Effects of Air Pollutants on Nicotiana Cultivars and Species Used for Virus Studies

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ABSTRACT

Three tobacco cultivars (*Nicotiana tabacum*) and two *Nicotiana* species, used for virus identification studies, were tested for susceptibility to air pollutants. The air pollutants used were (i) ozone; (ii) sulfur dioxide; and (iii) a mixture of both gases. *Nicotiana rustica* var. *brasilia* was the most susceptible cultivar to injury from individual gases, a gas mixture, and ambient polluted air. *Nicotiana rustica* var. *brasilia* displayed from 25 to 70% leaf injury when exposed to increasing ozone doses (10 to 30 pphm). Ozone injury to *N. glutinosa* fumigated at the same doses ranged from 10 to 60%. Samsun, Samsun (NN), and Xanthi cultivars were more resistant to ozone than *N. rustica* var. *brasilia* or *N. glutinosa*. Sulfur dioxide (ca. 45 pphm) added to an ozone (ca. 3.0 pphm) atmosphere induced a synergistic response evidenced as injury to all cultivars and species. Mixed gas injury was more acute on *N. glutinosa* and *N. rustica* var. *brasilia* than on Samsun, Samsun (NN), and Xanthi. Samsun and Samsun (NN) were more susceptible to sulfur dioxide (125 pphm) than *N. glutinosa* and Xanthi. Fumigations produced two injury syndromes: (i) light or dark punctate flecks, on upper leaf surfaces; or (ii) bifacial tissue collapse. Phytopathology 61:945-950.

Additional key words: smog, oxidants, reductants, stipples, bronzing.

A number of cultivars and species of *Nicotiana* routinely are used as indicators in plant virology studies. An acute problem of rearing healthy plants for virus research has developed in recent years at Beltsville, Md. Throughout the year, but especially in the summer, leaves of healthy, growing tobacco (*N. tabacum* L.) plants develop various types of injuries, rendering the plants unsuited for certain virus studies. Agents responsible for the problem apparently are abiotic, as we observe no evidence of pathogens as primary causes. Symptoms in domestic cultivars consist of leaf flecking and yellowing of leaves injured severely, but two *Nicotiana* species, *N. glutinosa* and *N. rustica* var. *brasilia*, exhibit glazing and bronzing of leaf undersurfaces.

Air pollutants are a possible cause of most of these symptoms. Ozone causes flecking and premature leaf chlorosis (13). Peroxyacetyl nitrate (PAN) induces lower surface bronzing of immature leaves (15), and exposure to ozonated hydrocarbons can produce lower-surface glazing of mature leaves (4). Sulfur dioxide causes bifacial tissue collapse and chlorosis (16), and ozone and sulfur dioxide mixtures act synergistically to injure leaves (9). Tobacco plants, grown in air passed through activated carbon to remove oxidants, show none of these injury symptoms (10).

A selection of tobacco cultivars and *Nicotiana* species, used to key the identity of viruses, were exposed to air pollutants. The purposes of the tests were to determine the injury thresholds of various toxicants and to compare injury syndromes obtained in fumigations and in greenhouses subject to polluted ambient air.

MATERIALS AND METHODS.—*Nicotiana tabacum* 'Xanthi', 'Samsun', and 'Samsun (NN)', *N. rustica* var. *brasilia* Shrank, and *N. glutinosa* L. were exposed to controlled levels of pollutants in a walk-in fumigation chamber. Tobacco seedlings were transplanted into 8-cm pots that contained equal portions (v:v) of a soil and vermiculite mixture. Two g of 7:7:7 fertilizer were added in solution to each pot 10 days later. Exposures to fumigants began about 10 to 12 weeks after seeding. Plants grew under natural lighting in the greenhouse at 30-C day and 24-C night temperatures. Equal numbers of tests were done in summer and winter months. None of the plants used for fumigations showed any symptoms of previous injury, although some grew in a naturally ventilated greenhouse and others grew in air passed through charcoal filters to remove pollutants.

The air pollutants used to determine susceptibility to leaf injury of the *Nicotiana* cultivars and species were ozone, sulfur dioxide, and a mixture of ozone and sulfur dioxide. Ozone fumigations were for 1 and 2 hr at 30 pphm, 1.5 and 3 hr at 20 pphm, and 3 and 6 hr at 10 pphm. Sulfur dioxide exposures were 2 and 4 hr at 110 to 125 pphm. The ozone and sulfur dioxide mixture was 3 pphm and 40 to 50 pphm, respectively, for 2- and 4-hr intervals. Fumigations were done in a chamber illuminated at 1,000 ft-c (cool-white fluorescent) and operated at 27 C and 70 to 80% relative humidity. The fumigation chamber and apparatus used was described previously (8). We used a Mast 724-1 ozone meter to monitor ozone levels, and a Beckman 906 sulfur dioxide analyzer to measure sulfur dioxide concentrations. The West & Gaeke (17) manual sampling method was used to determine sulfur dioxide in earlier tests. A chromium trioxide scrubber to remove sulfur dioxide interference (14) was attached to the ozone meter in exposures of mixed gases.

Data represent estimated necrosis expressed on an individual plant basis. The evaluation is arbitrary, and attempts to judge injury to each leaf according to the area affected and the necrosis within the area. Leaves were rated on a 1 to 10 scale. Each cultivar or species

TABLE 1. Per cent leaf injury resulting from ozone fumigation of selected *Nicotiana* cultivars and species[a]

Cultivar or species	O_3 Dosage (pphm hr)[b] 30				60			
	O_3 Rate (pphm)				O_3 Rate (pphm)			
	10	20	30	$\bar{x}$[c]	10	20	30	$\bar{x}$[c]
Samsun (NN)	3	16	6	8 a	5	22	18	15 a
Samsun	10	21	15	15 a	12	30	31	24 a
Xanthi	6	27	24	19 a	16	34	38	29 a
N. glutinosa	10	52	52	38 b	17	58	62	46 b
N. rustica var. *brasilia*	24	48	49	40b	36	63	70	56 b
$\bar{x}$[c]	11 a	33 b	29 b		17 a	41 b	44 b	

[a] Mean estimated necrosis expressed on a single plant basis and obtained in four fumigations of five plants each.
[b] pphm = 100 pphm hr equals 100 pphm O_3 administered for 1.0 hr.
[c] Means within the same column not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).

was replicated 5 times in each fumigation. Individual doses were repeated 4 times.

RESULTS.—*Nicotiana rustica* var. *brasilia* and *N. glutinosa* were approximately 2-3 times more susceptible to ozone at high and intermediate rates (20 and 30 pphm) than Samsun, Samsun (NN), and Xanthi (Table 1). Samsun (NN) was a little more resistant to ozone than Samsun; however, both were more ozone-resistant than Xanthi. Ozone at the lowest concentration (10 pphm) caused more injury to *N. rustica* var. *brasilia* than to *N. glutinosa* or to the three tobacco cultivars. *Nicotiana glutinosa* showed about as much resistance to the 10-pphm rate as Samsun and Xanthi. Ozone at 20 pphm for 1.5 hr caused slightly more injury to the 3 cultivars than at 30 pphm for 1 hr; but in the same exposure comparison, the two species exhibited no more injury at the 30-pphm rate.

The susceptibility trends of the species and cultivars exposed to a mixture of ozone and sulfur dioxide were similar to the trends obtained in ozone tests (Table 2). *Nicotiana glutinosa*, exposed to an ozone and sulfur dioxide mixture for 2 hr, exhibited 26% leaf injury compared to only 10% injury after a 3-hr ozone fumigation at 10 pphm. Only *N. glutinosa* exhibited appreciably more injury when the mixed-gas fumigation period was extended to 4 hr.

TABLE 2. Per cent leaf injury caused by exposure of selected *Nicotiana* cultivars and species to an ozone and sulfur dioxide mixture[a]

Cultivar or species	Leaf injury per plant, %[b] Exposure interval, hr		
	2	4	$\bar{x}$[c]
Samsun (NN)	2	1	2 a
Samsun	5	9	7 a
Xanthi	2	5	4 a
N. glutinosa	26	39	32 b
N. rustica var. *brasilia*	35	33	34 b

[a] Mean concentrations of O_3 and SO_2 in pphm were 3.0 and 45.0, respectively.
[b] Mean necrosis of 20 plants fumigated in four experiments.
[c] Means not followed by the same letter are significantly different at the 5% level.

Sulfur dioxide used at 125 pphm for 2, 3, and 4 hr injured *N. rustica* var. *brasilia* extensively (Table 3). *Nicotiana glutinosa* and the three cultivars showed substantial resistance to sulfur dioxide in 2- and 3-hr exposures, but a 4-hr fumigation resulted in 17 to 28% necrosis. The susceptibility of *N. rustica* var. *brasilia* to sulfur dioxide used at the 3 exposure intervals was significantly greater than the susceptibilities of *N. glutinosa* and the three cultivars.

Symptoms displayed by *N. rustica* var. *brasilia* in a greenhouse without charcoal filters occurred oftener and were more diverse than symptoms on the three cultivars and *N. glutinosa*. Xanthi, Samsun, and Samsun (NN) exhibited injury almost as frequently as *N. rustica* var. *brasilia*, but not as extensively. Leaf markings seldom developed on *N. glutinosa*.

Symptoms on plants grown in evaporatively cooled greenhouses without filters appear in Fig. 1. Syndromes observed on *N. rustica* var. *brasilia* included a grayish glazing on the lower surface (Fig. 1-A) and flecking on the upper surface (Fig. 1-B) of the oldest leaf. Xanthi and *N. rustica* var. *brasilia* exhibited bronzing of apical-leaf lower surfaces (Fig. 1-C). Samsun, Samsun (NN), and Xanthi showed punctate flecks on the upper surface and a chlorosis of mature leaves. On one occasion, mature leaves of young Samsun plants displayed bifacial tissue collapse. Injury to *N. glutinosa* was infrequent, but during early May, symptoms similar to ozone fleck appeared a day after operation of exhaust cooling fans (Fig. 1-D).

Fumigations produced four types of injury: punctate flecks on upper leaf surfaces; bifacial tissue collapse; lower surface glazing; and bleaching of upper surfaces. Symptoms were more extensively distributed on mature leaves than on young, apical leaves.

Ozone caused lower surface glazing, upper surface flecking, and complete bifacial necrosis on leaves of *N. rustica* var. *brasilia* (Fig. 2). The glazing of the lower surface appeared on the oldest leaf, but no upper surface involvement developed (Fig. 2-A). Ozone flecks on upper surfaces occurred on younger leaves at mid-plant positions. Bifacial necrosis was induced by high

TABLE 3. Per cent leaf injury to selected *Nicotiana* cultivars and species fumigated with 125.0 pphm sulfur dioxide[a,b]

Cultivar or species	Exposure interval, hr		
	2	3	4
Samsun (NN)	3 a	3 ab	28 a
Samsun	1 a	1 a	20 a
Xanthi	4 a	9 b	17 a
N. glutinosa	3 a	3 ab	18 a
N. rustica var. *brasilia*	35 b	48 c	76 b

[a] Mean necrosis of 15 plants exposed in groups of five each on three dates.
[b] Means within the same column not followed by the same letter are significantly different at the 5% level.

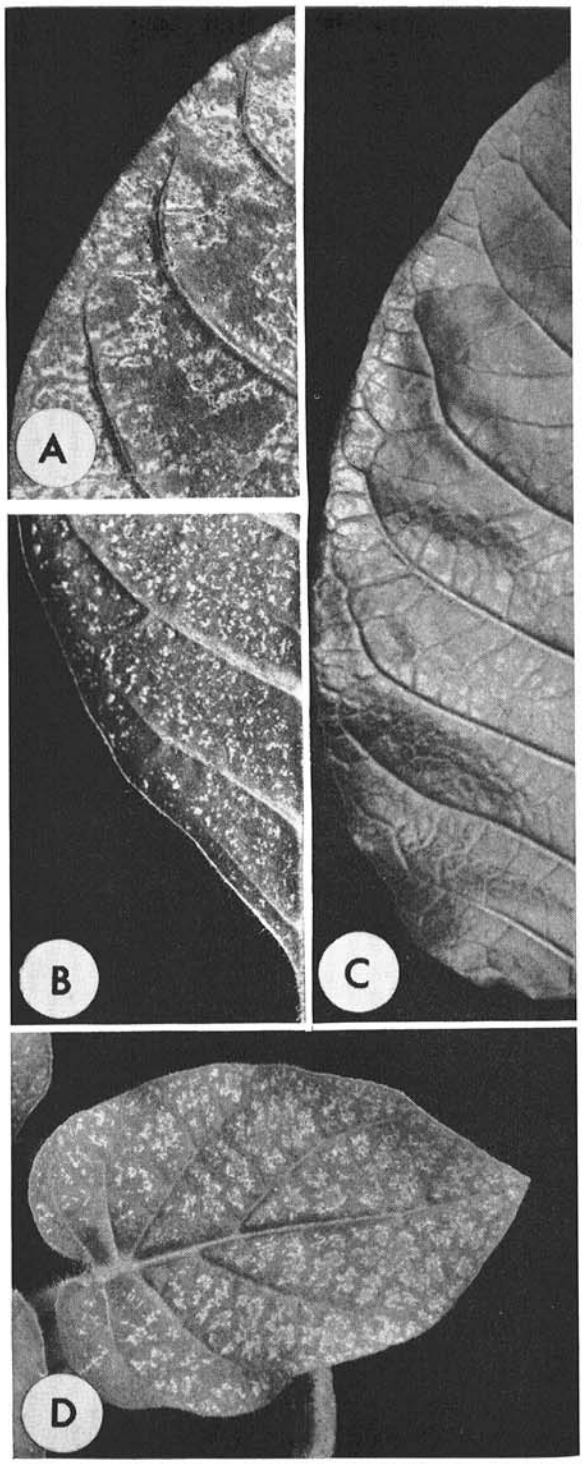

Fig. 1. Leaf injury observed on *Nicotiana* species and cultivars grown for virus studies at Beltsville, Md., in greenhouses cooled evaporatively; **A, B)** *N. rustica* var. *brasilia* **C)** *N. tabacum* 'Xanthi' **D)** *N. glutinosa*.

concentration exposures to ozone (Fig. 2-B). *Nicotiana glutinosa* showed two types of ozone injury. One type consisted of a very diffuse bleaching of the upper surface that did not have the distinctively punctate ozone fleck character (Fig. 2-C). Bifacial necrotic lesions constituted the other syndrome (Fig. 2-D). Samsun, Samsun (NN), and Xanthi exposed to phytotoxic ozone concentrations displayed flecks almost indistinguishable from those observed on these cultivars grown in ambient air (Fig. 1-B).

An ozone and sulfur dioxide mixture produced a general flecking or diffuse bleaching of upper leaf surfaces on all cultivars and species (Fig. 3). *Nicotiana glutinosa* symptoms resembled ozone flecks, but lacked the clearly defined punctate nature of ozone injury. The lesions seemed to consist of aggregations of ozone flecks, and appeared only on the upper surface of midplant leaves. The oldest leaf on *N. rustica* var. *brasilia* showed upper surface flecks (Fig. 3-B) and lower surface glaze (Fig. 3-C). One of the mixed-gas experiments caused a rather unusual display of symptoms on *N. rustica* var. *brasilia*. Bleached tissues closely outlined the distribution of the smallest veins (Fig. 3-D). The pattern resembled an etching or streaking of the leaf surface.

Sulfur dioxide caused mature leaves to collapse bifacially. The syndrome displayed by the cultivars and species was identical to that exhibited by Samsun (Fig. 4).

Discussion.—Ozone, sulfur dioxide, and a low-concentration mixture of the two gases administered, under controlled conditions, caused leaf injury similar to symptoms observed in greenhouses that had been subjected to polluted air from an evaporative cooling system. *Nicotiana rustica* var. *brasilia* was the most susceptible to all experimental toxicants, and usually exhibited the most frequent and extensive symptoms when grown in ambient air. We observed three syndromes in unfiltered air. Partially expanded apical leaves displayed a copper or bronze effect along the lower surface of the leaf margin. Midplant leaves showed upper surface white or tan flecks. The older, basal leaves developed glazed white or silver areas with irregular outlines. Ozone fumigations produced flecks on midplant leaves and lower surface glazing on basal leaves. An ozone and sulfur mixture caused flecks on the upper surface and glazing of the lower surface of leaves that were injured only on the lower surface when exposed to ozone. The ozone and the ozone-sulfur dioxide syndromes differed enough to be useful in distinguishing injury from ozone and a mixture of ozone and sulfur dioxide. The bronzing of apical leaves suggests phytotoxic levels of peroxyacetyl nitrate (PAN) (15), at times, in Beltsville greenhouses.

Nicotiana glutinosa was not especially susceptible to the lowest ozone concentrations, but was injured about as much as *N. rustica* var. *brasilia* at higher ozone rates. We conclude that the threshold for conspicuous ozone injury to *N. glutinosa* is greater than the general ozone pollution level during summer months. Glater et al. (3) attributed a very characteristic *N. glutinosa* injury syndrome to Los Angeles smog. They apparently believed that ozone was not a primary cause of the injury on greenhouse plants because there were no char-

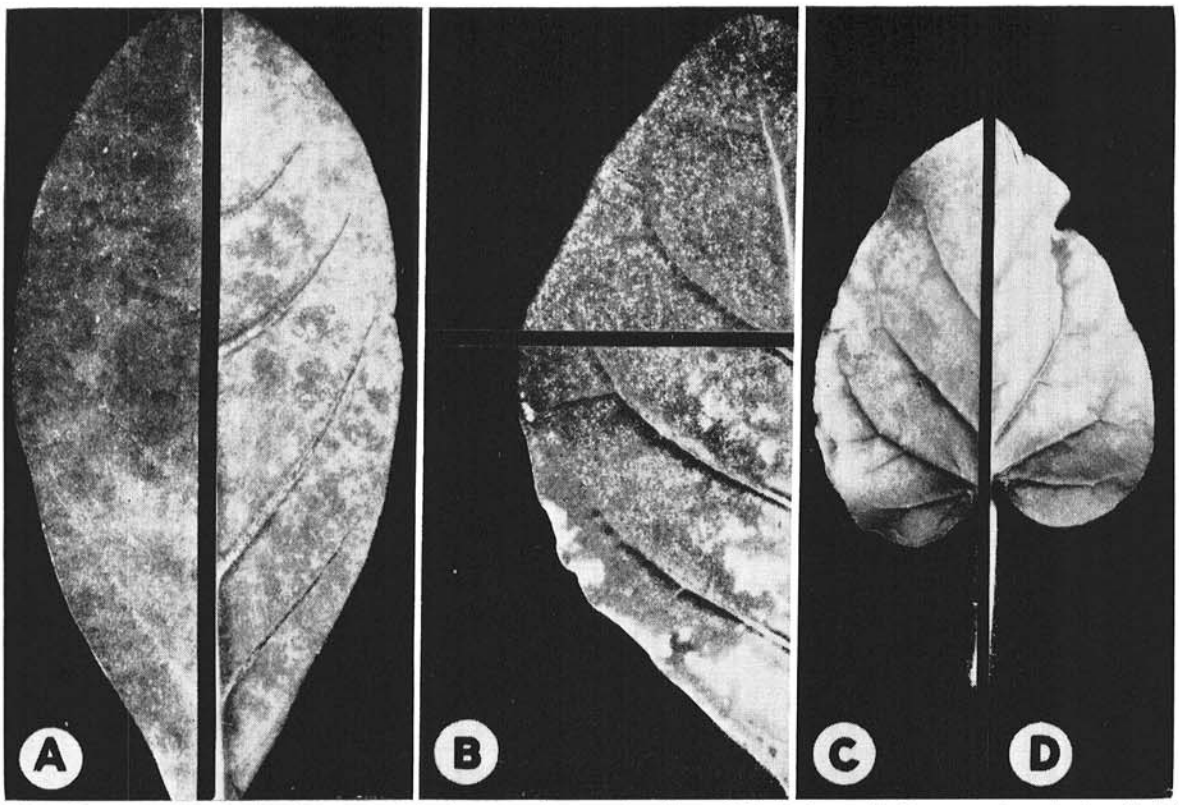

Fig. 2. Ozone fumigation injury to *Nicotiana rustica* var. *brasilia* and *N. glutinosa*. **A)** Lower surface glazing (right) and uninjured upper surface (left) of the oldest leaf from 8-week-old *N. rustica* var. *brasilia* plant. **B)** Upper surface flecking and bifacial necrotic lesions on a midplant leaf of *N. rustica* var. *brasilia*. **C)** Bleached upper surface and **D)** bifacial necrosis as depicted on *N. glutinosa*.

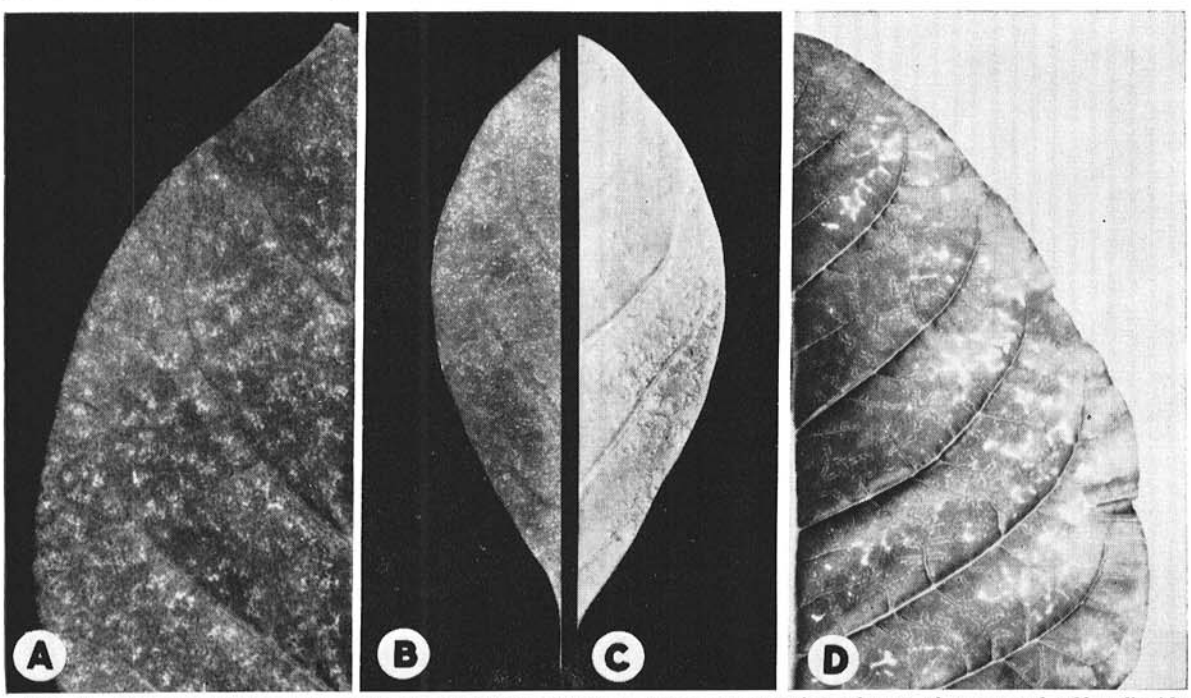

Fig. 3. Symptoms induced by exposure of tobacco plants to a low-concentration mixture of ozone and sulfur dioxide. Shown are a midplant leaf of **A)** *Nicotiana glutinosa*; **B)** the upper and **C)** lower surface of the oldest leaf; and **D)** a midplant leaf of *N. rustica* var. *brasilia*.

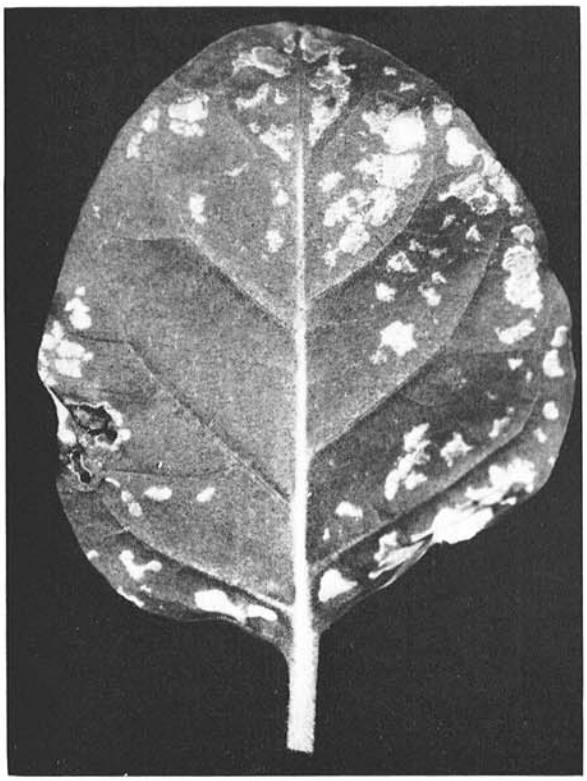

Fig. 4. Injury to *N. tabacum* 'Samsun' inflicted by a 2-hr exposure to 125.0 pphm sulfur dioxide.

acteristic ozone symptoms such as discrete, blanched lesions. Our experiments suggest that *N. glutinosa* reacted synergistically to a low-concentration mixture of ozone and sulfur dioxide. Menser & Heggestad (9) believed that the injury they caused by fumigating ozone-resistant cultivar Bel-B with subthreshold quantities of an ozone-sulfur dioxide mixture represented a synergistic response. Bel-B, and certain burley cultivars in other experiments (11), showed upper surface markings similar to (but not identical to) ozone fumigation injury. Based on sensitivity to the two gases in our fumigations, *N. glutinosa* may have potential use as a bio-indicator to detect synergistic concentrations of ozone and sulfur dioxide. When plants show injury after low ozone concentrations are recorded, this may indicate that ozone and sulfur dioxide acted synergistically to cause symptoms. Incidents of this nature may have occurred at Beltsville because of the strong resemblance between symptoms on *N. glutinosa* fumigated with mixed gases and symptoms observed on plants grown in ambient air.

Individual and mixed-gas fumigations revealed that Samsun, Samsun (NN), and Xanthi were much less susceptible to injury than *N. glutinosa* and *N. rustica* var. *brasilia*. During the summer, however, these more fleck-resistant cultivars develop extensive upper surface injury in evaporatively cooled greenhouses, and, except for *N. glutinosa,* they show very little difference in susceptibility. Plants retained in the greenhouse for 3 or 4 months show about 100% upper surface injury to leaves that reach full expansion.

At least two factors probably contribute to this apparent reduction in susceptibility thresholds of tobacco cultivars basically resistant to fleck. Although the ozone concentration is nearly the same inside and outside the greenhouse, moving the air through the greenhouse to produce cooling may increase the amount of ozone absorbed by the leaf. Brennan & Leone (1) demonstrated that air, polluted by ozone or sulfur dioxide at a given concentration, caused more plant injury when supplied at a high flow rate as compared to a low flow rate. They cited Fick's equation for gas diffusion into plants as a principle that favored increased pollutant uptake, and questioned whether injury, observed in portable chambers supplied with forced air (5), would have occurred in the field where air was not forcibly moved over the plants. Indeed, their results, and those reported by Howell et al. (6), create doubt concerning the magnitude of differences in crop growth obtained in filtered vs. ambient air comparisons that are not done with appropriate field controls.

The relative humidity of greenhouses cooled by the fan-pad system usually is quite high due to the moisture evaporation produced by the moving air stream. Otto & Daines (12) showed that ozone injury to Bel-W3 tobacco increased concomitantly with an increase in relative humidity and stomatal aperture as determined from porometer readings.

Ozone and mixed-gas fumigations of Samsun, Samsun (NN), and Xanthi caused upper surface flecking virtually identical to symptoms observed in ambient air. Lower surface injury such as glazing of mature leaves or bronzing of apical leaves has been very infrequent at Beltsville. Xanthi exhibited lower surface glazing similar to symptoms observed by Heggestad et al. (4) on Samsun tobacco exposed in California to the reaction products of an ozone:hexene-1 mixture.

Our sulfur dioxide tests caused only one injury syndrome; viz, complete bifacial necrosis of mature, basal leaves. The symptoms were similar regardless of the relative susceptibility of the cultivar or species. Bifacial necrosis is the principal expression of acute sulfur dioxide injury to most plant species (16), although MacDowall et al. (7) reported a fleck-type variation on Canadian tobacco. It is more likely that occasional incidents of bifacial injury to tobacco at Beltsville are caused by photochemical oxidants than by phytotoxic concentrations of sulfur dioxide.

The literature contains little information concerning the effects of ozone on diseased plants. Brennan & Leone (2) studied the effects of ozone on healthy and TMV-infected *N. sylvestris* plants. Ozone toxicity symptoms were suppressed in systemically infected plants exposed to 30 pphm for 3 to 6 hr; but at 40 pphm, healthy and diseased plants were injured by ozone. Plants fumigated 2 days after TMV inoculation failed to develop virus symptoms, but their reaction to ozone was similar to that of healthy plants. Biochemical studies did not disclose the mechanism of the ozone protection that was afforded by virus-infected plants. Their work does reveal, however, that ozone-polluted

air may alter the results of virus investigations, when *N. sylvestris* and perhaps other *Nicotiana* species and cultivars are used as indicators for the taxonomic identification of unknown viruses.

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