Determination of Methyl 2-Benzimidazolylcarbamate in Black Walnut Fruit

An analytical method for determining the systemic fungicidal compound methyl 2-benzimidazolylcarbamate (MBC) was developed for black walnut fruit by using a modified gas chromatographic procedure. Black walnut (*Juglans nigra* L.) trees were treated by soil injection or foliar sprays with benomyl (Benlate 50WP) or trunk infused with Lignasan BLP. Analysis of MBC residue in walnut fruit collected in August and September during one growing season determined low concentrations (≤0.030 ppm) present in all treatments. Only the August collection contained residue above the minimum detectable quantity (MDQ) of 0.010 ppm. The present allowable tolerance of MBC on or in black walnut fruit is designated at 0.13 ppm.

Satisfactory control of walnut anthracnose, a fungal disease of black walnut, has been achieved with applications of soil-injected (Neely, 1977) or foliar-sprayed (Neely and Funk, 1975; Berry, 1977) benomyl. At present benomyl is not registered for use on nut-bearing trees due to the uncertainty of its potential systemic movement into floral parts and fruits. Because residue data are not available for registration purposes, this study was initiated to determine residue levels in mature fruit of methyl 2-benzimidazolylcarbamate (MBC), a bioactive metabolite of benomyl.

Several different methods have been used to determine the residues of benomyl and its degradation products in foods including UV spectrophotometry (Mestres et al., 1971; Polzhofer, 1977; White and Kilgore, 1972), direct fluorometry (Aharonson and Ben-Aziz, 1973; Pease and Gardiner, 1969; Pease and Holt, 1971), colorimetry (Miller et al., 1974; Pease and Gardiner, 1969; Pease and Holt, 1971), high-speed liquid chromatography (Kirkland et al., 1973), and gas chromatography (Pyysalo, 1977; Rouchaud and Decallonne, 1974; Tjan and Jansen, 1979). The combination of insufficient analytical sensitivity, inadequate amounts of crop material available for analysis, and complicated analytical methodology eliminates many of these techniques for routine residue analysis of some crop materials. Because limited quantities of walnut fruit were available for this study and detection at low levels was required (<0.13 ppm of MBC), a modified electron capture, gas chromatographic technique by Tjan and Jansen (1979) was found appropriate for MBC analysis of walnut fruit. The present paper reports MBC residue concentrations detected in black walnut fruit from trees treated for disease control; an abbreviated preparation procedure for the pentafluorobenzyl derivative of MBC detected in GC and mass spectrometric analysis is also presented.

MATERIALS AND METHODS

Fungicide Treatments. Five to eight black walnut (*Juglans nigra* L.) trees grown in a plantation at Southern Illinois University, Carbondale, IL, were treated on May 31 by soil injection (570 g of a.i. (190 L) (18 m^2)$^{-1}$ tree^{-1}) with a hydraulic sprayer (John Bean Division, FMC Corp.) at 14 kg/cm^2 pressure, by foliar spray at 0.6 g of a.i./L repeated in July, or by trunk infusion of 3 L of a 1400-ppm Lignasan BLP (0.7% EC) solution gravity fed via plastic bags suspended from each tree trunk, remaining until no further uptake occurred.

On Aug 24, 10–15 nuts were collected from each tree with additional nuts collected on Sept 13 prior to anticipated fall. Nuts from all trees within each treatment were pooled together and frozen in plastic bags at −10 °C until analysis.

Chemicals. All solvents used were pesticide grade. The analytical-grade MBC was supplied by E. I. du Pont de Nemours & Co., Inc., Wilmington, DE. Pentafluorobenzyl bromide was obtained from Pfaltz and Bauer, Inc., Stamford, CT, and was used under a well-ventilated hood. **CAUTION**: This reagent is a strong lachrymator.

Extraction of Residue. From each pooled collection of nuts per collection date, three or four samples of nut meat tissue (25 g) were extracted for MBC residue with ethyl acetate as described by Tjan and Jansen (1979) in a Waring blender. In a 250-mL separatory funnel, three to four 50-mL washes with distilled water were used, and a 5% sodium lauryl sulfate solution was used to reduce emulsions between washings. MBC was partitioned into an acidified aqueous phase with two 50-mL aliquots of 0.1 N HCl. This was followed by adding 4.5 mL of 6.5 N NaOH to adjust the solution to pH 8–10 prior to the partitioning of MBC into ethyl acetate with two 25-mL portions. The extract was washed with 50 mL of distilled water and the organic fraction dried over anhydrous Na_2SO_4. Each sample was evaporated on a steam bath to 10 mL. Recovery efficiency of MBC was determined by extraction of untreated nut meat samples fortified at 0.88 ppm of MBC.

Derivatization. One milliliter of the ethyl acetate extract in a 15-mL graduated tube was dried over nitrogen gas, redissolved in 2 mL of acetone, and derivatized with the addition of 20 μL of 30% potassium carbonate and 50 μL 1% of pentafluorobenzyl bromide (PFB-Br) in acetone, refluxing for 3 h in a 60 °C water bath. Two milliliters of isooctane was added following derivatization and evaporated to 1 mL over nitrogen gas in a sand bath to remove the acetone. Each derivatized sample was then brought to 5 mL with isooctane.

GLC and Mass Spectrometry. A Varian Model 2100 gas chromatograph equipped with an electron capture detector was used for derivatized MBC analysis. The 1.8 m × 2 mm i.d. borosilicate glass column was packed with 3% OV-17 on Chromosorb W-HP. The following GC operating conditions were used: nitrogen carrier gas flow rate, 35 mL/min; column temperature, 240 °C; detector temperature, 280 °C; injector temperature, 260 °C. One to three microliters was injected from the MBC-derivatized samples.

An injection of derivatized MBC standard immediately following the nut extraction was used for quantitative as well as qualitative analysis by routine external standardization procedures. All peak heights measured were within the linear range of the detector.

The suspected peak corresponding to the derivatized MBC product was verified by GC–mass spectrometric analysis. A Varian MAT CH7 mass spectrophotometer coupled with a Varian Model 1700 gas chromotograph operated at conditions identical with those used in the GLC analysis was used. The ionizing voltage was 70 eV, the filament current, 300 μA, and the source temperature, 200 °C.

RESULTS AND DISCUSSION

Pentafluorobenzylation of MBC was effective in pro-

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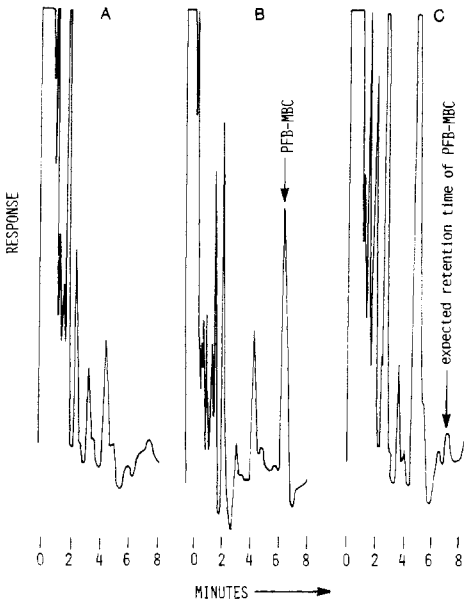

Figure 1. Gas chromatogram of (A) the pentafluorobenzyl bromide reagent, (B) derivatized MBC, and (C) the unfortified, derivatized nut extract.

ducing a stable derivative highly sensitive to electron capture gas chromatography. As little as 0.01 ppm could be detected in walnut fruit with a minimum detectable quantity (MDQ) of 10 pg as observed by Tjan and Jansen (1979). When walnut fruit was fortified with MBC, recoveries of 93–96% were recorded. Published MBC derivatization procedures using trifluoroacetic anhydride (Rouchaud and Decallonne, 1974) and acetic anhydride (Pyysalo, 1977) were tested but resulted in an incomplete and unstable product. Others (Pyysalo, 1977; Tjan and Jansen, (1979) have similarly described trifluoroacetylation of MBC as variable.

Following derivatization of MBC with the PFB-Br reagent, Tjan and Jansen (1979) employed a column cleanup procedure to remove unreacted derivative which may otherwise overload the detector. This step was eliminated in the modified procedure without loss of sensitivity by reducing the final ethyl acetate extract from 50 to 10 mL over steam and diluting the derivatized sample to 5 mL with isooctane prior to injection. Thus, unreacted PFB derivatives were diluted below the interference level while the relative MBC concentration in both procedures would have remained identical. The success of this modification was further enhanced by the longer retention time of the MBC derivative relative to the interferences produced by unreacted derivative and extraneous plant material (Figure 1).

Confirmation of the PFB-MBC derivative was established by GC–mass spectroscopy. The proposed molecular ion is a dipentafluorobenzyl derivative of MBC (Figure 2), occurring at m/e 551. Although all observed ion fragments agreed with those reported by Tjan and Jansen (1979), a molecular structure was not given.

MBC residues in walnut fruit above the MDQ was observed only in the August nut collections (Table I). The greatest concentrations (0.016–0.030 ppm of MBC) were seen in nuts from Lignasan BLP trunk-infused trees, while detectable residues also occurred in foliar and soil injection treatments with benomyl. The absence of detectable quantities in the September collections may have resulted from a dilution factor related to increased fruit growth or metabolic complexing of MBC which has been observed by others in various crops (Rouchaud and Decallonne, 1974; Siegel and Zabbia, 1972).

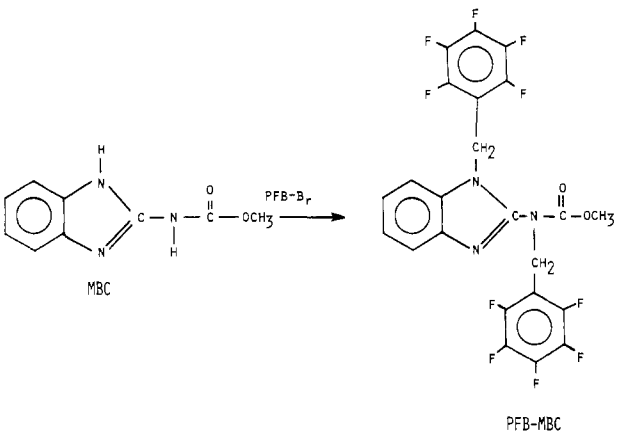

Figure 2. Structural configuration of MBC and the proposed dipentafluorobenzyl derivative of MBC.

Table I. Highest MBC (ppm) Residues Detected in Nut Meat Tissue of Black Walnut Trees Treated with Benomyl Foliar Sprays, Soil Injections, or Trunk Infusions in 1979 at Carbondale, IL

	collection dates	
treatment	Aug 24	Sept 13
1× foliar spray	0.013	<MDQ[a]
soil injection	0.010	<MDQ
trunk infusion	0.030	<MDQ

[a] The minimum detectable quantity (MDQ) was set at 0.010 ppm.

The proposed registration of benomyl for use on nut-bearing trees of black walnut is based on occurrence of residues ≤0.2 ppm of benomyl (0.13 ppm of MBC) on or in the fruit. The MBC residues observed from three different applications of benomyl fungicide, in the present study, were well below this level in nut meat tissue.

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