

Effect of Ozone and Tobacco Streak Virus Alone and in Combination on *Nicotiana tabacum*

R. A. Reinert and G. V. Gooding, Jr.

Plant Pathologist, Agricultural Research Service, U. S. Department of Agriculture, North Carolina State University, Raleigh, NC 27607, and Plant Pathologist, Department of Plant Pathology, North Carolina State University, respectively.

Cooperative investigations of the U. S. Department of Agriculture and the North Carolina State University. Paper No. 4983 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh.

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the U. S. Department of Agriculture or the North Carolina Agricultural Experiment Station and does not imply approval of it to the exclusion of other products or vendors that also may be suitable.

Thanks are extended to J. B. Young and Hershell Carter for technical assistance in this study. Appreciation is also extended to Hans Hamann, Associate Statistician, North Carolina State University.

Accepted for publication 14 June 1977.

ABSTRACT

REINERT, R. A., and G. V. GOODING, JR. 1978. Effect of ozone and tobacco streak virus alone and in combination on *Nicotiana tabacum*. *Phytopathology* 68:15-17.

The percentage foliar injury on *Nicotiana tabacum* L. 'Bel W₃' infected by tobacco streak virus (TSV) and exposed to ozone (O₃) was greater than the additive percentage foliar injury to either TSV-infected or O₃-exposed plants. The fresh and dry weights of leaves, stems and roots of Bel W₃ exposed

to O₃, infected with TSV, or both were significantly lower than the weights of the same parts from control plants. The growth-inhibiting effects of O₃ and TSV were only additive.

Additional key words: interaction, air pollution, oxidants.

The sensitivity of virus-infected plants to ozone (O₃) has been the subject of recent concern. Tobacco mosaic virus (TMV) suppressed visible foliar injury from O₃ in tobacco, *Nicotiana tabacum* L. (2). The suppression was evident when the tobacco was exposed to O₃ about 12 days after inoculation with TMV. Suppression was not apparent when tobacco was exposed to O₃ 2 days after inoculation and during the summer months when the systemic symptoms of the virus were somewhat masked.

Sensitivity to O₃ was less in *Phaseolus vulgaris* L. 'Pinto' inoculated with bean common mosaic virus (BCMV) when compared with virus-free, O₃-exposed pinto bean (3). This protection was observed 4 and 5 days following inoculation, but not at 3 days. Studies with three other plant viruses: alfalfa mosaic virus (AMV), tobacco ringspot virus (TRSV), and tomato ringspot virus (TOMRSV) also decreased sensitivity of 10-day-old pinto bean to O₃ 5 days following inoculation (4). Tobacco etch virus (TEV) reduced O₃ injury on tobacco cultivars exposed 10 or 12 wk after transplanting and 9 days following inoculation (9).

Observations of field-grown burley tobacco have suggested that symptoms produced by viruses and by weather fleck on the same plant are sometimes difficult to separate. The two may interact to decrease yield and market value of commercial cultivars. Plant viruses alone decrease tobacco yields (6) and O₃ inhibits the growth of tobacco grown under both greenhouse (10, 12) and field conditions (7).

The purposes for this study were: (i) to determine

whether the sensitivity to O₃ of the tobacco cultivar Bel W₃ infected with tobacco streak virus (TSV) was greater or less than the sensitivity to O₃ of virus-free Bel W₃, and (ii) to determine the effects of TSV and O₃ on weight of plants. Ozone was selected as the oxidant because of its known effect on tobacco (9, 10). Tobacco streak virus (TSV) was used because leaves developing after infection are essentially symptomless except for the first two to three leaves which show severe symptoms (8). Bel W₃ was chosen as the host cultivar because of its known sensitivity to O₃ (7, 10, 12).

MATERIALS AND METHODS

Experiments were conducted in a charcoal-filtered greenhouse with Bel W₃ tobacco plants that were grown in a soil, peat, perlite, and sand (1:1:0.5, v/v) mixture and fertilized with a slow release fertilizer. Greenhouse temperature was 26 ± 3 C and relative humidity ranged from 50-80%. Forty-eight 7-wk-old plants in 10-cm diameter clay pots were divided into two equal groups. One group was inoculated with TSV, while the other group served as noninoculated controls. Three to four weeks after inoculation, half of each group of plants (12 TSV-inoculated and 12 noninoculated plants) were randomly selected, and exposed to 588 µg/m³ (30 parts per hundred million) O₃ for 3 hr in greenhouse exposure chambers (5). The remaining control and inoculated plants were exposed for 3 hr to charcoal-filtered air. Each of the four treatment groups (12 plants each) was then split into two sets of six plants. One set from each group was exposed to O₃ the next day or to charcoal-filtered air.

The O₃ was generated by silent electrical discharge in dry oxygen (O₂) and continuously measured with a Mast oxidant meter. The readings were corrected to a 1% neutral buffered KI standard (11). The temperature at exposure was 26 ± 3 C and the relative humidity, 50-60%.

Two wk after exposure to O₃ the percentage leaf surface injured was estimated in increments of 5% and fresh and dry weights of leaves, stems and roots were determined. All plant weight data were subjected to analysis of variance with least significant difference values at *P* = 0.05.

RESULTS

Virus-infected plants developed characteristic TSV symptoms of necrosis and poorly developed leaves beginning about 3-4 days after inoculation. The first two or three leaves to develop above the inoculated leaves

showed severe virus symptoms, but leaves that formed later were symptomless. Before the O₃ treatments, leaves of TSV-inoculated plants at position 7 and above (counting from the first inoculated leaf) were fully expanded and nearly free from TSV symptoms. Virus-free control plants developed normally before exposure to O₃ or charcoal filtered air. The total number of leaves above position 7 averaged the same (6.0) for both virus infected and noninfected plants.

At the time of symptom evaluation, foliar injury was observed on both noninoculated and virus-infected, ozonated plants. Noninoculated Bel W₃ plants developed O₃ injury at leaf positions 3 through 9, (counting from a comparable leaf position in the healthy plants at the time of virus inoculation), whereas, TSV-infected plant leaves were injured by O₃ at positions 6-11. The mean percentage of O₃ only cumulative leaf injury was greater on the TSV-infected, ozonated plants than on the

TABLE 1. Foliar injury on tobacco streak virus-infected and virus-free Bel W₃ tobacco plants 2 wk after exposure to ozone^a

Treatment	Ozone concentration (μg/m ³)	Exposure time (hr) ^b	Cumulative leaf injury	
			Leaves 6-11 (%)	Leaves 9-11 (%)
Control	0	3	0	0
	0	3+3	0	0
O ₃	588	3	18	0
	588	3+3	73	8
TSV	0	3	5	0
	0	3+3	24	0
TSV + O ₃	588	3	91	5
	588	3+3	172	25

^aEach value is the mean of six plants.

^bPlants not exposed to O₃ were exposed to charcoal-filtered air; "3 + 3" represents 3-hr exposures on successive days.

TABLE 2. Weight of tobacco streak virus-infected and virus-free Bel W₃ tobacco plants 2 wk after exposure to ozone^a

Treatment	Ozone concentration (μg/m ³)	Exposure time (hr) ^b	Leaf weight (g) ^a		Stem weight (g) ^a		Root weight (g) ^a	
			Fresh	Dry	Fresh	Dry	Fresh	Dry
Control	0	3	90.1	8.3	31.8	3.2	17.1	1.8
	0	3 + 3	89.4	7.8	30.3	2.9	17.3	1.7
O ₃	588	3	69.5*	6.9*	22.7*	2.2*	12.3*	1.2*
	588	3 + 3	73.5*	7.4	24.2*	2.3*	13.6*	1.2*
TSV	0	3	48.7*	3.8*	16.4*	1.1*	8.3*	0.8*
	0	3 + 3	51.6*	4.7*	16.1*	1.3*	9.1*	0.8*
TSV + O ₃	588	3	39.8*	3.4*	12.4*	0.9*	5.8*	0.5*
	588	3 + 3	35.8*	3.4*	9.3*	0.7*	5.2*	0.5*
LSD (P = 0.05)			5.8	0.7	2.7	0.3	1.8	0.2

^aEach value is the mean representative of six plants.

^bPlants not exposed to O₃ were exposed to charcoal filtered air; "3 + 3" represents 3-hr exposures on successive days.

^cAsterisk (*) indicates significant differences (*P* = 0.05) from the control.

virus-free, ozonated plants (Table 1). The two 3-hr exposures to 588 $\mu\text{g}/\text{m}^3$ O_3 were more injurious than one 3-hr exposure. Depending on the dose of O_3 , the total percentage of injury on leaves at positions 9-11 ranged from 5-25% for TSV-infected, ozonated plants and from 0-8% for virus-free, ozonated plants. The percentage of foliar injury on TSV-infected, ozonated plants was greater than the sum of the percentages on plants only infected with TSV and plants only exposed to O_3 .

Bel W₃ tobacco infected with TSV, exposed to O_3 , or both weighed significantly less than control plants grown in charcoal-filtered air (Table 2). Additional exposure to O_3 on the second day did not produce significant additional weight reduction. Fresh leaves, stems and roots of TSV-infected, ozonated tobacco weighed significantly less than those of the control, O_3 -exposed, or TSV-infected plants. However, the growth inhibiting effects of O_3 and TSV were only additive.

DISCUSSION

The observation that sensitivity of tobacco to O_3 was greater on leaves systemically infected with a virus (TSV) than on virus-free leaves has not been previously reported. Although, an inhibition of O_3 injury by certain systemic viruses (TMV and BCMV) has been demonstrated, the inhibition occurred a few days after virus inoculation (1, 2, 3). Our study differed in both the virus used and the time after inoculation (3 to 4 weeks) at which the plants were exposed to O_3 . When bean leaves inoculated separately with several viruses causing local lesions were exposed to O_3 , sensitivity to O_3 was reduced. Since local lesions were produced the O_3 exposure of systemically infected leaves could not be tested (4).

Our findings suggest that tobacco infected with TSV may be more sensitive to O_3 than virus-free tobacco grown under field conditions. This interaction could be very important to the farmer. Under field conditions long periods of virus development in tobacco might influence the physiology enough to produce additive or possibly greater than additive foliar injury upon exposure to O_3 . The additive effects on plant weight and greater than

additive effects on leaf injury produced by O_3 and TSV suggest the importance of further study of other virus pollutant-host associations in relation to foliar injury and yield loss in tobacco and other plant species.

LITERATURE CITED

1. BRENNAN, E. 1975. On exclusion as the mechanism of ozone resistance in virus-infected plants. *Phytopathology* 65: 1054-1055.
2. BRENNAN, E., and I. LEONE. 1969. Suppression of ozone toxicity symptoms in virus-infected tobacco. *Phytopathology* 59:263-264.
3. DAVIS, D. D., and S. H. SMITH. 1974. Reduction of ozone-sensitivity of pinto bean by bean common mosaic virus. *Phytopathology* 64:383-385.
4. DAVIS, D. D., and S. H. SMITH. 1974. Reduction of ozone sensitivity of pinto bean by virus induced local lesions. *Plant Dis. Rep.* 60:31-34.
5. HECK, W. W., J. A. DUNNING, and H. JOHNSON. 1968. Design of a simple plant exposure chamber. *Natl. Ctr. Air Pollution Publ. APTD-68-6*. Cincinnati, Ohio. 24 p.
6. GOODING, G. V., JR., and H. ROSS. 1970. Effect of tobacco etch virus on yield and market value of commercial cultivars of burley tobacco in North Carolina. *Tobacco Sci.* 14:55-57.
7. HEAGLE, A. S., D. E. BODY, and W. W. HECK. 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environm. Qual.* 2:365-368.
8. LUCAS, G. B. 1975. Diseases of tobacco. H. E. Parker and Sons, Fuquay-Varina, N. C. 621 p.
9. MOYER, J., and S. H. SMITH. 1975. Oxidant injury reduction on tobacco induced by tobacco etch virus infection. *Environ. Pollut.* 9:103-106.
10. REINERT, R. A., D. T. TINGEY, W. W. HECK, and C. WICKLIFF. 1969. Tobacco growth influenced by low concentrations by sulfur dioxide and ozone. *Agron. Abstr.* 61:34 (Abstr.).
11. SALTZMAN, B. E. 1965. Selected methods for the measurement of air pollutants. *Public Health Service Publ. 999-AP-11*, p. D1-D5.
12. TINGEY, D. T., and R. A. REINERT. 1975. The effect of ozone and sulfur dioxide singly and in combination on plant growth. *Environ. Pollut.* 9:117-125.