Provenance and Family Variation in Response of *Fraxinus americana* and *F. pennsylvanica* to Ozone and Sulfur Dioxide

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ABSTRACT

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Two-year-old seedlings representing 10 provenances and 50 half-sib families of *Fraxinus americana* and 16 provenances and 59 half-sib families of *F. pennsylvanica* were exposed to 0.5 ppm ozone or 1.0 pp. sulfur dioxide (both as $\mu g/m^3$) for 7.5 hr. The *F. americana* seedlings exposed to ozone were later reexposed to the same concentration, but for 7.5 hr on two consecutive days to increase the injury level so differences in pollutant tolerance could be detected. Differences in severity of foliar injury among families were significant for both species and both pollutants and accounted for 16–49% of the total variation. Variation among provenances was greater than variation within provenances for response to ozone, but the reverse was true for response to sulfur dioxide. Provenance means tended to vary with geographic origin; trees from near-coastal provenances of *F. americana* were generally more tolerant to ozone than those from interior provenances, and trees from southern provenances of *F. pennsylvanica* were generally more tolerant to both ozone and sulfur dioxide than those from northern provenances. Our results suggest that both species offer considerable potential for genetic improvement in air pollution tolerance, that improvement programs should incorporate both provenance selection and family-within-provenance selection, and that selected individual trees of these two species may serve as sensitive bioindicators of ozone and sulfur dioxide.

Additional key words: green ash, white ash.

Fraxinus americana L. (white ash) and *F. pennsylvanica* Marsh. (green ash) are commonly planted as urban trees and are also important forest trees in much of the eastern United States. Unfortunately, they are also among the most sensitive to both ozone and sulfur dioxide (3,12).

It is well known that the responses of trees to air pollutants vary between and within species and that the variation is under strong genetic control (7). However, studies of trees have not adequately described the extent of genetic variation within a given species. For example, the pioneering studies of the response of lodgepole pine to sulfur dioxide (8) and the response of red maple (11) and green ash and white ash (9) to ozone were done on rather limited samples. Seedlings from four single-tree families were used in both the lodgepole pine and red maple studies. The ash study consisted of seedlings from 10 single-tree families of green ash and two single-tree families of white ash. Thus, none of these studies compared to extent of between- vs within-provenance variation. The majority of the other studies examining intraspecific variation of tree responses to air pollutants have dealt with describing tree-to-tree variation within local populations (4). Similarly, programs to test the variation in air pollution tolerance in agricultural and horticultural crops have examined only a limited sample of the natural populations (ie, cultivars and varieties).

The purpose of this study was twofold. First, it was designed to be the most complete examination of intraspecific variation in air pollution tolerances of plants yet attempted. Second, it was intended to be the initial step in a program to select green ash and white ash trees tolerant to ozone and sulfur dioxide for planting in areas with known pollutant problems and to identify air pollutant-sensitive trees of these two species to serve as bioindicators of ozone or sulfur dioxide or both. Most forest tree improvement efforts are based on selecting superior genotypes from unimproved wild populations; thus, this program offers a traditional, but greatly broadened, approach to selecting trees with variations in air pollution tolerance.

MATERIALS AND METHODS

Fraxinus americana. In February 1977 2-yr-old seedlings of five half-sib families in each of 10 provenances (Table 1), were brought into a greenhouse from a cold-storage building and grown in a mixture of peat, sterilized soil, and perlite (1:1:1, v/v) in 4.5-L plastic containers. The plants, which ranged in height from 0.2 m to 0.8 m, were fertilized biweekly with "Rapid Gro" (Rapid Gro Corp., Dansville, NY 14437) fertilizer, watered as needed, and watered before each fumigation.

Fraxinus pennsylvanica. Seeds of two to four half-sib families in each of 16 provenances (Table 1) from the junior author's *Fraxinus pennsylvanica* progeny testing program were germinated in the spring of 1976, and the seedlings were grown for two growing seasons in the greenhouse. The soil mix and the fertilizing and watering regimes were as described above for *F. americana*. Two-year-old seedlings, ranging in height from 0.3 to 1.0 m, were brought into the greenhouse from cold storage in February 1978.

Chamber fumigations. Fumigations of each species were begun approximately 4 wk after the last plants of that species had broken bud and the majority of their leaves had expanded to nearly mature size. They consisted of 7.5-hour exposures to 0.5 ppm ozone or 1.0 ppm sulfur dioxide (both as $\mu g/m^3$) and were done in a 2.4-m-tall by 4.3-m-diameter cylindrical chamber located in the Cary Arboretum greenhouse. The chamber and its pollutant generating and monitoring systems were described by Karnosky (5). Chamber temperature was maintained at between 20 and 25 C, and relative humidity between 40 and 60%. All fumigations were done from approximately 0830 to 1600 hr.

Different sets of seedlings of each species were fumigated with each pollutant on either five (*F. americana*) or six (*F.

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pennsylvanica) different occasions. For *F. americana*, the sulfur dioxide fumigations were run from 25 to 29 April 1977, and the ozone fumigations were run from 13 to 17 April 1977. Because *F. americana* seedlings were not sufficiently injured following one day of exposure to ozone, they were again exposed beginning 20 June 1977 to the same concentration for the same time, but on two consecutive days. For *F. pennsylvanica*, the sulfur dioxide fumigations were run from 3 to 8 April 1978 and the ozone fumigations were run from 14 to 19 April 1978.

In each series of fumigations, an additional set of control seedlings was exposed to filtered air in the chamber. Families were represented in each fumigation by randomly located plots of either two (*F. americana*—ozone, *F. pennsylvanica*—ozone and sulfur dioxide) or three (*F. americana*—sulfur dioxide) seedlings. However, the plants of all families of a species could not be accommodated in each fumigation because the chamber was too small. Thus, the experiments were designed as randomized incomplete blocks and either three or five complete replications were accomplished in five or six fumigations.

TABLE 1. Origins of *Fraxinus americana* and *F. pennsylvanica* provenances used in study

Species and provenance number	County, state (or province)	North latitude	West longitude
Fraxinus americana			
2102	Williamson, IL	37°48′	89°06′
2301	Forest, WI	45°42′	89°00′
3201	Wayne, OH	40°48′	81°54′
3302	Madison, AL	34°30′	86°30′
3501	Boone, AR	36°24′	93°00′
3701	George, MS	49°30′	88°48′
7103	Effingham, IL	39°00′	88°24′
8501	Penobscot, ME	44°54′	68°36′
9205	Hopkins, KY	37°18′	87°36′
9401	New Haven, CT	41°18′	73°00′
Fraxinus pennsylvanica			
093P	Manitoba, Canada	49°52′	97°05′
165P	Adams, IL	39°58′	91°25′
169P	Jersey, IL	39°00′	90°30′
177P	Mississippi, MO	36°41′	89°12′
181P	Tipton, TN	35°35′	89°37′
201P	Dutchess, NY	41°50′	73°33′
205P	Columbia, NY	42°02′	73°37′
253P	Kalamazoo, MI	42°20′	85°20′
271P	Custer, SD	43°39′	103°02′
277P	Perry, PA	40°33′	77°09′
309P	Anderson, TN	36°10′	84°04′
345P	Jackson, IL	37°43′	89°30′
373P	Onondaga, NY	43°02′	76°04′
409P	Madison, NE	42°01′	97°25′
437P	McKenzie, ND	47°10′	103°22′
441P	Thomas, NE	41°53′	100°19′

Measurement and analysis. The plants were observed for foliar injury symptoms for 1 mo after fumigation. At 1 wk after fumigation, the plants were scored for foliar injury by using a modification of the system described by Davis and Coppolino (2) according to the formula:

$$\text{Injury Index} = (\%\ \text{leaves injured in plot}) \times (\text{average degree of severity per leaf})$$

Degree of severity followed a scale of 0 to 5, depending on percentage of leaf surface injured (ie, 0 = no injury, 1 = 1–20%, 2 = 21–40%, etc.).

Statistical analyses were performed after the data were logarithmically transformed to make means and variances independent of one another. Because of the unbalanced designs, a least-squares solution to a linear model of the sources of variation was used to calculate analyses of variance with unbiased estimates, as described by Barr et al (1). Product moment correlations were used to determine relationships between provenance means and latitude or longitude of origin. Family and provenance means were retransformed to the original form for presentation.

RESULTS AND DISCUSSION

The most common symptom of ozone injury on both *F. americana* and *F. pennsylvanica* was purple stipple, which appeared on the upper leaf surface within 1 wk after fumigation. Black stipple and large areas of purple coloration on the upper leaf surface, sometimes occurring along the veins but generally across as well as between the veins, were also common. Bifacial necrotic stipple or large patches of bifacial necrosis, leaflet curling, and premature leaflet drop occurred on some of the most sensitive trees. Some of the purple and black color tended to fade to tan or grey within 1 mo after fumigation. Older and middle-aged leaves were injured in almost all cases where injury was found; younger leaves were only occasionally injured.

For sulfur dioxide, the most common symptoms for both *F. americana* and *F. pennsylvanica* were classical ones including bifacial, interveinal, tan, necrotic areas on older and middle-aged leaves. As with ozone, the younger leaves also were most tolerant to sulfur dioxide. Injury symptoms developed within the first week after fumigation. Leaflet curl and premature leaflet drop occurred on the most sensitive seedlings.

Substantial between-provenance variations in the foliar responses of *F. americana* and *F. pennsylvanica* to both ozone and sulfur dioxide were detected (Tables 2 and 3). For example *F. americana* family mean ozone injury indices varied from 0 (no injury) to 201.3 (injury symptoms covering approximately 40% of the total leaf surface). Differences among provenance means were significant for all treatments except *F. americana* exposure to sulfur dioxide (Table 4). These differences accounted for 10–33% of total variation in injury index scores for the *F. americana* seedlings

TABLE 2. Mean response of *Fraxinus americana* provenances to ozone and sulfur dioxide exposure

Ozone				Sulfur dioxide			
Provenance	State	Mean injury index[z]	Range of family means	Provenance	State	Mean injury index[z]	Range of family means
3501	AR	57.6 a	2.6–201.3	2102	IL	163.7 a	108.6–212.8
9205	KY	35.0 ab	13.8– 60.7	2301	WI	136.4 a	90.2–203.2
2301	WI	34.5 ab	10.9–113.3	8501	ME	108.1 ab	56.5–185.2
3201	OH	27.7 abc	12.6– 70.1	3501	AR	89.6 ab	23.5–233.4
2102	IL	20.2 abc	6.5– 48.0	3302	AL	88.4 ab	36.2–198.5
7103	IL	17.2 abc	3.9– 51.2	9205	KY	68.8 abc	25.9–189.5
3302	AL	14.0 abc	7.2– 33.7	3701	MS	62.6 abc	20.4–140.3
9401	CT	12.2 bc	5.1– 57.3	3201	OH	62.0 abc	24.7–130.8
3701	MS	7.3 c	2.0– 37.7	7103	IL	41.1 bc	16.4– 99.0
8501	ME	1.4 d	0– 7.1	9401	CT	30.3 c	13.1– 76.6
	Species mean = 22.7				Species mean = 85.1		

[z]Means not followed by the same letter are significantly different at $P \leqslant 0.05$, based on Duncan's "new multiple range test" (10) applied to transformed data values. Note: analysis of variance indicated no significant differences among sulfur dioxide means at overall experiment error rate of $P \leqslant 0.05$.

TABLE 3. Mean response of *Fraxinus pennsylvanica* provenances to ozone and sulfur dioxide exposure

Ozone				Sulfur dioxide			
Provenance	State or province	Mean injury index[z]	Range of family means	Provenance	State or province	Mean injury index[z]	Range of family means
093P	Man.	196.7 a	165.0–228.1	205P	NY	48.4 a	29.9– 65.1
437P	ND	125.9 ab	76.6–185.2	093P	Man.	41.5 a	18.5–116.5
201P	NY	105.7 abc	78.4–137.0	309P	TN	41.4 a	21.4– 82.2
271P	SD	64.5 abcd	40.7–103.7	201P	NY	37.2 a	10.0– 99.0
205P	NY	54.7 abcd	29.2–101.3	437P	ND	34.2 a	24.7– 55.2
373P	NY	43.9 bcde	27.2– 90.2	271P	SD	32.7 a	23.0– 45.8
253P	MI	41.2 bcde	8.1–103.7	165P	IL	30.9 a	10.5– 78.4
309P	TN	40.8 bcde	14.1– 92.3	277P	PA	28.8 a	19.0– 54.0
277P	PA	40.1 bcde	13.5–119.2	373P	NY	28.8 a	9.0– 59.3
441P	NB	38.3 bcde	20.4– 55.2	345P	IL	26.5 a	3.7– 94.5
165P	IL	25.9 cde	11.9– 63.6	409P	NB	23.6 a	15.6– 50.3
169P	IL	25.5 cde	4.9–157.5	169P	IL	21.0 ab	9.0– 52.7
409P	NB	21.8 de	4.8– 50.3	253P	MI	16.8 ab	2.3– 43.7
345P	IL	10.9 ef	7.9– 19.0	441P	NB	14.8 ab	2.2– 37.9
181P	TN	3.6 f	0.2– 15.6	181P	TN	4.9 b	1.4– 26.5
177P	MO	3.1 f	0.8– 9.7	177P	MO	4.8 b	3.6– 7.3
	Species mean = 51.4				Species mean = 27.2		

[z]Means not followed by the same letter are significantly different at $P \leqslant 0.05$, based on Duncan's "new multiple range test" (10) applied to transformed data values.

TABLE 4. Variance components, expressed as percentage of total variance, for ozone and sulfur dioxide injury to *Fraxinus americana* and *F. pennsylvanica*

Species and sources of variation	Ozone df	Ozone Variance component[a]	Sulfur dioxide df	Sulfur dioxide Variance component[a]
Fraxinus americana				
Blocks[b]	4	4.9**	4	27.4**
Provenances	9	19.2**	9	3.0
Families within provenances	40	14.7**	40	13.2*
Error	192	61.2	96	56.4
		100.0		100.0
Fraxinus pennsylvanica				
Blocks[b]	5	4.7**	5	5.1**
Provenances	15	33.1**	15	10.4*
Families within provenances	43	15.8**	43	18.0**
Error	231	46.4	225	66.5
		100.0		100.0

[a]*,** Statistically significant $P = 0.05$ and $P = 0.01$, respectively.
[b]Blocks = fumigations.

fumigated with ozone and sulfur dioxide treatments. Family-within-provenance differences in response to ozone and sulfur dioxide were significant for all treatments (Table 4) and accounted for 13–18% of total variation in the seedling responses to these pollutants. Thus, combined provenance and family effects accounted for 34–49% and 16–28% of the total variation in response of seedlings from both species to ozone and sulfur dioxide, respectively. Provenances were a relatively larger source of variation in response to ozone than families within provenances, but the reverse was true for response to sulfur dioxide.

As a species, *F. pennsylvanica* was considerably more sensitive to ozone than was *F. americana*. While *F. pennsylvanica* had a mean injury index of 51.4 (Table 3) following 7.5 hr exposure to 0.5 ppm ozone, the *F. americana* seedlings were not sufficiently injured by exposure to the same dosage to enable detection of provenance differences in ozone tolerance. Even after the doubled dose exposure (two consecutive days of 0.5 ppm ozone for 7.5 hr per day), the mean injury index for *F. americana*, 22.7 (Table 2), was substantially less than that for *F. pennsylvanica*. In contrast, *F. americana* was generally more sensitive to sulfur dioxide ($\overline{x} = 85.1$) than was *F. pennsylvanica* ($\overline{x} = 27.2$).

TABLE 5. Correlations between latitude or longitude of provenance origins and transformed injury indices of *Fraxinus americana* and *F. pennsylvanica* seedlings exposed to ozone and sulfur dioxide

Variables		Correlation coefficient[a]	
Injury index	Provenance	*F. americana*	*F. pennsylvanica*
O_3 injury index	North latitude	−0.491	+0.829**
	West longitude	+0.734*	+0.029
SO_2 injury index	North latitude	−0.016	+0.531*
	West longitude	+0.261	−0.155

[a]*,** Statistically significant, $P = 0.05$ and $P = 0.01$, respectively.

Mean ozone injury to trees from *F. americana* provenances was significantly correlated with longitude of origin (Table 5), but longitude in this case appears to be more accurately interpreted in terms of distance from the Altantic and Gulf Coasts. The most sensitive trees were from provenances in the central and western portions of the species' range, while the least sensitive were all native to near-coastal locations in Maine, Mississippi, and Connecticut (Tables 1 and 2). Mean sulfur dioxide injury to trees from *F. americana* provenances followed no recognizable geographic pattern.

Provenance variation in *F. pennsylvanica* tended to follow a latitudinal pattern for both ozone and sulfur dioxide injury (Table 5). Trees from two of the southernmost provenances (181P and 177P) were most tolerant to both pollutants, while trees from northern provenances (particularly 093P from Manitoba) tended to be the most sensitive (Table 3). The latitudinal pattern was particularly strong for ozone injury. Interestingly, five of the six *F. pennsylvanica* provenances with trees most tolerant to ozone (177P, 181P, 345P, 169P, and 165P) were all collected in or very near the Mississippi River Valley, from central Illinois to Tennessee. However, these were also the southernmost provenances except for 309P from eastern Tennessee (Table 1). This pattern of variation cannot be directly compared to that of *F. americana*, since no near-coastal provenances of *F. pennsylvanica* were sampled.

Steiner and Davis (9) observed a phenological cause for part of the variation pattern for Fraxinus seedling's response to ozone. In general, older leaves sustained greater injury than younger leaves. Since northern seed sources of *F. pennsylvanica* tend to break dormancy earlier than southern seed sources subjected to the same conditions (K. C. Steiner, *unpublished*), an examination of the relationship between bud flushing and sensitivity to pollutants was made for the *F. pennsylvanica* seedlings in this study. Weekly observations were made to determine the dates of bud flushing. A correlation was run on the number of days after 1 March when two thirds of the seedlings for a family had flushed and the family's foliar injury score for both ozone and sulfur dioxide. No relationship was found between flushing date and sulfur dioxide sensitivity ($r = +0.12$). The magnitude of the correlation coefficient for ozone injury and date of bud flushing ($r = -0.24$) was larger but still nonsignificant. There appeared to be a tendency for the earliest flushing families to have moderate to severe ozone injury. However, the families with the most-injured and least-injured trees by ozone in the entire experiment burst bud at virtually the same time. Thus, it appears that our results for *F. pennsylvanica* were primarily attributable to factors other than leaf age.

The majority of air pollution tolerance studies involving trees have been done in chambers in either the greenhouse or laboratory. Thus, there is a lack of information regarding the relative responses of many tree species to air pollutants in the field, where pollutant exposures are more complex and where numerous other environmental factors may be important. Karnosky (6) reported that several tree cultivars were more tolerant to oxidants in the field than they were to ozone in chamber exposures. The authors have begun to field test the responses of the *F. americana* and *F. pennsylvanica* seedlings by outplanting selected tolerant and sensitive families in the greater New York City area. The results of this field test will be evaluated before selections are made for ozone- and sulfur dioxide-tolerant and -sensitive trees of these two species.

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