Systemic Activity of Methyl 2-Benzimidazolecarbamate (MBC) in Almond Blossoms Following Prebloom Sprays of Benomyl + MBC

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ABSTRACT

Prebloom sprays of proprietary benomyl 50W afforded excellent control of *Monolinia laxa* blossom and twig blight of almond (cultivar 'Drake'). Sprays of 2.80 and 1.40 kg/hectare (2.5 and 1.25 lb/acre) benomyl 50W with or without oil were equally effective. Bioassays performed with conidia of *M. laxa* following prebloom sprays showed all blossom parts were protected through full bloom. Methyl 2-^{14}C-benzimidazolecarbamate (^{14}C-MBC) plus benomyl 50W applied with or without oil to branches of covered trees at green-bud or pink-bud stages resulted in radioactivity in all blossom parts at full bloom. Labeled and unlabeled MBC were the only residues recovered from such blossoms, indicating degradation of benomyl to MBC under field conditions. Benomyl and MBC were similar in degree of fungitoxicity to *M. laxa* conidia.

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Hamilton et al. (4) reported that the oxime derivative of cycloheximide acted systemically in cherry leaves to protect against the cherry leaf spot disease. Recently, benomyl has provided systemic protection of apple, cherry, and citrus leaves formed after spray application (1, 5). In spite of these findings, there has been no definitive report concerning the use of benomyl for systemic protection against fungal diseases of orchard tree blossoms. Two purposes of this study, therefore, were: (i) to determine the effectiveness of prebloom sprays of benomyl in controlling blossom and twig blight caused by *Monilinia laxa*, and (ii) to assess the degree of systemic fungicidal activity of benomyl in almond blossoms.

Also subject to question, is whether the systemic disease protection in trees is afforded by benomyl itself, or by its principal degradation product, methyl 2-benzimidazolecarbamate (MBC). The breakdown of benomyl to MBC has been reported to occur in aqueous (2, 9, 10) and chloroform (7) solutions. Peterson & Edgington (10) reported that stem and leaf sections of bean plants, fed benomyl in hydroponic solutions, contained only MBC. Therefore, another objective of this study was to determine the proportion of blossom blight control attributable to benomyl and to MBC.

MATERIALS AND METHODS.–*Commercial field-scale prebloom benomyl applications to almond trees.*–Air blast, low-volume, sprays of proprietary benomyl 50W at 1.40 and 2.80 kg/hectare (1.25 and 2.5 lb/acre) with and without Volck Supreme Oil at 46.77 liters/hectare (5 gal/acre) were applied in 1.17 kliters/hectare (125 gal/acre) to mature almond trees (cultivar 'Drake') at Durham, California, at green-bud or pink-bud prebloom stages (Fig. 1). A similar field plot, consisting of mature Drake trees at Ripon, California, was sprayed with a handgun at the same stages as described above. Benomyl 50W was applied at 113.4 and 226.8 g/378.5 liters (4 and 8 oz/100 gal) water, with and without Volck Supreme Oil at 3.79 liters/378.5 liters (1 gal/100 gal) water, to the drip stage. Both plots were set up in a randomized complete block design (6). The frequency of blighted twigs resulting from blossom infections, was determined as an estimate of disease control. Relative humidity, temperature, and rainfall were recorded at both sites during the course of the experiment.

Bioassay of systemic fungitoxic activity in field-sprayed blossoms.–At early full-bloom stage, ten closed blossoms were collected from each of the three replications of each treatment at the Durham plot and placed in plastic food crispers containing moist sand. When the blossoms opened they were sprayed with a spore suspension of *M. laxa* (12,500 conidia/ml) until thoroughly wet, then covered, and incubated in a room at 90% relative humidity and 20 C. Protection against blossom infection was measured for each treatment after 109 hr by a visual rating of the amount of infection exhibited by the individual blossoms. A rating scale of 1 to 5 was used, with 1 indicating no visual infection, and 5 indicating maximum infection.

Determination of benomyl degradation and distribution of fungitoxic residues in almond blossoms.–Spray mixtures of ^{14}C-MBC (methyl 2-^{14}C-benzimidazolecarbamate, sp act 2.7 mcuries/mmole–ICN Co., Inc., Irvine, Calif.) were diluted 10-fold with proprietary benomyl 50W on an active ingredient basis (1.8 mg ^{14}C-MBC : 34.2 mg benomyl 50W). The inclusion of ^{14}C-MBC helped to insure the detection of systemic residues in the blossoms. The presence of benomyl 50W enhanced the wettability and depositional properties of the ^{14}C-MBC, which by itself was impossible to spray. A water suspension of the mixed chemical powder was

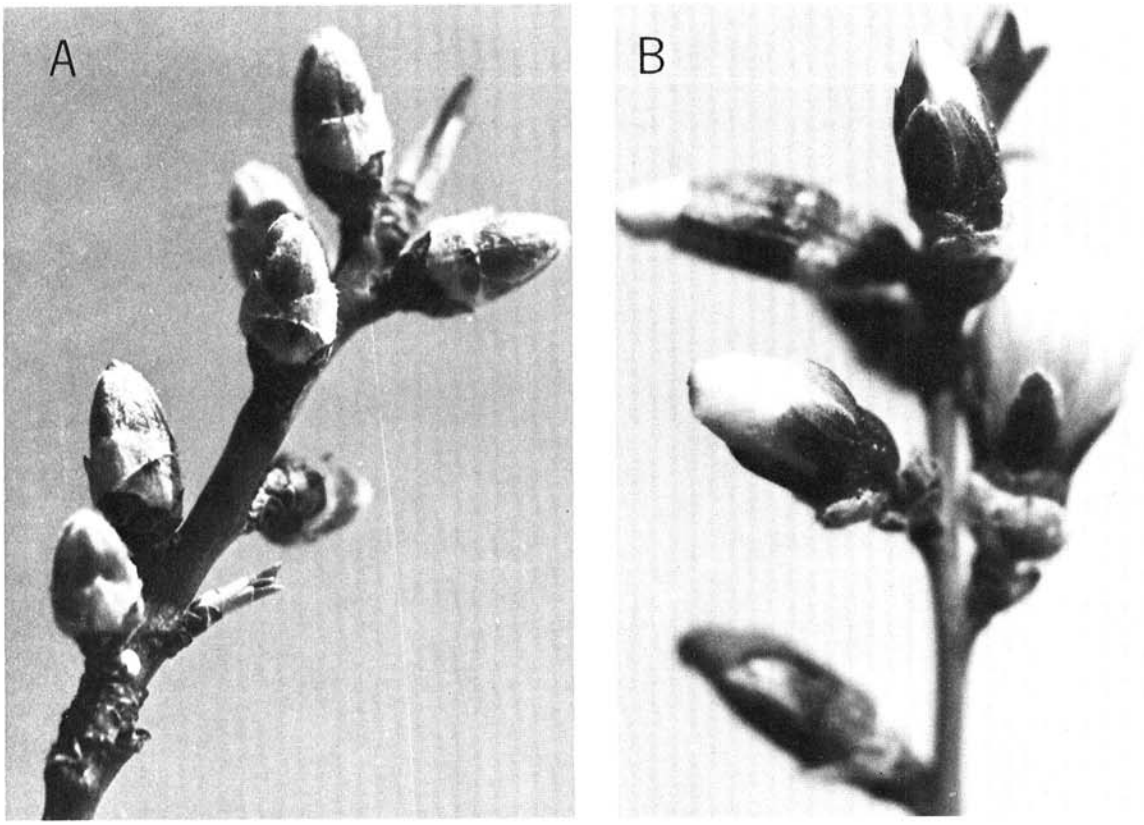

Fig. 1. A) Green-bud stage of almond bud development. Sepals have protruded through bud scales. **B)** Pink-bud stage. Tightly closed petals have protruded through the sepals.

made equivalent to a concentration of 113.4 gm (0.25 lb) active fungicide/378.5 liters (100 gal) water, which is an effective rate used in the field. To one half of this mixture, Volck Supreme Oil (Chevron Chemical Co., Richmond, Calif.) was added to make a 1% (v/v) emulsion. This oil was added to test its effectiveness in increasing the uptake of benomyl or MBC by almond tissues. Both mixtures contained ca. 1.2×10^6 dpm/ml. A DeVilbiss No. 15 hand atomizer was used to apply the sprays to drip stage to small branches of six mature Drake almond trees in the green-bud or pink-bud prebloom stages (Fig. 1). Three of the trees were covered with polyethylene (Monsanto GER-PAK 6-mil A-2400-644; Monsanto Chem. Co., St. Louis, Mo.) houses to prevent rain from redistributing the ^{14}C-MBC following application. The three additional trees were left uncovered.

Analysis of fungicide residues in blossoms.—Harvested blossoms which had been frozen (ca. 15 blossoms/replication) were thawed and separated into petals, pistils plus stamens, and calyces. The tissues were weighed (320, 100, and 560 gm fresh wt of petals, pistils plus stamens, and calyces, respectively). The blossom parts were frozen in liquid nitrogen, ground to a fine powder, and lyophilized. The frozen powder was placed in 20-ml scintillation vials containing 1 ml of water plus 0.3 gm Cabosil thixotrophic gel (Packard Instruments Co., Inc., Downers Grove, Ill.), and added to 10 ml Triton X-100 toluene scintillation fluid (PPO 6 g + POPOP 100 mg + 500 ml Triton X-100 surfactant/liter toluene). Radioactivity levels in the blossom parts were determined by liquid scintillation spectrometry. All counts were corrected for background and color quenching by the internal standard method (12).

The blossom parts from the uncovered trees (those in the more natural environment for benomyl degradation) were subjected to a more detailed chemical analysis of fungitoxic residues to determine the relative proportions of benomyl and/or MBC residues present after a 6-day period in the field. Aggregate samples of blossoms from the three replications of each treatment were divided into petals, pistils plus stamens, and calyces (fresh weights 1.0, 0.5, and 1.0 gm, respectively) which were frozen, ground, and lyophilized. The resulting powders were extracted with nanograde benzene (50 : 1, v/w) on a rotary shaker for 1.5 hr. The extracts were filtered through anhydrous sodium sulfate and taken to dryness by flash evaporation. The dry residue was dissolved in ethyl acetate and spotted on a preparative thin-layer plate (Sil plate-P5F-22,

Brinkman Instruments, Inc.). Analytical grade benomyl and MBC standards, control extracts, and fortified control extracts were also spotted to determine quantitative recovery factors. The plates were developed with a benzene-acetone solvent system (19 : 1, v/v) which separated benomyl and MBC (R_F = 0.62 and 0.0, respectively) from the natural residues also extracted from the blossom parts. MBC and benomyl were eluted from the plate with ethyl acetate and respotted on a polyamide 11_{UV}-254 thin-layer plate (Brinkman Instruments, Inc.) which was developed with chloroform-ethyl acetate-acetic acid (190 : 10 : 4, v/v) to further purify the MBC. The benomyl standard had an R_F of 0.62. Total MBC (labeled + unlabeled MBC), R_F = 0, was eluted and quantified spectrophotometrically at 286.5 nm. Percentage recoveries of MBC from MBC-fortified extracts of the blossom parts ranged from 36 to 53%. Benomyl-fortified controls yielded only MBC.

Effects of benomyl and MBC on growth of M. laxa from conidia in vitro.—Conidia of *M. laxa* were produced on oatmeal agar under continuous fluorescent light for 7-9 days. Three ml of spore suspension, containing 10^6 conidia/ml, were added to flasks containing 147 ml of autoclaved (10 min) Dion's liquid medium modified to give a stronger buffering capacity. The medium contained per liter of glass-distilled water: K_2HPO_4, 20 g; NaCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 10 mg; $ZnSO_4$, 10 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; dextrose, 30 g; l-asparagine, 5 g; and thiamine, 100 mg. Analytical grade benomyl or MBC in equal quantities of ethyl acetate was added to flasks to give concentrations of 0.05, 0.1, and 1.0 μg chemical/ml of nutrient solution. The cultures were grown at 25 C on a rotary shaker. The pH of the medium was 6.7 throughout the experiment. Samples were aseptically pipetted from the flasks at intervals over a 48-hr period. The fungus mats were filtered and washed with water, and dry weight determinations were used as a comparative index of growth. The rate of benomyl conversion to MBC in additional flasks that contained the medium plus spores and 10 μg analytical grade benomyl per flask was estimated by the TLC methods previously described.

RESULTS AND DISCUSSION.—*Blossom and twig blight control from prebloom benomyl sprays.*—All prebloom sprays applied by air-blast sprayer in the field at rates as low as 1.40 kg/hectare (1.25 lb/acre) of benomyl 50W, with or without oil, gave significant disease control (Table 1). Pink-bud treatments reduced twig blight by 81-93%, compared to 69-82% for green-bud treatments. Oil without benomyl, reduced disease severity by 57%. All of the prebloom applications protected blossoms during bloom.

Rainfall at the Durham plot (Table 1) was light 2.08 cm (0.82 inches) during the period between spray treatment and petal fall. Relative humidity reached 100% on eight occasions, and temperatures were sufficiently high during these periods to induce germination of *M. laxa* conidia and for blossom infection to occur.

TABLE 1. *Monilinia laxa* blossom and twig blight control following benomyl and benomyl plus oil prebloom sprays applied to mature 'Drake' almond trees at Durham, California[V]

Treatment	Amount per Hectare	Amount per (Acre)[W]	Time of application[X]	Total no. shoot strikes/ 4,500 shoots/ treatment[Y]	% Disease reduction	Blossom bioassay protection rating[Z]
Benomyl 50W + Supreme Oil	2.80 kg 46.77 liter	(2.50 lb) (5.00 gal)	pink bud	34 a	93	1
Benomyl 50W	2.80 kg	(2.50 lb)	pink bud	41 a	92	2
Benomyl 50W	1.40 kg	(1.25 lb)	pink bud	58 a	88	4
Benomyl 50W + Supreme Oil	1.40 kg 46.77 liter	(1.25 lb) (5.00 gal)	green bud	89 ab	82	2
Benomyl 50W + Supreme Oil	1.40 kg 46.77 liter	(1.25 lb) (5.00 gal)	pink bud	94 ab	81	4
Benomyl 50W	2.80 kg	(2.50 lb)	green bud	100 ab	80	4
Benomyl 50W + Supreme Oil	2.80 kg 46.77 liter	(2.50 lb) (5.00 gal)	green bud	129 ab	74	3
Benomyl 50W	1.40 kg	(1.25 lb)	green bud	154 ab	69	4
Supreme Oil	46.77 liter	(5.00 gal)	pink bud	214 b	57	3
Control				498 c		5

[V] Disease control evaluation done 4/13/71.

[W] Prebloom air-blast sprayer concentrate treatments applied at 1.17 kliter H_2O/hectare (125 gal H_2O/acre) and ca. 7.57 liter (2 gal)/tree. [All chemicals were used at per acre rates comparable to those used for dilute application of 4.68 kliter H_2O/hectare (500 gal H_2O/acre). 2.80 kg (2.50 lb) benomyl 50W/hectare (acre) = 226.8 gm (0.5 lb) benomyl 50W/378.54 liters (100 gal) H_2O-dilute application].

[X] Green-bud stage application date was 2/13/71. Pink-bud stage application date was 2/17/71.

[Y] 750 shoots/tree counted, two trees/replication evaluated, three replications total. Duncan's multiple range test (P = .05) used (3). Treatments followed by the same letter do not differ significantly.

[Z] Blossoms harvested at full bloom following prebloom spray application and inoculated with *M. laxa* conidia. (1 = no disease; 5 = no protection).

TABLE 2. *Monilinia laxa* blossom and twig blight control following benomyl and benomyl plus oil prebloom sprays applied to mature 'Drake' almond trees at Ripon, California[W]

Treatment	Amount/ 378.54 liter H_2O[X]	Amount/ (100 gal)	Time of application[Y]	Total no. shoot strikes/ treatment[Z]	% Disease reduction
Benomyl 50W + Supreme Oil	113.4 gm 3.79 liter	(0.25 lb) (1.00 gal)	pink bud	80 a	98
Benomyl 50W	226.8 gm	(0.50 lb)	pink bud	159 a	96
Benomyl 50W + Supreme Oil	226.8 gm 3.79 liter	(0.50 lb) (1.00 gal)	pink bud	499 a	88
Benomyl 50W	113.4 gm	(0.25 lb)	pink bud	556 a	87
Benomyl 50W	226.8 gm	(0.50 lb)	green bud	860 a	79
Benomyl 50W + Supreme Oil	226.8 gm 3.79 liter	(0.50 lb) (1.00 gal)	green bud	926 a	78
Supreme Oil	3.79 liter	(1.00 gal)	pink bud	2,329 ab	44
Benomyl 50W + Supreme Oil	113.4 gm 3.79 liter	(0.25 lb) (1.00 gal)	green bud	3,030 b	27
Control				4,154 b	

[W] Disease control evaluation done 3/30/71.
[X] Handgun application, ca. 18.93 liters (5 gal)/tree.
[Y] Green-bud stage application, 2/15/71; pink-bud stage application, 2/24/71.
[Z] All shoot strikes counted on each tree. Seven single tree replications/treatment. Duncan's multiple range test ($P = .05$) used (3). Treatments followed by the same letter do not differ significantly.

For the Ripon plot sprayed with a handgun, pink-bud stage applications reduced disease 87-98% (Table 2). Green-bud stage treatments were variable, reducing disease 27-79%. Oil at 46.77 liter/hectare (5 gal/acre) reduced disease 44%. The Ripon plot had 4.17 cm (1.64 inches) of rain during the period between application dates and 100% petal fall. Warm temperatures during periods of rain favored high levels of disease in the untreated control. Partial run-off of the dilute spray could account for the lesser degree of control compared to the Durham plot (Table 1) where the semiconcentrate air-blast application resulted in no run-off of spray. Additions of oil did not affect the degree of control obtained at either plot site and may have increased run-off during application.

Bioassay of systemic fungitoxic activity in field-sprayed blossoms.—Blossoms taken from the Durham plot were incubated in food crispers for 109 hr after they had been spray-inoculated with *M. laxa*

TABLE 3. Methyl-2-^{14}C-benzimidazolecarbamate residue from parts of 'Drake' almond blossoms sprayed at green- and pink-bud stages at Davis, California[U]

Treatment[V]	Time of application[W]	Blossom tissue analyzed at full bloom	Per gm tissue (fresh wt)[Y, Z] Covered trees dpm	Covered trees μg ^{14}C-MBC	Uncovered trees dpm	Uncovered trees μg ^{14}C-MBC
^{14}C-MBC + benomyl 50W	green bud	petals	1,589	0.05	1,411	0.04
		P+S[X]	947	0.03	1,768	0.05
		calyx	3,057	0.16	25,357	0.72
^{14}C-MBC + benomyl 50W + oil	green bud	petals	1,528	0.04	927	0.03
		P+S	1,046	0.03	1,323	0.04
		calyx	34,157	0.97	19,292	0.66
^{14}C-MBC + benomyl 50W	pink bud	petals	8,887	0.22	14,513	0.41
		P+S	3,067	0.09	3,422	0.08
		calyx	37,024	1.05	38,274	1.14
^{14}C-MBC + benomyl 50W + oil	pink bud	petals	4,154	0.13	4,693	0.13
		P+S	1,046	0.03	1,356	0.04
		calyx	35,517	1.00	11,664	0.33

[U] Three trees were covered with an ultraviolet light-transmitting polyethylene house to prevent external redistribution of chemical on blossoms following prebloom treatment. Three trees were left uncovered to allow external redistribution.
[V] ^{14}C-MBC 30 μg (1.2×10^6 dpm) + benomyl 50W 0.57 mg/ml H_2O. Oil = Volck Supreme Oil 1% v/v.
[W] Treatment dates: covered trees–green-bud spray, 2/15/71; pink-bud spray, 2/23/71. Uncovered trees–green-bud spray, 2/16/71; pink-bud spray, 3/2/71.
[X] P+S = pistils + stamens.
[Y] Values shown are the mean of three replications. LSD .05 = 0.37.
[Z] No benomyl recovered by thin-layer methods from blossom parts from any treatments; only labeled and unlabeled MBC found.

conidia and then rated as previously described. The pink-bud stage application of benomyl at the higher rate with or without oil gave the highest degree of protection (Table 1). The lower rate of benomyl applied at pink-bud stage and the green-bud stage sprays gave less protection.

Comparison of recoveries of benomyl, unlabeled MBC, and ^{14}C-MBC from blossom parts.—Radioactivity was recovered from all blossom parts susceptible to infection by *M. laxa* following all prebloom spray treatments (Table 3). Oil did not significantly affect the levels of ^{14}C-MBC recovered from blossom parts. The radioactivity levels in blossom parts from covered and uncovered trees were similar, and timing of prebloom applications did not give rise to significant differences in radioactivity in blossom parts. Labeled and unlabeled MBC were the only fungicide residues recovered from the parts of blossom treated with MBC plus benomyl, even though relatively non-nucleophilic solvents were used in extraction and chromatography.

It must be noted here that MBC residues from calyces following green-bud stage sprays and from calyces and petals following pink-bud stage sprays would be partly external, since these tissues were exposed at treatment time. Because external distribution of the fungicide by rain was precluded on the covered trees, it is reasonable to assume that MBC in blossom parts from covered trees would result from internal systemic movement. Ogawa (8) has shown that *M. laxa* conidia on almond blossoms causes the greatest amount of infection in petals, followed by the anthers, stigmas, and filaments. He also showed that sepals and floral tubes are rarely infected. Results of in vitro growth studies (Fig. 2-B) indicate that 0.05 μg MBC/ml nutrient medium effectively inhibited growth of *M. laxa* from conidia (although germination was allowed to proceed for a period of ca. 12 hr first). ^{14}C-MBC residues found in covered blossom parts at full bloom (Table 3) ranged from 0.03 - 1.05 μg/gm tissue. Since the labeled MBC was added in a 1 : 10 ratio to formulated benomyl

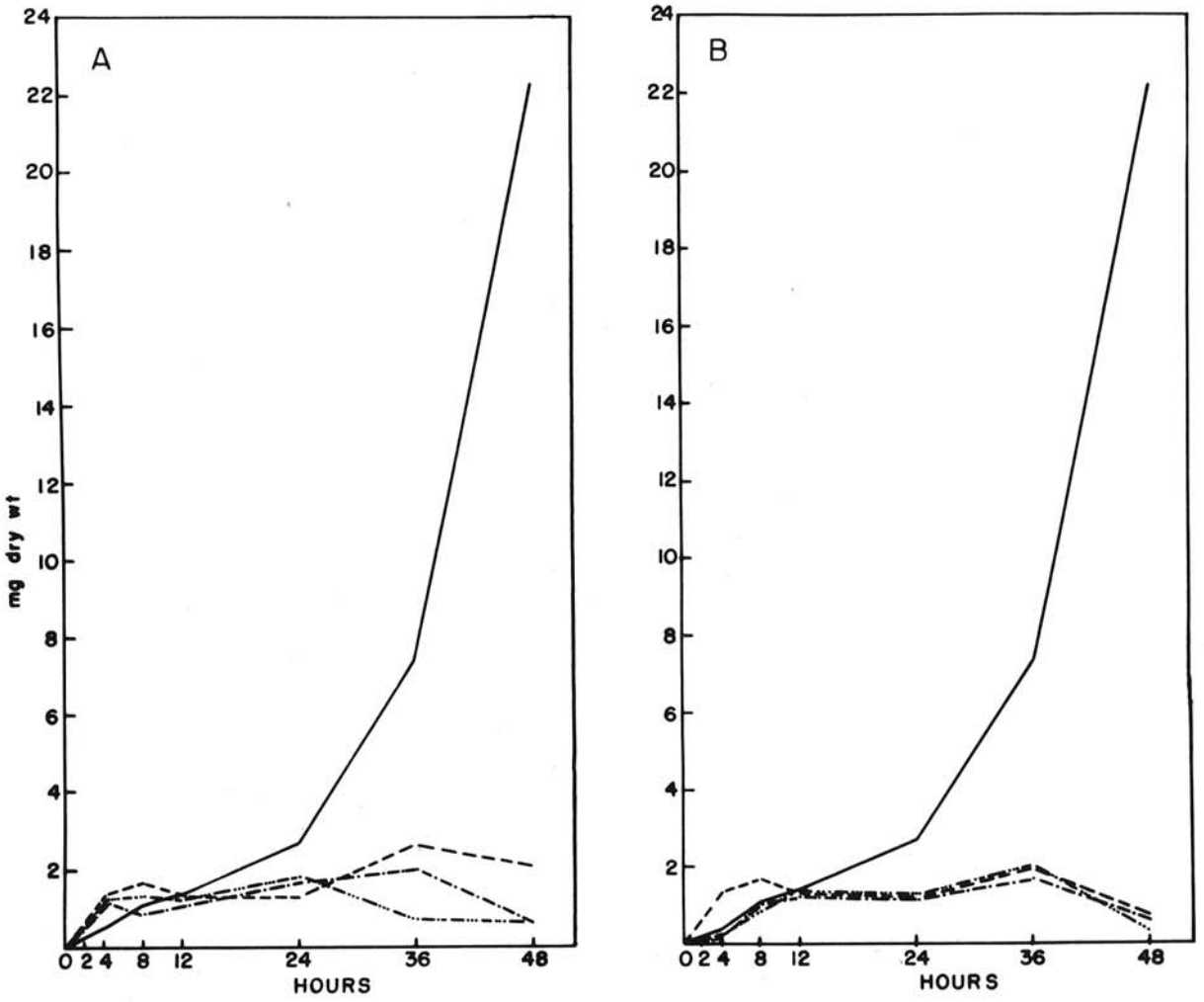

Fig. 2. A) Benomyl 0.05 μg/ml = ——; 0.1 μg/ml = —·—·—; 1.0 μg/ml = —···—···—. Control = ———. B) Methyl 2-benzimidazolecarbamate (MBC) 0.05 μg/ml = ——; 0.1 μg/ml = —·—·—; 1.0 μg/ml = —···—···—. Control = ———.

50W to make the spray mixture of 113.4 g (4 oz) active fungicide per 378.5 liters (100 gal) water, it is reasonable to assume that sufficient MBC would be present in blossom parts to inhibit the growth of *M. laxa*.

Little rainfall occurred on the uncovered trees in this experiment. Only 0.30 cm (0.12 inches) of rain fell between the green-bud application and blossom harvest at full-bloom stage and 0.10 cm (0.04 inches) fell between the pink-bud application and blossom harvest. Some additional free moisture did occur on blossoms because relative humidity reached 100% for periods during 8 days, mostly because of fog. Had there been more rain, greater differences between fungicide levels of parts of covered and uncovered blossom would have resulted, and a truer indication of the amount of natural external redistribution of the fungicide would have been obtained.

In vitro studies of the effects of benomyl and MBC on the growth of M. laxa from conidia.—Benomyl at 0.05, 0.1, and 1.0 μg/ml stimulated growth from conidia for the first 4 hr (Fig. 2-A) and inhibited growth after 12 hr. After 48 hr, 0.1 and 1.0 μg benomyl/ml inhibited growth 97%, whereas 0.05 μg/ml inhibited growth 88%. Only the 0.05 μg/ml level of MBC stimulated initial growth (Fig. 2-B), but MBC, like benomyl, at all rates tested inhibited growth after 12 hr. After 48 hr, MBC at 0.05, 0.1, and 1.0 μg/ml inhibited growth 96, 97, and 99%, respectively. No benomyl was recovered from the medium, even though samples were taken immediately after 10 μg of analytical grade benomyl were added to the test flasks. Total MBC in the benomyl-amended medium decreased from 9.6 μg at zero time to 1.0 μg at 48 hr. This decrease was largely due to uptake by the fungus, since in a previous similar experiment, ca. 50% of the radioactivity from .21 μcuries of ^{14}C-MBC (0.1 μg/ml medium) added to the medium was taken up by the fungus spores within 1 hr after addition of the labeled toxicant.

Other workers (10, 11) have reported that MBC is the main fungitoxic compound found in crop plants as a result of benomyl treatment through the roots. This is the first published report of the degradation of formulated benomyl 50W to MBC under field conditions as a result of topical spray application. Further, we have shown that prebloom spray applications of benomyl and MBC result in fungitoxic levels of only MBC in the parts of blossoms opened 6-14 days after application. This is the first case reported of a fungicide giving systemic fungicidal protection in the blossom parts of fruit trees as a result of prebloom application. These findings could result in earlier, and thus more flexible timing of protectant spraying of orchards for control of blossom diseases with benomyl by both ground and aircraft spray equipment.

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