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INTERACTION OF OZONE EXPOSURE AND *Fusarium subglutinans* INOCULATION ON GROWTH AND DISEASE DEVELOPMENT OF LOBLOLLY PINE SEEDLINGS

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Abstract

*Seedlings from ten half-sib families of loblolly pine (*Pinus taeda*) were exposed in open-top chambers to carbon-filtered air (CF), non-filtered air (NF), or air amended with ozone to 1·7 or 2·5 times ambient. After 105 days of exposure, half the seedlings within each family were wounded but not inoculated and half were wounded and inoculated with the pitch canker fungus,* Fusarium subglutinans, *to which five families were relatively resistant. After an additional 50 days of ozone treatment, seedling growth and canker development were recorded. Cankers were significantly ($\sigma \leq 0{\cdot}05$) smaller among resistant compared to susceptible families, and were significantly larger among seedlings receiving the highest (2·5) compared to the ambient (NF) ozone treatment. The wound scars of non-inoculated seedlings were also significantly larger among seedlings receiving the 2·5 compared to the NF treatment, but these dimensions did not differ significantly with seedling family or resistance. The weights of needles and large roots were significantly smaller at the 2·5 compared to the 1·7 ozone treatment for inoculated but not for non-inoculated seedlings; this resulted in a significant interaction for ozone and inoculation effects. Among resistant families, root weights were significantly smaller for inoculated seedlings. Diameter growth and dry weights of needles were significantly smaller among inoculated compared to non-inoculated seedlings, but did not differ between NF and 2·5 ozone treatments.*

INTRODUCTION

Ozone is the most important and widespread air pollutant affecting agriculture (Heagle, 1973) and forests in the USA (USEPA, 1986). Its role in the decline of white pine (*Pinus strobus* L.) has long been known (Berry & Ripperton, 1963; Linzon, 1960), and the effects of ambient exposure doses on southern pines have been demonstrated (Kress & Skelly, 1982). In pine needles exposed to ozone, respiration is increased and photosynthesis is reduced (Barnes, 1972) and chronic exposure, even at doses which produce no acute symptoms, can alter plant structure in ways that affect fitness. Premature needle loss is a common symptom of chronic exposure (Berry & Hepting, 1964; Linzon, 1961). Loss of needles affects both stored energy and early-season photosynthesis, which is important to height growth (Allen, 1965; Ursino *et al.*, 1968). Ozone at near ambient levels affects both the ratio of above- to below-ground biomass and the percentages of roots with mycorrhizae (Qiu *et al.*, in press) in loblolly pine (*P. taeda* L.). Such structural and physiological changes are likely to affect the susceptibility to biotic pathogens.

Fusarium subglutinans, the causal agent of pitch canker, is an important pathogen of loblolly and several other pine species. It affects seed production (Blakeslee *et al.*, 1987*b*; Kelley & Williams, 1982), growth of seedlings in nurseries (Barnard & Blakeslee, 1980), and trees throughout their development (Schmidt & Underhill, 1974; Arvanitis *et al.*, 1980; Blakeslee *et al.*, 1987*a*). Resistance varies quantitatively between (Barrows-Broaddus & Dwinell, 1983; Dwinell, 1978) and within susceptible pine species (Blakeslee & Rockwood, 1978; Dwinell & Barrows-Broaddus, 1978; Dwinell *et al.*, 1977). Observed differences in virulence among isolates of *F. subglutinans* have also been quantitative (Dwinell, 1978), and apparently are not directly related to host genotype.

Interactions of ozone and biotic diseases have varied among the few tree diseases studied (Smith, 1990). For example, exposure of cottonwood leaves that were otherwise maintained in carbon filtered air to 0·20 ppm ozone for 5 h beginning 40 h before inoculation decreased spore production of an obligate (*Melamspora medusae* Thum.) (Coleman *et al.*, 1988) but not a facultative (*Marsonia brunnea* (Ell. et Ev.) P. Magn.) foliar pathogen (Coleman *et al.*, 1987). Exposing *P. sylvestris* L. to 0·20 ppm ozone for 6 h 5 days before inoculation increased the numbers of needle lesions of *Scirrhia acicola* (Dearn.) Sigg. compared to an equal exposure 30 min after inoculation (Weidensaul & Darling, 1979). Impacts of ozone exposure vary among hosts and for various aspects of disease development by a single pathogen within a host. For example, when seedlings were exposed to either CF air or ozone at 0·22 or 0·45 ppm for 12 h per day root colonization by *Heterobasidion annosum* (Fr.) Bref. increased with ozone exposure for both *P. jeffreyi* Grev. & Balf. and *P. ponderosa* Laws, seedlings, but inoculation success increased significantly only for *P. jeffreyi* (James *et al.*, 1980*a*).

Because ozone concentrations tend to be similar over large areas it is difficult to assess its effects on plants and their diseases in the field (Heagle, 1973; Smith, 1990). Differences in severity of ozone symptoms have been assumed to indicate differences in ozone impacts within and between areas. Although this serves as a practical approach to field surveys numerous factors contributing to individual tree vigor can obscure the true effects of ozone. In a California study, the growth rate of *H. annosum* in freshly cut stumps of *P. ponderosa* and *P. jeffreyi*, into which it was inoculated, was proportional to the severity of oxidant injury symptoms in the trees before felling (James *et al.*, 1980*b*). Although faster growth in oxidant-stressed trees would increase the rate of infections in stands and make the fungus generally more abundant (James & Cobb, 1988), the incidence of *H. annosum* in North Carolina stands of *P. strobus* did not appear to be associated with differences in oxidant symptoms (Leininger, 1989).

The objectives of this study were to determine the effects of ozone on the development of pitch canker and to determine the combined effects of the fungal pathogen and ozone on the host plant.

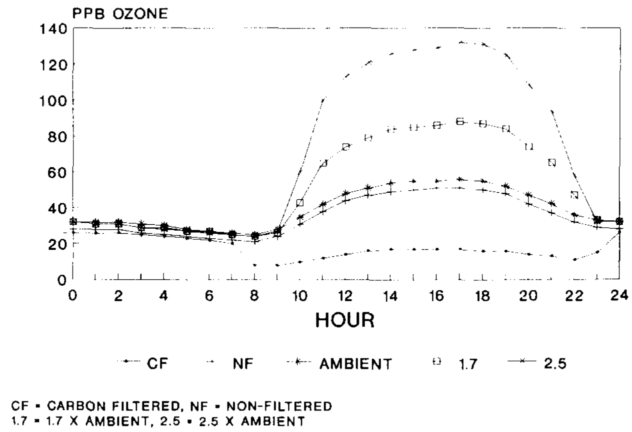


Fig. 1. Hourly means by treatment for the 1990 growing season from May to November.

MATERIALS AND METHODS

Seedlings

Seedlings from ten half-sib families of loblolly pine were used in this study. In seed orchard tests, relative pitch canker resistance had been determined for the mature, maternal parents of six of the families (Kelley & Williams, 1982). These six families were designated as source 'SO' (Seed Orchard). Relative resistance for the other four families was determined for half-sib seedlings and immature trees (from progeny tests, B. Runion, Personal communication) and these were designated as 'PT' source seedlings. One half of the families from each group were rated as resistant, but relative differences in resistance between groups were unknown.

Recently-emerged seedlings were transplanted (between March 23 and April 4 1990) to separate 6·35 cm diameter by 51 cm deep pots containing an autoclaved forest soil amended with a fresh commercial preparation of *Pisolithus tinctorius* ((Pers.) Cok & Couch) to insure uniform mycorrhizal inoculation. Seedlings were fertilized (complete fertilizer with micronutrients) on April 16 and June 8. Within families, seedlings were randomly distributed among ozone (O_3) treatments.

Ozone treatment

The ozone treatments and characteristics of the field site have been described in detail (Chappelka *et al.*, 1989). The concentrations of ozone used in this study cause quantitative (plant weights) and, in sensitive genotypes, qualitative (needle flecking) differences in loblolly pine seedlings among treatments (Qiu *et al.*, in press). Beginning June 20, ozone was administered 12 h per day (0900–2059 hours) in 4·8 m diameter × 4·5 m tall cylindrical open-top chambers (OTCs) using a single pass system (Heagle *et al.*, 1973). Treatments were: air filtered through charcoal (CF); nonfiltered air (NF); and nonfiltered air in which the ozone level was increased to 1·7 or 2·5 times that of ambient. Ozone treatments are characterized as hourly means, cumulative doses, and 24 h averages per month, respectively, in Figs 1, 2 and 3. Two OTCs were used for each ozone treatment. Chambers received simulated acid rain (pH 5·3) twice weekly, at a rate based on historic averages, and each seedling received an additional 100 ml of tap water twice weekly.

Each chamber also contained four three-year-old pines from another study. Seedlings were placed around these pines to receive maximum exposure to sunlight. Within chambers, seedling positions were shifted weekly to minimize position effects.

Inoculation

Seedlings were inoculated after 68 days of exposure to ozone (August 27). Inoculum was prepared using the

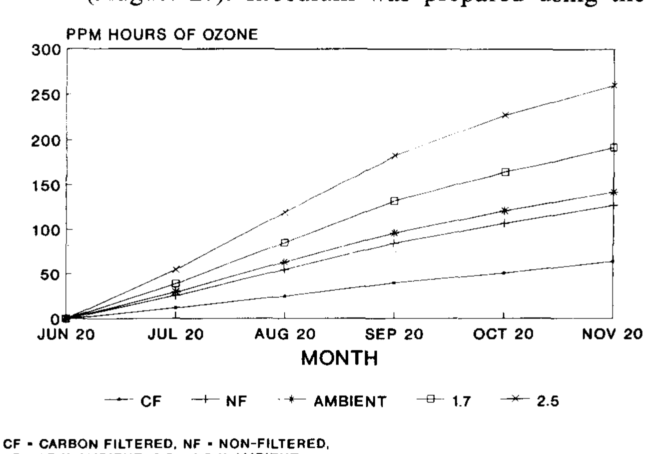


Fig. 2. Cumulative ozone dose by treatment and month for seedlings exposed beginning June 20, 1990.

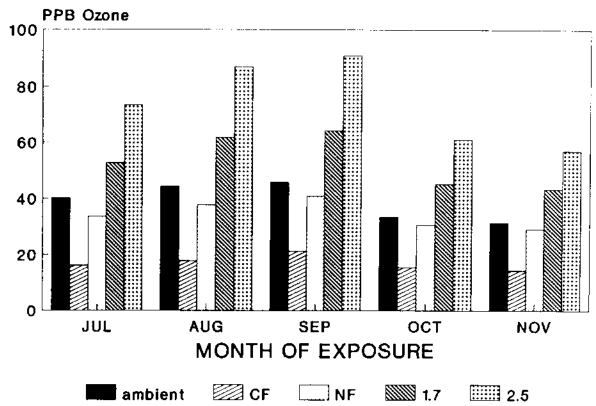


Fig. 3. Ozone concentrations (24 h) by month for open-top chambers during seedling exposure.

technique of Kelley & Williams (1982) from two *F. subglutinans* isolates recently reisolated from loblolly seedlings. Conidia from each of the two isolates were suspended in sterile water, suspensions were combined and concentrations were adjusted to approximately 15 000 spores per ml with sterile water. One-half of the seedlings per family in each OTC were randomly assigned for inoculation with conidia and half to inoculation with sterile water. Seedlings were wounded by removing a secondary needle fascicle from within 2 cm of the terminal bud and a drop of conidial suspension or sterile water was immediately placed on the wound. Seedlings were then misted with sterile water and maintained at 100% relative humidity and 20°C for 24 h before being returned to their respective OTCs. Ozone treatments were then continued for another 50 days.

Disease development

Seedlings were removed from OTCs on November 20. Lengths and widths of affected areas around inoculation wounds were recorded. Most inoculations with *F. subglutinans* resulted in small (4–6 mm in length) cankers delimited by callus ridges but in a few seedlings the fungus expanded without apparently eliciting a host response producing, in young loblolly, a slightly sunken, macerated area termed a diffuse canker. If the maximum extent of a diffuse canker appeared to be below the surface some tissue was scraped away to better estimate the canker margins. A few non-inoculated wounds also produce raised, callus ridges but most healed without them. Where cankers were less than approximately 3 mm in length but bordered by callus ridges the surface of the canker was excised and the canker recorded as infected if the tissues appeared water soaked. Diffuse cankers and callus formations were recorded. Canker tissues from a randomly selected subsample of seedlings receiving each treatment were transferred to selective media (Agrawal *et al.*, 1973) to confirm the presence of *F. subglutinans*.

Seedling growth

Seedling heights and diameters were recorded before, during, and after ozone treatments (at 0, 68 and 156 days of treatment). After recording seedling and canker dimensions, seedlings were separated into roots, stems and needles. Roots, stems and needles were pooled by family, OTC and inoculation treatment and oven dried. Fine roots were removed from larger roots after drying and oven-dry weights of stems, needles, and large and fine roots were recorded.

Design and analysis

The four levels of ozone and inoculated and non-inoculated seedlings produced eight treatment combinations. Each ozone treatment was replicated in two OTCs and half the seedlings per family per OTC were inoculated with conidia of *F. subglutinans*. In all, 18 seedlings (nine in each OTC) from the six families for which resistance had been determined for maternal parents (SO families) were exposed at all four ozone treatments ($6 \times 4 \times 18 = 432$ seedlings); the limited numbers of seedlings from the other four families (PT families) were divided equally between NF and 2·5 treatments ($4 \times 2 \times 18 = 144$ seedlings) selected as representative, respectively, of current conditions in Alabama or near Los Angeles, California. Analyses for all four ozone treatments were restricted to the six families receiving all treatments, and analyses for all ten families were restricted to the NF and 2·5 ozone levels. In addition to the effects of ozone, inoculation, and resistance to pitch canker, the data for all ten families were tested for differences associated with family source; that is, among the four PT and the six SO source families. Variables were averaged by chamber, family and inoculation treatment and analyzed using SAS ANOVA (except for analyses including effects of resistance by source for all ten families, which used SAS GLM).

RESULTS AND DISCUSSION

Effects of ozone and inoculation on seedling growth

Our hypothesis was that ozone would affect disease development indirectly by impacting seedling physiology. Because susceptibility to facultative parasites often increases with plant stress, canker size should be inversely proportional to ozone-induced reductions in seedling growth. Canker dimensions and seedling growth compared among ozone treatments was expected to indicate that ozone was a simple stress factor (a toxic substance).

Seedling dimensions

Seedling heights and diameters were the only variables measured before, during and after treatment. Mean seedling height averaged over all treatments increased from 11·70 cm to 19·05 cm (63% increase) during the 67 days of ozone treatment before inoculation (June 20 to Aug 27); but height increased only 1·50 cm in the next 50 days. For *P. taeda*, this is normal phenology. Height growth for the first year is typically 90% complete by August for seedlings planted when these were planted (Boyer & South, 1989). The timing of treatment application combined with the normal pattern of height growth limited the scope for treatment effects.

Table 1. Effects of ozone exposure, purported pitch canker resistance, and inoculation with *F. subglutinans* on dimensions of loblolly seedlings inoculated 50 days before the end of 155 days of ozone exposure

Variable	Level	Response[a]			
		Height (CM)		Diameter (MM)	
		Final	Growth	Final	Growth
Six families at four ozone levels					
Ozone	CF	20·95a	1·54a	3·95ab	1·13a
	NF	20·40a	1·42a	4·05a	1·15a
	1·7	20·62a	1·60a	3·87b	0·98b
	2·5	20·32a	1·56a	3·89b	1·12a
	LSD	0·93	0·33	0·13	0·12
Inoculation	Water	20·45a	1·51a	3·91a	1·07a
	Conidia	20·69a	1·56a	3·97a	1·12a
	LSD	0·66	0·09	0·23	0·09
Resistance	Resistant	21·21a	1·77a	3·92a	1·13a
	Susceptible	19·93b	1·30b	3·96a	1·07a
	LSD	0·66	0·23	0·07	0·09
Ten families at two ozone levels					
Ozone	NF	20·78a	1·74a	4·14a	1·22a
	2·5	20·77a	1·64a	4·03a	1·19a
	LSD	0·54	0·24	0·12	0·12
Inoculation	Water	20·69a	1·71a	4·02a	1·14a
	Conidia	20·85a	1·67a	4·15b	1·27b
	LSD	0·54	0·24	0·12	0·12
Resistance[c]	Resistant	20·82a	1·71a	4·04a	1·23a
	Susceptible	20·73a	1·61a	4·13a	1·17a
	LSD	0·54	0·24	0·12	0·12
Source	Progeny	21·40a	1·98a	4·26a	1·30a
	Seed orchard	20·36b	1·49b	3·97b	1·14b
	LSD	0·65	0·29	0·14	0·13

[a] Dimensions followed by the same letter are not significantly different (α = 0·05 SAS ANOVA for 4 × 6 and GLM for 2 × 10).
[b] NF, not filtered; CF, carbon filtered; 1·7 = 1·7 × ambient; 2·5 = 2·5 × ambient.
[c] SAS determined a significant interaction for the variables' resistance and source.

Seedling heights differed significantly among families, both before and after treatments (data not presented) but not for either ozone or inoculation treatments (Table 1). Unlike height, diameter increased uniformly throughout the growing season. Mean diameter increased at an average rate of approximately 0·02 mm per day, both before and after inoculation, increasing from 1·63 mm to 2·85 mm by August 27 and to 3·94 mm by November 23. For the 4 ozone by 6 family comparison, diameters and diameter growth differed significantly among ozone treatments, seedling heights differed significantly with purported family resistance, and no dimension differed significantly for inoculation with *F. subglutinans* (Table 1).

Seedling dimensions for the 2 ozone by 10 family comparison also are presented in Table 1. Dimensions did not differ between ozone treatments. Diameter growth after inoculation was significantly larger among inoculated seedlings. Significant interactions were determined for purported resistance and family source (the models for the statistics in Table 1 did not include interaction). Separated by family source, final seedling heights were larger and diameters smaller for resistant compared to susceptible families from both PT and SO sources. Unfortunately, the seedling sources were obtained from different companies, and their differences in growth cannot be precisely ascribed.

Although slowing stem growth (particularly height) as a function of normal seedling phenology after August should reduce differences between treatments, significant near-term differences may have been missed by the timing of harvest. Once a canker girdles a seedling, neither the width nor distal extent of the canker can be accurately determined. To avoid this, all seedlings were sampled when a small number of cankers had recently girdled their hosts. This action maximized near-term differences for cankers but reduced potential seedling-growth-impact which would result from top death. Stem dieback and mortality are not uncommon with seedling inoculations (Blakeslee & Rockwood, 1978; Dwinell, 1978), and could have increased the measured impact of disease on stems.

Seedling mass

Normal seedling phenology and the timing of treatment applications predisposed that root mass more than stem mass would be sensitive to the effects occurring after inoculation. Although stem growth normally slows during the period that the treatments were applied (the fall), root mass continues to increase (Boyer & South, 1989).

When component masses of inoculated and non-inoculated seedlings were analyzed together, those masses which differed significantly among ozone treatments (needles and large roots) also differed significantly with

Table 2. Effects of ozone exposure, purported pitch canker resistance, and inoculation with *F. subglutinans* on weights of loblolly seedlings inoculated 50 days before the end of 155 days of ozone exposure

Variable	Level	Response (g dry wt)[a]					
		Total	Leaf	Stem	Roots	Fine	Large
Six families at four ozone levels							
Ozone[b]	CF	4·94a	1·35a	1·07a	2·53a	1·43a	1·09a
	NF	4·72a	1·23cb	1·03a	2·45a	1·48a	0·97cb
	1·7	4·92a	1·30ab	1·04a	2·58a	1·53a	1·05ab
	2·5	4·60a	1·20c	1·01a	2·39a	1·46a	0·93 c
	LSD	0·33	0·10	0·08	0·24	0·21	0·10
Inoculation	Water	4·89a	1·32a	1·04a	2·53a	1·47a	1·06a
	Conidia	4·70a	1·22b	1·04a	2·45a	1·48a	0·97b
	LSD	0·23	0·07	0·05	0·17	0·15	0·07
Resistance	Resistant	4·89a	1·28a	1·04a	2·37b	1·42a	0·95b
	Susceptible	4·69a	1·26a	1·03a	2·60a	1·53a	1·07a
	LSD	0·24	0·07	0·05	0·17	0·15	0·07
Ten families at two ozone levels							
Ozone	NF	5·03a	1·33a	1·08a	2·61a	1·54a	1·07a
	2·5	4·97a	1·26a	1·06a	2·63a	1·60a	1·04a
	LSD	0·31	0·08	0·05	0·23	0·2	0·09
Inoculation	Water	5·20a	1·40a	1·07a	2·74a	1·60a	1·14a
	Conidia	4·79b	1·20b	1·08a	2·52a	1·55a	0·97b
	LSD	0·31	0·08	0·05	0·23	0·20	0·09
Resistance	Resistant	4·92a	1·32a	1·04b	2·56a	1·52a	1·04a
	Susceptible	5·07a	1·28a	1·10a	2·69a	1·63a	1·07a
	LSD	0·31	0·08	0·05	0·23	0·20	0·09

[a] Weights followed by the same letter are not significantly different ($\alpha = 0{\cdot}05$ SAS ANOVA).
[b] NF, not filtered; CF, carbon filtered; 1·7 = 1·7× ambient; 2·5 = 2·5 × ambient.

inoculation (Table 2) and with the interaction of ozone and inoculation. The source of the interaction is apparent when these data are analyzed by inoculation treatment (Table 3). Significant differences in seedling masses among ozone treatments occurred only among inoculated seedlings. The differences among ozone treatments for inoculated but not for non-inoculated seedlings are the source of the significant interaction for inoculation with ozone. The biotic disease apparently increased sensitivity to the effects of ozone (Table 3), and ozone apparently enhanced those of pitch canker (Table 4). Both disease agents, within the resolution of this experiment, appeared to effect the seedlings simultaneously.

Table 3. Effects of ozone exposure on weights of loblolly seedlings inoculated or not inoculated with *F. subglutinans* 50 days before the end of 155 days of ozone exposure

Inoculated	Ozone level[a]	Response (g dry wt)[b]					
		Total	Leaf	Stem	Roots	Fine	Large
Six families at four ozone levels							
Yes	CF	5·02a	1·35a	1·06a	2·81a	1·64a	1·17a
	NF	4·69ab	1·17bc	1·05a	2·47ab	1·50a	0·97b
	1·7	4·85a	1·26ab	1·01a	2·58a	1·61a	0·97b
	2·5	4·25b	1·07c	1·01a	2·17b	1·36a	0·82c
	LSD	0·48	0·14	0·11	0·34	0·32	0·14
No	CF	4·87a	1·32a	1·06a	2·46a	1·41a	1·07a
	NF	4·74a	1·29a	1·02a	2·43a	1·46a	0·97a
	1·7	5·00a	1·35a	1·06a	2·59a	1·45a	1·14a
	2·5	4·95a	1·33a	1·01a	2·60a	1·55a	1·05a
	LSD	0·46	0·12	0·11	0·35	0·29	0·16
Ten families at two ozone levels							
Yes	NF	4·96a	1·25a	1·08a	2·63a	1·60a	1·02a
	2·5	4·61a	1·15a	1·06a	2·41a	1·50a	0·91a
	LSD	0·45	0·13	0·11	0·31	0·27	0·11
No	NF	5·09a	1·41a	1·07a	2·60a	1·50a	1·11a
	2·5	5·31a	1·38a	1·06a	2·87a	1·71a	1·16a
	LSD	0·47	0·09	0·07	0·37	0·32	0·14

[a] NF, not filtered; CF, carbon filtered; 1·7 = 1·7 × ambient; 2·5 = 2·5 × ambient.
[b] Weights followed by the same letter are not significantly different ($\alpha = 0{\cdot}05$ SAS ANOVA).

Table 4. Effects of ozone exposure, purported pitch canker resistance, and inoculation with *F. subglutinans* on dimensions of cankers or wound scars resulting from fascicle removal 50 days after wounding

Variable	Level	Response[a] (mm) for length and width			
		Inoculated		Non-inoculated	
		Length	Width	Length	Width
Six families at four ozone levels					
Ozone[b]	CF	5·58b	3·44ab	1·77b	1·49b
	NF	6·53ab	3·95a	2·07b	1·75b
	1·7	5·41b	3·12b	2·04b	1·57b
	2·5	7·92a	4·05a	3·19a	2·41a
	LSD	1·43	0·59	0·44	0·26
Resistance	Resistant	5·81b	3·36b	2·36a	1·85a
	Susceptible	6·91a	3·92a	2·17a	1·75a
	LSD	1·01	0·42	0·31	0·18
Ten families at two ozone levels					
Ozone	NF	6·91b	4·07a	1·99b	1·69b
	2·5	8·49a	4·03a	3·19a	2·49a
	LSD	1·50	0·57	0·33	0·20
Resistance	Resistant	6·59b	3·69b	2·67a	2·14a
	Susceptible	8·83a	4·69a	2·51a	2·04a
	LSD	1·50	0·57	0·33	0·20

[a] Dimensions followed by the same letter are not significantly different (α = 0·05 SAS ANOVA).
[b] NF, not filtered; CF, carbon filtered; 1·7 = 1·7 × ambient; 2·5 = 2·5 × ambient.

For the combined data of inoculated and non-inoculated seedlings, needle and large root masses for the 4 × 6 comparison varied significantly for both ozone and inoculation (Table 2) and with their interaction. For non-inoculated seedlings, none of the component masses varied significantly with ozone treatment (Table 3) or with resistance to pitch canker (Table 5). For inoculated seedlings, the distributions of ozone treatment effects were largely proportional to concentration; seedling masses which differed significantly among ozone treatments (those for large roots, total roots, needles and whole seedlings) were smallest at 2·5 times ambient and largest in the CF treatment (Table 3). That is, mass was inversely proportional to ozone concentration. Although component masses did not differ significantly between the two intermediate (NF and 1·7) treatments, the proportionality with ozone concentration was opposite that for CF and 2·5 treatments for needles, roots and total seedling masses (Table 3). Further, differences between the intermediate treatments were large enough that 2·5 and 1·7 but not 2·5 and NF treatments differed significantly for large roots, total roots, needles and whole seedlings (Table 3). Non-proportional response to ozone dosage has been observed by others (Kress *et al.*, 1988), but the precise cause is unknown. Except for needle mass, the masses which differed significantly among ozone treatments also differed with purported pitch canker resistance (Table 2).

Inferences for effects of ozone, resistance and inoculation were complicated by a significant interaction between ozone and inoculation on the mass of needles (p = 0·005) and large roots (p = 0·004). Data for large roots and needles were separated by seedling resistance and inoculation treatment to make four single-degree-of-freedom comparisons. Each level of resistance was analyzed for effects of inoculation, and each level of inoculation was analyzed for effects of resistance. The means for these treatment combinations, and the significance for these differences are presented in Fig. 4. The masses of large roots from inoculated resistant seedlings were significantly smaller than those of either inoculated susceptible seedlings or non-inoculated resistant seedlings (Fig. 4). Root mass differences were not significant for resistance among non-inoculated seedlings or for inoculation among susceptible seedlings. For needle mass, the only significant difference was a reduction for inoculated susceptible compared to non-inoculated susceptible seedlings (Fig. 4).

Unfortunately, four seedling families were exposed only to the NF and 2·5 treatments, which, respectively, represent current ambient conditions in parts of the South and those in the San Bernardino Valley of California. None of the seedling variables differed significantly between the NF and 2·5 ozone treatments for either the six or ten family comparisons (Tables 2 and 3). Needle, large root and total seedling masses varied significantly only between inoculated and non-inoculated seedlings for the 10 family comparison (Table 2).

Needles in direct contact with cankers and those distal to girdling cankers die and subsequently are shed. This reduction of needle mass, as a direct result of canker expansion, was minimized in the present experiment by harvesting all seedlings when only six, which still retained their dead needles, had been girdled. Needle dry weights should have changed little due to direct canker effects. Also, cankers (Table 4) but not needle masses (Table 5) were significantly smaller among resistant, compared to susceptible seedlings. We conclude that the

Table 5. Effects of purported family resistance to pitch canker on weights of loblolly seedlings that were inoculated or not inoculated with *F. subglutinans* 50 days before the end of 155 days exposure in open-top chambers

Inoculated	Resistance	Response[a] (g dry wt)					
		Total	Leaf	Stem	Roots	Fine	Large
Six families averaged for four ozone levels							
Yes	Resistant	4·55a	1·24a	1·04a	2·27a	1·39a	0·88a
	Susceptible	4·97b	1·19a	1·03a	2·74b	1·66b	1·08b
	LSD	0·38	0·10	0·08	0·30	0·27	0·10
No	Resistant	4·84a	1·32a	1·04a	2·47a	1·45a	1·02a
	Susceptible	4·93a	1·32a	1·03a	2·58a	1·49a	1·09a
	LSD	0·32	0·09	0·08	0·24	0·20	0·12
Ten families averaged for two ozone levels							
Yes	Resistant	4·70a	1·21a	1·03a	2·46a	1·51a	0·95a
	Susceptible	4·88a	1·19a	1·12b	2·57a	1·59a	0·99a
	LSD	0·45	0·13	0·09	0·31	0·27	0·11
No	Resistant	5·13a	1·42a	1·05a	2·66a	1·53a	1·13a
	Susceptible	5·27a	1·37a	1·08a	2·81a	1·67a	1·15a
	LSD	0·45	0·09	0·07	0·37	0·32	0·14

[a] Weights followed by the same letter are not significantly different ($<\alpha = 0{\cdot}05$ SAS ANOVA).

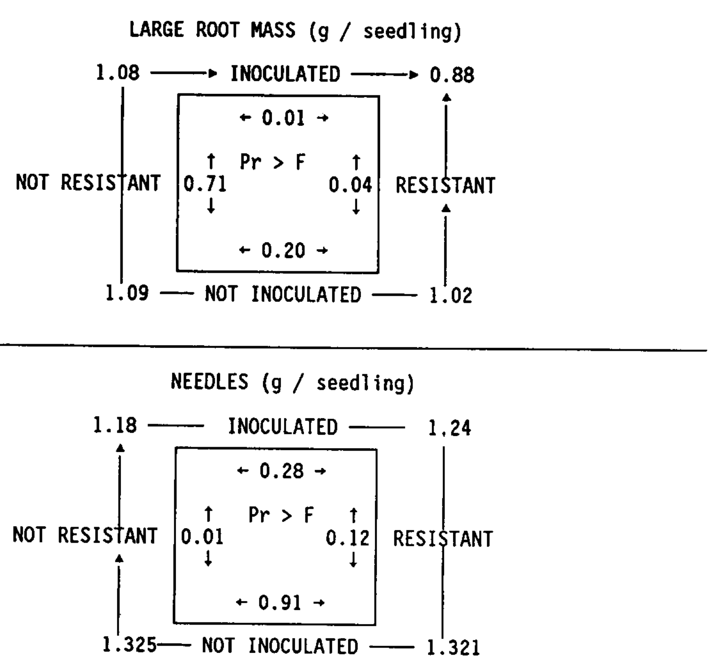
significant differences in needle mass attributable to inoculation, to ozone, and to the interaction of these effects are due to affects on seedling physiology and not needle mortality associated with cankers.

Canker development

Only three (one non-inoculated and two inoculated) of 576 seedlings died during the study. For non-inoculated and inoculated seedlings, respectively, 1 and 256 were recorded as infected; 11 and 130 developed callus; and 0 and 75 developed diffuse cankers. For inoculated seedlings, neither the number recorded as infected nor the numbers of callused or diffuse cankers varied significantly with ozone treatment. *Fusarium subglutinans* was successfully reisolated from 37 of 41 cankers sampled.

The trend for canker growth among ozone treatments was opposite to that for the accumulation of root mass. At the highest ozone concentrations (2·5 times ambient), cankers were largest and root masses smallest (Tables 3 and 4).

Average lengths and widths of affected stem around inoculated and non-inoculated wounds, respectively, were 7·1 by 3·8 mm and 2·3 by 1·9 mm. Ozone treatment significantly affected dimensions of both inoculated and non-inoculated wounds (except width for the two ozone treatment comparison), but only inoculated



Significant reductions between treatment means are indicated by arrows (—▸) toward the lower mean.

Fig. 4. Large root or needle masses of resistant or not resistant families that were or were not included with *F. subglutinans*.

seedlings differed significantly among families and between pitch canker resistance designations (Table 4).

Since it was not necessary to establish pathogenicity of *F. subglutinans*, data for cankers were separated by inoculation treatment. The length and width of cankers or scars resulting from fascicle removal were evaluated for effects of resistance ($N = 2$), family (nested in resistance, $N = 6$) and ozone ($N = 4$). For inoculated seedlings, highly significant differences both for length and width were recorded for all effects. The interaction of ozone with resistance was not significant. Significant smaller cankers among the families designated resistant reaffirmed previous determinations of relative resistance both for the 6-family (length 1·10 mm, LSD 1·01; width 0·56 mm, LSD 0·42) and 10-family comparisons (length 2·25 mm, LSD 1·5; width 0·73 mm, LSD 0·57). This indicates that some aspects of resistance to pitch canker are similar between seedlings and young (four families selected from provenance trials) or mature (six families selected from seed orchard) trees. For non-inoculated seedlings, lengths and widths of scars created by fascicle removal were significantly greater at the highest ozone treatment (Table 4).

SUMMARY

Ozone reduces energy available from needles as a function of decreased photosynthesis and increased respiration (Barnes, 1972). Needle and root growth and disease resistance appear to be competing energy sinks. The distribution of effects for both ozone and pitch canker among plant organs and seasonal demands and contributions of these organs to the energy pool could effect susceptibility to biotic and abiotic diseases.

Inference for the effects of ozone and inoculation with *F. subglutinans* on needle masses are complicated by the significant interaction between these effects but might logically be explained by the following hypothesis. Increased ozone dosage reduced the energy available to restrict cankers (through effects on needle physiology), and larger cankers reduced the energy available for root growth. This assumes that canker suppression requires energy. This assumption is supported by the fact that non-inoculated resistant and both inoculated and non-inoculated susceptible families accumulated more root mass than inoculated resistant families. Disease resistance (canker restriction among resistant families) correlated positively with reduced root growth.

Changes in needle and root masses between resistant and susceptible families inoculated with *F. subglutinans* may indicate different strategies of energy investment relative to the costs and potential benefits of canker suppression. Whether a seedling benefits most from increased root growth (susceptible strategy) or canker suppression (resistant strategy) depends in part on future environmental conditions.

Scar length and width were significantly larger at the highest concentration of ozone. Ozone effects on wound healing should be investigated for their potential to increase opportunities for wound pathogens such as *F. subglutinans*.

Attempts to correlate ozone exposure with production of agricultural crops are sometimes improved by emphasizing exposures above a critical concentration (Lee *et al.*, 1988; Musselman *et al.*, 1988). This implies that a physiological threshold exists for some plants above which ozone sensitivity increases. In the present experiment, most significant differences among ozone treatments occurred for only inoculated seedlings. This indicates that pitch canker increased the ozone sensitivity of loblolly seedlings.

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