Phytotoxicity of Benomyl to Crucifers

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ABSTRACT

Phytotoxicity of benomyl to crucifers was influenced by cultivar, seedling age, chemical concentration, and the presence or absence of a surfactant. Seedlings of cabbage cultivar Eastern Ballhead, and cauliflower cultivars Clou and Idol were highly sensitive to benomyl (0.154 mg/g oven dry soil); whereas seedlings of cabbage cultivars Red Rock, Early Greenball, King Cole, and Chieftain Savoy, broccoli cultivars Spartan Early and Green Comet, and brussels sprouts cultivars Jade Cross and Catskill were tolerant. Seedlings of cabbage cultivar Eastern Ballhead were severely stunted when seeds were sown in benomyl-treated soil, but were only slightly affected when seedlings were treated at 8 weeks of age. Plant growth was not affected by benomyl at

rates below 0.154 mg/g soil; cabbage, cauliflower, and brussels sprouts were stunted at rates above this concentration. When surfactants GAFAC RA-600 or GAFAC RS-710 (mixtures of mono- and diphosphate esters) (1:99, v/v) was added to benomyl or applied to filter paper and soil, seed germination and seedling emergence of cabbage cultivar Eastern Ballhead were less than when the filter paper and soil were treated with benomyl alone. This effect was not observed when 1% of the surfactant Tween 20 (polyoxyethylene sorbitan monolaurate) was added to benomyl.

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Since its introduction, the systemic fungicide benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] has been an effective fungicide against a wide range of plant pathogens (2). Recently, we (10) reported it to be effective in controlling clubroot in crucifers when drenched into infested soil, but found that it injured cabbage cultivar Jersey Queen. Further observations (Reyes, unpublished) of benomyl phytotoxicity on cabbage cultivar Eastern Ballhead in the greenhouse revealed that symptoms developed within 1 week of treatment. Affected plants exhibited irregular depressions, and chlorosis at the margin, tip, and central portions of the leaves. Chlorosis in the central part of the leaf was interveinal. Chlorosis started as isolated watersoaked (bleached) spots or bands, and was associated with a general loss in turgidity which resulted in wilting during hot, sunny days. Severe phytotoxicity included marginal leaf chlorosis that rapidly spread inward to cause whitish, brittle, dried leaves, and death of plants. Within a few days several dead leaves usually hung from the still greenish stem. When new leaves developed they also showed phytotoxic symptoms. Resultant plant weight was inversely related to the degree of phytotoxicity.

Benomyl has been reported to be phytotoxic to other crops also, including cauliflower (13), cucumber (11, 13), muskmelon (14, 15), pea (3), squash (11), and tomato (3, 5). Because the variables that affect benomyl phytotoxicity were unknown, a study of factors such as cultivar, seedling age, chemical concentration, and surfactant was initiated.

MATERIALS AND METHODS.—Seedlings and chemicals used.—Fifty-four seeds of each cultivar were sown in a steamed mixture of peat moss, sandy loam soil, and sand (1:3:1, v/v, pH 7.3) in a $6 \times 33 \times 51$ cm plastic tray in the greenhouse (24 ± 2 C and 65-75% relative humidity). Tap water was applied to the soil surface and the seedlings were treated with demeton (Chemagro, Kansas City) for insect control (7). Except where indicated, seedlings were 6 weeks old when transplanted. All transplants were grown in the soil mixture (2,710 g, 33% moisture) in 18-cm diameter peat pots.

Except where indicated, benomyl (Benlate 50% Wettable Powder, Du Pont of Canada Ltd., Toronto) was applied as an aqueous suspension [0.28 g active ingredient (a.i.) in 177 ml/pot, 0.154 mg/g oven dry soil] with care that the chemical did not contact the leaves of

TABLE 1. Effect of benomyl on fresh weight of crucifer seedlings 4 weeks after treatment in the greenhouse

Cultivar	Average plant	Weight ratio between benomyl and		
Cultivat	Benomyl treated	Nontreated	nontreated plants	
Cabbage				
Chieftain Savoy	39.8 z ^b	32.2 z	1.25	
King Cole	40.2 z	37.4 z	1.08	
Early Greenball	32.8 z	31.6 z	1.03	
Red Rock	28.8 z	39.2 z	0.74	
Eastern Ballhead	24.4 y	35.2 z	0.69	
Cauliflower				
Idol	17.6 y	27.8 z	0.64	
Clou	10.4 y	30.0 z	0.33	
Broccoli				
Green Comet	45.0 z	40.0 z	1.12	
Spartan Early	35.4 z	34.0 z	1.03	
Brussels sprouts				
Catskill	35.0 z	28.2 z	1.25	
Jade Cross	42.6 z	49.4 z	0.88	

"Values are means of three plants per pot of five replicates. Benomyl was applied at 0.154 mg/g soil at transplanting.

TABLE 2. Effect of benomyl treatment at different plant ages on fresh weight of cabbage cultivar Eastern Ballhead seedlings in the greenhouse

Age at planting ^a (weeks)	Average plant	Weight ratio between benomyl and	
	Benomyl treated	Nontreated	nontreated plants
0 (seed)	0.15	0.41	0.36 z
-1	0.34	0.86	0.39 z
2	1.32	2.45	0.46 y
4	9.15	12.30	0.75 x
8	24.65	30.69	0.79 x

Seedling age was determined from seed.

^hAny two figures for each cultivar followed with a common letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

^hValues are means of three plants per pot of five replicates, except for the 0-week level where 15 seeds were planted per pot. Benomyl was applied at 0.154 mg/g soil at planting.

^{&#}x27;Any two figures followed with a common letter are not significantly different (P=0.05) according to Duncan's multiple range test.

TABLE 3. Fresh weight of crucifer cultivars 4 weeks after application of various concentrations of benomyl in the greenhouse

Benomyl (mg/g soil) ^b	Average plant weight (g) ^a				
	Cabbage		Brussels sprouts		Cauliflower
	King Cole	Chieftain Savoy	Catskill	Jade Cross	Clou
0.0	76.5 xy ^c	39.4 y	39.9 y	43.2 x	38.6 y
0.039				•••	38.8 y
0.077	***	***	***	***	31.1 yz
0.154	79.5 x	38.3 y	40.0 v	42.5 x	27.9 z
0.308	67.6 xy	33.0 yz	38.0 y	40.4 xy	***
0.616	58.3 yz	26.6 yz	28.0 z	32.8 yz	•••
1.232	44.7 z	20.3 z	26.2 z	24.9 z	***

"Values are means of three plants per pot of a cultivar.

^bEach of five replicate pots received benomyl in 177 ml of suspension.

TABLE 4. Seed germination and seedling growth of cabbage cultivar Eastern Ballhead treated with benomyl alone, surfactants, and in combinations in the laboratory and field

Treatment ^a	In vitro experiment		Field experiment		
	Seed germination (%)	Length of radicle (mm)	Seedling emergence (%)	Final seedling stand (%)	Seedling fresh weight (g)
Benomyl	95.2 vw ^b	16.3 y	40.1 x	39.8 x	1.9 y
Benomyl + GAFAC RA-600 Benomyl + GAFAC RS-710 Benomyl + Tween 20		0.0 z 9.7 y 16.4 y	5.7 z 14.3 yz 42.0 wx	5.7 z 12.6 yz 41.6 x	0.3 z 0.9 z 1.9 y
GAFAC RA-600 GAFAC RS-710 Tween 20	0.0 z 27.2 y 88.0 w	0.0 z 9.5 y 36.0 x	20.3 y 11.6 yz 49.1 wx	20.3 y 11.2 yz 48.7 x	1.0 z 0.7 z 3.1 x
Water	97.6 v	49.9 w	55.3 w	53.8 x	2.6 xy

"Water = tap water; benomyl = 1.58 mg/ml; Tween 20, GAFAC RA-600, or GAFAC RS-710 = (1:99, v/v). All treatments employed 177 ml suspension. Data under In Vitro Experiment were obtained 5 days after treatment and each treatment involving 25 seeds was replicated five times. Data under Field Experiment were obtained I week (emergence) and 2 weeks (final seedling stand and fresh weight) after treatment, and each treatment involving 125 seeds was replicated six times.

^bAny two figures in the column followed with a common letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

the seedlings. This treatment provided the minimum quantity needed for effective control of clubroot in the field (10).

The surfactants polyoxyethylene sorbitan monolaurate (Tween 20, Atlas Chemical Co., Ont.), and GAFACRA-600 and GAFAC RS-710 (mixtures of mono- and diphosphate esters, Chemical Developments of Canada, Ltd., Ont.) were added to the benomyl suspension in the ratio of 1:99 (v/v) when needed. Suspensions of benomyl, and mixtures of benomyl and surfactants, were vigorously stirred before use.

Greenhouse experiments.—In greenhouse tests, three seedlings were transplanted into each of five replicate pots and then drenched with benomyl suspension. Water served as the control treatment. Treatments were randomized, and plants were watered and fertilized as required. Fresh weight was recorded as a measure of phytotoxicity 4 weeks after treatment because the resultant weight of the plant was inversely related to the extent of benomyl injury.

Under these conditions the effect of benomyl was

determined on the growth of five cultivars of *Brassica oleracea* L. var. *capitata* L. (cabbage), and two each of *B. oleracea* L. var. *botrytis* L. (cauliflower), *B. oleracea* L. var. *italica* Plenck (broccoli), and *B. oleracea* L. var. *gemmifera* Zenker (brussels sprouts). Also, the effect of benomyl on growth of cabbage cultivar Eastern Ballhead 1, 2, 4, and 8 weeks of age was determined. Pots were newly seeded (15 seeds/pot). The effect of benomyl concentrations (0.039, 0.077, 0.154, 0.308, 0.616, and 1.232 mg a. i./g oven dry soil) on growth of two cultivars of cabbage and brussels sprouts, and one of cauliflower was likewise determined.

In vitro experiment.—The interaction between surfactant and benomyl on seed germination and seedling growth was tested with cabbage cultivar Eastern Ballhead on double layers of filter paper (Whatman No. 54, 9-cm diameter) in petri dishes (10×1.5 cm). The treatments were benomyl alone, benomyl with each of the surfactants GAFAC RA-600, GAFAC RS-710, and Tween 20, and each surfactant alone. Water only served as the control. Four milliliters of each suspension were added per dish to provide moisture for germination and seedling growth.

^{&#}x27;Any two figures in a column followed with a common letter are not significantly different (P = 0.05) according to Duncan's multiple range test. (...) indicates that no data were collected.

Twenty-five seeds were exposed to each treatment. Randomized treatments, replicated five times, were maintained on a laboratory bench at room temperature. Germination percentage and radicle length of seedlings were recorded on the fifth day.

Field experiment.—Direct seeding is a potential development in the field culture of crucifers, and surfactants are known to increase movement of benomyl in soil (8, 9). Hence the treatments described above under In Vitro Experiment were applied to direct-seeded cabbage cultivar Eastern Ballhead to determine the effect of benomyl with and without surfactant on seedling emergence and growth. Rows of plants of this cultivar, 15 m long and 71 cm apart, were established at Jordan, Ontario (Vineland silt loam, pH 5.9). Hills, 60 cm apart in the row, were sown (five seeds per hill) on 18 June 1973. Immediately after seeding, each hill was drenched with 177 ml of the appropriate suspension. Treatments were randomized and replicated six times. Overhead irrigation was applied as required. Seedling emergence was recorded I week after seeding, and the final seedling stand and fresh weight were recorded 1 week later.

RESULTS AND DISCUSSION.—Cultivar.—The effect of benomyl on crucifers differed with cultivar (Table 1). Benomyl treatment reduced fresh weight of cabbage cultivar Eastern Ballhead, and cauliflower cultivars Clou and Idol; the leaves were severely burned. No growth reduction occurred on cabbage cultivars Red Rock, Early Greenball, King Cole, and Chieftain Savoy or on the cultivars of broccoli and brussels sprouts. Using the technique described by Jacobsen and Williams (4), bioassay of benomyl-treated cabbage cultivar Eastern Ballhead showed that benomyl was present in the roots, stems, and leaves. More chemical was found in the leaf margins and tips than in other parts of the leaves. No benomyl was found in the nontreated plants. These results supported those of Thomas (12) and Wensley (14), respectively, who reported varying reaction to benomyl in cultivars of celery and muskmelon.

Age of seedlings.—Phytotoxicity of benomyl on cabbage cultivar Eastern Ballhead was inversely related to age of the seedlings (Table 2). Seedlings grown from seeds in benomyl-treated soil weighed only 36% as much as did those grown in nontreated soil. By contrast, 8-week-old seedlings transplanted into treated soil weighed 79% as much as did those in nontreated soil 4 weeks later. These results appear comparable to those of Schroeder and Provvidenti (11) who observed that benomyl was taken up faster by younger squash seedlings (first-leaf stage) than by older seedlings.

Chemical concentration.—The severity of stunting of plants increased with the concentration of benomyl drenched into the soil (Table 3). None of the cultivars was stunted by benomyl at rates below 0.154 mg/g soil 4 weeks after treatment. Stunting occurred only with cauliflower cultivar Clou at 0.154 mg/g soil. Both cabbage and brussels sprouts were stunted at 1.232 mg/g soil. Similar results also were observed by other workers (4, 5, 6, 11, 13, 15).

Surfactant.—The combination of GAFAC RS-710 + benomyl was more phytotoxic to cabbage cultivar Eastern Ballhead seeds and seedlings on filter paper than benomyl alone (Table 4). This effect was due largely to GAFAC RS-710 because this surfactant alone was more

phytotoxic than when added to benomyl. The effect of Tween 20+ benomyl on germination and length of radicle was similar to that by benomyl alone. GAFAC RA-600 with or without benomyl prevented germination.

The combination of benomyl + either surfactant GAFAC RA-600 or RS-710 reduced seedling emergence, final stand, and fresh weight of direct seeded cabbage cultivar Eastern Ballhead in the field more than did benomyl alone (Table 4). No such effect was observed with the combination of benomyl + Tween 20. Both GAFAC surfactants applied alone were more phytotoxic than benomyl alone.

These results may be explained by reports of other workers. Booth and Rawlins (1) observed that GAFAC RA-600, GAFAC RS-710, and Tween 20 increased the diffusion of benomyl in agar. Pitblado and Edgington (8) found that the solubility of methyl-2-benzimidazole-carbamate (the breakdown product of benomyl) was increased from 10 to 1,000 μ g/ml by GAFAC RA-600, to 840 μ g/ml by GAFAC RS-710, and to 20 μ g/ml by Tween 20.

Benomyl, although an effective fungicide for clubroot control (10), is not yet registered in Canada for use with crucifers. The present results indicate that phytotoxicity must be carefully assessed when evaluating efficacy data.

LITERATURE CITED

- BOOTH, J. A., and T. E. RAWLINS. 1970. A comparison of various surfactants as adjuvants for the fungicidal action of benomyl on Verticillium. Plant Dis. Rep. 54:741-744.
- DELP, C. J., and H. L. KLOPPING. 1968. Performance attributes of a new fungicide and mite ovicide candidate. Plant Dis. Rep. 52:95-99.
- DESAI, B. G., and C. VAN ASSCHE. 1971. The effect of benomyl on Pythium myriotylum Dreschler an incitant of damping off of tomato and pea. Meded. Rijksuniversiteit Fac. Landbouwwet. Gent 36:1178-1187.
- JACOBSEN, B. J., and P. H. WILLIAMS. 1970. Control of cabbage clubroot using benomyl fungicide. Plant Dis. Rep. 54:456-460.
- LOCKE, J. C., and R. J. GREEN, JR. 1971. Systemic activity of benomyl fungicide against Cladosporium leaf mold and Verticillium wilt of greenhouse tomatoes. Indiana Acad. Sci. Proc. 80:104-109.
- MALOY, O. C., and R. MACHTMES. 1974. Control of onion white rot by furrow and root-dip application of fungicides. Plant Dis. Rep. 58:6-9.
- ONTARIO MINISTRY OF AGRICULTURE AND FOOD. 1973. Vegetable production recommendations. Ont. Min. Agric. Food Publ. 363, 71 p.
- PITBLADO, R. E., and L. V. EDGINGTON. 1972. Movement of benomyl in field soils as influenced by acid surfactants. Phytopathology 62:513-516.
- PITBLADO, R. E., and L. V. EDGINGTON. 1972. Preventative and curative control of Cladosporium leaf mold of greenhouse tomatoes with benomyl and surfactant additives. Can. J. Plant Sci. 52:459-462.
- REYES, A. A., T. R. DAVIDSON, and C. F. MARKS. 1974. Races, pathogenicity and chemical control of Plasmodiophora brassicae in Ontario. Phytopathology 64:173-177.
- SCHROEDER, W. T., and R. PROVVIDENTI. 1968. Systemic control of powdery mildew on cucurbits with fungicide 1991 applied as soil drenches and seed treatments. Plant Dis. Rep. 52:630-632.
- THOMAS, T. H. 1973. Growth regulatory effect of three benzimidazole fungicides on the germination of celery (Apium graveolens) seeds. Ann. Appl. Biol. 74:233-238.

- VAN ASSCHE, C., and A. VANACHTER. 1970. Systemic fungicides to control fungal diseases in vegetables. Parasitica 26:117-125.
- 14. WENSLEY, R. N. 1972. Effects of benomyl and two related systemic fungicides on growth of Fusarium wilt-

susceptible and resistant muskmelon. Can. J. Plant Sci. 52:775-779.

 WENSLEY, R. N., and C. M. HUANG. 1970. Control of Fusarium wilt of muskmelon and other effects of benomyl soil drenches, Can. J. Microbiol. 16:615-620.