

## On Exclusion as the Mechanism of Ozone Resistance in Virus-Infected Plants

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### ABSTRACT

The resistance to O<sub>3</sub> of TMV-infected plants cannot be attributed to stomatal closure such as occurs in resistant cultivars of onion. Leaf resistance, as measured with a diffusion porometer, was similar in virus-infected and

noninfected tobacco and bean plants both before and after exposure to the pollutant. Exclusion of O<sub>3</sub> is therefore not the principal factor in reducing O<sub>3</sub> toxicity symptoms.

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*Additional key words:* air pollution, stomata, *Nicotiana*, TMV, bean.

Since Brennan and Leone reported suppression of ozone (O<sub>3</sub>) toxicity symptoms in *Nicotiana sylvestris* plants infected with tobacco mosaic virus in 1969 (1), the phenomenon has been well substantiated. Moyer and Smith (10) observed reduction in oxidant injury in tobacco plants infected with tobacco etch virus, and Davis and Smith (2) reported it in bean plants infected with bean common mosaic virus. Davis and Smith (3) also found that local lesion infections in pinto bean induced by alfalfa mosaic, tobacco ringspot, tobacco mosaic virus, and tomato ringspot virus were effective in minimizing O<sub>3</sub> damage.

The mechanism responsible for the reduction of O<sub>3</sub> sensitivity in virus-infected plants has not been determined. Consideration of three lines of evidence led me to the hypothesis that exclusion of O<sub>3</sub> from virus-infected plant tissue might be responsible for resistance to O<sub>3</sub> damage. In their classic paper Engle and Gabelman (4) reported that certain genetic selections of *Allium cepa* owed their resistance to rapid stomatal closure in the presence of O<sub>3</sub>. In a recent review, Mansfield (9) concluded that "the balance of evidence suggests that stomatal apertures are of importance in determining the sensitivity of plants during exposure to air pollutants". That virus in infected tissue can induce stomatal closure was reported by Hall (5) in his experiments related to the reduction of photosynthesis in beet yellows-infected sugar beets. Therefore, I tested the hypothesis that reduced O<sub>3</sub> sensitivity of TMV-infected plants was related to increased diffusion resistance to the pollutant.

**MATERIALS AND METHODS.**—Two species, *Nicotiana sylvestris* Speg. & Comes and *Phaseolus vulgaris* L. 'Pinto 110', were grown in sterilized soil in a greenhouse during September and October, the tobacco plants singly and the bean plants two per pot. At the 12-13 leaf stage, approximately 6 weeks after the seedlings were transplanted, eight uniform tobacco plants were selected for the experiment. The selection of 20 uniform pinto bean plants was made when the unifoliate leaves were 50% expanded, 7 days after germination.

Half of each group of plants was dusted with Carborundum and inoculated with TMV-infected leaves ground in 0.02M phosphate buffer and 0.02M 2-mercaptoethanol at pH 7.0. The remaining plants were rubbed with a homogenate prepared from healthy tobacco leaves following an application of Carborundum.

The tobacco plants were ozonated 1 week following inoculation, and the bean plants, 3 days after inoculation. The reason for the time difference is that systemic infection requires about 1 week for full development of viral symptoms, and local lesion development only 3 days.

Ozonation was conducted in a 6 m<sup>3</sup> glass chamber located within a conventional greenhouse. Ozone was evolved by passing a metered stream of pure dry oxygen through a commercial O<sub>3</sub> generator (Instrument Development Co., Salt Lake City, Utah). The resulting O<sub>3</sub> was introduced into a charcoal-filtered air stream that passed through a mixing chamber before entering the fumigation chamber. Air in the chamber was exchanged once every 45 seconds. Ozone concentration was monitored continuously by an O<sub>3</sub> meter (Mast Development Co., Davenport, Iowa) which had been calibrated by the buffered potassium iodide method (7). The temperature in the chambers was kept at 27-30 C and the relative humidity at 50-60%. Because of their differing sensitivity, tobacco and bean plants were exposed to 669 μg/m<sup>3</sup> O<sub>3</sub> (0.35 ppm) for 4 hours, and 478 μg/m<sup>3</sup> (0.25 ppm) for 3 hours, respectively.

Before and immediately after ozonation, the relative resistance of virus-infected and uninfected foliage of tobacco and bean was measured with a diffusive resistance meter (Model LI-60, Lambda Instrument Co., Lincoln, Nebraska). The sensor was calibrated by the method of Kanemasu et al. (8) in units of sec cm<sup>-1</sup>. The fifth to ninth tobacco leaves were used for the measurement, since they were in a developmental stage most conducive to O<sub>3</sub> injury. The unifoliate bean leaf was used. The porometer was applied in such a manner to measure diffusion through the abaxial surface since it contained the greater number of stomata. The data were subjected to analysis of variance.

Plants were held for 3 days following ozonation to insure complete development of toxicity symptoms. At that time the degree of ozone injury was estimated visually for each leaf on an injury scale of 0-4, with the relative reading 0 = none, 1 = slight, 2 = slight to moderate, 3 = moderate, 4 = severe. The average rating for each age of leaf on all plants was reported.

The experiment with tobacco was repeated three times and that with bean twice.

**RESULTS.**—Tobacco plants inoculated with TMV developed characteristic symptoms of systemic virus

TABLE 1. Leaf resistance ( $\text{sec cm}^{-1}$ ) of leaves of different ages from TMV-infected and uninfected tobacco plants before and immediately after ozonation

Leaf No. <sup>a</sup>	Before O <sub>3</sub>		After O <sub>3</sub> <sup>b</sup>	
	+ TMV	- TMV	+ TMV	- TMV
5	3.8 <sup>c</sup>	4.9	6.2	9.0
6	4.0	4.5	5.1	5.2
7	4.2	4.8	7.8	7.8
8	4.4	4.5	5.7	6.5
9	4.1	4.9	6.9	5.6
$\bar{X}$	4.1	4.7	6.3	6.8

<sup>a</sup>Position of leaf from tip of the shoot.

<sup>b</sup>Plants exposed to 669  $\mu\text{g}/\text{m}^3$  O<sub>3</sub> for 4 hours.

<sup>c</sup>Mean of eight measurements taken on four plants. There were no significant differences by the "F" test for treatment, leaf age, or treatment  $\times$  leaf age. Results are from one of three experiments, all with similar results.

infection 1 week after inoculation. At the time of ozonation young leaves were mottled and deformed, old leaves were chlorotic, and the oldest leaves were prematurely shed. Ozonation did not produce any visible symptoms of O<sub>3</sub> toxicity. Ozonation of uninfected tobacco plants caused extensive necrosis of the fifth to tenth leaves from the top of the plant. Fifth and sixth leaves had an average rating of 3, and the seventh to tenth leaf a rating of 4, while all other leaves were rated 0.

Leaf resistance measurements are presented in Table 1. There was no significant difference between the resistance of virus-infected and uninfected leaves at any time. While there was a trend toward higher resistance in the noninfected plants before they were exposed to O<sub>3</sub>, it did not persist after ozonation. Variations in respect to leaf age or treatment  $\times$  leaf age were not significant. These results were confirmed in three separate experiments.

Pinto bean plants inoculated with TMV developed local lesions equivalent to about 10% of the total leaf area within 3 days. After ozonation, the infected leaves developed symptoms of O<sub>3</sub> toxicity consisting of bleaching of the adaxial leaf surface. However, they were not as severe as on the uninfected bean plants. The average injury score was 3 on uninfected plants compared to a rating of 1 on TMV-infected plants.

Leaf resistance measurements averaged 3.8  $\text{sec cm}^{-1}$  for virus-infected leaves and 4.3 for uninfected leaves before ozonation, and 6.7 compared to 6.3  $\text{sec cm}^{-1}$  after ozonation. Statistical analysis of the data again revealed no significant difference in resistance of the virus-infected and uninfected leaves at either time.

DISCUSSION.—Both tobacco and bean, when infected with TMV showed suppression of O<sub>3</sub> toxicity symptoms following ozonation. Porometer readings

made on foliage of infected and uninfected plants were essentially similar. Leaf resistances were higher after ozonation, as reported by Hill and Littlefield (6) for a range of species, but the resistances were high whether the plant was infected or not. Diffusion porometer readings are generally accepted as a measure of stomatal aperture as the instrument measures the rate of diffusion of water vapor from the interior of the leaf into a dry chamber. Since gaseous pollutants in general are considered to enter plants through stomata, one would infer that an equivalent amount of pollutant is entering virus-infected and uninfected plants. Rich et. al. (11) contend that O<sub>3</sub> follows the very same path as water vapor between the leaf interior and the external atmosphere. I conclude therefore that virus infection does not reduce O<sub>3</sub> damage by an exclusion mechanism.

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