

Phytotoxic Effects of Benzimidazole Fungicides on Bedding Plants

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Abstract. Benzimidazoles are effective and widely used fungicides, but they may be phytotoxic. We studied the effects of a single drench application of six benzimidazoles and one acetanilide fungicide on photosynthetic gas exchange, growth, development, and nutrient levels of four species of bedding plants in twenty growth-chamber and four greenhouse studies. Daily carbon gain and carbon-use efficiency were calculated from continuous crop gas-exchange measurements in the growth chambers. The maximum labeled rate of Benlate DF caused a 7- to 10-day decrease in net photosynthesis and daily carbon gain in transplants of all species. It also caused pronounced interveinal chlorosis and a 2- to 3-day delay in flowering. Growth of Benlate DF-treated plants was reduced more at high (90%) than at low (60% to 80%) relative humidity. Benlate DF had severe effects on 2-week-old petunia (*Petunia ×hybrida*) seedlings in plug flats, reducing photosynthesis 25% to 57%. Cleary's 3336 WP decreased photosynthesis in some trials. Benlate DF reduced photosynthesis within 24 hours, but 3336 WP effects did not become apparent until 1 week after the treatment. This suggests different modes of inhibition. 3336 WP also caused leaf-tip and marginal chlorosis in impatiens (*Impatiens wallerana*). Mertect 340-F was extremely phytotoxic but is not labeled for drench applications (it was included because of its chemical similarity to other benzimidazoles). The only benzimidazole fungicide that did not reduce photosynthesis was Derosal, but it caused slight interveinal chlorosis in some studies with petunia. Benlate DF and Derosal decreased leaf Ca levels. Subdue (or metalaxyl), an acetanilide fungicide, did not affect photosynthesis or cause any visual symptoms. Our results indicate that some benzimidazole fungicides can cause growth reductions and visual damage in bedding plants.

Benzimidazoles and thiophanates are a class of systemic fungicides that control a wide range of fungi at relatively low doses (Delp, 1987). Benomyl, the active ingredient in Benlate WP and Benlate DF (Du Pont) is especially effective because it penetrates plants better than carbendazim (MBC; methyl 2-benzimidazolecarbamate), its fungitoxic breakdown product (Upham and Delp, 1973). However, since the introduction of benomyl (Delp and Klopping, 1968), there have been many reports about side effects on plants. Carbendazim delays senescence in wheat (*Triticum aestivum* L.) (Tripathi et al., 1982), while benomyl also has cytokinin-like activity in soybean (*Glycine Max* (L.) Merr.) callus, radish (*Raphanus sativus* L.) cotyledons, (Skene, 1972), celery germination (*Apium graveolens* L.), and betacyanin production in *Amaranthus* (Thomas, 1974).

Benomyl can also be phytotoxic. Benomyl reduced the growth of cucumber (*Cucumis sativus* L.), loblolly pine (*Pinus taeda* L.), lettuce (*Lactuca sativa* L.), American elm (*Ulmus americana* L.), marigold (*Tagetes* sp.), and sycamore (*Platanus occidentalis* L.) (Rouchaud et al., 1985; Schreiber and Hock, 1975; Stumpff and South, 1991; Woo and Wick, 1995). Not all species seem to be equally sensitive to benomyl. Corn (*Zea mays* L.) and pea (*Pisum sativum* L.) were not affected, while turfgrass growth was stimulated by benomyl in the soil (Schreiber and Hock, 1975). Benomyl

caused veinal discoloration in Swedish ivy (*Plectranthus australis*) (Baxter et al., 1975), while tomato (*Lycopersicon esculentum* Mill.) seedlings developed chlorosis and stunting at higher rates (Mihuta-Grimm et al., 1990).

Benomyl breaks down to MBC and BIC (*n*-butyl isocyanate) (Tang et al., 1993). BIC can subsequently react to produce *n*-butylamine or DBU (*N,N'*-dibutylurea), and DBU concentrations of up to 8.85% have been found in unopened Benlate boxes (Moye et al., 1994). Dibutylurea reduces growth and inhibits photosynthesis of hydrilla (*Hydrilla verticillata* L. f. Royle) (Shilling et al., 1994) and may be partly responsible for benomyl effects on plant growth.

In the late 1980s, many commercial greenhouse and foliage growers reported crop injury after the use of Benlate DF. These claims have renewed research interest into the possible phytotoxic effects of benomyl. Although there is ample evidence that Benlate can reduce plant growth, it is not clear what physiological processes are affected. To understand the effects of benomyl on plants, we conducted a series of studies to quantify the effects of Benlate DF and related fungicides on gas exchange, growth, development, and nutrient levels of different crops.

Materials and Methods

Plant material. Impatiens (*Impatiens wallerana*, 'Accent Lilac'), cucumber ('Dasher 11'), celosia (*Celosia plumosa*, Kimono mix), and petunia (*Petunia ×hybrida*, several cultivars) were used to study the possible phytotoxicity of fungicides. Seeds were planted in soilless media [peat: perlite, 1:1 (v/v) with added limestone] or seedlings in plug flats were obtained from commercial growers. Seedlings were transplanted into black plastic flats with soilless media and grown in the greenhouse or growth chamber. None of the plants was exposed to any pesticides before the fungicide treatments.

Fungicides. An overview of the fungicides used in this research is given in Table 1. All Benlate lots were analyzed for DBU content at the Pesticide Research Laboratory of the Univ. of Florida,

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Table 1. The active ingredient, chemical name, and dibutylurea (DBU) content of the fungicides used in these studies.

Trade name	Active ingredient ^c	% DBU
Benlate DF, lot 311	Benomyl (50%)	0.36
Benlate DF, lot 193	Benomyl (50%)	0.41
Benlate DF, lot 688	Benomyl (50%)	0.28
Benlate DF, lot AG29098048	Benomyl (50%)	--- ^y
Benlate WP	Benomyl (50%)	0.42
Derosal	Carbendazim (59.4%)	---
Mertect 340-F	Thiabendazole (42%)	---
Cleary's 3336 WP	Thiophanate methyl (50%)	---
Banrot	Thiophanate methyl (25%) and etridiazol (15%)	---
Subdue	Metalaxyl (25.1%)	---

^aBenomyl = methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, carbendazim = methyl 2-benzimidazolecarbamate, thiabendazole = 2-(4-thiazolyl) benzimidazole, thiophanate methyl = dimethyl 4-4'-o-phenylenebis-3-thioallophanate, etridiazol = 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, metalaxyl = *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester.

^yNot analyzed for DBU; lot AG29098048 was used only in petunia study 6.

following their standard procedures for DBU analysis (Moye et al., 1994). All fungicides, except Derosal and Mertect 340-F, are labeled for drenches of bedding plants. Derosal and Mertect 340-F were used because of the chemical similarity of their active ingredient to benomyl. Benlate DF and WP were used at 12 g·m⁻² (1.2 g·L⁻¹, 10 L·m⁻²; or 0.01 lb/gal, 2 pints/ft), the maximum labeled drench rate for ornamentals and bedding plants. At this rate, 6.0 g or 0.022 mol of benomyl was applied per square meter. Mertect 340-F was used at a rate of 14.3 g·m⁻² (6.0 g or 0.030 mol of active ingredient/m²). The fungicide 3336 WP was initially used at 13.7 g·m⁻² (6 g a.i., 0.020 mol·m⁻²), but later at 14.9 g·m⁻² (0.022 mol·m⁻²), because the activity is more closely related to the molar amount of chemical than to the mass. Derosal was always applied at the same molar amount of active ingredient as Benlate DF, 6.7 g·m⁻² (0.022 mol·m⁻²). Banrot, a mixture of atriadiazole and thiophanate fungicides, was used at 10.4 g·m⁻². Subdue, an acetanilide, was included in our studies because it is commonly used in conjunction with thiophanates. It was used at the maximum labeled rate of 0.2 mL·m⁻² for seedlings and 0.82 mL·m⁻² for transplants. Fungicide applications to the plants were made only once during an experiment.

Since Benlate DF contains 10% (w/w) sucrose and 27.6% insoluble starch, which can be metabolized by plants and microorganisms, equivalent amounts of sucrose and insoluble starch were added to the control treatments. Sucrose and starch were not added to any of the fungicide treatments. Calculations and gas-exchange measurements indicated that metabolism of the sugar and starch was <3% of plant metabolism and lasted for about 24 h. All fungicides were received in unopened containers and opened in our laboratory. Samples of the Benlate lots were placed in sealed, desiccated storage at 4 °C to prevent chemical changes.

Plugs (4.4 cm³ root-zone volume) were drenched 20 to 25 d after seeding, while transplants (50 to 730 cm³ root-zone volume) were drenched 30 to 40 d after seeding.

Studies on cultural practices. The interaction between cultural practices and fungicide phytotoxicity was studied in growth chambers where environmental conditions could be controlled. A summary of the studies and environmental conditions is given in Table 2. Different lots of Benlate DF were compared in studies 1, 2, 3, 5, and 10 with cucumber and petunia. Different fungicides were compared in studies 4, 6–9, 11–13, and 16–20. These studies included petunia, cucumber, celosia, and impatiens.

Although Benlate DF does not have separate label rates for plugs and transplants, growers are likely to use lower rates on plugs; different rates of Benlate DF were compared on petunia plugs in study 1–3.

Since developmental stage of the plants may affect their susceptibility to fungicides, we compared the effects of fungicides on petunia plugs to those on transplants (studies 1–4 vs. 10–17).

Container size greatly influences how much fungicide is applied per plant, and different pot sizes were compared in studies 5–9 for cucumber and studies 10–17 for petunia.

Study 14–15 were used to determine whether relative humidity affects Benlate DF phytotoxicity to petunia, while studies 5–17 were used to determine possible interactions between temperature and phytotoxicity of fungicides.

Greenhouse studies were conducted to compare the phytotoxicity of different fungicides under conditions that more closely resemble the gradual temperature and light changes in commercial greenhouses.

Growth chamber gas exchange measurements. Continuous measurements of crop gas exchange were made with an open system, based on the principles reviewed by Bugbee (1992). Sealed, transparent chambers (DuPont Lucite; 0.47 m long × 0.36 m wide × 0.61 m high, 101 L) were placed in a growth chamber, and 12 to 60 transplants or 350 seedlings were enclosed in each chamber. Studies 1–16 and 18–20 were conducted in a three-chamber system, which included two treatments and a control. Study 17 was conducted in a ten-chamber system, which included four fungicide treatments and a control, each with two replications. Gas exchange in each chamber was measured once every 450 s, for a 15-s period. Daily averages of net photosynthesis during the light period (P_{net}) and respiration during the dark period (R_{dark}) were calculated from these data.

Daily carbon gain (DCG, mol·m⁻²·d⁻¹), gross photosynthesis (P_g , μmol·m⁻²·s⁻¹), and carbon use efficiency (CUE, dimensionless), the ratio of C stored in biomass to total C fixed in photosynthesis (Yamaguchi, 1978; Amthor, 1989), were determined from the gas exchange data:

$$DCG = (LP \times P_{net} + DP \times R_{dark}) \times 10^{-6} \quad [1]$$

$$P_g = P_{net} - R_{dark} \quad [2]$$

$$CUE = DCG / (LP \times P_g \times 10^{-6}) \quad [3]$$

where LP = light period (s), and DP = dark period (s).

Cumulative carbon gain (CCG, mol·m⁻²) was calculated as the integral of DCG over time and is especially useful since it is directly proportional to dry mass (DM) increase. In these equations, it is assumed that R_{dark} and the dark respiration during the light period were equal.

Temperature, monitored with shielded, aspirated thermocouples,

Table 2. Treatments, temperature, and number of plants per chamber in growth chamber trials. Benlate DF lot 311 was used unless otherwise indicated. All studies included at least one control treatment.

Species	Study no.	Treatment	Air temp °C (day/night)	Plant no.	Container vol (cm ³ /plant)	
<i>Studies with plug flats</i>						
Petunia	1	Benlate DF lots 311 and AG29098048	30/25	350	4.4	
	2	Benlate DF (0.5×) lots 311, 213	30/25	350	4.4	
	3	Benlate DF lots 311, 193	30/25	350	4.4	
	4	Subdue Banrot	30/25	350	4.4	
<i>Studies with transplants</i>						
Cucumber	5	Benlate DF lots 311, 193	35/30	60	50	
	6	Benlate DF Subdue	35/30	60	50	
	7	Benlate DF	35/30	12	730	
	8	Benlate DF	30/27	36	170	
	9	Benlate WP Benlate DF Mertect 340F	31/25	36	170	
	Petunia	10	Benlate DF lots 311, 193	30/25	36	170
		11	Benlate DF	35/30	36	170
		12	Benlate DF	30/25	60	50
		13	3336 WP	30/25	60	50
14		Benlate DF (80% and 90% RH)	30/25	60	50	
15		Benlate DF (60% and 85% RH)	33/28	60	50	
16		Benlate DF 3336 WP	25/20	60	50	
17		Benlate DF Derosal Subdue 3336 WP	29/23	60	50	
Celosia	18	Benlate DF 3336 WP	35/31	60	50	
	19	Benlate DF 3336 WP	35/30	60	50	
Impatiens	20	Benlate DF 3336 WP	32/26	60	50	

was maintained at 25 to 35 °C day and 20 to 31 °C night, depending on the study (Table 2). Relative humidity, measured with capacitance-type sensors (Vaisala Sensor Systems, model HMP 35A), was typically 70% to 80%. Photosynthetic photon flux at the canopy level was between 300 and 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ depending on the study, except for experiment 17 with petunia, when light intensity was 780 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Photoperiod was either 14 or 16 h.

Gas-exchange system performance and accuracy were determined by reacting a known amount of NaHCO_3 with acid and measuring the evolved CO_2 . The CO_2 recovery of the system was $102\% \pm 6\%$ (mean \pm standard deviation).

Greenhouse experiments. A system with 10 identical chambers was used in a greenhouse. The incoming air was enriched with CO_2 to keep the CO_2 concentration in the chambers at $400 \pm 20 \mu\text{mol}\cdot\text{mol}^{-1}$. Flow rates were adjusted to maintain similar CO_2 concentrations in all chambers. Samples of pre- and postchamber air were taken with 5-mL syringes. The CO_2 concentrations were determined by injecting a 4-mL sample into the CO_2 -free air

stream to the sample cell of an infrared gas analyzer (IRGA) (model 225; ADC), connected to a chart recorder. Peak height was used to determine CO_2 concentrations. A similar technique has been described in detail by Saltveit and Strike (1989). The reference cell of the analyzer was continuously supplied with CO_2 -free air. The IRGA was calibrated by injecting 4 mL of a standard gas into the air stream to the reference cell. Net photosynthesis was calculated as explained above. DCG, CCG, and CUE calculations were not possible in the greenhouse, because continuous measurements are needed for these computations. Temperature inside the chambers was maintained between 22 and 35 °C, with the relative humidity between 70% and 95%. Total daily photon flux was between 25 and 40 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Three studies with impatiens and one study with petunia were conducted in the greenhouse.

Whole-plant measurements. Shoot DM was determined at the end of the experiments (11 to 24 d after treatment), after drying the plant material at 80 °C to a constant mass. Total N in shoots was determined with a CHN-analyzer (model CHN-1000; LECO).

Table 3. Growth chamber trials. The effect of different fungicides on the cumulative carbon gain (CCG), shoot dry mass (DM), and net photosynthesis (P_{net}) at 1, 2, or 7 d after drench. Data are expressed as a percentage of untreated control plants \pm standard error among studies. Plug flat trials are not included in these means. Note the good correlation between CCG and shoot DM in all but the Benlate treatments. Benlate-treated plants probably had smaller root systems, causing a discrepancy between CCG and shoot DM (see text).

Crop	Fungicide	n	Cumulative carbon gain	Shoot DM	P_{net}		
					Day 1	Day 2	Day 7
<i>Percent of control plants</i>							
Celosia	Benlate DF 311	2	63.0 \pm 19.6	---	42.0 \pm 1.8	67.4 \pm 3.8	75.5 \pm 9.3
	3336 WP	2	81.8 \pm 1.8	---	81.6 \pm 11.5	81.8 \pm 0.1	79.5 \pm 0.6
Cucumber	Benlate DF 311	5	80.1 \pm 3.1	---	72.5 \pm 2.4	83.1 \pm 4.1	93.1 \pm 6.5
	Benlate DF 193	1	68.5	---	57.1	69.2	87.5
	Benlate WP	2	76.2 \pm 2.5	---	79.0 \pm 8.1	83.8 \pm 1.1	83.0 \pm 2.1
	Mertect 340-F	1	-5.3	---	-1.0	-2.2	-5.8
	Subdue	1	106.9	---	107.9	108.9	102.3
Impatiens	Benlate DF 311	1	79.0	100.1	25.5	34.7	92.4
	3336 WP	1	68.0	89.7	86.1	78.2	56.7
Petunia	Benlate DF 311	11	83.5 \pm 2.8	103.2 \pm 2.6 ^y	77.2 \pm 3.9	75.6 \pm 2.1	89.1 \pm 3.4
	Benlate DF 193	1	88.4	86.1	63.7	80.0	102.9
	Benlate WP	1	75.5	78.5	61.7	76.3	62.3
	Derosal	2	110.0 \pm 2.1	110.4 \pm 0.9	103.4 \pm 2.3	106.4 \pm 1.1	110.2 \pm 2.5
	3336 WP	5	96.3 \pm 4.1	97.9 \pm 4.1 ^x	101.9 \pm 3.5	100.7 \pm 4.2	94.4 \pm 5.5
	Subdue	2	102.8 \pm 0.8	100.6 \pm 3.5	102.0 \pm 0.6	99.4 \pm 0.8	103.0 \pm 1.6

²Data not collected.

^yMean of 10 measurements.

^xMean of four measurements.

Nitrate was analyzed using $\text{Ca}(\text{OH})_2$ extractant and chromotropic acid color development. Color intensity was measured with a spectrophotometer (Spectronic 1001; Bausch & Lomb). Reduced N was calculated as the difference between total N and nitrate. Calcium, K, P, and S were measured with an inductively-coupled plasma spectrometer (ICAP 9000; Thermo-Jarrel Ash).

Results

Gas exchange. Table 3 summarizes the results from the 20 growth chamber studies, while data from the most comprehensive study (study 17 with petunia) are shown in Figs. 1 and 2. Treatment effects in study 17 were less than average (compare Figs. 1 and 2 to Table 3). Benlate DF drenches typically resulted in an initial decrease in P_{net} (Fig. 1, Table 3). This effect was generally greatest at 1 to 2 d after the treatment, after which the plants slowly recovered (Table 3). Results were similar in the greenhouse experiments (Fig. 3). In addition to reducing net photosynthesis, Benlate DF also reduced CUE for a 3-d period after the drench. Derosal, 3336 WP, and Subdue slightly increased CUE (Fig. 2 A and B). The combination of reduced photosynthesis and CUE by Benlate DF resulted in a decrease in DCG for a period of 7 to 10 d after the drench application (Fig. 2 C and D). Because of the initial reduction in DCG, CCG never recovered to the control level (Fig. 2 E and F, Table 3). Benlate DF and Benlate WP responses were similar during the first few days after treatment, but Benlate WP drenches resulted in a larger decrease in P_{net} 7 d after treatment (Table 3, Fig 3). Higher relative humidity (85% to 90%) caused a larger decrease in CCG (25%) than the treatment at lower humidity (60% to 80%), which caused a 13% decrease CCG. Temperature did not appear to have an important influence on the phytotoxicity of Benlate DF.

Benlate DF was more phytotoxic to petunia seedlings in plug flats (4.4 cm^3 root-zone volume) than to older, transplanted plants (50 cm^3 root-zone volume). Net photosynthesis of petunia seedlings 24 h after the drench was reduced by 57% in petunia study 1

and by 25% to 35% in petunia study 3 (Fig. 4). Petunia study 2 was conducted at half the maximum label rate (5 $\text{L}\cdot\text{m}^{-2}$ or 1 pt/ft^2) and both Benlate DF lots decreased net photosynthesis by 18%, which was about half of the decrease observed at the 1 \times drench rate. The photosynthetic rate of Benlate treated plug flats had not recovered at the end of any of the studies (Fig. 4), although the plants produced normal leaves in the greenhouse about 20 d after the Benlate DF application. Banrot and Subdue did not significantly affect petunia plugs, with average photosynthetic rates of 91% and 110% of the control, respectively (petunia study 4). These differences in photosynthesis were the result of small differences in initial plant size; photosynthetic rate before and after the fungicide

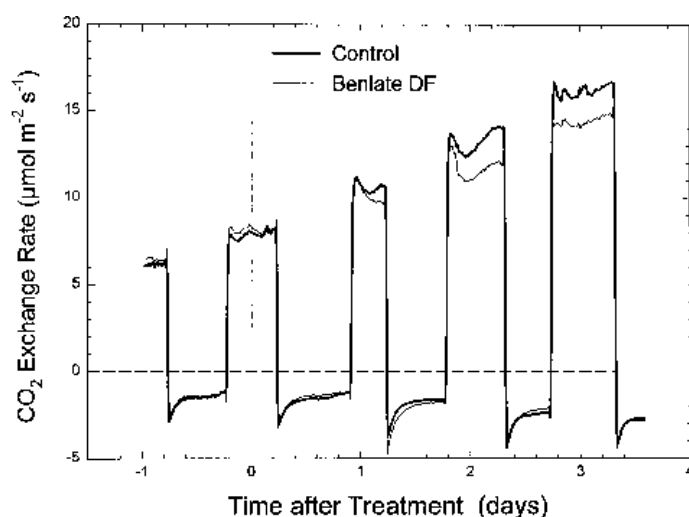


Fig. 1. An example of the time course for the effect of Benlate DF on net photosynthesis and dark respiration of petunia (study 17). Plants were treated with the maximum labeled drench rate of Benlate DF (12 $\text{g}\cdot\text{m}^{-2}$). Data represent the mean of two gas-exchange chambers with 60 plants in each chamber. Benlate DF effects on gas exchange were smaller than those in comparable studies.

drench was changed by <4% in both treatments. Increased rooting volume appeared to aggravate Benlate DF damage in cucumber. Benlate DF reduced CCG by 11%, 16%, and 26% with rooting volumes of 50, 170 and 730 cm³, respectively. We did not see a similar correlation with transplanted petunia, where Benlate DF reduced CCG by about 16%, regardless of rooting volume.

3336 WP decreased net photosynthesis of celosia and impatiens but had little effect on petunia (Table 3). The effect was different from that of Benlate DF. The initial decrease in net photosynthesis was usually small but became more pronounced over time (Table 3 and Fig. 3). In experiments where 3336 WP decreased P_{net}, the plants did not recover within 2 weeks after the drench. This suggests that the mode of photosynthesis inhibition is different for 3336 WP and Benlate DF.

Mertect 340-F was extremely phytotoxic to cucumber, but is not labeled for a drench treatment. Gross photosynthesis was less than the respiration rate, resulting in a negative P_{net} (Table 3). In preliminary studies, Mertect 340-F had similar effects on petunia and impatiens and was therefore not included in other studies. Subdue did not significantly affect the photosynthetic rate of transplanted petunia or cucumber, while Derosal had either no or a small stimulating effect on P_{net} of petunia.

Fungicide treatments altered CCG of all species examined. Mertect 340-F, Benlate WP, and Benlate DF consistently decreased CCG. 3336 WP decreased CCG of celosia and impatiens but did not affect CCG of petunia, while Derosal and Subdue slightly increased CCG of petunia. Differences in CCG were

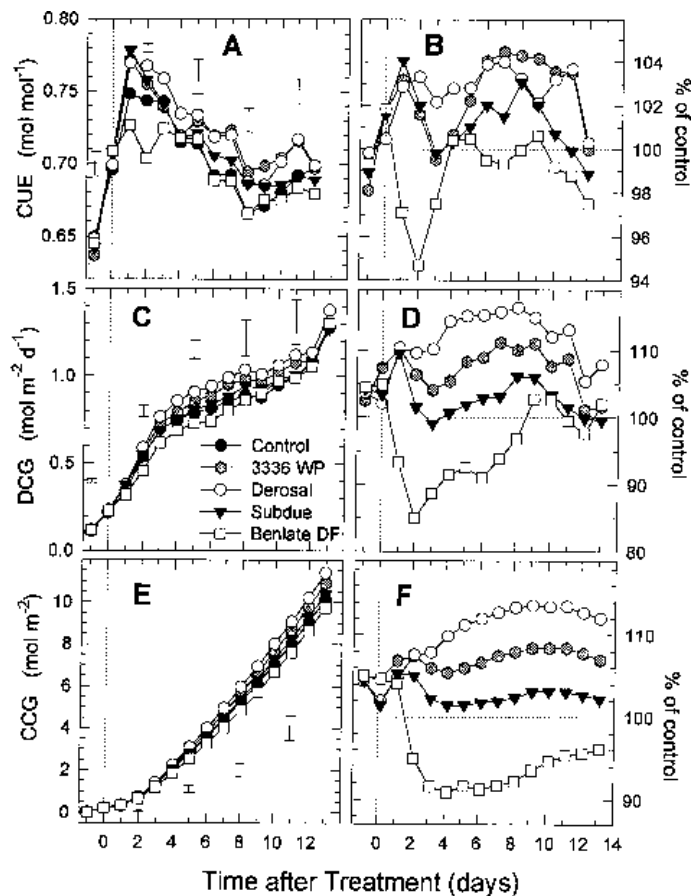


Fig. 2. Carbon use efficiency (CUE), daily carbon gain (DCG), and cumulative carbon gain (CCG) of petunia (study 17) as affected by four fungicides. Data represent the mean of two gas-exchange chambers with 60 plants each. Both absolute values (A, C, E) and data expressed as a percentage of the control plants (B, D, F) are shown. Error bars represent selected LSD_{0.05}s.

reflected in shoot DM accumulation, except when Benlate DF was used (Table 3; see discussion).

Although CCG calculations were not possible in the greenhouse studies, there was a correlation between 3336 WP effects on photosynthesis and shoot DM. 3336-WP treated plants had lower shoot DM in two out of three impatiens studies. The first study (Fig. 3A) was probably too short to detect any effects of 3336 WP on photosynthesis or shoot DM of impatiens. As in the growth chamber studies, Benlate decreased photosynthesis in the greenhouse (Fig. 3), but these differences were generally not reflected in shoot DM (Table 4).

Nutrient composition. Fungicide effects on the concentration of nutrients in study 17 are shown in Table 5. Benlate DF increased the total concentration of N in the plants but not the concentration of reduced N. Differences in N levels were caused by nitrate concentrations in the plants. Calcium levels were reduced by Benlate DF and Derosal but not by the similar fungicide 3336 WP. Benlate DF reduced Ca levels in five out of six experiments where nutrients were analyzed, while Derosal decreased Ca in three out

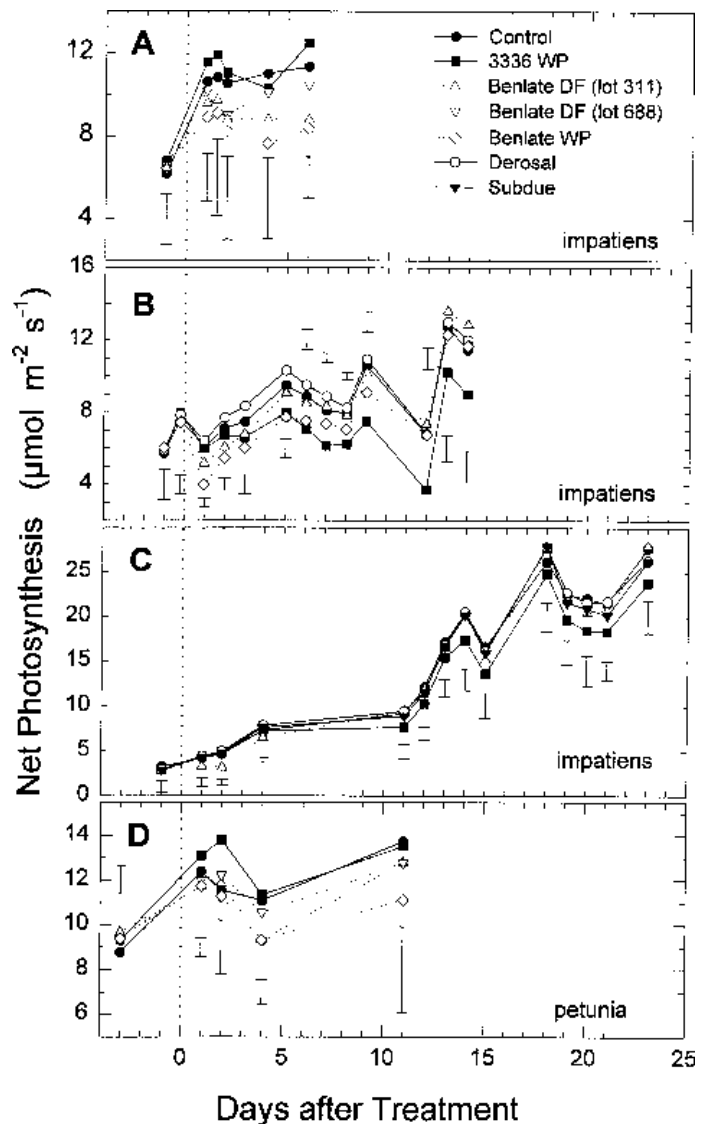


Fig. 3. The effect of different fungicides on the net photosynthesis of impatiens (A-C) and petunia (D) under greenhouse conditions. Data represent the mean of two gas-exchange chambers with 60 plants each. Error bars represent LSD_{0.05}. Note: only five of the seven treatments in the legend (A) were used in each individual study.

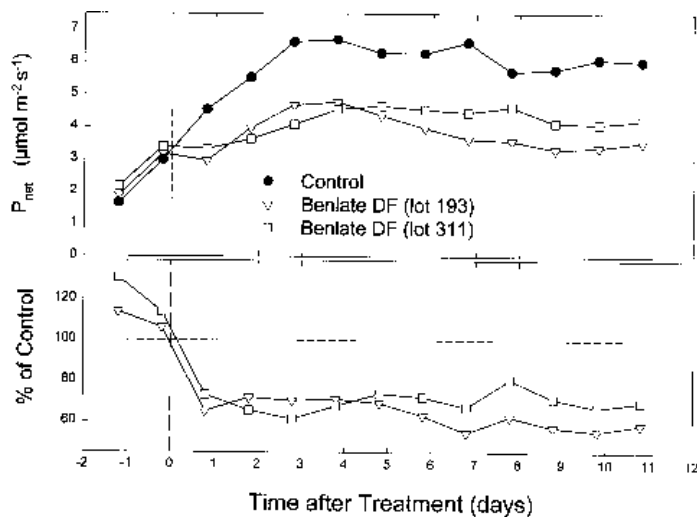


Fig. 4. The effect of two different Benlate DF lots on net photosynthesis of petunia seedlings (study 3). Plants were drenched with a 1× rate (10 L·m⁻² of fungicide solution with 1.2 g·L⁻¹ of Benlate DF). Data were collected in gas-exchange chambers with 350 plants/chamber. Both absolute values (A) and data expressed as a percentage of the control plants (B) are shown.

of four trials. Potassium, P, and S levels were sometimes affected by the fungicide treatments, but these effects were not consistent among studies. Benlate DF and WP had similar effects on nutrient levels (results not shown).

Visual symptoms: petunias. There were no phytotoxic visual symptoms associated with Subdue or Banrot drenches. Benlate DF often caused chlorotic or necrotic leaf edges, leaf deformation, or interveinal chlorosis in petunia. Chlorosis normally started about 3 d after the drench in the young leaves, while leaves that started expanding about 1 week after the fungicide drench often looked healthy. 3336 WP and Derosal sometimes also caused chlorosis in petunia, but the symptoms were less pronounced than with Benlate DF. The visual effects of Benlate DF on plugs were much more severe than on transplants. Plugs became extremely chlorotic, but like transplants, seedlings eventually produced healthy looking new leaves.

Visual symptoms: other species. The visual effects of 3336 WP were most pronounced in impatiens. Leaf tips became chlorotic and chlorosis slowly spread around the leaf edges of the plants. Benlate DF sometimes caused leaf tip necrosis and interveinal chlorosis. Benlate DF also caused necrotic spots on the leaves of celosia in one of the two experiments and resulted in chlorosis of cucumber leaves. Mertect 340-F caused chlorosis in cucumber starting 4 d after treatment, and the plants eventually died.

Development. Benlate DF delayed flowering by 2 to 3 d in petunia and impatiens. Celosia plants were already flowering at the start of the trials, so fungicide effects on flowering could not be determined. None of the other fungicides delayed flowering.

Discussion

Benlate DF and WP drenches decreased net photosynthesis by an average of 20% to 30% for about 1 week following treatment. This may partly be caused by benomyl breakdown and the subsequent formation of dibutylurea (DBU). Unopened boxes of Benlate DF and WP often contain DBU, while additional DBU can be formed on wet plant surfaces after application (Moye et al., 1994). Aqueous solutions of Benlate can produce *n*-butyl isocyanate (BIC) (Tang et al., 1992, 1993), which can subsequently react to

produce DBU (Moye et al., 1994). Dibutylurea completely inhibits photosynthesis of hydrilla (Shilling et al., 1994) and also inhibits the photosynthesis of petunia and impatiens (van Iersel and Bugbee, unpublished data). The mode of action of DBU appears to be similar to that of the substituted urea herbicide diuron, a photosystem II inhibitor (Shilling et al., 1994). This agrees with the finding that benomyl can inhibit the Hill reaction in isolated chloroplasts (Kristeva and Kristev, 1971). Previous reports on the effect of Benlate on P_{net} are inconsistent. Ferree and Hall (1975) did not see any effect of Benlate on the gas exchange of apple leaves, while Stamps and Chase (1988) saw a 10% to 14% decrease in P_{net} of leatherleaf fern after Benlate WP sprays, but the differences were not statistically significant. Wood et al. (1984) found a 25% decrease in P_{net} of pecan after a single foliar application of Benlate WP. The differences between these previous studies may be due to differences between production lots of Benlate. We found similar effects of the different Benlate DF production lots, but all of the lots were low in DBU (Table 1).

There have been no published reports of the effects of DBU on the gas exchange of higher plants, but DBU decreased the dry matter production and shoot height of cucumber (Shilling et al., 1994). This corroborates reports that Benlate DF can decrease the mass of a variety of species (Cole et al., 1970; Ishii, 1973; Reyes, 1975; Rouchaud et al., 1985; Schreiber and Hock, 1975). Wensley (1972) and Wensley and Huang (1970) report that benomyl suppresses growth of muskmelon for 12 to 14 d, but that plants recover eventually. This agrees with our finding that Benlate DF causes a temporary decrease in P_{net}.

Our studies did not indicate large interactions between temperature and Benlate phytotoxicity. Benlate DF decreased P_{net} in all environments tested. The reduction in P_{net} increased with humidity, and CCG was reduced by 25% at 85% to 90% RH, and by only 13% with lower RH (studies 14 and 15).

Benomyl and MBC are taken up passively by the roots, transported apoplastically, and accumulate in leaf tips and margins (Peterson and Edgington, 1970). Based on this, it would be expected that higher accumulation of phytotoxic compounds would occur under conditions of high evaporative demand. However, CCG of Benlate DF-treated plants was higher at low humidity than that at high humidity. It is possible that the lower CCG at high humidity was caused by a direct effect of the humidity on the growth rate of the plants.

Since the ratio between the amount of applied fungicide and rooting volume is higher for seedlings (4.4 cm³ root volume) than for transplanted plants (50 to 200 cm³ root volume), it is likely that seedlings can accumulate higher concentrations of benomyl and/

Table 4. The effect of different fungicides on the shoot dry mass (DM) of impatiens and petunia. Data were collected in four separate greenhouse experiments.

Fungicide	Shoot DM (g/plant)			
	Impatiens		Petunia	
Control	0.20 a ^z	0.44 ab	0.48 a	0.40 a
Benlate DF (311)	0.18 a	0.46 a	0.45 b	0.38 ab
Benlate DF (688)	0.19 a	---	---	0.38 ab
Benlate WP	0.17 a	0.44 ab	---	0.35 b
3336 WP	0.20 a	0.41 b	0.42 c	0.36 ab
Derosal	---	0.47 a	0.44 b	---
Subdue	---	---	0.45 ab	---

^zMeans within a column followed by the same letter are not significantly different (*P* = 0.05).

Table 5. The effect of fungicide drenches on the nutrient composition of petunia in growth chambers. Data are from petunia study 17.

Treatment	Total N	Nitrate	Reduced N	Ca	K	P	S
				%			
Control	2.58 b ^z	0.15 b	2.42 a	0.74 a	4.70 ab	0.52 a	0.28 a
BenlateDF	2.86 a	0.36 a	2.51 a	0.58 b	4.98 a	0.52 a	0.28 a
Subdue	2.54 b	0.20 b	2.34 a	0.73 a	4.96 a	0.52 a	0.26 b
3336 WP	2.55 b	0.20 b	2.35 a	0.74 a	4.49 b	0.51 a	0.29 a
Derosal	2.53 b	0.17 b	2.36 a	0.62 b	4.44 b	0.50 a	0.24 b

^zMeans within a column followed by the same letter are not significantly different ($P = 0.05$).

or its breakdown products in their leaves. This could explain the more pronounced effect of benomyl on seedlings as compared to transplants. Since benomyl and MBC are not phloem-mobile (Peterson and Edgington, 1970), it is unlikely that they are redistributed within plants. Benomyl is relatively immobile in soil (Baude et al., 1974; Johnson and Lavy, 1994; Rhodes and Long, 1974) and uptake is reduced by organic matter in the planting medium (Schreiber et al., 1971). Benomyl that is not rapidly taken up by plants may thus become unavailable to the plants. Immobility of benomyl and MBC in plants and soils may prevent accumulation in leaves that expand several days after treatment. These leaves are therefore not expected to show phytotoxicity. New leaves generally appeared healthy and were probably essential in the recovery of P_{net} after the drench.

Differences in CCG between controls and Benlate DF treated plants were not reflected in shoot DM, but there was a close correlation between CCG and shoot DM for the other fungicides (Table 3). Since CCG integrates carbon gain in the roots and shoots together, the lower CCG and similar shoot mass of Benlate-treated plants suggests that these plants had a smaller root mass than control plants. For example, based on CCG and shoot DM data from study 17 with petunia, Benlate DF treated plants would be expected to have a root DM of 0.053 g/plant, compared to 0.112 g for control plants. Estimated root DM in the other treatments varied from 0.129 g to 0.105 g/plant.

Benomyl indeed has been shown to affect root growth and development. Hocking and Thomas (1979) showed that it suppresses adventitious root development of cuttings and severely reduced root quality and dry weights. Root dry weight of *Chrysanthemum morifolium* and *Choysia ternata* cuttings was reduced up to 40%, depending on the Benomyl concentration. Benomyl also can inhibit rooting of poinsettia (*Euphorbia pulcherrima* Willd.) cuttings (Lee et al., 1983), while benzimidazole reduced root elongation of cucumber (Klingensmith, 1961). Benomyl interferes with cell division of root tip cells of cucumber (Woo and Wick, 1995) and onion (Richmond and Phillips, 1975). Aragaki et al. (1994) showed that a volatile component of Benlate suspensions (probably BIC) can inhibit root growth of cucumber. Benlate inhibition of root growth could also be the result of the breakdown product *n*-butylamine, which can reduce root growth of *Hyoscyamus albus* and *Datura stramonium* (Hibi et al., 1992).

Benomyl significantly altered nutrient composition of tomato, depending on the NH_4^+/NO_3^- ratio in the nutrient solution (Somda et al., 1990). Calcium levels were doubled by a benomyl treatment with NH_4^+ as the sole N source, halved with a 1:1 ratio, and not affected with NO_3^- as the sole N source. Effects on other cations (K, Mg, Fe, Zn, Mn, Cu, and Mo) were similar, indicating that benomyl may stimulate cation uptake when NH_4^+ is used as an N source, but inhibit uptake, when NH_4^+ and NO_3^- are present in equal amounts. The NH_4^+/NO_3^- ratio in our nutrient solution was 2:7. Benlate effects in our experiments would thus be expected to be intermediate between the 1:1 and 0:1 ratios in Somda et al. (1990).

This was indeed the case for Ca (22% decrease), but we did not see consistent effects on other cations.

The interaction between nutrient levels and N source of the nutrient solution may be related to the effects of Benlate on nitrification. Nitrification can be stimulated (van Faassen, 1974) or inhibited by Benlate, while Derosal does not affect nitrification (Ramakrishna et al., 1979). The reason for the different effects of Benlate on nitrification may depend on soil properties and experimental conditions (Ramakrishna et al., 1979). Nitrification processes were not studied in our experiments, but could explain the nitrate accumulation occurring in Benlate-treated plants. A decrease in Ca, Mn, and Na and an increase in Fe and Al as the result of benomyl applications was seen in cucumber (Cole et al., 1970). Machado-Neto et al. (1994) suggested that benomyl may interfere with nutrient uptake by inhibiting the establishment of mycorrhizae on roots. Benlate DF decreased Ca levels in five out of six of our studies, but effects on other nutrients were variable. Benlate effects on nutrient composition may depend on interactions between growing medium, nutrient availability, and soil microbes.

Visual symptoms of Benlate phytotoxicity were similar to those previously reported. Marginal chlorosis as the result of Benlate applications has been observed in cucumber, *Cassia occidentalis*, crucifers, muskmelon, and pumpkin (Delp and Klopping, 1968; Hammett, 1968; Ishii, 1973; Reyes, 1975; Schroeder and Provvidenti, 1968; Van Assche and Vanachter, 1970; Wensley, 1972). A literature search revealed little information on possible phytotoxicity of the other fungicides used in our studies. Severe phytotoxicity (marginal chlorosis, necrosis, and inhibited root growth) occurred on azaleas drenched multiple times with an experimental fungicide (CGA-48988 (50WP)) with the same active ingredient as Subdue (Benson, 1979). However, rates in these experiments were about 10 times as much active ingredient as the maximum labeled rate of Subdue, and the experimental fungicide may have contained different inactive ingredients than Subdue. Banrot decreased the growth of douglas fir [*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco] seedlings (Dumroese et al., 1990). Two thiophanate fungicides caused marginal chlorosis in cucumber (Van Assche and Vanachter, 1970). Apoplastically transported compounds accumulate in leaf margins, where symptoms would be expected to be most severe.

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