# Translocation of 2-(4-Thiazolyl) Benzimidazole in Maturing Cotton Plants

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ABSTRACT

Evidence of upward translocation of Thiabendazole [2-(4-Thiazolyl)benzimidazole] to morphologically different parts of maturing cotton plants was obtained when the fungicide was applied by a stem puncture method. Recovery of Thiabendazole was approximately 90% of the quantity applied to plant tissue dried at 80-85 C and extracted with methanolic HCl. Ten days after application, the fungicide was identified chemically and quantitatively determined in the stem, in young leaves at the top of the plant, and in growing bolls. No fungicide was detectable in the lower, old leaves and in roots.

Thiabendazole uniformly labeled in the benzene ring with $^{14}C$ and injected into the lower stem translocated to the upper stem, leaves, and bolls of maturing cotton plants. Radioactivity was detected in boll walls and ovules of the bolls present at the time of application, but it was not detectable in those formed after application. Radioactivity was detectable in both young and old leaves.

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*Additional key words:* systemic fungicides, spectrophotometry, thin-layer chromatography, liquid scintillation counting, radioautography.

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Systemic activity of Thiabendazole [2-(4-Thiazolyl)benzimidazole or TBZ] for control of Phymatotrichum root rot and Verticillium wilt of cotton has been reported (3, 6). It was also demonstrated that this fungicide is absorbed by the roots and translocated to the shoots of cotton (2, 9) and soybean (4). The evidence of upward translocation of TBZ in cotton was obtained with young plants in the vegetative stage of development (2, 9).

Thus far, the systemic activity of TBZ has not been established for cotton plants having bolls in various developmental stages at which the susceptibility to these diseases is most profound. Symptoms of Phymatotrichum root rot generally do not appear until the cotton plants begin squaring, or later, and wide-spread development of Verticillium wilt usually does not occur until after plants reach the flowering stage. Presley & Taylor (7) showed that ontogeny of xylem vessels in cotton plants influences wilt development. End walls in xylem vessels preclude systemic movement of conidia in young plants, but conidia move freely throughout older cotton plants in which these end walls have disappeared.

We were interested in quantitatively ascertaining the distribution of TBZ in maturing cotton plants following injection of a known quantity into the vascular system of the stem. Injecting the chemical into the stem bypasses the process of uptake; however, other investigators found that roots do absorb TBZ from the soil (2, 4).

MATERIALS AND METHODS.—Cotton plants, var. Stoneville 7A, grown in a greenhouse, were used for the experiments. Two delinted seeds were planted at a depth of about 3 cm in each plastic pot (25 cm in diam) containing a mixture of peat moss, perlite, and sand (2:1:1, v/v). After emergence, the seedlings were thinned to one plant/pot. The plants were fertilized with full-strength Hoagland nutrient solution No. 1 at weekly intervals. When the plants had bolls at various stages of development (after 3 to 4 months), they were transferred to a controlled-environment room with the following conditions: temperature, 27 C; photoperiod, 15 hr: illumination (mixed fluorescent and incandescent), 2,000 ft-c at the middle of the shoot system. The plants were kept under these conditions for a 24-hr period of adaptation and for the subsequent test period.

*Translocation of unlabeled TBZ.*—Unlabeled technical TBZ (supplied by Merck Chemical Division, Rahway, N.J.) dissolved in 0.1 N HCl was used. We injected ten mg TBZ in 1 ml solvent/plant into the xylem of the stem about 5 cm above the soil surface after puncturing the stem with a needle to a depth of 3 to 4 mm. Injection into the xylem of the stem as a mode of plant application has been used for other pesticides (1). After an experimental period of 10 days, each plant was separated into the following parts: (i) three stem segments, one 15 cm long, starting about 2 cm above the soil surface and including the area of TBZ application (lower), a second 15 cm segment from the middle of the plant (middle), and a 25-cm portion from the top of the plant (upper); (ii) young and old leaves; (iii) bolls; and (iv) roots. The bolls were grouped according to age, the average age of young and medium bolls selected for analysis being 14 and 24 days after anthesis, respectively. In the case of young bolls, the

entire bolls including ovules and fiber were used for analysis, whereas the medium-boll samples comprised only the boll walls. The roots were thoroughly rinsed with running tap water before extraction for removal of residual potting medium.

Plant samples were cut into small pieces, dried at 80-85 C for 48 hr, and weighed. They were then ground to 40 mesh in a Wiley mill. For extraction of TBZ, the ground samples were shaken with methanolic HCl (0.2 N HCl in 50% methanol, 25 ml/g dry weight) for 60 min on a rotary laboratory shaker at a speed of 350 rpm. After centrifugation of the samples, the extracts were filtered through Whatman No. 1 filter paper. Twenty-ml samples of the clear extracts were then purified for removal of interfering substances according to the method of Szalkowski & Kanora (8).

TBZ in the purified extracts (in dilute HCl) was determined spectrophotometrically. The absorbance of the samples in a 1-cm quartz cell was measured at 302 nm with a Model DB-G Beckman spectrophotometer. Untreated check samples were included for all treatments, and their absorbances were subtracted to give corrected absorbance values for the treated samples. A known standard solution of TBZ and a reference blank (methanolic HCl) were processed with the samples.

The validity of the extraction and spectrophotometric determination of TBZ in plant tissue was tested as follows: (i) the ultraviolet-absorption spectrum of a standard TBZ solution in dilute HCl showed a peak at 302 nm (Fig. 1-A); (ii) when known concentrations of TBZ were added to purified HCl extracts from untreated leaves, the absorbance at 302 nm was found to be directly proportional to the concentration of TBZ in the range of 0.5 to 4 μg/ml (Fig. 1-B); (iii) the recovery of TBZ from plant tissue by our extraction method was checked with excised cotton leaves to which known quantities of the fungicide were applied. The treated leaves were kept at 25 C under moist conditions for 24 hr after which extraction and quantitation was made as described. Recovery was approximately 90% of the quantity applied.

The purified plant extracts were also subjected to thin-layer chromatography (TLC) to determine whether TBZ remained chemically intact in the plant tissues. TLC-plates were prepared by coating glass plates (20 X 20 cm) with a 0.25-mm layer of the adsorbent Silicar TLC-7GF (Mallinckrodt) followed by activation at 105 C for 1 hr. The chromatograms were developed with the solvent system ethyl acetate-acetic acid-$H_2O$ 35:15:2 (9), dried, and the spots viewed with ultraviolet light.

*Translocation of labeled TBZ.*—Thiabendazole uniformly labeled with $^{14}C$ in the benzene moiety of the molecule, 0.15 μCi $^{14}C$-TBZ dissolved in 0.1 N HCl, was applied to the stem as described for the unlabeled fungicide. After a period of 10 days, each plant was separated into samples from the stem, leaves, bolls, and roots. The first three stem samples consisted of 9-cm-long segments taken around the area of TBZ-application (lower) and at intervals of

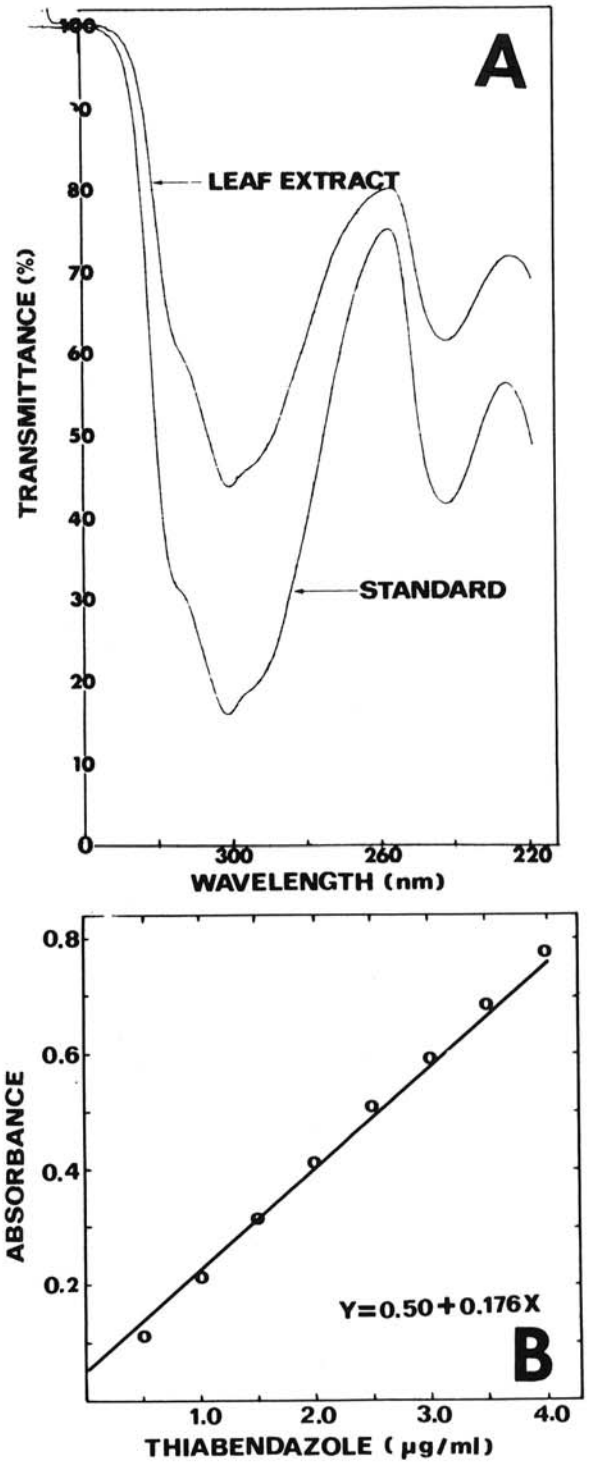
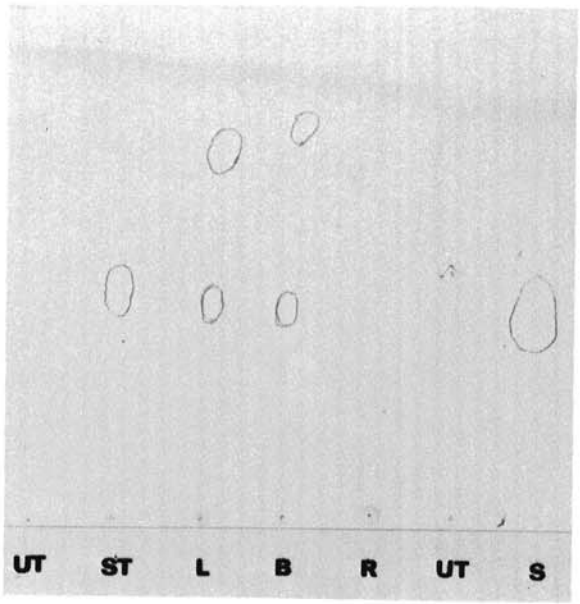



Fig. 1. A) Ultraviolet-absorption spectra of a standard Thiabendazole [2-(4-Thiazolyl)benzimidazole] solution in dilute HCl and of a purified leaf extract in diluted HCl from a Thiabendazole-treated cotton plant. B) Absorbance at 302 nm of a purified leaf extract in dil. HCl from an untreated cotton plant as a function of the concentration of Thiabendazole added.



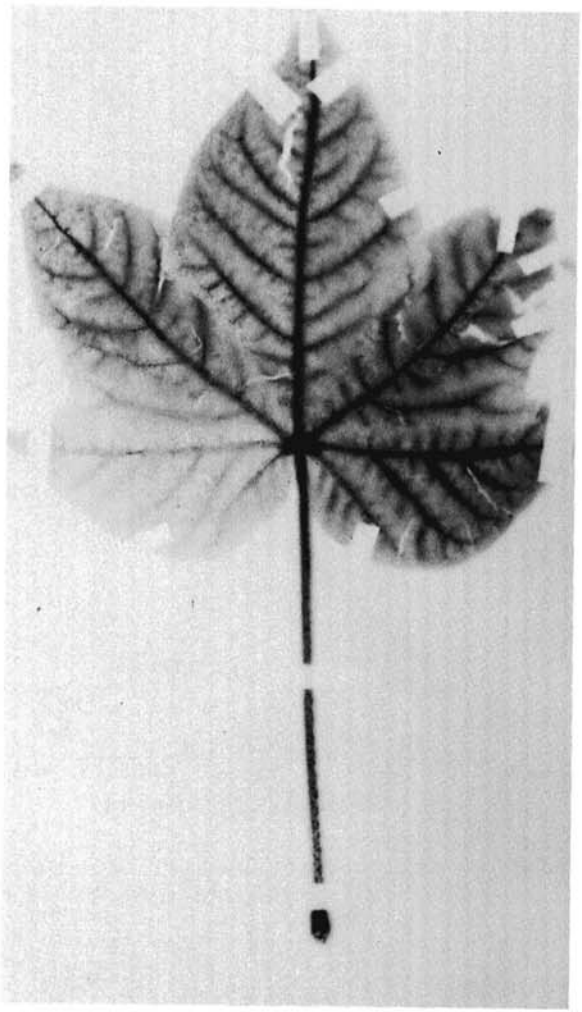



TABLE 1. Distribution of 2-(4-Thiazolyl)benzimidazole (TBZ) in maturing cotton plants after application of 10 mg of the fungicide to the lower stem

| Tissue | Sample wt[a] Fresh (g) | Sample wt[a] Dry (g) | TBZ[a] μg/g dry wt | TBZ[a] Total μg |
|---|---|---|---|---|
| Stem, lower | 7.33 | 3.37 | 1,126.7 ± 104.8 | 3,797.0 |
| Stem, middle | 6.12 | 2.69 | 25.7 ± 8.7 | 69.1 |
| Stem, upper | 3.41 | 1.50 | 17.0 ± 0.0 | 25.5 |
| Leaves, young | 20.39 | 5.15 | 115.5 ± 9.7 | 594.8 |
| Leaves, old | 30.59 | 5.73 | ND[c] | ND |
| Bolls, young | 30.45 | 5.50 | 194.5 ± 104.5 | 1,069.7 |
| Bolls, medium | 74.24 | 14.07 | 134.2 ± 72.8 | 1,888.2 |
| Roots | 12.70 | 5.17 | ND[c] | ND |
| Total | 185.23[b] | 43.18 | | 7,444.3 |

[a] Means of three replications.

[b] Mean plant weight was ca. 222.0 g; therefore recovery was $\frac{222.0 \times 7{,}444.3 \times 100}{185.23} = 89.2\%$.

[c] ND = no TBZ detectable.

about 9 cm from the first segment (lower middle, upper middle). The top 15-cm portion of the stem represented the upper stem. The bolls were separated into boll walls and ovules with the fibers. The fresh weight of all the samples was determined; then the samples were frozen and stored in a freezer until extraction.

For extraction of $^{14}$C-TBZ, the samples were cut into small pieces and homogenized in a Sorvall Omni-Mixer for 5 min, using 25 ml methanolic HCl (0.2 N HCl in 50% methanol) per sample. After centrifugation and filtration, the filtered extracts were made to volume. The radioassay of the samples was done by liquid scintillation counting. From each sample, 2.5 ml was pipetted into a scintillation vial; 15 ml of scintillation fluid containing 5 g of 2,5-diphenyloxazole (PPO), 100 g of naphthalene, 10 ml of water, and dioxane to 1 liter were added; and the samples counted in a Beckman LS-200B liquid scintillation system. The count rates were corrected for quenching by internal standardization (10).

RESULTS.—The quantitative distribution of TBZ in different parts of maturing cotton plants 10 days after injection into the xylem of the stem is shown in Table 1. The values represent the means from three replicate samples, and the standard deviations are also given. The results clearly indicate that the samples from the lower stem contained the highest TBZ

Fig. 2-3. 2) A thin-layer chromatogram for identification of Thiabendazole [2-(4-Thiazolyl)benzimidazole]. S = standard TBZ-solution. UT = leaf extract from untreated cotton plant; ST = lower stem; L = young leaves; B = young bolls; R = roots. Spots were located and circled under ultraviolet lighting. 3) Radioautograph of a leaf from the middle part of a cotton plant to which 0.15 μc $^{14}$C-Thiabendazole was applied by stem injection. The leaf was freeze-dried and exposed to No-Screen Medical X-ray film for 3 weeks.

concentration; that is, a major fraction of the TBZ applied remained in the lower portion of the stem and was not translocated upwards. Some TBZ apparently moved all the way up the stem, as is indicated from the TBZ contents of the middle and upper stem samples. There was also considerable translocation of TBZ laterally from the stem into the branches with the fungicide detectable in moderate amounts in young expanding leaves at the top of the plants and in the bolls. The old leaves were senescing and taken from lower branches without bolls. The large standard deviations for the values of the boll samples can probably be ascribed to the variation in age within the two groups of bolls. No TBZ was detectable in the lower, old leaves and in the roots.

The presence of TBZ was confirmed in samples of stem, young leaves, and bolls by means of spectrophotometry and TLC. The upper curve in Fig. 1-A represents an ultraviolet-absorption spectrum of an extract from young leaves exhibiting a peak in absorbancy at the same wavelength as the standard TBZ-sample; that is, at 302 nm. The identification of TBZ in plant extracts was also made by TLC (Fig. 2-3). Extracts from untreated plants did not produce any ultraviolet-visible spots on the chromatogram. Hence, the purified plant extracts did not contain substances that would interfere with the detection of TBZ. There were spots on the chromatogram from samples of lower stem, young leaves, and young bolls at the same position as the spot from the TBZ-standard, thus confirming the presence of unaltered TBZ in these plant samples. In addition, the leaf and boll samples contained a compound different from TBZ which moved faster on the chromatogram than TBZ. This unknown compound may represent a degradation product of TBZ as a result of the metabolic activity of the growing leaves and fruits of the cotton plant. There was no spot visible from the root extract, in accordance with the result in Table 1.

The quantitative distribution of labeled TBZ in maturing cotton plants 10 days after injection into the xylem of the stem is shown in Table 2.

Using $^{14}$C-TBZ, it was possible to ascertain a more detailed picture of the distribution of TBZ in maturing cotton plants, mainly as far as the bolls are concerned (Table 2). The results confirm that TBZ was translocated upwards in the stem to the top of the plant and moved also laterally into the branches. No unlabeled TBZ was detectable by spectrophotometry in the old, senescing leaves; however, all the leaves including the senescing and subtending ones contained $^{14}$C-activity in the experiments with $^{14}$C-TBZ. This suggests that TBZ in leaves is chemically altered during senescence. That such a pattern of TBZ-distribution is the consequence of upward translocation in the xylem is borne out in the radioautograph of a leaf from the middle part of a plant to which $^{14}$C-TBZ was applied by stem injection (Fig. 3). The activity is mostly in the veins, but some lateral transport into the mesophyll occurred as well and an area of high activity is evident in the glands at the base of the leaf blade. The results in Table 2 further reveal that radioactivity could be detected in bolls of various ages up to those approaching maturity (36 days of age). Both the boll walls and the ovules with the fibers contained activity. In general, the activity on a fresh weight basis was greater in the ovules than in the boll walls. No activity was found in bolls which had developed after the application of $^{14}$C-TBZ.

DISCUSSION.—By definition, a systemic pesticide must exhibit the feature of a compound that is transportable in the host plant and is able to exert biological action at sites removed from those of application. We confirmed the systemic activity of the fungicide TBZ in the cotton plant, as was reported for plants in the vegetative stage of development by Erwin et al. (2) and Wang et al. (9), and demonstrated that it is also systemic in maturing plants. There is evidence of upward translocation of TBZ from the point of application at the base of the stem to the top of the plant within 10 days, as a graded concentration of TBZ existed along the main axis at the end of the experimental period. Unlike the situation in young plants reported by Wang et al. (9), where little radioactivity was detected in leaves after application of $^{14}$C-TBZ to roots or stems, we found an accumulation of TBZ in young, growing leaves at

TABLE 2. Distribution of $^{14}$C-Thiabendazole [2-(4-Thiazolyl)benzimidazole] in maturing cotton plants after application of the fungicide to the lower stem. Boll samples are listed with the days after anthesis at time of harvest in parentheses

| Tissue | $^{14}$C-Thiabendazole[a] | Tissue | $^{14}$C-Thiabendazole |
|---|---|---|---|
| | *dpm/g fresh wt* | | *dpm/g fresh wt* |
| Stem, lower | 36,341 | Boll walls (14 days) | 62 |
| Stem, lower middle | 1,886 | Ovules (14 days) | 156 |
| Stem, upper middle | 1,118 | Subtending leaves | 914 |
| Stem, upper | 506 | Boll walls (18 days) | 183 |
| Leaves, lower (senescing) | 1,137 | Ovules (18 days) | 272 |
| Leaves, lower middle | 981 | Subtending leaves | 463 |
| Leaves, upper middle | 737 | Boll walls (36 days) | 101 |
| Leaves, upper (top of plant) | 609 | Ovules (36 days) | 126 |
| Boll walls (5 days) | ND[b] | Subtending leaves | 453 |
| Ovules (5 days) | ND[b] | Roots | 30 |

[a] 0.15 μc $^{14}$C-Thiabendazole applied; means from duplicate samples.
[b] ND = no $^{14}$C-activity detectable.

the top of the maturing plant in much higher concentrations than in portions of the stem bearing these leaves. Furthermore, we detected $^{14}$C-activity in all the leaves of the maturing plant after application of $^{14}$C-TBZ to the lower stem. Such a distribution pattern indicates strongly that the upward translocation of TBZ proceeds through the xylem and follows the transpiration stream into the transpiring leaves (5).

In this study, no downward movement of TBZ to the root system was detectable. Thus, TBZ may not be mobile in the phloem, regardless of whether it is applied to young (9) or maturing cotton plants.

A result of paramount importance is the accumulation of TBZ in growing bolls. The concentration of TBZ in the fruits was about as high as in the young leaves. In the experiments with $^{14}$C-TBZ, radioactivity was detectable in boll walls and ovules of the bolls present at the time of application. Thus, the systemic activity of TBZ encompasses also the reproductive organs of the cotton plant and may be of practical significance for protection from many boll-rotting fungi.

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