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Toxicity of Benlate® to Cucumber and Evidence for a Volatile Phytotoxic Decomposition Product

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Abstract. Drench applications of the fungicide Benlate DF® (dry flowable) or WP (wettable powder) in greenhouse studies resulted in stunted cucumber seedlings. Exposure of cucumber seedlings to suspension of Benlate DF or WP in sealed glass dishes, severely inhibited primary and secondary root development. Root inhibition occurred not only when seedlings were in contact with Benlate, but also when seedlings were not in contact with the fungicide, implicating the effect of a volatile toxicant(s). The presence of butyl isocyanate (BIC) in the gaseous phase was confirmed in the glass dishes containing Benlate and stunted cucumbers. Cucumber roots not in contact with Benlate suspensions (76 mg/2 ml) were severely inhibited when Benlate suspension depths were 1.7 mm or less, but virtually unaffected when depths were 3 mm or more, indicating that the evolution of volatile phytotoxicant(s) is inversely related to depths of Benlate suspensions, which further suggests that the volatile phytotoxicant reacts with water, thereby reducing the amount released. Moistened Benlate DF stored for 14 days continued to evolve a volatile substance toxic to cucumber seedling roots. No volatile phytotoxic substance from methyl thiophanate fungicides was detected.

Many ornamental and foliage plant growers in Florida and other states have implicated Benlate DF as the cause of severe plant damage. Benlate DF allegedly caused stunting, chlorosis, deformities, and other types of plant abnormalities.

In the agricultural community, Benlate has been recognized as a premier fungicide for plant disease control. Delp and Klopping (1968) found high efficacy and broad spectrum activity for benomyl or methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate, the active ingredient in Benlate formulations. Since this first report, several products, including Benlate 50WP, Tersan 1991, and Agrocit became available, and the fungicide was rapidly approved for use on many crops (Kirby 1972). Numerous studies (reviews by Erwin 1973; Kirby 1972) on efficacy, chemistry, and mode of fungicidal action, supported the immense popularity of benomyl. The principal decomposition product and fungitoxic substance from benomyl is methyl-2-benzimidazolecarbamate, known by its trivial name carbendazim, or by the acronym MBC (Erwin 1973; Kirby 1972). Several other byproducts are known, including the highly reactive and volatile butyl isocyanate or BIC.

In the 1970s, methyl thiophanate, a fungicide chemically distinct from benomyl was developed. In water, methyl thiophanate also produces MBC as the primary decomposition product, but apparently no BIC. In 1987, Benlate DF, a dry flowable formulation of benomyl was introduced by E. I. Du Pont de Nemours and Co, Inc.

In the past two decades of benomyl use, there have been a number of reports attributing plant damage to benomyl. Benomyl at the high rate of 40 lb/A caused slight marginal chlorosis of potted cucumber seedlings (Delp and Klopping 1968). Toxicity of benomyl was also recorded on cucumber (Cole *et al.* 1970; Hammett 1968; Schroeder and Provvidenti 1968), muskmelon (Wensley 1972), squash (Schroeder and Provvidenti 1968), pumpkin (Hammett 1972), cabbage (Reyes 1975; Jacobsen and Williams 1970), brussels sprouts (Reyes 1975), cauliflower (Reyes 1975), chrysanthemum (McCain 1971), *Cassia occidentalis* (Ishii 1973), and Swedish ivy (Baxter *et al.* 1975). Schreiber and Hock (1975) reported phytotoxicities to marigold, pepper, tomato, buckthorn, American elm, sycamore, and silver maple. Severe chlorosis of maranta leaves caused by benomyl was reported by Chase and Simone (1985) and repeated applications of benomyl to spathiphyllum, resulted in gray leaves which then become tan, with marginal chlorosis and necrosis (Chase 1985).

Solel (1971) reported liberation of fungitoxic vapors from benomyl for 11 days following spray applications. Hammerschlag and Sisler (1972) demonstrated the evolution of volatile BIC from moistened benomyl or a benomyl slurry, and its fungitoxicity to *Saccharomyces cerevisiae* and *Ustilago maydis*. Recently, the evolution of BIC from an aqueous suspension was also confirmed by Tang *et al.* (1992).

In our literature search, no studies were found on the toxicity of volatile decomposition products from benomyl to higher plants. This study was undertaken to determine the role, if any, of Benlate DF, WP, or their byproducts, in causing plant damage.

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Materials and Methods

Studies in greenhouses were conducted on cucumber seedlings to determine possible phytotoxic effects of several Benlate formulations and several methyl-thiophanate fungicides. *In vitro* bioassays were conducted to detect possible phytotoxic volatile products released from Benlate, determine effects of increasing amounts of Benlate on production of phytotoxic substances, monitor continuous release of these phytotoxic products, and study evolution of these products relative to depths of aqueous Benlate suspensions.

The following is a list of Benlate specimens, lot numbers where available, and status when received. Methyl thiophanate fungicides used in the study are also listed:

Benlate DF (dry flowable)		
K1:	Lot Z 10610-C	(unopened, original container)
K2:	Lot Z 10611-A	(unopened, original container)
K3-1:	U-91889 0204 Pallet 04	(unopened, original container)
K3-2:	identical to K3-1	(opened container)
K4:	U 0022190 0143	(unopened, original container)
K11:	Unknown	(opened container)
Benlate WP (wettable powder)		
K6:	unknown	(acquired in mid-1970s)
K7:	unknown	(acquired in 1988)
K8:	F 304011	(unopened, original container)
Methyl thiophanate fungicides		
K12:	Cleary 3336 50 WP, W. A. Cleary Chemical Corp., Somerset, NJ	(unopened, original container)
K15:	Fungo 50W, Grace-Sierra Horticultural Products Co, Milpitas, CA	(unopened, original container)
K16:	Topsin M 70 WP, Pennwalt Corp, Philadelphia, PA	(acquired in mid-1970s)
Methyl benzimidazole carbamate		
K14:	MBC (99.6%), E. I. Du Pont de Nemours and Co, Wilmington, DE	(acquired in 1992)

The Benlate application rate used in greenhouse tests was 1 lb/100 gal dispensed at 2 pt/ft^2 (1.2 g/L dispensed at 1.02 ml/cm^2) unless otherwise stated. Methyl thiophanate fungicides Cleary 3336 and Fungo 50 WP were applied at 1.4 g/L, and Topsin M was applied at 1.0 g/L, each dispensed at 1.02 ml/cm^2. Methyl thiophanate concentrations were adjusted to provide the theoretical yield of MBC expected from Benlate.

Cucumber was selected as the bioassay plant of ready availability, rapid seed germination, uniform and rapid growth, and easily observed symptoms which are reproducible. Greenhouse studies were conducted primarily with a bush cucumber cultivar "Spacemaster"; early tests also involved "Straight Eight," a vining cucumber cultivar. All laboratory studies were conducted with "Spacemaster."

For greenhouse studies, uniform, healthy seeds were selected, washed in running tap-water for 30 min, surface disinfested with 0.5% sodium hypochlorite for 1 min, and planted in Sunshine Blend #1, a commercial potting mix. Five days after seeding, cucumber seedlings were drenched with one of five lots of Benlate DF (K1, K2, K3-2, K4 or K5) or one of three lots of WP (K6, K7, or K8). Young cucumber was also sprayed with Benlate DF (K3-2) or WP (K6).

Tests to determine production of phytotoxic volatile substance(s) from Benlate DF (K1, K3-1, K4 or K11) and Benlate WP (K6 or K8) were conducted under laboratory conditions. Cucumber seeds were washed and surface sterilized as described above, and placed in sterilized, moistened, seed-germination pouches for 2 days. Seedling roots (including hypocotyl) were then measured to establish lengths at the time of initial exposure to potential phytotoxicants. Eight seedlings of uniform root length were transferred to a sterile, moist, 9 cm filter paper placed in a 150 × 20 mm glass Petri dish. Seventy-six mg of each Benlate specimen (*in vitro* rate throughout the study unless otherwise stated) was placed in 2 ml of sterile water in separate 60-mm Petri dishes. One of the 60-mm dishes with Benlate was placed within each 150-mm dish containing the germinated seeds. The 150-mm dish was sealed with Parafilm. There was no contact between cucumber seedlings and the Benlate suspension in the 60-mm dish. Control chambers received water only in the 60-mm dish. After 3 days, seedling root length was again measured to determine growth during the exposure period.

The response of cucumber seedlings to possible phytotoxic, volatile substance(s) produced by increasing amounts of Benlate DF (K3-1) was tested in the laboratory, using 0.32, 1.0, 3.2, 10.0, or 32 mg added to 1.5 g of sterile potting mix, and 5 ml sterile water in 60-mm Petri dishes. The 60-mm Petri dishes were placed within sealed 150-mm dishes containing eight cucumber seedlings as described above, and seedlings were measured after 3 days exposure. Control chambers received water only in the potting mix.

The duration for effective release of volatile substance(s) from moistened Benlate, phytotoxic to bioassay cucumber seedlings, was explored in laboratory tests. Benlate DF preparations (32.0 mg of K3-1 in 1.0 ml of water) in 60-mm sealed Petri dishes were stored at 24°C for 0, 4, 7, 11, and 14 days prior to cucumber seedling exposure. Each of the stored Benlate preparations or a water control was placed in a 150-mm dish containing cucumber seedlings as described above, and sealed with Parafilm. After 3 days exposure, seedlings were examined for symptoms of phytotoxicity, and roots were measured.

The effect of suspension depth on the release of volatile phytotoxic substances from Benlate was examined in a cucumber bioassay test in the laboratory. Freshly prepared Benlate DF suspensions (76 mg of K3-1 in 2 ml of water) were placed in glass cylinders of different diameters, resulting in suspension depths of 0.8, 1.0, 1.7, 3.0, 5.0, 9.5, and 17.4 mm. Each cylinder was placed in a 95 (diam) × 75 mm glass storage dish which contained six 2-day-old cucumber seedlings on sterile moist filter paper. Storage dishes were sealed with Parafilm. After 3 days exposure, seedlings were evaluated for phytotoxic symptoms and root length.

Benomyl breaks down to methyl benzimiadzole carbamate (MBC) which is the principal fungitoxic byproduct (Erwin 1973, Kirby 1972, Tang *et al.* 1992). Similarly, methyl thiophanate also decomposes to form MBC (Kirby 1972). To investigate the possibility that MBC is phytotoxic, cucumber seedlings, prepared as above *in vitro*, were placed in direct contact with methyl thiophanate fungicides (K12, K15, or K16), and MBC (K14). Responses were compared to Benlate DF (K3-1) and an untreated control. To investigate the possibility of phytotoxic volatile decomposition products from methyl thiophanate or MBC, the *in vitro* methods utilized for Benlate were employed. All tests were repeated at least once.

Results and Discussion

In greenhouse studies, bioassay cucumber plants treated with Benlate DF or WP were stunted 3 days following application. Six days after treatment, stem length of the DF treatments was reduced by 21%, whereas in the WP treatments, the reduction was approximately 50% (Table 1). Benlate-treated plants produced cotyledons or leaves which turned downwards, were chlorotic or bleached, and exhibited various degrees of marginal necrosis. Results were less consistent when Benlate was applied as a foliar spray on cucumber seedlings, where necrosis of cotyledons and leaf margins was the most severe symptom.

In sealed glass dish studies, hypocotyls and cotyledons of cucumber with no direct contact with Benlate suspensions were

Table 1. Effect of Benlate formulations on greenhouse cucumber[a] seedlings

Treatment	Test 1 seedling ht (mm)	Test 2 seedling ht (mm)
Benlate DF		
K1	97 ± 13[b]	76 ± 10
K2	101 ± 10	86 ± 17
K3-2	103 ± 10	79 ± 16
K4	110 ± 12	71 ± 10
K5	113 ± 11	77 ± 13
Benlate WP		
K6	—	41 ± 7
K7	66 ± 11	34 ± 4[c]
K8	93 ± 15	41 ± 6[c]
Control		
Untreated	137 ± 14	96 ± 15

[a] Test 1: cultivar "Straight Eight"
Test 2: cultivar "Spacemaster"
[b] Mean ± SD, n = 20 unless otherwise noted
[c] Mean ± SD, n = 19

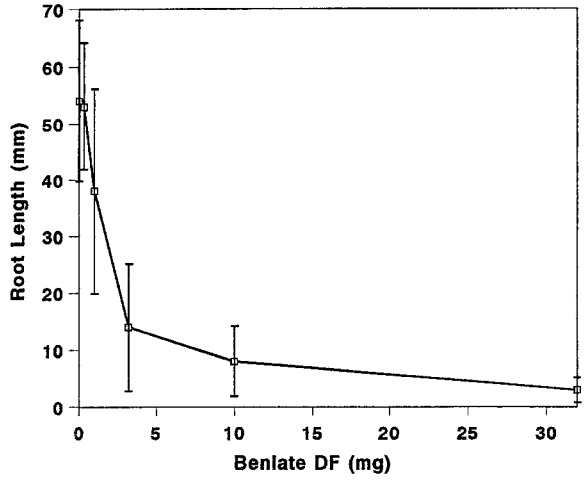

Fig. 1. Cucumber root-elongation response to Benlate DF (K3-1) dosage. Data points represent means ± SD, n = 24

Table 2. Inhibitory effects of stored, moistened Benlate DF preparations on cucumber[a] root growth

No. of days Benlate DF stored in water (76 mg/2 ml H_2O)	Test 1 root length (mm)	Test 2 root length (mm)
0	14 ± 7[b]	13 ± 7
4	16 ± 6	17 ± 8
7	14 ± 13	10 ± 4
11	16 ± 11	15 ± 11
14	15 ± 7	27 ± 14
Control		
Untreated	61 ± 7	61 ± 4

[a] "Spacemaster"
[b] Mean ± SD, n = 8

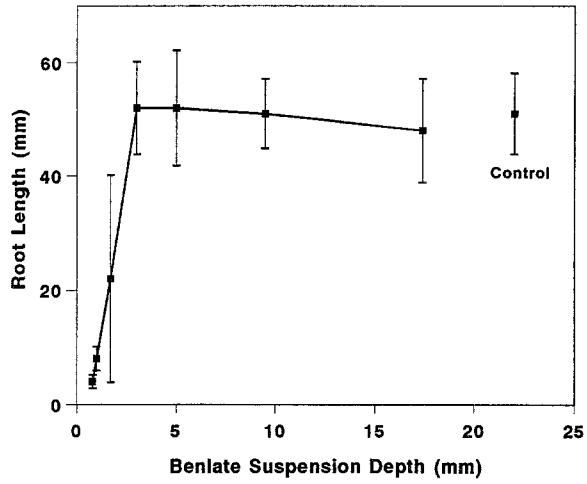

Fig. 2. Cucumber root-elongation response to suspension depths of Benlate DF; 76 mg of K3-1 and 2 ml of water in each cylinder. Data points represent means ± SD, n = 8

reduced in size, but without noticeable necrosis. The principal adverse effects were severely stunted primary and poorly developed lateral roots and drastically reduced numbers of lateral roots. Root growth inhibition of approximately 90% was observed in laboratory tests of six different lots of Benlate DF or WP formulations, irrespective of formulation. These results implicate benomyl as the potential source of plant damage that can be caused by either DF or WP formulations.

Increasing rates of Benlate in sealed glass dishes also demonstrated the evolution of phytotoxic, volatile compound(s). Cucumber root development decreased as the amount of Benlate (Figure 1) increased. Root growth was reduced by 30% with 1.0 mg Benlate DF and by 75% with 3.2 mg.

Studies with moistened Benlate stored for varying periods demonstrated that volatile toxic substance(s) continued to be produced for up to 14 days (Table 2). Root development was reduced by 77% with freshly prepared Benlate DF, and from 56% to 75% with 14-day-old Benlate suspensions. The continued release of volatile substances at phytotoxic concentrations for up to 14 days from moistened Benlate suggests a potential for plant damage for an extended period; furthermore, since the Benlate label allows for reapplication after 14 days, residue accumulation with increasing toxic effects can develop.

Seedlings placed beside Benlate suspensions in glass cylinders of different diameters revealed significant effects of suspension depths. Cucumber roots were severely restricted when suspension depths were 1.7 mm or less, indicating release of a phytotoxic substance. In contrast, Benlate suspension depths of 3.0 mm or more, did not have any effect on cucumber roots (Figure 2), reflecting minimal release of volatile phytotoxicants. This is in concert with the known reactivity of BIC and its reaction with water (Hammerschlag and Sisler 1972; Tang 1992). These results also explain the need for N_2 purging to facilitate the release of BIC from an aqueous medium. Thus, by increasing the depth of the water layer, reaction with water is increased and BIC release is reduced.

Methyl-benzimidazole carbamate (K14) and methyl thiophanate fungicides (K12 and K16), applied directly to cucumber roots did not inhibit growth (Table 3); however, methyl thiophanate fungicide (K15) caused 83% stunting, compared to

Table 3. Effect of benzimidazole fungicides in contact with cucumber[a] seedlings

Treatment	Test 1 Root length (mm)	Test 2 Root length (mm)
Benlate DF		
K3-1	1 ± 1[b]	1 ± 1
Methyl thiophanate		
K12	64 ± 12	65 ± 15
K15	13 ± 22	12 ± 22
K16	54 ± 8	48 ± 12
MBC		
K14	65 ± 6	62 ± 4
Control		
Untreated	68 ± 8	71 ± 10

[a] "Spacemaster"
[b] Mean ± SD, n = 8

Table 4. Effect of volatile phytotoxic substance(s) from benzimidazole fungicides on cucumber[a] seedlings

Treatment	Test 1 root length (mm)	Test 2 root length (mm)
Benlate DF		
K3-1	2 ± 1[b]	2 ± 3
Methyl thiophanate		
K12	77 ± 7	77 ± 7
K15	78 ± 8	71 ± 12
K16	79 ± 8	80 ± 7
MBC		
K14	73 ± 8	72 ± 12
Control		
Untreated	78 ± 9	71 ± 11

[a] "Spacemaster"
[b] Mean ± SD, n = 8

98% stunting caused by Benlate DF. In sealed dishes containing methyl thiophanate fungicides, none of the thiophanate treated cucumber seedlings showed signs of being affected, in contrast to seedlings exposed to Benlate DF (Table 4). It is therefore concluded that, at these levels, methyl thiophanate and MBC are not phytotoxic and, furthermore, do not generate phytotoxic, volatile, decomposition products based on cucumber bioassay. The stunting induced by K15 in direct contact with cucumber seedlings must be caused by a unique ingredient(s) in the formulation of this particular fungicide, since other data eliminate methyl thiophanate or MBC as the cause.

Air samples collected from sealed glass dishes containing moistened Benlate DF and root-damaged seedlings were confirmed to have BIC by gas chromatography-mass spectrometry (Tang, unpublished data, 1992) using previously described methods (Tang *et al.* 1992).

In summary, phytotoxic symptoms on greenhouse cucumber were readily and consistently reproduced by drenches with either Benlate DF or WP. On seedlings, these symptoms included stunting, limited root development, chlorosis or bleaching, leaf margin necrosis, and a downward curling of cotyledons. Phytotoxic symptoms were also repeatedly demonstrated *in vitro* on cucumber seedlings exposed to volatile products generated by moistened Benlate DF or WP. Cucumber damage increased as the amounts of Benlate increased in tests designed to detect volatile phytotoxicant(s). Evidence was also found for moderately long-term production of a volatile, phytotoxic substance(s). It was further demonstrated that production and/or release of such phytotoxic substance(s) were reduced by Benlate suspension depth. No evidence was seen that methyl thiophanate fungicides or MBC were phytotoxic to cucumber. This is the first study which documents damage to a higher plant caused by a volatile, toxic product, formed from the decomposition of Benlate in water.

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