Variable Effects of Ozone on Pinto Bean Internodes

Ida A. Leone and Eileen Brennan

Associate Research Professors, Department of Plant Biology, Cook College, Rutgers University – The State University of New Jersey, New Brunswick 08903.

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ABSTRACT

Ten- to 14-day-old Pinto bean seedlings exposed to ozone exhibited positive, negative, or no effects on internodal elongation according to ozone dosage. At the foliage-marking dosage of 600 $\mu g/m^3$ (0.30 ppm) ozone for 3 hours there was an inhibition; whereas, at sublethal dosages of 200 $\mu g/m^3$ (0.10 ppm) for 48 or 72 hours, there was a stimulation of growth in internodes which were starting to elongate during the ozone exposures. Intermediate dosages had no effect. The initiation of new internodes also appeared to be stimulated by the lower-level ozone exposures.

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While attention is generally focused on the detrimental effects of atmospheric contaminants on vegetation, certain pollutants have been shown to have stimulatory effects on plant growth at sublethal levels. Such effects have been reported for fluorides (1, 7, 10, 11, 20, 21), for sulfur dioxide (3, 5, 6, 9, 17), for ozone (2, 4, 8, 15), and more recently for nitrogen dioxide (22).

Since 1969, in experiments designed to determine whether or not inhibition in stem height would occur in *Phaseolus vulgaris* L. Pinto exposed to sublethal ozone dosages, we frequently observed increased rather than decreased growth. With a view toward further elucidation of this phenomenon, a study was conducted to determine the effect of prolonged low-level ozone exposures on the

elongation of Pinto bean seedlings.

MATERIALS AND METHODS.—Pinto beans were germinated either in 10.2-cm diameter clay pots (two plants per pot) or directly in wooden flats (18 to 36 seedlings per flat) in sterilized greenhouse soil to which no fertilizer was added. The plantings were maintained in a charcoal-filtered greenhouse. Ten to 14 days later, when most of the seedlings had developed to the primary leaf stage, measurements were made of the length of primary stems (hypocotyl and first internode), and of any developing internodes. Replicate plants were then exposed to ozone at 200 $\mu g/m^3$ (0.10 ppm) for 48 or 72 hours; 300 $\mu g/m^3$ for 3, 6, or 12 hours; and 600 $\mu g/m^3$ for 3 hours in a glass-walled fumigation chamber by methods previously described (12). Temperatures in the chamber were maintained at 23.9-26.7 C (75-80 F), relative humidity at 65 to 70%, and supplementary light was supplied by a battery of fluorescent lights. Ozone concentration was monitored by a Mast Ozone Meter. A similar number of seedlings was placed in a corresponding charcoal-filtered control chamber.

After the given exposure period, seedlings were placed in the charcoal-filtered greenhouse for 14-17 days. At the end of this period, primary stems and subsequently developed internodes were measured. Primary leaves were detached, examined for visible injury, and their fresh and dry weights determined. In all, six experiments were conducted during the months of September through March, 1973. Statistical analyses were made by Student's *t*-test.

RESULTS.—*Foliage injury.*—Injury was observed only in Experiment VI on primary leaves of plants exposed to ozone at 600 $\mu g/m^3$ for 3 hours, the standard dosage used to elicit typical ozone symptoms (16).

Green and dry weight of foliage.—Fresh and dry weights of the primary leaves of control plants (Table 1) were fairly uniform, except for those in Experiment VI which, having been grown during March as opposed to January or February for the others, enjoyed a more favorable light regime which could have led to the production of more green, and hence more dry, matter. This experiment was also the only one in which weight alterations were produced by fumigation (600 $\mu g/m^3$ for 3 hours). The effect was a reduction in dry weight at $P = 0.01$, and consequently in percent dry ($P = 0.01$), which parallelled the occurrence of injury in the primary leaves.

Length of primary stem.—Table 2 presents data for the length of primary stem (hypocotyl plus first internode) of control and ozone-treated bean seedlings immediately before and 14-17 days after exposure. Variability in length of these stem sections previous to exposure may be accounted for by variation in age at the time of treatment, or in light conditions at the time of year in which fumigation took place. Increase in length varied from 2 to 19% of the original, but there was no difference in length of this stem section between control and fumigated plants two weeks after fumigation, the length probably having been established prior to ozone exposure.

Length of internodes.—Table 3 contains data for length of internodes of control and treated plants 14-17 days after the termination of each exposure. In Experiment VI, at the ozone dosage which produced visible injury (600 $\mu g/m^3$ for 3 hours), as might be expected from an injurious ozone dosage, there was a significant decrease ($P = 0.01$) in length of the second internode. Exposures at 200 $\mu g/m^3$ O_3 for 48 and 72 hours (Experiments I and II), on the other hand, produced a significant increase at $P = 0.01$ in length over controls in the third internode only. Fumigations at 300 $\mu g/m^3$ for 3, 6, or 12 hours produced no growth alterations in any internode.

By the end of the post-exposure period, a number of seedlings had produced a rudimentary fifth internode which was too small to be measured. However, a count of the frequency of such occurrences (Table 4) revealed an increased number among plants exposed to 200 $\mu g/m^3$ O_3 for 48 or 72 hours and to 300 $\mu g/m^3$ O_3 for 3 hours, over the controls. Plants exposed to 300 $\mu g/m^3$ O_3 for 6 or 12 hours exhibited no increase in number of fifth internodes and plants exposed to 600 g/m^3 O_3 for 3 hours produced no fifth internodes nor did their controls. The latter group of plants was only 24 days old at the completion of this experiment, whereas plant age in the other experiments was 27-30 days. This might possibly have been the reason for total lack of fifth internodes in Experiment VI.

DISCUSSION.—The results in these experiments provide evidence for inhibitory, stimulatory, or no effect of ozone on internodal elongation in Pinto bean seedlings depending on ozone dosage. The inhibitory effect in Experiment VI (600 $\mu g/m^3$ for 3 hours) appeared in the second internode while the stimulatory effects in Experiments I and II were exerted on the third internode. These internodes were most probably initiating elongation during the respective fumigations. Apparently, these effects were exerted only during the actual exposure period, since neither pre-formed nor subsequently formed tissues were involved. In the case of internode length, cell elongation was apparently

TABLE 1. Fresh weight, dry weight, and percent dry weight of primary leaves of control and ozone-treated Pinto bean seedlings

Exp.	Plants in each treatment group (no.)	Ozone dosage: Concn. ($\mu g/m^3$)	Ozone dosage: Duration (hours)	Fresh weight (g per leaf): Control	Fresh weight (g per leaf): Treated	Dry weight (g per leaf): Control	Dry weight (g per leaf): Treated	Dry weight (%): Control	Dry weight (%): Treated
I	20	200	48	...	...	...	...	...	...
II	36	200	72	0.68	0.70	.076	.073	11.21	10.40
III	18	300	3	0.68	0.59	...	...	...	...
IV	24	300	6	0.61	0.60	.070	.070	11.50	11.67
V	24	300	12	0.63	0.63	.073	.078	11.58	11.45
VI	30	600	3	0.99	0.97	.117	.096*[a]	11.80	9.90*

[a] Asterisk (*) indicates significant at $P = 0.01$.

TABLE 2. Length of primary stem of control and ozone-treated Pinto bean seedlings immediately before and 14 to 17 days after ozone exposure

Exp.	Ozone dosage: Concn. ($\mu g/m^3$)	Ozone dosage: Duration (hours)	Length of primary stem (cm): Control: Pre-expos.	Control: Post-expos.	Control: Comparison with control (%)	Treated: Pre-expos.	Treated: Post-expos.	Treated: Comparison with control (%)
I	200	48	8.60	8.77	102	8.89	8.95	101
II	200	72	10.42	12.20	117	10.56	12.08	115
III	300	3	8.70	10.36	119	8.95	10.44	118
IV	300	6	11.50	12.83	111	11.52	12.71	110
V	300	12	11.04	12.87	117	11.21	12.73	113
VI	600	3	9.47	10.46	110	9.25	10.05	108

TABLE 3. Average length of internodes and total length of control and ozone-treated Pinto bean seedlings 14 to 17 days after ozone exposure

Exp.	Ozone dosage: Concn. ($\mu g/m^3$)	Ozone dosage: Duration (hours)	Length (cm): Internode 2: Control	Internode 2: Treated	Internode 3: Control	Internode 3: Treated	Internode 4: Control	Internode 4: Treated	Total: Control	Total: Treated
I	200	48	2.98	2.88	2.68	3.09*[a]	2.06	2.25	17.11	18.00
II	200	72	3.73	3.51	2.76	3.11*	2.99	2.33	20.32	21.66
III	300	3	3.11	2.94	1.68	1.64	1.85	1.65	16.34	15.33
IV	300	6	3.13	3.00	2.13	2.09	1.90	1.80	19.47	19.30
V	300	12	3.41	3.05	2.34	2.03	1.94	1.88	20.06	19.29
VI	600	3	3.60	2.88*	1.57	1.54	1.02	1.07	15.81	14.89

[a]Asterisk (*) indicates significant at $P = 0.01$.

involved, much as in the case with growth-promoting substances such as gibberellic acid.

The reason for the greater tendency of fumigated plants to produce a fifth internode during the experimental period is not known at this time.

Stimulatory effects of ozone were first reported by Engle and Gabelman (8) in 1967. Whereas they too used Pinto bean as a test plant, growth parameters in their experiments were elongation and fresh weight of lateral buds rather than of primary leaves or internodes and their ozone dosage was 100 $\mu g/m^3$ (0.05 ppm) ozone for 3-5 days as opposed to 200 $\mu g/m^3$ used in our investigation. Comparisons, therefore, are not feasible. Craker and Feder (4), in 1972, reported increases in fresh weight of petunia and geranium flowers as the result of ozone exposures at 200-240 $\mu g/m^3$. Whereas their dosage was equivalent to ours, species and growth parameters varied. Neil et al. (15), in 1973, observed significant increases in shoot elongation in tomato plants after a 2-hour treatment with 600 $\mu g/m^3$, a dosage which in our experience has been known to cause foliar injury in both tomato and Pinto bean. However, the tomatoes in the Neil et al. investigation had developed in a growth chamber under a 16-hour photo period which changed abruptly, constituting a stressed condition in the plants. In addition, relative humidity in their exposure chamber was maintained at 50-55% as compared to 70-75% in the growth chamber. The authors alluded to previous occasions on which ozone combined with stress conditions in plants had induced growth stimulation. Finally, Bennett et al. (2), in 1974, reported increases in dry weight of stems, primary leaves, and middle leaflets of wax bean cultivar Pure Gold treated with only 60 $\mu g/m^3$ (0.03 ppm) ozone for 12 days. Whereas this concentration was less than one-third of that used in our experiments, the 12-day duration might conceivably have compensated for the lower concentration in inducing growth stimulation.

TABLE 4. Frequency of occurrence of fifth internode in control and treated bean seedlings 14 to 17 days after ozone exposure

Exp.	Ozone dosage: Concn. ($\mu g/m^3$)	Ozone dosage: Duration (hours)	Ratio of number of fifth internodes to total plant number: Control	Treated
I	200	48	6:20	11:20
II	200	72	6:36	20:36
III	300	3	2:18	11:18
IV	300	6	2:24	3:24
V	300	12	4:24	4:24
VI	600	3	0:30	0:30

In spite of the wide variation in conditions and results among the preceding investigations, a common link with ours was the absence of foliar injury at the dosages associated with stimulatory effects. The results presented in this report, therefore, add corroborative evidence for the hypothesis that prolonged sublethal ozone concentrations may tend to produce growth stimulation in certain plant species.

Ozone is neither a classic metabolic poison (as are fluorides) nor a possible plant nutrient (as are SO_2 and NO_2); therefore, its role in promoting growth is not as easily explained. Perhaps at low concentrations it possesses a hormonal activity similar to that of gibberellic

acid. Engle and Gabelman (8) explored auxin-like activity as an explanation for ozone effects in increasing fresh weight and length of Pinto bean buds, and found that the stimulation was not associated with an increase in indoleacetic acid activity.

The significance of this stimulatory phenomenon is not quite clear. Although Bennett et al. (2) pointed out that the levels which cause stimulation are close to those that occur in nature and hence plants grown in clean air are at a disadvantage, it must be borne in mind that most of the evidence supporting stimulatory effects of pollutants has been the result of experimentation involving a single pollutant injected into filtered air. The actual situation in ambient air, a mixture of many known, and possibly a number of as yet unknown pollutants, may be of an entirely different nature, particularly if one considers the possibility of synergistic action among two or more such pollutants at low levels, as has been suggested by Menser and Heggestad (14) and others (18, 19). At any rate, experiments performed at Rutgers University, through the use of circular, open-top chambers have shown the sum total effect of sublethal concentration of all pollutants in ambient air in New Jersey during the summer of 1973 to be inhibitory rather than stimulating (13).

This phenomenon should not be considered as a license to relax secondary air quality standards, but rather as a possible additional factor in the complex picture of ambient oxidants which warrants further investigation.

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