Response of Several Eastern Forest Tree Species to Chronic Doses of Ozone and Nitrogen Dioxide

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ABSTRACT

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Two- to four-week-old seedlings of 10 eastern forest species were exposed to ozone at 0, 0.05, 0.10, or 0.15 ppm, and 2- to 4-wk-old seedlings of seven tree species were exposed to ozone and/or nitrogen dioxide at 0.10 ppm in 6 hr/day exposures for 28 consecutive days. Loblolly pine and American sycamore exhibited significant growth suppressions, whereas white ash and yellow poplar exhibited significant growth stimulations when exposed to ozone at 0.05 ppm. Yellow poplar and Virginia pine were the only species that failed to show any significant adverse growth effects in response to the ozone treatment at 0.15 ppm. In several instances, significant growth effects were noted in the absence of foliar injury. Nitrogen dioxide alone significantly suppressed root and total dry weight of sweetgum. The only significant interaction effects noted between ozone and nitrogen dioxide were less than additive.

The data base for the effects of low oxidant doses to indigenous vegetation is somewhat limited. Many of the data were obtained from studies of visible foliar response to short-term exposures, and the relationship between foliar injury and growth and yield was not clear. Only one study utilized near-ambient ozone (O_3) concentrations in a long-term exposure to measure the responses of tree species. Wilhour and Neely (14) measured growth and dry weight of seedlings of nine western conifer species exposed to O_3 at 0.10 ppm for 6 hr/day for 122 consecutive days. Ponderosa pine and western white pine exhibited significant growth reductions in dry weight of roots and dry weight of foliage, respectively. Based on foliar injury, ponderosa pine was determined to be very sensitive, and western white pine was considered to be insensitive (14).

The sensitivity to air pollution of several eastern forest tree species has been estimated using short-term exposures and measurements of resulting foliar injury (5), evaluation of the growth of trees exposed to low doses of O_3 (10,11), evaluation of tree growth in ambient air (11,13), evaluation of physiologic or metabolic alterations (1,4), and the presence or absence of plant species near a pollution source (12).

Based on studies by Santamour (13) and Kress (11), growth evaluations may provide more relevant information than foliar injury measurements for determining the sensitivity of plants to air pollution because leaf injury is not always related to growth reduction.

The objectives of this study were to determine the effects of low O_3 concentrations in long-term exposures on the growth of 10 eastern U.S. forest tree

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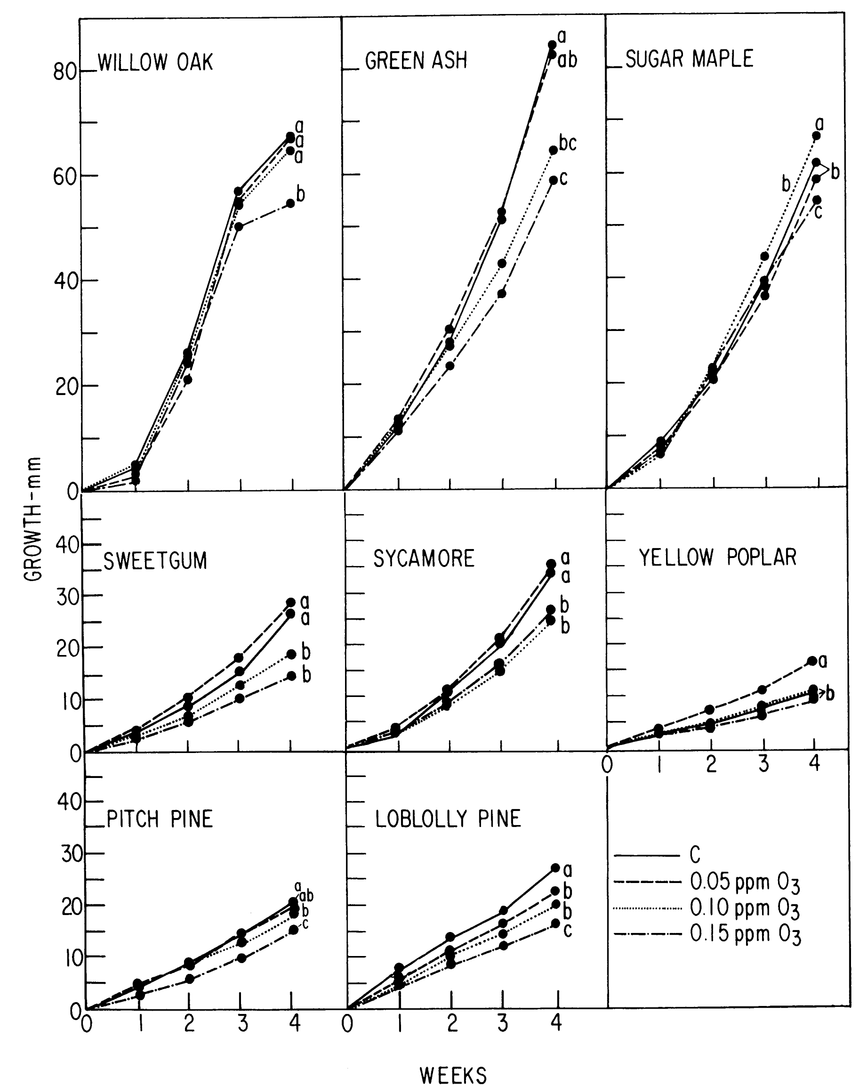

Fig. 1. Height growth of eight tree species exposed to ozone (O_3) at 0 (C), 0.05, 0.10, or 0.15 ppm in 6 hr/day exposures for 28 consecutive days. Within species, values followed by the same letter at week 4 do not differ at $P = 0.05$ according to Duncan's new multiple range test.

species and to determine the potential impact of long-term exposures to O_3 and nitrogen dioxide (NO_2) in combination on the growth of seven eastern tree species.

MATERIALS AND METHODS

Plant material. Seed of 10 forest tree species was collected from the wild in Virginia and North Carolina: Virginia pine (*Pinus virginiana* Mill.), yellow poplar (*Liriodendron tulipifera* L.), and sugar maple (*Acer saccharum* Marsh.) from the North Carolina Piedmont; white ash (*Fraxinus americana* L.) from the North Carolina mountains; willow oak (*Quercus phellos* L.), green ash (*F. pennsylvanica* Marsh.), and sweetgum (*Liquidambar styraciflua* L.) from the Virginia coastal plain; pitch pine (*P. rigida* Mill.) from the Virginia mountains; and loblolly pine (*P. taeda* L.) from the Virginia Tidewater area. In addition, a half-sib collection of American sycamore (*Platanus occidentalis* L.) was gathered from a phenotypically superior tree in Burke County, NC, and a full-sib collection of loblolly pine (6-13 × 2-8) was gathered from the tree improvement seed orchard at the New Kent Forestry Center, Providence Forge, VA. The seeds were sown in mid-August 1979 in flats of sandy loam soil, sphagnum peat, vermiculite, and Weblite (expanded shale product of the Webster Brick Co., Roanoke, VA 24061), 1:1:1:1 by volume. Beginning on 30 August, 2- to 3-day-old seedlings were transplanted four to a pot in 0.45-L plastic pots with the same potting mix. The soil in each pot was drenched prior to transplanting with 20 ml of Ortho garden fungicide (Orthocide; 50% captan) mixed at the recommended rate. The seedlings were maintained in a greenhouse supplied with charcoal-filtered air. The charcoal filtration system was approximately 70% efficient for O_3, so all plants were exposed to O_3 at 0.01–0.03 ppm daily. They were watered daily and supplied with supplemental light (15 klux) from high-pressure sodium-vapor lamps before sunrise and after sunset for a total photoperiod of 15 hr. One week after transplanting, the seedlings in each pot received 1 g of Osmocote 14-14-14 slow-release fertilizer.

Treatments. The exposures were performed from 23 September to 18 October when the trees were 2–4 wk old. The hardwoods were in the one- to two-leaf stage when the exposures began. Two separate experiments were performed. In the first experiment, 16 trees of each of 10 species were exposed to O_3 at 0 (charcoal-filtered air), 0.05, 0.10, or 0.15 ppm for 6 hr/day for 28 consecutive days. There were eight exposure chambers available, which provided two replicates per treatment and eight trees per replicate. In the second experiment, eight trees of each of seven species were exposed to charcoal-filtered air, O_3 at 0.10 ppm, NO_2 at 0.10 ppm, or O_3 and NO_2 at 0.10 ppm each for 6 hr/day for 28 consecutive days in four exposure chambers. Each pot of plants was treated as a replicate for the second experiment, so there were two replicates per treatment and four trees per replicate. In both experiments, the trees were exposed from 0800–1400 EDT and were maintained in the charcoal-filtered air greenhouse for the remainder of each day.

Fumigation procedures. Indoor

Table 1. Height growth, dry weight, and foliar symptom evaluation expressed as percentage of control for seedlings of 10 tree species exposed to ozone (O_3) at 0, 0.5, 0.10, or 0.15 ppm for 6 hr/day for 28 consecutive days[x]

Tree species	O_3 treatment (ppm)	Percentage of control: Height growth	Dry weight: Top	Dry weight: Root	Dry weight: Total	Symptom[y]
Loblolly pine (*Pinus taeda* L.)	Control	100 a[z]	100 a	100 a	100 a	1.0
	0.05	82 b	85 ab	90 ab	86 ab	1.0
	0.10	73 b	79 b	72 bc	78 b	1.0
	0.15	59 c	74 b	64 b	72 b	1.3
Loblolly pine (6-13 × 2-8) (*P. taeda* L.)	Control	100 a	100 ab	100 a	100 ab	1.0
	0.05	91 ab	108 a	102 a	107 a	1.0
	0.10	91 ab	95 ab	93 a	94 ab	1.0
	0.15	80 b	86 b	84 a	86 b	1.4
Pitch pine (*P. rigida* Mill.)	Control	100 a	100 a	100 a	100 a	1.0
	0.05	96 ab	94 a	84 ab	92 ab	1.0
	0.10	87 b	83 a	77 ab	81 ab	1.0
	0.15	74 c	78 a	68 b	76 b	1.4
Virginia pine (*P. virginiana* Mill.)	Control	100 a	100 a	100 a	100 a	1.0
	0.05	95 a	98 a	120 a	102 a	1.0
	0.10	89 a	97 a	93 a	97 a	1.0
	0.15	86 a	87 a	86 a	87 a	1.0
Sweetgum (*Liquidambar styraciflua* L.)	Control	100 a	100 a	100 a	100 a	2.1
	0.05	109 a	91 ab	88 ab	90 a	8.4
	0.10	71 b	76 ab	65 bc	74 ab	13.3
	0.15	55 b	60 b	52 c	58 b	14.8
Sycamore (*Platanus occidentalis* L.)	Control	100 a	100 a	100 a	100 a	1.3
	0.05	104 a	84 a	57 b	77 a	1.2
	0.10	73 b	43 b	27 b	39 b	4.5
	0.15	79 b	36 b	19 b	31 b	3.8
White ash (*Fraxinus americana* L.)	Control	100 a	100 b	100 ab	100 b	1.2
	0.05	112 a	125 a	111 a	122 a	1.1
	0.10	91 a	92 b	87 b	91 bc	4.4
	0.15	85 a	83 b	81 b	83 c	6.5
Green ash (*F. pennsylvanica* Marsh.)	Control	100 a	100 a	100 a	100 a	2.9
	0.05	98 ab	86 ab	86 a	86 a	4.1
	0.10	76 bc	71 ab	75 a	72 a	6.7
	0.15	70 c	64 b	75 a	67 a	6.8
Willow oak (*Quercus phellos* L.)	Control	100 a	100 a	100 a	100 a	1.3
	0.05	99 a	99 a	94 a	98 a	1.0
	0.10	96 a	92 a	83 a	89 a	2.1
	0.15	81 b	89 a	83 a	87 a	1.4
Sugar maple (*Acer saccharum* L.)	Control	100 b	100 a	100 ab	100 a	1.8
	0.05	95 b	88 a	161 a	97 a	1.3
	0.10	108 a	90 a	116 ab	93 a	2.8
	0.15	88 c	58 b	69 b	59 b	9.6
Yellow poplar (*Liriodendron tulipifera* L.)	Control	100 b	100 b	100 a	100 a	3.7
	0.05	160 a	147 a	133 a	141 a	3.3
	0.10	108 b	111 ab	96 a	105 a	3.9
	0.15	88 b	108 b	121 a	118 a	5.6

[x] Height growth was measured as the growth in millimeters during the 28 days of exposure. Conifers were measured to the tip of the needles and hardwoods to the base of the petiole of the newest leaf. Each value is the average of 16 trees.

[y] Symptom is the value for symptom type (1 = no injury, 2 = chlorosis, 3 = pigmented stipple, 4 = necrosis) multiplied by the value for percentage of leaf affected (1 = <5%, 2 = 5–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%). Each value is the average for the most severely injured leaf on each of eight trees.

[z] Values for each column/species followed by the same letter are not different at $P = 0.05$ according to Duncan's multiple range test. The analysis was performed on the actual height growth measurements and converted to percentages for presentation.

exposure chambers were constructed similar to those of Heck et al (8). The major differences were the addition of a steam inlet in each chamber and the use of high-pressure sodium-vapor lamps as the light source. There was one air change every 1.5 min. The air inside each chamber was sampled continuously through a tube (FEP Teflon) placed at plant height. O_3 was generated by passing oxygen through a silent electric discharge and metered to the chambers by flow control rotometers. The pollutant concentrations were monitored with a chemiluminescent O_3 analyzer (Bendix model 8002, Bendix Process Instruments Division, Lewisburg, WV 24970) calibrated by a constant O_3 source that was verified by the EPA Quality Assurance Branch at Research Triangle Park, NC 27711. Nitrogen dioxide was obtained as bottled gas diluted in nitrogen (1.09% NO_2) and was monitored with a chemiluminescent NO-NO_2-NO_x monitor (Bendix model 8101-B) calibrated by NO_2 permeation tube and a known NO source.

Illumination in the chambers was 22–24 klux at plant height. Temperature during exposure was 30–33 C for experiment 1 and 33–35 C for experiment 2. Relative humidity was 67–75% for experiment 1 and 62–67% for experiment 2.

Evaluation procedures. Height growth was measured weekly beginning with the day prior to exposure initiation. Conifer height growth was measured from the cotyledons to the tip of the needles, whereas hardwood height growth was measured from the cotyledons to the base of the petiole of the newest leaf. At the termination of the 28th exposure, the trees were evaluated for foliar injury. Symptom type was rated as 1 = no injury, 2 = chlorotic symptoms (chlorosis, chlorotic stipple), 3 = stipple (pigmented), and 4 = necrotic symptoms (fleck, necrosis). Percentage of each leaf affected was rated as 1 = <5%, 2 = 5–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%. The value for symptom type was multiplied by the value for percentage of injury for the most severely injured leaf per tree. Following injury evaluation, the trees were harvested, bulked by pot (four trees), and the dry weights of tops and roots were determined.

An analysis of variance was performed for weekly height growth, top dry weight, root dry weight, and total dry weight. The results of the analysis were then tested using Duncan's new multiple range test to determine treatment differences at $P = 0.05$. The $O_3 + NO_2$ interaction was also tested by partitioning treatment variance into O_3, NO_2, and interaction effects.

RESULTS

Ozone exposures. There were no significant height differences between plants in each treatment group within species when the exposures began. The 10 tree species varied appreciably in their height growth responses to O_3, with eight species exhibiting a significant effect in at least one treatment (Fig. 1). In response to O_3 at 0.05 ppm, loblolly pine (wild type) exhibited a significant height growth suppression and yellow poplar exhibited a significant height growth stimulation. The O_3 treatment at 0.10 ppm resulted in significant height growth suppressions of loblolly pine (wild type), pitch pine, sweetgum, sycamore, and green ash. Sugar maple exhibited a significant height growth stimulation with the O_3 treatment at 0.10 ppm. All species except white ash and Virginia pine exhibited significantly suppressed height growth in response to the O_3 treatment at 0.15 ppm.

In general, height growth suppressions or stimulations were accompanied by similar dry weight suppressions or stimulations (Table 1). However, for some species in some treatments, the dry weight effects were the opposite of the height growth effects. The height growth of loblolly pine (6-13 × 2-8) and Virginia pine exposed to O_3 at 0.05 ppm was less than that of the control trees, whereas the dry weight was greater than that of the control trees. Sweetgum and sycamore exhibited stimulated height growth but suppressed dry weight in response to O_3 at 0.05 ppm. Yellow poplar exhibited suppressed height growth and stimulated dry weight growth in response to O_3 at 0.15 ppm. All three O_3 treatments had considerably greater impact on sycamore dry weight growth than on height growth. Sycamore root growth was by far the most O_3-sensitive parameter of any species.

The pine species and willow oak did not exhibit appreciable foliar injury in any treatment. Only sweetgum exhibited a significant difference in foliar injury between the control and trees treated with O_3 at 0.05 ppm.

Ozone and nitrogen dioxide exposures. Only two of the seven species exhibited significant height growth effects in response to O_3 and/or NO_2 at 0.10 ppm (Table 2). Virginia pine and loblolly pine wild type and 6-13 × 2-8) height growths were significantly suppressed by the $O_3 + NO_2$ treatment. Loblolly pine (6-13 × 2-8) height growth was also significantly suppressed by O_3 alone.

The dry weight responses to some of the pollution treatments differed from the height growth responses for some species (Table 2). The $O_3 + NO_2$ treatment caused a greater height growth than dry weight suppression for loblolly pine (wild type and 6-13 × 2-8) and Virginia pine. Pitch pine responded to the O_3 treatment with a height growth suppression and a stimulation in dry weight. Root growth appeared to be the most sensitive parameter for sweetgum and white ash.

The variance partitioning analysis revealed some significant effects not apparent in the analysis of variance. Height growth of loblolly pine (wild type), Virginia pine, green ash, and white ash was significantly suppressed by O_3. Loblolly pine (wild type) and Virginia pine height growth was also suppressed by NO_2. The NO_2 treatment also induced less foliar injury than the control for green ash. The test for $O_3 + NO_2$ interaction revealed significantly less than additive suppressions for sweetgum root and total dry weight and white ash root dry weight.

DISCUSSION

Three species in this study exhibited a threshold for significant suppressions with O_3 between 0.06 and 0.10 ppm, whereas three other species exhibited a threshold for significant effects between 0.10 and 0.15 ppm. However, two species exhibited a threshold for significant growth suppressions at 0.05 ppm. Those two species, loblolly pine and American sycamore, are probably the most important of the species tested to the forest industry (3,7). The significant effects on plant growth of O_3 at 0.05 ppm were not accompanied by foliar injury, which suggests that there may be a potential impact to the forest industry in the south heretofore generally unrecognized.

In a previous study, there were some indications that low concentrations of NO_2 might be stimulatory to tree growth or alleviate O_3 phytotoxicity (11). Similar indications were noted for some of the tree species in this study. Two species (white ash and green ash) exhibited greater height growth (albeit non-significant) in the NO_2 treatment than the control, and the only significant interactive effects were less than additive (sweetgum and white ash). The ratio of the two pollutants to one another may have an impact on potential interaction. This study only utilized one ratio (1:1), and the implications of the study may have been different had other ratios been tested.

Comparing growth response with foliar injury response supports past research, which suggests that foliar injury alone is not an adequate method of evaluating plant sensitivity (9,11,13). Yellow poplar was one of the most sensitive species in terms of foliar injury and is listed as sensitive in the literature (5), but it was the most tolerant in terms of height and dry weight suppression. Other species sensitive in terms of growth impact were tolerant (loblolly pine) or intermediate (sycamore and white ash) in terms of foliar injury.

Evaluation of the height growth and dry weight effects revealed that for some species different interpretations could be made, depending upon which variable was measured. O_3 had a much more severe effect on sycamore and sugar

Table 2. Height growth, dry weight, and foliar symptom evaluation expressed as percentage of control for seedlings of seven tree species exposed to ozone (O_3) and/or nitrogen dioxide (NO_2) at 0.10 ppm for 6 hr/day for 28 consecutive days[x]

		Percentage of control				
		Height	Dry weight			
Tree species	**Treatment**	**growth**	**Top**	**Root**	**Total**	**Symptom**[y]
Loblolly pine						
(*Pinus taeda* L.)	Control	100 a[z]	100 a	100 a	100 a	1.0
	O_3	83 ab	79 a	87 a	80 a	1.0
	NO_2	85 ab	78 a	83 a	78 a	1.0
	$O_3 + NO_2$	61 b	74 a	74 a	74 a	1.0
Loblolly pine (6-13 × 2-8)						
(*P. taeda* L.)	Control	100 a	100 a	100 a	100 a	1.0
	O_3	75 b	89 a	69 a	86 a	1.0
	NO_2	89 ab	90 a	86 a	89 a	1.0
	$O_3 + NO_2$	76 b	96 a	83 a	94 a	1.0
Pitch pine						
(*P. ragida* Mill.)	Control	100 a	100 ab	100 a	100 ab	1.0
	O_3	86 a	114 a	100 a	112 a	1.0
	NO_2	84 a	80 b	89 a	81 b	1.0
	$O_3 + NO_2$	74 a	89 b	85 a	88 ab	1.0
Virginia pine						
(*P. virginiana* Mill.)	Control	100 a	100 a	100 a	100 a	1.0
	O_3	89 ab	98 a	81 a	95 a	1.0
	NO_2	87 ab	99 a	93 a	98 a	1.0
	$O_3 + NO_2$	77 b	99 a	81 a	96 a	1.0
Sweetgum						
(*Liquidambar*						
styraciflua L.)	Control	100 a	100 a	100 a	100 a	3.0
	O_3	73 a	70 b	55 c	67 b	11.3
	NO_2	68 a	75 ab	73 b	74 b	2.6
	$O_3 + NO_2$	72 a	79 ab	52 c	73 b	9.8
White ash						
(*Fraxinus americana* L.)	Control	100 a	100 a	100 a	100 a	1.0
	O_3	80 a	63 b	45 b	58 b	5.5
	NO_2	105 a	99 a	63 b	89 a	1.0
	$O_3 + NO_2$	84 a	63 b	48 b	59 b	5.3
Green ash						
(*F. pennsylvanica*						
Marsh.)	Control	100 a	100 a	100 a	100 a	3.3
	O_3	81 a	83 a	88 a	84 a	8.8
	NO_2	101 a	90 a	82 a	88 a	2.0
	$O_3 + NO_2$	78 a	71 a	81 a	74 a	7.0
Willow oak						
(*Quercus phellos* L.)	Control	100 a	100 a	100 a	100 a	1.0
	O_3	105 a	101 a	111 a	103 a	1.0
	NO_2	90 a	76 a	86 a	78 a	1.0
	$O_3 + NO_2$	86 a	87 a	88 a	87 a	1.0

[x] Height growth was measured as the growth in millimeters during the 28 days of exposure. Conifers were measured to the tip of the needles and hardwoods to the petiole of the newest leaf. Each value is the average of eight trees.

[y] Symptom is the value for symptom type (1 = no injury, 2 = chlorosis, 3 = pigmented stipple, 4 = necrosis) multiplied by the value for percentage of leaf affected (1 = <5%, 2 = 5–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%). Each value is the average for the most severely injured leaf on each of eight trees.

[z] Values for each column/species followed by the same letter are not different at $P = 0.05$ according to Duncan's new multiple range test. The analysis was performed on the actual height growth measurements and converted to percentages for presentation.

(11), but it was less sensitive than the wild seed lot in this study. The full-sib seed lot was secured from a tree improvement seed orchard of superior trees. Although not purposely selected for pollution tolerance, because selections were made from trees exposed to ambient air, sensitive trees would have been at a competitive disadvantage.

Stimulation of growth at low O_3 concentrations has been noted in the past (2), and some species exhibited growth stimulation in this study. In some cases, stimulation was noted for height growth but not for dry weight (especially root dry weight) or vice versa. Generally, the maple in terms of dry weight suppression compared with height growth suppression, whereas the reverse was true for loblolly pine. The $O_3 + NO_2$ interaction affected the dry weight growth of white ash more than the height growth, and vice versa for loblolly and pitch pine. This supports the idea that, for certain species, dry weight (particularly root dry weight) may be the most reliable indicator of pollution sensitivity (14).

Two seed lots of loblolly pine were used in this study, and they responded differently. The 6-13 × 2-8 seed lot had been determined to be the most sensitive of 18 full-sib families in a previous study hardwood species exhibited some growth stimulation in height and/or weight with O_3 at 0.05 ppm, whereas the conifer species did not.

The relationship that data such as these have to ambient field conditions is tenuous. However, sensitive and insensitive families of loblolly pine maintained growth differences and exhibited significant growth suppression without foliar injury when outplanted in filtered and unfiltered air near a pollution source (11). There is also some evidence that relative (between sensitive and insensitive trees) tree response to air pollution is consistent between seedlings and 2-yr-old (11) and up to 7-yr-old (6) trees. This study demonstrates the potential for adverse effects to young seedlings grown in artificial environments when exposed to O_3 and NO_2 at near-ambient concentrations. Future research is required to determine whether such effects can be demonstrated in the field.

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