Influence of Benomyl Soil Treatment on Pinto Bean Plants Exposed to Peroxyacetyl Nitrate and Ozone

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ABSTRACT

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The ability of soil drench applications of benomyl to protect pinto bean plants from injury by peroxyacetyl nitrate (PAN) or combinations of PAN and ozone was tested. Following a 7-day treatment with 0, 60, 80, or 100 μg benomyl/g soil, 15-day-old pinto beans were exposed to 745 $\mu g/m^3$ PAN or 745 $\mu g/m^3$ PAN and 492 $\mu g/m^3$ ozone for 3 hours. Benomyl did not protect the young primary leaves of these plants from either pollutant regime. When 20-day-old plants were exposed to the same dose of ozone and PAN following a 7-day benomyl treatment, the older primary leaves were protected.

Additional key words: air pollution, *Phaseolus,* systemic fungicide.

The systemic fungicide benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] was observed to protect tobacco plants from "weather fleck" which was of air pollution origin (16). Since ozone was considered responsible for the "weather fleck" symptom (2) it was logical that the effectiveness of benomyl to prevent ozone damage be tested under controlled conditions. Benomyl, when applied as a soil drench or soil amendment, was demonstrated to reduce ozone injury to pinto bean, annual blue grass, and petunia in the laboratory (5, 7, 8, 13). In conjunction with these studies, a number of field trials were established. Ozone-sensitive species, viz. bean cultivars Tempo and Pinto 111, azalea cultivar Snow, tobacco cultivar Conn. 7272, and grape cultivars Ives and Concord were planted at a number of locations with acknowledged air pollution problems; untreated plants exhibited typical ozone injury symptoms whereas benomyl-treated plants were protected (4, 6, 9, 17). When protection of ozone-sensitive species was observed in the field, researchers speculated that benomyl prevented oxidant damage per se (4, 6). The field experiments discussed above were all conducted in the northeastern United States where ozone has been detected at phytotoxic levels (3). There are two hypotheses which could explain prevention of air pollution injury to ozone-sensitive plants by benomyl treatments. (i) Ozone was the only oxidant present in phytotoxic concentrations at the time these studies were conducted. (ii) Benomyl protected plants against all phytotoxic oxidants that were present.

There has been indirect evidence of the presence of another phytotoxic oxidant, viz. peroxyacetyl nitrate (PAN). There have been a number of reports of "PAN-like" plant injury in the northeastern United States and Eastern Canada (1, 10, 11). The presence of PAN in the northeastern United States and Canada never has been confirmed by air monitoring. Conclusive diagnoses of symptoms observed in the field could not be made. Furthermore, without data to document whether PAN was present during the field trials in which benomyl protection of ozone-sensitive plants was observed, interpretation of experimental results remains a matter of speculation. The possibility that benomyl would protect plants from PAN alone or from PAN and ozone together provided the rationale for conducting the experiments described below.

MATERIALS AND METHODS

Phaseolus vulgaris L. 'Pinto 111 plants were grown in sand:Hagerstown silty clay loam soil mix (2:1, v/v), two per 10.2-cm (4-inch) diameter pot in an air-filtered greenhouse. Eight or 13 days after the seeds were sown, a soil drench of 0, 60, 80, or 100 μg benomyl in distilled water per gram dry weight of soil was applied. Seven days after benomyl application, plants were exposed to one of the pollutant regimes. Benomyl presence in the tissue was verified at the time of fumigation by a leaf disk bioassay method (12).

Plants were placed in the fumigation chamber 24 hours prior to PAN fumigation. All plants received a minimum of 3 hours of pre- and post-fumigation exposure to light to insure optimum plant sensitivity (19). Plants were exposed to the pollutants in a modified growth chamber described by Wood et al. (22). PAN generation and fumigation techniques were modified from procedures previously described (14, 21). The method of ozone exposure followed procedures outlined by Wood et al. (22). Plants were either exposed to 745 $\mu g/m^3$ (0.15 ppm) PAN or simultaneously to 745 $\mu g/m^3$ (0.15 ppm) PAN and 492 $\mu g/m^3$ (0.25 ppm) ozone for 3 hours.

Forty-eight hours after fumigation, leaf surface injury was evaluated on a scale of 0-100 where 0 = no injury, 10 = 1-10 percent injury, 20 = 11-20 percent injury, etc. Individual leaves and adaxial and abaxial leaf surfaces were rated separately; an average was then calculated so that each plant would have one rating which would not exceed 100%. Each experiment discussed below was replicated four times with 20 plants per treatment per replicate. The data were analyzed by an analysis of variance and significant differences between treatment means were determined by a Duncan's Modified (Bayesian) Least Significant Difference Test (20).

RESULTS

Benomyl afforded no protection from a toxic dose of PAN (Table 1). In fact, there was a significant increase in PAN injury to the primary leaves of plants treated with benomyl at 100 $\mu g/g$ soil. Benomyl was ineffective in preventing injury to the primary leaves of pinto bean plants exposed simultaneously to ozone and PAN (Table 1).

In the two experiments discussed above the primary leaves were young (trifoliolate leaves not yet apparent) and ostensibly more sensitive to PAN than to ozone. In a second series of experiments, with plants that were 20 days old, primary leaves were middle-aged and trifoliolate leaves were young but fully expanded. Benomyl, at all three rates tested significantly reduced injury to the primary leaves exposed to PAN and ozone (Table 2). When the trifoliolate leaves of these plants were examined, the level of injury was always below 10 percent. There was a statistically significant reduction in PAN plus ozone phytotoxicity in trifoliolate leaves of those plants treated with benomyl at 80 and 100 $\mu g/g$ soil when compared with nontreated plants (Table 2). Leaf disk bioassay results showed that benomyl was present in primary and trifoliolate leaves of treated plants in all experiments. From the assay, it was apparent that concentrations of benomyl in the leaves were proportional to concentrations applied to the soil.

DISCUSSION

From the experimental results reported above it is clear that benomyl does not protect primary leaves of pinto bean plants from PAN injury. In fact, benomyl at high concentrations may stimulate PAN phytotoxicity. A plant species more sensitive to PAN than pinto bean will have to be studied in order to evaluate the possible stimulatory effects of benomyl.

When plants were exposed simultaneously to PAN and ozone the younger primary leaves were afforded no protection by benomyl, but the older primary leaves were protected. Benomyl has been shown to protect pinto bean plants from ozone (13), but apparently not from PAN. It is not surprising that benomyl effectively protected the older primary leaves, which are sensitive to ozone and not PAN (18), from a simultaneous exposure to both gases. Following the same reasoning young primary leaves which are sensitive to PAN and not ozone would be damaged by the two gases in spite of chemical treatment. The ability of benomyl to protect plants from PAN and ozone injury seems to be related to plant age. When more mature leaves are susceptible to ozone there is protection; when the younger leaves are susceptible to PAN there is no protection.

The decrease in PAN and ozone injury to trifoliolate leaves treated with benomyl at 80 and 100 $\mu g/g$ soil was statistically significant, but was of questionable biological significance. The statistical significance could be attributed to the insensitivity of the plants and the large number of leaves which received little or no injury. A difference in injury ratings of 8.1 percent in untreated plants and 4.3 and 4.5 percent in benomyl-treated plants is not of biological significance. A plant several days older may have been more sensitive to one or both pollutants.

The concentration of PAN used in these experiments was higher than would be anticipated in the ambient atmosphere. Pinto bean is ordinarily sensitive to lower concentrations of PAN than used in these experiments. The heavy sand-soil mixture minimized plant sensitivity; it was necessary to use this type of mix, however, because peat and perlite, which are generally used to aerate soil mixes, bind benomyl. To verify these results, the experiments should be repeated with a more susceptible species.

The apparent difference in interaction between ozone and benomyl, PAN and benomyl, or both oxidants and benomyl is not surprising. Even though PAN and ozone are chemically similar (both are oxidizing agents), their biological effects are different. The macroscopic and microscopic symptoms of PAN and ozone on foliage are dissimilar (18). All plant species are not equally susceptible to the two gases. Furthermore, cultivars of some species (e.g., bean) are susceptible to one of the

TABLE 1. Effect of benomyl soil drench on visual injury rating of primary leaves of 15-day-old pinto bean plants exposed to 745 $\mu g/m^3$ (0.15 ppm) PAN alone or with 492 $\mu g/m^3$ (0.25 ppm) ozone for 3 hours

Benomyl ($\mu g/g$ soil)	Leaf surface injured (%)[a]	
	PAN	PAN and Ozone
0	20.7 x	27.0 xy
60	21.4 x	33.8 x
80	16.2 x	31.8 xy
100	30.4 y	25.3 y

[a]Each numerical value is the mean of four replications with 20 plants per treatment per replication. Means followed by the same letter are not significantly different according to a Duncan's Modified (Bayesian) Least Significant Difference Test Value (K = 100) approximating $P = 0.05$.

TABLE 2. Effect of benomyl soil drench on visual injury rating of primary and trifoliolate leaves of 20-day-old pinto bean plants exposed to 745 $\mu g/m^3$ (0.15 ppm) PAN and 492 $\mu g/m^3$ (0.25 ppm) ozone for 3 hours

Benomyl ($\mu g/g$ soil)	Leaf surface injured (%)[a]	
	Primaries	Trifoliolates
0	15.7 x	8.1 x
60	1.2 y	6.5 xy
80	1.5 y	4.3 y
100	2.8 y	4.5 y

[a]Each numerical value is the mean of four replications with 20 plants per treatment per replication. Means followed by the same letter are not significantly different according to a Duncan's Modified (Bayesian) Least Significant Difference Test Value (K = 500) approximating $P = 0.01$.

oxidants and tolerant to the other (15).

The effectiveness of benomyl in preventing oxidant injury to susceptible species in the field was illustrated in a number of studies (4, 6, 9). The conclusion that benomyl prevented oxidant injury rather than ozone injury, was based on one of two assumptions: (i) benomyl protected vegetation from all oxidants, including ozone and PAN, or (ii) PAN was not present in significant concentration to be phytotoxic. Since the first assumption is invalid based on the data presented here we must conclude either that assumption ii is valid or the plant species utilized were insensitive to PAN. Since these experiments were conducted in the northeastern United States, an area for which there is virtually no data concerning PAN concentration in the atmosphere, the second assumption cannot be made at present. Obviously, it is essential that the air be monitored for PAN to enable proper interpretation of the results of field experiments. The protection which benomyl affords bean plants to ozone but not to PAN may be a useful tool in field diagnosis of air pollution injury in areas where PAN and/or ozone are acknowledged problems.

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