Systemic Activity of Benomyl Fungicide against Cladosporium Leaf Mold and Verticillium Wilt of Greenhouse Tomatoes[1]

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Abstract

Verticillium wilt and *Cladosporium* leaf mold are two serious diseases of commercially grown greenhouse tomatoes in Indiana. Benomyl fungicide, has shown activity against species of the genera *Cladosporium* and *Verticillium*, and a combination of preventive, curative, and systemic fungicidal properties. This study utilized three methods in assaying for benzimidazole carbamic acid, methyl ester (BCM), the breakdown product of benomyl, in plant tissue: tissue plating, tissue incorporation, and vascular fluid incorporation. Vascular fluid incorporation was the most quantitative method. *Penicillium oxalicum* was completely inhibited *in vitro* at 0.5 ppm benomyl and *P. expansum* and *P. finiculosum* at 0.1 ppm. Benomyl at 25 ppm either as a soil incorporation or a soil drench was effective in protecting tomato from infection by *V. albo-atrum*. A 25 ppm benomyl soil incorporation also protected against *C. fulvum* infection. At this level of treatment, no detrimental effects were noted either on foliage or root system development. Although BCM accumulated in leaves at concentrations of at least 25 ppm, the compound could not be detected in the mature fruit.

Verticillium wilt, caused by *Verticillium albo-atrum* Rke. & Berth., and gray leaf mold, caused by *Cladosporium fulvum* Cke., are two serious diseases of commercially grown greenhouse tomatoes in Indiana due to the cultural conditions found in greenhouse operations. *Verticillium albo-atrum*, a soil borne pathogen, often builds up in greenhouse soil under constant cultivation, and can usually be controlled only by the costly procedure of steam sterilization or by fumigating the soil. Gray leaf mold causes severe leaf necrosis under humid conditions and effective control requires control of atmospheric humidity and application of protective foliar fungicides. Genetic resistance is available for both diseases but is not extensively used.

The systemic fungicide benomyl, 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester is effective against both of these pathogens and is thus a potential means for control of the diseases they cause in the greenhouse (2). Hine *et al.* (3) demonstrated that benomyl is unusually persistent in soil, compared to other soil fungicides, and recent studies (1, 4, 5) have shown that benomyl breaks down rapidly in aqueous solution to benzimidazole carbamic acid, methyl ester (BCM) and that his fungitoxic derivative is likely what is taken up systemically by treated plants. The uptake, translocation and accumulation of BCM has been demonstrated in other plants, including bean and cotton (4, 5).

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The objectives of this study included:

1) Determination of systemic uptake, translocation and accumulation of benomyl or its fungitoxic derivative BCM by the tomato plant.

2) Effectiveness of benomyl under greenhouse conditions to prevent, reduce or eradicate infection of tomato by *Cladosporium fulvum* and *Verticillium albo-atrum*.

Methods and Procedures

A bioassay was developed to determine the uptake, accumulation and persistence of BCM, the fungitoxic derivative of benomyl, in tomato. Conidiospores of *Penicillium oxalicum*, *P. expansum* and *P. funiculosum* were placed on solidified potato dextrose agar (PDA) in which benomyl was incorporated at varying concentrations. After incubation for 3 days at 28°C, spore germination and growth of the 3 test fungi when exposed to varying concentrations of the fungicide, were determined. The sensitivity of *P. oxalicum* was later used in the bioassay for BCM in host tissue.

The uptake, translocation and accumulation of BCM from soil by tomato seedlings was ascertained by tissue plating, tissue macerates and vascular fluids. In tissue platings, sections or discs of tomato stem and leaf tissue were placed directly on solidified PDA with the antibiotics aureomycin and streptomycin incorporated to reduce bacterial contamination and previously seeded with condiospores of *P. oxalicum*. The zones of inhibition of spore germination were used to determine the presence of BCM. In use of tissue macerates, leaf and stem tissues were first ground in a food blender and incorporated in PDA with antibiotics at known ratios of weight/volume. Condiospores of *P. oxalicum* were placed on the agar surface and concentrations of BCM in the tissues estimated.

The most accurate bioassay method for uptake and translocation of BCM in tomato tissues was by use of vascular fluids. The fluids were collected by excising the plants just below the first true leaf and inverting the plant container over a test tube for 24 hours. The fluids exuded by root pressure were incorporated at varying concentrations in PDA+antibiotics and the bioassay with conidiospores of *P. oxalicum* used.

The protection of tomato seedlings from infection by *Cladosporium fulvum* and *Verticillium albo-atrum* and the eradication of established infection by the latter by the systemic uptake and translocation of BCM was determined. Benomyl was either mixed into the soil at known concentrations prior to planting the test plants or by soil drench with the fungicide after planting. Inoculation of tomato with *C. fulvum* was by atomizing conidiospores onto the foliage under conditions favorable for infection. Tomato seedlings were inoculated with *V. albo-atrum* either by root dip into a suspension of spores-mycelium or by planting test plants into soil previously infested with known inoculum densities of microsclerotia of this fungus.

Results

The growth of *Penicillium expansum*, *P. funiculosum* and *P. oxalicum* on PDA in the presence of benomyl fungicide was completely inhibited at 0.5 ppm. This provided a highly sensitive group of test organisms for bioassay of host tissues for presence of benomyl or its fungitoxic derivatives. As indicated, *P. oxalicum* was used as the test organism in the bioassays throughout this study.

Uptake, Translocation, and Accumulation of Benomyl

Tissue Plating. BCM was detected in the stem tissue of tomato plants within 3 days after transplanting into 100 ppm (w/w) benomyl-incorporated soil. Stem sections from the treated plants produced zones of growth inhibition on PDA plates seeded with spores of *P. oxalicum*. After 6 days, growth inhibition zones occurred with both stem sections and leaf discs from these plants. The concentration of BCM in the plant tissues was determined to be in excess of 0.5 ppm, since this is the minimum dosage necessary for growth inhibition of this test organism.

Tissue Incorporation. To establish a more precise assay for BCM concentration in host tissues, stem and leaf samples from tomato seedlings grown in benomyl treated soil were macerated and incorporated into PDA on a weight/volume basis. Complete inhibition of growth occurred when 1 g of leaf tissue, from plants grown in soil treated with benomyl at 50 ppm for 6 days, was incorporated into 100 ml of PDA. Since germination of spores of *P. oxalicum* were inhibited at 0.5 ppm, it was estimated that the leaf tissue contained at least 50 ppm BCM, based on the dilution of the host tissue in the growth medium. Lower concentrations of host tissues failed to inhibit growth of the test organism, supporting the estimated concentration of BCM in the leaf tissue.

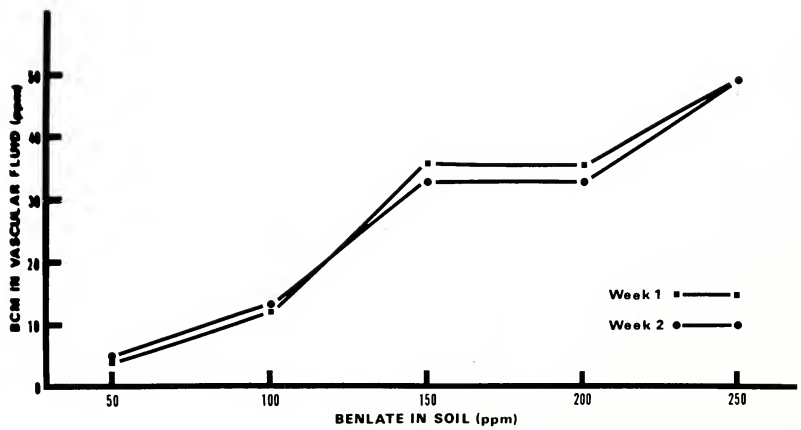

FIGURE 1. *Concentrations of BCM (2-benzimidazole carbamate, methyl ester) in vascular fluids of tomato plants in soil treated with Benlate (benomyl).*

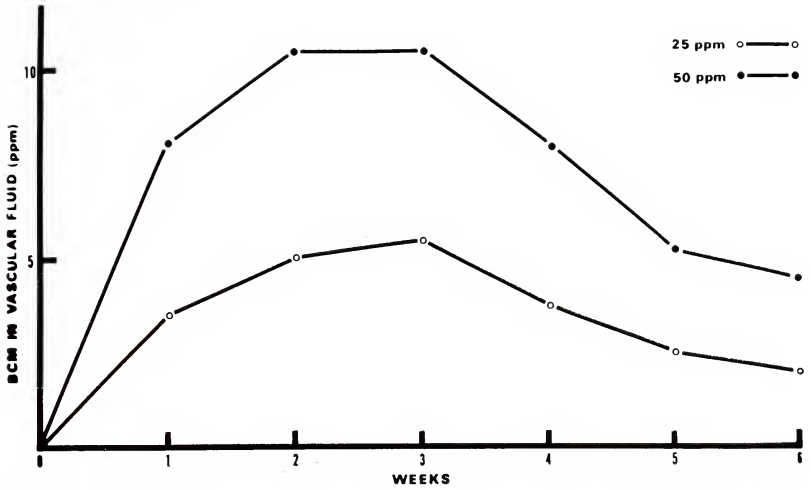

FIGURE 2. *Concentrations of BCM (2-benzimidazole carbamate, methyl ester) in vascular fluids of tomato plants over 6 weeks period.*

Vascular Fluid Incorporation. Using the vascular fluid analysis procedure, both the concentration of BCM uptake and the duration of uptake over a 6-week period were determined. Results presented in Figure 1 show that the concentration of BCM in the plant was linearly related to the concentration of benomyl incorporated into the soil. Second, the uptake of BCM reached a peak between the second and third weeks following treatment, and then dropped off gradually over the next 3 weeks (Fig. 2). The relation between soil concentration of benomyl and uptake concentration of BCM, as previously noted, also holds in this test.

Control of *Cladosporium* Leaf Mold on Tomato

Tomato seedlings were protected from infection by *C. fulvum* when grown in soil with 25 ppm benomyl incorporated prior to transplanting test plants. At soil concentrations of 100 ppm and 200 ppm benomyl there was slight to moderate marginal chlorosis of the leaflets of the lower leaves of tomato seedlings, indicating limited phytotoxicity.

When tomato seedlings with established infection by *C. fulvum* were treated by application of benomyl as a soil drench, no new infections occurred after treatment at 25 ppm or above. Foliar applications of benomyl by spraying at concentrations of 600 ppm also prevented further infection of plants that already exhibited symptoms of this disease. However, there was no evidence of eradication of established infections by either the soil drench or foliar with benomyl.

Protection/Eradication—*Verticillium* Wilt of Tomato

Tomato seedlings were transplanted to soil infested with *Verticillium albo-atrum* (microsclerotia—250/g soil) with varying concentra-

tions of benomyl from 10 ppm to 100 ppm. Incubation was for 5 weeks and infection was ascertained by plating stem sections on PDA regardless of presence of symptoms. The results are presented in Table 1.

TABLE 1. *Tomato plants infected by* Verticillium albo-atrum *after 5 weeks in soil containing microsclerotia and benomyl.*

Treatment (ppm)	Number of stems assayed	Number of stems infected	Percent infected
Control	7	5	71
10	7	2	29
25	8	0	0
50	8	0	0
100	8	0	0

Typical symptoms of Verticillium wilt (lower leaf necrosis, chlorosis, stunting and unilateral leaflet development) appeared on control untreated tomato seedlings in infested soil and on plants in soil with 10 ppm benomyl. Plants in all other treatments were normal and free from the pathogen.

Tomato seedlings were root-dip inoculated with *V. albo-atrum* and grown in sterile sand for varying periods to establish infection. After 3 to 15 days, inoculated seedlings were transplanted to soil with benomyl incorporated at 10 ppm and 50 ppm. Infection in the untreated inoculated seedlings was determined by attempting to recover the fungus from the stems. The effectiveness of BCM in the eradication of established infection by *V. albo-atrum* was determined by comparing the infection of treated plants to untreated checks. The results are presented in Table 2.

TABLE 2. *Eradication of* Verticillium albo-atrum *from infected tomato seedlings by transplant into soil with benomyl.*

Number of days between inoculation and transplant	Number of infected plants/total plants in the treatment		
	Control	10ppm	50ppm
3	6/6(100%)	5/5(100%)	0/6(0%)
6	6/6(100%)	6/6(100%)	3/5(60%)
9	6/6(100%)	6/6(100%)	5/6(83%)
12	6/6(100%)	5/5(100%)	6/6(100%)
15	6/6(100%)	6/6(100%)	6/6(100%)

The vascular pathogen *V. albo-atrum* was partially or completely eradicated from inoculated plants for a period up to 9 days after infection. After 12 days, the dysfunction of the invaded roots apparently reduced or inhibited uptake of BCM and the chemical was no longer effective.

Summary

A bioassay for the detection of benomyl fungicide or its fungitoxic derivative (BCM) in tomato plant tissues was developed using conidiospores of *Penicillium oxalicum*. The bioassay is sensitive to concentrations of 0.5 ppm. BCM was detected using tissue sections or tissue macerates and in vascular fluids. The latter method gave the most quantitative results. When benomyl was incorporated in soil, BCM accumulated in vascular fluids of test plants to levels of 50 ppm and there was a linear relationship between soil concentration and the accumulation in vascular fluids. The BCM concentration increased for 3 weeks after transplanting test plants into treated soil, followed by a gradual decline. Phytoxicity was limited to marginal chlorosis of leaflets of lower leaves and occurred only at the higher rates.

Incorporation of 25 ppm benomyl in soil protected tomato seedlings from infection by *Cladosporium fulvum* and *Verticillium albo-atrum*. Established infection by *V. albo-atrum* could also be partially or completely eradicated up to 9 days after inoculation with soil treatments of 25 ppm.

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