# Transport of the Systemic Fungicide, Benomyl, in Bean Plants

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ABSTRACT

Benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate) rapidly decomposed to methyl 2-benzimidazolecarbamate (MBC) in dilute aqueous solution and within plants. Only slight breakdown of the MBC occurred in bean leaflets during the month following treatment of the plants with benomyl. Benomyl and MBC were taken up passively by the roots, transported to leaves via the xylem, and accumulated in the leaf tips and margins. When the supply to the roots was discontinued for 15 days, no MBC was detected in stems or central areas of the leaves. MBC is not phloem-mobile, although it may appear in the bark because of lateral transfer from the xylem. Phytopathology 60:475-478.

The systemic fungicide benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate) has been used successfully for the control of many plant diseases in both field and greenhouse trials (5). When applied to plant roots, the fungicide controls diseases in the foliage, indicating that it is taken up by the roots and moved systemically in the plant (5). Benomyl also has been applied as a foliar spray (5), but movement downward to the roots has not been demonstrated. This paper presents results from a study of Benomyl uptake, translocation, and breakdown in bean plants. Since benomyl rapidly breaks down to methyl 2-benzimidazolecarbamate (MBC) in aqueous solution (3, 9) and in plants (9), this study deals mainly with the fungitoxic breakdown product, MBC.

MATERIALS AND METHODS.—*Plant culture.*—Bean (*Phaseolus vulgaris* L. 'Tender Green') seed were germinated in vermiculite, and plants were grown in half-strength modified Knop's nutrient solution (8). Unless otherwise indicated, they were treated with benomyl when the first trifoliate leaves were fully expanded.

*Bioassays.—Bioautography.*—Identification and quantitative determination of Benomyl and MBC was accomplished using a bioautograph technique. This involved thin-layer chromatography of a sample (solvent system, acetone), and spraying the dried chromatogram with warm agar seeded with *Penicillium* sp. spores. After a 20-hr incubation period in a moist chamber, the fungitoxic spots were seen as clear areas against the opaque growth of the fungus. The diam of the spots was linearly related to the log of the concentration of the fungicide. Details of this method have been presented elsewhere (9).

*Glomerella bioassay.*—A more conventional bioassay was used for the routine determination of MBC in small pieces of stem or leaf tissue. The tissue was frozen using a Freon gas spray, then placed on 1.5% potato-dextrose agar previously seeded with spores of *Glomerella cingulata* (Ston.) Spauld. & Schrenk (2.5 ml spore suspension with 0.3 absorbance at 450 mμ added/100 ml agar). Inhibition zones were measured after 48-hr growth at room temperature. When the method was used to assay the amount of MBC in leaf strips, the agar was trimmed to leave a band equal in width to the cut edge of the strip. This prevented lateral diffusion of the fungitoxic chemical at the leaf edges. Filter-paper strips (5 × 7 cm) were immersed in standard chloroform solutions of MBC, dried, and placed on *G. cingulata*-seeded plates. The widths of the resulting zones of inhibition were linearly related to the log of the fungicide concentration.

RESULTS.—*Uptake of benomyl and MBC.*—The root systems of four intact bean plants were treated with 5 ppm benomyl for 5 days. Treatment solutions were sampled 0, 2, and 5 days after initiation of the treatment. The samples were dried at room temperature under vacuum and dissolved in chloroform; amounts of MBC were determined by bioautography.

Although benomyl was dissolved in the treatment solutions, the only fungitoxic chemical recovered from a sample of the solution taken on the first day of treatment was MBC. The concentration of MBC in the treatment solutions remained constant in samples checked at 0, 2, and 5 days. A statistical analysis confirmed that no significant difference existed among the samples.

*Breakdown of Benomyl and MBC.*—It was shown previously that the leaflets of a trifoliate bean leaf did not differ significantly in MBC content (expressed on a fresh wt basis) after the plant had taken up a benomyl solution (40 ppm) for 4 days through the roots (9). Thus, it was possible to study the breakdown of the MBC in one leaf by sampling its leaflets at three time intervals following treatment. Four replicate plants were used. Each leaflet was extracted with chloroform, and amounts of MBC were determined by bioautography.

The amounts of MBC in trifoliate leaves harvested 7, 15, and 30 days after the last day of treatment decreased slightly, indicating a breakdown or complexing of some of the MBC in the leaf (Table 1).

*Distribution of MBC within leaves.*—The distribution pattern of MBC within leaflets of plants grown in hydroponics solution containing benomyl was compared to the pattern in plants grown in soil to which the fungicide was added. Benomyl was applied at 10 and 25 ppm (w/w). The three leaflets of the first

TABLE 1. Amounts of methyl 2-benzimidazolecarbamate (μg/g fresh wt) extracted from leaflets of the first trifoliate leaves of bean plants placed in hydroponics treated with 40 ppm benomyl for 4 days

| Day of harvest | Leaflet | Plant 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 7 | Right | 41 | 68 | 72 | 69 |
| 15 | Terminal | 23 | 48 | 52 | 42 |
| 30 | Left | 38 | 64 | 45 | 29 |

trifoliate leaf were sampled at 1, 2, 3, 4, and 5 days for each treatment. After 5 days, the hydroponic treatment solution was replaced by nutrient solution, and leaflet samples were taken at 7, 15, and 30 days. Three 7-mm strips were removed from the lamina at distances of 0.25, 0.50, and 0.75 of the total leaf length. These strips were assayed on *G. cingulata* plates as described above.

There was a progressive change in distribution of MBC within leaflets of the first trifoliate leaves after treatment with 10 or 25 ppm benomyl via hydroponic solution (Fig. 2). Initially, the chemical was present throughout the leaf and was most concentrated at the mid-vein and at the leaf base. During successive days, the MBC became more concentrated at the margins and apices of the leaflets. However, some chemical was always present in the central regions, since the leaflet was being continuously supplied from the roots. When plants were removed from their treatment solutions and returned to nutrient solutions, the MBC gradually became depleted in the centers of the leaflets as margin and tip accumulation increased. By day 15, no detectable amounts of the fungicide remained near the mid-rib in the basal section. Marginal accumulation became even more pronounced with longer time intervals. With soil treatment, the distribution of chemical within the leaflets during the first 5-day period was similar to that obtained with hydroponic culture (Fig. 2). However, at intervals longer than 5 days, no central depletion occurred in leaflets of plants grown in soil treated with 25 ppm benomyl, indicating that the source of available fungicide in the soil had not been depleted (Fig. 2). When the amount of benomyl in the soil was decreased to 10 ppm, the plants did show an absence of MBC in the central areas of their leaflets after 5 days.

*Distribution of MBC within stems.*—Bean plants were grown in hydroponics, as described earlier, until flower buds were initiated. These buds were removed, and 12 plants were treated with a 40-ppm solution of benomyl for a period of 5 days. Three plants were harvested 0, 7, 15, and 30 days after the last day of treatment. Stem segments (5 mm in length) were removed just below the primary, first trifoliate, and second trifoliate leaf nodes on each plant, and the bark was separated from the xylem. The amount of MBC in each segment was estimated using the *G. cingulata* bioassay, the bark being placed cambium-side down on the agar; the xylem was set on as a cylinder.

The bioassay of the xylem and bark samples taken on the last day of the 5-day treatment shows that both

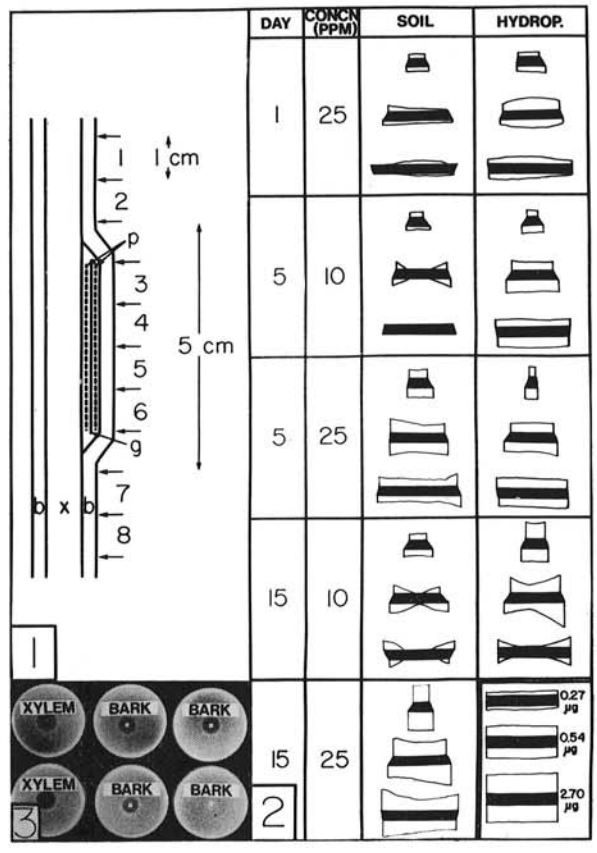


**Fig. 1-3.** Transport of Benomyl and methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate in bean plants. **1)** Diagram of a longitudinal section through a stripped bean stem showing insertion of moist papers (p) and glassine paper (g) between bark (b) and xylem (x). Arrows indicate points at which stem was sectioned. Sections were numbered as shown. **2)** Drawings showing zones of inhibition around leaf strips (shaded) taken near the apex (upper leaf strip), middle (middle leaf strip), and base (lower leaf strip) of terminal leaflets of the first trifoliate leaves of benomyl-treated bean plants grown in soil and hydroponics (hydrop.). Plants harvested at 15 days were treated for 5 days, then returned to nutrient solution. Insert (lower right) shows zones of inhibition around 7-mm paper strips dipped in standard solutions of MBC. **3)** Bioassay of MBC in xylem (left) and phloem (middle and right) segments on agar seeded with spores of *Glomerella cingulata*. Upper three plates contain sections from unstripped stem. Lower three plates contain sections from part of stem in which the bark on one side had been stripped; unstripped phloem (middle); stripped phloem (left).

tissues contain substantial amounts of MBC at all points along the stem examined (Table 2). Seven days later, however, very little remained in the xylem, and none in the bark. By 15 days, it also was no longer detectable in the xylem.

*Stripping method.*—A modified version of the stripping technique of Stout & Hoagland (12) was employed to determine whether MBC was transported in the phloem. Vertical incisions 5 cm in length were made in opposite sides of the lower stems of 2-month-old bean plants. The bark between the slits was separated

TABLE 2. Amounts of methyl 2-benzimidazolecarbamate (μg) in the bark and xylem of stem segments following a 5-day root treatment with 40 ppm benomyl in hydroponics[a]

| Time after termination of treatment (days) | Plant part | Location of segment[b] A | B | C |
|---|---|---|---|---|
| 0 | Xylem | 129 | 82 | 49 |
| | Bark | 125 | 52 | 29 |
| 7 | Xylem | 4 | 3 | 1 |
| | Bark | 0 | 0 | 0 |
| 15 and 30 | Xylem | 0 | 0 | 0 |
| | Bark | 0 | 0 | 0 |

[a] Data are the average of 4 replications.
[b] Stem segments (5 mm in length) were taken from immediately below the primary leaf node (A), the first trifoliate leaf node (B), and the second trifoliate leaf node (C).

from the xylem on one side of the stem so that the bark remained attached to the stem at both ends. Moist papers were placed next to the exposed tissues to prevent drying, and a strip of glassine paper was inserted between these, as shown in Fig. 1, to prevent lateral diffusion from xylem to phloem. The entire area was then wrapped in glassine paper. The plant roots were placed in a 40-ppm solution of benomyl, and the terminal leaflet of the first trifoliate leaf was treated with 5 μc of glucose-U-$^{14}$C applied as a droplet to the upper surface over the mid-vein. After 3 days, the center of the stripped area was segmented into 4 sections 1 cm in length, as shown in Fig. 1. The unstripped bark on the opposite side of the stem was separated from the xylem, and both were similarly segmented. Also, areas above and below the stripped area were segmented, as shown in Fig. 1. The xylem and bark from the upper half of each section was assayed for MBC on agar seeded with *G. cingulata* spores. The lower halves of the xylem and bark samples were finely chopped and extracted in 80% ethanol for 36 hr. Radioactivity in the extract was determined by means of a Unilux II scintillation counter.

MBC was not detected in segments of bark taken from the stripped area. It was present in bark segments located directly above and below the stripped area and in the unstripped bark on the opposite side of the stem (Table 3, Fig. 3). The phloem in the stripped area was considered functional, since the bark in that region transported $^{14}$C-assimilates from an upper leaflet (i.e., each section contained appreciable amounts of $^{14}$C). Therefore, it was concluded that the MBC had moved into the bark by lateral transfer from the xylem and not by vertical movement in the phloem.

DISCUSSION.—The study of benomyl transport in plants is complicated by its rapid breakdown in dilute aqueous solution. Initially, the fungicide solutions used to treat plants in this study were a mixture of benomyl and MBC; soon only MBC remained. For instance, the time required for drying a 5-ppm benomyl solution and for bioautography is sufficient for complete breakdown to occur. Most of the reported systemic fungicidal activity in plants is really due to that of the

TABLE 3. Amounts of methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (μg) in xylem and bark segments 3 days after stripping, treatment with 40 ppm benomyl in hydroponics, and treatment of the first trifoliate leaf with 5 μC glucose-U-$^{14}$C[a]

| Segment no.[b] | Plant Part Xylem | Unstripped phloem | Stripped phloem[c] |
|---|---|---|---|
| 1 | 34 | 18 | 18 |
| 2 | 32 | 18 | 14 |
| 3 | 38 | 18 | 0 |
| 4 | 38 | 16 | 0 |
| 5 | 33 | 18 | 0 |
| 6 | 27 | 17 | 0 |
| 7 | 31 | 18 | 18 |
| 8 | 36 | 19 | 17 |

[a] Results are averages of 4 replicate experiments.
[b] See Fig. 1 for location of segments.
[c] In segments 1, 2, 7, and 8, the phloem was not stripped, but samples were taken in vertical alignment with the stripped phloem of the other segments.

breakdown product, MBC. This conclusion was also reached by Clemons & Sisler (3) in a recent study of benomyl breakdown.

The bioassay of this fungicide was greatly enhanced by freezing the plant tissue. The use of unfrozen leaf tissue, as reported by Erwin et al. (6), was not nearly as sensitive. Apparently, freezing allows more rapid diffusion of the fungicide from the tissues as a result of disruption of cell structure.

Benomyl has given long-term protection against a variety of diseases when applied to soil (7) and to cut potato seed (11). The relative stability of MBC within the plant reported in the present work adds theoretical support to these findings. The tendency for accumulation of the fungicide at the leaf tips and margins with a concomitant tendency toward fungicide depletion in the central area of the leaf of bean was similar to that observed in tomato by Biehn & Dimond (2). This central depletion explains the report of Schroeder & Provvidenti (10) noting a lack of protection against powdery mildew in the central areas of cucumber leaves when a low dosage of benomyl was applied. The center of the leaf may be protected if sufficient quantities of benomyl are applied to the soil. However, the continual accumulation of MBC at the leaf margins may cause a phytotoxic response, as has been reported for cucumbers (7, 10) and pumpkins (7). The situation in stems is analogous to that of the central areas of the leaves. Without a continuous supply of fungicide, the stem also rapidly becomes depleted. This character probably applies to any systemic fungicide which moves primarily in the apoplast, a term referring to the continuum of nonliving cell-wall material of the plant and which contains the symplast (the continuum of interconnected protoplasts). Apoplastic movement of this fungicide would involve passive uptake by the roots, movement via cell walls to the xylem, movement through the xylem to the leaves, then movement to regions of evaporation via the cell walls of the leaf, resulting in accumulation at the tip and margins. Monuron, a herbicide transported in the

apoplast, also shows this characteristic accumulation pattern in leaves (4). Exceptions to this rule can occur, however, if the chemical becomes complexed and/or rendered insoluble along its path of transport as in the case of calcium (1).

Movement of a fungicide in the phloem could have certain advantages over movement via the apoplast. For instance, the chemical could be applied to leaves and exported to the roots and young growing areas of the plant, protecting roots and foliage that were not yet formed at the time of application. When benomyl was found in the bark as well as in the xylem during a study of its distribution within the stem, the possibility of phloem movement arose. However, data of a stripping experiment clearly showed that the MBC in the bark had arrived there by lateral movement from the xylem, and that it was not transported in a vertical direction within the bark.

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