Protective, Curative, and Eradicant Activity of Difenoconazole Against *Venturia inaequalis, Cercospora arachidicola*, and *Alternaria solani*

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ABSTRACT

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The protective and curative activities of difenoconazole against *Venturia inaequalis, Cercospora arachidicola*, and *Alternaria solani* were determined in the greenhouse on apple, peanut, and tomato, respectively. In addition, the effects of difenoconazole on established, visible scab lesions and on the production of spores from treated lesions were evaluated. Spray treatments with 12.5 μg a.i./ml of difenoconazole provided 90 and 100% disease control of *V. inaequalis* following 3 days of protective and 3 days of curative treatments, respectively. Applications 5 or 7 days after inoculation were too late to prevent symptom appearance, but they prevented sporulation. Against *C. arachidicola*, difenoconazole showed excellent protective and curative activity. The protective activity persisted for up to 21 days. Curative application with 75 μg a.i./ml of difenoconazole provided 89–100% disease control and 90–100% antisporulant activity. Eradicative treatments with 75 μg a.i./ml provided at least 80% antisporulant activity. In the few lesions that developed after protective treatments with the longest application intervals and lowest concentration, the compound had little antisporulant activity. Against *V. inaequalis*, with treatments 3 days prior to inoculation and a concentration of 12.5 μg a.i./ml, only 63% antisporulant activity was found. Against *C. arachidicola* difenoconazole worked similar to chlorothalonil, which was included as a standard. Treatment 21 days prior to inoculation with 250 μg a.i./ml of difenoconazole and 1,250 μg a.i./ml of chlorothalonil gave 80% disease control but almost no reduction in sporulation. Against *A. solani* the protective and curative activity of difenoconazole was superior to that of mancozeb. Treatments 7 days prior to and 1 day after inoculation gave 83–100% disease control. Eradicative treatments applied 2 days after inoculation provided little initial control, but 10 days after inoculation they provided about 60% control. Difenoconazole is the first sterol inhibitor compound with excellent activity against *A. solani*. These results have an important bearing on optimal treatment schedules and the selection of suitable mixture partners for difenoconazole.

Fungicides that inhibit ergosterol biosynthesis (SI fungicides) come from diverse chemical classes and can be subdivided into two groups according to their biochemical mode of action. The first group are inhibitors of sterol C-14 demethylation (DMIs), which affect cytochrome P-450 enzymes. Examples from this group are pyrimidines like fenarimol and nuarimol; imidazoles such as prochloraz; triazoles like bitertanol, triadimefon, propiconazole, penconazole, flutriafol, flusilazole, and cyproconazole; and piperazines such as triforine. The second group of SI fungicides includes the morpholines like fenpropimorph and tridemorph, which interfere with the C-14 reductase or the $\Delta^8 \rightarrow \Delta^7$ isomerase (1,2,11,15). The SI fungicides vary widely in their spectra and levels of disease control activity and in their systemic properties within the plant system. Field and greenhouse experiments have confirmed that SI fungicides combine protective and curative properties and thus interfere with target fungi throughout their infection cycles after penetration. These properties make SI fungicides highly suitable for use with various types of disease-warning systems (3,4,7,12).

The study reported here deals with difenoconazole, a new fungicide belonging to the triazole family that exhibits high activity against a large number of plant pathogenic fungi belonging to the classes Ascomycetes, Basidiomycetes, and Deuteromycetes. In the field, the compound provides long-lasting, preventive, and excellent curative activity against *Alternaria, Septoria, Cercospora, Cercosporidium, Ascochyta, Ramularia, Venturia, Guignardia, Phoma, Colletotrichum*, rust fungi, powdery mildew fungi, and several seedborne pathogens (5,8,9,14). Difenoconazole is the first SI fungicide with excellent activity against *Alternaria* spp. With its curative properties, it can interfere with target fungi throughout their infection cycles. Thus, it is suitable for adaptation to various disease-warning systems.

In this greenhouse study, controlled inoculations were used to determine more precisely the characteristics of difenoconazole relative to other DMIs and conventional protective fungicides. Protective, curative, and eradicative treatments were evaluated for the control of *Alternaria solani* Sorauer on tomato, *Cercospora arachidicola* S. Hori on peanut, and *Venturia inaequalis* (Cooke) G. Wint. on apple seedlings.

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MATERIALS AND METHODS

Antifungal compounds. The chemicals used in this study were difenoconazole (Score; 3-chloro-4-[4-methyl-2-(1*H*-1,2,4-triazol-l-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether), formulated as EC 250; propiconazole (Tilt, EC 250); penconazole (Topas WP25); chlorothalonil (Daconil WP75); and mancozeb (Mancozeb WP80). The concentrations used are given as active ingredient.

Host plants. Tomato plants (*Lycopersicon esculentum* Mill. cv. Roter Gnom), peanuts (*Arachis hypogaea* L. cv. Flory Giant), and apple seedlings (*Malus sylvestris* Mill. seed from cv. McIntosh) were grown in an organic standard soil (type TKS 1, Torfstreuverband GmbH, Oldenburg, Germany) in 8-cm pots in the greenhouse.

Inoculum production. *A. solani* (isolate 297) was cultured on PCA medium (2% potato, 2% carrot, and 1.8% Bacto Agar [Difco], pH 6.7). To induce sporulation, agar plates overgrown with mycelium of *A. solani* were placed under constant cool-white fluorescent light 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 juice agar (20% V8 juice, 4.5% Czapek-Dox agar, 1% agar no. 3, 0.3% calcium carbonate, pH 6.3). A sterile layer of filter paper was placed on the surface of the solidified agar medium and inoculated with the fungus. Then plates were incubated for 14 days under constant cool-white fluorescent light at 22 C. *V. inaequalis* was grown on 4-wk-old McIntosh apple seedlings in the greenhouse at 20–23 C and 12 hr of light per day. Fourteen days after inoculation conidia were washed off of sporulating lesions to inoculate the test plants.

Timing tests. The experiments were done on 3- to 4-wk-old seedlings. Spray treatments were made at various times before (protective tests) and after (curative tests) inoculation. For each pathogen the latest treatment was applied after symptoms were visible (eradicative tests). To inoculate tomato plants with *A. solani*, a conidial suspension (spore density of 40,000 conidia per milliliter) was atomized on both sides of the tomato leaves. The plants were then placed for 3–4 days under cheesecloth on a bench in a greenhouse chamber with intermittent misting and light for 14 hr/day.

Temperature was 24 C during the day and 18 C at night. The cheesecloth cover was removed and misting stopped during the remaining incubation time. The rating was done twice, beginning when about 60% of the leaf area on untreated plants was diseased. Spore density for *V. inaequalis* was 260,000 conidia per milliliter; for *C. arachidicola*, 90,000 conidia per milliliter. Incubation methods for both fungi were essentially the same as for *A. solani*. The inoculated peanut plants were transferred after the 3-day wetting period to a greenhouse with temperatures of 27 C during the day and 24 C during the night. Since older apple seedling leaves are more resistant to scab infection, only the youngest developed leaves were evaluated. They were marked prior to inoculation.

To rate disease control, the diseased leaf area was estimated in percent. From the same set of leaves, sporulation of *V. inaequalis* on individual leaves was determined by brushing the spores into 20 ml of water. For *C. arachidicola*, inhibition of sporulation was estimated. Since no sporulation of *A. solani* on tomato plants was obtained under our test conditions, only figures on disease progress 4 and 10 days after inoculation are presented. At least five single-plant replicates per treatment were used. The experiments were done twice, with similar results.

RESULTS

Control of *V. inaequalis*. Difenoconazole applied 3 days prior to inoculation provided 89–100% control of apple scab (Table 1). Protection with 25 and 50 μg a.i./ml was better than protection with 12.5 μg a.i./ml of difenoconazole. Normal sporulating lesions developed on seedlings treated with 12.5 μg a.i./ml of difenoconazole, but the very few lesions that developed on plants treated with 12.5 μg a.i./ml were not sporulating lesions.

Difenoconazole at 12.5, 25, and 50 μg a.i./ml and penconazole at 50 μg a.i./ml provided complete control of scab when applied 3 days after inoculation (Table 1). A large number of nonsporulating lesions were observed on seedlings treated 5 days after inoculation. Both sporulating and nonsporulating lesions were observed when the seedlings were sprayed 7 days after inoculation. The percentage of nonsporulating lesions increased as the rate of the fungicide increased. Nonsporulating lesions from difenoconazole treatments had a puffed-up appearance. These lesions were less well defined than those on penconazole-treated plants, which showed the typical triazole-induced chlorotic fleck lesions.

Control of *C. arachidicola*. Protective treatments with difenoconazole 21 days prior to inoculation were superior to propiconazole treatments and similar to chlorothalonil (Table 2). Exposure to 125 μg a.i./ml of difenoconazole and 1,250 μg a.i./ml of chlorothalonil gave 80 and 79% disease control, respectively. However, spray intervals of 14 and 21 days prior to inoculation were too long to control sporulation. Sporulation was observed on the few infection sites which developed. In contrast, 14-day protective treatments with propiconazole provided less disease control but almost 100% antisporulant activity. However, after 21 days protective treatments the antisporulant activity of propiconazole was also weak. Three-day curative treatment of both difenoconazole and propiconazole gave 100% disease control.

Control of *A. solani*. Protective and curative treatments at 7 days before and 1 day after inoculation on tomato against *A. solani* gave almost 100% disease control with concentrations of 125 μg a.i./ml and higher (Table 3). With treatments 14 days prior to inoculation, disease control was not as good compared with treatments 7 days before inoculation. Treatments 2 days after inoculation were eradicative. After such treatments with difenoconazole, lesions kept expanding for about another 2 days but were then stopped from developing further. Treatments with mancozeb, on the other hand, had no effect on already established lesions.

DISCUSSION

Data on optimal treatment time (Tables 1, 2, and 3) indicate that difenoconazole provides a long-lasting protective and excellent curative disease control activity, which is also reported by Ruess et al (9) against a number of other fungi. Results of curative treatments with difenoconazole were typical of that obtained with other DMI compounds. With lengthened time between inoculation and compound application, the effect on lesion development decreased, whereas the effect on sporulation of *V. inaequalis* and *C. arachidicola* remained high. Following difenoconazole treatments, the normal scab lesions on infected apple leaves were replaced by puffed-up undulating zones. Eradicative treatments with current DMI compounds resulted in nonexpanding chlorotic fleck lesions (7,13). In both cases, the appearance of puffed-up lesions or chlorotic fleck lesions indicate that further development of the fungus in the lesions is arrested by the compound following postsymptom treatments. Strong protective action is shown against all three pathogens tested. A possible reason for the failure of difenoconazole to act on sporulation when applied protectively is that the low concentrations applied at extended ap-

Table 1. Control of *Venturia inaequalis* lesion development and sporulation on apple seedlings with protective and curative spray treatments of difenoconazole under greenhouse conditions

Compound	Concentration (μg a.i./ml)	Fungal activity[a]	Control effects (%) by spray treatments[b]			
			Protective	Curative		
			Days before inoculation	Days after inoculation		
			3	3	5[c]	7[d]
Difenoconazole	12.5	Scab lesions	89 ± 4.1	100	0[e]	0
		Sporulation	63 ± 1.4	100	98 ± 0.4	62 ± 3.75
	25	Scab lesions	100	100	0	0
		Sporulation	100	100	96 ± 0.1	73 ± 2.5
	50	Scab lesions	98 ± 0.9	100	0	0
		Sporulation	100	100	99 ± 2.2	93 ± 2.2
Penconazole	50	Scab lesions	...	100	0	0
		Sporulation	...	100	93 ± 0.2	85 ± 1.1

[a] For scab lesions, disease rating 13 days after inoculation; diseased leaf area on untreated plants was 73%. For sporulation, reduction of spore production 15 days after inoculation compared to untreated leaves. The number of conidia on the untreated samples (26.26×10^5 conidia per leaf) was set as 100%.
[b] Statistics are standard error.
[c] Treatment into lesions just at the beginning of sporulation.
[d] Treatment into fully sporulating lesions.
[e] Nonsporulating or poorly sporulating puffed-up lesions; no control of symptom development.

Table 2. Control of *Cercospora arachidicola* lesion development and sporulation on peanut with a protective and curative spray schedule under controlled greenhouse conditions

Compound	Concentration (μg a.i./ml)	Fungal activity[a]	Control effects (%) by spray treatments[b]					
			Protective			Curative		
			Days before inoculation			Days after inoculation		
			21	14	7	3	5	7[c]
Difenoconazole	75	Symptoms	53 ± 9.6	88 ± 1.6	100	100	89 ± 1.7	18 ± 3.4
		Sporulation	0	0	100	100	90	80
	125	Symptoms	80 ± 9.7	87 ± 2.8	100	100	87 ± 3.2	17 ± 9.7
		Sporulation	0	0	100	100	90	90
	250	Symptoms	78 ± 9.5	96 ± 2.0	100	100	94 ± 3.1	28 ± 5.2
		Sporulation	10	0	100	100	100	90
Propiconazole	125	Symptoms	47 ± 7.7	96 ± 1.3	100	100	100	79 ± 3.1
		Sporulation	20	97	100	100	100	100
Chlorothalonil	1,250	Symptoms	79 ± 8.2	89 ± 3.2	100	NT[d]	NT	NT
		Sporulation	20	97	100	NT	NT	NT

[a] Diseased leaf area on untreated check plants was 93%; antisporulation activity was estimated in relation to the check.
[b] Statistics are standard error.
[c] Eradicant treatment.
[d] NT = not tested.

Table 3. Control of *Alternaria solani* on tomato with a protective and curative spray schedule under controlled greenhouse conditions

Compound	Concentration (μg a.i./ml)	Rating time (days after inoculation)	% Disease control[a]			
			Protective		Curative	
			Days before inoculation		Days after inoculation	
			14	7	1[b]	2[c]
Difenoconazole	125	4	25 ± 3.5	88 ± 0.6	83 ± 1.25	13 ± 2.5
		10	19 ± 11.0	94 ± 0.9	91 ± 1.0	56 ± 5.6
	250	4	37 ± 2.5	100	85 ± 1.1	33 ± 2.2
		10	50 ± 6.1	97 ± 1.1	92 ± 1.2	62 ± 3.5
	500	4	62 ± 2.5	100	87 ± 1.1	32 ± 4.1
		10	75 ± 3.5	91 ± 3.9	94 ± 1.9	62 ± 6.1
Mancozeb	60	4	20 ± 7.4	42 ± 5.4	25 ± 0.4	12 ± 2.5
		10	4 ± 2.2	16 ± 7.2	10 ± 4.1	10 ± 4.1
	200	4	25 ± 4.7	50 ± 3.5	20 ± 2.7	7 ± 2.7
		10	12 ± 4.7	56 ± 7.5	0	5 ± 2.7
	600	4	7 ± 2.7	82 ± 1.4	65 ± 3.2	42 ± 2.7
		10	4 ± 2.7	75 ± 4.7	41 ± 11.4	16 ± 7.2

[a] Diseased leaf area on untreated plants 4 and 10 days after inoculation was 40 and 80%, respectively.
[b] For difenoconazole, symptoms were tiny, necrotic, nonexpanding spot lesions.
[c] Eradicant treatment.

plication intervals resulted in too-low levels of compound on or in the leaves.

Studies under controlled conditions reported in this paper give valuable information on the biological profile of fungicides, but optimal treatment schedules and effective dosages for practical use must obviously be evaluated under field conditions. First results from field trials worldwide (9) report excellent long-lasting protective and strong curative activity of difenoconazole on apple scab applied at 2.5–10 g a.i./hl, which allows more flexibility in application timing.

The compound is likely to be a valuable tool for integrated pest management programs because its good protective, curative, and eradicative activities confers great flexibility in application timing. In recent years, considerable progress has been made with the establishment of scab-warning services using simple or computerized apple scab prediction systems for scheduling fungicide applications for scab control (6,10). Such systems are based on climatic factors like temperature, amount and duration of rainfall, duration of leaf wetness, and the period required for the pathogen to initiate infection. When the conditions are favorable for fungal infection, the recommendation to apply fungicides is made. The availability of fungicides with protective, curative, and eradicative properties against apple scab makes it possible to achieve reliable disease control using these scab-warning systems.

The most unique feature of difenoconazole in comparison to other SI compounds is its long-lasting protective activity against *Alternaria* spp. In our study this feature is shown for tomato early blight. Others have shown the long-lasting protection by difenoconazole against *Alternaria* spp. on potatoes, onions, apples (9), and brassicas (8).

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