

Effects of Propiconazole on *Exserohilum turcicum* in Laboratory and Field Studies

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ABSTRACT

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Propiconazole inhibited mycelial growth of *Exserohilum turcicum*, the causal organism of northern leaf blight, but did not inhibit conidial germination. Conversely, mancozeb inhibited only conidial germination. In greenhouse experiments, lesion numbers on plants and rates of lesion expansion were inversely proportional to the rate of propiconazole application. Propiconazole slowed lesion expansion for 17 days, and reduced the rate of disease development in the field when applied at 3-wk intervals, relative to weekly applications of mancozeb.

Systemic fungicides are absorbed into and translocated short distances through a plant (7). These fungicides can eradicate pathogens shortly after infection, are

less subject to weathering, and may need to be reapplied less frequently than some protectant fungicides (7). The systemic fungicide propiconazole (Tilt 3.6 EC, Ciba-Geigy, Greensboro, NC) inhibits sterol-biosynthesis and is effective against pathogens causing diseases such as rusts of wheat, scab of pecan, and powdery mildew on apple (3,4,11,17). Excellent control of southern and northern leaf blights of corn (*Zea mays* L.) also has been reported with propiconazole used in a protectant application schedule (18).

Northern leaf blight (NLB), caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs, is an important disease of corn,

causing as much as 40% loss in susceptible hybrids (13). Although sweet corn and dent corn inbreds are regularly sprayed with protectant fungicides (6), NLB is usually not controlled with fungicides in hybrid corn crops because of low crop value relative to high application costs. Most effective control of NLB with protectant fungicides such as maneb and mancozeb is achieved when the fungicide is applied as soon as disease is observed and reapplied every 4-7 days. A systemic fungicide that is effective against NLB for a longer period of time than maneb or mancozeb, and is not phytotoxic to corn, could reduce application costs and provide corn producers with a more economical option for controlling NLB.

The objective of this study was to define the activity and period of effectiveness of propiconazole against *E. turcicum* on corn. Preliminary results have been published (2).

MATERIALS AND METHODS

Fungicidal activity in vitro. The fungicidal activity of propiconazole

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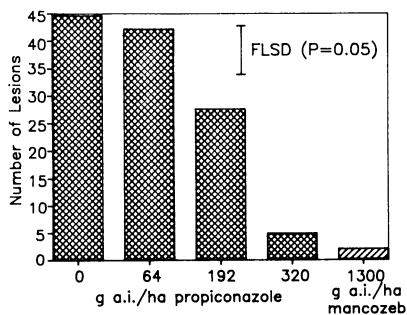


Fig. 1. Effects of propiconazole on numbers of northern leaf blight lesions when the fungicide was applied at 24 hr prior to inoculation with *Exserohilum turcicum* (lesion counts made at 20 days postinoculation).

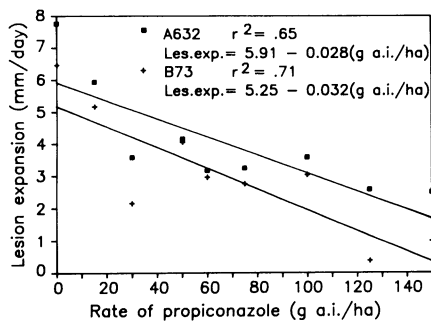


Fig. 2. Effects of propiconazole on rates of northern leaf blight lesion expansion when the fungicide was applied at 24 hr after inoculation with *Exserohilum turcicum*.

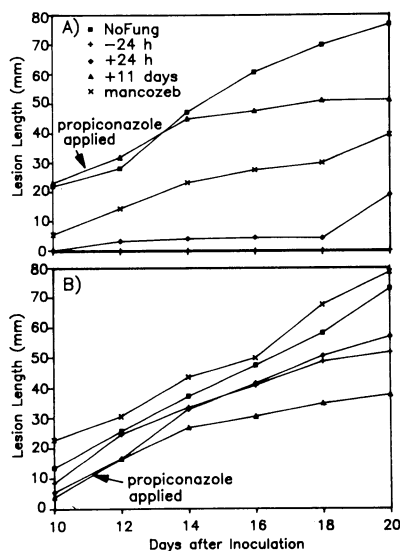


Fig. 3. Effects of timing of propiconazole application (60 g a.i./ha), relative to inoculation with *Exserohilum turcicum*, on development of northern leaf blight lesions on corn inbreds (A) A632, and (B) B73. Plants were grown in the greenhouse. Mancozeb (1.3 kg a.i./ha) treatment was made 24 hr after inoculation.

against *E. turcicum*, relative to the activity of mancozeb, was determined by incorporating each of the fungicides into lactose casein hydrosylate agar (LCA) and observing conidial germination and colony growth. All treatments consisted of five replicated plates, and the experiment was repeated twice. Fungicides were added to tempered LCA and approximately 25 ml of amended agar were poured into 100 × 15 mm petri plates. Fungicide amendments were: mancozeb (Dithane M-45, Rohm & Haas, Philadelphia, PA) at 30 μg a.i./ml agar and propiconazole at 0.001, 0.01, 0.1, 1.0, and 10 μg a.i./ml. Conidial germination was determined by transferring five conidia from sporulating leaf tissue to petri plates containing amended LCA. Conidia were visually studied daily for 10 days. Colony growth was assessed by removing agar plugs (5 mm diameter) from the margins of 10-day-old *E. turcicum* colonies growing on unamended LCA, and inverting plugs onto amended agar plates. Colony diameter was measured daily for 10 days.

Effect of propiconazole on lesion establishment. The corn inbred, A632, was grown in the greenhouse in 5-cm plastic cones for 2 wk, then fungicides were applied in 234 L/ha water, using a motor-driven spray boom. Fungicide treatments were: mancozeb at 1.3 kg a.i./ha and propiconazole at 0 (water control), 64, 192, and 320 g a.i./ha (manufacturer's suggested rate is 64 g a.i./ha). Treatments were applied to three replicate plants. Twenty-four hr after fungicide treatment, plants were inoculated by atomizing them with 75 ml of a conidial suspension containing approximately 10,000 conidia/ml to which 0.05 ml of a spreader-binder (Triton CS-7, Rohm & Haas) was added per 20 ml suspension. Plants were allowed to dry and then placed in a mist chamber at 100% humidity for 16 hr. Numbers of lesions were determined every 2 days from 10 to 20 days after inoculation (DAI) and averaged over replications.

Effect of propiconazole on lesion expansion. Plants of two corn inbreds, A632 and B73, were grown as described above and then inoculated with micro-drops of conidial suspensions (12 containing 200 conidia/20 μl drop (10,000 conidia/ml). Two replicate plants were sprayed with 0, 15, 30, 50, 60, 75, 100, 125, or 150 g a.i./ha propiconazole 24 hr after inoculation. In a second experiment, plants were inoculated as previously described and treated with 0, 30, 60, 120, or 240 g a.i./ha propiconazole after 24 hr. In both experiments, two or three lesions per plant were marked and measured at 2-day intervals, starting 8 DAI and continuing through 20 DAI. Lesion growth rates per day were calculated and averaged over replications. Regression models were developed with

lesion growth rate dependent on the rate of propiconazole application.

Duration of propiconazole effectiveness. B73 and A632 plants were grown and inoculated with micro-drops of conidial suspensions, as described above, and treated with water as a control; mancozeb (1.3 kg a.i./ha) 24 hr after inoculation; or propiconazole (60 g a.i./ha) 24 hr before, 24 hr after, and 11 days after inoculation. Each treatment consisted of four replicate plants. Lesion numbers and growth rates were evaluated as previously described.

Propiconazole effects on sporulation. Twenty days after treatment with mancozeb (1.3 kg a.i./ha), propiconazole (60 g a.i./ha), or water (control), leaf pieces with NLB lesions were removed from A632 plants. Leaves were pressed and dried for 7 days. Leaf tissue was cut to a uniform size (1 × 1 cm²), each with half necrotic/lesion area and half green leaf tissue, and incubated in a petri plate on moistened filter paper. *E. turcicum* conidial production was assessed by vortexing each piece in 5 ml of sterile water with 0.05 ml of spreader-binder. Five 20-μl samples were taken from each of three replicates and conidia were counted from different leaf pieces after 36 and 48 hr of incubation.

Field studies. Field experiments were conducted at the Agronomy and Plant Pathology South Farm in Urbana and at the Northwestern Research Center near Monmouth, IL, in 1985 and 1986. The experimental design was a randomized complete block with fungicidal treatments on each of two inbreds, A632 and B73. A632 was very susceptible to NLB and had an early maturity relative to B73. Treatments were replicated four times in each year. Plots were 4.6 m long at Urbana and 13.7 m long at Monmouth, and were four rows (76 cm apart) in width. Plots were planted between 29 April and 4 May at 74,000 plants/ha in Urbana and 52,000 plants/ha in Monmouth, and thinned to 52,000 plants/ha 1 mo after emergence at Urbana.

The two center rows of plots were inoculated with leaf tissue collected from NLB-diseased plants grown in the preceding year. Approximately 1.1 g (50 cc) of dried, ground leaf tissue was placed into whorls of plants when eight to twelve leaves were visible. Fungicides were applied at regular intervals starting 2 wk after inoculation (WAI). In Urbana, fungicides were applied using a tractor-driven boom applying 420 L/ha water. In Monmouth, fungicides were applied with a commercial high-boy sprayer in 280 L/ha water.

Fungicide treatments were: mancozeb at 1.3 kg a.i./ha applied weekly, and propiconazole at 125 g a.i./ha in 1985 and 60 g a.i./ha in 1986, applied at 3-wk intervals. The early mancozeb treatment was initiated 2 WAI in 1985 and 4 WAI in 1986, before 1% NLB severity was

observed in either year. An early propiconazole treatment was initiated 2 WAI in 1985 and 6 WAI in 1986. Two additional treatments included plots that were inoculated, but never treated, with a fungicide and plots that were neither inoculated nor treated with fungicide.

Average disease severities (the proportion of necrotic leaf tissue) of all plants in each plot were assessed weekly. After flowering, leaf areas were measured from 10 plants in each treatment plot in 1985. Sample yields were taken by harvesting 10 competitive plants (evenly spaced with neighbors) from every plot (1).

RESULTS

Activity of propiconazole in vitro.

Germination of *E. turcicum* conidia on LCA amended with propiconazole was not affected but germ mycelia from these conidia were shortened, thickened, and excessively branched compared with those of conidia germinated on unamended LCA. No conidia germinated on LCA amended with mancozeb, so colony growth on mancozeb-amended media was not evaluated. Colony growth from plugs of *E. turcicum* was increasingly inhibited with higher rates of propiconazole. The EC_{50} for *E. turcicum* growth was $0.01 \mu\text{g a.i./ml}$ propiconazole.

Lesion establishment and growth.

Numbers of NLB lesions were significantly reduced by propiconazole at 192 and 320 g a.i./ha, but not at 64 g a.i./ha (Fig. 1). The effect of propiconazole at 320 g a.i./ha was similar to that of mancozeb at 1,300 g a.i./ha.

Rates of NLB lesion expansion were reduced proportionally to the rate of propiconazole used on greenhouse-grown plants (Fig. 2). Regression models indicated that 65 and 71% of the variation in the lesion expansion rate on A632 and B73, respectively, was explained by g a.i./ha propiconazole in the first experiment. The second experiment was conducted under different environmental conditions, but also showed a decrease in the rate of lesion expansion with increasing rates of propiconazole. Regression models developed from data from the second experiment had coefficients of -0.006 and -0.014 and r^2 values of 34 and 46% for A632 and B73, respectively.

Duration of propiconazole activity.

Lesion expansion rates were affected by timing of fungicide applications relative to inoculation. No lesions developed on A632 treated with propiconazole 24 hr before inoculation with *E. turcicum*. Propiconazole applied to A632 24 hr after inoculation significantly reduced ($P=0.05$) the rate of lesion expansion for 17 days after fungicide application (18 DAI) (Fig. 3A). No significant effects on lesion expansion were observed on B73 when propiconazole was applied within 24 hr of inoculation (Fig. 3B). However, lesions on plants treated with propiconazole

remained smaller than those on control plants. Mancozeb reduced lesion expansion on A632 plants (Fig. 3A), but had no effect on lesions produced on B73 plants (Fig. 3B). Propiconazole applied to established and expanding NLB lesions (11 days) on either inbred had no effect on rate of lesion expansion for 3 days after fungicide treatment (11–14 DAI), but significantly reduced ($P=0.05$) subsequent expansion of the lesions.

Effects on sporulation. Numbers of conidia produced in NLB lesions on propiconazole-treated plants were 93–97% lower than those produced in lesions from untreated plants (Table 1). Numbers of conidia from lesions from mancozeb-treated plants were 50–72% lower than those from untreated plants.

Field studies. Northern leaf blight development was lower ($P=0.05$) on plants treated with propiconazole than on plants that had not been treated with a fungicide or that were treated with mancozeb. Disease progress curves on A632 at Urbana in 1985 were typical of treatment differences in both inbreds in any environment (Fig. 4). Northern leaf blight developed in all plots after anthesis (10 July), even in plots that were neither inoculated nor treated with fungicide. Inoculated plots that had been treated with fungicides had lower disease severities by the end of the season than plots that were neither inoculated nor treated with fungicide. Fungicide treated plots also had average yields of 5,113 kg/ha compared with 4,666 kg/ha from untreated plots.

In 1985 at Urbana, propiconazole was phytotoxic to the inbred B73. Ear height, total height, and total leaf area were lower ($P=0.05$) in plots of B73 treated with propiconazole than in plots left untreated (Table 2). No plant stunting was observed in A632 in 1985, or in either inbred in 1986.

DISCUSSION

Propiconazole failed to inhibit *E. turcicum* conidial germination in our in vitro tests, which is characteristic of sterol biosynthesis inhibiting fungicidal compounds (15). However, these compounds do inhibit fungal growth at low concentrations, as observed in this and other studies (5,9,19). The EC_{50} of $0.01 \mu\text{g a.i./ml}$ for propiconazole against *E. turcicum* falls within the range reported for this fungicide against other fungal pathogens (9,19).

Propiconazole has been reported to have after-infection activity in pecan against *Cladosporium caryigenum* (Ell. & Lang.) Gottwald (11), and curative activity in barley against *Puccinia graminis* Pers. (17) and in pecan against *Cristulariella moricola* (Hino) Redhead (10). Fungicidal activity has also been reported for more than 50 days against *Puccinia striiformis* Westend. (3) and *C.*

moricola (10). Our investigations showed that propiconazole had no curative effects when applied 24 hr after inoculation of corn with *E. turcicum*, nor did fungitoxicity persist beyond 17 days after the fungicide was applied. This interval of activity, however, is similar to the interval activity observed for pecan scab and net blotch of barley (11,16).

Fungistatic effects of propiconazole against *E. turcicum* were inversely proportional to the rate of application; fewer and smaller lesions resulted from progressively higher dosages. *E. turcicum* infections were not prevented by propiconazole, except when the compound was applied at three or more times the manufacturer's suggested application rate. Infection of pecan by *C. moricola* also was not prevented by propiconazole (10). The application rate of propiconazole that provided the same level of control as mancozeb against new infections by *E. turcicum* was higher than rates at which phytotoxicity occurred, as has been noted in other studies (5,19). However, at the suggested application rate, propiconazole affected two components of the monocycle: lesion expansion and conidia production.

Corn treated with propiconazole at 3-wk intervals had less severe epidemics than corn treated with mancozeb at 1-wk intervals. This was similar to the results reported by White (18), who obtained better control of southern and northern leaf blight with propiconazole than with mancozeb formulations.

Table 1. Production of conidia by *Exserohilum turcicum* in leaf lesions from corn plants treated with mancozeb (1.3 kg a.i./ha) or propiconazole (60 g a.i./ha)

Treatment	No. conidia/ cm ² (36-hr incubation)	No. conidia/ cm ² (48-hr incubation)
Control	22.3	57.8
Mancozeb	10.9	20.6
Propiconazole	0.6	4.4
LSD	13.5	32.0

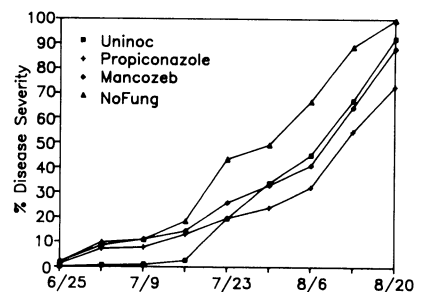


Fig. 4. Effects of propiconazole (125 g a.i./ha) and mancozeb (1.3 kg a.i./ha) applications on development of northern leaf blight on corn inbred A632, grown at Urbana in 1985.

Table 2. Effects of propiconazole (125 g a.i./ha) on growth and development of two corn inbreds grown at Urbana, IL in 1985

Inbred	Fungicide treatment	Ear height (cm) ^z	Total height (cm) ^z	No. leaves	Leaf area (cm ²) ^z
A632	None	11.15	24.05	13.25	568.79
	Propiconazole	10.60	22.60	13.88	555.39
B73	None	14.97 a	25.94 a	13.81	867.75 a
	Propiconazole	11.75 b	21.79 b	13.44	804.12 b

^zData are means for 10 randomly selected plants measured at completion of pollen shed. Means followed by different letters are significantly different ($P = 0.05$) according to Student's t test.

Management of plant diseases by regular applications of protectant fungicides is not economically feasible in many crops because of high application costs (\$3–5/acre) and the need for frequent applications. Numbers of applications could be reduced if a fungicide had eradivative effects and/or provided protection from pathogens over a longer period of time. Propiconazole is effective against NLB, and has a longer interval of effectiveness than some protectant fungicides. Therefore, the use of this fungicide at 3-wk intervals appears to be economically feasible. Propiconazole also appears to be a broad spectrum fungicide that could provide protection against several diseases of corn (18).

Reduction of components of the monocycle by ergosterol-inhibiting fungicides is similar to the effects of rate reducing host resistance (14). Some researchers have suggested that fungicides be used in combination with resistance to enhance disease control (8,14). Differential results with the two inbreds used in

our studies indicated that propiconazole could be effectively used with resistance to control NLB, and also to quantify polygenic resistance levels in corn in terms of fungicide equivalents.

LITERATURE CITED

1. Balko, L. G., and Russell, W. A. 1980. Response of maize inbred lines to N fertilizer. *Agron. J.* 72:723-728.
2. Bowen, K. L., and Pedersen, W. L. 1985. Investigations on the effects of Tilt against *Exserohilum turcicum*. (Abstr.) *Phytopathology* 75:1346.
3. Brown, J. S., Smith, E., Bell, C. J., and Price, T. V. 1986. Duration of control of stripe rust of wheat with foliar sprays. *Aust. Plant Pathol.* 15:9-10.
4. Cimanowski, J., and Szkolnik, M. 1985. Postsymptom activity of ergosterol inhibitors against apple powdery mildew. *Plant Dis.* 69:562-563.
5. Comstock, J. C., Ferreira, S. A., Ching, S. A., and Hilton, H. W. 1984. Control of pineapple disease of sugarcane with propiconazole. *Plant Dis.* 68:1072-1075.
6. Craig, W. F. 1977. Production of hybrid seed corn. Pages 671-719 in: *Corn & Corn Improvement*. G. F. Sprague, ed. American Society of Agronomy, Inc., Madison, WI. 774 pp.

7. Edgington, L. V., Martin, R. A., Bruin, G. C., and Parsons, I. M. 1980. Systemic fungicides: A perspective after 10 years. *Plant Dis.* 64:19-23.
8. Fry, W. E. 1975. Integrated effects of polygenic resistance and a protective fungicide on development of potato late blight. *Phytopathology* 65:908-911.
9. Jones, R. K., Belmar, S. B., and Jeger, M. J. 1987. Evaluation of benomyl and propiconazole for controlling sheath blight of rice caused by *Rhizoctonia solani*. *Plant Dis.* 71:222-225.
10. Latham, A. J. 1987. Laboratory and greenhouse techniques for evaluating selected fungicides against *Cristulariella moricola* on pecan leaves. *Plant Dis.* 71:1010-1014.
11. Latham, A. J., and Hammond, J. M. 1983. Control of *Cladosporium caryigenum* on pecan leaves and nut shucks with propiconazole (CGA-64250). *Plant Dis.* 67:1136-1139.
12. Leath, S., and Pedersen, W. L. 1983. An inoculation technique to detect the *HtN* gene in inbred lines of corn under greenhouse conditions. *Plant Dis.* 67:520-522.
13. Raymundo, A. D., and Hooker, A. L. 1981. Measuring the relationship between northern corn leaf blight and yield losses. *Plant Dis.* 65:325-327.
14. Schein, R. D., Nelson, R. R., Thomas, G. G., Royer, M. H., and Borges, O. 1984. Comparison of the effect of sublethal doses of triadimefon to those of rate-reducing resistance to *Erysiphe graminis* in wheat. *Phytopathology* 74:452-456.
15. Siegel, M. R. 1981. Sterol-inhibiting fungicides: Effects on sterol biosynthesis and sites of action. *Plant Dis.* 65:986-989.
16. Sutton, J. C., and Steele, P. 1983. Effects of seed and foliar fungicides on progress of net blotch and yield in barley. *Can. J. Plant Sci.* 63:631-639.
17. Urech, P. A., Pieta, S. D., and Speich, J. 1980. The use of CGA 64250 against cereal rust diseases. Pages 249-254 in: *Proc. Eur. Mediterr. Cereal Rust Conf.* 5th. 290 pp.
18. White, D. G. 1983. Control of northern corn leaf blight and southern corn leaf blight with various fungicides, 1982 (133). *Fungic. Nematic. Tests* 38:70-71.
19. Whitson, R. S., and Hine, R. B. 1986. Activity of propiconazole and other sterol-inhibiting fungicides against *Phymatotrichum omnivorum*. *Plant Dis.* 70:130-133.