TRANSLOCATION, DISEASE REMISSION AND PERSISTENCE OF THIABENDAZOLE AND 5-ETHOXY-3-(TRICHLOROMETHYL)-1, 2, 4-THIADIAZOLE IN TOMATO SEEDLINGS[1]

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Abstract

The systemic fungicides thiabendazole (2-(4-thiazolyl)-benzimidazole) and 5-ethoxy-3-(trichloromethyl)-1, 2, 4-thiadiazole (ETMT) were taken up by roots of tomato seedlings and moved unaltered into stems and leaves. Seedlings grown in soil treated with thiabendazole at 25 or 50 μg/g restricted development of wilt symptoms in seedlings following stem-inoculation with conidial suspensions of *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium albo-atrum*. Thiabendazole persisted in tomato leaves for 7 days and in stems for 5 days after root uptake. ETMT was not detected in any tissues after 1 day. Thiabendazole persisted in soil for 16-20 weeks and ETMT for 5 weeks.

INTRODUCTION

Uptake and translocation of thiabendazole (2-(4-thiazolyl)-benzimidazole) has been reported in cotton (6), corn (13), and soybeans (9). The compound is toxic *in vitro* to *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. (*Fol*) and *Verticillium albo-atrum* Reinke & Berth. (*Vaa*) (3, 5). 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole) (ETMT)(Terrazole) is systemic in cotton and protects seedlings against infection by *Rhizoctonia solani* Kuehn (1). This paper reports on: root uptake and translocation of thiabendazole and ETMT in tomato seedlings; systemic activity of these compounds against *Fol* and *Vaa*; and persistence of the fungicides in tomato tissues and soil.

MATERIALS AND METHODS

Suspensions of thiabendazole (60% wettable powder, Merck & Co.) in water and ETMT (95% emulsifiable concentrate, Olin Chemical Corp.) in water plus 1% Tween 20 surfactant (E. I. du Pont and Co.) were used at concentrations of 25, 50, and 100 μg/ml or μg/g. All experiments had four replications with five plants per replicate in a split-plot design unless otherwise indicated. Each experiment was conducted twice.

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Root uptake and translocation was studied by applying the two fungitoxicants separately around roots of 2-week-old tomato (Lycopersicon esculentum 'Bonny Best') seedlings growing in vermiculite (Terralite brand) in 5-g styrofoam cups. Treated and nontreated (control) plants were maintained in a growth chamber programmed for a 14-hr photoperiod with a light intensity of 1500-1600 ft-c, and a day temperature of 28-30°C. After 48 hr, 1 g (wet weight) samples of leaves, stems, and whole plants (without roots) from each treatment were extracted separately in a microblender with 30 ml of ethanol for 1 min. Pigments were removed by filtering through bone charcoal. Extracts were then reduced to 1 ml under vacuum and used for thin-layer chromatography and bioassay. Fluorescent spots from extracts were compared by location with those obtained from stock solutions of the two compounds. Rf values for thiabendazole and ETMT were 0.85-0.88 and 0.90-0.95, respectively.

Chromatographic assays were made using 50-100 μliter of fungitoxicant stock solutions or extracts spotted on Eastman chromatograms (No. 6060 with fluorescent indicator) developed for 10 cm in acetone, and observed under ultraviolet light (254 nm).

Disease remission by thiabendazole and ETMT against Fol and Vaa was studied in 4-week-old seedlings grown in 10-cm clay pots containing 500 g of autoclaved field soil. The fungitoxicants were applied separately as a soil drench. At 5 days after treatment, plants were stem-inoculated with conidial suspensions of Fol or Vaa (1×10^6 spores/ml) using a 1-μliter syringe and needle. Inoculated plants grown in nontreated soil served as controls. Plants were maintained in a greenhouse at 26-28°C, receiving weekly applications of Hoagland's solution (12). At 4 weeks after inoculation, the plants were rated for disease severity based on leaf chlorosis: 1 = no chlorosis, 2 = 1-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% leaves chlorotic.

The persistence of the fungitoxicants was determined by growing seedlings in clay pots containing autoclaved soil : sand (2 : 1) mixture drenched with water (control) or with one of the two compounds at the three rates. At weekly intervals, entire plants (except roots) were removed, 1-g samples extracted and chromatographically assayed as described above. In laboratory tests, seedlings were grown in styrofoam cups containing vermiculite to which was added 100 cc of either water (control), thiabendazole or ETMT suspensions. After 48 hr, plants were removed, roots washed under tap water, and replanted in nontreated vermiculite. Bioassays were made at 1, 3, 5, 7, and 9 days after transfer.

Bioassay for thiabendazole was made by using the paper-disc plane method of Thornberry (15) with Difco potato-dextrose agar (PDA) to which was added 0.5-ml spore suspension of Penicillium atrovenetum G. Smith with an optical density of 0.05 at 600 nm. Ten ml of the mixture was dispensed into 9-cm culture plates, incubated at room temperature (23-25°C) and diameters of the zones of inhibition recorded after 36 hr. Bioassay for ETMT was similar to that described by Allam, et al. (2). Tissue extracts were added to cool PDA adjusted to pH 5.0 with lactic acid. Culture plates were poured and inoculated with 6-mm PDA discs from cultures of Rhizoctonia solani Kuehn. Colony diameters were recorded in mm after 3-4 days' incubation at room temperature.

Persistence of the fungitoxicants in soil was studied by drenching 500 g of autoclaved field soil in clay pots with suspensions of the two materials at the three concentrations. Six pots were treated for each concentration, including the water controls. The pots were watered daily, except prior to sample removal. Soil samples were first taken at 1-cm intervals and extracted with methanol. Fungitoxic activity was detected by bioassay up to 10 cm below the soil surface; therefore, 8-cm cores of soil were removed weekly from the pots with a No. 10 cork borer and 10 g of air-dried soil extracted with 30 ml of methanol on a wristaction shaker for 10 min. Extractions longer than 10 min did not increase the amount of fungitoxicant recovered. The extracts were filtered, evaporated to 1 ml and assayed.

RESULTS AND DISCUSSION

P. atrovenetum was sensitive to thiabendazole at concentrations as low as 2.5 μg/ml and ETMT completely inhibited R. solani at 20 μg/ml.

Thiabendazole and ETMT were taken up by roots of tomato seedlings and translocated into stems and leaves. Bioassays of plant extracts from thiabendazole-treated seedlings showed the amount of toxicant in the tissues increased with increasing levels of fungicide treatment (Table). Stem extracts from seedlings treated with 100 μg/ml of thiabendazole showed significantly (0.01 level) greater inhibiton than extracts from 25 or 50 μg/ml and extracts from whole plants treated with 100 μg/ml showed significantly (0.05 level) greater inhibition than extracts from plants treated with 25 μg/ml.

ETMT was detected in all tissues by bioassay using R. solani. Extracts from stems, but not from whole plants whose roots were exposed to 25 μg/ml, gave significantly (0.05 level)