Role of Benomyl in the Systemic Control of Fungi and Mites on Herbaceous Plants

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ABSTRACT

Radioautographs, combustion analyses, and bioassays show that up to 20 times more active compound enters and moves within herbaceous plants when 2-^{14}C-benomyl, rather than methyl 2-^{14}C-benzimidazolecarbamate (MBC), is applied to the leaf surface. The improved systemic and curative control of plant diseases with benomyl is due to this increased penetration and indicates that benomyl remains intact on treated plant foliage. Selected surfactants increase penetration. Movement of active components in the plant is predominantly with the transpiration stream.

Cereal plants growing from treated seed have less chemical in each new leaf, and it is concentrated at the tips. Fungus spores and mites on plants treated systemically with ^{14}C-benomyl contain high ^{14}C concentrations.

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Some aspects of the penetration and systemic movement of Benlate® Benomyl Fungicide [50% methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and methyl 2-benzimidazolecarbamate (MBC), have been reported for several herbaceous plant species (1-16). Although best known as a fungicide, benomyl also has an inhibitory effect on mite populations (4, 7).

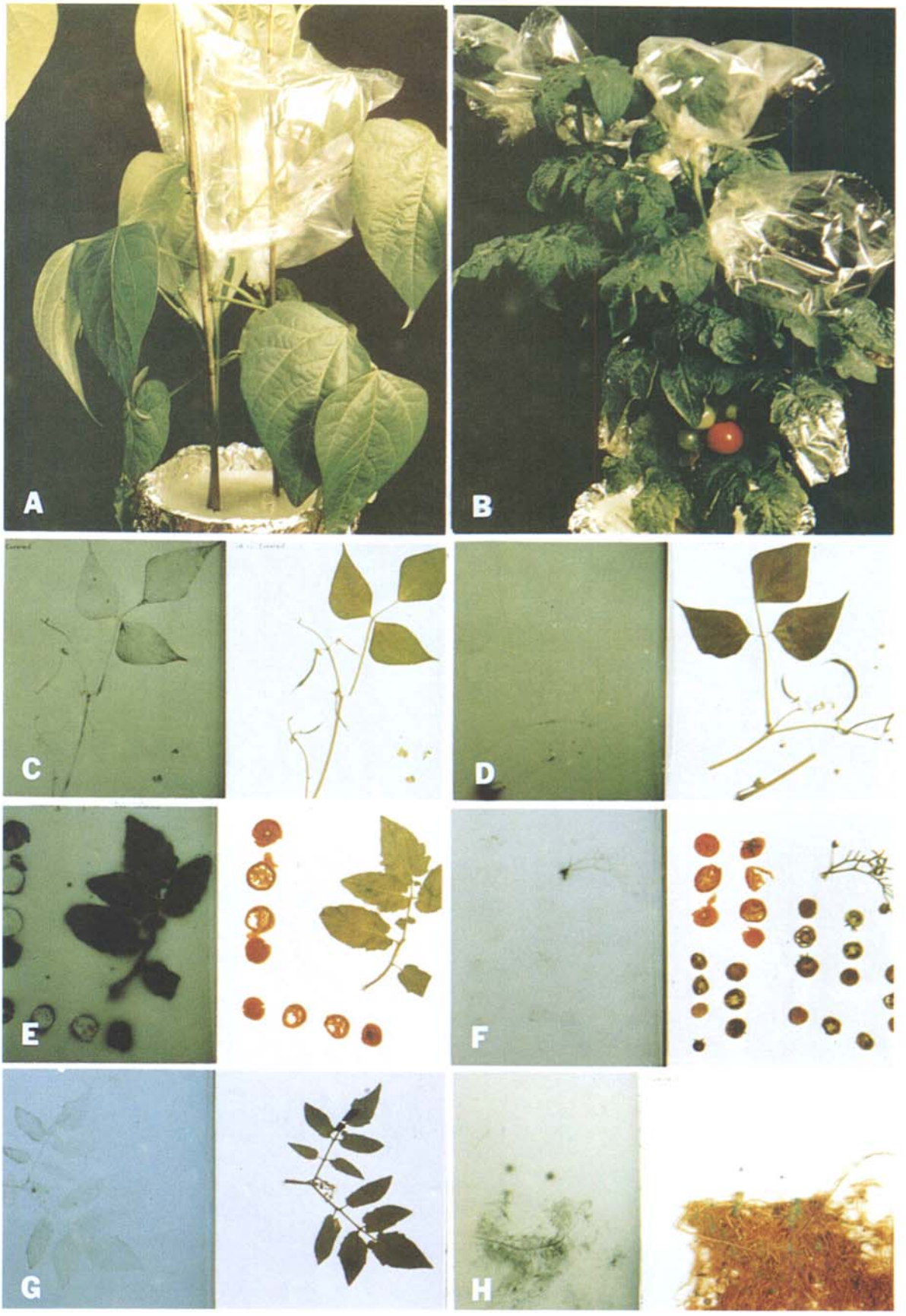
It has been shown that benomyl in suspension remains largely intact at recommended dilutions for spray applications and constitutes a major portion of the spray deposit on various leaf surfaces for at least three weeks (Baude, F. J., *unpublished*). Therefore, benomyl is considered to be an adequate reservoir for penetration and systemic fungicidal action under practical application conditions. In aqueous solution at very high dilution, benomyl apparently is hydrolyzed to MBC (3, 5, 10, 14). Previously reported studies (10, 11, 13 14) also have indicated that MBC is a fungicidal component at sites within the plant well removed from the point of application.

This paper will document the special function of benomyl in facilitating the penetration phase and providing for improved systemic activity. The results of radiotracer and supporting bioassay studies are presented to demonstrate the penetration and systemic movement in herbaceous plants of ^{14}C-tagged components following foliar sprays, spot treatments on leaves, seed treatments, and soil drenches. The radioactive materials used were benomyl and, in the case of the leaf spot work, also MBC. The accumulation of radioactivity within plant pathogens and mites along with the distinctive nature of the distribution in diseased host plants are given special attention.

MATERIALS AND METHODS.—All applications of 2-^{14}C-benomyl (0.418 mc mmole^{-1}, 1.44 μc mg^{-1}) and 2-^{14}C-MBC (0.378 mc mmole^{-1}, 1.98 μc mg^{-1}) were made using aqueous suspensions of very finely divided 50% wettable powder formulations and a surfactant where indicated. The ^{14}C was in the 2-position of the benzimidazole ring in both compounds. For radioautographs, the plant material was dried in a plant press, mounted on white bond paper, and exposed against X-ray film. Representative samples, critically selected from the radioautographs, were analyzed for total ^{14}C by combustion-liquid scintillation techniques. The samples were combusted in a Packard Model 305 Sample Oxidizer and counted in a Nuclear Chicago Model 6801 liquid scintillation spectrometer. Counting results were corrected for quenching by use of a channels ratio technique. These data were converted to fresh weight concentrations from moisture content readings made on representative plant parts.

Foliar sprays.—'Tiny Tim' (dwarf) tomatoes and 'Black Valentine' beans were grown to the blossom and immature fruit stage in the greenhouse. Special care was taken to protect soil, roots, selected leaf parts, terminal buds, and fruit from spray treatment so that ^{14}C in these parts could be only from systemic movement. Aluminum foil and paraffin wax were used to seal the soil and pots to avoid root contamination. Selected leaves, buds, blossoms, and

Fig. 1. Bean and tomato plants sprayed with 2-^{14}C-benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] at 1,520 μg/ml. **A, B)** Illustrations of the protective covering of selected leaves, leaf tips, blossoms, fruit, and soil of bean and tomato. **C-H)** Radioautographs on left. Exposed plant material on right. **C)** Protected portion of a bean plant which had been exposed in a saturated humidity chamber after spraying. **D)** Protected portion of a bean plant which had been allowed to dry immediately after spraying. **E)** Treated tomato foliage and fruit. **F)** Protected tomato fruit. **G)** Protected tomato foliage. **H)** Protected bean roots.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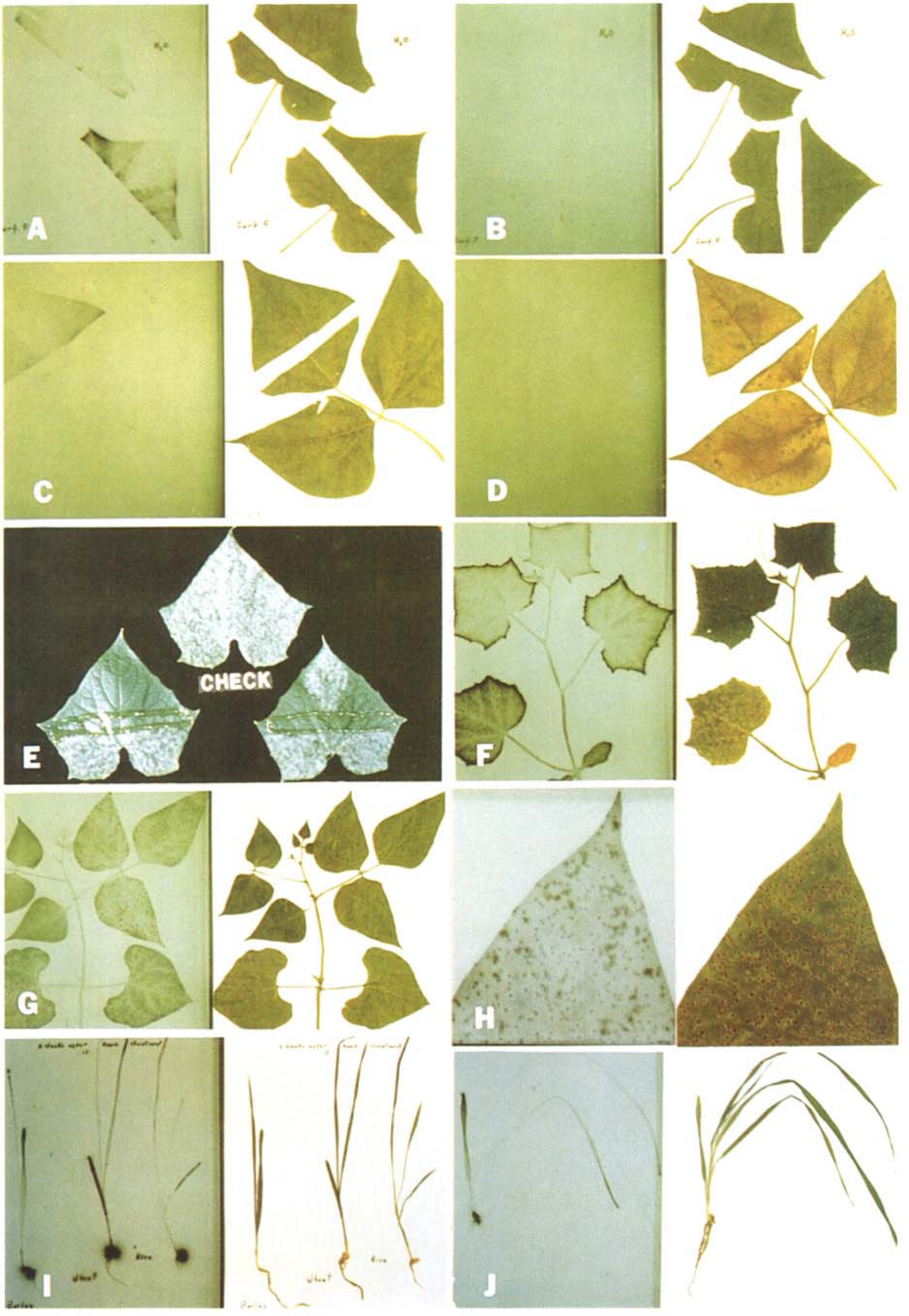
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immature fruit were protected with polyethylene bags. Leaf tips were covered with Saran® wrap using a lanolin band to make a seal (Fig. 1-A, B).

Tomatoes and beans were sprayed with 2-^{14}C-benomyl at 380 μg/ml of water, which is about the normal use rate, and at an elevated rate (four times the normal rate) to allow the greatest opportunity for detectable ^{14}C to appear in untreated plant parts. Two replicates of each of the treatments were placed in the greenhouse and harvested four days after treatment. One replicate of each crop sprayed at the elevated rate was grown to maturity (24 days) before sampling.

To accentuate possible downward movement, two replicates each of beans and tomatoes, sprayed at the elevated rate, were placed in a dark humidity chamber (100% relative humidity) for 48 hr. After an additional two days in the greenhouse, the above ground plant parts were removed, and the roots washed free of soil and dried.

Radioautographs were made by exposing plant material to the film for three weeks.

Spot treatments on leaves.—'Black Valentine' beans and 'Straight Eight' cucumbers were grown to the blossom stage in the greenhouse. A lanolin rectangle was placed across the middle of the leaf on the ventral side (Fig. 2-E). The fungicide was applied as an aqueous suspension in a row of small drops inside the lanolin barrier. The treated leaves were supported in a horizontal position to further hinder surface movement of the fungicide.

The plants were treated with 2-^{14}C-MBC at 250 μg/ml of water and also with an equimolar quantity of 2-^{14}C-benomyl. The very finely divided compounds were applied as suspensions in water alone and in a 1:1 ratio with Surfactant F (Nopco Trem®-014) or Atlas Brij® 78 (polyoxyethylene [20] stearyl ether). Two replicates of each crop were placed directly in the greenhouse after treatment. To determine the effect of a dark, moist environment, an additional replicate of beans and two replicates of cucumbers were placed in a dark humidity chamber at 100% relative humidity for 24 hr and then held in the greenhouse.

The treated leaves were removed from the plants two days after treatment and mounted for subsequent radioautographs and combustion analyses. The portions of the leaves with the lanolin rectangles containing the treatments were cut away before mounting. The film was developed after 39 days exposure.

A duplicate test on cucumbers using nonradioactive chemicals was conducted for bioassay with powdery mildew (*Erysiphe cichoracearum* DC.). Two days after treatment, all plants were inoculated with a conidial suspension and incubated in the greenhouse for eight days.

A study of the effect of leaf age on uptake and movement was conducted using this same isolated leaf spot technique with both radiotracer and bioassay on cucumbers. 2-^{14}C-MBC at 250 μg/ml and an equimolar quantity of 2-^{14}C-benomyl were compared in water and in a 1:1 ratio with Brij® 78 in water. Each treatment was replicated on four plants. A mature and an immature leaf were treated on each plant. Only ^{14}C-labeled chemicals were used, but all treatments were inoculated with *E. cichoracearum* conidia one day after treatment. After seven days incubation, leaves were sketched and photographed for bioassay and dried and mounted for radioautographs.

Seed treatments.—2-^{14}C-Benomyl was applied as a dust at a concentration of 5 g/kg (about the normal use rate) to 'Wong' barley, 'Redcoat' wheat, and 'Gulfrose' rice seed. The treated seed was planted in 12.8-cm diam (five-inch) pots, seven pots per treatment, and grown in the greenhouse. Two, four, and 12 weeks after treatment one whole plant was harvested from each pot, the roots washed free of soil and the plants dried. Radioautographs were exposed for four days.

Soil drenches.—'Pinto' beans were inoculated with *Uromyces phaseoli* (Pers.) Wint. and 'Straight Eight' cucumbers with *E. cichoracearum*. Infected plants were incubated in a greenhouse for a week. Bean rust pustules and cucumber powdery mildew colonies had just begun to sporulate when 2-^{14}C-benomyl was applied to the potted plants as a soil drench at 2.6 mg/8-cm pot (about five pounds per acre), as a soil drench to the potted plants. Care was taken to avoid contamination of above-ground plant parts. Spores were harvested four days after treatment by tapping the stems and allowing the spores to fall onto paper. The harvest was repeated with cucumber powdery mildew nine days after treatment. The spores were weighed and analyzed for total ^{14}C. Radioautographs (exposed four days) were made of the foliar portions of these plants removed ten days after treatment.

Fig. 2. Cucumber, bean, and cereal plants treated with 2-^{14}C-benomyl [methyl 1-(butylcarbamoyl)-benzimidazolecarbamate] or 2-^{14}C-MBC [methyl 2-benzimidazolecarbamate]. Radioautographs on the left (except bioassay E). **A-E)** Treatments applied in a band across the leaf. Note lanolin rectangle which contained drops of chemical shown in E has been removed from A-D prior to X-ray exposure. **A)** Cucumber leaves spot-treated with ^{14}C-benomyl in water (top) and Surfactant F (bottom). **B)** Cucumber leaves similarly spot-treated with ^{14}C-MBC in water (top) and Surfactant F (bottom). **C)** Bean leaflet spot-treated with ^{14}C-benomyl in Surfactant F. **D)** Bean leaflet similarly spot-treated with ^{14}C-MBC in Surfactant F. **E)** Cucumber leaves spot-treated with benomyl in Brij® 78 on left, untreated control in center, and MBC in Brij 78 on right. *Erysiphe cichoracearum* serves as a bioassay. **F)** Cucumber foliage from plant soil-drenched with ^{14}C-benomyl after *E. cichoracearum* was established on the cotyledons and first true leaf. Note the greater retention of radioactivity in the diseased tissues. **G)** Bean foliage from plant soil-drenched with ^{14}C-benomyl after *Uromyces phaseoli* was established on the primary and first trifoliate leaves. **H)** Close-up of the top leaflet in G, showing the concentration of ^{14}C in the rust pustules. **I)** Barley, wheat and rice grown for two weeks after seed treatment with ^{14}C-benomyl at 5 g/kg. **J)** Barley grown for four weeks after seed treatment with ^{14}C-benomyl at 5 g/kg.

TABLE 1. Translocation of ^{14}C in beans and tomatoes following foliar spray applications of 2-^{14}C-benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate]

Plant part	Total ^{14}C as benomyl[a]			
	Normal[b]	Elevated[b]		
	Greenhouse			Humidity chamber[e]
	4 days[c]		24 days[d]	
Bean leaflets exposed[f]	76	136	354	
Bean leaflets covered[g]	0.2	1.3	0.6	
Tomato leaflets exposed[f]	72	306	252	420
Tomato leaflets covered[g]	0.2	1.6	1.7	1.3
Tomato blossoms covered[g]		1.0		
Tomato fruit exposed[f]	0.2	27	11	
Tomato fruit covered[g]	0.04	0.3	0.06	

[a] Total ^{14}C calculated as benomyl (μg/g fresh wt tissue).
[b] Rate applied: Normal equals 380 μg/ml and elevated equals 1,520 μg/ml of benomyl-2-^{14}C.
[c] Plants held in greenhouse four days after treatment.
[d] Plants held in greenhouse 24 days after treatment.
[e] Plants held in dark saturated humidity chamber two days, then in the greenhouse two days after treatment.
[f] Assay from exposed and treated portion of plant.
[g] Assay from polyethylene-covered, thus untreated, portion of plant.

'Red Kidney' beans grown in soil drenched at planting with 2-^{14}C-benomyl at about 14 mg/10-cm pot (15 lb per acre) were used to feed female two-spotted mites, *Tetranychus urticae* Koch. Fifty mites, fed for three days on each leaf, were collected and analyzed for total ^{14}C.

RESULTS.—*Foliar sprays.*—Radioautographs were made of various plant parts from tomatoes and beans, treated at both concentrations, including covered versus exposed leaf tips, leaves, blossoms, and fruit along with protected roots. Representative samples were chosen for combustion analysis following critical examination of these radioautographs.

The combustion analyses (Table 1) showed that the exposed leaves on the treated plants contained 100 to 400 μg of benomyl per gram of fresh tissue when sprayed at the elevated rate and about 75 μg/g when sprayed at the normal use rate. Concentrations accumulated by systemic movement in the covered leaf tissue were 0.6 to 1.7 μg/g at the elevated rate and 0.2 μg/g at the normal rate. Radioautographs of representative bean leaves indicate a significant increase in systemic movement under high humidity conditions (Fig. 1-C, D). Accumulation in covered, untreated tomato fruit was only 0.04 μg/g from sprays at the normal rate. Radioautographs (Fig. 1-E, F, G) substantiated the data obtained by combustion analysis of covered and exposed tomato tissue. Also, the presence of ^{14}C was noted in roots of beans which had received elevated rate foliage treatments in such a way as to avoid root contamination and were subsequently held in the dark and in a saturated atmosphere (Fig. 1-H).

Spot treatments on leaves.—Replicated radioautographs were prepared from treated leaves representing all of the variables included in the test. Again, samples were selected for combustion analyses (Tables 2 and 3). The results of the combustion work compared well with the radioautographs of the same treated leaves. Movement of benomyl was generally upward with only traces found in the basal part of the leaf or petiole. The addition of a surfactant increased penetration and movement. Treatments with benomyl resulted in substantially more ^{14}C in untreated portions of the leaves than similar MBC treatments. For example, the cucumber leaf tip above the band treated with benomyl plus Surfactant F contained 2.5 μg of benomyl per gram of tissue. Leaf tips from MBC plus Surfactant F treatments contained only 0.1 μg of MBC per gram (Table 2). This difference is clearly shown in the radioautographs (Fig. 2-A, B).

Although there was generally less chemical movement in beans following the spot treatment, the greater movement with benomyl was confirmed (Fig. 2-C, D). Treatments which had been kept wet for 24 hr in a dark humidity chamber showed little increase in movement.

Where the fungicides were applied to cucumber leaves in combination with the surfactant Brij® 78, upward movement from the point of application was greater in young leaves than in old (Table 3). Under these conditions, the use of benomyl again resulted in 10 to 20 times greater movement than did MBC as determined by combustion studies. The advantage of benomyl over MBC was also demonstrated biologically by inoculating test leaves with conidia of *E. cichoracearum* and noting the relative development of the disease (Fig. 2-E).

Seed treatments.—The pattern of movement of ^{14}C from benomyl treated seed into the developing seedlings of representative cereal crops was demonstrated by radioautographs (Fig. 2-I, J). The amount of ^{14}C at any time was greatest in the leaves that had formed earliest and, within a given leaf, tended to concentrate in the distal portion. Roots in the vicinity of the treated seed contained ^{14}C but downward translocation was minimal.

Soil drenches.—Fungus spores, harvested from infected plants four days after soil drench treatments with benomyl, contained ^{14}C equivalent to 24 μg of benomyl per gram of fresh spore weight for *U. phaseoli* and 15 μg/g for *E. cichoracearum*. A second harvest of powdery mildew conidia nine days after treatment resulted in concentrations up to 62 μg/g. Mites accumulated ^{14}C up to 49 μg/g of body weight.

Radioautographs of infected bean leaves from plants that had been treated by a soil drench showed a concentration of ^{14}C in the rust pustules with an area of reduced concentration around them (Fig. 2-G, H). Radioautographs of cucumber foliage showed more retention of ^{14}C in the infected areas of cotyledons and primary leaves than in uninfected new growth (Fig. 2-F).

DISCUSSION.—Commercial applications of benomyl for plant protection are largely foliage sprays containing 100 to 300 μg/ml of active benomyl or about 28-112 g (1-4 oz) active material

TABLE 2. Translocation of ^{14}C from isolated band applications of 2-^{14}C-benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and 2-^{14}C-MBC [methyl 2-benzimidazolecarbamate] on mature cucumber and bean leaves

Plant	Carrier	Leaf part	Total ^{14}C calculated as benomyl or MBC (μg/g fresh wt tissue)	
			benomyl	MBC
Cucumber	Water	Above treated band	0.5	0.1
		Treated band	520	308
		Below treated band	0.01	0.004
Cucumber	Surf. F[a]	Above treated band	2.5	0.1
		Treated band	422	266
		Below treated band	0.02	0.004
Bean	Water	Above treated band	0.03	0.007
		Treated band	303	117
		Below treated band	0.001	0.007
Bean	Surf. F	Above treated band	0.7	0.04
		Treated band	268	374
		Below treated band	0.003	0.001

[a] Surf. F = "Surfactant F" = Trem®-014 (Nopco Chemical Company).

TABLE 3. Translocation of ^{14}C from isolated band applications of 2-^{14}C-benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and 2-^{14}C-MBC [methyl 2-benzimidazolecarbamate] on young and old cucumber leaves

Carrier	Age of leaf[a]	Leaf part	Total ^{14}C calculated as benomyl or MBC (μg/g fresh wt tissue)	
			benomyl	MBC
Water	Young	Above treated band	0.5	0.2
		Treated band	168	296
		Below treated band	0.03	0.01
		Petiole	0.003	0.002
Water	Old	Above treated band	0.5	0.2
		Treated band	505	561
		Below treated band	0.02	0.03
		Petiole	0.01	0.002
Brij® 78[b]	Young	Above treated band	5.1	0.4
		Treated band	306	214
		Below treated band	0.02	0.02
		Petiole	0.01	0.002
Brij® 78	Old	Above treated band	3.1	0.1
		Treated band	605	286
		Below treated band	0.03	0.01
		Petiole	0.02	0.003

[a] Old leaves were 9 to 12 from the base of the plant. Young leaves were 15 to 17 from the base of the plant. They were the youngest possible fully expanded leaves.

[b] Brij 78® = polyoxyethylene [20] stearyl ether.

per 100 gallons. This study with 2-^{14}C benomyl and MBC emphasized foliar treatments of herbaceous plants and provided radioautographs and combustion observations that were consistent with disease control experience. Following application, benomyl penetrated host plant leaves and provided local systemic disease control. The distribution of ^{14}C within the plant and the pattern of disease control suggests passive movement in the water transport system under most conditions. Distribution was predominantly acropetal with accumulation at the margins and tips of healthy leaves. This is in agreement with conclusions reported by several other investigators (2, 8, 10, 11). Under conditions minimizing the upward movement of the transpiration stream, some ^{14}C did move into the root system of bean plants from the above-ground parts (Fig. 1-H). Baron (1) reported evidence of basipetal movement out of treated banana leaves. Work by Siegel & Zabbia (13) indicates that the material actually moving within the plant may be mostly MBC plus smaller amounts of the fungicidally inactive 2-aminobenzimidazole. Nevertheless, our work shows that direct applications of MBC resulted in much less plant penetration of active component than similar treatments with benomyl. The ^{14}C accumulated in untreated tissue following the leaf applications was up to 20 times greater with benomyl than with MBC. This difference was also evident in disease control experiments. Under conditions where benomyl provided systemic control of *E. cichoracearum*, similar treatments with MBC were less effective. Most benomyl treatments resulted in sufficient penetration to provide local systemic control, whereas most MBC treatments at the same levels did not. These results substantiate the speculation in 1969 by Clemons & Sisler (3) that improved performance of benomyl over MBC might be due to superior penetration properties.

Earlier disease control studies had suggested that certain surfactants used with benomyl or MBC increased the concentration of active component within plant tissue. Other surfactants appeared to have no effect. In this work, using surfactants already known to have a beneficial influence, definitive physical evidence of the greater penetration and movement was indicated. The advantage of benomyl over MBC in penetration was greatest when both materials had been applied with an effective surfactant.

Seed treatments with benomyl provided an initial source of active component for systemic movement into the first few leaves of the developing seedling. As the roots grew away from the deposit of chemical, uptake was increasingly dilute and only trace amounts accumulated at the tips of leaves developing after a few weeks. Consistent with this pattern, experience with seed treatments has shown exceptional control of seedling diseases, even systemic infections of smuts (4, 5, 6, 12, 16). No chemical residue (<0.1 μg/g) has been detected in grain produced by plants that had developed from seed treated with benomyl.

This study demonstrated that ^{14}C was concentrated in the spores of fungi and in mites sustained on plant tissue systemically supplied with active component. It is possible that these greater levels were brought about by an increased water loss through spores and mites over that of normal leaf transpiration. In any event, the amount of ^{14}C was greatest at points of need for the active chemical.

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