Effect of Ozone on Infection of Wild Strawberry by Xanthomonas fragariae

J. A. Laurence and F. A. Wood

Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108. Present addresses of the authors: Boyce Thompson Institute for Plant Research, 1086 North Broadway, Yonkers, NY 10701 and Office of the Dean for Research, University of Florida, Gainesville, FL 32611.

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ABSTRACT

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Exposure of wild strawberry (*Fragaria virginiana*) to ozone concentrations of 294 $\mu g/m^3$ (0.15 ppm) for 2 hr resulted in necrotic flecks on the upper leaf surface. Exposure to higher concentrations for longer periods resulted in increased injury. Infection of wild strawberry by *Xanthomonas fragariae* was inhibited when plants were exposed to 392 $\mu g/m^3$ (0.20 ppm) O_3 for 3 hr before or after inoculation. Exposure to 157 $\mu g/m^3$ (0.08 ppm) O_3 for 3 hr did not result in consistent inhibition of infection.

Additional key words: pollutant-parasite interactions.

The response of woody plants to ozone (O_3) is well documented, but effects of O_3 on native herbaceous plants are not well known. Wild strawberry (*Fragaria virginiana* Duchesne) is common in open woods and meadows throughout Minnesota. It is found in and around urban areas where pollution levels may be high, as well as in rural and remote areas where low concentrations of pollutants exist. Usually, cultivated strawberry (*F. ananassa*) is considered to be resistant to O_3 (9). The response of wild strawberry to O_3 has not been reported. In addition, wild strawberry often is afflicted with a variety of leafspot diseases, the severity of which, if affected by O_3, might be used as an indicator of air quality or pollutant effects on plants.

Ozone interacts with plant parasites and thus influences other diseases. It has been shown that exposure to O_3 generally reduced infection, invasion, and sporulation by pathogenic fungi (2). In some instances (11, 12, 13) diseases caused by facultative parasites may be enhanced by O_3 exposure, as in the case of *Botrytis cinerea* on geraniums and field-grown potatoes.

Interactions of O_3 and bacterial plant pathogens have not been studied extensively. Ozone exposure reduces the number, size, and nodule weight of *Rhizobium* sp. on bean and soybean (10, 17). Also, it has been observed that O_3 symptoms can be lessened or modified in the presence of bacterial infections of leaves (6, 15).

Angular leafspot or bacterial blight of strawberry, caused by *Xanthomonas fragariae* Kennedy and King is found on strawberry in all areas of Minnesota, as well as in several other states (3, 4, 5, 16). Symptoms of the disease include small water-soaked angular lesions which may coalesce.

Studies were conducted to determine the response of wild strawberry to O_3 and effects of O_3 on the *X. fragariae–F. virginiana* interaction. In addition, it was of interest to determine if wild strawberry or the host-parasite interaction could be used as an indicator of O_3 air pollution.

MATERIALS AND METHODS

Culture of wild strawberry.—A random sample of wild strawberry was collected from Voyageurs National Park, near International Falls, Minnesota. Plants were grown from runners planted in sand and were transplanted to greenhouse soil mix in 10-cm square pots. Eleven days prior to exposure of plants to O_3, top growth was cut back to the emerging leaf; this provided leaf tissue of approximately the same age for exposure and inoculation. Prior to exposure, leaves were inspected to assure that neither O_3 nor angular leafspot symptoms were present. Plants were fertilized uniformly once per month with an all purpose liquid fertilizer (N:P:K 20-20-20).

Culture of Xanthomonas fragariae.—An isolate of *X. fragariae* obtained from infected leaves of locally grown strawberry was used to inoculate leaves of wild strawberry. About 14 days after inoculation, when angular leafspot symptoms had developed, leaves were collected, pressed, and stored in a refrigerator at 5 C. Inoculum was prepared by grinding an infected leaflet in 20 ml of sterile distilled water and diluting this suspension with an additional 100 ml of sterile distilled water. This technique was used to maintain the virulence of *X. fragariae*.

Exposure to O_3.—Two days prior to exposure to O_3,

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plants were transferred to, and randomly assigned positions in, a conditioning growth chamber supplied with charcoal-filtered air (Model M-2, Environmental Growth Chambers, Integrated Development and Manufacturing Co., Chagrin Falls, OH 44022). The plants were maintained at 21 C, 80% relative humidity, and 26 Klx illumination with a 12-hr photoperiod. All plants were watered uniformly and given a 1.5-hr light period immediately before exposure to O_3. Plants to be exposed were transferred to a modified growth chamber (18). This chamber was identical to the conditioning chamber except for the pollutant introduction and monitoring systems. Exposures were conducted from approximately 0900 to 1200 under the environmental regime previously described. Ozone was produced with an Orec generator (Model 03V5-O, Ozone Research and Equipment Corp., Phoenix, AR 85019) and measured continuously with a McMillan 1100 chemiluminescence monitor (McMillan Electronics Co., Houston, TX 77036). Concentrations during exposure never varied by more than 29 $\mu g/m^3$ (0.015 ppm) from the desired level. Immediately following exposure, plants were returned to the conditioning chamber. They were maintained in that chamber for 2 days and then transferred to the greenhouse.

Ozone injury was evaluated 7 days after exposure by visual estimates of symptom intensity similar to those used by Kohut et al. (7). A severity index [(the percentage leaf area affected) × (frequency of symptom type)] was calculated to quantify injury. The index ranged from 0 (no injury) to 100 (most severe).

Inoculation procedure.—Leaves of wild strawberry were inoculated with *X. fragariae* by applying 0.25 ml of inoculum suspension with a Jet-Pac aerosol sprayer (Sprayon Products, Inc., Cleveland, OH 44146). Noninoculated controls were treated similarly except that sterile distilled water was substituted for inoculum. Leaflets were inoculated at six times: 2 days, 1 day, and 1 hr before exposure; and 1 hr, 1 day, and 2 days after exposure. The number of lesions per leaflet was recorded

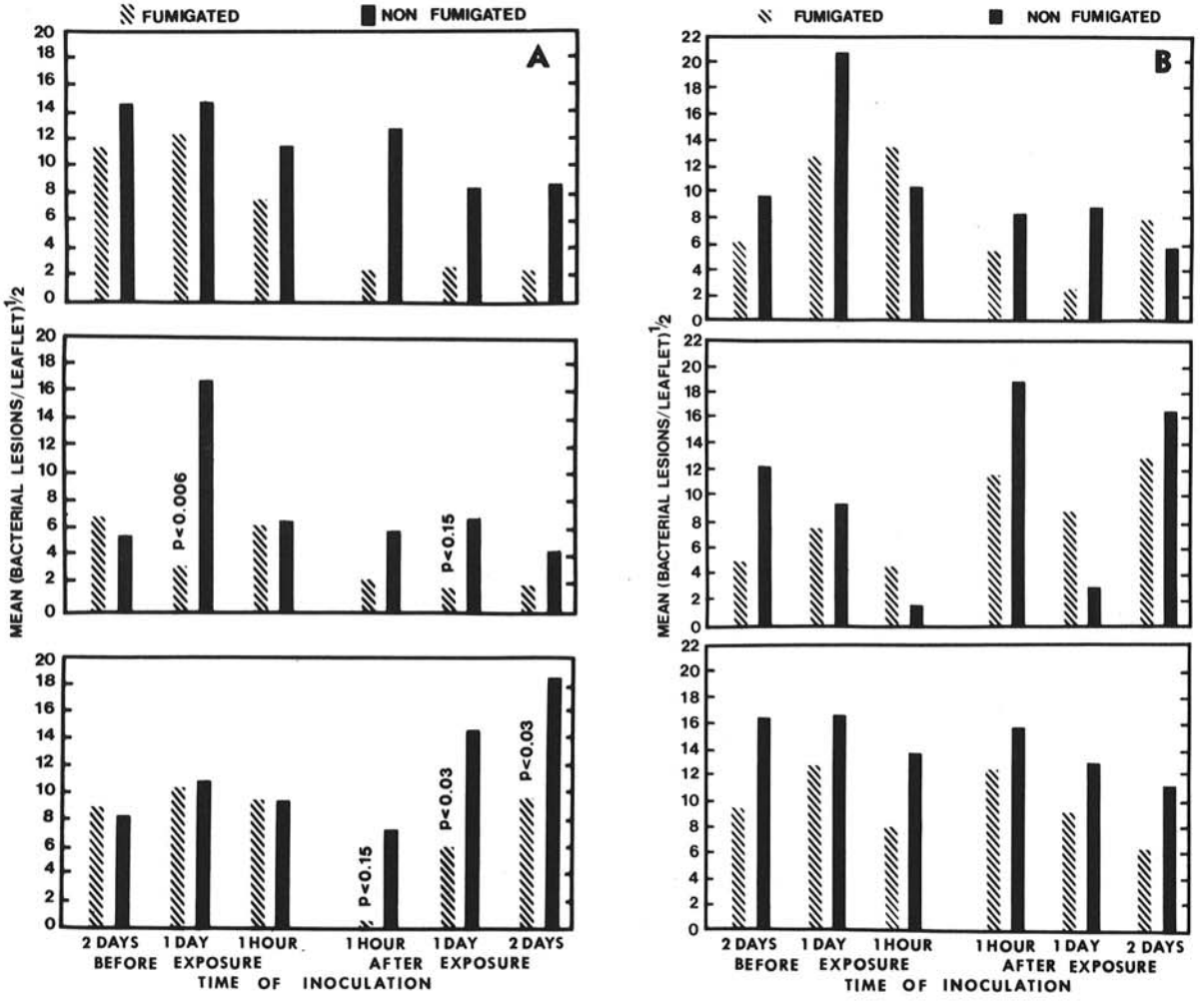

Fig. 1-A, B. Effect of ozone (O_3) exposure on infection of wild strawberry by *Xanthomonas fragariae*. Multiple graphs in each part of the figure represent repetitions of the experiment. The *P*-values indicate the significance of the difference. Absence of *P*-values indicates $P > 0.3$. **A)** Plants exposed to 392 $\mu g/m^3$ (0.20 ppm) O_3 for 3 hr. **B)** Plants exposed to 157 $\mu g/m^3$ (0.08 ppm) O_3 for 3 hr.

10 days after inoculation.

Experimental design.—Ozone–*Xanthomonas fragariae interaction studies.*—Factorial experiments in randomized, complete block design were utilized. Three leaflets per plant and four blocks resulted in 12 observations per treatment. Each experiment was repeated three times. To stabilize the variance, the data were re-expressed as the square root of the number of bacterial lesions per leaflet and were analyzed by factorial analysis of variance. Nontransformed symptom severities were analyzed similarly. Relationships of treatment means were examined using two sample *t*-tests and the Bonferroni inequality (1) which adjusts the test to reflect the number of treatment comparisons made.

Dose-response experiment.—A completely randomized design was used with five replications of three leaflets per replication at each of four concentrations and exposure lengths. The experiment was repeated twice, resulting in 480 observations (leaflets). Regression analysis was used to determine the relationship between O_3 concentration, length of exposure, and severity index.

RESULTS

Ozone-Xanthomonas fragariae interaction studies.—Fewer lesions per leaflet were observed on plants exposed to 392 $\mu g/m^3$ (0.20 ppm) for 3 hr and inoculated 1 day and 1 hr before or after exposure (Fig. 1-A). Comparisons were made only between exposed and nonexposed plants at the same time of inoculation because our inability to precisely quantify viable inoculum made meaningful comparisons between times of inoculation impossible. Differences and trends, although not always statistically significant, were consistent across repetitions although a significant experiment × time of inoculation interaction precluded combination of the data. There was no apparent spatial relationship between bacterial lesions and necrotic flecks on the leaves.

Similar groups of plants were exposed to 157 $\mu g/m^3$ (0.08 ppm) O_3 for 3 hr. The results were more variable than those obtained from exposures at the higher concentration. Inoculation at 2 days and 1 day before exposure and 1 hr after exposure resulted in fewer lesions per leaflet in all repetitions, but the differences were not significant (Fig. 1-B).

Throughout the study, O_3 injury was not observed on nonexposed plants, nor were bacterial lesions found on noninoculated plants. Differences in O_3 symptom severity between inoculated and noninoculated plants were not found, and there were no differences in O_3 symptom severity between times of inoculation in any experiment. Ozone symptom severity differed between repetitions at the higher concentration. Ozone injuury was not observed on plants exposed to 157 $\mu g/m^3$ for 3 hr.

The response of wild strawberry to four O_3 concentrations at four exposure durations was evaluated to determine the relationship between pollutant concentration, length of exposure, and symptom severity. Necrotic flecking was the predominant symptom following exposure to 294 $\mu g/m^3$ (0.15 ppm) and 392 $\mu g/m^3$ (0.20 ppm) O_3 (Fig. 2). Bifacial necrosis was observed following exposure to 490 $\mu g/m^3$ (0.25 ppm) O_3 for 2 hr or more. Stippling was not observed on plants at any concentration-time combination. The response of wild strawberry to O_3 was approximately linear; i.e., equal concentration × length of exposure products resulted in similar amounts of leaf injury (Fig. 3).

DISCUSSION

Data from the O_3–*X. fragariae* interaction studies indicate that bacterial infection was inhibited by O_3 exposure at concentrations causing visible injury. The basis for this inhibition is unknown although Mussell and

Fig. 2. Necrotic flecking of the adaxial leaf surface of wild strawberry induced by exposure to ozone (O_3).

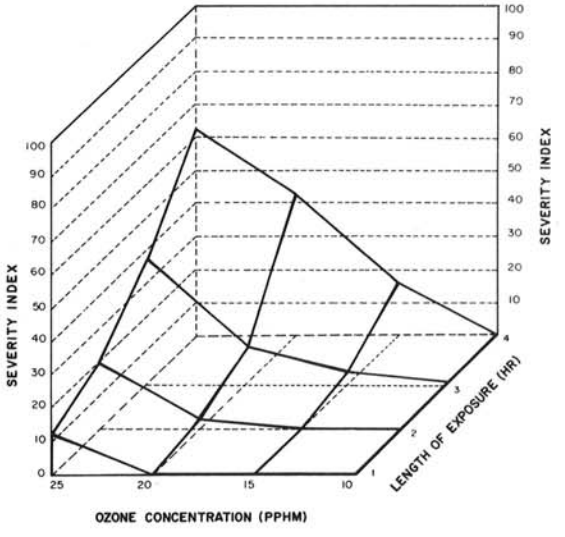

Fig. 3. Relationship of ozone (O_3) concentration and length of exposure to O_3 symptom severity. 1 pphm = 19.6 $\mu g/m^3$.

Staples (14) reported the production of two phytoalexinlike compounds in strawberry roots inoculated with *Phytophthora fragariae*. They also suggested that polyphenol and melanin compounds may be formed. The lower amount of infection observed on exposed plants may result from the presence of such compounds, produced in response to O_3 exposure.

The inhibition of bacterial lesion development probably was not caused by a reduction in leaf area available due to O_3 induced necrosis since plants inoculated 2 days before exposure had similar O_3 injury but no reduction in lesions.

The similarity of effects of O_3 on bacterial infection in soybean (8) and wild strawberry suggests inhibition of bacterial infection or growth following exposure to O_3. The effects of long term, low concentration exposures to O_3, and of occasional high concentration fumigations during these exposures, on bacterial disease development are unknown.

Wild strawberry was more sensitive to O_3 exposure than cultivated strawberry. The threshold for symptom development was higher than the current ambient air quality standard for O_3 but within the range of concentrations found both in urban and rural areas of Minnesota (8). This suggests the possible use of the plant as an indicator of ambient phytotoxic concentrations of O_3; however, the effects of O_3 on both sexual and asexual reproduction of wild strawberry should be studied since resistant individuals could eventually dominate a stand.

LITERATURE CITED

1. DAVID, H. A. 1970. Order statistics. John Wiley and Sons, New York. 272 p.
2. HEAGLE, A. S. 1973. Interactions between air pollutants and plant parasites. Annu. Rev. Phytopathol. 11:365-388.
3. HILDEBRAND, D. C., M. N. SCHROTH, and S. WILHELM. 1967. Systemic invasion of strawberry by Xanthomonas fragariae causing vascular collapse. Phytopathology 57:1260-1261.
4. KENNEDY, B. W., and T. H. KING. 1962. Angular leafspot on strawberry caused by Xanthomonas fragariae sp. nov. Phytopathology 52:873-875.
5. KENNEDY, B. W., and T. H. KING. 1962. Studies on epidemiology of bacterial angular leafspot on strawberry. Plant Dis. Rep. 46:360-363.
6. KERR, E. D., and R. A. REINERT. 1968. The response of bean to ozone as related to infection by Pseudomonas phaseolicola. Phytopathology 58:1055 (Abstr.).
7. KOHUT, R. J., D. D. DAVIS, and W. MERRILL. 1976. Response of hybrid poplar to simultaneous exposure to ozone and PAN. Plant Dis. Rep. 60:777-780.
8. LAURENCE, J. A. 1976. Ozone: Transport from an urban area and effects on infection of soybean and wild strawberry by bacterial plant pathogens, Ph.D. Thesis, University of Minnesota, St. Paul. 110 p.
9. LEDBETTER, M. C., P. W. ZIMMERMAN, and A. E. HITCHCOCK. 1959. The histopathological effects of ozone on plant foliage. Contrib. Boyce Thompson Inst. 20:275-282.
10. MANNING, W. J., W. A. FEDER, and P. M. PAPIA. 1972. Influence of long term low levels of ozone and benomyl on growth and nodulation of pinto bean plants. Phytopathology 62:497 (Abstr.).
11. MANNING, W. J., W. A. FEDER, and I. PERKINS. 1970. Ozone and infection of geranium flowers by Botrytis cinerea. Phytopathology 60:1302 (Abstr.).
12. MANNING, W. J., W. A. FEDER, and I. PERKINS. 1970. Ozone injury increases infection of geranium leaves by Botrytis cinerea. Phytopathology 60:669-670.
13. MANNING, W. J., W. A. FEDER, I. PERKINS, and M. GLICKMAN. 1969. Ozone injury and infection of potato leaves by Botrytis cinerea. Plant Dis. Rep. 53:691-693.
14. MUSSELL, H. W., and R. C. STAPLES. 1971. Phytoalexinlike compounds apparently involved in strawberry resistance to Phytophthora fragariae. Phytopathology 61:515-517.
15. PELL, E. J., F. L. LUKEZIC, and W. C. WEISSBERGER. 1976. Alteration of ozone injury of soybean foliage preinoculated with Pseudomonas sp. which elicits a hypersensitive response. Proc. Am. Phytopathol. Soc. 3:242 (Abstr.).
16. POWELL, D., and M. N. KHARE. 1967. Angular leafspot of strawberry in Kentucky. Plant Dis. Rep. 51:353.
17. TINGEY, D. T., and U. BLUM. 1973. Effects of ozone on soybean nodules. J. Environ. Qual. 2:341-342.
18. WOOD, F. A., D. B. DRUMMOND, R. G. WILHOUR, and D. D. DAVIS. 1974. An exposure chamber for studying the effects of air pollution on plants. Pa. Agric. Exp. Stn. Prog. Rep. 335. 7 p.