Benomyl in Soil and Response of Pinto Bean Plants to Repeated Exposures to a Low Level of Ozone

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ABSTRACT

Benomyl soil amendments provided short-term protection from ozone injury for 'Pinto' bean plants repeatedly exposed to ozone at 0.06 μliter/liter (6 pphm) for 5-day periods. Protection was accompanied by varying degrees of benomyl phytotoxicity. Benomyl amendments were not effective in overcoming the long-term deleterious effects of repeated exposures to a low level of ozone on the growth, reproduction, and nodulation of Pinto bean plants.

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Additional key words: benomyl toxicity.

Pellissier et al. (5, 7) have shown that benomyl, used as a soil drench or amendment in a soil-peat-perlite mixture, was effective in protecting 'Pinto' beans from injury due to a single exposure to ozone at 0.25 μliter/liter (25 pphm) for 4 hr. Since Pinto beans are also susceptible to ozone at lower levels (0.05 to 0.10 μliter/liter) which occur in ambient air for longer periods (2), we attempted to determine the effects of benomyl amendments in nonsterilized field soil on the response of Pinto beans to repeated exposures to a low level of ozone. A brief report of this work has been published (4).

MATERIALS AND METHODS.–Pinto bean plants (*Phaseolus vulgaris* L. strain 111) were grown in paired greenhouses from January through March in 15 -cm diam pots containing nonsterilized Merrimac fine sandy loam. One of the greenhouses contained charcoal-filtered, ozone-free air and the other filtered air plus ozone at 0.06 μliter/liter for 8 hr/day, 5 days/wk, beginning at date of seeding. Ozone was produced with Welbach generators and continuously monitored with Mast meters equipped with sulfur dioxide scrubbers.

Two experiments were conducted. Both consisted of three replications with six plants per treatment per replication. In experiment 1, plants were grown for 20 days in soil amended with benomyl at 25, 50, 75, and 100 μg/g soil. In experiment 2, plants were grown for 40 days in soil amended with benomyl at 50 μg/g soil. A modified leaf disk bioassay method (6) was used to determine the presence of benomyl in the primary leaves of 10-day-old bean plants. Leaf disks, 1-cm diam, were cut from washed leaves and placed on potato-dextrose agar plates seeded with spores of *Botrytis cinerea* Pers. Isolations from root nodules were made using methods and media described by Allen (1). Incidence of ozone injury, plant heights, dry weights of tops and roots, and number and weight of nodules and seed were recorded. Means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION.–Nodules were found on the roots of all plants in both experiments. Isolations from these nodules yielded bacteria with characteristics of the genus *Rhizobium*.

Leaf disk bioassays showed benomyl to be present in the primary leaves of all 10-day-old plants grown in benomyl-amended soil in both experiments.

In experiment 1, benomyl suppressed ozone injury at a concentration of 50 μg/g soil and above. This suppression lasted for 10 days and was accompanied by extensive yellow-green chlorosis and interveinal burning on the primary leaves and later on the first two trifoliolate leaves. Benomyl did not significantly affect nodule numbers on plants exposed to ozone (Table 1). In filtered air, benomyl at 25 μg/g soil increased nodule numbers when compared to benomyl at 100 μg/g soil. Plants exposed to ozone and grown in soil containing benomyl at 75 μg/g soil had fewer nodules than those grown in filtered air in soil containing 0, 25, or 75 μg benomyl/g soil. Benomyl also reduced nodule numbers at the 25 μg/g soil concentration in ozone compared to filtered air. Ozone alone did not significantly affect nodule numbers. Benomyl significantly decreased nodule weights on plants grown in filtered air only at the 100 μg/g soil level. Comparisons between ozone-supplemented and filtered air, however, showed that benomyl significantly decreased nodule wieghts at the 25, 50, and 75 μg/g soil levels. Ozone alone also reduced nodule weights. Differences in average plant heights and dry weights of tops and roots were not significant.

In experiment 2, benomyl caused moderate phytotoxicity and provided protection from ozone injury for 12 days. Benomyl and ozone did not significantly affect nodule numbers, but nodule weights were reduced by benomyl in both ozone-supplemented and filtered air (Table 2).

TABLE 1. Nodulation of 20-day-old 'Pinto' bean plants as affected by benomyl in soil and repeated exposures to a low concentration of ozone

	Rhizobium nodules[x]			
	Number		Weight (g)	
Benomyl levels (μg/g soil)	Ozone[y]	Filtered air[z]	Ozone	Filtered air
100	41.0 de	40.9 de	.22 d	.26 d
75	37.2 d	48.3 ef	.21 d	.43 e
50	43.3 de	45.8 def	.20 d	.42 e
25	42.8 de	52.4 f	.21 d	.43 e
0	39.8 de	38.9 ef	.29 d	.44 e

[x] Each numerical value is the mean of three replications with six plants per treatment per replication. Means followed by the same letters are not significantly different from each other at the $P = 0.01$ level, using Duncan's multiple range test.

[y] 0.06 μliter/liter (6 pphm) ozone, 5 days/wk, from planting date.

[z] Charcoal-filtered, ozone-free air.

TABLE 2. Growth, nodulation, and yield of 40-day-old 'Pinto' bean plants as affected by benomyl in soil and repeated exposures to a low concentration of ozone

Treatments	Plant measurements[x]						
	Dry wt (g)		Height (cm)	Nodules		Seed	
	Tops	Roots		No.	Wt (g)	No.	Wt (g)
Ozone[y]							
Benomyl (50 μg/g soil)	1.1 d	0.3 d	33.0 d	10.4 d	.117 d	40 d	4.3 d
Control (No benomyl)	1.5 d	0.4 de	46.3 d	18.1 d	.239 e	69 e	7.4 d
Filtered air[z]							
Benomyl (50 μg/g soil)	2.5 e	0.6 de	38.6 e	29.5 d	.611 f	146 f	16.9 e
Control (No benomyl)	2.9 e	0.8 e	61.7 e	29.0 d	.895 g	217 g	26.6 f

[x] Each numerical value is the mean of three replications with six plants per treatment per replication. Means followed by the same letters are not significantly different from each other at the $P = 0.01$ level, using Duncan's multiple range test for comparisons.

[y] 0.06 μliter/liter (6 pphm) ozone, 5 days/week, from planting date.

[z] Charcoal-filtered, ozone-free air.

Nodule weights were also reduced by ozone in the absence of benomyl. Benomyl and ozone did not reduce dry weights of the tops of plants grown in ozone or filtered air. Comparisons between the two air regimes, however, showed significant differences between results for benomyl and controls. Root dry weights showed differences only between the benomyl treatment in ozone and the control in filtered air. Benomyl and ozone did not significantly reduce heights of plants grown in ozone or filtered air, but differences were observed when comparisons were made between benomyl and controls in ozone-supplemented and filtered air. Benomyl did reduce average seed numbers in both ozone and filtered air. Seed numbers were also reduced by ozone in the absence of benomyl. Benomyl did not affect seed weights in ozone, but did affect them in filtered air. Comparison between ozone and filtered air showed significant differences in seed weights between benomyl treatments and controls.

Benomyl soil amendments provided temporary, short-term protection from ozone injury on Pinto beans repeatedly exposed to a low level of ozone. Repeated exposures to the low level of ozone, however, resulted in loss of the protection after 10-12 days. This loss, plus benomyl phytotoxicity at levels necessary for protection from ozone injury, resulted in general plant decline as measured by changes in growth, productivity, and nodulation. Similar results with other plants grown in benomyl-amended soil were obtained by Schreiber & Hock (8). Our results indicate that compounds being evaluated for suppression of ozone injury should be used with plants grown in nonsterilized mineral soils and exposed to low levels of ozone for long periods of time. Soil is particularly important as Hock et al. (3) showed that the organic matter in potting soils and artificial mixes restricts plant uptake of benomyl through immobilization.

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