

Growth Responses of two Tree Species Exposed to Simulated Acidic Rain and Ozone

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산성비와 오존에 대한 두 수종의 성장반응

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ABSTRACT

One-year-old yellow-poplar (*Liriodendron tulipifera* L.) and sweetgum (*Liquidambar styraciflua* L.) seedlings were exposed to $0.10 \mu\text{l} / \text{l O}_3$ and simulated acid rain at pH 3.0 for ten consecutive weeks. Shoot height growth (SHG), fresh weight (FWT), dry weight (DWT), apparent plastochron duration (APD) and foliar nutrient concentrations were measured.

None of growth measurements, except the apparent plastochron duration (APD), were significantly affected by any treatment in yellow-poplar seedlings. APD was approximately 30% higher in seedlings exposed to $0.1 \mu\text{l} / \text{l O}_3 + \text{pH } 5.6$ solution than any other treatment. Ozone significantly reduced SHG of sweetgum seedlings by 24% at the end of the ten-week fumigation. There were also significant effects of single and combined effects of ozone and simulated acid rain on APD in sweetgum. APD was significantly increased by 19.8% and 25.7% in seedlings exposed to $0.1 \mu\text{l} / \text{l O}_3$ and pH 5.6 solution, respectively, and resulted in 46.1% higher APD in seedlings exposed to $0.1 \mu\text{l} / \text{l O}_3 + \text{pH } 5.6$ solution compared with seedlings exposed to $0.0 \mu\text{l} / \text{l O}_3 + \text{pH } 3.0$ solution.

Phosphorus and sulphur were significantly greater in seedlings exposed to simulated rain at pH 3.0 compared with pH 5.6 for both species. Foliar S concentration was higher in seedlings exposed to $0.0 \mu\text{l} / \text{l O}_3 + \text{pH } 3.0$ than in seedlings exposed to any other treatment in sweetgum. Ozone significantly increased Ca in sweetgum seedlings, however, ozone reduced Ca in yellow-poplar. Ozone also reduced S and Mg in sweetgum seedlings.

Key words: Yellow-poplar, Sweetgum, Ozone, Acid rain, Growth, Foliar nutrients

INTRODUCTION

Ozone and acidic precipitation are the two most widespread airborne pollutants impacting forest ecosystems in the eastern United States (Cogbill and Likens 1974, Skelley *et al.*

1982). During the spring and early summer, when forest vegetation is in a rapid growth phase, mean hourly ambient ozone concentrations generally range from 0.050 to 0.065 $\mu\text{l}/\text{l}$ (Duchelle *et al.* 1982, Yang and Chevone 1982) and can exceed 0.09 $\mu\text{l}/\text{l}$ for several hours daily during ozone episodes (Duchelle *et al.* 1983). Ambient ozone concentrations are known to reduce growth of sensitive forest species under both laboratory and field experimental conditions (Kress and Skelly 1982, Miller *et al.* 1972, Skelly *et al.* 1982), probably through inhibition of photosynthesis and retention of photosynthate in foliage (Yang *et al.* 1983).

Acidic precipitation is considered as a major environmental concern, primarily in the eastern United States. The effect of acidic rain on the leaching of calcium and the mobilization of aluminum in soils has been implicated in the decline of German forest (Ulrich 1981). Feeder root length in Norway spruce was shown to decrease as the molar ratio of calcium to aluminum decreased in soil solution. A similar relationship between soil calcium and aluminum has not been found in declining spruce stands in the United States (Johnson 1983).

Ozone and simulated acid rain have been reported to induce magnesium and calcium deficiency in Norway spruce (*Picea abies* [L.] Karst.) (Bosh *et al.* 1986, Weiss and Agerer 1986) and magnesium and zinc deficiency in spruce and fir (*Abies alba*) (Zech and Popp 1983). These nutrient deficiencies resulted in the expression of symptoms similar to those in declining spruce stands and the application of fertilizer containing magnesium-calcium or magnesium brought about a revitalization of affected trees in Germany (Bosch *et al.* 1986, Weiss and Agerer 1986, Zech and Popp 1983, Kaupenjohann *et al.* 1987). Friedland *et al.* (1985) observed winter injury in a declining red spruce site, and proposed that this injury might be related to excessive nitrogen deposition which could retard the development of frost hardiness and predispose the foliage to freezing during winter time.

The potential exists for ozone and acid rain to interact with the nutrient status and growth of forest tree species. However, scientific evidence demonstrating effects of acid rain and ozone, alone and in combination, on terrestrial vegetation is inconclusive. The combined effects of ozone and acidic precipitation on tree growth and nutrient concentrations have not yet been investigated adequately.

The purposes of this research were to examine the effects of ozone and simulated acid rain, alone and in combination, on foliar nutrient concentrations and the growth of yellow-poplar, and sweetgum seedlings.

MATERIALS AND METHODS

One-year-old yellow-poplar (*Liriodendron tulipifera* L.) and sweetgum (*Liquidambar styraciflua* L.) seedlings were obtained from a commercial source (Hillis Nursery Co., Inc., McMinnville, TN, U.S.A.) and transplanted into 15 cm diameter plastic pots containing Altavista soil. Prior to transplanting, the fresh weight of each seedling was measured.

The soil was collected from the A horizon at the Reynolds Homestead Agricultural Experiment Station (Patrick County, VA, U.S.A.). Soil was screened through a 0.5 cm mesh and mixed with sand in ratio of 5:1 (v/v). The final soil mix had a pH of 5.6.

All seedlings were grown in a greenhouse supplied with charcoal-filtered air (mean hourly O₃ concentration < 0.025 μl/l). The photoperiod was supplemented with 1000 W sodium lamps to provide a 16-hour daylength with 580±30 μMol m⁻²s⁻¹ photosynthetic photon flux density (PPFD). O₃ fumigations and simulated rain applications were begun when the majority of seedlings started to break bud.

Seedlings were exposed to O₃ in a continuous stirred tank reactor system (CSTR) (Heck *et al.* 1978). Ozone fumigations occurred for four consecutive hours, three d/wk for ten continuous weeks at 0.00 or 0.10 μl/l. Exposure conditions within the CSTRs were maintained at 27±2°C, 55±10% RH, and 600±30 μMol m⁻²s⁻¹ PPFD at plant canopy height. O₃ was generated and monitored as described by Chappelka *et al.* (1985).

All seedlings were exposed to simulated acid rain in the greenhouse area using a rainfall simulator described by Chevone *et al.* (1984). Treatments consisted of simulated rain events at pH 3.0 (1000 μeq H⁺/l), and 5.6 (2.5 μeq H⁺/l). Major anionic and cationic concentrations approximated the mean concentrations found in rain in southwestern Virginia (Skelly *et al.* 1982). Applications of simulated rain were conducted for one hour, twice each week at a rainfall intensity of 0.75 cm/h. O₃ exposures and rain exposures occurred on different days during each week.

Height growth rate was determined by measuring seedlings to the top of the terminal bud, every two weeks, starting with the first day of fumigation. Ten seedlings per treatment were harvested for fresh and dry shoot and root weight after five and ten weeks of treatment. Dry weights were measured after oven drying at 60°C for 48 h, and these data were used for determining relative growth rates (RGR).

The growth rate was also determined by measuring an apparent plastochron duration (Besnard-Wilbaut 1981). Apparent plastochron duration (APD) is the time interval (d) between breaking two successive capsules of leaf primordia (yellow-poplar) or the time (d) which separates the formation of two 5 mm long successive leaves (sweetgum). The average number of APD among treatments was calculated for the entire ten-week exposure.

Needles were collected from seedlings used for dry weight measurements and ground with a Cyclone Sample Mill (UD Co., Boulder, CO, U.S.A.) through a 0.1 cm screen. One gram of ground needle was ashed at 550°C for 5.5 hours and dissolved in 25 ml of concentrated HCl. Concentrations of P, K, Ca, Mg, Al, and S were measured by using inductively coupled plasma (ICP) spectrometry, and total nitrogen was measured by the micro-Kjeldahl method in the Soil Testing and Plant Analysis Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA, U.S.A.

The experiments were designed as a randomized factorial combination of two O₃ (0.0 and 0.1 μl/l) and two pH (3.0 and 5.6) treatments. Standard analysis of variance (ANOVA) was used to analyze the single treatment effects and interactions between O₃ and

simulated rain. Analysis of covariance (ANCOVA) was performed to adjust the data by a significant covariate such as initial height or initial fresh weight. Duncan's new multiple range test was used to compare the data among combined treatments.

RESULTS

Growth response of yellow-poplar seedlings

No growth measurements, except the apparent plastochron duration (APD), were significantly affected by any treatment in yellow-poplar seedlings (Table 1). APD was approximately 30% higher in seedlings exposed to $0.1\mu\text{l/l O}_3$ + pH 5.6 solution than any other treatments.

Visible foliar injury developed on approximately 75% of the plants submitted to simulated rain at pH 3.0 across all ozone treatments. Symptoms began to appear primarily on new, fully expanded leaves five weeks after treatment initiation, and were characterized by necrotic circular lesions (0.4~1.4 mm diameter) on the adaxial leaf surface. However, no ozone symptoms were observed through the ten weeks of treatment.

Growth response of sweetgum seedlings

Ozone significantly reduced shoot height growth (SHG) of sweetgum seedlings (Fig. 1) and this reduction became apparent after six weeks of fumigation, and resulted in approximately 24% less than control seedlings at the end of the ten-week fumigation. There were no significant changes in FWT, RGR, total dry weight (DWT), and root to shoot ratio (RSR) as a result of ozone or simulated acid rain treatment.

There were significant effect of single and combined treatment of ozone and simulated acid rain on APD (Table 2). APD was significantly increased by 19.8% and 25.7% in seed-

Table 1. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), relative growth rate (RGR), and apparent plastochron duration (APD) of one-year-old yellow-poplar seedlings after treatment with ozone and simulated acid rain.

	OZONE ⁴		RAIN pH ⁵		INTERACTION			
	0.0	0.1	3.0	5.6	0.0+3.0	0.0+5.6	0.1+3.0	0.1+5.6
FWT ¹	6.58	5.96	6.54	6.00	6.70	6.47	6.38	5.54
DWT ¹	2.51	2.31	2.42	2.41	2.50	2.51	2.33	2.29
RSR	.428	.449	.432	.446	.405	.452	.459	.439
RGR ²	.103	.098	.102	.100	.103	.103	.100	.096
APD ³	6.68	7.97	6.97	7.68	7.30a	6.07a	6.63a	9.30b

¹ gm; ² gm gm⁻¹ wk⁻¹; ³ days; ⁴ Ozone ($\mu\text{l/l}$) treatment across all rain pHs; ⁵ Rain pH across all ozone treatments

Means within rows of interaction with common letters are not significantly different at .05 level by Duncan's new multiple range test. FWT, DWT, and RGR were adjusted for initial fresh weight.

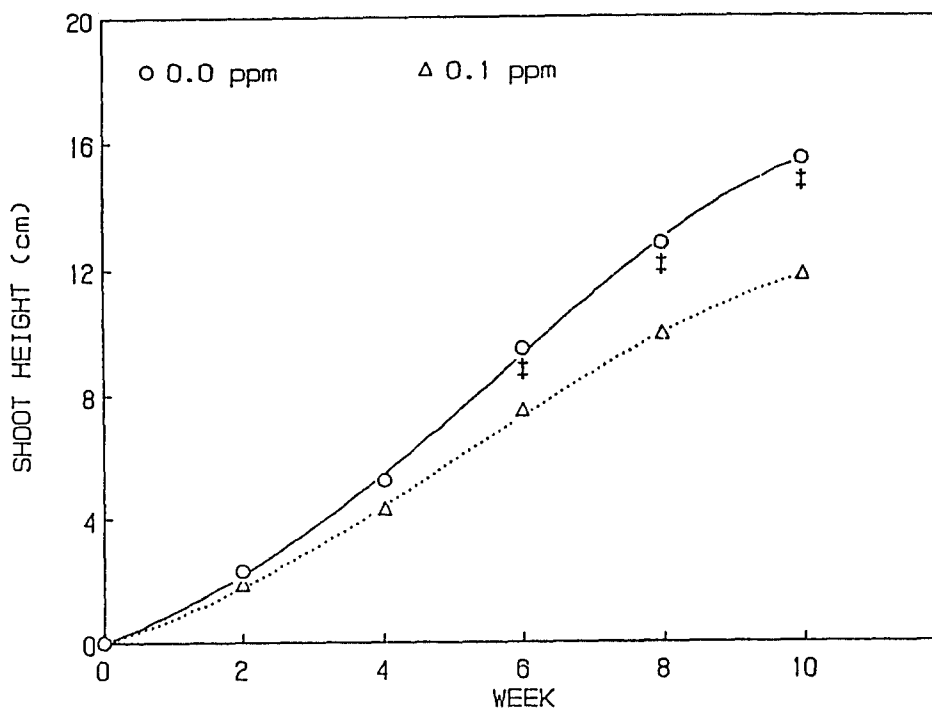


Fig. 1. Changes in cumulative shoot height growth (cm) of one-year-old sweetgum seedlings during 10 wks of treatment with ozone. Dagger (‡) indicates statistically significant differences at $P=0.01$. Data are adjusted for initial height.

Table 2. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), relative growth rate (RGR), and apparent plastochron duration (APD) of one-year-old sweetgum seedlings after treatment with ozone and simulated acid rain.

	OZONE ⁴		RAIN pH ⁵		INTERACTION			
	0.0	0.1	3.0	5.6	0.0+3.0	0.0+5.6	0.1+3.0	0.1+5.6
FWT ¹	27.32	24.94	26.57	25.69	28.10	26.54	25.05	24.84
DWT ¹	10.13	9.50	9.82	9.81	10.23	10.03	9.40	9.59
RSR	.361	.349	.357	.353	.353	.368	.360	.339
RGR ²	.164	.156	.163	.158	.169	.160	.157	.155
APD ³	4.91	5.88**	4.78	6.01**	4.77a	5.06a	4.79a	6.97b

¹ gm; ² gm gm⁻¹ wk⁻¹; ³ days; ⁴ Ozone (μ l/l) treatment across all rain pHs.; ⁵ Rain pH across all ozone treatments.

** indicate the significant differences at .01 level within single treatment.

Means within rows of interaction with common letters are not significantly different at .05 level by Duncan's new multiple range test. FWT, DWT, and RGR were adjusted for initial fresh weight.

lings exposed to $0.1\mu\text{l}/\text{l}$ O_3 and pH 5.6 solution, respectively, and resulted in 46.1% higher APD in seedlings exposed to $0.1\mu\text{l}/\text{l}$ O_3 + pH 5.6 solution compared with seedlings exposed to $0.0\mu\text{l}/\text{l}$ O_3 + pH 3.0 solution, which means additive combination effect of $0.1\mu\text{l}/\text{l}$ O_3 and pH 5.6 solution on APD (Table 2).

Visible foliar symptoms were observed on approximately 95% of the plants exposed to $0.1\mu\text{l}/\text{l}$ O_3 regardless of simulated rain pH. Visible foliar injury first appeared on the older leaves after four weeks of fumigation initiation, and was characterized by premature red pigmentation with small brown necrotic lesions on the adaxial leaf surface. There was no visible foliar injury due to the simulated acid rain.

Effects of ozone and simulated acid rain on foliar nutrient concentrations

The effects of ozone and simulated acid rain treatment on foliar nutrient concentrations are presented in Tables 3 and 4. Phosphorus and sulphur were significantly greater in seedlings exposed to simulated rain at pH 3.0 compared with pH 5.6 for both species, and significant interactions between ozone and solution pH occurred in S concentration in sweetgum seedlings. Foliar S concentration was higher in seedlings exposed to $0.0\mu\text{l}/\text{l}$ O_3 + pH 3.0 than in seedlings exposed to any other treatments in sweetgum. Ozone significantly increased Ca in sweetgum seedlings. In contrast, ozone fumigation significantly reduced Ca in yellow-poplar, and reduced concentrations of S and Mg in sweetgum seedlings.

Table 3. Summary of results of foliar analysis of one-year-old yellow-poplar after ten weeks of treatment with ozone and simulated acid rain. Values (% per gram leaf dry weight) are means of five samples.

		N			P			K		
		Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
		0.0	0.1		0.0	0.1		0.0	0.1	
pH	3.0	2.60	2.35	2.48	0.13	0.12	0.13*	1.19	1.06	1.12
pH	5.6	2.52	2.44	2.48	0.10	0.10	0.10	0.99	1.03	1.01
	X ²	2.56	2.40		0.11	0.11		1.09	1.05	
		Ca			Mg			S		
		Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
		0.0	0.1		0.0	0.1		0.0	0.1	
pH	3.0	1.02	0.90	0.96	0.34	0.31	0.32	0.088	0.100	0.094**
pH	5.6	0.96	0.84	0.90	0.33	0.29	0.31	0.065	0.072	0.069
	X ²	0.99	0.87*		0.33	0.30		0.077	0.086	

¹ Averaged across all ozone levels

² Averaged across all pH levels

*, ** Means indicate significant differences at 5% (*) or 1% (**) level within ozone or simulated acid rain treatments across all other treatments

Table 4. Summary of results of foliar analysis of one-year-old sweetgum after ten weeks of treatment with ozone and simulated acid rain. Values (%) per gram leaf dry weight are means of five samples.

		N			P			K		
		Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
		0.1	0.1		00.0	0.1		0.0	0.1	
pH	3.0	1.72	1.74	1.73*	0.16	0.16	0.16*	1.20	1.19	1.20
pH	5.6	1.50	1.66	1.63	0.14	0.14	0.14	1.16	1.17	1.17
	X ²	1.66	1.70		0.15	0.15		1.18	1.18	
		Ca			Mg			S		
		Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
		0.0	0.1		0.0	0.1		0.0	0.1	
pH	3.0	0.82a	0.82a	0.82	0.27	0.22	0.25	0.084a	0.071b	0.078**
pH	5.6	0.77b	0.86a	0.81	0.26	0.21	0.23	0.062c	0.060c	0.061
	X ²	0.79	0.84*		0.26	0.22*		0.073	0.066**	

¹ Averaged across all ozone levels

² Averaged across all pH levels

*, ** Means indicate significant differences at 5% (*) or 1% (**) level within ozone or simulated acid rain treatments across all other treatments

Means within interaction lacking (or with common) letters are not significantly different at 5% level by Duncan's new multiple range test

DISCUSSION

Ozone alone has been reported to have little effect on the growth of yellow-poplar seedlings in most laboratory studies (Jensen 1973, Kress and Skelly 1982, Mahoney *et al.* 1984, Chappelka *et al.* 1985), which is consistent with the current results. In contrast, Duchelle *et al.* (1982) found that ambient concentration of ozone (monthly average was 0.05 $\mu\text{l}/\text{l}$) significantly reduced the height growth of yellow-poplar by 44% in a field study. Jensen (1985) also observed suppression of growth in yellow-poplar with 0.1 $\mu\text{l}/\text{l}$ ozone in a laboratory study. In this present study, although FWT, DWT, and RGR of yellow-poplar were not affected significantly by ozone or simulated acid rain, in general, treatments with ozone and pH 5.6 solution resulted in lower biomass accumulation compared with 0.0 $\mu\text{l}/\text{l}$ ozone and pH 3.0 solution. The repressive effects of ozone and pH 5.6 solution were additive for all biomass variables. Therefore, the potential exists for a detrimental effect of ozone or for the interactive effects of ozone and simulated acid rain on growth of yellow-poplar.

In this present study, sweetgum is the more sensitive species to ozone in terms of SHG, APD, and foliar injury. Sweetgum was the only species exhibited significant foliar symptom when treated with 0.05 $\mu\text{l}/\text{l}$ ozone among ten eastern forest species examined by Kress and Skelly (1982). In this study, only sweetgum seedlings showed foliar ozone injury. APD was the most sensitive indicator for ozone and simulated acid rain in sweetgum and yellow-poplar.

Most of the previous studies on the effect of air pollutants on plant growth (Skeffington and Roberts 1985) have reported no significant interactions between ozone and simulated rain pH, which is supported by this study with yellow-poplar and sweetgum seedlings.

Application of simulated rain at pH 3.0 significantly increased P concentration for both species; however, none of them exhibited a significant increase in growth. This might be due to the higher concentration of P than the critical level. Wells *et al.* (1973) found, in the Southeastern Coastal Plain, U.S.A., that a concentration of 0.1 percent P was the critical level in loblolly pine needles; above this concentration, tree height was not increased by P fertilization whereas below this concentration growth was usually increased.

Although simulated acid rain contained very high concentrations of S and N, only foliar S concentration was increased in yellow-poplar only. The stimulation effect of simulated rain at pH 3.0 on S concentration supports the results reported by Abouguendia and Baschak (1987) for white spruce (*Picea glauca*). The increase in foliar S was not above the threshold value of 0.12 % (Zech *et al.* 1985), and did not result in any growth alteration.

German researchers found Mg and Ca deficiencies were induced by high SO₂ concentration (Zech *et al.* 1983) and by the combination of ozone and simulated acid rain (Bosch *et al.* 1986, Weiss and Agerer 1986). Magnesium and Ca fertilization resulted in a revitalization of trees which had exhibited Mg-deficiency symptoms by air pollutants (Kampenjohnann *et al.* 1987, Bosch *et al.* 1986, Weiss and Agerer 1986). Magnesium is known to play many essential roles in the processes of plant metabolism. Magnesium is a constituent of the chlorophyll molecule, and is necessary for full activity of many enzymes involved in carbohydrate metabolism and in the synthesis of nucleic acids. In this present study, the Mg content was significantly reduced in seedlings exposed to 0.1 $\mu\text{l/l}$ ozone compared with control plants. A reduced Mg concentration may affect photosynthesis and cause a reduction in SHG. The effects of simulated acid rain on foliar nutrient concentration were variable among tree species. Similar results have been reported in the literature. Wood and Bormann (1977) found declines of K, Mg, and Ca concentrations at pH's of 3.0 and below, and a positive correlation of nitrogen level in plant tissue with nitrate level in simulated rain. However, Tveite (1980) reported increases of concentrations of sulphate, P, Mn, and Fe by treatment with ground water acidified with only sulphuric acid. When peat moss (Abouguendia and Baschak 1987) or fertilizer (Takemoto *et al.*, 1987) were added to potting media, no significant alterations were observed for any foliar nutrients. Therefore, interpretation of the results should be performed with care based on soil characteristics, fertilizer, simulated rain constituents, and experimental environments (field, greenhouse, temperature, humidity, etc.).

The results from this study indicate that ozone and/or simulated acidic rain can alter the growth and foliar nutrient status of forest trees under laboratory conditions. However, additional research is necessary using different tree ages, soil types, and field tests to determine if such effects found in the laboratory conditions are occurring in natural forest ecosystems.

적 요

일년생 *Liriodendron tulipifera*와 *Liquidambar styraciflua*를 0.1 $\mu\text{l/l}$ 의 오존과 pH 3.0의 산성비에 10주간 폭로하면서 생장의 변화와 그에 따른 영양분의 변화를 측정하였다. *Liriodendron tulipifera*에서는 오직 apparent plastochron duration (APD)만이 0.1 $\mu\text{l/l}$ O_3 + pH 5.6 용액을 처리한 식물에서 30% 늘어났다.

반면에 *Liquidambar styraciflua*에서는 오존에 의해 줄기생장이 24% 감소되었으며, APD는 1 $\mu\text{l/l}$ O_3 와 pH 5.6에 의해 각각 19.8%와 25.7%가 증가되었으며, 이는 0.0 $\mu\text{l/l}$ O_3 + pH 3.0 산성비를 처리한 식물에 비하여 0.1 $\mu\text{l/l}$ O_3 + pH 5.6 용액을 처리한 식물에서 46.1%의 증가로 나타났다.

인과 황의 함량은 두 종에서 모두 pH 3.0의 산성비를 처리한 식물이 pH 5.6의 용액을 처리한 것에 비하여 높았다. *Liquidambar styraciflua*에서의 황함량은 0.0 $\mu\text{l/l}$ O_3 + pH 3.0을 처리한 식물에서 가장 높았다. 오존은 *Liquidambar styraciflua*의 칼슘량을 증가시켰으나, *Liriodendron tulipifera*에서는 반대로 감소시켰다. 오존은 또한 *Liquidambar styraciflua*의 황과 마그네슘의 함량을 감소시켰다.

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