Effectiveness of Benzimidazole, Benomyl, and Thiabendazole in Reducing Ozone Injury to Pinto Beans

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ABSTRACT

The hypothesis that benomyl protection against ozone injury is due to the benzimidazole moiety was tested. Three concentrations of benzimidazole, benomyl, and Thiabendazole [2-(4-Thiazolyl)-benzimidazole] were incorporated into a soil-peat-perlite (2:1:1) growth mixture. Plants grown in the treated soil mixtures were fumigated for 4 hr with 25 parts/hundred million (pphm) of ozone. Benzimidazole and benomyl protected the plants from ozone injury; Thiabendazole did not. A bioassay technique used in these studies indicated that failure of Thiabendazole to protect against ozone injury may have been caused by low uptake of the chemical. The technique involved measurement of zones of *Penicillium cyclopium* growth inhibition surrounding leaf discs placed on inoculated malt agar plates.

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Benomyl and Thiabendazole [2-(4-thiazolyl)-benzimidazole] are two commercially used systemic fungicides with related chemical structures and fungal toxicity spectra. It has been proposed that the selective toxicity of these two compounds depends on the benzimidazole portion of the molecule (2). However, Gilpatrick (4) and later, Erwin (3), noted that benzimidazole itself had no effect in reducing powdery mildew of apple and Verticillium wilt of cotton. Benzimidazole was also observed to lack fungitoxicity in in vitro tests (3). Benzazole compounds, of which benzimidazole is one, are known to be growth regulators and to have antisenescent properties (5). Leaves of plants treated with these compounds appear greener, possibly due to increased numbers of intergrana and grana lamellae of the chloroplast (10). Benzimidazole has also been reported to considerably enhance the uptake of potassium (1). The studies reported here were designed to test the hypothesis that previously reported protection from ozone (6, 7, 8) when potting mixtures were tested with benomyl was, in fact, due to the benzimidazole moiety.

MATERIALS AND METHODS.—Seeds of *Phaseolus vulgaris* L. 'Pinto III' were planted 3/pot, hilum down, at a depth of 0.5 cm in 500-ml plastic pots filled with a Hagerstown silty clay loam-peat-perlite (2:1:1) soil mix. Benomyl and Thiabendazole were incorporated by spraying a water suspension on the dry soil while it was being tumbled. Benzimidazole was dissolved in acetone, and the solution sprayed into the soil. The acetone was then allowed to evaporate from the soil mix. Concentrations used were based on the molecular weight of benzimidazole, and were applied at the rates of 20, 40, and 80 μg/g soil. A 40-μg/g dosage based on the benzimidazole moiety molecular weight was equivalent to a 98-μg/g benomyl dosage and a 68-μg/g Thiabendazole dosage. Commercial formulations of wettable powders, Benlate 50W and Mertect 160, were the sources of benomyl and Thiabendazole, respectively.

Bioassays involving zones of inhibition of *Penicillium* sp. of the cyclopium series, Raper & Thom (9), on malt agar plates were used to detect the presence of diffusible toxic substances in leaf discs cut from benomyl-, Thiabendazole-, and benzimidazole-treated bean plants. Leaf discs (6 mm diam) were cut from the lobe of the leaf before ozone fumigation. An agar medium was inoculated with a 22×10^3 spore/ml suspension of conidia. Sensitivity of the fungus to the three compounds was determined by using filter paper discs 6 mm in diam impregnated with different quantities of the material in a chloroform solution. Zones of inhibition were measured 2 days after plating. Bean plants were fumigated with 25 pphm of ozone for 4 hr 11 days after planting. Temperature during fumigation was maintained at 30 C; relative humidity, at 78%; and light intensity, ca. 650 ft-c. Percentage of damage on primary leaves was estimated 3 days after fumigation. A completely randomized experimental design was used, and the data obtained were subjected to analyses of variance and Duncan's multiple range test.

RESULTS.–In the in vitro petri dish tests, benzimidazole was not toxic to *P. cyclopium* (Table 1). Thiabendazole was slightly less fungitoxic to the fungus than benomyl. An inhibition zone was not observed around leaf discs from benzimidazole- or Thiabendazole-treated plants. Inhibition zones of 14 and 23 mm were observed around leaf discs cut from plants growing in soil mixtures containing 98 and 196 μg benomyl/g soil (40 and 80 μg on a benzimidazole moiety basis), respectively.

Nontreated fumigated plants showed ozone-induced flecks on 50-55% of the leaf surface (Table 2). One hundred per cent protection was obtained at a benzimidazole dosage of 40 μg/g or higher, and at a benomyl dosage of 196 μg/g. Ninety per cent protection was obtained at a benomyl dosage of 98 μg/g. For benomyl treatments of 49 and 98 μg/g, it was observed that injury occurred only along the veins. Thiabendazole resulted in a 50% reduction at the highest concentration (196 μg/g), and did not give any significant protection at the lower dosages used. Phytotoxicity or visible changes in the morphology of the plants were not apparent with any of these treatments.

TABLE 1. Sensitivity of *Penicillium cyclopium* to benzimidazole, benomyl, and Thiabendazole

Active ingredient in filter paper disc (μg)	Diameter of inhibition zone (mm)[a]		
	benomyl	Thiabendazole	benzimidazole
.05	9	0	0
.1	14	8	0
.2	20	12	0
.3	23	17	0
.4	24	20	0

[a]Average of 10 replications.

TABLE 2. Effect of benzimidazole, benomyl, and Thiabendazole soil amendments in reducing visible ozone injury to *Phaseolus vulgaris* 'Pinto III'

Chemical treatment	% Area of injured foliage[a] μg/g soil based on benzimidazole moiety		
	20	40	80
Control	55 a	50 a	50 a
Benzimidazole	15 b	0 b	0 b
Thiabendazole	40 a	45 a	23 c
Benomyl	15 b	5 b	0 b

[a]Means in the same column not followed by the same letter are significantly different from each other (Duncan's multiple range test .05 probability level). Each number is the mean of 24 isolations.

DISCUSSION.–Benzimidazole was effective in protecting Pinto beans from ozone injury when it was incorporated in the soil at concentrations as low as 20 μg/g. At benzimidazole-equivalent dosages, benomyl provided a level of protection equivalent to benzimidazole. This supports the view that the benzimidazole moiety was responsible for the induced protection from ozone damage obtained by a soil treatment with benomyl. It has not been determined whether protection was due to a change in the physiology of the plant induced by the treatment or by direct inactivation of ozone by the chemical. Bioassay did not detect fungitoxic substances in the leaves of beans treated with Thiabendazole, suggesting that the lack of protection from ozone damage in treated plants was probably due to limited translocation of the chemical throughout the plant. This assumption is made on the basis that with Thiabendazole and other plant species, uptake as determined by chemical analysis can usually be correlated with bioassay results as determined by inhibition zones with a sensitive fungus.

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