Transport of Benomyl into Various Plant Organs

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ABSTRACT

Benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate) was applied to the roots of flowering geranium and poinsettia plants. Fungicidal activity was later detected in the leaves, but none could be found in the geranium petals or in poinsettia bracts. Studies carried out with bean and tomato plants bearing fruit showed that the fungicide was transported primarily to the foliage. The concentration in tomato fruit (fresh weight basis) ranged from 0.03 to 2% of that in the foliage. In bean, the concentration in the fruit tended to be greater (0.3 to 3% of the concn in the foliage). Geranium petals had no stoma; poinsettia bracts had nonfunctional stoma; and tomato fruits had no stoma. Bean fruit which accumulated the most fungicide in relation to the leaves, however, had functional stoma. The capacity of an organ to transpire apparently governs its ability to accumulate benomyl when it is used as a systemic fungicide. Marginal and apical accumulation of fungicide in plant leaves can be mimicked by dye movement on filter paper cut to resemble leaves. Thus, the distribution of fungicide within the plant can be explained on the basis of physical forces. Phytopathology 61: 91-92.

Additional key words: methyl 2-benzimidazolecarbamate, eosin, *Glomerella cingulata* bioassay.

Benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate) has been successfully employed as a systemic fungicide for the control of various plant diseases (3). In aq solution, benomyl forms a fungicidal breakdown product, methyl 2-benzimidazolecarbamate (MBC) (2, 4), which is quite stable in bean plants (4, 5). Previous investigations of the translocation of benomyl have shown that MBC is transported through the xylem and moves acropetally within leaves resulting in an accumulation of fungicide at the margins and tips (1, 5). This transport pattern is characteristic of materials moving in the apoplast.

Such a distribution pattern could be the result of physical forces alone, as it also could be demonstrated by eosin dye movement on filter paper. Filter papers were cut in the shapes of leaves, saturated with water, and suspended so that their "petioles" dipped into a small container of 0.1% eosin. When the dye had ascended about half-way up the "leaf", the eosin solution was replaced with water. With time, the dye moved toward the margins and apex of the "leaf", where it accumulated (Fig. 1-a). Lobed "leaves" accumulated the dye in the tips of the lobes (Fig. 1-b, c).

The relative uptake of benomyl from root treatments into various plant organs was investigated. Benomyl was applied as a drench (1.25 g in 500 ml water) to potted geranium (*Pelargonium hortum* [Bailey]) and poinsettia (*Euphorbia pulcherrima* Wild) plants. Three days later, as determined by bioassay with *Glomerella cingulata* (Ston.) Spauld. & Schrenk (5), geranium leaves had a concn of 50 μM fungicide, while petals contained no detectable fungicide. When poinsettia bracts and leaves were similarly bioassayed 7 days after treatment, no fungicide could be detected in the bracts, but leaves had a concn of 209 μM fungicide. Microscopic examination of the epidermis of the geranium petals revealed that no stomates were present. Poinsettia bracts had only one-tenth as many stomates as the leaves, and the bract stomates were apparently nonfunctional, as they were never open during the day, as verified by the silicone rubber cast-cellulose acetate impression method described by Sampson (6).

Tomato plant cuttings bearing fruit were placed in a 100 μM solution of benomyl for 5 days. The fruits were removed and extracted in chloroform for 1.5 min in a Waring Blendor. The extracts were centrifuged at 1,000 *g* and the pulp and water layers were extracted with chloroform twice. The chloroform extracts were combined and concd to a volume of 0.5 ml. Aliquots (20 μliters) were assayed for fungicide by the bioautograph method described previously (4).

Although the fruit and foliage contained varying concn of MBC, the fungicide was always much more concd in the foliage than in the fruit (Table 1). Both ripe fruit and green fruit (which had increased in volume as much as 86% during the treatment period) contained very low concn of the chemical. Values range from 0.03 to 2% of the concn in the foliage. The variation noted may have been due to differences in fruit size and position on the plant as well as differences in over-all transpiration rates of the cuttings due to their position in the growth chamber. The tomato fruits had no stomates and no detectable transpiration (measured in a Siemens Sirigor gas exchange chamber). The small amount of fungicide in the fruit may have moved there by diffusion in the apoplast similar to that found between the xylem and bark of bean plants reported earlier (5).

A study of the distribution of MBC within bean pods following a 5-day treatment of the plants with 100 μM benomyl in a hydroponic solution was made by removing 5-mm slices from the distal, middle, and proximal ends of the pods. These sections were bio-

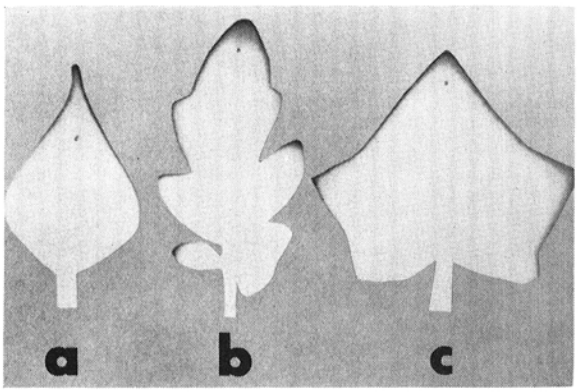

Fig. 1. Distribution of eosin dye on filter paper cut to resemble leaves of **a)** bean; **b)** tomato; and **c)** cucumber. See text for details of preparation.

assayed on *G. cingulata* plates. The remainder of the pod, as well as the foliage and roots, was extracted with chloroform as described above and assayed for fungicide using the bioautograph method. Again, the considerable variation in the amounts of fungicide present in the fruit, foliage, and roots of individual bean plants was probably due to variations in growth chamber conditions. In all cases, the fruits were found to contain a very low concn of the fungicide as compared to the foliage and roots (Table 2). The concn of MBC in the fruit was from 0.3 to 3% of the concn in the foliage, and from 0.3 to 7% of the concn in the roots. The fungicide was most concd at the proximal end of the pod, indicating that the organ transpired so little that no marginal accumulation of the fungicide had occurred. Although functional stomates were found on the surfaces of bean pods, one would not expect organs such as fruits to transpire as rapidly (on a wt basis) as leaves.

Although both bean and tomato fruits contained much less fungicide than the foliage, there was often enough fungicide present to inhibit the growth of a sensitive fungus. For example, the ED_{50} of benomyl for *Colletotrichum lagenarium* is 1.1 μM. The results obtained from this study are consistent with the hypothesis that MBC moves through the plant in the apoplast, and that its distribution among plant organs is dependent on the rate of transpiration of the organ in question.

TABLE 1. Concentrations (μM) of methyl 2-benzimidazolecarbamate in fruits and foliage of tomatoes as determined by bioautography of chloroform extracts of plant homogenates. Plants were grown in soil and severed just above ground level, and the severed stems placed in 100 μM benomyl solutions for 5 days prior to harvest

Plant[a]	Fruit	Foliage	% Increase in fruit size
1	4.48	234	0
2	0.30	234	0
3	0.03	334	0
4	0.01	264	0
5	1.90	757	19
6	0.91	895	86
7	4.78	516	27

[a] Plants 1-4 had ripe fruit; plants 5-7 had green fruit.

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TABLE 2. Distribution of methyl 2-benzimidazolecarbamate (MBC) within pods, and concn of MBC in the fruit, foliage, and roots of bean plants following a 5-day root treatment with 100 μM benomyl

	A[a]			B[b]		
Plant	Distal	Middle	Proximal	Fruit	Foliage	Roots
1	0	2.36	23.30	0.74	215	237
2	0	3.57	15.55	2.94	184	251
3	2.43	3.43	11.30	14.60	522	196
4	0	2.84	16.20	6.30	214	608

[a] Concentration of MBC (μM) in 5-mm slices of bean pod (determined by bioassay with *Glomerella cingulata*).
[b] Concentration of MBC (μM) in parts of bean plants (determined by bioautography).