Residues of Benomyl (Determined as Carbendazim) and Captan in Postharvest-Treated Pears in Cold Storage[†]

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Residues of carbendazim (applied as benomyl) and captan, applied to pears prior to cold storage, were examined during 6 months of storage. Total carbendazim residues were below the 7 ppm tolerance, initial residues averaging 0.37 ppm, and no reduction of residues was detected during cold storage. In another study, three postharvest application techniques were compared and again no significant differences in the quantities of the residues found. Captan residues on Anjou pears were insignificant. The analytical procedure used for these analyses was an acetone extraction of acidified pear puree followed by partitioning with ethyl acetate to separate total carbendazim (benomyl plus carbendazim) and captan residues. For captan residue, charcoal and silica gel cleanup of the organic extract was followed by electron capture GLC. The pH of the aqueous extract containing carbendazim was adjusted to about 9 with sodium hydroxide, and the carbendazim residue was partitioned into ethyl acetate and analyzed by HPLC.

Large quantities of fungicides are used to control postharvest decays of fruits that are tightly packed, stored for long periods, handled in bulk, and shipped for long distances (Ogawa and Manji, 1984). The use of chemicals for the control of postharvest diseases has been reviewed by Eckert and Ogawa (1988). Two of the fungicides found useful for the control of postharvest rots and molds are benomyl [methyl [1-(butylcarbamoyl)-1H-benzimidazol-2-yl]carbamate] and captan [N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide]. Since there is very little information available in the literature describing the fate of these fungicides after postharvest application to fruit, information about the magnitude of residues of these compounds in postharvest-treated pears is reported here.

The study described here required determination of residues of both carbendazim as degradation compound of benomyl and captan in each sample of fruit. The method developed in this laboratory involves initial extraction of acidified pear puree with acetone. Captan residues were then partitioned into ethyl acetate, treated with charcoal, and passed through a silica gel column before quantitation by electron capture GLC. Benomyl and carbendazim residues were isolated by adjusting the pH of the initial extract with sodium hydroxide to about 9 followed by extraction into ethyl acetate and analysis by HPLC.

EXPERIMENTAL PROCEDURES

Treatment of Fruit. *Anjou Pears, 1982.* The pears were placed in cold storage after harvest September 15, 1982, until November 29, 1982, when they were removed from cold storage and treated with a suspension consisting of 0.45 kg of 50% Benlate WP, 0.45 kg of 85% captan WP, and 1 kg of ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, used for scald control) in 189 L of waxing solution (Primafresh 30, Johnson's Wax, Racine, WI). This suspension was sprayed onto the fruit followed by sponging to achieve uniform coverage. The fruits

were then passed through a drying tunnel at 40–50 °C for about 1 min before being packed into boxes for storage. The application rate for pears was 3.8 L of the suspension/~6200 kg of fruit. The pears were stored at −1 °C in ambient atmosphere at 94–95% relative humidity.

Anjou and Bosc Pears, 1983. The effect of three different benomyl treatments on postharvest residues was studied during the 1983 season. All treatments were made immediately after harvest, September 29, 1983. The first treatment consisted of an aqueous line spray of 0.45 kg of 50% Benlate WP in 379 L of water; the fruits were dried after the spray but not waxed. Pears receiving the second treatment were passed through a line spray of 0.45 kg of 50% Benlate WP followed by waxing with Primafresh 30, sponging, and drying. The third postharvest treatment studied was similar to the 1982 treatment where benomyl was applied with the waxing solution consisting of 0.45 kg of 50% Benlate WP and 1.0 kg of ethoxyquin in 189 L of waxing solution (Primafresh 30). The pears were then sponged, dried, and stored at −1 °C in ambient atmosphere at 94–95% relative humidity.

The postharvest treatment of Bosc pears was identical with the 1983 first treatment of Anjou pears.

Reagents. Analytical grade benomyl (99+% purity) was supplied by the Biochemicals Department, E. I. du Pont de Nemours and Co., Wilmington, DE, and captan (99% purity) by Ortho Division, Chevron Chemical Co., Richmond, CA. All solvents were distilled-in-glass grade and determined to be suitable for use without further treatment. Anhydrous sodium sulfate was thermally treated for 15 h at 500 °C. The charcoal powder (Norite A, Alkaline Decolorizing Carbon, Fisher Scientific Co., Pittsburgh, PA) was used as received and silica gel (Grade 950, 60–200 mesh, Davidson Chemical Division, W. R. Grace and Co., Baltimore, MD) was stored at 130 °C and then cooled and deactivated by addition of 7% water no less than 24 h before use.

Apparatus and Operating Conditions. *High-Performance Liquid Chromatography.* A Waters liquid chromatograph consisting of a Model 6000A solvent delivery system, a Model U6K injector, and a Model 440 absorbance detector operated at 280 nm was used. A Model 660 solvent programmer was used to control the pumps and allowed the injector, column, and detector to be purged of aqueous solvents daily. A Waters Z-Module radial compression separation system equipped with a 10 cm × 5 mm i.d. 10 μm μBondapak CN column was operated isocratically at 1.5 mL/min with 60% tetrahydrofuran, 40% water mobile phase.

Gas–Liquid Chromatography. A Varian Model 3740 gas chromatograph equipped with a [63]Ni electron capture detector was

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used. Chromatography was performed on a 90 cm × 1/8 in. (o.d.) × 1.5 mm (i.d.) Teflon column packed with a specially bonded and deactivated Carbowax 20M support prepared as follows. Carbowax 20M was thermally bonded at 270 °C to 100–200-mesh Chromosorb WHP. This was washed with ethyl acetate to remove the nonbonded coating followed by solution coating with 2% SP 2401 in ethyl acetate solution and fluidized bed drying. The column was operated isothermally at 175 °C; injector and detector temperatures were 180 and 280 °C, respectively. Nitrogen was used as carrier gas at 30 mL/min and as detector makeup gas at 7 mL/min.

Analytical Procedures. *Extraction of Pears.* All samples were placed in frozen storage at -20 °C upon receipt at the laboratory. Three frozen fruits were selected to represent the range of sizes of pears in each sample lot submitted for analysis. These were allowed to thaw in preweighed 800-mL beakers, and their weight was determined. Each weighed pear was blended in a high-speed blender with an amount of doubly distilled water to make the blended sample 60% fruit and 40% water; all three blended pears were then combined and blended before subsampling. This sampling procedure was performed in triplicate for each lot of pears.

A subsample of blended fruit equivalent to 50 g of fruit was transferred to a 400-mL Sorvall Omni-mixer cup and acidified with 1 mL of 85% phosphoric acid. Two hundred milliliters of acetone was added and the sample blended at high speed for 3 min. The macerate was then vacuum filtered on coarse filter paper and the filter cake resuspended two times in 100 mL of acetone, allowing 1 min each time before the vacuum was applied and the samples were refiltered. The combined acetone extracts were filtered through fine filter paper and transferred to a 1000-mL round-bottom flask for concentration.

The bulk of the acetone extract was removed under vacuum at 40 °C on a rotary evaporator, reducing the volume to 75–100 mL. Extracts were then transferred to 500-mL separatory funnels and partitioned once with 150-mL and twice with 75-mL portions of ethyl acetate. Adequate extraction efficiency was obtained by gently mixing the layers in a separatory funnel without vigorous shaking. Vigorous shaking can lead to emulsions, making the separation of layers time-consuming. After the ethyl acetate extracts containing the captan residues were combined in a second 500-mL separatory funnel, they were washed with three 50-mL portions of doubly distilled water. These water washes were added to the aqueous extract in the first separatory funnel. The organic and aqueous layers thus separated needed further cleanup.

Cleanup and Analysis of Carbendazim (Benomyl) Residues. After the extract was separated into two fractions, the aqueous extract containing the carbendazim residues and the organic extract containing the captan residues, the pH of the acidified aqueous fraction was adjusted to approximately 9 by addition of 0.5 N sodium hydroxide. Carbendazim residues were extracted by liquid/liquid partitioning into ethyl acetate using one 100-mL portion and two 50-mL portions of the solvent.

The combined ethyl acetate extracts were dried by passage through anhydrous sodium sulfate and then concentrated to a small volume on a rotary evaporator. A gentle jet of dry air was used to carefully remove the last traces of ethyl acetate. The residues were quantitatively transferred with methanol to 15-mL graduated centrifuge tubes, where they were concentrated under an air jet to about 1.0–5.0 mL. Final sample volumes were adjusted to give carbendazim peaks resulting from 15-μL injections of sample solutions very close in size to those obtained from 15-μL injections of 10 ng/μL benomyl standard solution in methanol after 6 h of preparation.

An injection of standard solution was made 2–3 min after each sample solution injection to yield pairs of peaks, closely matched in height, for direct external peak height comparison. Typically, a 100-ng injection of carbendazim produced a peak of 40–50% full-scale deflection at 0.02 AUFS and about 5.5-min retention time.

Cleanup and Analysis of Captan Residues. The combined ethyl acetate fractions, separated for captan analysis, were dried with anhydrous sodium sulfate and concentrated to dryness on a rotary evaporator. Residues were transferred to a 125-mL Erlenmeyer flask with 50 mL of benzene and 0.5 g of charcoal powder. The extract was then swirled for 1 min before vacuum filtration

Table I. Recovery of Added Benomyl and Captan from Pears

variety	no. of recov	level of fort., ppm	av recov, %	range of recov, %	SD, %
			Benomyl		
Anjou	17	0.25–0.75	82	70–97	±8
Bosc	6	0.5	78	68–89	±7
			Captan		
Anjou	6	0.05–1.0	90	82–96	±6

Table II. Residues of Benomyl and Carbendazim (Determined as Carbendazim) and Captan on Anjou Pears in Cold Storage[a]

storage, days	benomyl equivalent,[b] ppm		captan,[b] ppm	
	av residue	SD, ppm	av residue	SD, ppm
0	0.36	±0.06	0.027	±0.012
85	0.22	±0.05	<0.01	
108	0.26	±0.07	<0.01	
140	0.25	±0.05	<0.01	
185	0.29	±0.10	0.012	±0.007

[a] Treated in packing house Nov. 29, 1982, with 0.23 kg of benomyl and 0.38 kg of captan in 189 L of waxing solution. Stored at -1 °C.

[b] Average of three replicates.

on fine filter paper. Two 10-mL benzene rinses of the flask and the filter funnel were used to effect quantitative transfer of the residues. The benzene extract was concentrated to 5 mL on a warm water bath with a gentle jet of dry air, and 5 mL of hexane was added. CAUTION: Sample preparations involving benzene must be performed in a hood with adequate ventilation. Chromatography on 7% deactivated silica gel was performed in a 14.5 mm i.d. × 250 mm glass column equipped with a Teflon stopcock. The column was partially filled with hexane, and then 5 g of the deactivated adsorbent was added, allowed to settle, and topped with anhydrous sodium sulfate. The sample extract was added and eluted with the following solvent series: 50 mL of 1:1 hexane/toluene, 50 mL of 10% chloroform in 1:1 hexane/toluene, and 50 mL of 1:1 hexane/chloroform. These solvents were eluted as column wash fractions and discarded. The captan residues were eluted with 200 mL of chloroform.

Complete removal of chloroform was required for electron capture GLC. This was accomplished by concentrating the chloroform fraction just to dryness, redissolving the residue in 10 mL of toluene, and concentrating to dryness again. Hexane was used to redissolve the residues for GLC analysis.

Gas chromatographic analysis was by direct external peak height comparison. Captan standards were in hexane solution at concentrations of 0.01 and 0.05 ng/μL, and sample extract volumes were adjusted to about 10–50 mL to give peak heights very close in size to those obtained from standard injections of similar size (2–5 μL). An injection of 30 pg of captan typically yielded a peak of 12% full-scale deflection at 5.3 min. At least three pairs of sample and standard injections, closely matched in injection volume and peak size, were made for each analysis.

Limit of Detection. Untreated pears yielded no detectable residues of any extraneous interferences in chromatography either for carbendazim (benomyl) or for captan. The sensitivity limits of the analytical methods were therefore estimated on the basis of the HPLC or GLC response to the analytical standards, baseline noise level, and sample size. The sensitivity limit of the analytical method was estimated to be 0.1 ppm for carbendazim (benomyl) and 0.01 ppm for captan.

RESULTS AND DISCUSSION

The reliability of the analytical method was tested by adding known amounts of benomyl and captan to the slurry formed by blending untreated fruit with water before acetone extraction and analysis. The results of the recovery studies are shown in Table I. The average recovery for benomyl was 79% and for captan 90%.

Numerous methods have been published for the analysis of benomyl residues in fruits and vegetables, including

Table III. Residues of Benomyl and Carbendazim (Determined as Carbendazim and Reported as Benomyl Equivalents, ppm ± SD[a]) during Cold Storage of Anjou and Bosc Pears Receiving Various Postharvest Treatments[b]

treatment	storage, days				
	0	42	76	127	188
Anjou					
line spray, no wax	0.42 ± 0.06	NA[c]	0.46 ± 0.09	0.60 ± 0.05	0.90 ± 0.07
line spray, waxed	0.39 ± 0.05	NA[c]	0.42 ± 0.02	0.44 ± 0.05	0.45 ± 0.04
benomyl in wax	0.38 ± 0.07	NA[c]	0.42 ± 0.08	0.34 ± 0.16	0.49 ± 0.07
Bosc					
line spray, no wax	0.29 ± 0.02	0.35 ± 0.04	0.31 ± 0.01	0.31 ± 0.04	0.33 ± 0.03

[a] Average of three replicates. [b] Treated in the packing house Sept. 29, 1983, with 0.23 kg of benomyl in 379 L of water or 189 L of waxing solution. [c] NA, not analyzed.

fluorometry and colorimetry (Pease and Gardiner, 1969), UV spectrophotometry (Chiba, 1977), thin-layer chromatography (Tjan and Burgers, 1973), gas–liquid chromatography (Rouchaud and Decallone, 1974), and high-performance liquid chromatography (Kirkland, 1973; Chiba and Veres, 1980; Zweig and Gao, 1983).

Refluxing of samples in acid solution prior to extraction recommended by some authors (Kirkland et al., 1973; Farrow et al., 1977) was shown to generate large amounts of interfering plant coextractives and was therefore unsuitable for the analysis of pears. However, adding acid before solvent extraction allowed separation of benomyl and carbendazim from captan, and sufficient cleanup was achieved by washing the acidified extracts of benomyl followed by pH adjustment and organic solvent extraction.

The quantitative conversion of benomyl to methyl 1*H*-benzimidazol-2-ylcarbamate (carbendazim, MBC) during analytical procedures is well documented (Austin and Briggs, 1976; Kirkland, 1973; Kirkland et al., 1973). Benomyl is also decomposed to carbendazim in organic solvents as reported by Chiba and Cherniak (1979), who found that only 0.6% of the benomyl remained intact after 5.6 h of storage in methanol, the solvent used in this study for HPLC determinations. Carbendazim deliberately produced during the analytical procedure, both in analytical standards and in the sample extract, cannot be distinguished from carbendazim that was present in the sample as natural degradation product. Benomyl residues reported here, using this analytical procedure, therefore represent the total residue of benomyl and its primary breakdown product, carbendazim, expressed as benomyl equivalents. This approach has been used by most investigators because the analysis for intact benomyl is difficult (Chiba and Singh, 1986).

Methods described for the determination of captan residues include the use of thin-layer chromatography (Sherma and Stellmacher, 1985), gas–liquid chromatography (Baker and Flaherty, 1972), and high-performance liquid chromatography (Büttler and Hörmann, 1981).

Chromatography on both Florisil (Gilvydis and Walters, 1984) and silica gel (Baker and Flaherty, 1972) has been optimized for cleanup of captan extracts from crops for analysis by electron capture GLC. Considerable band spreading was observed on a fully activated silica gel column, and 7% water deactivation was required before the desired chromatography was obtained. Further treatment of captan extracts was required, however, to prevent overloading of the silica gel column. Adding charcoal to the benzene solution containing captan was found to adequately prepare the extracts of pear samples for chromatography on silica gel. Cleanup was also improved by washing the silica gel column with different solvent mixtures before elution with chloroform.

Several commercially available GLC liquid phases including DC-200, OV-1, OV-210, and Ucon 550 were tested for captan analysis but were rejected because of too much bleeding or lack of sensitivity or linearity. The use of the specially prepared column packing resulted in satisfactory chromatography.

The results of the residue decline studies are reported in Tables II and III. It can be seen from Table II that the combined residues of benomyl and carbendazim, reported as benomyl equivalents, were considerably below the 7 ppm tolerance established for pears. It is also evident that the combined residues did not decline over the 6-month storage period; residues after 185 days were still about 81% of the initial residue. It has been shown that benomyl, if applied as benomyl and determined as carbendazim, slowly penetrates into the pulp of the fruit and that there is a more than 3-fold increase in residue in the inner core with time. If the fungicide is applied as carbendazim, there is less penetration but more of the fungicide is retained on a whole fruit basis (Cano et al., 1987). Captan residues, however, remain on the surface of the fruit. Captan residues found on pears were very low, insignificant when compared to the 25 ppm tolerance for captan on pears. Captan residues were also much lower than those of benomyl even though the treatment rate was higher.

Degradation of benomyl to carbendazim was first reported by Clemons and Sisler (1969), who observed formation of the degradation product in formulated technical benomyl (Benlate) in aqueous suspension. Baude et al. (1973) have shown that benomyl is converted to carbendazim on plant surfaces under summer outdoor temperature and rainfall conditions. Twenty-three percent to 52% of the residue on leaf surfaces was carbendazim 21–23 days after treatment with benomyl. Although the breakdown of benomyl is temperature dependent, Chiba (1979) observed no degradation of benomyl in organic solvents at 1 °C for short time periods. In our studies pears were stored at −1 °C and were exposed to ambient or elevated temperatures only for short periods of time; therefore, a slowdown of the degradation of benomyl in or on stored pears should be expected.

The effect of different benomyl treatments on the magnitude and fate of residues on pears was studied in 1983. The results in Table III indicate that there was no significant difference in the magnitude and decline of total carbendazim residues between the two treatments using wax. An increase of total residue with time was observed in the treatment where wax was not used. It is not possible at this time to determine the reason for the increase but a treatment error or breakdown of the treatment equipment seems to be the most likely explanation.

The packing house fruit-processing equipment was set to deliver approximately 14–16 mL of the treatment solution containing 0.12% of benomyl per box of fruit. If all the benomyl applied was deposited on the fruit, the concentration would have been approximately 1 ppm.

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However, the initial concentration found on pears was only 0.37 ppm, which indicates that the rest of the benomyl was lost during the application, sponging, or drying.

Little information is available in the literature on the fate of benomyl or captan residues on fruit kept in cold storage. Bioassays by Ben-Arie (1975) showed a decline in benomyl residues resulting from postharvest treatment of pears and also a slow penetration of the residue into the core area of the fruit. An unpublished study by George and Maitlen (1984) using apples showed results similar to those reported here. In their study benomyl was applied as a drench or in waxing solution, and the initial residues ranged from 0.86 to 1.2 ppm. There was no evidence of decline in benomyl residues over 2–6 months of cold storage. Mónico-Pifareé and Xirau-Vayreda (1987) reported initial residues of 0.44 ppm on apples and observed no reduction of residues over a 200-day storage at 0 °C. Cano et al. (1987), in contrast to the previous studies, observed a decline of benomyl and carbendazim residues during cold storage; only 28–34% of the initial residue was still present 169 days after treatment.

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