Activity of Cyprodinil: Optimal Treatment Timing and Site of Action

G. Knauf-Beiter, H. Dahmen, U. Heye, and **T. Staub,** Ciba-Geigy Ltd., CIBA Crop Protection, Business Unit Disease Control, CH-4002 Basel, Switzerland

ABSTRACT

Knauf-Beiter, G., Dahmen, H., Heye, U., and Staub, T. 1995. Activity of cyprodinil: Optimal treatment timing and site of action. Plant Dis. 79:1098-1103.

Cyprodinil, a fungicide with a new mode of action, was recently introduced by Ciba-Geigy for foliar application on cereals, grapes, vegetable crops, and deciduous fruits. In greenhouse studies, the protective and curative activity of cyprodinil was determined against *Erysiphe graminis* f. sp. *tritici* on wheat, *Drechslera teres* on barley, and *Venturia inaequalis* on apples. Cyprodinil significantly reduced fungal infection stages that are formed after penetration of the leaf. Foliar applications of 600 mg a.i./liter of cyprodinil provided 90 to 100% control of *E. graminis* leaf symptoms after 7-day protective and 5-day curative applications. Curative applications did not completely prevent symptom development; however, sporulation was reduced 100%. Histological studies showed that secondary haustoria were the stage in the infection cycle of *E. graminis* most sensitive to cyprodinil. Symptom development of *V. inaequalis* was reduced 90 to 100% following 6-day protective and 3-day curative applications with 100 mg a.i./liter. This was mainly based on reduction in the growth of the subcuticular stroma. Application of 200 mg a.i./liter of cyprodinil provided 80 to 90% reduction in lesion area of *D. teres* within an effective application period lasting from 7-day protective to 2-day curative. Histological observations of late infection stages of *D. teres* were difficult, but it is hypothesized that the activity of cyprodinil against *D. teres* is based on the inhibition of intra- and intercellular mycelial growth.

Additional keywords: anilinopyrimidines, CGA 219417, methionine biosynthesis

Cyprodinil (CGA 219417, Ciba-Geigy Ltd., Basel) was recently introduced by Ciba-Geigy as a new fungicide against various diseases (6). The compound offers a broad spectrum of activity on cereal diseases, including eyespot, powdery mildew and leaf spot diseases, and their pathogens, such as *Drechslera teres* (Sacc.) Shoemaker, *Rhynchosporium secalis* (Oudem.) J. J. Davis and *Septoria nodorum* (Berk.) Berk in Berk. & Broome (1,6,7,9), and on seedborne diseases (10). Another strength of cyprodinil is its activity against *Venturia inaequalis* (Cooke) G. Wint. on pome fruit and on *Botrytis cinerea* Pers:Fr. on grapes, vegetables, and field crops. Cyprodinil belongs to a new fungicide class, the anilinopyrimidines, which includes pyrimethanil (4,13) and mepanipyrim (11,14). It is hypothesized that both fungicides act on the secretion of hydrolytic enzymes in *Botrytis* spp. In 1994, Masner et al. (12) reported a possible interference of all three compounds with methionine biosynthesis in *B. cinerea*.

Cyprodinil is readily taken up into leaves and systemically distributed within them. Site of action studies, which are reported in this paper, show that the biological activity is based on this systemic effect. Greenhouse studies were carried out with *Erysiphe graminis* DC f. sp. *tritici* Ém. Marchal, *D. teres,* and *V. inaequalis* to define the protective and curative activity under controlled conditions and to describe the site of action of the compound at the cytological level.

Corresponding author: G. Knauf-Beiter
E-mail: Gertrude.Knauf-Beiter@chbs.mhs.ciba.com

Accepted for publication 17 July 1995.

MATERIAL AND METHODS

Chemicals and application. Cyprodinil was applied as a WP 50 formulation with the exception of the *D. teres* histology study, in which a WG 75 formulation was used. Reference compounds included fenpropimorph (EC 750) against *E. g.* f. sp. *tritici*, difenoconazole (EC 250), flusilazole (EC 400), captan (WP 83) against *V. inaequalis* and propiconazole (EC 250) against *D. teres*. Concentrations are given as milligram of active ingredient (a.i.) per liter. The formulated fungicides were suspended in double-distilled water immediately before application.

Plants treated with double-distilled water served as check plants. Foliar applications were made both protectively (prior to inoculation) and curatively (after inoculation) following individual time schedules for each pathogen.

Plant production. Wheat (*Triticum aestivum* L. 'Kanzler') was grown in TKS-2 organic standard soil (Torfstreuverband GmbH, Oldenburg, Germany) in 9-cm pots at 20°C and a light period of 16/8 h for 14 days in the greenhouse.

Apple seedlings (*Malus* × *domestica* Borkh. 'McIntosh') were grown in TKS-1 organic standard soil in 8-cm pots at 20/18°C and a light period of 16/8 h for 5 weeks in the greenhouse. Maintenance treatments were conducted once with 0.4% Fongarid (25% furalaxyl) and weekly thereafter with sulfur vapor until 5 days before inoculation.

Barley (*Hordeum vulgare* L. 'Triton') was grown in TKS-2 organic standard soil in 9-cm pots at 20/18°C and a light period of 16/8 h for 10 days in the greenhouse.

Inoculum production, inoculation and incubation. *Erysiphe graminis* f. sp. *tritici* (strain 387, Ciba-Geigy culture collection) was propagated on 3-week-old wheat plants, cv. Kanzler, at 20°C and a light period of 16/8 h in a growth chamber. Freshly formed conidia were harvested 10 days after inoculation and dusted over the trial plants in an inoculation tower until a spore density of approximately 170 conidia/cm^2 leaf area was reached (checked by light microscopy on different leaf samples). After inoculation, plants were kept in a growth chamber at 20°C and a light period of 16/8 h (250 μE s^{-1} m^{-2} light intensity) until evaluation. To rate disease control, the percent nonsporulating and sporulating leaf areas were estimated separately on the first secondary leaf 11 days after inoculation.

Venturia inaequalis (strain 409, Ciba-Geigy culture collection) was propagated on 3-week-old apple seedlings, cv. McIntosh, in the greenhouse at 20 to 23°C and a light period of 16/8 h. Fourteen days after inoculation, conidia were washed off sporulating lesions with double-distilled water and passed through cheesecloth. A conidial suspension of 1 × 10^5 conidia/ml was atomized on the upper leaf surface of the youngest developed leaves. The plants were incubated for 2 days under cheesecloth with intermittent misting at 20 to 23°C and a light period of 16/8 h in the greenhouse. After the incubation period, plants were kept in the greenhouse at 20/18°C and a light period of 16/8 h without misting until evaluation. Disease control was determined by estimating the percent sporulating leaf area 10 days after inoculation. Since older apple seedling leaves are more resistant to scab infections, only the youngest treated leaves, marked prior to inoculation, were evaluated.

Drechslera teres (strain 412, Ciba-Geigy culture collection) was grown on V8-medium (20% V8 juice, 0.3% $CaCO_3$, 2% Bacto Agar [Difco, Detroit, Mich.], pH

6.3). For the first 3 days, cultures were incubated at 18°C in the dark. Afterwards, they were transferred to a light period (cool white light Philips TLF 40 W/33RS) of 8/16 h dark and a temperature of 22/18°C for 10 days. For inoculation, conidia were harvested into double-distilled water containing 0.1% Tween 20 and filtered through cheesecloth. A conidial suspension of 2×10^4 conidia/ml was prepared and atomized onto the plants. The plants were incubated for 2 days under plastic hoods covered with cheesecloth with intermittent misting at 20/18°C and 16 h day length. After the incubation period, plants were kept in the greenhouse at 20/18°C and a light period of 16/8 h without misting until evaluation. Disease control was determined by estimating the percent chlorotic and necrotic symptoms 8 days after inoculation on the first secondary leaf.

Light microscope investigations. *Erysiphe graminis* f. sp. *tritici* infected leaf segments, approximately 5 mm long, were cleared by the vapor phase exchange method (5). The decolorized samples were either mounted on glass slides and stained with 0.05% Trypan Blue (Fluka, Buchs, Switzerland) in lactophenol/ethanol (1:2) by careful heating (determination of germination and mycelial growth) or they were immersed in 0.05% Trypan Blue in lactophenol/ethanol (1:2) and stained by boiling in a water bath for 2 min (determination of haustoria formation). They were mounted on glass slides as described above for evaluation. The mycelial circumference was measured with an image analyzing system (MOP Videoplan, Kontron, Munich) combined with an interference contrast microscope (Zeiss, Oberkochen, Germany). Disease control rating was performed as described above.

Leaves infected by *V. inaequalis* were cut into 5 × 5 mm leaf pieces and decolorized by the vapor phase exchange method (5). Cleared leaf segments were mounted on glass slides and stained with 0.5% Trypan Blue in lactophenol/ethanol (1:2) under careful heating to observe germination. To observe penetration, the Trypan Blue solution was removed by aspiration, replaced by 0.05% Coomassie Brilliant Blue R 250 (Fluka) in lactophenol/ethanol (1:2) and the samples were carefully heated. For the evaluation of subcuticular growth, the leaf segments were first boiled in 0.05% Trypan Blue in lactophenol/ethanol (1:2) for 1 min. Afterwards they were transferred into 0.05% Coomassie Brilliant Blue and boiled for 30 s. Leaf segments were mounted on glass slides as described above. The subcuticular stroma were measured with an image analyzing system (MOP Videoplan, Kontron) combined with an interference microscope (Zeiss). Disease control rating was carried out as described above.

Leaves infected by *D. teres* were cut into 5-mm-long segments and cleared in methanol for 2 h at room temperature. The leaf segments were transferred into 0.1% diethanol (Ciba-Geigy) in double-distilled water. After 10 min, leaf sections were mounted with 50% glycerol on glass slides and were evaluated with an epifluorescence microscope (Zeiss) equipped with the filter combination G 356/FT 395/LP 397 (Zeiss). The percent affected leaf area was determined as described above.

Three to four hundred conidia from four to six leaves per pathogen and treatment were evaluated to determine germination and penetration. The formation of primary haustoria of *E. g.* f. sp. *tritici* was evaluated at 400 infection sites with appressoria, and 200 infection sites with primary haustoria were checked to determine the number of secondary haustoria. The mycelial growth of *E. g.* f. sp. *tritici* and *V. inaequalis* was determined from 20 to 30 colonies per treatment.

Statistical analysis. Six to eight single plant replicates were evaluated per treatment and test. All experiments were conducted at least twice. Results of individual experiments are presented as mean values with standard deviation of the mean (±).

RESULTS

Disease control. Foliar infections of *E. g.* f. sp. *tritici* were completely controlled with 600 mg a.i./liter of cyprodinil applied

Table 1. Percent control of *Erysiphe graminis* f. sp. *tritici* with protective and curative spray applications of cyprodinil under controlled greenhouse conditions

			Percent control and treatment time[a]					
			Days prior to inoculation			Days after inoculation		
Compound	Dosage (mg a.i./liter)	Lesion site of action	14	7	1	1	3	5
Cyprodinil	60	Visible	25 ± 3	0	54 ± 4	53 ± 4	0	28 ±7
		Sporulating	25 ± 3	0	54 ± 4	74 ±4	85 ± 3	13 ± 4
	200	Visible	0	17 ± 2	85 ± 3	53 ± 4	0	34 ± 15
		Sporulating	0	17 ± 2	85 ± 3	100	100	59 ± 5
	600	Visible	8 ± 8	100	100	58 ± 7	4 ± 6	34 ± 8
		Sporulating	8 ± 8	100	100	100	100	92 ± 1
Fenpropimorph	60	Visible	32 ± 6	0	92 ± 2	0	0	25 ± 7
		Sporulating	32 ± 6	0	95 ± 0.6	100	98 ± 9	92 ± 1
	200	Visible	61 ± 9	75 ± 3	96 ± 0.8	53 ± 5	0	13 ± 6
		Sporulating	61 ± 9	75 ± 3	99 ± 0.4	100	99 ± 0.4	92 ± 1
	600	Visible	73 ± 9	100	98 ± 0	72 ± 3	0	25 ± 8
		Sporulating	83 ± 7	100	100	100	100	92 ± 1

[a] Disease rating was carried out 11 days after inoculation. Values are expressed as percent control ± standard deviation compared with check, which had approximately 70% visible lesions and sporulating lesions. Developmental stages of the fungus: 1 day after inoculation = penetration; 3 days = 3 to 5 haustoria; 5 days = beginning of sporulation.

Table 2. Percent control of *Venturia inaequalis* with protective and curative spray applications of cyprodinil under controlled greenhouse conditions

		Percent control of sporulating leaf area and treatment time[a]					
		Days prior to inoculation			Days after inoculation		
Compound	Dosage (mg a.i./liter)	6	4	2	1	3	5
Cyprodinil	5	25 ± 17	17 ± 12	2 ± 2	79 ± 6	52 ± 12	21 ± 6
	25	10 ± 9	36 ± 8	88 ± 4	100	99 ± 0.8	58 ± 9
	100	100	97 ± 1	100	100	100	52 ± 8
Difenoconazole	5	100	98 ± 0.8	100	100	100	26 ± 9
Flusilazole	5	82 ± 5	90 ± 6	90 ± 4	100	100	22 ± 5
Captan	600	46 ± 16	61 ± 14	80 ± 8	35 ± 13	7 ± 2	7 ± 3

[a] Disease rating was carried out 10 days after inoculation. Values are expressed as percent control ± standard deviation compared with check, which had approximately 71% sporulating leaf area. Developmental stages of the fungus: 1 day after inoculation = germination; 3 days = extension of subcuticular stroma approximately 630 µm; 5 days = circumference of subcuticular stroma >2,700 µm.

Table 3. Percent control of *Drechslera teres* with protective and curative spray applications of cyprodinil under controlled greenhouse conditions

		Percent control of affected leaf area and treatment time[a]						
		Days prior to inoculation				Days after inoculation		
Compound	Dosage (mg a.i./liter)	11	7	3	1	1	2	3
Cyprodinil	50	43 ± 3	63 ± 8	88 ± 0.2	89 ± 1	87 ± 0.9	85 ± 2	56 ± 11
	100	12 ± 5	77 ± 8	94 ± 0.7	91 ± 0.4	88 ± 0.8	86 ± 1	33 ± 10
	200	57 ± 2	90 ± 0.9	94 ± 0.8	89 ± 1	87 ± 1	81 ± 1	49 ± 11
Propiconazole	50	51 ± 4	85 ± 0.4	99 ± 0.2	97 ± 0.4	91 ± 0.2	76 ± 1	78 ± 2

[a] Disease rating was carried out 8 days after inoculation. Values are expressed as percent control ± standard deviation compared with check, which had approximately 54% affected leaf area. Developmental stages of the fungus: 1 day after inoculation = formation of primary vesicles; 2 and 3 days = mycelial growth, first symptoms visisble.

Table 4. Effect of cyprodinil in the infection cycle of *Erysiphe graminis* f. sp. *tritici* after protective and curative treatments

Application time	Dosage (mg a.i./liter)	Appressorium formation[a]	Formation of primary haustoria (%)[b]	Number of secondary haustoria[c]	Mycelial circumference (μm)[d]	Percent control[e]	
						Visible lesions	Sporulation
1-day protective	Check	93 ± 2	71 ± 4	23 ± 0.1	3,426 ± 277		
	60	93 ± 1	67 ± 3	0.4 ± 0.3	816 ± 62	0	35 ± 4
	200	91 ± 3	51 ± 3	0.3 ± 0.1	467 ± 26	56 ± 5	100
	600	84 ± 5	18 ± 6	0	534 ± 16	62 ± 4	100
10-h curative	Check	NE[f]	57 ± 8	>500	2,334 ± 257		
	60	NE	24 ± 5	0	339 ± 77	100	100
	200	NE	4 ± 1	0	0	100	100
	600	NE	0	0	0	100	100
2-day curative	Check	NE	NE	>500	>3,000[g]		
	60	NE	NE	7 ± 3	1,035 ± 51	0	94 ± 1
	200	NE	NE	6 ± 2	840 ± 76	0	100
	600	NE	NE	0	414 ± 42	0	100

[a] Percentage of infection sites evaluated; sampling time 24 h after inoculation.
[b] Relative to number of appressoria; sampling time 48 h after inoculation.
[c] Sampling time after protective application 4 days after inoculation; after curative application, 6 days after inoculation.
[d] Sampling time after protective application 5 days after inoculation; after curative application, 6 days after inoculation.
[e] The values are expressed as percent control ± standard deviation compared with check, which had approximately 35% visible lesions and sporulating lesions.
[f] Not evaluated.
[g] Circumference at time of application approximately 150 μm.

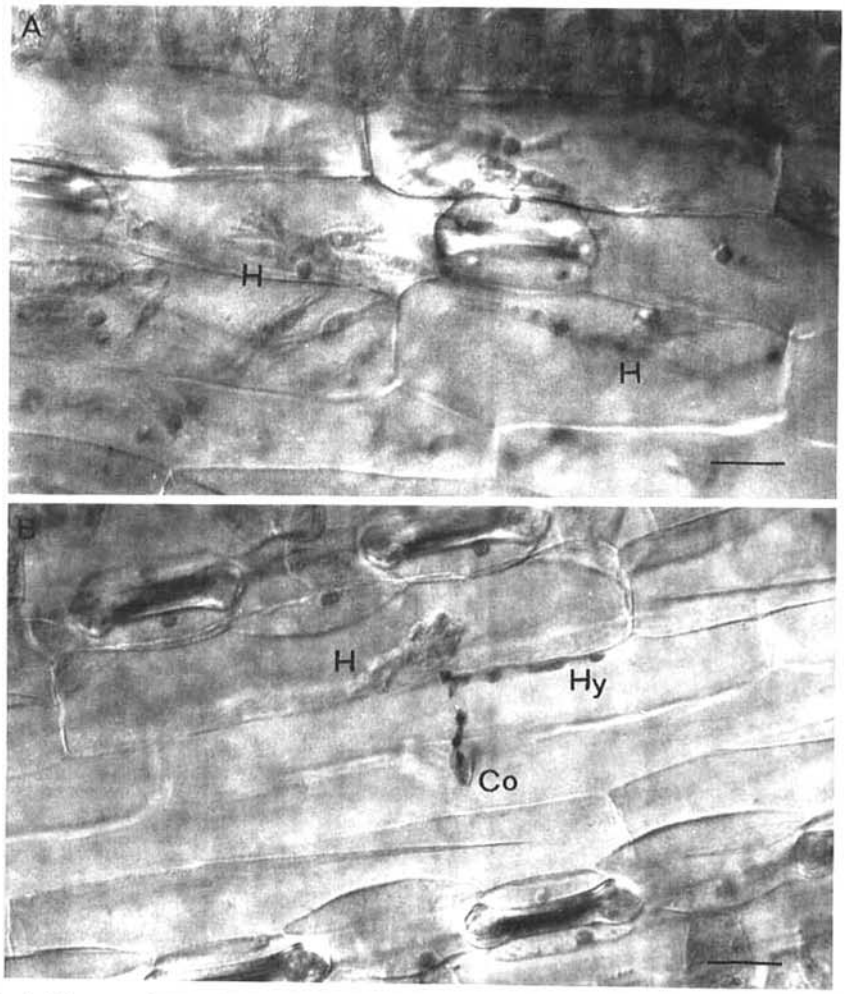

Fig. 1. Primary and secondary haustoria of *Erysiphe graminis* f. sp. *tritici* 5 days after inoculation. **(A)** An untreated plant following removal of the surface mycelium; H = haustoria. **(B)** A plant treated with cyprodinil (60 mg a.i./liter) 1 day before inoculation (bar represents 25 μm); H = haustorium, Co = conidia, Hy = hyphae.

up to 7 days before inoculation (Table 1). The standard fenpropimorph at the same rate provided approximately 70 and 100% activity in controlling symptom development with 14- and 7-day protective sprays, respectively. Neither compound completely prevented symptom development after curative applications; the treated plants showed chlorotic spots indicating that infections were established but no sporulation was observed. Symptom development was reduced to approximately 50 and 70% with 1-day curative application of cyprodinil and fenpropimorph at 600 mg a.i./liter. After 5-day curative treatment both compounds provided only 25 to 35% control of visible symptoms. However, cyprodinil at 600 mg a.i./liter reduced sporulation by more than 90% when applied up to 5 days after inoculation. Strong suppression of sporulation was also achieved with 60 or 200 mg a.i./liter of cyprodinil when applied 3 days after inoculation while only 600 mg a.i./liter of cyprodinil was highly effective after 5-day curative applications.

The protective and curative activity of cyprodinil at 100 mg a.i./liter against *V. inaequalis* was equally as good as difenoconazole at 5 mg a.i./liter within the period between 6 days before, and 3 days after inoculation, and superior to flusilazole (5 mg a.i./liter) in protective application schedules (Table 2). All three compounds lost activity in 5-day curative applications, although cyprodinil (25 and 100 mg a.i./liter) still provided 50% activity, in contrast to 5 mg a.i./liter difenoconazole and flusilazole, which lost more than 70% of their efficacy. The activity of cyprodinil at 100 mg a.i./liter was superior to captan at 600 mg a.i./liter, which provided acceptable disease control only in the 2-day protective application (Table 2).

Cyprodinil provided excellent control of *D. teres* lesion area at rates between 50 and 200 mg a.i./liter. At 200 mg a.i./liter the application time window extended from 7 days before to 2 days after inoculation (Table 3). Increasing the rate from 50 to 200 mg a.i./liter did not improve the performance of cyprodinil in curative trials, in contrast to protective applications in which 200 mg a.i./liter prolonged the effective application period from 3 days to 7 days. Similarly, the protective activity of propiconazole (50 mg a.i./liter) did not last

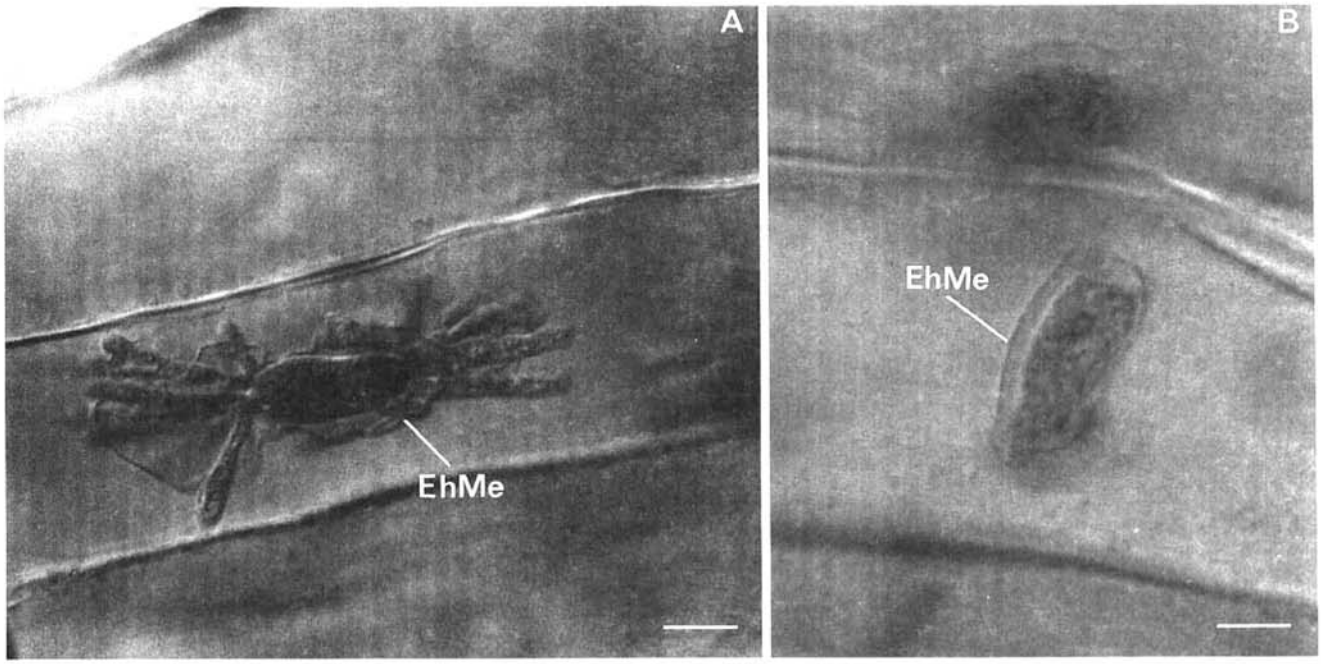

Fig. 2. Haustoria of *Erysiphe graminis* f. sp. *tritici* 6 days after inoculation. **(A)** An untreated plant (bar represents 10 µm); EhMe = extrahaustorial membrane. **(B)** A plant treated with cyprodinil (600 mg a.i./liter) 2 days after inoculation (bar represents 6.5 µm).

beyond the 7-day protective treatment. Neither cyprodinil nor propiconazole arrested the development of symptoms completely. In all cases, infections were arrested after small necrotic lesions appeared.

Site of action. Within the concentration range tested, germination and appressorium formation by *E. g.* f. sp. *tritici* conidia were not significantly reduced by cyprodinil (Table 4). However, the formation of primary and secondary haustoria was reduced. The application of 200 mg a.i./liter the day before inoculation reduced the number of primary haustoria to 70% of the check while a dosage of 600 mg a.i./liter inhibited primary haustoria formation to 25% (Fig. 1A, B). The formation of haustoria and the growth of the surface mycelium were completely inhibited with 10-h curative applications at 200 mg a.i./liter (Table 4). Treatment with 200 mg a.i./liter of cyprodinil 2 days after inoculation retarded colony growth and reduced the formation of secondary haustoria to approximately 1% of the check (Table 4). Morphological changes of the primary haustoria were visible after 1-day protective and 10-h curative applications. Applications at rates of 20 mg a.i./liter or greater resulted in incomplete lobe development at approximately 30% of the primary haustoria (Fig. 2A, B). Generally, the deformation was visible 3 to 4 days after inoculation and mycelia did not develop from these haustoria. After 2-day curative application, secondary haustoria also showed signs of deformation, which were visible 4 to 6 days after inoculation.

Cyprodinil did not reduce germination and only had a weak effect on the penetration of *V. inaequalis* germ tubes when applied 1 day before inoculation. One-day curative application of 370 mg a.i./liter had little influence on germination but reduced the penetration of the fungus by more than 50% (Table 5). However, the most obvious effect of cyprodinil was on growth of the subcuticular stroma after protective, as well as curative, applications (Table 5). Growth was reduced to a minimum with dosages as low as 23.1 mg a.i./liter and visible symptoms were completely suppressed after 1-day protective and 1-day curative treatment. After 3-day curative treatment the circumference of the stroma was significantly reduced but not sufficiently to suppress symptom development (Table 5).

Percentage of germination and penetration success of *D. teres* were not influenced by cyprodinil in the concentration range tested (up to 200 mg a.i./liter) with either protective or curative applications. Successful penetrations were indicated by the formation of primary vesicles in the epidermal host cells (Table 6). Primary lesions developed at the infection sites in spite of treatment with cyprodinil; however, a delay in their formation was observed (data not shown). The formation of the primary vesicles in the host epidermal cells coincided with a rapid necrosis of the penetrated cells. Approximately 90% of the penetration attempts were accompanied by formation of primary necrotic lesions on the check plants as well as on treated plants. The application of cyprodinil did not prevent initial lesion formation; however, the effect on further symptom development was obvious (Table 6).

DISCUSSION

Data on optimal timing indicate excellent protective and curative activity of cyprodinil against various pathogenic fungi. Timing experiments with *E. g.* f. sp. *tritici* showed that the greatest effect of cyprodinil was achieved with 600 mg a.i./liter applied between 7 days before, and 5 days after inoculation. *Drechslera teres* was controlled with cyprodinil at 200 mg a.i./liter within a period spanning from 7 days before inoculation to 2 days after inoculation. The rather short curative application period against *D. teres* may be explained by the rapid infection by the fungal strain in our greenhouse trial; first visible symptoms developed within 2 to 3 days after inoculation. Cyprodinil at 100 mg a.i./liter protected apple seedlings against *V. inaequalis* by applications within a period from 6 days before inoculation to 3 days after inoculation. When applied 1 day curatively, cyprodinil was comparable to the standards (rate for rate at 5 mg a.i./ liter). However, difenoconazole and flusilazole performed better when applied in curative applications after 1 day (rate for rate at 5 mg a.i./liter). When applied 3 days after inoculation, 100% control of *V. inaequalis* symptoms was achieved by increasing the rate of cyprodinil to 25 mg a.i./liter.. When applied 5 days curatively, the performance of cyprodinil was unsatisfactory. In contrast to difenoconazole (3) no activity was provided by cyprodinil when applied to sporulating scab lesions (data not shown). Thus, to assure good performance against apple scab, cyprodinil should be applied mainly in protective schedules. Cautious exploitation of the curative properties of cyprodinil is also advised as an antiresistance measure.

The light microscopy studies revealed a strong inhibitory effect on powdery mil-

Table 5. Effect of cyprodinil in the infection cycle of *Venturia inaequalis* after protective and curative treatments

Application time	Dosage (mg a.i./liter)	Germination (%)[a]	Penetration (%)[b]	Stroma circumference (µm)[c]	Percent control[d]	
					Visible lesions	Sporulation
1-day protective	Check	63 ± 3	63 ± 5	>2,700[e]		
	23.1	58 ± 3	49 ± 3	101 ± 15	100	100
	92.5	55 ± 3	42 ± 0.8	49 ± 3[f]	100	100
	370	61 ± 4	47 ± 4	45 ± 2[f]	100	100
1-day curative	Check	70 ± 2	79 ± 3	>2,700[e,f]		
	23.1	62 ± 4	71 ± 4	50 ± 3	100	100
	92.5	51 ± 5	41 ± 4	36 ± 1[f]	100	100
	370	57 ± 4	32 ± 4	40 ± 2[f]	100	100
3-day curative	Check	NE[g]	NE	>2,700[e,h]		
	23.1	NE	NE	5,145 ± 253	14 ± 11	100
	92.5	NE	NE	2,816 ± 120	1 ± 2	100
	370	NE	NE	1,530 ± 430	24 ± 6	100

[a] Sampling time 2 days after inoculation.
[b] Relative to number of germinated conidia; sampling time 4 days after inoculation.
[c] Sampling time 10 days after inoculation.
[d] Disease rating was carried out 14 days after inoculation. Values are expressed as percent control ± standard deviation compared with check, which had 86% affected leaf area.
[e] Check only measurable up to 4 days after inoculation.
[f] Growth of the stroma already stopped 4 days after inoculation.
[g] Not evaluated.
[h] Stroma circumference at date of application approximately 630 µm.

Table 6. Effect of cyprodinil in the infection cycle of *Drechslera teres* after protective and curative treatments

Application time	Dosage (mg a.i./liter)	Germination (%)[a]	Primary vesicle formation (%)[b]	Percent control[c]
3-day protective	Check	92 ± 3	63 ± 1	
	12.5	90 ± 4	67 ± 3	80 ± 3
	50	88 ± 3	60 ± 4	77 ± 3
	200	87 ± 4	60 ± 3	90 ± 1
1-day curative	Check	88 ± 3	62 ± 5	
	12.5	89 ± 5	64 ± 2	78 ± 3
	50	85 ± 3	57 ± 3	90 ±2
	200	88 ± 4	70 ± 3	95 ± 1

[a] Sampling time after protective treatment 24 h after inoculation; after curative treatment 48 h after inoculation.
[b] Relative to the number of germinated conidia; sampling time 4 days after inoculation.
[c] Values are expressed as percent control ± standard deviation compared with check, which had 95% affected leaf area.

dew haustoria, especially on the formation of secondary haustoria. In addition, disorganized primary haustoria were found. The growth of conidia-producing mycelium is to a great extent dependent on functional haustoria that serve as a nutrient supply for the fungus (2). Consequently, a decrease in the number of functional haustoria lacking lobes should result in a reduction of mycelial growth, and, to a much greater extent, in a decrease in sporulation. Following protective and curative applications, the subcuticular stroma of *V. inaequalis* was found to be the main target site of cyprodinil, resulting in a reduction of sporulating leaf area. The intra- and intercellular growth of *D. teres* was impossible to quantify by light microscopy because the proceeding necrosis of the tissue resulted in a dense network of necrotized epidermal and parenchymal host cells (8). However, because there were no indications of an early effect of cyprodinil on *D. teres* infection stages, and based on its high efficacy against the disease symptoms, we assume that cyprodinil acts mainly on the expansion of the mycelium in the host tissue.

Cyprodinil was shown to act after germination of the fungi. Although pathogen dependent, a moderate effect on the penetration process was observed. However, the major target sites of cyprodinil were inter- and intracellular infection stages. This indicates that the antifungal activity is based on systemic activity, which explains the curative effect of cyprodinil. A similar situation occurs with the highly systemic sterol demethylase inhibitors acting very specifically on the haustoria formation of *E. g.* f. sp. *tritici* and inducing more or less strong morphological changes in the haustorial structure (16,17). Furthermore, cyprodinil seems to be taken up rapidly in order to reach the major target sites, because it is also effective after curative applications against pathogens with rather short infection cycles. Even though the *D. teres* strain used in our study was able to establish infection on barley very rapidly (first visible symptoms 2 days after inoculation), the 1- to 2-day curative application of cyprodinil restricted the fungal growth to a minimum.

Our findings are in part contradictory to results reported for the chemically related pyrimethanil, which was shown to act on germ tube extension and penetration of *Botrytis* spp. and reduce the number of dead epidermal cells (13,15). Necrotization of host cells generally coincides with the growth of *Botrytis* in the host tissue. Therefore, the reduction of the number of dead cells may indicate a reduction of intercellular fungal growth. A reduction of growth of the subcuticular stroma was reported to be the major target site in *Venturia inaequalis* after curative pyrimethanil sprays (4). Effects of cyprodinil on later infection stages were confirmed in various pathosystems but the effect of cyprodinil on penetration was less pronounced. However, one cannot exclude the possibility that in addition to the basic mechanism described above there is a specific activity on germ tube extension and penetration of *Botrytis*.

Cyprodinil is a fungicide with a new mode of action providing excellent field performance against a broad spectrum of diseases (1,6,7). Excellent protective and some curative activity was demonstrated against various diseases under greenhouse conditions thus underscoring a certain flexibility in application timing of this compound.

ACKNOWLEDGMENTS

Many thanks for the technical assistance of L. Probst, M. Schroth ,and M. Theiler. Thanks also to Jim Ligon for critically reviewing the manuscript.

LITERATURE CITED

1. Bocquet, G., Sylvestre, M., and Speich, J. 1994. Le cyprodinil, fongicide cereales. Phytoma - La Défense des végétaux 458:53-55.
2. Bushnell, W. R. 1972. Physiology of fungal haustoria. Ann. Rev. Phytopathol. 10:151-176.
3. Dahmen, H., and Staub, T. Protective, curative, and eradicant activity of difenoconazole against *Venturia inaequalis*, *Cercospora arachidicola,* and *Alternaria solani*. Plant Dis. 76:774-777.
4. Daniels, A., Birchmore, R. J., and Winter, E. H. 1994. Activity of pyrimethanil on *Venturia*

inaequalis. Proc. Brighton Crop Prot. Conf. 2: 525-532.
5. Gerlach, D. 1969. Botanische Mikrotechnik. Georg Thieme Verlag, Stuttgart.
6. Heye, U. J., Speich, J., Siegle, H., Steinemann, A., Forster, B., Knauf-Beiter, G., Herzog, J., and Hubele, A. 1994. CGA 219417 - a novel broad spectrum fungicide. Crop Prot. 13:541-549.
7. Heye, U. J., Speich, J., Siegle, H., Wohlhauser, R., and Hubele, A. 1994. CGA 219417 - a novel broad spectrum fungicide. Proc. Brighton Crop Prot. Conf. 2:501-508.
8. Keon, J. P. R., and Hargreaves, J. A. 1983. A cytological study of the net blotch disease of barley caused by *Pyrenophora teres*. Physiol. Plant Pathol. 22:321-329.
9. Leadbeater, A. J., Speich, J., Knauf-Beiter, G., Kühl, A., and Rambach, O. 1994. CGA 219417 - disease control in Western Europe. Proc. Brighton Crop Prot. Conf. 1:333-338.
10. Leadbitter, N. J., Steck, B., Frank, L. R., and Leadbeater, A. J. 1994. CGA 219417: A novel fungicide for the control of *Pyrenophora* spp. on barley. Br. Crop Prot. Council (BCPC) Monog. 57:73-78.
11. Maeno, S., Miura, I., Masuda, I., and Nagata, T. 1990. Mepanipyrim (KIF-3535), a new pyrimidine fungicide. Proc. Brighton Crop Prot. Conf. 2:415-422.
12. Masner P., Muster, P., and Schmid, J. 1994. Possible methionine biosynthesis inhibition by pyrimidineamine fungicides. Pestic. Sci. 42:163-166.
13. Milling, R. J., Richardson, C. J., and Daniels, A. 1993. Pyrimethanil inhibits hydrolytic enzyme secretion by *Botrytis* spp. and prevents lysis of host cells. (Abstr. 3.7.15) Int. Plant Cong. Plant Pathol., 6th.
14. Miura, I., Kamakura, T., Maeno, S., Hayashi, S., and Yamaguchi, I. 1994. Inhibition of enzyme secretion in plant pathogens by mepanipyrim, a novel fungicide. Pestic. Biochem. Physiol. 48, 222-228.
15. Neumann, G. L., and Winter, E. H. 1992. Pyrimethanil: A new fungicide. Proc. Brighton Crop Prot. Conf. 1:395-402.
16. Smolka, S., and Wolf, G. 1986. Cytological studies on the mode of action of systemic fungicides on the host pathogen complex barley-powdery mildew (*Erysiphe graminis* f. sp. *hordei* Marchal). Pestic. Sci. 17:249-255.
17. Staub, T., and Dahmen, H. 1984. Effect of propiconazole and etaconazole on the infection cycles of their target fungi. Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR 222:75-83.