Biological Characterization of Uptake, Translocation, and Dissipation of Difenoconazole (CGA 169374) in Wheat, Peanut, and Tomato Plants

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ABSTRACT

Dahmen, H., and Staub, T. 1992. Biological characterization of uptake, translocation, and dissipation of difenoconazole (CGA 169374) in wheat, peanut, and tomato plants. Plant Dis. 76:523-526.

Uptake and translocation of the triazole fungicide difenoconazole (CGA 169374) were evaluated on peanut, wheat, and tomato plants with *Alternaria solani, Cercospora arachidicola,* and *Erysiphe graminis* f. sp. *tritici* as test organisms. Persistence and penetration studies were carried out on peanut and tomato plants with *Cladosporium cucumerinum* as indicator fungus for the bioautographic in vitro assay. Sufficient quantities of difenoconazole penetrated into plants sprayed with 50 μg a.i./ml to give excellent disease control. Translaminar movement after leaf band application as well as acropetal translocation out of the treated zone towards the leaf tip were demonstrated. Uptake by the root system and upward translocation to foliage, however, was low. After leaf-dipping and stem-painting treatments, the symplastic and apoplastic transport of difenoconazole was also insignificant when judged by disease development on upper leaves. Persistence of difenoconazole on the surface of leaves seems to be plant-dependent. At the last sampling, 21 days after treatment, 50% of the applied activity was found on peanut leaves, whereas on tomato leaves only 10% was observed. Rate of penetration was similar for both plant systems.

Difenoconazole (CGA 169374) was introduced as a new experimental fungicide with a broad spectrum of activity against pathogens belonging to the classes Ascomycetes, Basidiomycetes, and Deuteromycetes (7). It has excellent protective and curative as well as persistent activities (*unpublished*). Protective treatments with 12.5 μg a.i/ml 21–14 days before inoculation with *Venturia inaequalis* (Cooke) G. Wint. and *Cercospora arachidicola* S. Hori provided 90–100% disease control. Curative treatments showed excellent antisporulant activity and the viability and virulence of conidia of *V. inaequalis* from treated lesions were clearly reduced (*unpublished*). Spore germination and initial establishment of the fungus inside the leaf were not greatly suppressed, but fungal growth was obviously inhibited after initial penetration.

Difenoconazole belongs to the newer sterol-demethylation inhibitors that possess significantly longer-lasting activity than the conventional protective fungicides. They also differ from the earlier sterol-demethylation inhibitor compounds such as propiconazole, penconazole, bitertanol, and triadimenol in their uptake, penetration, and systemic behavior in the plant. The penetration behavior of bitertanol, a triazole, is similar to that observed for tebuconazole and triadimenol. However, its systemic translocation in the transpiration stream is very low and comparable to that observed with prochloraz, an imidazole (2,6). With tebuconazole, a newer triazole, translocation is slower in comparison to triadimenol. However, it is much more mobile than the locosystemic compound bitertanol (4,6).

Knowledge of the dynamics of uptake, translocation, and metabolism are important for the performance of penetrating systemic fungicides and relates to their optimal use in the field. This study describes the behavior of difenoconazole under controlled conditions on three important target crops.

Accepted for publication 10 September 1991 (submitted for electronic processing).

MATERIAL AND METHODS

Antifungal compound. The compound used in this study was CGA 169374, with the common name difenoconazole, known under the trade name Score and formulated as EC 250. All concentrations given are based on a.i.

Host plants. Tomato plants (*Lycopersicon esculentum* Mill. 'Roter Gnom'), peanut plants (*Arachis hypogaea* L. 'Flory Giant'), and wheat plants (*Triticum aestivum* L. 'Kanzler') were grown under greenhouse conditions in an organic standard soil in 8-cm pots at 20–23 C.

Inoculum production. *Alternaria solani* Sorauer (isolate 297) was cultured on PCA medium (2% potato, 2% carrot, and 1.8% Bacto-Agar, pH 6.7). To induce sporulation, agar plates overgrown with mycelium of *A. solani* were placed under constant cool white fluorescent light (Philips TMLF 40W/33RS) for 24 hr and then transferred to the dark for 3 days at 22 C. *C. arachidicola* (isolate 59) was cultured on Czapek Dox V8 agar (20% V8 juice, 4.5% Czapek Dox agar [Oxoid], 1% agar no. 3 [Oxoid], 0.3% calcium carbonate [Fluka], pH 6.3). A sterile layer of filterpaper was placed on the surface of the solidified agar medium and inoculated with the fungus. Then the plates were incubated for 14 days under constant cool white fluorescent light at 22 C. *Cladosporium cucumerinum* Ellis & Arth. (isolate 9) was grown on PCA in flat bottles in the dark at 22 C for 7 days. Conidia of *Erysiphe graminis* DC. f. sp. *tritici* Ém. Marchal were multiplied on 4-wk-old wheat plants. Ten days after inoculation, sufficient conidia were produced to inoculate the test plants.

Uptake and translocation in tomato and peanut plants. To assess the uptake and translocation of difenoconazole, 4-wk-old tomato and peanut plants with four to five fully expanded leaves were treated with the test suspension 1 or 5 days before inoculation. Four different application procedures were used: a) spraying the aboveground plant parts with concentrations of 0.5, 5, 50, and 250 μg/ml; b) dipping the first true leaf for 5 s into the compound solution with concentrations of 250, 500, and 1000 μg/ml; c) painting the lower stem part from the stem base to the second leaf stipule four times with concentrations of 250, 500, and 1000 μg/ml; and d) a soil drench treatment with concentrations of 250, 500, and 1000 μg/ml.

Until inoculation the treated plants were kept in a growth chamber at 22 C, well separated to avoid possible gas phase activity. The leaves treated by dipping into the compound solution were removed before inoculation, to avoid redistribution of the chemicals under the wet incubation conditions. Five plants per treatment were inoculated by spraying on the lower leaf surfaces a spore suspension of *A. solani* or *C. arachidicola* containing approximately 5×10^4 conidia per milliliter and covered for the first 2–4 days with a plastic hood that was cooled by intermittent misting to maintain a temperature of 20–22 C. The plants were then transferred to a growth chamber and were kept at 25–27 C and 12 hr of light per day until the end of the experiment. Disease was rated at 5 and 10 days after inoculation by estimating the percentage of the diseased leaf area. The experiment was repeated twice.

Translaminar, acropetal, and basipetal translocation after leaf band treatment

of wheat. To assess the translaminar, acropetal, and basipetal movement of difenoconazole, 2-wk-old wheat plants were treated by distributing ten 5-μl drops in a 1-cm band running across the upper surface of the first true leaf halfway between the leaf base and leaf tip (band treatment). A total of eight plants per treatment were used. The concentrations per band were 0.005, 0.05, and 0.5 μg. Two days later, freshly formed conidia of *E. g. tritici* from heavily infected leaves were dusted over the lower surface of the horizontally arranged primary leaves. The experiment was conducted in a growth chamber at 20 C and a light intensity of 250 μmol m^{-2} s^{-1} for 16 hr/day and repeated three times with similar results. Disease was rated by estimating the diseased leaf area per cm of length beginning from leaf base to leaf tip 7 days after inoculation.

Penetration and dissipation studies. To study the penetration and degradation of difenoconazole, 4-wk-old tomato and peanut plants were treated with a suspension containing 500 μg a.i./ml in a spray tower. Fifteen-gram samples of treated leaf tissue were taken for the extraction 1 hr, 1, 3, 7, 14, and 21 days after treatment. To remove the residues of difenoconazole on the leaf surface, the leaves were washed three times for 2 s each in 150 ml ethyl acetate. The 450-ml wash solution was concentrated by evaporating the solvent at 40 C and resuspended in 5 ml acetone. To extract the compound from the washed leaves, leaves were homogenized in a 300-ml beaker with a blender (Ultra-Turrax) in the presence of 200 ml of ethyl acetate for 5 min. The beaker was kept on crushed ice. The homogenized material was shaken for 2 hr at 6 C on a rotary shaker at 150 rpm. After settling, the supernatant was decanted and filtered through one layer of cheesecloth. The settled homogenized plant material was resuspended in 200 ml of ethyl acetate, and the procedure was repeated once more. Finally, the filtered plant extracts were combined and evaporated to dryness at 40 C, and the residues were redissolved in 5 ml of acetone. The acetone solutions were stored at −80 C.

To identify the active fungicidal material and to estimate its amount, a semiquantitative in vitro bioautographic technique was used. The amounts of 0.1, 0.01, 0.001, and 0.0001 μg and individual plant extracts were spotted onto 0.25-mm thin-layer chromatography plates of silica gel. The plates were developed in chloroform:isopropanol (9:1, v/v). Plates were kept at room temperature for 24 hr in order to evaporate the solvents from the silica gel layer. Conidia of *C. cucumerinum*, which had been stored in liquid nitrogen, were thawed and suspended in 5% broth (Sabouraud Maltose) amended with 300 μg a.i. of dicloran per milliliter to suppress contamination with *Rhizopus* and adjusted to 5×10^5 conidia per milliliter. Twelve milliliters of the conidial suspension were sprayed uniformly on each chromatographic plate. The plates were incubated for 3 days at 22 C in a closed plastic box lined with moist filter paper. The inhibition zones, which appeared as clear white spots against a dark green background of growing fungus mycelium, were measured. A plot of the diameter of the inhibition zones vs. the log of difenoconazole concentration served as a standard curve (Fig. 1). The amount of extracted difenoconazole was estimated by comparing the diameters of the inhibition zones obtained with plant extracts and with the standard curve. The total amount of estimated activity on and in the plants 1 hr after treatments was set as 100% recovered activity. The experiments were done twice. The results shown in the figures are the data from one experiment.

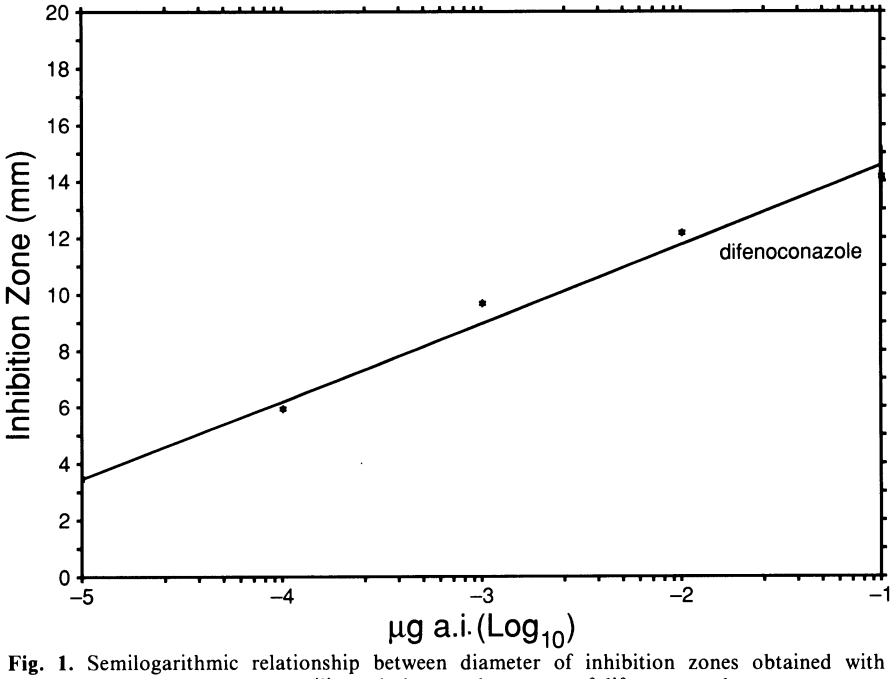

Fig. 1. Semilogarithmic relationship between diameter of inhibition zones obtained with *Cladosporium cucumerinum* on silica-gel plates and amounts of difenoconazole.

Table 1. Fungicidal activity of difenoconazole in growth-chamber trials after different types of application

Concentration (μg a.i./ml)	Disease control (%)			
	Leaf treatments		Stem treatment[a] by painting	Soil treatment[a] drench
	Spray[a]	Dipping[b]		
Alternaria solani on tomato plants, 1 day protective treatment				
0.5	20 ± 5[c]	nt[d]	nt	nt
5.0	60 ± 6	nt	nt	nt
50.0	95 ± 2	nt	nt	nt
250.0	100[e]	3 ± 0.1	3 ± 0.1	37 ± 6
500.0	nt	3 ± 0.1	3 ± 0.1	70 ± 6
1,000.0	nt	0[e]	5 ± 2	100
Cercospora arachidicola on peanut, 5 days protective treatment				
0.5	14 ± 2	nt	nt	nt
5.0	78 ± 3	nt	nt	nt
50.0	100	nt	nt	nt
250.0	100	0	0	14 ± 10
500.0	nt	0	0	14 ± 8
1,000.0	nt	2 ± 2	0	82 ± 6

[a] Disease rating on all aboveground plant parts.
[b] Disease rating on the untreated leaves.
[c] Standard error.
[d] Not tested.
[e] Either 100% or no disease control of *A. solani* and *C. arachidicola*. No statistical analyses possible.

RESULTS

Uptake and translocation of difenoconazole in tomato, peanut, and wheat plants. Biological data on uptake and translocation of difenoconazole in tomato and peanut plants are shown in Table 1. The protective activity after foliar spray treatments against *A. solani* and *C. arachidicola* reached 95 and

100%, respectively, at a concentration of 50 μg a.i./ml (Table 1). To obtain a similar level of activity with the soil drench treatment 1,000 μg a.i./ml or more were needed for both plant systems. Dip treatments of the lowest major leaf and painting of the lower stem resulted in no disease control on the upper untreated plant parts even at the highest concentration employed (Table 1).

Translaminar, acropetal, and basipetal movement within a leaf after band treatment are shown in Fig. 2. After treatment of wheat leaf segments on one side with 0.005 μg per segment, about 70% disease control was observed on the opposite side because of translaminar movement of the compound (Fig. 2). The local activity on the treated side was almost the same (78%). The compound is translocated out of the treated zone acropetally and, to a lesser extent, basipetally. Towards the leaf tip, about 10 cm away from the treated zone, approximately 50% disease control was observed with 0.005 μg, 80% with 0.05 μg, and 100% with 0.5 μg of difenoconazole. The pattern of the graph (Fig. 2) indicates that the compound is present from the treated zone to the leaf tip. Towards the bases of the leaf, 35% disease control was found with the highest concentration (Fig. 2). The repeat experiment produced similar results.

Penetration and dissipation studies. The persistence of difenoconazole on and in peanut and tomato plants, as well as its penetration and degradation in leaves over a period of 21 days, is shown in Fig. 3. Approximately 45% of the applied difenoconazole was still found on the surfaces of peanut leaves 21 days after treatment (Fig. 3A), whereas on tomato leaves only 8% was found (Fig. 3B).

Almost half of the activity on the leaf surface of peanut plants was lost within the first 3 days after application (Fig. 3A); on tomato plants almost half of the activity on the leaf surface was lost within the first 24 hr (Fig. 3B). Penetration of the parent compound into the leaf was similar for both plants (Fig. 3). Seven days after treatment, about 20% of the applied difenoconazole was recovered in the tomato leaf, but 21 days after application the concentration had decreased to 10%. In peanut leaves the highest recovered concentration never exceeded 20%, but the level was stable from 7 to 21 days. The repeat experiment produced similar results.

DISCUSSION

Conventional spray treatments of the aboveground plant parts with 5 μg/ml gave excellent disease control. Translaminar movement and acropetal translocation out of the treated leaf zone is significant and is distributed over the leaf tip. Basipetal movement within a leaf is much lower than acropetal translocation. The systemic activity of difenoconazole

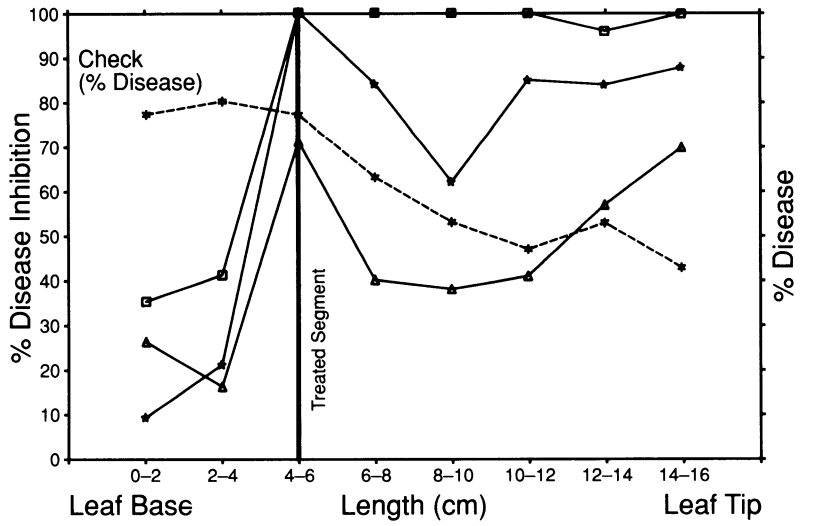

Fig. 2. Distribution of difenoconazole in leaves after segment treatment. Bioassay on wheat with *Erysiphe graminis* f. sp. *tritici*. Open squares = 0.5; stars = 0.05; and open triangles = 0.005 μg of a.i. per treated segment. Dashed line = disease on nontreated checks.

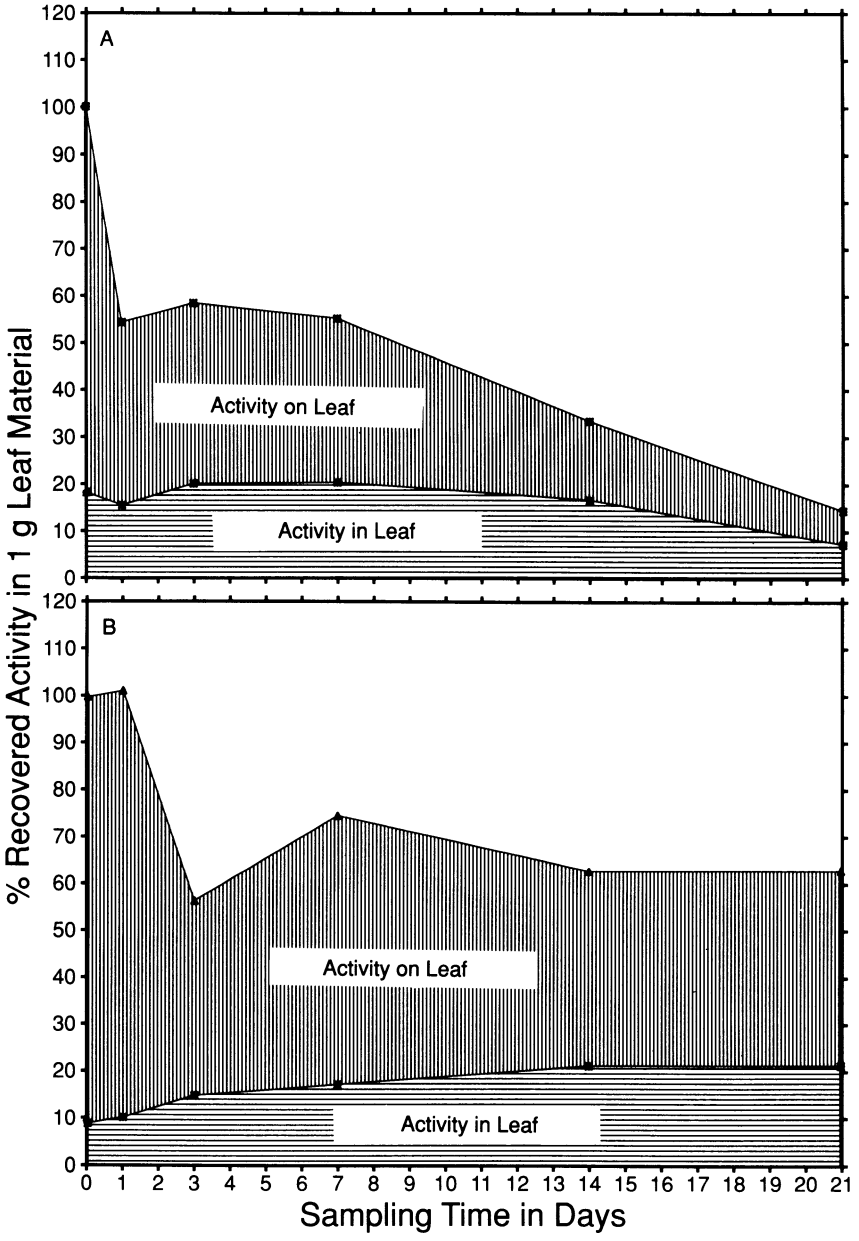

Fig. 3. Persistence and penetration of difenoconazole on and in peanut leaves **(A)** or tomato leaves **(B)**.

shown in Table 1 indicates that only small amounts of the compound are taken up by the root system and transported into the leaves of tomato and peanut plants after soil drench treatments with 1000 μg a.i./ml.

These observations are quite different from those made with other sterol inhibitor compounds, such as propiconazole, penconazole, triadimenol, fenarimol, and nuarimol. These fungicides are readily taken up by the roots and translocated to new growth, which results in excellent disease control after a soil drench treatment (1,4,5). Lack of control after stem treatment with difenoconazole also indicates weak apoplastic transport. Symplastic transport out of lower treated leaves to new growth did not seem to occur.

The persistence of difenoconazole on the surface of leaves seems to be plant-dependent. On peanut leaves, about 50% of the applied activity disappeared during the first 3 days. Thereafter, the concentration remained constant until the last sample was taken 21 days after treatment. This may explain the long protective activity observed in the greenhouse (*unpublished*). The large decrease in recovered activity within the first 1–3 days may be attributable to evaporation or degradation into nonfungicidal metabolites. The persistence of propiconazole on leaves is much shorter. Kelley (3) reported that only 10% of the applied propiconazole was found on apple leaves 12 hr after treatment, whereas 90% penetrated into the leaves. In contrast, 60% of the activity of bitertanol was still present on the leaves and only 40% had penetrated 24 hr after treatment. No further accumulation of propiconazole and bitertanol in the leaves was observed. These observations are similar to difenoconazole.

With both plant systems, 10–20% of the applied difenoconazole penetrated within the first hour, and no further accumulation of the parent compound in the leaves was observed over the entire sampling time. The rate of degradation of difenoconazole seems to be plant-specific. The reason is possibly differential degradation of the parent to nonfungicidal products that cannot be detected with our bioassay. The persistence of difenoconazole on and in the leaf, and its penetration behavior, may be reasons for its activity against a wide range of plant pathogenic fungi from the classes Ascomycetes, Basidiomycetes, and Deuteromycetes, despite their differences in the speed of disease development. The period of time required for conidial germination, germ tube penetration, fungal establishment inside the leaves, symptom development, and sporulation varies enormously. It took 3 days under greenhouse conditions for symptoms to develop after inoculation with *A. solani*, whereas with *C. arachidicola* 11 days were needed. The persistence of difenoconazole on and in the plant were longer than the infection cycle of the target fungus with the longest disease cycle and enabled it to remain active against the range of fungi tested.

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