Control of *Cladosporium caryigenum* on Pecan Leaves and Nut Shucks with Propiconazole (CGA-64250)

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ABSTRACT

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After-infection control of *Cladosporium caryigenum* on pecan (*Carya illinoinensis*) was obtained for 5 days on leaves sprayed to runoff with 270 mg propiconazole per liter of water. Application of propiconazole 6 or 8 days after inoculation resulted in survival of *C. caryigenum* 3 or 23% as great, respectively, as on lesions of unsprayed leaves. Development of scab lesions on nut shucks inoculated at intervals after propiconazole treatment indicated fungicide deterioration was significant between 15 and 18 days. Propiconazole was not translocated from one leaflet to another in sufficient quantity to control scab.

Scab caused by *Cladosporium caryigenum* (Ell. et Lang.) Gottwald (=*Fusicladium effusum*) (2) is the most important disease of pecans (*Carya illinoinensis* (Wang.) C. Koch) in humid areas such as the southeastern United States. Growers must apply a protectant fungicide such as triphenyltin hydroxide (TPTH, Du-Ter 47.5WP) (1,9) from April through August for control of scab. Excellent control has been reported for propiconazole (CGA-64250, Tilt 3.6EC) used in a protectant application schedule (11,12).

These investigations were conducted with propiconazole to better define the protective activity and the period of time fungicide treatment is effective after infection.

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MATERIALS AND METHODS

Three-year-old Schley pecan trees were maintained at a shoot-tip height of 40–50 cm above the soil line in the greenhouse in 12.3-L pails. Conidia of *C. caryigenum* were washed from scabby pecan leaves of these plants and, unless stated otherwise, adjusted with a hemacytometer to a concentration of 3.2×10^6 conidia per milliliter of water. Three drops of Tween 20 were added per liter and the conidial suspension was sprayed to runoff on the leaves of test plants. Immediately after inoculation, the pecan trees were placed in a Percival Dew Chamber (Percival Mfg. Co., Boone, IA 50036) programmed to 25 C and 100% relative humidity for 12 hr (8) with no lights. The time of inoculation, ie, beginning of the wet period, was used as time zero for subsequent fungicide application schedules (15). All propiconazole applications were sprayed to runoff with a Handi-Sprayer (Voluntary Purchasing Groups, Inc., Bonham, TX 75418) using a solution containing 270 mg propiconazole per liter of water.

For identification, compound leaves of test plants were assigned numbers, one corresponding to the youngest (terminal) leaf at the time of treatment. Leaflets of a compound leaf were identified by numbers followed by lowercase letters (a or b), starting with the basal (most mature) leaflets. The letters identified the positions of opposite leaflets at each numerical position.

Systemic activity. A suspension of *C. caryigenum* was sprayed on leaves of potted Schley pecan trees with leaves in the yellow-green stage of growth (susceptible to infection). Immediately after inoculation, the trees were placed in a dew chamber for 12 hr of incubation, then returned to the greenhouse. At 24, 72, and 120 hr after inoculation, a plastic envelope (3 × 8 cm) was placed around a lateral leaflet on a compound leaf and sealed with cellophane tape. On another compound leaf, the three terminal leaflets were enclosed in a plastic bag sealed around the rachis of the compound leaf. The exposed leaves on the shoot were sprayed with propiconazole solution, using four one-tree replicates for each time interval. Similar treatments using sterile tap water instead of propiconazole served as controls. After treatment, the trees were returned to the greenhouse and the plastic envelopes or bags were removed after 12 hr. At 30 days after inoculation, lesions on each leaflet were counted.

All inoculated leaves were scored as follows: 1 = stroma discernible by reflected light, no spores; 2 = pale brown lesions <1 mm diameter, some spores; 3 = lesions moderately developed, brown to black, 1–1.5 mm diameter, spores abundant; 4 = lesions well developed, purple-black, 1.6–3 mm diameter; 5 = large spreading lesion >3 mm diameter; 6 = fungicide-treated leaves free of disease symptoms; and 7 = tissue collapsed, shiny dark brown fleck.

After-infection control. After inoculation and a 12-hr infection period as described, plants were maintained on the laboratory bench with a 12-hr photoperiod at 21 C. The fourth compound leaf from the top of the shoot of each tree was covered with a plastic bag, which was sealed around the rachis. The three younger compound leaves were sprayed with propiconazole 48, 96, 144, or 192 hr after inoculation. Six trees were sprayed after each incubation time and six control trees were sprayed with sterile tap water. Subsequently, the trees were held for 30 days in the greenhouse and lesions on each leaflet were counted.

Viability of *C. caryigenum*. Potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI 48232) was modified by adding 10 g agar per liter of medium. After the sterilized medium had cooled to 50 C, 2.8 ml concentrated lactic acid per liter was added; final pH was 3.6. Pecan leaflets treated with propiconazole or water were surface-sterilized in 0.525% sodium hypochlorite for 5 min, rinsed six times with sterile water, and embedded in PDA in petri dishes. Leaflets were incubated for 8 days at 24 C, at which time small black colonies of *C. caryigenum* growing from the leaves were recorded. Sporulation of *C. caryigenum* was verified after 6 wk.

Protectant activity in leaves. The fourth leaf from the top of the shoot on each of 18 pecan trees was covered with a plastic bag and sealed around the rachis. The youngest three leaves of 12 trees were sprayed with propiconazole; the remaining six trees were sprayed with sterile tap water. After drying, the plants were placed on a laboratory bench at 21 C. The plastic bags were removed 6 hr after spraying. At 48 hr after spraying, a suspension of *C. caryigenum* was applied to six trees sprayed with propiconazole and to six sprayed with water. The remaining trees were inoculated after 144 hr. After a 12-hr infection period in the dew chamber, the trees were moved to a greenhouse. Thirty days later, lesions on each leaflet were counted. Lesion numbers on leaves inoculated 144 hr after propiconazole treatment were analyzed statistically.

Protectant activity in nut shucks. Nut clusters on pecan trees of the USDA selection 56-10-6 were labeled to identify treatments. To prevent infection before treatment with propiconazole, 0.35 g TPTH per liter of water was applied to the nut clusters with an AHB sprayer (American Hardware Buyers, manufactured by R. E. Chapin, Mfg., Batavia, NY 14020) on 5 and 19 May and 2 June. This procedure was similar to that of Gottwald and Bertrand (3), who maintained scabfree nuts with 0.79 g TPTH per liter applied at 2-wk intervals; after two more weeks, they inoculated pecan nut clusters with *C. caryigenum*. The rate of TPTH used in our tests is minimal for scab control on established cultivars on a 21-day schedule in Alabama; therefore, control on the USDA 56-10-6, a highly scab-susceptible selection, would be inadequate. Also, aqueous TPTH was readily degraded when exposed to sunlight (14).

On 21 June, propiconazole was applied to all nut clusters. Fifteen days later (12 July), 10 nut clusters were inoculated with 2.8×10^6 conidia of *C. caryigenum* per milliliter of sterile water. Immediately after inoculation, a plastic bag containing a 3-cm^3 sponge saturated with sterile water to promote high humidity was secured around each cluster; the bag was removed after 3 days. This inoculation procedure was repeated on newly selected clusters every 3 days for 30 days. Lesions were counted when they became macroscopic.

RESULTS

Systemic activity. Lesions caused by *C. caryigenum* developed on lateral and terminal leaflets that were covered with plastic when the other leaflets on the compound leaves were sprayed with propiconazole (Table 1). At the rate tested, translocation of toxic quantities of propiconazole from treated leaflets through the compound leaf rachis or leaflet petioles to covered leaflets did not occur. Activity of propiconazole after infection had occurred was evidenced by necrotic flecks where incipient infections by *C. caryigenum* were stopped in leaves sprayed 72 to 120 hr after inoculation. Reductions in lesion numbers of control leaves at position 4A from 233 at 24 hr to 89 at 120 hr possibly reflects the development of scab resistance with leaflet maturation. Because basal leaflets mature before terminal ones, the latter were still highly susceptible on the fifth

Table 1. Effects of propiconazole on *Cladosporium caryigenum* in pecan leaflets

Fungicide application after inoculation (hr)	Disease severity on lateral leaflets[x]						Disease severity on terminal leaflets[x]				
	3a[y]	b	4a	b	5a	b	4a	b	5a	b	Term.
	F[z]	F	NF	F	F	F	F	F	NF	NF	NF
24	0 (6)	0 (6)	233 (1.8)	0 (6)	0 (6)	0 (6)	0 (6)	0 (6)	148 (2.4)	178 (2.4)	195 (2.6)
72	0 (6)	0 (6)	146 (2)	0 (6)	0 (6)	0 (6)	0 (6)	0 (6)	155 (3.3)	136 (3.3)	142 (3.6)
120	80 (7)	99 (7)	89 (2.4)	66 (7)	19 (7)	18 (7)	178 (7)	150 (7)	160 (3.8)	158 (3.8)	136 (3.8)

[x] Mean number and type rating (in parentheses) of lesions counted in four leaflets. Rating key: 1 = stroma discernible by reflected light, no spores; 2 = pale brown lesions <1.0 mm diameter, some spores; 3 = lesions moderately developed, brown to black, 1–1.5 mm diameter; 4 = lesions well developed, purple-black, 1.6–3 mm diameter; 5 = large spreading lesion >3 mm diameter; 6 = fungicide-treated leaves free of disease symptoms; and 7 = tissue collapsed, shiny dark brown fleck.

[y] Leaflets numbered from base of compound leaf; a or b indicates position of opposite leaflets; Term. = terminal leaflet.

[z] F = propiconazole, 270 mg/L; NF = no fungicide; leaves infected with *C. caryigenum* were covered with plastic when propiconazole was applied.

Table 2. Control of *Cladosporium caryigenum* by propiconazole applied 2–8 days after inoculation

Leaf treatment[a]	Leaf no. from top of shoot	Check	Disease severity[b] on lateral leaflets treated after				Colonies in PDA on leaflets[c] from postinoculation treatments		
			2 days	4 days	6 days	8 days	4-day	6-day	8-day
Covered	4	46.4 (2.5)	55.2 (2.6)	53.4 (2.6)	67.1 (2.4)	57.8 (2.9)	43.3	53.3	53.9
Uncovered	3	57.1 (2.6)	0 (6)	22.2 (7)	51.4 (7)	57.9 (7)	0	1.5	0.8
Uncovered	2	48.1 (3.0)	0 (6)	26.0 (7)	55.8 (7)	55.7 (7)	0	2.3	19.8
Uncovered	1	35.0 (2.8)	0 (6)	17.6 (7)	35.6 (7)	38.4 (7)	0	0.5	11.7
Mean of leaves 1–3		46.7	...	...	...	...	0	1.4	10.8

[a] Leaves inoculated with 3.2×10^6 *C. caryigenum* conidia per milliliter, then covered with plastic when propiconazole was applied (or uncovered).
[b] Mean number and type rating (in parentheses) of lesions counted on 42 leaflets. Rating key: 1 = stroma discernible by reflected light, no spores; 2 = pale brown lesions < 1.0 mm diameter, some spores; 3 = lesions moderately developed, brown to black, 1–1.5 mm diameter; 4 = lesions well developed, purple-black, 1.6–3 mm diameter; 5 = large spreading lesion >3 mm diameter; 6 = fungicide-treated leaves free of disease symptoms; and 7 = tissue collapsed, shiny dark brown fleck.
[c] Mean number of *C. caryigenum* colonies from 30 leaflets in potato-dextrose agar.

Table 3. Protectant activity of propiconazole against infection of pecan leaves by *Cladosporium caryigenum*

Treatment	Leaf no. from top of shoot	Number of lesions[y] Time from treatment to inoculation		
		48 Hr	144 Hr	Control
Water	4	103.3 a[z]	71.6 b	113.7 a
Propiconazole, 270 mg/L	3	0 b	0 b	106.3 a
Propiconazole, 270 mg/L	2	0 b	0 b	89.0 a
Propiconazole, 270 mg/L	1	0 b	0 b	49.1 a

[y] Values are mean number of lesions on seven leaflets on each of six plants.
[z] Means followed by the same letter in a row are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

day (Table 1).

After-infection control. The fourth leaf from the top of the pecan shoot served as a scab-susceptibility indicator for inoculated leaves (Table 2). The slightly reduced average number of lesions in the fourth leaf of check plants in relation to the third leaf indicated greater maturity and reduced susceptibility of the fourth leaves. Lesion numbers on the fourth leaf of all treatments indicated the amount of scab that could have occurred without propiconazole treatment. Control of *C. caryigenum* on leaves treated 2, 4, 6, and 8 days after infection compared with check leaves 1, 2, and 3 show the excellent control obtained with propiconazole. Leaves treated with propiconazole 2 days after inoculation remained free of symptoms. Observations of leaf scab control 30 days after treatment showed that propiconazole applications made 4 days after inoculation stopped incipient growth of *C. caryigenum*, leaving flecks that were discernible with a stereomicroscope and rated 7 (Table 2). Similar observations of fungicide applied after 6 or 8 days of incubation revealed a leaf spot or fleck distinguishable without a microscope.

Viability of *C. caryigenum*. Small black colonies of *C. caryigenum* developed within 8 days in PDA from leaves sprayed with sterile water. No colonies developed from leaves treated with fungicide 4 days after infection. Leaflets sprayed with propiconazole 6 and 8 days after inoculation yielded averages of 1.4 and 10.8 colonies, respectively, compared with 46.7 colonies for the untreated control (Table 2). *C. caryigenum* produced typical conidia on these colonies.

Protectant activity in leaves. Propiconazole protected pecan leaves from infection by *C. caryigenum* when inoculations were made 2 and 6 days after treatment (Table 3). Although plants for the 6-day treatment were held at 21 C during that period to suppress leaf maturity, significantly fewer lesions developed in the fourth leaves. This indicates that leaves can lose susceptibility rapidly and suggests the need for internal susceptibility controls. The low number of lesions on leaf 1 compared with leaf 2 or 3 reflects the small surface area at the time of inoculation.

Protectant activity in nut shucks. Significantly fewer lesions developed after inoculation at 15 days after propiconazole treatment than at 18 or 21 days posttreatment (Table 4). This indicated that fungitoxicity of the propiconazole (single application) had deteriorated by the 18th day. When lesions were counted on 25 July, nut shucks that had received no fungicide had an average of 194 lesions.

DISCUSSION

Management of plant diseases by applying protective fungicides according to prescriptions based on meteorological measurements has received much attention (5,7). Disease control may also be obtained by applying a fungicide after significant spore dispersal and subsequent infection have occurred. The major drawback for the successful implementation of such a system has been the limited availability of fungicides with curative properties for after-infection use. Kelley and Jones (6) showed that CGA-64251 was effective for 96 hr in after-infection control of *Venturia inaequalis* in apple leaves. Early testing of CGA-64251 against *C. caryigenum* resulted in erratic control (10). However, propiconazole, an analog of CGA-64251, provided 100% control when used at rates of 247 g/ha (11,12). Systemic acropetal movement of propiconazole has been reported in peanuts, soybeans, and wheat (4,16). The after-infection activity of propiconazole in peanuts against *Cercospora arachidicola* and *Cercosporidium personatum* has been reported (13) as has curative activity in wheat against *Puccinia graminis* (16).

Our investigations have shown that the activity of propiconazole in pecans when applied to foliage is of a local nature. Either the fungicide did not move from one leaflet to another or did so in insufficent quantity to be fungitoxic.

The curative activity of propiconazole was absolute when treatment was made within 2 days of inoculation. We did not observe either microscopic or macroscopic symptoms of disease for the 2-day postinfection treatment. When propiconazole treatment was delayed 4 days after inoculation, flecks developed in numbers about 50% as great as control lesions. Flecks could be seen only with the aid of a stereomicroscope, and subsequent incubation of treated leaves in PDA failed to produce colonies. When propiconazole treatment was delayed 6 or 8 days after inoculation, a shiny dark brown fleck appeared, apparently caused by *C. caryigenum*. Based on the numbers of lesions on unsprayed check leaves, only 3% of *C. caryigenum* infections survived treatment 6 days and 23% survived treatment 8 days after infection. Transfer of the 6- and 8-day postinfection propiconazole-treated leaflets to PDA resulted in colonies with typical conidia.

Propiconazole provided good protection of leaves when applied 5 days after inoculation. Because maturing leaves

Table 4. Propiconazole activity against *Cladosporium caryigenum* on nut shucks[x] of USDA selection 56-10-6

Inoculation[y] (days posttreatment)	Lesions on nut shucks[z]
15	9.25 a
18	13.83 b
21	43.03 c

[x]Shucks sprayed with propiconazole at 270 mg/L on 21 June.
[y]Inoculation by spraying shucks with 2.8×10^6 *C. caryigenum* conidia per milliliter.
[z]Means from 40 nut shucks. Means followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

rapidly develop resistance to *C. caryigenum*, investigations to further define the length of propiconazole protectant activity were conducted on pecan nut shucks, which have an extended period of susceptibility. Because *C. caryigenum* had been controlled with propiconazole on a 21-day schedule (11,12), these investigations were started 15 days after treatment. Lesion counts made after the 15-day treatment indicated propiconazole protection had begun to break down. Both the 18- and 21-day inoculations resulted in significantly more scab lesions than inoculations 15 days after fungicide treatment did.

Development of propiconazole is significant for control of pecan scab and should allow growers to make fungicide applications after leaf wetness or nut-shuck wetness conditions have occurred. Thus, improved disease management procedures should be possible when propiconazole is registered for use on pecans.

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