Systemic Uptake of ^{14}C-labeled 2-(4′-thiazolyl)benzimidazole in Soybean

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ABSTRACT

Direct evidence of the uptake and translocation of TBZ [2-(4′-thiazolyl)benzimidazole] was obtained using ^{14}carbon-labeled TBZ and nonlabeled TBZ. Both compounds were absorbed by roots of soybean seedlings, and appeared to move unaltered into all aboveground tissues. The accumulation of ^{14}C-TBZ increased in epicotyl and root tissues with increase in exposure time, while radioactivity in hypocotyl tissues was not affected by exposure time.

TBZ was fungitoxic for *Cephalosporium gregatum* both in vitro and in vivo. TBZ, used as a soil drench in clay pots (25 ml of 200 μg/g in 750 g soil), restricted the development of internal browning in soybeans which were wound-inoculated with *C. gregatum* when plants were 8 weeks old. The fungus could not be reisolated from treated plants. Phytopathology 61:523-525.

Additional key words: brown stem rot, fungicide.

Systemic activity of TBZ [2-(4′-thiazolyl)benzimidazole], Merck & Co.'s Mertect, 60 WP) has been shown in several plants (1, 2, 3, 4, 5) other than soybean. Gray & Sinclair (6) detected a fungitoxic material in extracts of hypocotyls, cotyledons, and epicotyls of soybean seedlings exposed to TBZ, but did not ascertain whether the fungitoxicity was due to TBZ or a breakdown product.

In the experiments with soybean seedlings reported here we studied (i) the effect of exposure time on the distribution of ^{14}C-TBZ; (ii) stability of TBZ; and (iii) protection of seedlings with TBZ against *Cephalosporium gregatum* Allington & Chamberlain.

MATERIALS AND METHODS.—Soybean, *Glycine max* (L.) Merr. 'Amsoy' and 'Clark-63', were used. TBZ and ^{14}C-TBZ were provided by Merck & Co. The radioactive compound was uniformly labeled in the benzene ring with ^{14}C, and had a specific activity of 1.59 mc/mmole. Thirty-two μc of the labeled fungicide were diluted with 2 ml of ethyl alcohol and maintained in a freezer (—20 C) until needed. When the labeled material was used it was mixed with distilled water.

The effect of exposure time on distribution of ^{14}C-TBZ was studied using 7-day-old Amsoy soybean seedlings transplanted into 30-ml glass vials containing 25 ml of distilled water without (control) or with 0.94 μc of ^{14}C-TBZ. A single seedling in each vial was held in place with a foam rubber ring so that only the roots were immersed in the water. The vials were aerated at all times with forced air and maintained at room temperature (23-25 C) under 200 ft-c of light. The seedlings were either exposed continuously for 7 days to the labeled solution, or transferred after 3 days' exposure to vials containing distilled water, then grown for an additional 4 days. Roots of seedlings were thoroughly washed in distilled water at the time of transfer and at the end of the experiment (7 days). There were three replications of each treatment. The experiment was performed twice. The root, hypocotyl, cotyledon, and epicotyl tissues were collected separately from each treatment, and the tissues were extracted in acetone using Gray & Sinclair's technique (6), except that extracts were resuspended in acetone to volume. Radioactivity in the tissue extracts was determined with a gas-flow detector, and sample activity was corrected for self-absorption.

In an additional experiment, seedlings were exposed to 0.2 μc of ^{14}C-TBZ for 7 days, harvested, and rinsed thoroughly with distilled water, then dried between absorbent papers, placed between cardboard sheets, and dried for 3 days in an oven at 65 C. The plants were prepared for radioautography and exposed for 7 weeks to Kodak no-screen X-ray film.

Two types of experiments were conducted to determine if TBZ was altered in soybean seedlings. Seven-day-old Amsoy seedlings were either exposed to 0.94 μc of ^{14}C-TBZ for 7 days by using the glass vial technique; or seedlings were transplanted into vermiculite without or with 200 μg/g active TBZ and exposed for 7 days. There were five replications with three seedlings/replication. At the end of the exposure period the seedlings were removed, washed thoroughly in distilled water, and separated into roots, hypocotyls, cotyledons, and epicotyls. The tissues were extracted in acetone. Extracts from both the ^{14}C-TBZ-treated and TBZ-treated plants were spotted on thin-layer chromatographic plates (TLC). Extracts were spotted in duplicate on each plate together with a TBZ standard (technical TBZ in methanol). A ^{14}C-TBZ standard was also included on plates spotted with extracts from plant tissues treated with the labeled material. Three plates were prepared for each tissue extract. The plates were developed for a distance of 10 cm in an acetone-saturated solvent system, then air-dried and sprayed with 0.05% Na-fluorescein in methanol. Plates were examined under ultraviolet light (2570 A) to locate the TBZ standard, which appeared as a reddish-brown spot. Three bands were marked on the TLC plates, then scraped

off: (i) a 0.5-cm band corresponding in R_F to the TBZ standard; (ii) a band from the origin of the plate to the TBZ standard; and (iii) a band from the TBZ standard to the solvent front. Radioactivity was determined with a gas-flow detector, and self-absorption corrections were made on all samples. The bands removed from plates spotted with tissue extracts from plants treated with TBZ were bioassayed with *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman).

In vitro studies showed that TBZ was fungitoxic to *C. gregatum* at concentrations as low as 0.2 μg/g (Gray & Sinclair, *unpublished data*). The fungitoxicity of TBZ in vivo to *C. gregatum* was determined, using Clark-63 seedlings. Seedlings were grown in 4-inch clay pots containing 750 g of pasteurized soil. When the plants were 8 weeks old, one of the following solutions of TBZ was applied as a soil drench: (i) 25 ml of 200 μg/g; (ii) 50 ml of 200 μg/g; (iii) 50 ml of 400 μg/g; and (iv) 100 ml of 400 μg/g. Seedlings drenched with distilled water served as a nontreated control. There were four replications of each treatment. All plants were inoculated 3 days after treatment by puncturing the stem at a point 2 cm above the soil line with a sterile scalpel and then placing a plug of mycelium of *C. gregatum* in the wound. After inoculation, the wound was covered with pasteurized soil inside a plastic ring. Nontreated, noninoculated plants served as controls. The plants were maintained in the greenhouse at 21 C for 3 weeks. At 3 weeks, stems of all plants were split longitudinally with a scalpel, and extent of internal stem browning was measured in cm (7).

RESULTS AND DISCUSSION.—The count/min per g of tissue $\times 10^4$ after 3 days' exposure to ^{14}C-TBZ were: root, 13.0; hypocotyl, 0.83; cotyledon, 3.3; and epicotyl, 3.5; after 7 days, the count/min per g of tissue $\times 10^4$ were: root, 28.0; hypocotyl, 0.97; cotyledon, 1.4; and epicotyl, 8.4. There was no activity in the control extracts. These results provided more direct evidence from that presented earlier (6) that TBZ moved systemically into all tissues of treated soybean seedlings. The highest radioactivity among tissues was in the roots of treated seedlings, and the lowest in the hypocotyl extracts regardless of exposure time.

Radioautographs showed that ^{14}C-TBZ was distributed in all soybean tissues. The highest activity was noted in the roots and the lowest in hypocotyl (Fig. 1). (This distribution pattern of TBZ in soybean seedlings is similar to the pattern reported for carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilido, UniRoyal's Vitavax) in cotton seedlings by Kirk et al. (8). In contrast, Erwin et al. (5) reported that the radioactivity of ^{14}C-TBZ-treated cotton seedlings decreased with increasing height above the roots. These differences may be due to environmental factors, host species, age of plant, and the techniques used.

Radioactivity on TLC plates spotted with tissue extracts from plants treated with ^{14}C-TBZ was confined to the same R_F of the TBZ standard, and no breakdown products were detected after 7 days. Bioassays of various bands on TLC plates, spotted with tissue extracts from plants exposed to nonlabeled TBZ, showed complete inhibition of the test fungus only from bands

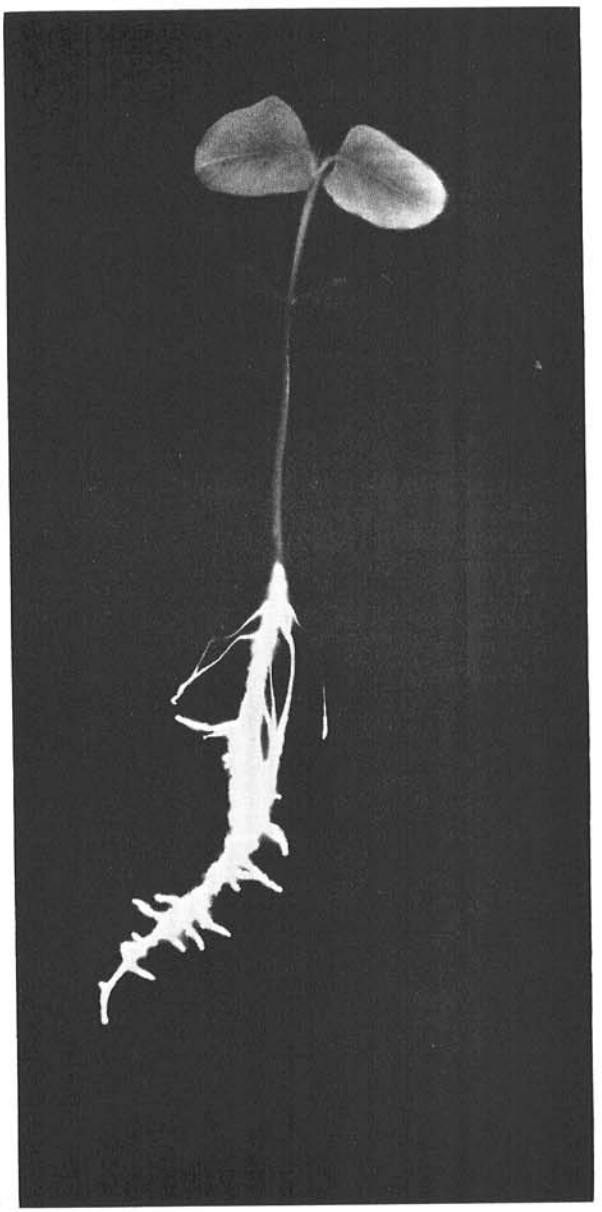

Fig. 1. Seven-week radioautograph of soybean seedling following 7-day root uptake of ^{14}C-labeled TBZ [2-(4'-thiazolyl) benzimidazole].

which corresponded in R_F to the TBZ standard. There was no inhibition from nontreated plant extracts. These results indicate that TBZ is present in the soybean tissues in its original form, and is not broken down for at least 7 days in soybean seedlings. Erwin et al. (5) were not able to detect any breakdown products of TBZ in 3-week-old cotton seedlings after 3 days' exposure to ^{14}C-TBZ.

Soybean plants treated with TBZ as a soil drench showed restricted development of internal stem browning caused by *C. gregatum* at all rates. Internal stem browning for the inoculated control was 113 cm; for 25 ml of 200 μg/g, 67 cm; for 50 ml of 200 μg/g, 66

cm; for 75 ml of 400 μg/g, 40 cm; and for 100 ml of 400 μg/g, 32 cm. Internal browning was confined to the first node of all plants treated with TBZ, while it extended to the third and fifth nodes of nontreated, inoculated controls. *Cephalosporium gregatum* was reisolated only from the inoculated control plants. TBZ appeared to be fungitoxic for *C. gregatum* both in vitro and in vivo. Thapliyal (9) has shown similar results using benomyl. However, field studies in which TBZ was applied at 100 and 200 μg/g active as an in-the-furrow treatment showed no control of internal browning of soybean caused by *C. gregatum* (Gray & Sinclair, *unpublished data*).

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