Detection and Translocation of Benomyl in Postharvest-treated Peaches and Nectarines

Douglas J. Phillips

Plant Pathologist, Market Quality and Transportation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 2021 South Peach Avenue, Fresno, California 93727.

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ABSTRACT

Diffusion-plate bioassays with *Monilinia fructicola* detected benomyl residues on peaches and nectarines treated after harvest with benomyl and 2,6-dichloro-4-nitroaniline suspended in emulsified fruit wax. The weight of wax-fungicide mixture per treated fruit was positively correlated with the inhibition zone of the bioassay and was used to estimate the amount of benomyl residue on stone fruits. Benomyl estimated to be 2-4 μg/g, based on the fresh weight of the fruit, controlled brown rot on inoculated peaches treated experimentally, and on noninoculated peaches treated commercially in packing houses in California. Decay of inoculated peaches was controlled effectively during 3 weeks of storage at 4 C and during subsequent ripening at 21 C. The assay of residues upon or within these fruits indicated that benomyl or a derivative of this fungicide was translocated 4 mm into the peach mesocarp during storage.

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Additional key words: stone fruits, fungicides.

Postharvest application of benomyl effectively controls brown rot of peaches, nectarines, and plums incited by *Monilinia fructicola* (Wint.) Honey (6, 13, 14). Diffusion plate bioassay (3) bioautography (8), or chemical analysis (7, 15) may be used to determine benomyl residue of treated plants. I report on (i) an adaptation of the diffusion-plate bioassay for detection of benomyl in postharvest-treated stone fruits; (ii) the fungitoxic residue required for effective control of brown rot during short or prolonged storage; and (iii) the movement of either fungicide, or fungitoxic breakdown products, into the peach during storage at 4 C for 3 weeks.

MATERIALS AND METHODS.—*Technique for diffusion plate bioassay.*—Cultivars of freshly harvested peaches (cultivar Sunkist, and an unknown cultivar) or nectarine (cultivar, Regular Sun Grand) were obtained from orchards where fungicides had not been applied. One cultivar was used per test. Each treated fruit was measured (two major axes) and dipped for 10 seconds into a 1:10 (v/v) water dilution of Decco Peach Wax WT-52 (Pennwalt Corp., Monrovia, Calif.), that contained (i) no fungicides, (ii) 300 μg/ml active benomyl formulated from a 50% wettable powder (WP), (iii) 300 μg/ml active 2,6-dichloro-4-nitroaniline (DCNA) from a 75% WP, or (iv) 300 μg/ml benomyl and 300 μg/ml DCNA. In order to measure the fungicide applied by each dip treatment, single fruits were suspended from strings (5 fruit per treatment), weighed, and dipped into the various mixtures of fungicide. Several seconds after excess liquid had dripped off the fruit each fruit was re-weighed. The fruit was not touched, or the string wetted, during dipping and weighing. Treated and weighed fruits were placed on trays, covered with polyethylene, and placed at 2 C for 24 hours prior to the bioassay.

A 1.3-cm-diameter cylinder was cut with a cork borer from an area on each treated fruit not touched by the tray

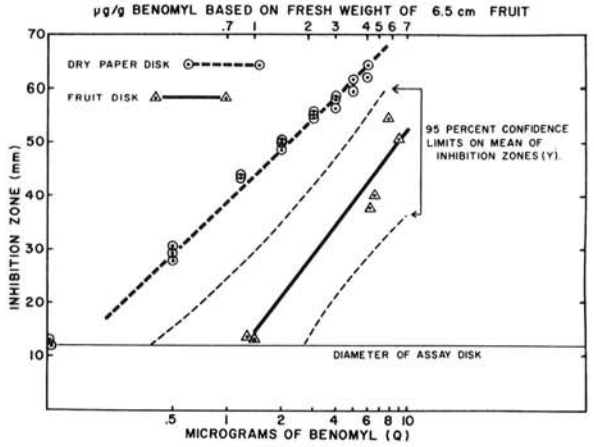

Fig. 1. The effect of different concentrations of benomyl applied to fruit or paper disks on inhibition of *Monilinia fructicola* on agar plates. A spore suspension was sprayed on the medium and disks after 24 hours at 4 C. Benomyl was solubilized by conversion to MBC before adding to the paper disks. Nonsolubilized benomyl was applied to whole peaches or nectarines in a wax emulsion 24 hours before disks were cut from the fruit. $Log_{10}Q$ (abscissa) was used for regression analysis where Q = μg of benomyl on the surface of the fruit, fruit line [IZ = 45Q + 7, correlation coefficient (r) = 0.97], paper line (IZ = 31Q + 40, r = 0.99). Each point represents the mean of at least five fruit or paper disks. The micrograms of benomyl was calculated for each fruit separately.

or plastic cover. Disks, 1-mm thick, were cut from the cylinder with a curved fruit-peeling knife equipped with a slice-thickness guide. The disks were placed upper-surface-downward on a solid agar medium in plastic petri dishes (diameter 100 mm). Then an additional 13-ml of the medium (which contained 20 g strained peach baby food, 20 g agar, and 0.1 g chloramphenicol per liter) was dispensed into each plate. The peeling knife and cork borer were rinsed in water after each use. The plates with disks were held at 0 to 4 C for 24 hours, then sprayed with a conidial suspension (ca. 5,000 conidia/ml) derived from 1-week-old cultures of *M. fructicola* grown on potato-dextrose agar. The disks were then incubated 5 to 6 days at ca. 22 C, after which the diameters of the inhibition zones (IZ) were recorded.

Inhibition zones obtained on plates with fruit disks were compared with zones of known residues of benomyl placed on 1.3-cm diameter filter paper disks. Because preliminary tests with freshly suspended benomyl on the paper disks produced erratic results, the benomyl was solubilized to form benzimidazolecarbamate (MBC) by autoclaving (5 minutes at 1.0 atmosphere) a suspension containing 1.0 g of actual benomyl formulated from a 50% WP with 1.0 ml of concentrated HCl per liter of water (7, 12, 15). This MBC was diluted in water and added to the paper disks. Seven dilutions were prepared to give a range of from 0.5 μg to 60 μg benomyl per paper disk. Three tests using five disks per dilution established the dosage response of one isolate of *M. fructicola* (Fig. 1). This isolate was used in all tests; it did not differ in benomyl sensitivity from nine other isolates collected in 1971 from various orchards in Fresno and Tulare Counties of California.

Inoculation, treatment, and storage of peaches for bioassay.—Before treatment, the fruit was inoculated by spraying the surface of the fruit with a water suspension of 200 conidia/ml. The fruit was loosely packed in wood lugs covered with plastic and held 12 to 18 hours at 21 C before being treated. In the first test, inoculated 49er peaches were dipped for 10 seconds into: wax alone (1:10 dilution of WT-52 wax), wax plus 300 μg/ml DCNA, wax plus 300 μg/ml DCNA plus 300, 600, or 900 μg/ml benomyl, and one lot neither inoculated nor dipped into the wax-fungicide. This procedure resulted in a total of six treatments (440 peaches per treatment). In a second test, Summerset peaches were similarly treated, but 50, 150, and 300 μg/ml benomyl was applied with wax and DCNA.

After the treatments, all fruit was repacked into lugs and stored at 4 C. One hundred and ten fruits from each treatment were removed from storage after 1, 7, 14, or 21 days. At each holding time, four serial disks of peach pericarp were cut from 10 fruits from each treatment for bioassay. The outer disk from each fruit was placed on

TABLE 1. Fruit rot (brown rot) in peaches ripened 4 days at 26 C after storage for 1, 7, 14, and 21 days at 4 C, and a postharvest dip treatment of benomyl and 2,6-dichloro-4-nitroaniline (DCNA) suspended in a wax-emulsion[a]. Peaches were inoculated with conidia of *Monilinia fructicola* 12-18 hours before treatment

Fungicide concentration in dip (μg/ml)[a]		Incidence of rot (%)[b,c] after 4 days' ripening following storage at 4 C for							
		1 day		7 days		14 days		21 days	
Benomyl	DCNA	BR	O	BR	O	BR	O	BR	O
0[d]	0	2	2	2	2	2	2	6	1
0	0	64	1	46	2	55	2	48	2
0	300	40	1	40	1	42	1	56	0
50	300	0	2	2	2	2	0	22	0
150	300	0	3	0	7	2	2	0	9
300	300	1	3	1	9	0	8	0	10
600	300	0	4	0	2	0	13	0	21
900	300	0	3	0	2	0	5	0	11

[a] Fungicides were added to a 1:10 (v/v) dilution of Decco WT 52 peach wax.
[b] BR = brown rot; O = other rots.
[c] The results of two tests: test 1, cultivar 49 er; and test 2, cultivar Summerset. Each datum represents at least four samples of 25 fruit.
[d] Top line is noninoculated control. Fruit for all other treatments was inoculated.

one plate, and the remaining disks on a second bioassay plate.

The 100 remaining fruits from the treatment samples were placed in fruit lugs (25 fruit per lug) fitted with plastic trays. The lugs were then held in random blocks for 4 days at 26 C, after which the fruit was evaluated for decay or injury.

RESULTS.—*Benomyl residue on treated fruit (string dipped).*—An increase in the weight of wax-fungicide suspension remaining on fruit after it was dipped resulted in an increased IZ around disks taken from the fruit (Fig. 1). The residue on each cultivar differed because of the size and texture of the fruit. Peaches held four to seven times more residue than nectarines. The actual amount of benomyl on the 1.3-cm disk was calculated for each fruit and cultivar from the weight of the fungicide-mixture per fruit and the surface area of the fruit; area = $\pi[1/2\,(\text{long axis of fruit} + \text{short axis of fruit})]^2$. The amount of benomyl applied, based on the fresh fruit weight (μg/g fresh wt) also was calculated.

A plot of the points of the logarithms of benomyl concentrations on the surface of the fruit disks (Q) against IZ, fits a straight line reasonably well (correlation coefficient = .97, Fig. 1). A similar plot of the bioassay data obtained with paper disks containing MBC from benomyl, formed a second line that was nearly parallel to that based on the fruit disks. However, at any given concentration, the IZ from a paper disk was greater than the IZ from a fruit disk (Fig. 1). Addition of 300 μg/ml DCNA to the wax benomyl dip did not affect the size of the IZ and the results of the benomyl-DCNA treatments were included in the fruit-disk analysis (Fig. 1).

Decay of treated fruit after storage.—Peaches dipped into 50 μg/ml benomyl developed little brown rot after 2 weeks of storage at 4 C, and those treated with 150 μg/ml were protected for 3 weeks (Table 1). Loss from other rots, mainly Alternaria rot, increased with time of storage, where benomyl treatments controlled brown rot.

Benomyl activity was found in assay disks from 1, 2, 3, or 4 mm deep after the fruit had been held 1, 7, 14, or 21 days, respectively (Fig. 2). The bioassay indicated that activity (inhibition ring = IZ − diameter of disk) increased as the concentration of benomyl in the dip treatment increased. Activity decreased with depth into the fruit. While activity in the upper 1.0 mm did not vary greatly with time, the total activity in the top 4-mm increased with time. When fungitoxic activity was found in the uppermost disk, brown rot was controlled.

Three hundred μg/ml DCNA applied in wax resulted in a 16% reduction of brown rot. While DCNA has been reported to penetrate peach flesh (11), the bioassay did not detect it, probably because of the low solubility and poor movement of DCNA in the agar medium. Application of 300 μg/ml DCNA to fruit in this test resulted in an average residue of 2-4 μg/g DCNA, based on the weight of fresh fruit, as determined by gas chromatographic techniques (M. Uota, *unpublished*). Commercial postharvest treatments often result in residues within this range and are applied to control Rhizopus rot.

DISCUSSION.—Our data indicate that the bioassay standardized in these tests can be used to estimate initial benomyl residues ranging from 1 to 7 μg/g fresh wt on

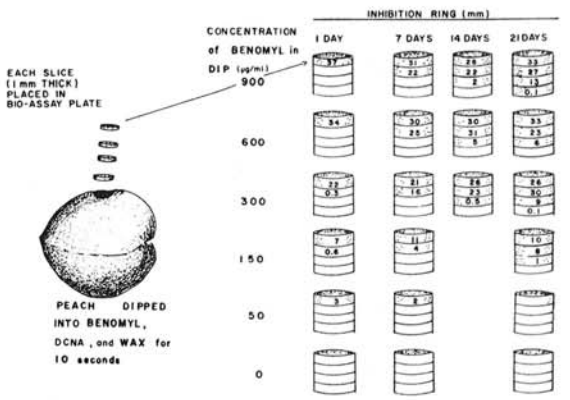

Fig. 2. Benomyl activity found by bioassay of disks taken from postharvest treated peaches after 1, 7, 14, and 21 days of storage at 4 C. Each datum represents at least 10 observations at each time and depth. Data are from two tests; one test used peaches (cultivar, 49er) dipped 10 seconds in 900, 600, or 300 μg/ml benomyl with 300 μg/ml 2,6-dichloro-4-nitroaniline (DCNA) diluted in emulsified wax; the second test used Summerset peaches dipped in 300, 150, or 50 μg/ml benomyl in the DCNA and wax.

postharvest-treated peaches (Fig. 1, upper scale). However, since no chemical analysis was used to confirm the estimates of residue based on the bioassay, and because of variation in fruit size, the bioassay should be used only as a guideline to the residue on stone fruits. By monitoring fruit treated commercially in 1972 and 1973 in Fresno and Tulare Counties, California, we found the assay to be of value as an indicator of benomyl residue and brown rot control.

A 2-4 μg/g benomyl residue, indicated by the bioassay, controlled the postharvest development of brown rot. This was true for peaches, nectarines, or plums treated with DCNA-benomyl-wax postharvest sprays in our tests or commercially in 1972 and 1973. The reported conversion of benomyl to MBC (2, 8, 9, 10, 11) suggests that the activity detected in the bioassay and the control of brown rot are largely due to MBC. The rate of loss of the fungitoxic product on peaches appears to be slow enough at 4 C to maintain a relatively high concentration of active material on the fruit surface. A 2-4 μg/g fresh wt initial residue controlled brown rot development for 3 weeks, whereas a 0.5 to 1.5 μg/g fresh wt residue protected the peaches only 2 weeks in 4 C storage. Translocation into the fruit reported here and by others (4, 11) may lower the active concentration on the surface of the peach to a point where control is lost and rot develops. The active substance found by the bioassay was translocated at least 4 mm into the mesocarp, but lower concentrations not detected in the assay may have occurred deeper in the peach. Our data suggest that an active component from benomyl moves more slowly in peach fruit than in water (8), but at a rate similar to that in the peel of oranges (1).

Transpiration and growth are important in the distribution of MBC through plant organs (5, 10). Transpiration or growth of peach fruit would be minimal in cold storage, particularly where a high relative humidity is maintained. Translocation of benomyl, or a

derivative of it, into peaches in storage seems to be by passive diffusion into the mesocarp.

Rot incited by *Alternaria* sp. is not controlled with benomyl or DCNA (6). Alternaria rot occurred in our test only after the storage life for peaches at 4 C had been approached or exceeded, and probably would not be a problem unless the fruit had been injured by handling or storage.

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