Distribution and Metabolism of Methyl 2-Benzimidazolecarbamate, the Fungitoxic Derivative of Benomyl, in Strawberry Plants

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ABSTRACT

The distribution and metabolism of methyl 2-benzimidazolecarbamate (MBC) was studied in strawberry plants grown in Hoagland's solution and root-treated with ^{14}C fungicide. There was little accumulation of label in mature fruit when plants were treated either prior to, or at the start of, flower initiation. When non-fruiting plants were treated, the label accumulated in the roots and foliage produced before and during the treatment period. Much less label was found in the foliage produced after cessation of treatment. While MBC and 2-aminobenzimidazole (2-AB) were present in both root and foliage tissue of treated plants, MBC was most concentrated in the foliage. The amount of ^{14}C MBC in whole plants treated for 36 and 88 days was 54 and 32%, respectively. The amount of 2-AB was 10-18% for the same period. The remaining label (36-58%) was in unidentified metabolites which were either water-soluble or bound to the residue produced after exhaustive organic solvent extraction. Analysis of the unidentified water-soluble metabolites suggested that conjugation of the benzimidazole nucleus occurred. The data indicate that, although MBC is more extensively metabolized in strawberry plants than in other species, the benzimidazole nucleus appears to be relatively stable to degradation in strawberry plants.

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According to a previous report (12), benomyl could not be recovered from dwarf pea plants grown in a nutrient solution containing ^{14}C fungicide. The fungitoxic derivative, methyl 2-benzimidazolecarbamate (MBC), was present in large quantities (78%) in plants 52 days after treatment. The remaining label was in unknown metabolites which were either water soluble (5%) or bound to the plant residue (14%). MBC has been recovered from other plant species treated with either benomyl (3, 8, 9, 13) or the benzimidazole fungicide thiophanate-methyl (3, 14, 16). A number of metabolites of the benzimidazole pesticides have been recovered as water-soluble products from plants and animals (1, 2, 4, 15). The acaricide fenazaflor (5,6-dichloro-1-phenoxycarbonyl-2-trifluoromethyl-benzimidazole) is extensively metabolized in apples and pears to trifluoromethyl benzimidazole (1, 2). In addition a β-glycoside of 5,6-dichloro-4-hydroxy-2-trifluoromethylbenzimidazole is formed by hydroxylation and conjugation of the benzimidazole nucleus. The apparent metabolism of the benzimidazole nucleus of fenazaflor in species of the family Rosaceae suggested that MBC might undergo similar reactions.

In this study, the metabolic fate and distribution of ^{14}C MBC in strawberry plants was investigated. MBC, rather than benomyl, was studied because benomyl has low water solubility, rapidly decomposes to MBC in aqueous solutions, and is the compound which is fungitoxic in plants. Portions of this study were reported previously (10).

MATERIALS AND METHODS.–*Plant culture*.–Strawberry plants (*Fragaria ananosa* 'Superfection') were planted in Weblite®, a shale product (Weblite Corp., Roanoke, Va.), and watered daily with half-strength Hoagland's solution (6). After 2-3 weeks the plants were removed from the Weblite, their roots were washed with water to remove most of the particles, and then placed in full strength Hoagland's solution (1,800 ml) in 2-quart, blackened, plastic containers. The stem of each plant was fitted into a hole in the container top and held in place by two thin circular strips of cork. The solution was aerated 4,219 kg/m^2 (6 lb/in^2) through tubing placed in a small hole at a corner of the plastic top.

Plants were grown in controlled environment cabinets with temperatures at 22 C (day) and 16 C (night). The day-length was 16 hr 12,900-15,070 lux (1,200-1,400 ft-c). The relative humidity was 30-50%. The nutrient solution was changed every 4 days, and water was added each day to replace that lost by evaporation and transpiration. After the plants had four to five new leaves, the nutrient solution was fortified with an additional concentration of iron which was added on the first and third days. Flowers and flower stalks were detached from all plants, unless otherwise stated.

Fungicide.–^{14}C benomyl, labeled in the 2 position of the imidazole ring with a specific activity of 2.01 mc/mmole, was purchased from the New England Nuclear Corp., Boston Mass. ^{14}C benomyl was converted to ^{14}C MBC according to the method of Pease & Holt (7).

Treatment, extraction, and distribution of label from ^{14}C MBC.–Strawberry plants, grown hydroponically for 4 weeks were placed in 1,800 ml of Hoagland's solution which contained 1.7 μmole (3.5 μc) of ^{14}C MBC. Fungicide was added from a methanol stock solution. Fungicide and nutrient solution were changed every 4 days and the pH adjusted daily with KOH to 4.7-4.9.

To determine whether strawberry fruits accumulate fungicide, some plants were treated with ^{14}C MBC when the first flowers were in full petal. Other plants were treated for 42 days prior to flower initiation. In both cases, the plants were harvested when the berries ripened. The roots were washed, and the plants were sectioned into three tissue-type samples: roots, stem and foliage, and berries.

To determine whether fungicide is translocated to foliage produced after the cessation of treatment, plants were grown in the presence of ^{14}C MBC for 32 days, and then for an additional 35 days without fungicide. The roots were washed and the plants divided into four samples: roots, foliage produced before treatment, foliage produced during treatment, and foliage produced after treatment. All samples were freeze-dried, ground in a Wiley mill, and the distribution of radioactivity determined (12).

The study of the distribution of radioactivity, and the metabolism of MBC in plants continously exposed to ^{14}C MBC, was performed as previously described (12), except for the following modifications. Lower leaves of all plants were removed and frozen when they showed signs of senescence. These leaves were processed when the plants were harvested 36 and 88 days after the start of the treatment. The roots were washed and plant was sectioned into two samples: roots, and stems and foliage. These samples were quickly frozen in plastic bags in an alcohol-dry ice mixture, and stored at −20 C.

The frozen plant tissue was allowed to thaw slightly and was homogenized for 2-3 min with cold 90% acetone in a Waring Blendor cup. The residue was separated from the extract by filtration (under vacuum). Homogenization and extraction of the residue was repeated twice. The resulting residue was further extracted with 1:1:1 benzene:methanol:chloroform (v/v) mixture (BMC) and the water partitioned from the acetone-water solvent by chloroform extraction. The chloroform fraction was put through a glass column containing Na_2SO_4 to remove water.

Purification and isolation of ^{14}C compounds in the organic and aqueous fractions.–The organic and aqueous extracts were further treated to remove substances which interfere with TLC. Both fractions were reduced to 20-40 ml by vacuum evaporation, and then passed through individual 1.5 × 15-cm columns packed with prepared (11) Dowex 50 × 8-200 cation exchange resin, 100-200 mesh (Sigma Chem. St. Louis, Mo.). The columns were washed with methanol and the resin removed and placed in a

TABLE 1. Distribution and amount of radioactivity in strawberry plants grown in Hoagland's solution containing ^{14}C methyl 2-benzimidazolecarbamate[a]

Tissue	Treated 33 days[b]		Treated 75 days[c]	
	% ^{14}C	Sp. Act.[d]	% ^{14}C	Sp. Act.
Roots	32	3.4×10^{-4}	30	1.1×10^{-3}
Foliage	63	2.9×10^{-4}	70	5.7×10^{-4}
Berries	1.0	2.2×10^{-5}	0.001	3.4×10^{-6}

[a]Hoagland's solution containing 1.7 μmole (3.5 μc) ^{14}C MBC added to 35-day-old plants. Solutions changed at 4-day intervals. Plants harvested when fruit ripened, freeze dried, and the amount of radioactivity determined by oxygen combustion of the tissue.

[b]Flower formation occurred on the first day of treatment.

[c]Flower formation occurred 42 days after the start of treatment.

[d]Sp. Act. (Specific Activity) - μc/mg dry wt tissue, average of two plants.

beaker with 75 ml of 4 N NH_4OH and stirred for 2 hr at room temperature. The NH_4OH was removed by allowing the resin to settle and then decanting the solution. The extraction of the radioactivity from the resin was repeated 3-4 times, or until 80-95% of the radioactivity was eluted from the resin. The extracts were cooled to 1 C in an ice bucket and then combined quick-frozen, and lyophilized with a freeze dryer. The lyophilized extracts were stored in a desiccator at −20 C.

The lyophilized samples containing either the organic-soluble or water-soluble compounds were extracted with small portions of methanol until 90-95% of the radioactivity was solubilized. MBC and other labeled compounds in the aqeuous and nonaqueous extracts were resolved by a combination of TLC and bioassay. Solvents and R_F values for MBC and 2-AB were the same as those previously listed (12). Identification of 2-AB was confirmed by bromination and analysis of the products by TLC (12).

Analysis of the ^{14}C label in the water-soluble fractions.—The methanol extract of the water-soluble fraction was divided into four equal portions which were treated as follows: 1) One portion was mixed with one-fourth volume of 6.5 N NH_4OH and extracted three times with equal portions of ethyl acetate. The ethyl acetate was reduced in volume and stored at 1 C. Perchloric acid (PCA) was added to the aqueous phase to a final concentration of 36% v/v and the solution placed in a 100 C oven for 2 hr. The mixture was cooled and adjusted to approximately pH 7.0 with concentrated KOH. The resulting $KClO_4$ precipitate was removed by filtration and washed with water until all the original radioactivity was recovered in the filtrate. The filtrate was made alkaline with KOH or NaOH and extracted with ethyl acetate as previously described. 2) The three remaining methanol portions were added to tubes containing:(i) 10 ml 0.2 M citrate-phosphate buffer (pH 4.8), one drop of toluene, and either 0.5 ml of β-glucuronidase (3×10^4 Fishman units) - sulfatase (4.5×10^4 Whithead units), or (ii) 20 mg. of β-glucosidase (2×10^4 units), or (iii) no enzymes. Enzymes were purchased from Calif. Biochem. Corp., Los Angeles, Calif. Samples were incubated for 12-18 hr at 35 C. Each sample was diluted to 20 ml and mixed with 7 ml of 6.5 N NaOH. The solutions were extracted with ethyl acetate. The ethyl acetate extracts were reduced in volume and analyzed by TLC.

Analysis of the ^{14}C label in the residue.—The radioactive material remaining in the stem and root residue after BMC extraction was treated with boiling NaOH (12), for 2 hr and the supernatant solution after centrifugation was extracted three times with equal volumes of ethyl acetate. The distribution of radioactivity in aqueous and nonaqueous fractions and in the residue was determined.

RESULTS.—*Translocation and distribution of label in roots, foliage, and fruit.*—Very little label was present in the fruit of plants treated for 33 and 75 days with ^{14}C MBC and harvested when the berries matured (Table 1). Although the fruit (8-10 berries/plant) represented 14% of the dry weight of the plant, it did not contain a proportional amount of the radioactivity. When non-fruiting plants were treated, label accumulated in foliage produced before and during the treatment period (Table 2). Much smaller amounts of label (1.1%) were in the new foliage produced after cessation of treatment. Even though the new foliage represented 33% of the dry weight of the plant, the specific activity was approximately 10-25 times less than that of the older foliage and roots.

Distribution and identification of label in tissue extracts of roots and foliage.—The distribution of label in tissue extracts and residue from plants treated with ^{14}C MBC is illustrated in Table 3. As will be shown, the label in the water-soluble fraction and residue is in products other than MBC. This suggests that considerable decomposition of the fungicide occurred with treatment time.

TABLE 2. Distribution and amount of radioactivity in strawberry plants grown for 32 days in Hoagland's solution containing ^{14}C MBC, followed by an additional 35 days of growth in the absence of fungicide[a]

Tissue	Dry wt %	^{14}C %	Sp. Act.[b]
Roots	23	36	2.2×10^{-3}
Foliage[c]	17	44	2.5×10^{-3}
Foliage[d]	27	18	1×10^{-3}
Foliage[e]	33	1.1	1.1×10^{-4}

[a]Conditions the same as in Table 1.

[b]Sp. Act. - μc/mg dry wt tissue; average of three plants.

[c]Foliage produced before treatment with ^{14}C MBC.

[d]Foliage produced during treatment with ^{14}C MBC.

[e]Foliage produced after treatment with ^{14}C MBC.

Following the cation-exchange purification, TLC and bioassay procedures, the compounds present in the organic-soluble and water-soluble fractions from plants treated for 36 and 88 days with ^{14}C MBC were determined (Table 4). Not all the label in the extracts from treated plants was recovered during the purification procedures. Label not recovered from the resin (2-8%), or which did not bind to the resin (5-10%), was considered to be contained in the unidentified products. The percentages reported in Table 4 were corrected to represent the amounts of radioactivity extracted from the plants prior to purification. To prove that the stability of MBC and 2-AB was maintained during the analytical procedure, labeled, known MBC or 2-AB was mixed with a 90% acetone extract of a non-treated plant and the organic and water phase separated by partition with chloroform. The two fractions were purified using the cation-exchange resin, NH_4OH elution, freeze drying and TLC techniques. Both MBC and 2-AB were recovered as such from the resin. Apparently, inactivation of MBC only occurs upon heating at alkaline pH (3). None of the product(s) bound to the residue was identified. The organic-soluble and water-soluble fractions from foliage and roots of plants treated for 36 and 88 days contained both MBC and 2-AB. However, the amount of MBC found in the water-soluble fraction was negligible compared to that in the organic-soluble fraction. The organic-soluble fraction contained only small amounts of unidentified labeled products (0-9%). The unidentified products were associated primarily with the water-soluble fractions and bound to the residues.

The percent distribution of MBC, 2-AB, and unidentified products in foliage and roots of plants treated for 36 and 88 days is summarized in Table 5. MBC and 2-AB were present in both foliage and root tissue from all plants. The highest concentration of MBC was in the foliage. Unidentified compounds increased over the entire treatment period, but the increase was more pronounced in the roots.

Further analysis of the ^{14}C compounds in the water-soluble fraction.—The water-soluble fractions from roots and foliage of treated plants contained high concentrations (58-71%, Table 4) of unidentified compounds. By treating the water-soluble fraction with base, acid, and specific enzymes, certain

TABLE 3. Percentage distribution of label in tissue extracts and residue from strawberry plants treated with ^{14}C MBC for 36 and 88 days[a]

Time (days)[b]	Organic-soluble		Water-soluble		Residue	
	Foliage	Root	Foliage	Root	Foliage	Root
36	65	40	19	33	16	27
88	51	35	28	34	21	31

[a]Conditions same as in Table 1, except plants were not freeze dried, prior to solvent extraction.
[b]Average of two plants.

TABLE 4. Identification and percentage distribution of compounds in extracts from strawberry plants treated with ^{14}C MBC for 36 and 88 days[a]

Interval	Distribution of ^{14}C (%)			
	Foliage		Roots	
	Organic soluble	H_2O soluble	Organic soluble	H_2O soluble
36 days				
MBC	95	4	93	4
2-AB	5	29	7	38
Unidentified	0	67	0	58
88 days				
MBC	73	4	63	2
2-AB	18	25	29	32
Unidentified	9	71	8	66

[a]Conditions same as in Table 1, except plants were not freeze dried, prior to solvent extraction. The acetone-water extracts were partitioned with $CHCl_3$. The resulting $CHCl_3$-acetone and water mixtures represent the organic-soluble and water-soluble fractions, respectively.

products in the unidentified group could be isolated and separated by TLC (Table 6). Less than 5% of the label partitioned into ethyl acetate when there was no treatment of the water-soluble fractions. The NaOH-PCA treatment resulted in a partitioning, into the organic solvent, of 75 and 63% of the label from the foliage and roots, respectively. Treatment of a separate aliquot of the water-soluble fraction with β-glucosidase resulted in a partitioning into the ethyl acetate of 79 and 65% of the label from the foliage and roots, respectively. The control was similar to the NaOH treatment.

The label partitioned into the ethyl acetate solvent from the treated water-soluble fractions was separated into ^{14}C compounds by TLC (Fig. 1). Both PCA and enzyme treatments of the root and foliage water-soluble fractions produced similar chromatographic patterns. In the water-soluble foliage fraction three compounds were isolated, whereas, in the root fraction, two compounds were present. The only compounds isolated from the base and control treatments were 2-AB (compound number 1) and very small amounts of MBC. The concentrations of these compounds approximated the amounts estimated in the original water-soluble fraction (Table 4). The amount of 2-AB present in the enzyme treatment also approximates that in the water-soluble fractions treated with NaOH and in the control. Treatment with β-glucuronidase - sulfatase enzymes yielded results similar to those described in Table 6 and Fig. 1. Compounds 2 and 3 remain unidentified and represent 15% and 29% of the total label present in the water-soluble fraction from foliage, respectively. Compound 3 represents 35% of the total label present in the water-soluble fraction from roots. No zones of inhibition were found for these compounds when the TLC plates were bioassayed. Similar TLC patterns were also found for

TABLE 5. Identification and percentage distribution of labeled compounds in foliage and root tissue from strawberry plants treated with ^{14}C MBC for 36 and 88 days[a]

Compound[c]	Distribution of ^{14}C (%)					
	36 days			88 days		
	Foliage	Roots	Total[b]	Foliage	Roots	Total
MBC	62	38	54	37	19	32
2-AB	8	14	10	15	23	18
Unidentified	30	48	36	45	58	50

[a]Conditions of growth and plants the same as in Tables 3 and 4.

[b]^{14}C distribution in products for the entire plant (foliage and roots).

[c]Compounds isolated by TLC on 5 × 20-cm plates coated with 0.250-mm thick silica gel Hf-254 (Brinkman Instruments, Inc., Westbury, N.Y.) and developed to 17 cm in solvents B-E (ref. 1). MBC and 2-AB identified by bioassy and co-chromatography with known compounds.

the ^{14}C-labeled products in either acid or enzyme reacted water-soluble fractions, from foliage and root tissue of plants treated with fungicide for 36 days.

Further analysis of the residue fractions.—The residue fractions (after organic solvent extraction) were subjected to exhaustive NaOH extraction. The release of label from foliage residues of plants treated 36 and 88 days was 61 and 63%, respectively. From the root residue of the same plants, the label released was 62 and 80%, respectively. Extraction of the NaOH solutions with ethyl acetate resulted in a partitioning of about 10% of the label into the organic solvent. This indicated that the released products were primarily water-soluble. Treatment of the residues with dilute HCl or concentrated H_2SO_4, either before or after base treatment, did not result in a release of appreciable amounts of label.

TABLE 6. The effect of base, acid, and enzyme treatment on the partitioning characteristics of the label in the water-soluble fractions from roots and foliage of plants treated for 88 days with ^{14}C MBC[a]

Treatment[b]	Foliage, % ^{14}C		Roots, % ^{14}C	
	Ethyl acetate	Water	Ethyl acetate	Water
None	3	97	5	95
NaOH[c]	33	66	39	61
PCA	75	25	63	37
Enzyme[d]	79	21	65	35
Control[e]	34	66	32	68

[a]Water-soluble fractions from the same plants as in Tables 3-5.

[b]Original water-soluble fractions after column exchange purification and lyophilization were in methanol. Methanol diluted with water (50-fold) and treated.

[c]Fraction mixed with NaOH(2 N, v/v) and extracted with ethyl acetate. The resulting water fraction was treated for 2 hr at 100 C with PCA (36%, v/v). Fraction was made basic and extracted with ethyl acetate.

[d]Aliquot of the water-soluble fraction treated with β-glucosidase for 18 hr at 35 C. Mixture made basic with NaOH and extracted with ethyl acetate.

[e]Control contained no enzyme, but fraction was otherwise the same as described in footnote d.

DISCUSSION.—*Distribution.*—^{14}C label from MBC was translocated from the roots to green portions of the plant where it accumulated in foliage produced before and during the treatment period. When the supply of fungicide was discontinued, only small amounts of label were translocated to new foliage. These results are similar to those previously reported for pea plants (12) when ^{14}C benomyl was the source of fungicide. The situation with respect to roots was different for the two species of plants, in that high concentrations of label remained in the strawberry roots, but not in the pea roots. The lack of depletion of the label from strawberry roots may be explained as follows: 1) Greater decomposition of the fungicide occurred in strawberry root tissue than in treated root tissue from pea plants. The products in the water-soluble and residue root tissue, from plants treated for 36 days, represented 60% of the total label present in this tissue (Table 3). These products may not be translocated. 2) The high concentration of label present was due to the longer treatment period and to higher total concentrations of ^{14}C fungicide. 3) Distribution patterns of MBC and hence any metabolites may be controlled by whether benomyl or MBC is the source of fungicide. Fuchs et al. (3) demonstrated that only in benomyl root-treated plants was there a gradual release of fungitoxicant (MBC) into all the aerial parts. In the MBC treated plants depletion of fungicide from the roots and stems occurred so rapidly that leaves formed after cessation of treatment contained little MBC. The amount of disease control and identification of MBC were not determined in the strawberry plants harvested 35 days after cessation of treatment. Therefore, all or parts of the above hypotheses could explain the distribution data.

The lack of accumulation of label in the fruit is also similar to results reported for the fruit of dwarf peas (12). These data support the hypothesis that since the fungicide moves in the xylem, accumulation in plant organs would be controlled by the transpiration rate. The low levels of fungicide in fruit which may have few or no functional stomates is probably dependent, therefore, on the amount of water required for growth and not for transpiration (5, 8, 9, 12).

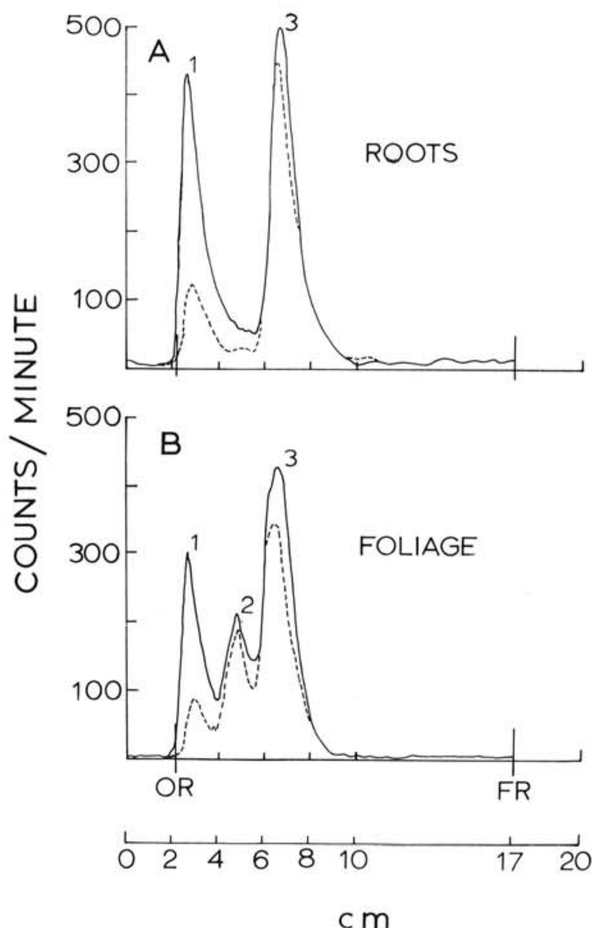

Fig. 1. Radioactivity scans of thin-layer chromatography (TLC) plates of the products formed by reactions of the water-soluble fractions from plants treated for 88 days with ^{14}C fungicide, with perchloric acid, or β-glucosidase. Enzyme treatment ————, PCA treatment − − − − − − − −, OR=origin; FR=solvent front. TLC the same as in Table 5, plates developed in $CHCl_3$:ethyl acetate:acetic acid (1:1:.04) solvent with MBC and 2-AB known compounds. Compound number 1 identified as 2-AB, R_F of known MBC, from the origin, 0.35 (8 cm).

Metabolism.—The assembled data indicate that MBC is more rapidly metabolized in strawberry than in pea plants (12). In the pea experiments only 22% of the MBC was metabolized when the plants were treated for 4 days and then analyzed 52 days later. In the continuous treatment of strawberry plants for 36 and 88 days, 46 and 68% of the fungicide was metabolized, respectively.

The highest concentrations of MBC were in the foliage tissue. The only product of MBC metabolism identified was 2-AB which was present in both foliage and root tissues. The formation of 2-AB could occur directly from MBC by demethylation and decarboxylation of the methyl carbamate side chain. The unidentified products of MBC metabolism were either water-soluble or bound to the residue tissue. These products increased with treatment time and were in the greatest concentration in the root tissue (Tables 3 and 5).

The data suggest that metabolism of the benzimidazole nucleus occurs and that some of the resulting products are either water-soluble *N*- and *O*-glycoside, or glucuronide and sulfate conjugates. Two nonfungitoxic hydrolysis products of these conjugates were isolated from the water-soluble fractions by treatment with either acid or β-glycosidase, glucuronidase, and sulfatase. Formation of the *N* and *O* conjugates could arise by conversion of both MBC (4) and 2-AB.

The liberation of a large percentage of the radioactivity from the residual tissue by NaOH, but not by acid treatment, suggests that a portion of these unidentified product(s) were associated primarily with hemicelluloses of the plant cell walls (17, 18). This would be expected since the fungicide moves in the xylem.

The benzimidazole nucleus appeared to be relatively stable to degradation in plants treated up to 88 days with ^{14}C MBC. If degradation had occurred one might have expected to find label in $^{14}CO_2$ and products of intermediary metabolism (amino acids, sugars, organic acids, etc.). Evolution of CO_2 was not studied in these experiments. However, there was no indication of label in products of intermediary metabolism as determined by TLC. Benomyl-treated foliage, stems, and roots, will probably contain the intact benzimidazole nucleus for long periods of time. If these portions of the plant are not harvested but return to the soil, then degradation may depend primarily on the interactions with soil and soil microorganisms.

LITERATURE CITED

1. BOWKER, D. M., & J. E. CASIDA. 1969. Metabolism of the acaricide chemical, Fenazaflor (5,6-dichloro-1-phenoxycarbonyl-2-trifluoromethyl-benzimidazole), and related 2-trifluoromethylbenzimidazoles in certain mammals, insects, and plants. J. Agr. Food Chem. 17:956-966.
2. CROFTS, M., R. J. HARRIS, & R. J. WHITEOAK. 1972. Determination of residues in apples and pears following treatment with the acaricide fenazaflor 1. Routine method for fenazaflor and major metabolites. Pest. Sci. 3:29-37.
3. FUCHS, A., G. A. VAN DEN BERG, & L. C. DAVIDSE. 1972. A comparison of benomyl and thiophanates with respect to some chemical and systemic fungitoxic characteristics. Pest. Biochem. Physiol. 2:191-205.
4. GARDINER, J. A., R. BRANTLEY, & H. SHERMAN. 1968. Isolation and identification of a metabolite of methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate in rat urine. J. Agr. Food Chem. 16:1050-1052.
5. HINE, R. B., L. J. ASHWORTH, JR., A. O. PAULUS, & J. L. MC MEANS. 1971. Absorption and movement of benomyl into cotton bolls and control of boll rot. Phytopathology 61:1134-1136.
6. HOAGLAND, D. R., & D. I. ARNON. 1938. The water culture method for growing plants without soil. Calif. Agri. Exp. Sta. Circ. 347, 39 p.
7. PEASE, H. L., & R. F. HOLT. 1971. Improved method for determining benomyl residues. J. Assoc. Off. Agric. Chem. 54:1399-1402.

8. PETERSON, C. A., & L. V. EDGINGTON. 1970. Transport of the systemic fungicide, benomyl, in bean plants. Phytopathology 60:475-478.
9. PETERSON, C. A., & L. V. EDGINGTON. 1971. Transport of benomyl into various plant organs. Phytopathology 61:91-92.
10. SIEGEL, M. R. 1972. Distribution and metabolic fate of methyl-2-benzimidazolecarbamate in strawberry. Phytopathology 62:789 (Abstr.).
11. SIEGEL, M. R., & H. D. SISLER. 1964. Site of action of cycloheximide in cells of Saccharomyces pastorianus. 1. Effect of the anitbiotic on cellular metabolism. Biochim. Biophys. Acta 87: 70-82.
12. SIEGEL, M. R., & A. J. ZABBIA, JR. 1972. Distribution and metabolic fate of the fungicide benomyl in dwarf pea. Phytopathology 62:630-634.
13. SIMS, J. J., H. MEE, & D. C. ERWIN. 1969. Methyl 2-benzimidazolecarbamate, a fungitoxic compound isolated from cotton plants treated with methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl). Phytopathology 59:1775-1776.
14. SOEDA, Y., S. KOSAKA, & T. NOGUCHI. 1972. Identification of alkyl 2-benzimidazolecarbamates as major metabolites of thiophanates fungicide in/on the bean plant. Agr. Biol. Chem. 36:817-823.
15. TOCCO, D. J., C. ROSENBLUM, C. M. MARTIN, & H. J. ROBINSON. 1966. Absorption, metabolism, and excretion of thiabendazole in man and laboratory animals. Toxicol. Appl. Pharmacol. 9:31-39.
16. VONK, J. W., & A. KAARS SIJPESTEIJN. 1971. Methyl benzimidazol-2-ylcarbamate, the fungitoxic principle of thiophanate methyl. Pest. Sci. 2:160-164.
17. WHISTLER, R. L., ed. 1965. Methods in carbohydrate chemistry, Vol. V. General polysaccarides. 463 p.
18. WHISTLER, R. L., & E. L. RICHARDS. 1970. Hemicelluloses. p. 447-467. *In* K. Ward & P. A. Steib [ed.]. The carbohydrates. Academic Press. New York.