Grove Application of Benomyl and its Persistence in Orange Fruit

G. Eldon Brown and L. Gene Albrigo

Plant Pathologist III, Florida Department of Citrus; and Assistant Horticulturist, University of Florida, Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, P.O. Box 1088, Lake Alfred, Florida 33850, respectively.

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ABSTRACT

Benomyl, detected as methyl 2-benzimidazolecarbamate (MBC), was found in the peel and juice of Hamlin and Valencia oranges as early as 1 day after application. Translocation of MBC from the foliage into the fruit was not detected. Residues of MBC in the orange peel were affected by rainfall which stimulated additional movement of the fungicide into the peel. MBC was still detected in the peel of Hamlin oranges 70 days after the application of benomyl (300 μg/ml) and could be detected in Valencia fruit 86 days after spraying the trees with 500 μg/ml of benomyl. MBC levels in the peel of both Hamlin oranges 70 days after benomyl application, and Valencia oranges 84 days after application, were sufficient to reduce the incidence of green mold. Disease control with benomyl was usually improved by adding oil, Biofilm, Nu-Film 17, or Vapor Gard to the spray mix.

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Grove applications of benomyl markedly reduced postharvest decay of citrus fruit caused by *Diplodia natalensis*, *Phomopsis citri*, and *Penicillium digitatum* (3, 4). Best control was obtained when benomyl was applied 1 week before harvest, but significant control was still achieved with a spray applied 4 months before picking (4). A single application of benomyl in June or July has shown promise for the control of citrus greasy spot, *Mycosphaerella citri* (16). This spray might serve a dual purpose by also reducing postharvest decay. Incorporating benomyl into a miticide spray (15) in September or October for control of postharvest decay may also be practical. The value of these benomyl sprays may be enhanced by adding spray adjuvants to improve or extend the activity of benomyl (6, 7, 13).

Details are given of the distribution of benomyl detected in the form of its breakdown product, methyl 2-benzimidazolecarbamate (MBC) (5, 9, 10, 11, 12, 14), on and in oranges after the application of benomyl to trees in the grove. The effects of spray adjuvants on distribution were also studied, and the adjuvants were evaluated according to their ability to enhance the fungitoxic activity of benomyl against *Penicillium digitatum* Sacc. and *Diplodia natalensis* P. Evans, the causal organisms of green mold and stem end rot, respectively.

MATERIALS AND METHODS.–Mature trees of *Citrus sinensis* (L.) Osbeck 'Hamlin' or 'Valencia' were sprayed with ca. 75 liters of a dilute suspension of benomyl (50% WP). Benomyl was sprayed alone and in combination with emulsified oil (Chevron Chemical Co., Richmond, Calif.), Biofilm (a mixture of alkylarylpolyethoxy ethanol, free and combined fatty acids, glycol ethers, di-alkyl benzenedicarboxylate, and isopropanol) (Colloidal Products Corp., Petaluma, Calif.), Wilt-Pruf (polyvinyl chloride) (J. A. Hartman Corp., Greenwich, Conn.), and Nu-Film 17 and Vapor Gard, two formulations of Pinolene (β-pinene polymer) (Miller Chem. & Fertilizer Corp., Hanover, Pa.).

We collected benomyl from the surface of samples of 10 unwashed fruit by immersing each fruit for 1 min in 300 ml of heated (65 C) hexane and CCl_4 (1:1), concentrating this to dryness under vacuum at 50 C, and dissolving the residue in 25 ml of chloroform. After removing the benomyl from the fruit surface, the peel was quartered longitudinally and removed. A 0.85 × 3 cm segment of peel (flavedo and albedo) was removed from the equatorial portion of each quarter. The flavedo was separated from the albedo, and each was extracted separately in chloroform. The extracts were allowed to stand 24 hr at 26 C so that the emulsion, formed between the peel oil and water, would separate from the chloroform. Each extract was then mixed with 0.4 g Celite No. 545, filtered through Whatman No. 41 filter paper in a Büchner funnel, and concentrated to dryness under vacuum at 50 C. The residue was dissolved in 5 ml of chloroform. After removal of the peel, the 10 fruit of some of the samples were comminuted in a blender, and the juice was separated from the coarser pulp by a straining through cheesecloth. Acetone at the rate of 183 ml was added to a 250-ml sample of the juice. After 5 min, the pulp precipitated by the acetone was separated by filtration from the juice, with 0.4 g Celite No. 545 as a filter aid. The pulp was extracted again with boiling chloroform which was filtered. The juice was extracted twice with 100 ml of chloroform, and then this chloroform was combined with the chloroform extract of the pulp and concentrated to dryness under vacuum at 50 C. The residue was dissolved in 25 ml chloroform. Varying amounts of the chloroform extracts of benomyl were spotted on Eastman Chromagram sheets 6061 (silica gel without fluorescent indicator) and developed in acetone (10) or ethyl acetate/chloroform (60:40, v/v) (14). MBC on the chromatograms was determined quantitatively by the bioautograph technique of Peterson & Edgington (10).

Fruit sprayed with benomyl and held in storage for decay evaluations were degreened when necessary by treatment with ethylene (5 μliters/liter) for 72 hr at 30 C and 88-92% relative humidity. Immediately after harvest or after degreening when required, the fruit were washed and waxed with a commercial solvent wax. We mechanically injured fruit inoculated with *P. digitatum* by rolling them over a board containing seven wire nails protruding 1-2 mm spaced 10 mm apart. We dipped the injured fruit in an aqueous spore suspension prepared by washing spores from 7- to 10-day-old cultures grown on Bacto potato-dextrose agar (PDA) in petri dishes held at 24

TABLE 1. Distribution of methyl 2-benzimidazolecarbamate (MBC) in the peel of Hamlin oranges during the 14 days following treatment with benomyl by dipping individual fruit in the spray mix or spraying the tree[a]

Days after treatment	Rainfall since last sampling (cm)	μg MBC/g peel: Surface		Flavedo		Albedo	
		Dipped	Sprayed	Dipped	Sprayed	Dipped	Sprayed
1		11.01 q[b]	14.85 p	2.23 uvw	2.78 tuv	0.14 z	0.16 z
3	0.0	8.94 r	14.14 p	1.50 vwxyz	1.91 vwxy	0.16 z	0.22 z
7	0.8	5.73 s	6.66 s	2.07 uvwx	3.63 tu	0.33 yz	0.73 wxyz
14	1.0	4.19 t	5.61 s	1.85 vwxy	2.27 uvw	0.47 xyz	0.73 wxyz

[a] Average of two replications of 10 fruit each treated with 1,000 μg of benomyl/ml.
[b] Values followed by unlike letters are significantly different at the 5% level of probability.

C. Noninoculated and inoculated fruit were stored at 21 C in 0.03-m^3 ventilated fiberboard cartons.

RESULTS.–Benomyl was degraded rapidly to MBC during residue analysis. Therefore, any benomyl which may have been present on or in orange fruit at sampling was identified as MBC on the chromatograms.

We evaluated distribution of MBC on and in the peel of Hamlin oranges during the 14 days after the application of benomyl by taking fruit from sprayed trees or unsprayed trees where only the fruit were dipped in benomyl (Table 1). Fruit from sprayed trees usually contained significantly more MBC on the surface than did dipped fruit from unsprayed trees. Decreasing quantities of MBC were usually recovered from fruit surfaces of both dipped and sprayed fruit at each sampling following the application of benomyl. A significant part of the decrease in surface residues of MBC was associated with rainfall that fell in two showers between the 3rd and 7th days from treatment. Levels of MBC in the flavedo of both dipped and sprayed fruit remained relatively unchanged after benomyl application during a period of nightly dews but no rainfall. However, at 7 days after treatment and after rainfall, MBC in the flavedo of sprayed fruit exceeded the amount found at 3 days. By 14 days, MBC residues in the flavedo seemed to be declining even though additional rainfall had occurred. MBC moved into the albedo of both dipped and sprayed fruit within 1 day, and gradually accumulated to a relatively constant level at 7 and 14 days. Small quantities of MBC (0.005 μg/ml) were detected in the juice 1 day after treatment. By the 14th day, this had increased to 0.065 μg/ml in dipped fruit and 0.109 μg/ml in sprayed fruit.

Translocation of MBC from the foliage into the fruit was not detected in any fruit protected with plastic bags during spraying and rainfall.

We observed redistribution of MBC 15 days after the application of benomyl by attaching untreated fruit to limbs of the sprayed trees, which were then sprayed with 114 liters of water. MBC in the amount of 0.126 μg/cm^2 of peel was detected 1 day later on the surface of the untreated fruit.

TABLE 2. Surface residues of methyl 2-benzimidazolecarbamate (MBC) on Hamlin oranges following a grove application of benomyl[a]

Days from application	μg MBC/cm² of fruit surface: Benomyl	Benomyl + Wilt-Pruf[b]	Benomyl + Nu-Film 17[b]
1	0.52 x[c]	0.53 x	0.70 w
14	0.17 z	0.18 z	0.34 y
28	0.10 z	0.06 z	0.19 z
56	0.05 z	0.09 z	0.14 z
70	0.03 z	0.10 z	0.13 z

[a] 300 μg/ml.
[b] 0.5 ml/liter.
[c] Values followed by unlike letters are significantly different at the 5% level of probability.

TABLE 3. Changes in methyl 2-benzimidazolecarbamate (MBC) content of the flavedo and albedo of Hamlin oranges after a grove application of benomyl[a]

Days from application	μg MBC/g tissue: Flavedo	Albedo
1	0.40 x[b]	0.10 z
14	0.56 w	0.27 xy
28	0.40 x	0.19 yz
56	0.32 xy	0.11 z
70	0.08 z	0.05 z

[a] 300 μg/ml.
[b] Values followed by unlike letters are significantly different at the 5% level of probability.

Surface residues of MBC on Hamlin oranges in a commercial grove receiving overhead irrigation were determined at various intervals for 70 days after application of benomyl (Table 2). The addition of Nu-Film 17 to benomyl resulted in an increase in surface residues of MBC at 1 and 14 days after application. By 70 days, 94% of the MBC detected at 1 day after treatment had disappeared from the surface of fruit receiving only benomyl, whereas 81% had been lost from fruit surfaces receiving benomyl with Wilt-Pruf or Nu-Film 17. MBC content of the peel was increased only slightly by the addition of either Wilt-Pruf or Nu-Film 17 to the benomyl spray, and at 1 day from application, these adjuvants retarded the movement of MBC into the albedo. Residues of MBC in the flavedo from the application of benomyl were usually greater than those in the albedo (Table 3). The highest residues of MBC in the flavedo and albedo were found 14 days after spraying, and some MBC could still be detected in the peel at 70 days.

At 42 days after application, sufficient MBC to reduce the incidence of green mold (Table 4) was still present in the peel from the application of benomyl or benomyl with any one of the spray adjuvants. However, at 70 days after spraying, control of green mold was improved where benomyl had been applied with oil, Nu-Film 17, or Biofilm (Table 4). Levels of MBC in the flavedo and albedo at 70 days (Table 3) were near the calculated ED_{50} value (0.08 μg/g) of benomyl for activity against the growth of *P. digitatum* in PDA. Oil, Nu-Film 17, or Biofilm applied with benomyl apparently increased the MBC residue sufficiently to reduce the occurrence of green mold (Table 4). The addition of adjuvants to benomyl had no significant effect on the control of stem end rot with benomyl (Table 4).

We studied the influence of Nu-Film 17 and Biofilm on benomyl activity, based on residue tests and on decay control, with Valencia orange trees using applications of benomyl at 300 μg/ml with or without the addition of these adjuvants at 0.5 ml/liter. We slightly increased quantities of MBC on the fruit surface by adding Nu-Film 17 or Biofilm to the benomyl spray; but by 57 days after application, these differences were negligible. At 57 days after application, 0.10, 0.12, and 0.14 μg/g of MBC were

TABLE 4. Postharvest decay of Hamlin oranges harvested 42 and 70 days after spraying with benomyl and benomyl plus spray adjuvants[a]

	% decay			
	42 days[b]		70 days	
Treatment	Stem end rot[c]	Green mold[e]	Stem end rot[d]	Green mold[f]
Check	32.8 y[g]	97.2 y	20.0 y	99.6 w
Benomyl (300 μg/ml)	5.8 z	38.9 z	7.1 z	95.8 wx
Benomyl + oil (2.5 ml/liter)	9.4 z	36.1 z	11.8 yz	73.8 z
Benomyl + Wilt-Pruf (0.5 ml/liter)	4.7 z	39.5 z	12.9 yz	89.6 wxy
Benomyl + Nu-Film 17 (0.5 ml/liter)	7.5 z	27.2 z	7.5 z	76.7 yz
Benomyl + Biofilm (0.5 ml/liter)	2.5 z	33.9 z	5.8 z	72.5 z

[a] Treatments were applied to three replicates of single trees.
[b] Degreened.
[c] One hundred twenty fruit/replication.
[d] Eighty fruit/replication.
[e] Sixty fruit/replication artificially inoculated with a suspension of *Penicillium digitatum* spores (0.03 absorbance at 450 mμ).
[f] Eighty fruit/replication artificially inoculated with a suspension of *Penicillium digitatum* spores (0.07 absorbance at 450 mμ).
[g] Values within each column followed by unlike numbers are significantly different at the 5% level of probability.

TABLE 5. Distribution of methyl 2-benzimidazolecarbamate (MBC) in the peel of Valencia oranges following a grove application of benomyl[a] with and without the addition of Vapor Gard

	Rainfall	μg MBC/g peel[b]								
Days after treatment	since last sampling (cm)	Benomyl Surface	Benomyl Flavedo	Benomyl Albedo	Benomyl + 1% Vapor Gard Surface	Benomyl + 1% Vapor Gard Flavedo	Benomyl + 1% Vapor Gard Albedo	Benomyl + 3% Vapor Gard Surface	Benomyl + 3% Vapor Gard Flavedo	Benomyl + 3% Vapor Gard Albedo
7		4.60 h[c]	1.31 q	0.09 z	6.13 g	2.30 m	0.39 uvwx	4.65 h	2.06 n	0.54 u
30	1.9	2.90 l	0.50 uv	0.03 z	4.37 i	0.41 uvwx	0.09 z	3.43 k	0.33 wx	0.08 z
61	7.3	0.44 uvw	0.85 t	0.35 wx	3.60 j	1.16 r	0.54 u	2.40 m	1.03 s	0.37 vwx
86	4.5	0.41 uvwx	0.27 xy	0.00 z	1.60 p	0.15 yz	0.03 z	1.88 o	0.39 uvwx	0.05 z

[a] 500 μg/ml.
[b] Treatments were applied to two replications of three-tree plots.
[c] Values followed by unlike letters are significantly different at the 5% level of probability.

present in the flavedo of oranges sprayed with benomyl, benomyl + Nu-Film 17, and benomyl + Biofilm, respectively. Green mold was significantly reduced in fruit harvested 57 days after spraying with benomyl, but no improvement in control was obtained by the addition of either Nu-Film 17 or Biofilm.

The effect of high rates (1 and 3%) of the antitranspirant, Vapor Gard, on the persistence and distribution of benomyl on and in the peel of Valencia oranges was determined for the 86 days after application (Table 5). During the 59 days following spraying, rain of only 3.0 cm fell during 11 showers; but on the 2 days preceding sampling on day 61, a total of 6.3 cm fell in two heavy showers. Applications of benomyl with either 1 or 3% Vapor Gard left deposits of MBC on fruit surfaces which persisted more readily during the 86 days than did deposits of MBC from benomyl applications alone. At 7 days after application, more MBC was present in the peel of fruit receiving the two Vapor Gard and benomyl combinations than in fruit receiving benomyl alone; but this difference was not observed 30 days after application. Significantly more MBC was present in the flavedo and albedo of fruit from all treatments at 61 days than was present at 30 days (Table 5). This increase seemed to be associated with the 6.3 cm of rain that fell just preceding sampling at 61 days. At 86 days, levels of MBC in the flavedo of fruit from all treatments had again decreased, with only slight or no MBC being detected in the albedo (Table 5).

A mixture of benomyl and Vapor Gard was generally more effective against green mold than was benomyl alone (Table 6). At 84 days after a spraying, fruit were picked and the flavedo was injured, then inoculated with *P. digitatum*. Green mold developed in 52.5% of the fruit sprayed with benomyl, but only in 37.5% of the fruit sprayed with benomyl and 3% Vapor Gard. Inoculations into injuries made into the albedo where residues of MBC were quite low resulted in higher decay than where inoculations were made into injuries confined to the flavedo where somewhat higher MBC levels existed (Table 6).

DISCUSSION.—*Distribution and uptake of MBC.*—The MBC detected within the fruit apparently

TABLE 6. Development of green mold in Valencia oranges sprayed with benomyl with and without the addition of Vapor Gard and artificially inoculated with *Penicillium digitatum*

	% Decay[a]			
	Depth of injury (mm)			
Treatment	1[b]	1.5[b]	1.5[c]	2[c]
Check	62.5 y[d]	91.3 y	93.8 x	100.0 y
Benomyl (500 μg/ml)	21.3 z	30.0 z	52.5 y	80.0 z
Benomyl + 1% Vapor Gard	11.3 z	21.3 z	43.8 z	75.0 z
Benomyl + 3% Vapor Gard	11.3 z	22.5 z	37.5 z	66.3 z

[a] Average of two replicates of 40 fruit each inoculated with a spore suspension of 0.09 absorbance at 450 mμ.
[b] Picked and inoculated 76 days after spraying.
[c] Picked and inoculated 84 days after spraying.
[d] Values within each column followed by unlike letters are significantly different at the 5% level of probability.

moved in from the fruit surface, as the highest residues of MBC were always found on the fruit surface with decreasing amounts in the flavedo, albedo, and juice. Also, translocation of MBC from the foliage into the fruit could not be shown. After the application of benomyl, residues of MBC on the fruit surfaces decreased, but those within the peel usually increased for a period of time as further movement of MBC occurred from the fruit surface. Rainfall appeared to be more effective in stimulating additional uptake of MBC from the fruit surface into the peel than did dew. Since some areas of the surfaces of oranges are covered with epicuticular wax platelets (1), dew may not as effectively redistribute MBC laterally off these platelets to uptake sites of the cuticle as does rainfall. Redistribution of MBC from the foliage to the fruit could also occur during rainfall. In this study, we did not eliminate the possibility of some uptake of MBC through the roots from runoff at application or through subsequent erosion of the foliar deposits.

Persistence of MBC and its effect on decay.—Within plant tissues, MBC has been shown to decrease with time due to breakdown or complexing (2, 8, 11). In the peel of Hamlin oranges after 70 days, the quantity of MBC had decreased to a level which did not effectively control green mold. In Valencia oranges, however, some inhibition of *P. digitatum* did occur in fruit inoculated 84 days after spraying. Some control of postharvest decay due to the persistence of MBC in the fruit from benomyl applied in September or October could be expected in citrus cultivars harvested in November or December. However, decay control would be limited from benomyl applied in June or July for greasy spot control because MBC would not be expected to persist until November or December.

Most spray adjuvants evaluated in this study slightly extended the effectiveness of residues of MBC, particularly those on the fruit surfaces. The use of adjuvants usually improved decay control over that obtained with benomyl alone.

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