Susceptibility of Tree and Shrub Species and Response of Black Cherry Foliage to Ozone

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ABSTRACT

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Plants of 12 species were exposed to 0.20 ppm of ozone for 5 hr periodically throughout the growing season of 1976. Species exhibiting symptoms, in approximate descending order of susceptibility, were Hinodegiri azalea (*Rhododendron obtusum* 'Hinodegiri'), black cherry (*Prunus serotina*), American sycamore (*Platanus occidentalis*), hybrid poplar (*Populus maximowiczii* × *trichocarpa* 'USDA Forest Service clone NE-388'), yellow poplar (*Liriodendron tulipifera*), black walnut (*Juglans nigra*), Delaware Valley white azalea (*Rhododendron mucronatum*), black elder (*Sambucus nigra*), and spreading cotoneaster (*Cotoneaster divaricata*). Species failing to show symptoms were Austrian pine (*Pinus nigra*), eastern white pine (*P. strobus*), and Virginia pine (*P. virginiana*). During the summer of 1977, black cherry seedlings were exposed for 2, 4, 6, and 8 hr to 0.10 and 0.19 ppm of ozone. Visible foliar injury followed exposure for 4 hr at 0.10 ppm and for 2 hr at 0.19 ppm. The Larsen-Heck dose-response equation was used to model the effects of different concentrations and times of exposure. The predicted response of black cherry to the dosage used in the 1976 study was in good agreement with what had been observed.

Field workers are aided in diagnosing ozone injury on woody vegetation by examining highly susceptible species whose symptoms are well known. Susceptibility rankings and symptom descriptions for many species are available (1–5,7,10–12). In 1976 experiments, we exposed species already recognized as susceptible to ozone to a lower dosage than previously used for the purpose of differentiating further among them. In 1977 experiments, we studied in detail the response of black cherry to various dosages of ozone. This dose-response information may be useful to agencies involved in setting standards.

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MATERIALS AND METHODS

In the summer of 1976, 12 species (Table 1) of 1- or 2-yr-old cuttings and 2- or 3-yr-old seedlings were potted in a peat:perlite (2:1 by volume) mixture in 10- or 15-cm pots (depending on plant size) and maintained in outdoor beds. Budbreak was defined as the date by which more than half of the individuals of a species began to grow shoots.

Ten individuals of each species growing in 10-cm pots and five of each growing in 15-cm pots were brought inside very 2 wk beginning 2 wk after budbreak (Table 1). For all species, a different set of plants was used at each interval. The plants were preconditioned in an exposure chamber (13) for 18 hr at 23 C, 75% relative humidity, and 33 klux, with a 12-hr photoperiod beginning at 0600. Plants were then exposed to 0.20 ppm of ozone for 5 hr at the same conditions.

We measured ozone concentrations continuously during exposure with a REM chemiluminescence ozone monitor (REM, Inc., Santa Monica, CA) connected to a strip-chart recorder. The ozone monitor was calibrated using 1% neutral buffered potassium iodide. Temperature and relative humidity inside the chamber were monitored using wet- and dry-bulb thermocouples connected to a recorder.

After exposure, we transferred plants to another chamber maintained as during preconditioning. Plants were held in this chamber for 5 days, after which we evaluated symptoms on the current year's growth. Symptoms were described and their location with respect to leaf surface and leaf position noted. We estimated the proportion of leaves injured, relative to the total number of leaves on the plant, at intervals of 5%.

Because all plants of a set were exposed together, each set of trees of a given species represented an experimental unit. Consequently, measures of the response of the set as a unit were derived and analyzed statistically. We determined the mean proportion of injured foliage (PIF) per plant in each set by adding the individual proportions for each tree and dividing by the number of trees in the set. We computed a second variable, the proportion of injured individuals (PII) in the set, by dividing the number of injured individuals by the total number of trees in the set. Because of missing observations, the experiment was analyzed without accounting for variation caused by time since budbreak. The Kruskal-Wallis test and Dunn's multiple comparison procedure were used to assess differences among species (6).

We ranked species by susceptibility by calculating the seasonal averages (arithmetic means for all sets exposed) of PIF and PII for each species. Species were also ranked by the average rank sums (scores) from the Kruskal-Wallis tests for each response variable. Rankings were compared using Spearman's rank correlation coefficient (6).

In the 1977 dose-response experiment, we potted 2-yr-old black cherry seedlings in 15-cm pots and maintained them in outdoor beds. Every week when the foliage was 3–15 wk old, four sets of five individuals each were exposed to ozone. The concentration was 0.10 ppm at even numbered weeks after budbreak and 0.19 ppm at odd numbered weeks.

Fumigation began on the four sets of plants at the same time, but one set was withdrawn every 2 hr. This pattern created exposure durations of 2, 4, 6, and

Table 1. Foliar injury on 12 species exposed to 0.20 ppm of ozone for 5 hr

Species	Plants per set	Weekly values of PIF (%)[a] at weeks since budbreak[b]									Seasonal means (%)	
		2	4	6	8	10	12	14	16	18	PIF	PII[c]
Hinodegiri azalea (*Rhododendron obtusum* 'Hinodegiri')	10	…[d]	…	…	…	…	…	24	32	43	33 (1)[e]	97 (1)
Black cherry (*Prunus serotina*)	5	0	33	2	79	24	44	17	17	…	27 (2)	70 (4)
American sycamore (*Platanus occidentalis*)	5	0	16	38	34	21	27	…	…	…	26 (3)	73 (3)
Hybrid poplar (*Populus maximowiczii* × *trichocarpa* 'USDA Forest Service clone NE-388')	5	0	5	34	7	5	30	34	41	…	20 (4)	65 (5)
Yellow poplar (*Liriodendron tulipifera*)	5	0	0	9	19	50	35	…	…	…	19 (5)	57 (7)
Black walnut (*Juglans nigra*)	5	0	12	0	17	0	…	43	…	…	12 (6)	30 (8)
Delaware Valley white azalea (*Rhododendron mucronatum*)	10	…	…	3	5	5	1	26	24	20	12 (7)	77 (2)
Black elder (*Sambucus nigra*)	5	12	5	12	22	12	13	12	0	…	11 (8)	58 (6)
Spreading cotoneaster (*Cotoneaster divaricata*)	10	0	9	0	15	0	8	0	0	…	4 (9)	21 (9)
Austrian pine (*Pinus nigra*)	5	0	0	0	0	0	0	0	0	…	0 (11)	0 (11)
Eastern white pine (*P. strobus*)	5	0	0	0	0	0	0	0	0	…	0 (11)	0 (11)
Virginia pine (*P. virginiana*)	5	0	0	0	0	0	0	0	0	…	0 (11)	0 (11)

[a] PIF = mean proportion of injured foliage per plant in a set.

[b] For pines, add 1 to the number of weeks to get the correct age.

[c] PII = mean proportion of injured individuals in a set.

[d] … = missing observation.

[e] Numbers in parentheses are ranks of the seasonal mean (average ranks used for ties).

8 hr. The proportion of injured foliage on each tree was measured by counting the number of injured leaves and the total number of leaves, and by forming the quotient. In other details, the 1977 and 1976 experiments were the same.

We examined the effects on response of concentration and exposure duration by using a variation of the Larsen-Heck regression model (8). PIF was transformed by the probit transformation (inverse cumulative normal distribution function) and used as the dependent variable. Whenever PIF was 0.00, it was arbitrarily assigned the value 0.0001 before transformation to keep it within the domain of the probit. The natural logarithms of concentration (in parts per million) and exposure duration (in hours) were the independent variables.

RESULTS

Symptoms. Leaf stipple was the predominant symptom in the 1976 study, but necrosis, chlorosis, and premature abscission also occurred. Ozone induced a dark stipple, as illustrated for black cherry (3), on the adaxial leaf surface of all broad-leaved species except hybrid poplar, which exhibited black, bifacial necrosis. Stipple was generally most severe on the interveinal leaf tissue, but on American sycamore it occurred adjacent to the larger veins and along the leaf margin. On cotoneaster, stipple typically appeared along the margin. Stipple was most evident on several species 24–48 hr after exposure, and then it faded. This fading was most noticeable on black cherry, which also exhibited chlorosis. Severely blackened hybrid poplar leaves and chlorotic black cherry leaves abscised prematurely.

In the 1977 dose-response study, the location of the stipple on black cherry changed as the plants aged. Four weeks after budbreak, stipple was most severe at the margin near the apex of the oldest leaves. Six weeks after budbreak, symptoms appeared along the midvein and margin. Beginning 8 wk after budbreak, interveinal stipple occurred widely across the adaxial surface of leaves located on the basal two-thirds of the stem. Throughout the season, immature leaves located on the apical one-third of the stem were seldom injured. In general, stipple became more severe as leaf position progressed from stem apex to base.

Two other phenomena were evident at higher dosages of ozone. Basal leaves often abscised following exposure 6 or more weeks after budbreak to 0.19 ppm. Foliar symptoms often appeared immediately after exposure to the highest dosage (0.19 ppm for 8 hr).

Symptoms also faded on black cherry during the dose-response study. The dark reddish brown to black stipple rapidly faded, becoming a chlorotic to tan stipple that was increasingly hard to detect as time passed after fumigation. This fading often resulted in a sharp reduction in symptom severity if a rating 1 day after exposure was compared with one taken 5 days after exposure. Fading was most pronounced on those trees fumigated 10 or more weeks after budbreak.

Susceptibility. The 12 species exhibited a wide range of susceptibility following exposure to 0.20 ppm of ozone for 5 hr (Table 1). All nine broad-leaved species showed visible symptoms, whereas the three conifers showed none. Among broad-leaved species, the seasonal mean PII ranged from 21 to 97% and the seasonal mean PIF from 4 to 33% (Table

1). Damage to a high proportion of individuals in a species was not necessarily accompanied by severe symptoms. For example, 77% of the Delaware Valley white azaleas exhibited visual symptoms, but the seasonal mean PIF was only 12%.

Significant differences between pairs of species and a susceptibility ranking by Kruskal-Wallis scores for each response variable are presented in Table 2. Susceptibility rankings were also assigned according to the seasonal means of the response variables (Table 1). However, the statistical significance of differences between species based on seasonal means could not be assessed because of the inability to meet the normality assumption implicit in parametric analysis of variance procedures.

The four rankings had no major discrepancies. In fact, the probability of achieving a higher rank correlation coefficient by chance was never more than 0.0004 in any of the six possible correlations of pairs.

Table 2. Susceptibility rankings of the species based on the mean rank sums (Kruskal-Wallis scores) for the two variables

	Ranks[w]	
Species	**PIF**[x]	**PII**[y]
Hinodegiri azalea	1 a[z]	1 a
Black cherry	3 a	4 a
American sycamore	2 a	3 a
Hybrid poplar	4 a	5 ab
Yellow poplar	6 ab	7 ab
Black walnut	8 ab	8 ab
Delaware Valley white azalea	5 ab	2 a
Black elder	7 ab	6 ab
Spreading cotoneaster	9 ab	9 ab
Austrian pine	11 b	11 b
Eastern white pine	11 b	11 b
Virginia pine	11 b	11 b

[w] Average ranks used for ties.

[x] PIF = mean proportion of injured foliage per plant in a set.

[y] PII = mean proportion of injured individuals in a set.

[z] Different letters indicate significant differences at $P \leq 0.0015$ using Dunn's multiple comparison rule. The error rate for all comparisons on a single variable is approximately 0.20.

The 12 species can be classified into four groups. Hinodegiri azalea, black cherry, and American sycamore were the most susceptible. They had moderately high damage levels and could be separated from the undamaged conifers using Dunn's procedure on both response variables. The conifers—Austrian pine, eastern white pine, and Virginia pine—never showed any symptoms, and were thus the least susceptible. Species in the upper intermediate group—hybrid poplar and Delaware Valley white azalea—could be separated from the conifers for only one response variable. The remaining species—yellow poplar, black walnut, black elder, and spreading cotoneaster—formed the lower intermediate group.

Time since budbreak. In the study of relative susceptibility, foliage of most species was least sensitive during early growth and became more sensitive as the season progressed (Table 1). Of seven species exposed 2 wk after budbreak, only black elder exhibited symptoms. Two weeks later, only yellow poplar failed to exhibit symptoms.

In the dose-response study, black cherry foliage exposed to 0.19 ppm was also least sensitive immediately after growth began (Table 3). Further, the black cherry data suggest a peak of sensitivity 5–7 wk into the season. In both studies, week-to-week variability obscured any distinct evidence of a seasonal pattern in plant response.

Dose-response of black cherry. Black cherry foliage was more sensitive to ozone as concentration and length of exposure increased (Table 3). At 0.10 ppm, 4 hr of exposure was necessary to induce visible foliar symptoms; at 0.19 ppm, symptoms appeared after only 2 hr.

The pattern of black cherry response to ozone may be summarized using the Larsen-Heck regression model (8): $\text{probit}(\text{PIF}) = b_0 + b_1 \ln(c) + b_2 \ln(t)$, where c is the ozone concentration in parts per million, t is the exposure duration in hours, PIF is the mean proportion of injured foliage per plant in each set (expressed as a fraction), ln(x) is the natural logarithm function, probit(x) is the inverse cumulative normal distribution function, and b_0, b_1, and b_2 are the unknown coefficients.

This model was fit to the black cherry data using ordinary least squares regression techniques on the transformed variables. The resulting equation is: $\text{probit}(\text{PIF}) = -0.2174 + (2.2457)\ln(c) + (2.1378)\ln(t)$. The model provides a significant reduction in the variation ($P \leq 0.0001$ for the overall F-statistic), with $R^2 = 0.769$. Both concentration and exposure duration are required; dropping either significantly reduces the fit ($P \leq 0.0001$ for each variable). A lack-of-fit test (9) showed no evidence ($P > 0.50$) of model shortcomings. Thus, no other terms are required in the model.

We assessed the predictive ability of the model. The conditions of the susceptibility study (0.20 ppm of ozone for 5 hr) were substituted into the regression equation, resulting in a predicted mean PIF of 30.6% with 95% confidence limits of 21.4 and 41.4%. The seasonal mean PIF for black cherry in 1976 was 27%, which is well within the confidence interval.

DISCUSSION

Symptoms reported in this study are similar to those previously observed (1–5,7,10–12). Descriptions of ozone-induced foliar symptoms based on laboratory exposures have helped us to identify ozone injury on wild grape (*Vitis* spp.), common milkweed (*Asclepias syriaca*), sassafras (*Sassafras albidum*), white ash (*Fraxinus americana*), green ash (*F. pennsylvanica*), and other bioindicators. Most of these species exhibit foliar injury every year in western Pennsylvania, where ozone concentrations up to 0.19 ppm have been recorded (Davis, *unpublished data*).

Species that are highly susceptible in the laboratory commonly exhibit symptoms in the field. Black cherry, ranked in the most susceptible group, is often injured by ozone in western Pennsylvania. Hybrid poplar and American sycamore, also ranked as highly susceptible, exhibit symptoms under ambient conditions.

Table 3. The influence of concentration, length of exposure, and time since budbreak on the response of black cherry foliage to ozone

Ozone concentration (ppm)	**Length of exposure (hr)**	**PIF (%)**[a] **at weeks since budbreak**													**Row means**
		3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
0.10	2		0		0		0		0		0		0		0
	4		0		9		4		0		2		1		3
	6		0		17		6		6		4		17		8
	8		30		15		27		17		25		7		20
	Column means		8		10		9		6		8		6		8
0.19	2	0		0		5		1		0		6		2	2
	4	1		55		71		8		10		36		9	27
	6	36		66		85		55		42		47		48	54
	8	28		76		83		61		72		64		55	63
	Column means	16		49		61		31		31		38		29	37

[a] PIF = mean proportion of injured foliage per plant in a set.

Although we have seen symptoms on yellow poplar in the field, we believe that the remaining species, ranked as less susceptible, are unlikely to be injured by ambient ozone in the northeastern United States.

In the field, ozone symptoms on black cherry first appear during early July and become more severe during the following weeks. Severe stippling, chlorosis, and premature defoliation are common on black cherry and other indicator species by late August. This phenology differs from laboratory experience, where peak sensitivity occurs earlier in the season. However, under ambient conditions, plants experience chronic exposure, and the resultant stipple is probably cumulative.

Species previously classified as susceptible to ozone showed differing susceptibility in the 1976 study. The three conifer species that did not develop symptoms following exposure to 0.20 ppm of ozone for 5 hr in this study had been injured after exposure to 0.25 ppm for 8 hr in the same chamber under identical conditions (4). The threshold for visible foliar injury to the conifers under these conditions is evidently somewhere between the two dosages.

Seasonal pattern in sensitivity. Plants were exposed periodically throughout the summer to minimize the bias induced by changes in sensitivity as the plants developed. This precaution was applied fully in the dose-response study, but not all species were exposed at every 2-wk interval in the study of relative susceptibility. For example, Hinodegiri azalea, the most extreme case, was exposed only three times (three sets of 10 trees each). If these fumigations coincided with a time of particular sensitivity, the estimate of susceptibility might be inflated.

Another reason for exposing the plants throughout the summer was to identify seasonal patterns in response. Earliest growth stages in the spring were resistant to ozone. This finding is consistent with the pattern of injury on individual trees, in which the youngest leaves were seldom damaged. In spring, all leaves are young and resistant. After the beginning of the season, evidence of seasonal patterns in plant response was clouded in both studies by week-to-week variability. Although there are other possibilities, we believe that the primary cause of the variability was the fluctuating outdoor environment where the plants were grown before exposure.

Dose-response of black cherry. We analyzed the black cherry dose-response data by Larsen and Heck's method (8), using mean response rather than median to express the existence of a small degree of injury in a set. We also used the mean from each replicate as a separate observation, rather than pooling all replicates of each concentration-duration combination. Multiple observations for each treatment combination are valuable because they allow a formal test for lack of fit in the regression model.

The theory used by Larsen and Heck includes the assumption that the terms ln(c) and ln(t) are adequate and that higher order terms such as $[\ln(c)]^2$ or $[\ln(c)][\ln(t)]$ are not necessary. They use multiple correlation coefficients from several data sets to evaluate their model, but they fail to determine whether the inclusion of higher order terms would enhance the fit. The multiple correlation coefficient achieved by fitting our black cherry data ($R = 0.88$) is comparable to the values they report. Moreover, we tested whether higher order terms would improve the model and found no need for them. This finding provides limited support for the Larsen-Heck assumption. Had the black cherry data included more than two ozone levels, this support would be stronger.

The regression equation can be used to predict foliar injury. We sucessfully used the equation derived from the 1977 data to predict the seasonal mean PIF for black cherry from the 1976 experiment. The predictive validity of the black cherry dose-response equation is limited to the range of concentrations and durations and to the exact conditions of the experiment. If these limitations could be circumvented, a modified dose-response equation with a Larsen-Heck skeleton might be used to predict injury for a variety of plant species under a wide range of environmental conditions and pollutant dosages.

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