Dibutylurea Reduces Photosynthesis, Growth, and Flowering of Petunia and Impatiens

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ABSTRACT. Dibutylurea (DBU), a breakdown product of benomyl, may be partially responsible for the previously reported phytotoxicity of the fungicide Benlate DF. We quantified the effect of DBU on the growth of two popular bedding plant species, petunia (*Petunia x hybrida*) and impatiens (*Impatiens wallerana* Hook. f.). DBU reduced photosynthesis of both species, and its effect strongly depended on the amount of DBU applied. The effects of DBU were most apparent 2 to 4 days after treatment, at which time 1.20 g·m⁻² (corresponding to 10% DBU in Benlate DF at maximum labeled drench rate) inhibited photosynthesis completely. DBU also decreased flower number and caused marginal necrosis. DBU effects were more pronounced in low relative humidity. Benlate DF containing 3.1% DBU and an equivalent amount of reagent grade DBU had similar effects on photosynthesis and petunia necrosis. Our results showed that DBU is responsible for at least part of the phytotoxic symptoms that can be caused by Benlate DF. However, other ingredients or breakdown products may also contribute to the phytotoxic symptoms of Benlate DF.

Drenches with the benzimidazole fungicide Benlate DF decrease photosynthesis and cause pronounced visual symptoms in a variety of bedding plants (van Iersel and Bugbee, 1996). Visual symptoms that have been reported include veinal discoloration, chlorosis, and stunted growth (Baxter et al., 1975; Mihuta-Grimm et al., 1990).

The active ingredient in Benlate DF, benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), can break down to produce carbendazim (MBC, methyl 2-benzimidazolecarbamate) and a variety of other products. The half-life of benomyl in aqueous solution is ≈7 h (Singh et al., 1990). Carbendazim is believed to be the active fungitoxic ingredient in Benlate suspensions (Upham and Delp, 1973) and is also formed in suspensions of other benzimidazole and thiophanate fungicides.

Other benomyl breakdown products include n-butyl isocyanate (BIC), n-butylamine, *N,N'*-dibutylurea (DBU), *N*-butylacetamide, and *N*-butylformamide (Moye et al., 1994; Singh et al., 1990; Tang et al., 1992). DBU is an important breakdown product of benomyl, and newly opened boxes of Benlate DF can contain concentrations as high as 8.85% (w/w) (Moye et al., 1994). Moye et al. (1994) also reported that additional DBU can be formed on or in leaves. DBU completely inhibits photosynthesis of the hydrophyte hydrilla (*Hydrilla verticillata* L. f. Royle) and reduces cucumber (*Cucumis sativus* L.) growth by 60% at rates of 0.28 g·m⁻² (Shilling et al., 1994). These findings led to the hypothesis that DBU is responsible for the drop in photosynthesis associated with Benlate DF applications (van Iersel and Bugbee, 1996). The objectives of our current studies were to 1) quantify the effect of DBU on photosynthesis of petunia (*Petunia x hybrida*) and impatiens (*Impatiens wallerana*), 2) determine the interaction between DBU effects and

humidity, and 3) compare the effects of Benlate DF and DBU on photosynthesis.

Materials and methods

PLANT MATERIAL. 'Ultrapink' petunia and 'Accent Lilac' impatiens were seeded in soilless medium (1 peat : 1 perlite, v:v) and placed on a misting bench. Seedlings were transplanted into 1206 flats (50 cm³ cell volume) ≈4 weeks after planting. Plants were grown at 25/20 °C day/night and ambient CO₂ concentrations until the start of the experiments.

APPLICATION OF DBU AND BENLATE DF. Application rates of DBU were based on the amount of DBU present in Benlate DF drenches at the maximum labeled drench rate of 12 g·m⁻² (1.2 g·L⁻¹ and 10 L·m⁻²). Based on the stoichiometry of benomyl breakdown, the maximum amount of DBU that can be produced from Benlate DF is 15.6% (w/w), resulting in 1.87 g·m⁻² DBU. Based on this, DBU amounts for dose response curves were kept between 0 and 1.8 g·m⁻², except for the impatiens study in the greenhouse. In this study, the highest rate was 2.32 g·m⁻² to mimic the DBU amounts that could be applied to plants in repeated drench applications (the label allowed for Benlate DF applications at 2-week intervals). Reagent grade DBU (Acros Organics, Pittsburgh) was dissolved in acetone, which was subsequently evaporated. The DBU was then dissolved in deionized water. DBU solutions and Benlate DF suspensions were applied to the plants as a soil drench. Benlate DF (lot _0220 p 02) was applied at the maximum labeled rate. A sample of the Benlate DF from this lot was artificially aged in an open container at 35°C and 95% relative humidity (RH) at the Univ. of Florida for 4 weeks to increase the DBU content. Benlate samples were analyzed for DBU concentration at the Univ. of Florida Pesticide Research Laboratory (Moye et al., 1994). Unaged and aged Benlate DF contained 0.3% and 3.1% DBU (w/w), respectively.

Plants were inspected for visible damage at the end of each study. Dry mass was measured after the plants had been oven dried at 70 °C to a constant mass.

EXPERIMENT 1—DBU DOSE RESPONSE CURVES. Dose response curves were determined with plants on greenhouses benches and in gas-exchange chambers in the greenhouse. Seedlings were grown in the greenhouse and transplants were transferred either to

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greenhouse benches or gas-exchange chambers ($0.47 \times 0.36 \times 0.61$ m³, $l \times w \times h$) and treated with DBU amounts ranging from 0 to $2.32 \text{ g} \cdot \text{m}^{-2}$. The CO₂ concentration inside the gas exchange chambers was maintained at $370 \pm 20 \text{ } \mu\text{mol} \cdot \text{mol}^{-1}$ by adding pure CO₂ to the line supplying air to the chambers. Air inside the chambers was mixed by fans (micronel V624L) circulating $500 \text{ L} \cdot \text{min}^{-1}$. Supplemental light was provided by 1000-W high-pressure sodium lights. Air temperature was measured with aspirated, shielded thermocouples, and RH was monitored with humidity probes (model HMP 35A; Vaisala sensor systems). Photosynthetic photon flux density outside of the chambers was measured with a quantum sensor (model LI-190SB; LI-COR). Total daily photon flux was 30 to $50 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$.

Whole crop photosynthesis (P_{net}) was measured several times a week in the gas exchange chambers. It was calculated from the mass flow rate of air going into the chamber ($\text{mol} \cdot \text{s}^{-1}$), soil area (m^2) and the difference in CO₂ concentration between the incoming air (C_{pre} , $\mu\text{mol} \cdot \text{mol}^{-1}$) and air in the chambers (C_{chamber}). Air flow through the chambers was regulated and measured with rotometers (model RMB-SSV; Dwyer). The CO₂ concentration of the prechamber and chamber air was determined from syringe samples. Four-milliliter samples were taken from the incoming air line and the ten chambers and injected into an infrared gas analyzer (model 225; ADC). Six replicate samples were taken from each chamber. Photosynthesis was calculated using the following equation $P_{\text{net}} = (C_{\text{pre}} - C_{\text{chamber}}) \times \text{air mass flow rate/soil area}$ ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

Two experiments with petunia and one with impatiens were conducted in the greenhouse and in the gas-exchange chambers, for a total of six experiments. The greenhouse experiments lasted for 18 (petunia Expt. 1), 35 (petunia Expt. 2), and 33 d (impatiens), while the experiments in the gas-exchange chambers lasted until 11 d (both petunia experiments) and 18 d (impatiens) after the DBU applications. The experimental design was a randomized complete block with two replications. The experimental unit was a group of 60 plants.

EXPERIMENT 2—DBU $\times$ HUMIDITY INTERACTIONS. DBU effects in the open greenhouse differed from those in gas exchange chambers. The main difference in environmental conditions was RH, so a growth chamber experiment was designed to determine a possible interaction between DBU, humidity, and dry mass increase of petunias. Two growth chambers were used in this study. Both growth chambers were maintained at $28/22 \text{ } ^\circ\text{C}$ day/night, a photosynthetic photon flux of $440 \text{ } \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the top of the canopy, and a 14-h photoperiod. Total daily photon flux was $22.2 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. RH in one of the growth chambers was $15\% \pm 5\%$, while RH in the other growth chamber was kept at $95\% \pm 5\%$ with a humidifier. In the humidity chamber, water condensed on the plants, greatly reducing transpiration. The plants in the 95% RH treatment did not need water during the 8 days of this experiment. On the first day, the growing medium was drenched with DBU at rates of 0, 0.6, or $1.2 \text{ g} \cdot \text{m}^{-2}$. Treatments were applied to groups of six plants, with 14 replications per treatment. Replicates were completely randomized within

each growth chamber, and shoot dry mass was collected at the end of the experiment.

EXPERIMENT 3—BENLATE DF AND DBU COMPARISON. To determine whether the decrease in P_{net} as the result of Benlate DF applications can be attributed to DBU in the fungicide, we compared Benlate DF and DBU effects on photosynthesis and growth of petunia. Treatments were an untreated control, Benlate DF ($12 \text{ g} \cdot \text{m}^{-2}$), aged Benlate DF ($12 \text{ g} \cdot \text{m}^{-2}$), and DBU (0.036 and $0.37 \text{ g} \cdot \text{m}^{-2}$). These DBU levels were similar to the amount of DBU in the fresh and aged Benlate DF samples, respectively. The experiment was conducted in a 10-chamber continuous gas-exchange system, as described by van Iersel and Bugbee (1996). Plants (60 plants per gas exchange chamber) were treated on the fifth day of the gas-exchange measurements and the experiment was continued until 13 d after the start of the treatments. The experimental design was a randomized complete block with two replications. Temperature inside the gas-exchange chambers was $29/26 \text{ } ^\circ\text{C}$ day/night. The light period was 14 h with a photosynthetic photon flux of $780 \text{ } \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, resulting in a total photon flux of $39 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$.

Results

Experiment 1—DBU dose response curves

DBU DOSE RESPONSE CURVES IN GAS-EXCHANGE CHAMBERS. Net photosynthesis of petunia and impatiens decreased as the applied amount of DBU increased (Fig. 1). The largest decrease in photosynthesis generally occurred 2 to 4 d after the drench, after which the photosynthetic rate slowly recovered. The differences in photosynthetic rate were reflected in the final shoot dry mass of the plants (Fig. 2A), which decreased linearly with increased rates of DBU.

Fig. 1. The effect of growing mix drenches with reagent grade dibutylurea (DBU) on the gas exchange of impatiens (A) and petunia (B and C) (Expt. 1). Light interception by flowers in the control and $0.12 \text{ g} \cdot \text{m}^{-2}$ treatment caused the photosynthetic rate to drop during the last two measurement days of petunia Expt. 2 (C). Each data point represents the mean of two gas-exchange chambers with 60 plants each. Error bars represent the $\text{LSD}_{0.05}$ to compare treatments within a day.

DBU-treated plants also developed pronounced visual symptoms. Impatiens treated with 1.2 g·m⁻² started developing marginal leaf necrosis 2 d after the drench, while plants treated with 0.72 g·m⁻² did not develop symptoms until 7 d after treatment. Flowering of DBU-treated impatiens was not different from that of the control plants (Table 1).

Petunia developed visual symptoms similar to those of impatiens. After DBU applications of 0.72 and 1.20 g·m⁻², necrosis first appeared at the leaf tips and spread to the leaf edges. When 0.36 g·m⁻² was applied to petunia, some leaf tips became necrotic, while many leaves developed chlorotic tips. The leaf blades in all three treatments were chlorotic. Necrosis was most obvious in leaves that were in the upper part of the canopy at the time of treatment. Leaves that appeared after the DBU drench showed little effect. Plants treated with 0.12 g·m⁻² did not develop any visual symptoms. In petunia Expt. 1, the number of petunia flowers was reduced by 46% by 1.20 g·m⁻² DBU, while the lower DBU levels had little effect on flowering (Table 1). In petunia Expt. 2, the 0.36 g·m⁻² and higher treatments reduced the numbers of flowers.

DBU DOSE RESPONSE CURVES ON GREENHOUSE BENCHES. Dry mass of petunia and impatiens was significantly reduced by DBU (Fig. 2). A DBU application of 2.3 g·m⁻² stopped the growth of impatiens almost completely and ≈25% of the plants eventually died. Young leaves of impatiens treated with 2.3 or 1.5 g·m⁻² did not expand normally and many leaves developed marginal necrosis. DBU levels of 0.92 and 0.47 g·m⁻² caused marginal chlorosis and some necrotic leaf tips. A DBU drench of 0.16 g·m⁻² did not cause visual symptoms in impatiens leaves. Impatiens treated with 2.3 or 1.5 g·m⁻² DBU did not have flowers at harvest, while the treatments with 0.92, 0.47, and 0.16 g·m⁻² had far fewer flowers than the control (Table 1).

Leaf edges of petunia treated with 1.2 or 1.8 g·m⁻² DBU showed some necrosis on the second day after the treatment and the symptoms became more pronounced over time. The plants eventually became severely chlorotic and stopped growing. One severely deformed flower eventually opened in the 1.8-g·m⁻² treatment, and there were no flowers in the 1.2-g·m⁻² treatment. The first visual symptoms in the 0.6-g·m⁻² treatment appeared 4 d after the drench when plants developed necrotic leaf tips. Leaves were chlorotic at the end of the experiment. Flowering was reduced by >50% compared to the untreated control (Table 1). Plants in the

0.12-g·m⁻² treatment did not show visual symptoms on the leaves, but flowering was slightly reduced compared to the control. The visual symptoms and growth reduction associated with DBU were more pronounced in these studies than in the comparable studies in gas-exchange chambers.

Experiment 2, DBU × humidity interactions

Untreated control plants grown at 15% RH had a healthy dark green color, while plants at 95% RH were chlorotic and some of the younger leaves were curled. However, dry mass of the control plants from both RH treatments was similar (Fig. 3). The reduction in growth and visual deformation associated with DBU were both more pronounced at low humidity. A DBU drench with 0.6 g·m⁻² decreased dry mass by 60% at 15% RH, and by only 15% at 95% RH. Humidity also differentially affected treatments effects evaluated visually. At 95% RH, the control and 0.6-g·m⁻² treatment had some leaf deformation and chlorosis. The plants from the 1.2-g·m⁻² treatment had necrotic leaf tips and edges. Visual symptoms of the treatments were far more pronounced at 15% than at 95% RH. In the 15% RH treatment, the 0.6- and 1.2-g·m⁻² treatments caused severe necrosis in the leaf tips and margins. About 50% of the total leaf area treated with 1.2 g·m⁻² DBU at 15% RH was necrotic at the end of the experiment, while necrosis was limited to the leaf tips and margins at 95% RH.

BENLATE DF AND DBU COMPARISON. Aged Benlate DF and DBU at a rate of 0.37 g·m⁻² (treatments contained the same amount of DBU) quickly affected the photosynthetic rate of petunia (Fig. 4). The photosynthetic rate of both treatments did not recover to that of the control within 8 days. The effect of aged Benlate DF and DBU (0.37 g·m⁻²) on the photosynthesis of petunia was nearly identical (Fig. 4), suggesting that DBU was responsible for the decrease in photosynthesis caused by Benlate DF. However, unaged Benlate DF caused a much bigger decrease in photosynthesis than a DBU application of 0.036 g·m⁻², while these two treatments contained similar amounts of DBU. Dark respiration was initially unaffected by any of the treatments, but dark respiration in the aged Benlate DF and DBU (0.37 g·m⁻²) treatments was lower (closer to zero) than in the other treatments; dark respiration started to decline the fourth night after the drench.

The drench with DBU at 0.036 g·m⁻² did not cause visual symptoms, while Benlate DF caused severe interveinal chlorosis and some extremely chlorotic leaves.

Fig. 2. The effect of growing medium drenches with reagent grade di-butylurea (DBU) on the dry mass of impatiens and petunia (Expt. 1). Plants were either grown on greenhouse benches (A) or in gas-exchange chambers in the greenhouse (B). The growth chamber experiments lasted for 18 (impatiens) and 11 d (both petunia experiments) and the greenhouse experiments for 18 (petunia Expt. 1), 35 (petunia Expt. 2), and 33 d (impatiens) after the drench with DBU. Data points represent the mean of two replications of 60 plants each. Error bars indicate ±1 standard error.

Table 1. The effect of dibutylurea (DBU) on flowering of impatiens and petunia. Plants were grown in acrylic gas-exchange chambers in the greenhouse, or on greenhouse benches. Flowers were counted at the end of the experiments.

DBU rate (g·m ⁻²)	Gas-exchange chambers			Greenhouse		
	Impatiens	Petunia Expt. 1	Petunia Expt. 2	Impatiens	Petunia Expt. 1	Petunia Expt. 2
Open flowers (no.) per 60 plants						
0.00	19.5	40.0	20.0	20.5	14.5	11.0
0.12	24.0	33.0	22.5	---	11.0	5.0
0.16	---	---	---	10.5	---	---
0.36	16.5	34.5	7.0	---	---	---
0.47	---	---	---	4.0	---	---
0.60	---	---	---	---	7.0	1.0
0.72	24.5	35.0	12.0	---	---	---
0.92	---	---	---	3.0	---	---
1.20	27.0	21.5	12.0	---	0.0	0.0
1.55	---	---	---	0.0	---	---
1.80	---	---	---	---	0.5	0.0
2.32	---	---	---	0.0	---	---
LSD _{0.05}	8.2	12.4	9.0	9.1	7.4	7.8

Chlorosis was more severe with regular Benlate DF than with aged Benlate DF, but aged Benlate DF caused leaf tip necrosis. This necrosis developed in leaves present at time of the drench, while chlorosis was especially apparent in the younger leaves. A DBU application of 0.37 g·m⁻² caused similar leaf necrosis as aged Benlate DF and some chlorosis, but chlorosis was less pronounced than in the Benlate DF-treated plants. Necrosis in the 0.37-g·m⁻² DBU and aged Benlate DF treatments started appearing at 3 d after the drench.

Discussion

DBU levels of 0.36 g·m⁻² (comparable to 3% DBU in Benlate DF when applied at the maximum labeled rate of 12 g·m⁻²) significantly decreased photosynthesis and dry mass of petunia and impatiens. The inhibition of photosynthesis and dry matter increase became more severe with higher concentrations of DBU. In petunia Expt. 2, treatment differences were less pronounced during the last 2 d of this experiment (Fig. 1C). Light interception

by the many flowers in the control and 0.12-g·m⁻² treatment of petunia Expt. 2 may have been the cause of the decrease in P_{net} , reducing these values to those of the 0.36- and 0.72-g·m⁻² treatments. Although these measurements cannot distinguish between direct and indirect effects on photosynthesis, the fast decrease in photosynthesis (within 15 min after DBU applications) suggests that DBU may have affected photosynthesis directly. This is supported by the findings of Shilling et al. (1994), who reported that the effects of DBU on photosynthesis were similar to those of diuron, a substituted urea herbicide that inhibits photosynthetic electron transport. Chlorophyll a fluorescence and oxygen production of hydrilla shoot tips were affected similarly by DBU and diuron, suggesting that they have similar modes of action. Kristeva and Kristev (1971) showed that benomyl inhibits the Hill reaction in isolated chloroplasts, which is consistent with the findings of Shilling et al. (1994).

Shilling et al. (1994) also found that the effect of DBU on cucumber biomass was dose dependent, although they reported a larger growth inhibition than seen in our studies. This difference may have been the result of different growing media. Shilling et al. (1994) grew plants in silica sand, which did not contain organic matter. Our media consisted of 50% peat, which probably bound some of the DBU in the root zone and decreased the effective dosage for the plants. We found that Benlate DF damage to petunia increases as the organic matter fraction of the media decreases from 60% to 0% (van Iersel and Bugbee, unpublished data).

There was a greater decrease in dry matter and visual symptoms were more severe in the greenhouse than in the gas-exchange chambers. Visual symptoms were always most pronounced in leaf tips and margins, suggesting that DBU is transported apoplastically; solutes in the xylem tend to accumulate at leaf margins (Canny, 1990). This is consistent with the observation that damage was more pronounced in the greenhouses than in the gas exchange

Fig. 3. The shoot dry mass of petunia, as affected by dibutylurea (DBU) and relative humidity (Expt. 2). Plants were grown in growth chambers and dry mass data were collected at 7 days after the application of DBU. Data points are the mean of 14 groups of six plants. Error bars indicate ± 1 standard error.

chambers. RH was generally between 80% and 90% in the growth chambers, and only 30% to 40% in the greenhouse. The greater vapor-pressure deficit in the greenhouse undoubtedly caused higher transpiration rates than in the gas-exchange chambers, since other environmental conditions were similar. This may have caused a faster accumulation of DBU in the leaves.

The results from our growth chamber study confirmed that vapor-pressure deficit is an important variable in the severity of DBU damage. The reduction in dry matter production and visual symptoms following DBU applications were more severe at low RH. This corroborates the hypothesis that DBU is transported apoplastically and accumulates in the leaf margins, where it can then cause necrosis. Shilling et al. (1994) reported identical symptoms of DBU after application to cucumber. Marginal necrosis is a typical symptom of substituted urea compounds, possibly because the inhibition of photosynthetic electron transport can cause photo-oxidations in chloroplasts. This can induce chlorophyll degradation and cell death (Stanger and Appleby, 1972).

The greater severity of DBU damage in low humidity is seemingly inconsistent with the fact that most problems with Benlate DF were reported in the southeastern United States and Hawaii, where RH is generally high. This apparent discrepancy can be explained by two factors. Water is required in the reaction that produces DBU from BIC (Moye et al., 1994), and Benlate DF stored under high humidity would probably produce higher levels of DBU than when it is stored under low humidity. In addition, high humidity favors many diseases, which would lead to more frequent applications of fungicides.

Our comparison between Benlate DF and DBU did not clearly confirm that DBU is fully responsible for the effect of Benlate DF

on the photosynthesis of bedding plants. One Benlate DF sample used in this part of our study contained 0.3% DBU and one contained 3.1% DBU. The effect of a DBU application of 0.37 g m^{-2} was similar to that of a drench with aged Benlate DF, containing the same amount of DBU. This suggested that DBU is responsible for the inhibition of photosynthesis. However, a DBU application of 0.036 g m^{-2} did not decrease photosynthesis, while a Benlate DF drench containing the same amount of DBU distinctly decreased photosynthesis. This apparent discrepancy can be explained by the fact that Benlate DF breakdown may produce more DBU after it is suspended in water (Moye et al., 1994), and the surfactant in Benlate DF may facilitate DBU uptake into the plant. It is also possible that Benlate DF inhibited growth and photosynthesis through some other mechanism. It has been shown, for example, that Benlate DF can alter bacteria populations in the rhizosphere of leatherleaf ferns [*Rumohra adiantiformis* (Forst.) Ching], promoting a bacterial component with phytotoxic properties (Kremer et al., 1996).

Continuous gas exchange measurements showed that DBU rapidly affected photosynthesis of petunia, but did not affect dark respiration until later in the trial. The effect of DBU on dark respiration may have been an indirect effect of the growth reduction caused by DBU. The growth rate of plants treated with either aged Benlate DF or DBU at 0.37 g m^{-2} was lower than that of plants in the other three treatments. Smaller plants generally have lower maintenance respiration rates, while the growth reduction may also have decreased growth respiration. In addition, respiration rates in these two treatments may have been limited by the amount of available carbohydrates, due to the lower photosynthetic rates. The finding of Shilling et al. (1994) that DBU does not affect the respiration of hydrilla shoot tips also supports the hypothesis that the effect of DBU on respiration was indirect.

DBU was highly phytotoxic and appears to be responsible for at least part of the phytotoxicity of Benlate DF. There may be other phytotoxic compounds in Benlate DF. Butylamine, for example, inhibits root growth in *Hyoscyamus albus* and *Datura stramonium* (Hibi et al., 1992). Butyl isocyanate is phytotoxic to Boston fern and can cause chlorosis and brown spots in cucumber leaves (Moye et al., 1994). In moistened Benlate DF, a volatile substance (probably BIC) evolved that is toxic to cucumber roots (Aragaki et al., 1994). There is little or no information available on possible phytotoxicity of the benomyl breakdown products 2-aminobenzimidazole, *N*-butylacetamide, and *N*-butylformamide.

Literature Cited

- Aragaki, M., J.Y. Uchida, and C.Y. Kadooka. 1994. Toxicity of Benlate to cucumber and evidence for a volatile phytotoxic decomposition product. *Arch. Environ. Contam. Toxicol.* 27:121-125.
- Baxter, L.W., W. Witcher, and M.G. Owens. 1975. Benomyl injury of swedish ivy (*Plectranthus australis*). *Plant Dis. Rpt.* 59:686.
- Canny, M.J. 1990. Tansley review no. 22. What becomes of the transpiration stream? *New Phytol.* 114:341-368.
- Hibi, N., T. Fujita, M. Hatano, T. Hashimoto, and Y. Yamada. 1992. Putrescine *N*-methyltransferase in cultured roots of *Hyoscyamus albus*. *n*-butylamine as a potent inhibitor of the transferase both in vitro and in vivo. *Plant Physiol.* 100:826-835.

Fig. 4. A comparison between the effect of Benlate DF and dibutylurea (DBU) on the gas exchange of petunia (Expt. 3). Fresh Benlate DF contained 0.3% DBU, while aged Benlate DF contained 3.1% DBU. When Benlate DF is used at the maximum labeled rate of 12 g m^{-2} , these DBU levels correspond to 0.036 and 0.37 g m^{-2} , respectively. Data represent the mean daytime photosynthesis and nighttime respiration. Each data point is the mean of two gas-exchange chambers, containing 60 plants each. Error bars represent the $\text{LSD}_{0.05}$ to compare treatments within a day.

- Kremer, R.J., D.N. Sasseville, and H.A. Mills. 1996. Promotion of phytotoxic bacteria in rhizospheres of leatherleaf fern by Benlate DF. *J. Plant Nutr.* 19:939–953.
- Kristeva, M. and K. Kristev. 1971. Respiratory and photosynthetic rates in apple leaves treated with the systemic fungicide benomyl. *Acta Phytopathol. Acad. Sci. Hung.* 6:365–369.
- Mihuta-Grimm, L., W.A. Erb, and R.C. Rowe. 1990. Fusarium crown and root rot of tomato in greenhouse rock wool systems: Sources of inoculum and disease management with benomyl. *Plant Dis.* 74:996–1002.
- Moye, H.A., D.G. Shilling, H.C. Aldrich, J.E. Gardner, M. Buszko, J.P. Toth, W.S. Brey, B. Bechtel, and J.K. Tolson. 1994. *N,N'*-dibutylurea from n-butyl isocyanate, a degradation product of Benomyl. 1. Formation in Benlate formulations and on plants. *J. Agr. Food. Chem.* 42:1204–1208.
- Shilling, D.G., H.C. Aldrich, H.A. Moye, J.F. Gaffney, J.K. Tolson, R. Querns, M.A. Mossler, and B.L. Russell. 1994. *N,N'*-Dibutylurea from n-butylisocyanate, a degradation product of benomyl. 2. Effects on plant growth and physiology. *J. Agr. Food Chem.* 42:1209–1212.
- Singh, R.P., I.D. Brindle, C.D. Hall, and M. Chiba. 1990. Kinetic study of the decomposition of methyl [1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate (Benomyl) to methyl 1*H*-benzimidazol-2-yl carbamate (MBC). *J. Agr. Food Chem.* 38:1758–1762.
- Stanger, C.E. and A.P. Appleby. 1972. A proposed mechanism for diuron induced phytotoxicity. *Weed Sci.* 20:357–363.
- Tang, C.S., K. Yanagihara, and Y. Zhang. 1992. 1-Butylisocyanate from aqueous Benlate formulations. *Arch. Environ. Contam. Toxicol.* 23:270–272.
- Upham, P.M. and C.J. Delp. 1973. Role of benomyl in the systemic control of fungi and mites on herbaceous plants. *Phytopathology* 63:814–820.
- van Iersel, M.W. and B. Bugbee. 1996. Phytotoxic effects of benzimidazole fungicides on bedding plants. *J. Amer. Soc. Hort Sci.* 121:1095–1102.