Ozone and Sulfur Dioxide Mixtures Cause a PAN-Type Injury to Petunia

Elizabeth Lewis and Eileen Brennan

Graduate Student and Professor, Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903.

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ABSTRACT

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Foliage of petunia plants exposed to a mixture of ozone (O_3) and sulfur dioxide (SO_2) in a controlled environment developed a symptom that fits the description of classic peroxyacetylnitrate (PAN) injury. The undersurface of newly matured leaves became glazed, especially at the apex of the youngest susceptible leaf, at the middle of the intermediate-aged leaf and at the base of the oldest susceptible leaf. As with PAN, injury was more severe on a white-flowered cultivar than on a red-flowered one. In contrast to the result reported for PAN-fumigated plants, prior treatment with benomyl afforded some protection against injury from the mixture of O_3 and SO_2 in controlled fumigations. In a field study conducted in New Jersey in the summer of 1975, white petunia cultivars responded to ambient air pollution in a manner identical to that observed in controlled fumigations with the mixture of O_3 and SO_2. We question the generally accepted notion that petunia damage in the northeastern USA is a consequence of toxic levels of PAN and we suggest an alternative cause, a mixture of O_3 and SO_2.

Petunia (*Petunia hybrida* Vilm.) is an important bedding plant in New Jersey and in the northeastern USA. Injury caused by air pollution often is severe enough to inhibit normal growth of seedlings and to conspicuously disfigure attractive foliage, especially on white cultivars. Characteristically the injury appears on susceptible leaves as an undersurface glazing that is sometimes accompanied by chlorosis or necrosis of the upper leaf surface (2, 4, 13). Since the symptom is consistent with that described by researchers for peroxyacetylnitrate (PAN) (15, 16), the injury generally is attributed to the presence of PAN in the ambient air. In the absence of accompanying air-monitoring data for PAN in any part of the northeastern USA, the conclusion is tenuous.

Tingey et al. (17) have shown that glazing of leaf undersurfaces can be produced on radish, tomato, and tobacco foliage by a mixture of ozone (O_3) and sulfur dioxide (SO_2). We hypothesized that the mixture also could produce the same PAN-type symptom on petunia. We exposed two petunia cultivars, one sensitive, and one relatively tolerant to ambient air pollution to O_3, SO_2, or a mixture of the two pollutants in a controlled environment. Additionally, we planted the same cultivars in the field to compare the symptoms resulting from ambient air pollution and monitored ambient O_3 and SO_2 levels. Since benomyl enhances PAN damage to plants (10, 11), treatment with the fungicide was included to determine: (i) its effect on plants exposed to a mixture of O_3 and SO_2, and (ii) its possible use as a tool to differentiate between the two sources of injury.

MATERIALS AND METHODS

Plant material for controlled fumigations.—Red and white petunias of cultivar Cascade (Park Seed Co.) were started from pelleted seed in a 1:1 (v/v) mixture of peat moss and perlite. At the four-leaf stage, seedlings were transplanted to 15 cm diameter plastic pots containing a 1:1:1 (v/v) mixture of peat, perlite, and loam previously steam sterilized and adjusted to pH 6.5. When a benomyl treatment was to be used, peat and perlite were omitted from the mixture since they tend to bind the fungicide (11). Plants were grown in a greenhouse equipped with charcoal air filtration and were watered and fertilized as required. At the time of exposure, plants were succulent and in flower.

Ten days prior to fumigation, a group of plants was treated with a benomyl soil drench (50% active ingredient) at the rates of 0, 40, 60, 80, or 120 $\mu g/g$ soil. The presence of benomyl in the foliage was confirmed by bioassay (12) before each fumigation.

Fumigation method.—Plants were exposed to a single pollutant or a mixture in a 6-m^3 glass-enclosed fumigation chamber located in the greenhouse. Ozone was evolved by passing a metered stream of pure dry oxygen through a commercial ozone generator. It was dispersed into a charcoal-filtered air stream which passed through a mixing chamber before entering the fumigation chamber. Air flow in the chamber caused a complete air

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change every 45 sec, temperature was maintained at 24-27 C, and relative humidity ranged 75-80%. Use of a turntable within the chamber assured that all plants received equal exposure. Ozone concentrations were monitored with a Mast Ozone Meter (Mast Development Co., Inc., Davenport, IA 52802) calibrated by the buffered neutral KI method (5). A chromic acid scrubber on the Mast meter eliminated interference by SO_2.

Sulfur dioxide was dispensed from commercial cylinders (Matheson Co., East Rutherford, NJ 07073) containing 3% SO_2 and 97% N_2 under the same conditions described above. Sulfur dioxide concentration in the chamber was measured by the West and Gaeke method (19) at 30 min intervals. When a mixture of two pollutants was used, they were introduced in the same manner as when used individually.

Controlled fumigations.—Plants were placed in the chamber immediately preceding fumigation. Each fumigation with O_3, SO_2, or the O_3 and SO_2 mixture included four plants of each cultivar, and fumigations at each pollutant level or ratio were repeated five times. Additional fumigations with the O_3/SO_2 mixture were carried out with benomyl treated plants; each fumigation utilized one plant at each of the five benomyl rates. These fumigations were repeated 20 times.

The individual pollutants were used at the following concentrations: O_3–580, 680, and 780 $\mu g/m^3$ for 4.0 to 6.0 hr and SO_2–3,900, 5,200, 6,300, and 7,800 $\mu g/m^3$ for 4 hr. The O_3/SO_2 mixture was used at ratios ranging from 190/780 $\mu g/m^3$ for 6 hr to 680/3,900 $\mu g/m^3$ for 4.5 hr (1 ppm SO_2 = 2,617 $\mu g/m^3$ SO_2, 0.1 $\mu g/m^3$ O_3 = 200 $\mu g/m^3$ O_3 at 25 C and 760 mm Hg).

Plants were rated after 48 hr for severity of injury on the following scale: 0 = no leaves affected; 1 = glazing on one-to-three leaves; 2 = glazing on four-to-six leaves, often accompanied by bifacial necrosis; and 3 = glazing and necrosis on more than six leaves.

Field experiment.—In addition to White and Red Cascade, the cultivar White Sails also was included in the field experiment. These plants were purchased from a local nursery and transplanted to the field 1 June 1976. Seventy-two plants were set out in groups of 12 plants per replicate per variety. They were irrigated, fertilized, and weeded as required. On 8 June a soil drench of benomyl was applied to half of all replicates. By this time plants were lush and in flower, conditions which were maintained by periodic flower removal. The drench was prepared with 2.3 g of benomyl per 3.78 liters (1 gal) of water, as recommended for ornamental plants, and 7.57 liters (2 gal) were drenched evenly over a 14.5 m^2 area at 3-wk intervals. Plants were rated weekly for severity of injury as described above.

The percentage of plants in each injury category, both treated with benomyl and nontreated, was determined for three cultivars. Ambient levels of O_3 and SO_2 for the duration of the experiment were obtained through courtesy of the Exxon Corp., Linden, NJ 07036 which monitors in Linden, New Jersey approximately 16 km from New Brunswick. Ozone was measured by a chemiluminescent method (7) and SO_2 by the Wusthoff conductivity method (5).

Swiss chard (*Beta chiliensis*, Hort.) and corn (*Zea mays* L. 'Golden Security') also were planted in the field because they are considered to be PAN-sensitive species (1).

TABLE 1. Symptoms exhibited by White and Red Cascade petunia plants following exposure to ozone

		White Cascade		Red Cascade	
Ozone ($\mu g/m^3$)	Time (hr)	Symptom	Severity	Symptom	Severity
580	4.5	none	0.0[a]	none	0.0
680	4.5	bleaching	1.2	none	0.0
780	4.0	flecking, bleaching	2.5	none	0.0
780	6.0	flecking, bleaching	3.0	bleaching	2.4

[a]Each value represents the average injury level based on the exposure of 20 plants. Plants were exposed in groups of four per fumigation. Severity ratings are described under "Materials and Methods", and ranged from 0 = no injury, to 3 = glazing and bifacial necrosis on more than six leaves.

TABLE 2. Symptoms exhibited by White and Red Cascade petunia plants following exposure to sulfur dioxide

		White Cascade		Red Cascade	
Sulfur dioxide ($\mu g/m^3$)	Time (hr)	Symptom	Severity	Symptom	Severity
3,900	4.0	none	0.0[a]	none	0.0
5,200	4.0	bifacial necrosis	1.5	bifacial necrosis	1.1
6,300	4.0	bifacial necrosis	3.0	bifacial necrosis	2.5
7,800	4.0	bifacial necrosis	3.0	bifacial necrosis	2.8

[a]Each value represents the average injury level based on the exposure of 20 plants. Plants were exposed in groups of four per fumigation. Ratings as in Table 1.

RESULTS

Controlled fumigations.—White Cascade was injured at lower levels of pollution than Red Cascade in every instance (Tables 1, 2, and 3). Ozone injury on both White and Red Cascade was characteristic, appearing either as flecking or bleaching of the upper leaf surface. White Cascade was injured at 680 $\mu g/m^3$ for 4.5 hr, but Red Cascade required 780 $\mu g/m^3$ for 6 hr (Table 1). Sulfur dioxide injury was visible on both cultivars at 5,200 $\mu g/m^3$ SO_2 for 4 hr. Injury was typically bifacial necrosis (Table 2).

The O_3/SO_2 mixture produced undersurface glazing of the intermediate-aged leaves. At times even the sepals of the flowers in bloom were affected (Table 3). At the lowest exposure, 190 $\mu g/m^3$ O_3 and 780 $\mu g/m^3$ SO_2 for 6 hr, White Cascade suffered moderate undersurface glazing. To produce the same degree of injury on Red Cascade, 190 $\mu g/m^3$ O_3 and 2,000 $\mu g/m^3$ SO_2 for 6 hr was required. At no time did the symptoms appear to be a simple mixture of typical O_3 and SO_2 injury, but were unique in that they were initiated on the lower leaf surface.

Plants treated with benomyl at 120 μg of benomyl/g soil were protected completely while lower levels of benomyl provided varying degrees of protection against the mixture of pollutants (Table 4).

Field experiment.—The effects of ambient air pollution were evident on field-grown plants twice during the experiment, in early July and mid-August (Table 5). Benomyl treatment afforded some protection to all three cultivars; for Red Cascade and White Sails at $P = 0.05$ and for White Cascade at $P = 0.10$.

On the 3 days prior to the appearance of plant symptoms, the ambient O_3 concentration exceeded the Federal secondary air quality standard (0.08 $\mu g/m^3$) (3) for 5 hr each day in epidose 1 and 9 hr in episode 2. The maximum hourly O_3 level exceeded 0.14 $\mu g/m^3$ and 0.30 $\mu g/m^3$, respectively. The SO_2 concentration never approached the secondary air quality standard (0.50 $\mu g/m^3$ for 3 hr). However, the maximum hourly average during each epidose, 0.04 and 0.05 $\mu g/m^3$ was greater than the monthly average, 0.02 $\mu g/m^3$.

TABLE 3. Symptoms exhibited by White and Red Cascade petunia plants following exposure to ozone/sulfur dioxide mixtures

O_3/SO_2 ($\mu g/m^3$)	Time (hr)	White Cascade Symptom	White Cascade Severity	Red Cascade Symptom	Red Cascade Severity
190/780	6.0	glazing	2.2[a]	none	0.0
190/2,000	4.5	none	0.2	none	0.0
190/2,000	6.0	glazing	2.5	glazing	2.2
290/1,300	4.5	glazing, flecking	2.0	glazing	1.5
390/2,600	4.5	glazing	2.7	glazing	1.7
390/5,200	4.5	glazing, bifacial necrosis	2.9	glazing	2.6
680/3,900	4.5	glazing, bifacial necrosis	3.0	glazing flecking	2.8

[a]Each value represents the average injury level based on the exposure of 20 plants. Plants were exposed in groups of four per fumigation. Ratings as in Table 1.

DISCUSSION

The response of petunia to a mixture of O_3 and SO_2 was remarkably similar to that reported for PAN. A foliar symptom consisting of undersurface glazing characteristically was produced under controlled fumigations with a mixture of O_3 and SO_2. Leaves of intermediate age were most sensitive. Within a group of sensitive leaves, the portion of the leaf injured varied with the age of the leaf. Younger leaves often were injured at the apex, intermediate-aged leaves toward the center and sometimes over the entire lower leaf surface, and older leaves were affected principally at the base or marginally. This pattern of injury is precisely that described for PAN injury on petunia (14). Moreover, sensitivity with respect

TABLE 4. Degree of injury on benomyl-treated White Cascade (WC) and Red Cascade (RC) petunia plants following exposure to O_3/SO_2 mixtures in a controlled environment

O_3/SO_2 ($\mu g/m^3$)[a]	Cultivar	Benomyl concentrations (μg/g soil) 0	40	60	80	120
390/2,600	WC	2.0[b]	0.0	0.0	0.0	0.0
	RC	2.1	0.0	1.5	0.0	0.0
39/3,100	WC	2.0	0.0	0.0	1.5	0.0
	RC	2.3	1.5	2.1	1.6	0.0
390/3,900	WC	2.1	2.3	1.5	1.6	0.0
	RC	3.0	2.5	1.7	2.4	0.0
39/5,200	WC	3.0	2.5	2.4	1.2	0.0
	RC	2.5	3.0	3.0	2.8	0.0

[a]All fumigations were of 4.5 hr duration.
[b]Each value represents the average injury level for 20 plants. Plants were exposed in groups of four per fumigation. Ratings as in Table 1.

TABLE 5. Air pollution injury on nontreated and benomyl-treated petunia plants in the field, indicated as percent of plants in each injury category[a]

Cultivar	Plants in injury category: 0 (%)	1 (%)	2 (%)	3 (%)
Nontreated controls:				
White Cascade	21[b]	29	25	24
White Sails	16	16	42	25
Red Cascade	52	39	8	0
Benomyl-treated				
White Cascade[c]	34	19	9	37
White Sails[c]	53	9	18	19
Red Cascade[c]	73	14	7	5

[a]Severity ratings of 0 to 3, as in Table 1.
[b]Each value represents the average for 72 plants.
[c]By analysis of variance, difference in percent plants injured by benomyl treatment on cultivars Red Cascade and White Sails was significant at $P = 0.05$, and on cultivar White Cascade at $P = 0.10$.

to cultivars was the same for the O_3/SO_2 mixture as that reported for PAN.

Symptoms observed on petunia in the field paralleled those produced with an O_3/SO_2 mixture in a chamber. The appearance of injury could be related to higher-than-normal ambient levels of O_3 and SO_2. The concentration, however, was much lower than that used in our controlled fumigations. It is not unusual that higher concentrations or longer exposures are necessary to produce a given amount of plant damage in controlled chambers than in field situations. In 1968, Menser and Hodges (8) reported that Bel W-3 tobacco developed flecks when ambient air O_3 levels ranged from 0.05-0.07 $\mu g/m^3$ for 2 to 3 hr, but a concentration of 0.10 for 6 hr was required to produce an equivalent amount of injury in a controlled chamber. Thompson et al. (18) supplied O_3 to navel oranges growing in a filtered-air greenhouse in concentrations equal to that found in the ambient air at Riverside, California, for 8 mo. Although the decrease in yield was significant, it was not equivalent to that sustained by trees growing in the field. The authors concluded that peroxyacetylnitrate and oxides of nitrogen in ambient air had an additional deleterious effect. For our experiment, we might speculate that another gaseous or aerosol contaminant in ambient air either predisposed the plant to injury by the O_3/SO_2 mixture or acted as a synergist to the mixture.

Experiments with benomyl provided additional evidence for the implication of O_3 and SO_2 in petunia injury. In both the controlled fumigations with the mixture and in the field study, benomyl protected the plants. Had PAN been the pollutant responsible for injury, one would have predicted an enhancement of injury (11).

In view of our findings, observations of field injury may be subject to reassessment. The PAN-type injury reported on tomatoes in Canada during 1972 and 1973 by Pearson et al. (19) may have resulted from elevated levels of O_3 and SO_2, which would not have been unusual in that location. Damage to petunia in the northeastern USA may be due not to PAN, but predominantly to a mixture of O_3 and SO_2. A critical test of our speculation obviously lies in ambient air analysis for PAN (7).

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