Reduction in Tobacco Pollen Germination and Tube Elongation, Induced by Low Levels of Ozone

Abstract. *Pollen of the ozone-sensitive tobacco variety Bel W-3 undergoes a reduction in germination rate and tube elongation when exposed to ozone. As little as 0.1 part of ozone per million for a 5.5-hour exposure period is sufficient to cause a 40 to 50 percent reduction in germination and a 50 percent reduction in pollen-tube elongation. Ozone causes these effects to approximately the same degree whether the pollen is exposed to ozone in vitro or agar disks, or in vivo when the intact plant is exposed. Exposure to ozone at a concentration of 1.0 part per million for more than 3 hours in vitro completely prevents germination of Bel W-3 pollen.*

Concentrations of ozone of 0.025 to 0.1 part per million (ppm), measured coulombmetrically or iodometrically, markedly damage the leaves of certain cigar-wrap tobacco varieties, especially the U.S. Department of Agriculture variety Bel W-3 (*1*). The effects, if any, of ozone upon pollen production, germination, and tube elongation in this plant are not known. Ozone damage to these processes could result in decreased fertility and consequent reduction in seed production.

Bel W-3 tobacco plants were grown to maturity in charcoal-filtered, ozone-free air in a greenhouse during the fall and winter of 1967 to 1968. Pollen was harvested from individual flowers of the same plant when the flowers were slightly open and showed some color, and just as the anthers were beginning to dehisce.

The pollen was removed by crushing the anther in a drop of sterile distilled water. The pollen grains were picked up with a sterilized camel's hair brush and brushed onto the surface of a disk of 1 percent agar fortified with 10 percent sucrose. Fifteen to 20 such disks were divided at random; some were placed in the ozonating chamber, and the remainder served as untreated controls. Ozone was generated by passing 1 liter of scrubbed, washed compressed air per minute over a GE S411 bulb enclosed in an aluminum pressure cooker. The bulb voltage was regulated by a transformer. The resultant ozone-containing air was passed into a plexiglass box where it contacted the pollen grains and was then passed outdoors through an exhaust port. The plexiglass cube was equipped with two other larger ports, one of which was used for insertion of the monitoring probe of the ozone meter; the other was used for the insertion and removal of the agar disks. The plexiglass reaction box was kept at 26.7°C in a temperature-controlled chamber during the fumigation. After fumigation, disks were removed from the box and placed into plastic petri plates which served as humidity chambers. A high humidity was maintained in the dishes with a piece of moistened filter paper placed in the dish lid. The dishes were stored in the dark at 26.7°C for 24 hours after which the percentage of germination of groups of 100 pollen grains was recorded (Table 1).

In a first series of tests, pollen was exposed to ozone (1 ppm) for up to 6 hours. Disks were removed from the chamber after exposure of 30, 60, 120, 180, 240, or 360 minutes. In the second series, the pollen was exposed to ozone (0.1 ppm) for 5.5 and 24 hours.

A third series consisted of intact flowering plants exposed to ozone (0.1 ppm) for 24 hours in a larger ozonating chamber in which ozone was generated by a Welsbach generator from charcoal-filtered ambient air. Pollen was removed and allowed to germinate as described above.

Germination among the untreated pollen grains ranged between 85 and 90 percent. Tube lengths of pollen grains treated for up to 2 hours with ozone (1 ppm) were not significantly different from those of untreated grains. The percentage of germination in grains exposed for longer than 2 hours was markedly reduced (Table 1), and there was essentially no pollen tube elongation.

The germination rate of pollen grains exposed to ozone (0.1 ppm) for periods of 5.5 to 24 hours was reduced by 40 to 50 percent (Table 1), and pollen tube length was about one-half that of the untreated, control pollen. Maximum reduction in germination percentage and tube growth occurred after only a 5.5-hour exposure to the ozone. Untreated pollen tubes invariably discharged their contents on the agar after 24-hour growth. Discharge never occurred in the shorter tubes arising from ozonated pollen.

Pollen grains taken from plants which had been exposed intact to ozone (0.1 ppm) for 24 hours also showed reduction in germination rate and exhibited reduced tube growth. These reductions were in the same order of magnitude as those observed when pollen was exposed in vitro on agar disks.

A concentration of ozone of 0.1 ppm reflects the concentration of this air pollutant encountered repeatedly at our monitoring station 12 miles west of Boston, Massachusetts, during the summer of 1967. This fact plus the data presented in this paper should alert those interested in the effects of air pollutants upon plant life to the possibility that through a reduction in pollen viability and vigor, ozone and related oxidant-like pollutants may be exerting a subtle stress on plant populations in the Northeast.

Table 1. Effect of concentration and duration of exposure to ozone on the germination rate of pollen from tobacco variety Bel W-3. Values are expressed as percentages.

Value	Exposure time (hours)									
	½	1	2	3	4	5	0	5½	24	0
	Concentration of 1 ppm									
Mean	59	41	8.0	1.8	0.0	0.0	89			
Standard deviation	5.0	2.5	1.8	1.3	0.0	0.0	3.5			
Range	13	6.0	5.0	3.0	0.0	0.0	9.0			
	Concentration of 0.1 ppm									
Mean								54.2	52.2	86.6
Standard deviation								5.2	5.6	4.4
Range								13	15	11

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References and Notes

1. H. E. Heggestad, F. R. Burleson, J. T. Middleton, E. F. Darley, *Int. J. Air Water Pollution* **8**, 1 (1964); H. E. Heggestad and H. A. Menser, *Phytopathology* **52**, 735 (1962); H. A. Menser and O. E. Street, *Tobacco Sci.* **6**, 165 (1962).
2. I thank F. Sullivan and B. Udin for technical assistance.

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