Translocation of Benomyl in Creeping Bentgrass

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ABSTRACT

Root uptake and translocation of benomyl and its breakdown product, MBC (methyl-2-benzimidazole carbamate), were studied in Toronto creeping bentgrass (*Agrostris palustris*) stolons having either one or two root systems. The roots of stolons with a single root system were either nontreated (control) or treated with 5, 12.5, or 25 mg/liter benomyl for 24, 48, or 96 hr. Bioassay and thin-layer chromatography showed presence of fungistatic compounds throughout all tissues of stolons with a single root system. This showed translocation of benomyl upward in the transpiration stream to the growing point. When benomyl at 100, 500, or 1,000 mg/liter was sprayed on leaves, upward translocation was found but no downward translocation. Using a double-cup technique, a second and separate root system was established five nodes from the first. When this system was exposed to either 25, 100, 500, or 1,000 mg/liter benomyl for 4 days, the fungistats were found to be translocated back into the crown of the first root system. This indicated "lateral" movement of the fungistats. Phytopathology 61:1198-1200.

Additional key words: Sclerotinia homeocarpa, Penicillium atrovenetum.

Benomyl was shown to control several diseases of creeping bentgrass (1, 2, 4). When blended with the soil, benomyl controlled *Sclerotinia homeocarpa* and *Rhizoctonia solani* (1); as a soil drench, it controlled *Erysiphe graminis, Urocystis agropyri,* and *Ustilago striiformis* (2); and as a spray to the foliage, it controlled *U. striiformis* (4). Only presumptive evidence was presented in these reports for the systemicity of benomyl in creeping bentgrass. This paper provides evidence of the uptake and translocation of benomyl (and its breakdown product, MBC [methyl-2-benzimidazole carbamate]) by roots and leaves of creeping bentgrass, as well as for lateral movement of the fungicides in stolons.

MATERIALS AND METHODS.—The isolate of *Sclerotinia homeocarpa* Bennett used was subcultured from a stock culture supplied by the American Type Culture Collection, ATCC-10943. The commercial formulation of benomyl was used. Stolons of Toronto creeping bentgrass (*Agrostris palustris* Huds.) used in these experiments were harvested from a pure stand maintained in the University of Illinois plant pathology greenhouses. All experiments were conducted in a growth chamber at 22 C, 65% relative humidity, with 12 hr of light (2,500 ft-c).

Root-uptake studies.—Single nodes from stolons of creeping bentgrass were planted in moist vermiculite (Terralite brand) for rooting. They were fertilized with one-half strength Hoagland's solution (3) once a week. After 3 weeks, plants were cut to a height of 3 cm and transplanted into a series of 258 cc (9-oz) plastic (Styrofoam) cups containing vermiculite. To each cup was added either 110 cc of distilled water (control) or 110 cc of a distilled water suspension of benomyl at concentrations of either 5, 12.5, or 25 mg/liter. There were three replicates for each treatment-exposure time combination and two performances of the experiment. Plants were placed in the growth chamber for either 24, 48, or 96 hr, then removed from the cups, washed thoroughly in tap water, and prepared for bioassay.

The plant parts bioassayed were roots, crown, and leaves. Crown pieces consisted of a 1-cm section of stolon which included the node where roots were formed. Each section from each treatment was ground separately in 10 ml of acetone, using sterile mortars and pestles. Plant extracts were filtered through cheesecloth and reduced in volume in a flash evaporator. Six ml of distilled water were added to each extract and placed in test tubes for autoclaving (121 C/15 min). Two ml of each extract were added to 9 ml of molten (70 C) commercial potato-dextrose agar (PDA), and equal quantities poured into two 9-cm culture plates. A single PDA plug (6 mm) containing mycelium of *S. homeocarpa* was placed in the center of each plate after solidification. Radial diameters were measured when colonies on control plates reached 9 cm. Measurements were taken in millimeters, and the average diameter from the two axes was recorded.

Leaf blade application.—The tips of leaf blades at the end of stolons on bentgrass plants in the three-node stage were atomized with either distilled water (control) or with distilled water suspensions of benomyl at 100, 500, or 1,000 mg/liter, with or without 100 mg/liter of surfactant (E. I. DuPont's Spreader-Sticker). There were either three or six plants/treatment. After 4 days, plants were removed from the growth chamber and the stolons thoroughly washed to remove any surface benomyl, then placed in plastic bags and placed in a freezer (−18 C) for 2 days. Care was taken to prevent roots from coming into contact with leaf blades. For bioassay, the roots were removed from the stolons, and stolons were cut into four sections, three nodal tissues, and the tip portion after the third node. Each portion was placed separately in culture plates with PDA and sprayed with a sterile, distilled water suspension of *Penicillium atrovenetum* G. Smith. Plates were refrigerated (0 C) for 1 day, then incubated for

4 days at 20 C. Zones of inhibition were measured in millimeters, and means determined.

Lateral transfer.—Individual bentgrass stolons of eight nodes were planted in two plastic cups containing vermiculite in such a way that the second node at either end was in contact with the moist vermiculite in the respective cup (Fig. 1, above). At the end of 3 weeks, root systems were formed at the respective nodes, each in a separate cup, separated by four nodes. The root system farthest from the growing point was considered the first root system; the system closest to the growing point was considered the second. Water suspensions without (control) or with benomyl at 25, 100, 500, or 1,000 mg/liter were added to the cups containing either the primary or secondary root systems, but not both. There were three and four replicates/treatment. After 4 days in the growth chamber, the plants were removed and thoroughly washed. They were prepared for bioassay in a manner described above. The tissues of the four nodes between the two root systems, new growth which had developed after planting, and each root system were bioassayed. Zones of inhibition were measured in millimeters and the means recorded.

Bioautographic studies were made using the technique of Peterson & Edgington (5). One-g samples of creeping bentgrass treated with benomyl were used and the extracts compared with a 25 mg/liter commercial formulation.

RESULTS AND DISCUSSION.—Bioautographs showed the presence of both benomyl and its breakdown product, MBC, in creeping bentgrass tissue. Therefore, readings from bioassays reflect the activity of either or both these compounds throughout these experiments. Benomyl and MBC were taken up by the roots of creeping bentgrass and translocated in the stolons to the leaves (Table 1). Activity was detected with bioassays of extracts of leaves from plants treated for 48 hr at 5, 12.5, and 25 mg/liter. Activity was detected also in the crowns and roots of plants treated with benomyl at 25 mg/liter after 48 hr. The data collected at 96 hr shows the fungistats in the leaves, with no significant difference in the treated crowns and roots from the control. This was evidence for upward translocation.

Bioassays of various tissues of creeping bentgrass plants atomized with benomyl showed no activity in any tissue of either controls or plants treated with 100 mg/liter benomyl after 4 days. Extracts from leaf blades atomized with 500 and 1,000 mg/liter showed zones of inhibition of 4.6 and 10.8 mm, respectively, whereas extracts of the new growth showed inhibition zones of 7.5 and 6.2 mm, respectively. No inhibition zones were obtained when roots, crowns, and lower leaf blades were bioassayed. This was evidence for leaf absorption and upward translocation into new growth, but no downward movement.

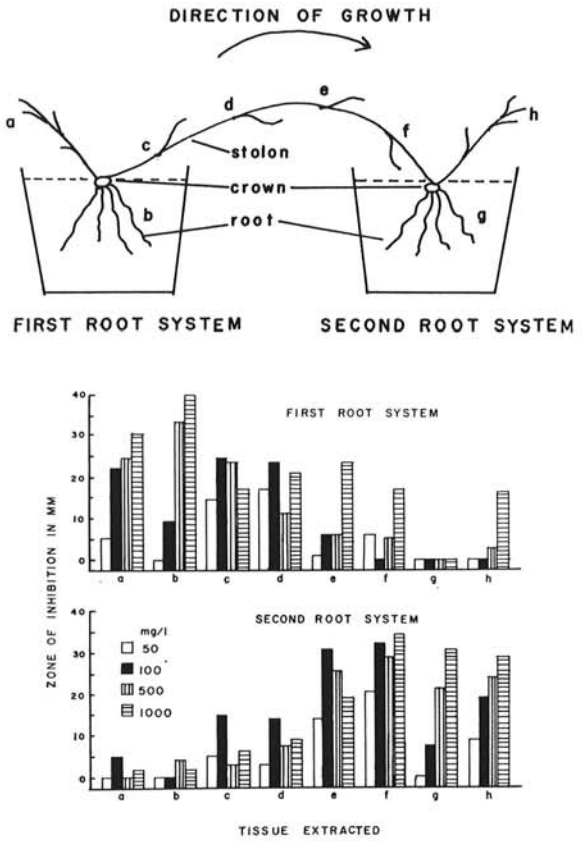

Fig. 1. (Above) Diagrammatic representation of double-cup technique used to study uptake and translocation of systemic fungicides in creeping bentgrass stolons with two root systems. Lower case letters indicate location of tissues used in bioassay studies. (Below) Zones of inhibition in millimeters in bioassay plates against *Penicillium atrovenetum* from extracts of eight tissues of creeping bentgrass with two root systems, either of which was treated with 50, 100, 500, or 1,000 mg/liter, benomyl. a = New growth from first root system; b = first root system; c = first node; d = second node; e = third node; f = fourth node; g = second root system; and h = new growth from second root system.

TABLE 1. Mean[a] growth of *Sclerotinia homeocarpa* in millimeters on potato-dextrose agar mixed with extracts from either leaf, crown, or roots of creeping bentgrass grown in vermiculite without (control) or with 5, 12.5, or 25 mg/liter benomyl for 24, 48, and 96 hr

Rate of benomyl in mg/liter	Tissue bioassayed	Exposure time in hr[b]		
		24	48	96
0 (Control)	Leaf	50 a	47 a	41 a
	Crown	50 a	45 ac	40 a
	Root	50 a	47 a	42 a
5	Leaf	46 a	22 e	6 b
	Crown	50 a	45 ac	42 a
	Root	50 a	45 ac	41 a
12.5	Leaf	37 a	8 b	6 b
	Crown	50 a	42 c	39 a
	Root	50 a	44 ac	40 a
25	Leaf	27 a	6 b	6 b
	Crown	50 a	33 d	32 a
	Root	50 a	36 d	32 a

[a] Mean growth of six replicates. Average of two experiments.

[b] Duncan's multiple range test used for 5% level. Means with the same letter are not significantly different.

When two root systems were allowed to develop on a single stolon, however, translocation of benomyl and MBC was noted in both directions (Fig. 1, below). When the first root system was treated with 500 and 1,000 mg/liter of benomyl, fungistatic activity by bioassay was noted in all tissues except of the second root system. There was activity detected also in all tissues from plants whose roots were treated with 50 and 100 mg/liter, except the second root system and the new growth from that system.

When the second root system was treated with 1,000 mg/liter, fungistatic activity was noted by bioassay in all tissues (Fig. 1, below). When treated with 500 mg/liter, activity was found in all tissues except the new growth from the first root system. When treated with 100 mg/liter, activity was recorded in all tissues but the first root system. Activity was noted in all tissues when the second root system was treated with 25 mg/liter, except the first and second root systems and the new growth from the first root system.

Although evidence is presented for the movement of benomyl and MBC against the direction of growth of creeping bentgrass, it does not seem that this can be presented as evidence of downward movement. The establishment of a second root system on the stolons establishes a different system from that of a plant with a single root system. It is noteworthy, however, that the fungicide can be translocated (laterally, presumably) in the xylem of these stolons. It indicates that perhaps there is a reverse flow in the xylem vessels after a second root system is established.

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