Reduction of Ozone-Sensitivity of Pinto Bean by Bean Common Mosaic Virus

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ABSTRACT

Bean common mosaic virus infection reduced the sensitivity of pinto bean to ozone (O_3) damage. Immature primary leaves inoculated with the virus 5 days prior to exposure with 25 μl O_3/liter of air for 4 h showed less O_3 injury than the noninfected control plants. Protection was influenced by the initial inoculum dilution and by length of time between inoculation and exposure to O_3.

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Ozone (O_3) is one of the major air pollutants affecting plants in the USA and O_3 injury has been reported on many plant species (5, 6, 8, 9). Results of laboratory investigations with tobacco have demonstrated that virus infection reduces O_3 injury (1, 2, 7).

Observation of commercial bean plantings indicated that infection with bean common mosaic virus (BCMV) might protect against air pollution injury. Experiments reported herein were designed to determine whether such an interaction exists between BCMV on pinto bean (*Phaseolus vulgaris* L. 'Pinto 111') and O_3 injury. This model was selected because of the known high sensitivity of pinto bean to O_3, the knowledge of factors affecting the sensitivity of this bean variety to O_3, and the sensitivity of this variety to BCMV.

MATERIALS AND METHODS.—Five beans per pot were planted in a steam-sterilized (1:1, v/v) peat:perlite mix and maintained in a greenhouse for 5-7 days until epicotyl emergence. Plants were then transferred to ISCO (Instrument Specialities Co., Lincoln, Nebraska) controlled environment chambers set at 21 C, 75% relative humidity (RH), and a 0600 to 1800 photoperiod of 25,000 lux. After 2-3 days of growth, the two plants most uniform in size were selected, and the remaining three removed.

Plants were inoculated with BCMV 10-14 days after seeding. BCMV-infected leaves were ground in 0.05 M phosphate buffer, pH 7.2, and the homogenate brushed on Carborundum-dusted primary leaves.

Ozone was generated and transported to the chamber as previously described (3). Ozone exposures were conducted at 0.25 μl/liter (ppm) for 4 h at 21 C, 75% RH, and a light intensity of 25,000 lux from 1000 to 1400. During exposures O_3 levels were continually monitored with a REM (REM, Inc., Santa Monica, Calif.) chemiluminescent O_3 meter. Ozone values of the REM meter were compared to the determination of oxidants by the neutral buffered potassium iodide method. Meter efficiencies were approximately 100%. Intermittent temp and RH measurements were made during each exposure

using copper-constantan wet- and dry-bulb thermocouples connected to a 24-point recorder. The exposure chamber was a modified version of a commercially available chamber (Environmental Growth Chamber Co., Chagrin Falls, Ohio) which has been described elsewhere (10). Following exposure, plants were returned to the ISCO chambers and were maintained for 72 h under conditions similar to those prior to exposure, when the symptoms were evaluated. Symptoms were rated in terms of percentage upper leaf surface of tissue injured by O_3. Injury classes of 0, 10, 30, 50, 70, 90, and 100% were recorded. Untreated control plants were included in each experiment.

RESULTS.—Typical symptoms developed on O_3-injured plants, consisting of small tan flecks on the upper leaf surface. On leaves with more extensive injury, the symptom was observed on both leaf surfaces. Occasionally pigmented stipple was observed on the upper leaf surface. Ozone injury was not observed on unexposed control plants.

TABLE 1. Ozone sensitivity of bean leaves after inoculation with bean common mosaic virus

Treatment	leaf injury[a] (%)
Noninoculated + O_3[b]	55.8
72 AI[c] + O_3	52.5
96 AI + O_3	5.0
120 AI + O_3	0.0
144 AI + O_3	0.0
144 AI	0.0

[a] Percentage of total leaf area showing O_3 injury. Each value is the average of 12 treatments.

[b] Plants were exposed to 0.25 μl O_3/liter of air for 4 h.

[c] AI (after inoculation) values indicate time (hr) between inoculation with BCMV and exposure to O_3.

Experiment 1.—In an initial experiment involving 80 plants, immature primary leaves of 40 plants were inoculated with BCMV, 20 plants were brushed with Carborundum and healthy plant juice and 20 plants remained untreated. Five days later, all plants were exposed to O_3, except 20 of the plants inoculated with BCMV.

Noninoculated plants exhibited 25.5% leaf injury and BCMV infection reduced O_3 damage to 0.2%. Plants brushed with extracts from noninfected beans prior to O_3 exposure showed 34.0% injury and the BCMV infected (but nonexposed) plants exhibited no symptoms of O_3 injury. All the noninoculated plants and those brushed with the extract from noninfected beans showed O_3 injury on both leaves. Only a single leaf on one infected plant developed O_3 symptoms.

Experiment 2.—A second experiment was performed to determine the length of time after inoculation necessary to impart protection against O_3 injury. Primary leaves were inoculated with BCMV at various time periods prior to a single exposure. Twelve plants were used in each of six treatments as follows: BCMV only; O_3 only; exposed to O_3 72-h after inoculation (AI); exposed to O_3 96-h AI; exposed to O_3 120-h AI; and exposed to O_3 144-h AI.

TABLE 2. Relationship between size of virus inoculum and ozone sensitivity of bean leaves

Treatment	% leaf tissue injured by O_3			
	Rep 1	Rep 2	Rep 3	Av.
Noninoculated	77.1[a]	78.9	31.4	62.5
1:1[b]	14.0	1.4	0.0	5.1
1:2	6.8	0.7	0.0	2.5
1:4	4.6	8.9	0.0	4.5
1:16	0.0	2.1	0.7	0.9
1:64	35.0	4.6	6.1	15.2

[a] Percentage of total leaf area showing injury after 4-h exposure to 0.25 μl O_3/liter air 120 h after inoculation. Each value is the average of 14 treatments.

[b] Leaves were inoculated with a homogenate from BCMV-infected leaves ground in 0.05 M phosphate buffer, pH 7.2. Numbers indicate the proportion of virus-infected leaves (g fresh wt) per volume (ml) of buffer used to prepare the virus inoculum.

Table 1 represents results obtained from experiment 2. All primary leaves on noninoculated plants exposed to O_3, and on those plants exposed 72-h after inoculation, were injured by O_3. Ten leaves on six plants showed O_3 injury when exposed 96-h after inoculation. The remaining plants had no injury. No O_3 injury was observed on plants treated with virus only. A waiting period of at least 120 h between inoculation and exposure to O_3 was utilized in further experiments because of these findings.

Experiment 3.—This experiment, which involved 252 plants, was initiated to determine the level of inoculum necessary to protect primary leaves against O_3 injury. Fourteen plants were used in each of the following six different treatments: noninoculated, O_3 only; and a dilution series involving weight of virus-infected tissue: volume of buffer (1:1, 1:2, 1:4, 1:16, 1:64) + exposure to O_3 120 h after inoculation. Additional plants were inoculated with virus, and not exposed to O_3. This study was replicated three times.

Results from experiment 3 are given in Table 2. Although virus inoculation did not completely protect the plant from O_3 injury, virus infection drastically reduced the amount of leaf tissue injured by O_3. The most dilute inoculum (1:64) protected less effectively than did other members of the dilution series. Nonexposed, BCMV-infected plants did not exhibit O_3 injury.

DISCUSSION.—Bean common mosaic virus reduced the amount of injury on primary leaves of pinto beans exposed to 0.25 μl/liter O_3 for 4 h. This protection phenomenon is similar to that afforded virus-infected tobacco (1, 2, 7). It was postulated that tobacco mosaic virus (TMV) possibly afforded protection to the plant by altering its carbohydrate or nitrogen metabolism (1). The carbohydrate level is known to affect the sensitivity of pinto bean to O_3 (4). Thus, if BCMV alters the carbohydrate level, protection from O_3 may be afforded. However, virus infection alters many other aspects of plant metabolism, and a number of these may also be involved in protection against O_3 injury.

A 96-h waiting period after inoculation with BCMV was not enough time to alter the metabolism in such a way to afford protection. However, bean plants exposed to O_3 120- or 144-h after inoculation were completely protected

from O_3 injury. These findings are similar to a report that TMV-infected tobacco plants were not completely protected from O_3 injury when exposed 2 days after inoculation, but were protected when exposed 6-12 days after inoculation (2). Apparently, to protect against O_3 injury, the virus must multiply within the host for a specific time period. However, in the field a very long time period may elapse between inoculation and exposure to O_3. If this period is of long enough duration, such as 1-2 mo, the effect of a virus on a plant's physiology, and subsequently its sensitivity to O_3, may be quite different from the results reported here.

Similar physiological factors are probably responsible for the results observed in the dilution experiment. A threshold level of virus replication must be present within the leaf tissue to afford protection. This threshold is probably reached most quickly when a more concd inoculum is utilized.

The objective of this series of studies was to determine whether or not the phenomenon of virus protection from O_3 injury occurred with BCMV and pinto beans, and to develop techniques for using this as an experimental model in future studies. Results from these studies indicated that bean plants infected with BCMV of suitable inoculum concn and for a suitable period prior to exposure to O_3 are afforded protection from O_3 injury. These results provide a basis for future studies to determine the mechanism involved in the decreased sensitivity of pinto bean to O_3 damage.

These findings also indicate that researchers studying air pollution effects on plants must consider virus infection as a possible complicating factor when evaluating effects. For example, in screening a wide number of species as to susceptibility or resistance to O_3, a virus infection may alter the degree of resistance. The same plant without virus may respond to O_3 quite differently than when infected. Such complications might also affect field diagnoses involving O_3 injury to vegetation.

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