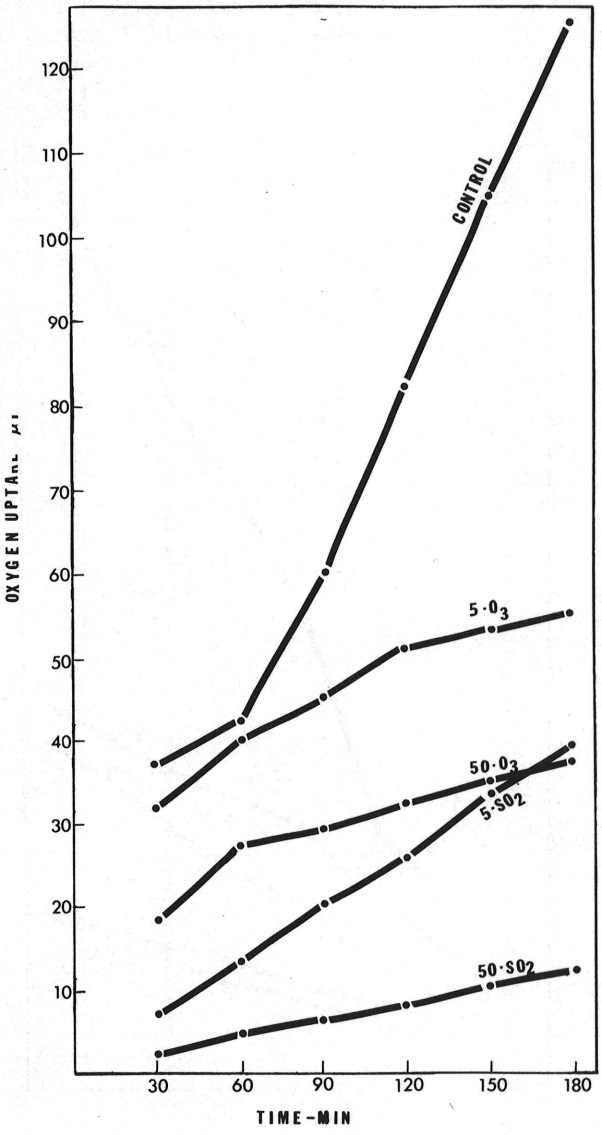

Fig. 2. Oxygen uptake in loblolly pine root segments with *Thelephora terrestris* ectomycorrhizae exposed to two concentrations of O_3 and SO_2 in pphm (1 pphm = 10 μliters/m^3).

uptake. The root segments exposed to 50 and 500 μliters/m^3 SO_2 each showed a 90% reduction in O_2 uptake. As observed with O_3, it appeared that a damage threshold limit also was evident with SO_2.

Nonexposed segments of roots with *T. terrestris* ectomycorrhizae were consuming O_2 at a level of 126 μliters at the 180-min time period (Fig. 2). When these root segments were exposed to 50 μliters/m^3 O_3 they underwent a reduction of 56% in O_2 uptake. At 500 μliters/m^3, O_2 uptake was reduced to 71%. The nonmycorrhizal roots showed a 75% reduction in O_2 uptake at 50 μliters/m^3, but the ectomycorrhizal roots exhibited only a 56% change indicated that some degree of protection was afforded by the ectomycorrhizae. At 500 μliters/m^3 there appeared to be only a slight difference in resistance between the two types of root segments, with segments of nonmycorrhizal and mycorrhizal roots showing O_2 uptake reductions of 74 and 71%, respectively. Root segments with *T. terrestris* ectomycorrhizae not exposed to SO_2 were consuming 117 μliters of O_2 at the 180-min time period. Following exposure to 50 μliters/m^3 SO_2, the level was reduced by 66%. At 500 μliters/m^3, the segments showed a reduction of 90%. The nonmycorrhizal root segments exposed to 50 μliters/m^3 SO_2 also showed a 90% reduction in O_2 uptake.

Nonexposed root segments with *P. tinctorius* ectomycorrhizae were consuming 109 μliters of O_2 at the 180-min time period (Fig. 3). At 50 μliters/m^3 O_3, O_2 uptake was reduced by 60% and at 500 μliters/m^3 by 81%. Ectomycorrhizal root segments formed by *P. tinctorius* appeared to be somewhat less resistant to the influence of O_3 than those formed by *T. terrestris*. Some protective influence was exerted by the presence of *P. tinctorius* at 50 μliters/m^3 O_3, but not at 500 μliters/m^3 when compared to the O_2 uptake by root segments without the ectomycorrhizae. Oxygen uptake by *P. tinctorius* ectomycorrhizal root segments also was influenced by exposure to 50 and 500 μliters/m^3 SO_2. The nonexposed controls were consuming 94 μliters of O_2 at the 180-min time period; the segments exposed to 50 μliters/m^3 SO_2 showed a 55% reduction. At 500 μliters/m^3, a reduction of 78% was recorded. This compared to 60 and 81% reductions with the nonmycorrhizal root segments exposed to 50 and 500 μliters/m^3 SO_2, respectively.

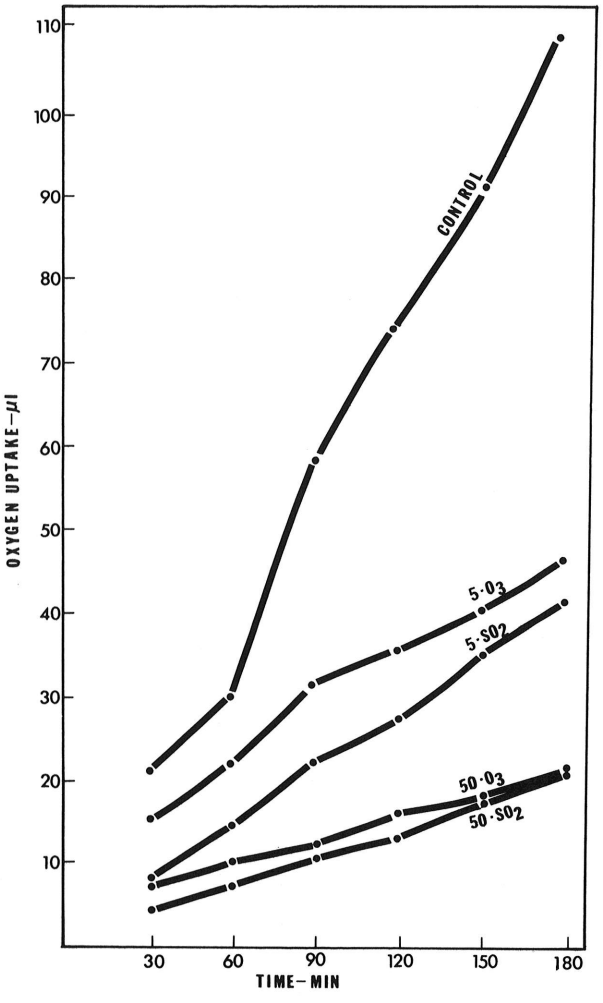

Fig. 3. Oxygen uptake in loblolly pine root segments with *Pisolithus tinctorius* ectomycorrhizae exposed to two concentrations of O_3 and SO_2 in pphm (1 pphm = 10 μliters/m^3.

DISCUSSION

Oxygen uptake by loblolly pine root segments without ectomycorrhizae was seriously affected by O_3 exposures of 50 and 500 μliters/m^3. However, SO_2 appeared to be more inhibitory to these structures than O_3. There appeared to be a threshold level at which further increases in gas concentration did not show further reduction in O_2 consumption. A similar effect was noted by Harley and Aprees (1) following addition of the metabolic inhibitors sodium azide and dinitrophenol to beech (*Fagus sylvatica*) roots without ectomycorrhizae. Tenfold increases in molar concentration of these inhibitors above 10^{-3} and 10^{-4}M failed to cause further reductions in respiratory activity.

The high level of O_2 uptake inhibition following exposure of loblolly pine root segments with ectomycorrhizae to O_3 and SO_2 may indicate a possible mechanism related to effects observed on plant foliage during chronic exposures to these same gases. Should newly developed feeder roots be exposed to these gases, they would be unable to develop mycorrhizae. Slankis (4) demonstrated that mycorrhiza formation is dependent upon the specific physiological conditions existing in the root, and that these associations will not develop in either moribund or dead tissues. Inevitably, exposure of roots without ectomycorrhizae to O_3 and SO_2 could result in an alteration in the physiological condition of these tissues. Whether root death or serious injury to the root segments would have resulted from the exposures used in the present study is not known.

Exposure of loblolly pine root segments with ectomycorrhizae provided evidence that these structures were more resistant to the deleterious influences of O_3 and SO_2 than were the roots without ectomycorrhizae. Root segments with *T. terrestris* ectomycorrhizae were more resistant to O_3 than those without the mycorrhizae.

Ectomycorrhizal root segments showed 19% greater O_2 uptake at 50 μliters/m^3 than did the segments without ectomycorrhizae, but only a 3% difference was noted at 500 μliters/m^3. Exposure of similar segments to SO_2 demonstrated 24% greater O_2 uptake at 50 μliters/m^3 in the presence of the fungus symbiont, but no difference in uptake was found at 500 μliters/m^3.

Root segments with *P. tinctorius* ectomycorrhizae appeared to react to O_3 in a manner similar to that for *T. terrestris* at the 50 μliters/m^3 level but the former were less resistant at the 500 μliters/m^3 level. With SO_2, *P. tinctorius* ectomycorrhizal segments showed 48% greater O_2 uptake than the segments without ectomycorrhizae at the 50 μliters/m^3 level, but only 12% at 500 μliters/m^3.

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