

Interaction of Fungicide Physical Modes of Action and Plant Phenology in Control of Stem Rust of Perennial Ryegrass Grown for Seed

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ABSTRACT

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Azoxystrobin provided protective and curative effects against stem rust (caused by *Puccinia graminis* subsp. *graminicola*) of inoculated perennial ryegrass under field conditions, significantly reducing disease severity compared with the nontreated check when applied as much as 15 days before infection or 14 days after infection. Propiconazole had a significant effect when applied 13, but not 15, days before infection or 7, but not 9, days after infection. Either fungicide was very effective when applied near the time of infection, and the effectiveness of each fungicide was well described by a second- or third-order polynomial with time (days or stem rust latent periods) as the independent variable. When symptomatic plants were sprayed with a fungicide, subsequent urediniospore production per pustule was reduced by 73% in propiconazole-treated plants and by 95% in azoxystrobin-treated plants. Azoxystrobin modestly but significantly reduced germinability of urediniospores from sprayed pustules, unlike propiconazole. These differences between the two fungicides in physical modes of action resulted in a marked difference in their effects on secondary, within-plant spread of the disease. In normal disease development, sporulation from the inner (adaxial) face of pustules on the flag-leaf sheath produces inoculum that leads to numerous contiguous secondary infections along the length of the emerging inflorescence, but only 7% of azoxystrobin-treated sheath pustules had sporulation from the adaxial surface compared with 72 and 90% of propiconazole-treated and nontreated pustules, respectively. Tillers treated with propiconazole early in the process of within-plant spread had significantly greater final stem rust severity than those treated with azoxystrobin at this time. Results of these experiments will allow effects of fungicide application to be incorporated into quantitative epidemic models that describe disease development as a function of environment and plant phenology.

Additional keywords: *Lolium perenne*

For plant diseases that are controlled with fungicides, a major goal for improving management is to optimize timing of fungicide applications, thereby reducing their number to the minimum required for acceptable yield and quality. Optimum timing depends on the overall effect of a fungicide on disease development, which includes toxicity to the pathogen and additional characteristics referred to as physical modes of action (7,16,18). The physi-

cal modes of action are defined by duration and degree of protective activity (fungicide applied prior to infection) and curative activity (fungicide applied after infection), effect of post-symptom application on spore production, and movement of the fungicide to nontreated parts of the plant. As disease warning and management models become more precise, information about fungicide physical modes of action is needed to make best use of the models for optimum fungicide timing.

In fields of perennial ryegrass (*Lolium perenne* L.) grown for seed, the major disease constraint in the United States and New Zealand is stem rust caused by *Puccinia graminis* Pers.:Pers. subsp. *graminicola* Z. Urban. This disease is managed with fungicides (6,17) that typically are applied three to five times during the growing season in the Pacific Northwest of the United States, where most perennial ryegrass seed is produced. The fungicides of choice in this region are triazoles (principally propiconazole and tebuconazole) and strobilurins (principally azoxystrobin).

Triazoles and strobilurins are known to have some degree of acropetal systemic movement in plants (4,8); however, the

levels of protective and curative activity can vary among fungicides and even among pathosystems for a single fungicide (10,18). Triazoles are sterol synthesis inhibitors and many have good activity against rust diseases (8). Propiconazole is protective for 2 to 3 days before infection and curative for up to 8 days after infection against chrysanthemum white rust (*P. horiana*) (3). Tebuconazole has moderate protective and curative activity against *P. recondita* on wheat (4). In a comparison of *Puccinia* spp. affecting ornamental plants, propiconazole was an effective protectant against daylily rust but not against geranium rust, and had different levels of curative activity among three rust diseases (10). Triazoles generally have little effect on fungal spore germination, due to the timing of sterol synthesis and precursor accumulation during germination (15). Spores of *P. graminis*, in particular, apparently use endogenous sterol reserves for the first 6 to 8 h of germination and, thus, are insensitive to triademefon (a triazole) at this point in the life cycle (15). Propiconazole reduced, but did not inhibit completely, germination of urediniospores of several rust pathogens of ornamental plants, and its activity was fungistatic rather than fungitoxic (11).

Strobilurins interfere with respiratory chain enzymes and generally are very effective against rust diseases (4). Azoxystrobin has significant protective effect (as much as 15 days prior to inoculation) and curative activity (7 days post inoculation) against three *Puccinia* spp. on their ornamental hosts (10). This fungicide also is effective against *P. recondita* in wheat, where it is acropetally translocated within the leaves and has curative activity 5 days after infection (4). The related strobilurin fungicide kresoxim-methyl is reported to be protective, but not curative, against the same pathogen (5). Strobilurins are strongly inhibitory to germination of fungal spores (2,9), and were shown to be toxic to urediniospores of several rust pathogens of ornamental crops after very short exposure times (11). In addition, these fungicides can inhibit sporulation from lesions in some pathosystems when applied post infection or post symptom development (18,19).

We currently are developing an epidemic model that uses weather data to estimate infection severity and disease development for stem rust of perennial

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ryegrass (12,13). To incorporate optimized fungicide use into this model, information about the physical modes of action for propiconazole and azoxystrobin is needed. Duration and degree of protective or curative activity would affect a fungicide's impact on the weather-related infection events specified in the epidemic model. In addition, the model specifies the dynamics of a unique type of within-plant disease spread related to grass phenology, in which any single lesion of stem rust on a leaf sheath can produce multiple, contiguous lesions on the enclosed stem or inflorescence as it elongates (14). This process can account for over 50% of final stem rust severity in a crop. Therefore, information about fungicide physical modes of action with respect to this secondary spread process is of overriding importance in optimizing fungicide application timing. There are no reports of physical modes of activity for these fungicides against stem rust on any host. Experiments described here were conducted to provide quantitative information about physical modes of action for the fungicides used to manage stem rust of perennial ryegrass grown for seed, and the resultant effect of fungicide timing on within-plant spread of the pathogen.

MATERIALS AND METHODS

Protective and curative activity of fungicides. A field experiment was conducted twice in 2001 and once in 2003 to determine protective and curative properties of propiconazole and azoxystrobin when applied at various durations before and after an infection event. These three trials of the experiment were conducted in the field, to include the effect of natural growing conditions on the persistence and activity of the fungicides. Disease severity from an inoculation in the field is highly dependent upon weather conditions (13). Therefore, it was decided to conduct each trial with a single infection event (all treatments inoculated on the same date) and various fungicide application times, rather than a single fungicide application time with several infection events.

Perennial ryegrass cv. Morningstar was planted in rows 30 cm apart on 10 October 2000 (for the 2001 trials) and 6 October 2002 (for the 2003 trial). For each trial, a plot 3 by 26 m within the planting was

used. In the plot, alternate rows of the crop were mowed, as were 42-cm-wide strips perpendicular to the rows, so as to leave 55-cm lengths of nonmowed row available for treatment. Each 55-cm section served as a replicate and received a single treatment (one fungicide treatment at one application date); there were four replicates per treatment in a completely randomized design. There were two check treatments (four replicates each) in which no fungicide was used; one was inoculated with the pathogen and the other was left noninoculated as a check on background levels of disease. In the three trials of this experiment, fungicide application timings ranged from 15 days before inoculation to 14 days after inoculation (Table 1), with the actual intervals depending upon when conditions were favorable for fungicide sprays and for inoculation with the pathogen. To apply fungicide to a replicate, the 55-cm section of grass was enclosed with a fence (enclosure 55 cm long by 30 cm wide by 80 cm tall) of solid plastic to prevent fungicide spray reaching other replicates. Fungicide was applied through a single flat-fan Tee-Jet nozzle (no. 8002VS) attached to a CO₂-powered sprayer operating at 138 kPa pressure. The nozzle was attached to a boom resting across the top of the rectangular enclosure and was pushed across the rectangle at the rate of 2 s per 55 cm, delivering fungicide solution at 50 ml/m² to the replicate. Fungicide solutions were prepared in water as follows: propiconazole (Tilt 428C; Syngenta, Inc., Basel, Switzerland) 374 mg of active ingredient per liter (a.i. liter), and azoxystrobin (Quadris 2.08EC; Syngenta, Inc.) 325 mg a.i. liter amended with 1% (vol:vol) non-ionic surfactant (Agridex, Helena, MT). Thus, the rates of fungicide application were equal to standard, labeled rates of Tilt at 189 g a.i. ha and Quadris at 165 g a.i. ha.

The inoculum consisted of urediniospores of *P. graminis* subsp. *graminicola* that had been collected from field plantings of perennial ryegrass the prior year, dried overnight, stored at -60°C, and heat shocked before use as described previously (14). The field populations of *P. graminis* subsp. *graminicola* from which the inoculum was collected were in a seed-production area where triazole fungicides have been used for over 25 years and stro-

bilurins have been in common use for about 8 years. A suspension of urediniospores (approximately 3.8 × 10⁶ spores/ml) was prepared for each replicate by mixing 9 mg of spores in 750 µl of light mineral oil (Soltrol; Phillips Petroleum Co., Bartlesville, OK). Each 750-µl aliquot was mixed thoroughly immediately before being sprayed onto a replicate, with the use of a small, handheld Venturi atomizer (1) operating at 34 kPa. To contain the inoculum on the target replicate, the rectangular enclosure described previously was set around it before application; a covering of plastic sheeting with a slit for the operator's hand was placed on the top of the rectangular enclosure. Inoculations were done between 9:00 a.m. and 12:00 noon. To avoid the phytotoxicity that results from mixing of water with the oil before it dries, plants were kept clear of dew by covering the plot with a temporary tent during the night preceding the inoculation day. The tent was removed before inoculation if rain was not threatening; otherwise, it was removed as soon as the oil was dry (1 to 2 h after inoculation). The favorability of the weather for infection during the night and morning following inoculation was calculated (13) from temperature and leaf wetness data collected in the plot, and is shown in Table 1.

Approximately 1.6 latent periods (LPs) after inoculation (14 to 21 days after inoculation, as calculated [12] from temperatures during the interval), plants were scored for disease severity. The plants from a 20-cm-long section of row within the 55-cm replicate were cut as a sample, from which 50 tillers were taken at random to count the number of pustules per tiller. The average number of pustules per tiller was used as the replicate value in the statistical analysis. Mean and standard error were computed for each treatment, and Dunnett's test was used to compare each sprayed treatment with the inoculated check. In addition, a two-way analysis of variance (ANOVA) was done within each trial (fungicide and timing as factors). A regression analysis was done for the combined results of the three trials for each fungicide, to assess the relationship between time of application (before and after infection) with disease severity. Time was expressed as days or as LPs (a heat unit

Table 1. Field experiment to measure protective and curative activity of azoxystrobin and propiconazole against stem rust of perennial ryegrass

Trial	Planting date	Date	Inoculation ^x		
			Plant stage	Infection value ^y	Fungicide application (days) ^z
I	10 October 2000	16 May 2001	Feeke's 10.1	2.2	-13, -7, -1, +2, +5, +7
II	10 October 2000	5 June 2001	Feeke's 10.5.1	2.6	-15, -7, -1, +3, +7, +9
III	6 October 2002	20 May 2003	Feeke's 10.2	3.0	-7, -1, +1, +7, +14

^x Replicate rows, 55 cm long, of perennial ryegrass in field plots were inoculated by spraying with a suspension of 9 mg of urediniospores of *Puccinia graminis* subsp. *graminicola* in 750 µl of light mineral oil.

^y Favorability of overnight and early-morning weather conditions for infection, summarized by infection value calculated from temperature and leaf wetness duration (13). Value is on a log scale, and ranges from 0 to 3.0.

^z Each 55-cm-long replicate received a single fungicide application on the indicated number of days before (-) or after (+) the inoculation date.

calculation equal to the duration of one LP in this pathosystem) (12) and disease was normalized among trials by expressing it as a proportion of the disease severity on the respective inoculated check. All analyses were computed using SigmaStat software (SPSS, Inc., Chicago).

Production and germinability of spores from fungicide-treated plants. Fieldgrown perennial ryegrass plants were transplanted to pots at 2 months of age, then maintained outdoors until used in experiments. Potted plants were brought into a greenhouse and inoculated with urediniospores of *P. graminis* subsp. *graminicola* as described previously (14), using a spore suspension of 2 mg of spores/ml of Soltrol oil. Inoculated plants were kept in a mist chamber overnight at 16°C, then allowed to dry gradually while exposed to light and temperatures increasing to 25°C (13). Plants were maintained in a greenhouse with day and night temperatures of 18 and 15°C, respectively, until pustules erupted 9 days later. Pots were divided randomly into three treatment groups: two fungicides and the check (water-only) treatment. Potted plants were placed upright on the ground and the area was sprayed using a backpack sprayer operating at 138 kPa and groundspeed required to deliver propiconazole at 189 g a.i. or azoxystrobin at 165 g a.i. in 170 liters of water/ha. Two days after fungicide treatment, accumulated urediniospores were removed and discarded from randomly selected pustules by gently vacuuming with a miniature cyclone sampler (1), taking care not to damage the uredinia. This procedure removed spores that may have been produced before fungicide treatment, as well as fungicide that may have been deposited on the pustules. Then, 2 days later (4 days after fungicide treatment), newly produced urediniospores were collected with the cyclone sampler from each of five previously cleaned pustules per treatment, and the spores were counted with the aid of a hemacytometer after suspending them in light mineral oil. Germinability of urediniospores from five additional pustules per treatment also was assessed. For the germination test, a leaf segment bearing a single pustule was cut from the plant and gently tapped while holding it above a plate of water agar, to release spores onto the agar surface. Plates were kept in the dark at 24°C for 24 h, then germination was assessed by microscopic examination of 100 spores/pustule. Spores were considered germinated if the germ tube was at least as long as the spore diameter.

The experiment was conducted twice (two trials). Data were transformed to the logarithms of the observed values to equalize the variance before conducting a two-way ANOVA with fungicide treatment and trial as the factors.

Effect of fungicides on within-plant disease spread. Perennial ryegrass plants

undergoing reproductive growth were obtained as described previously (14), by transplanting vernalized plants from the field into pots in a greenhouse. When tillers reached the phenological stage of flag-leaf sheath exposure, they were inoculated to produce a single lesion on the flag leaf sheath. A spore suspension was prepared (6 mg of urediniospores in 1 ml of Soltrol oil) and a small amount was applied to one 5-mm-diameter spot on each flag leaf sheath by means of a small paintbrush. The inoculated spot was located on the opposite side of the sheath from the overlapping leaf edges and 1 to 2 cm below the junction of the leaf blade and sheath. Tillers were tagged individually for future identification, and flag leaf sheath lengths were recorded. After the Soltrol oil had evaporated, plants were exposed overnight to conditions favorable for infection (14), then maintained in a greenhouse where they were irrigated without allowing water to contact leaves or stems. A datalogger in the greenhouse recorded temperature every half-hour during the experiments, for calculating LP durations (12).

Previous research (14) had shown that urediniospores are released from the inner face of the infected sheath at the pustule site when the pustule erupts, one LP after the primary infection occurs on the sheath surface. These spores then initiate multiple secondary infections on the stem as it extends from within the enclosing sheath. In the experiment reported here, the fungicides were applied after the secondary infections had begun (i.e., >1 LP after inoculation), to determine fungicide curative effects on the secondary stem infections as well as fungicide effects on within-plant spread via effects on sporulation of the primary pustule or subsequent infection processes. Preliminary experiments suggested that fungicide activity on secondary infections could differ with their physical exposure to the fungicides and with the relative timings of the infection event and the fungicide application (Fig. 1). For example, secondary infections on that portion of the inflorescence that had extended above the flag leaf sheath by the time of fungicide application (Fig. 1, segment B) may respond differently to fungicide application than secondary infections on the portion of the inflorescence that has not yet emerged above the sheath (Fig. 1, segment C).

The experiment to measure effects of fungicide application on secondary, within-plant spread of stem rust was conducted twice. In the first trial, fungicide was applied to some plants at 1.1 LP after sheath inoculation and to others at 1.6 LP post inoculation. In the second trial, fungicide application was done at 1.2 and 1.8 LP post inoculation. To ensure that adequate fungicide coverage of the inoculation site would occur, fungicide was applied after laying the potted plants on their sides

on the ground, with the inoculated side of the tiller facing up. A CO₂-powered backpack sprayer was used to apply the fungicide through a TeeJet flat fan nozzle, with the operator walking at a speed to apply propiconazole at 189 g a.i. or azoxystrobin at 165 g a.i. in 170 liters of water/ha. The azoxystrobin solution was amended with surfactant as previously described. After the fungicide dried on the plants, they were returned to the greenhouse and maintained as described previously.

Measurements of stem lengths and lesion location on each inoculated tiller were taken periodically (including at times of inoculation, eruption of primary pustule, and fungicide application) to permit later identification of the segments illustrated in Figure 1. After inflorescence extension was complete (approximately 21 days after inoculation; Fig. 1, time 3), one additional LP was allowed to pass so that all latent secondary infections on the inflorescence had time to erupt. Then, 30 days after inoculation of the sheath, the length and location of secondary infections on the inflorescence were measured and mapped onto diagrams of the inoculated tillers. Locations and lengths of the three segments B through D (Fig. 1), derived as described in the previous paragraph, were superimposed on the disease diagrams. Disease severity, as the length (in centimeters) of inflorescence bearing pustules, was recorded for each segment.

The experimental unit was one pot and there were two inoculated tillers per pot. Measurements from the two tillers/pot were averaged to produce the replicate value for analysis, and there were eight replicate pots/treatment. The design of the experiment was completely randomized. ANOVA was used to compare full-tiller disease severity (percent total stem length diseased as a proportion of this value for inoculated, nontreated check plants) across fungicides and application times. For each date-of-application treatment within each trial, a *t* test was used to compare propiconazole-treated and azoxystrobin-treated tillers for disease severity (proportion of the stem length that was rusted) for each of the three stem segments (B, C, and D) shown in Figure 1.

RESULTS

Protective and curative activity of fungicides. In-field inoculation of perennial ryegrass with *P. graminis* subsp. *graminicola* was successful, but the disease severities (number of pustules per tiller) differed among the three trials of the experiment (Fig. 2), perhaps due to different weather conditions immediately following inoculation (Table 1). Inoculated, nontreated checks averaged 2.5, 6.2, and 17.1 pustules/tiller in the three trials, respectively. Noninoculated checks had very little stem rust (0.02, 0.3, and 0.1 pustules/tiller, respectively), indicating that

background levels of disease were not important compared with the severity produced by the single inoculation event in each trial. For each trial, most fungicide

treatments had significantly ($P < 0.05$, Dunnett's test) less disease than the respective inoculated check, except for propiconazole treatments 15 days before infec-

tion or ≥ 9 days after infection. Two-way ANOVA on the data for each trial showed a significant effect ($P < 0.05$) for day of treatment (i.e., fungicides were more effective when applied close to the time of infection than when applied earlier or later). The main effect for fungicide (propiconazole versus azoxystrobin) was significant ($P < 0.05$) in trials II and III, but not in trial I.

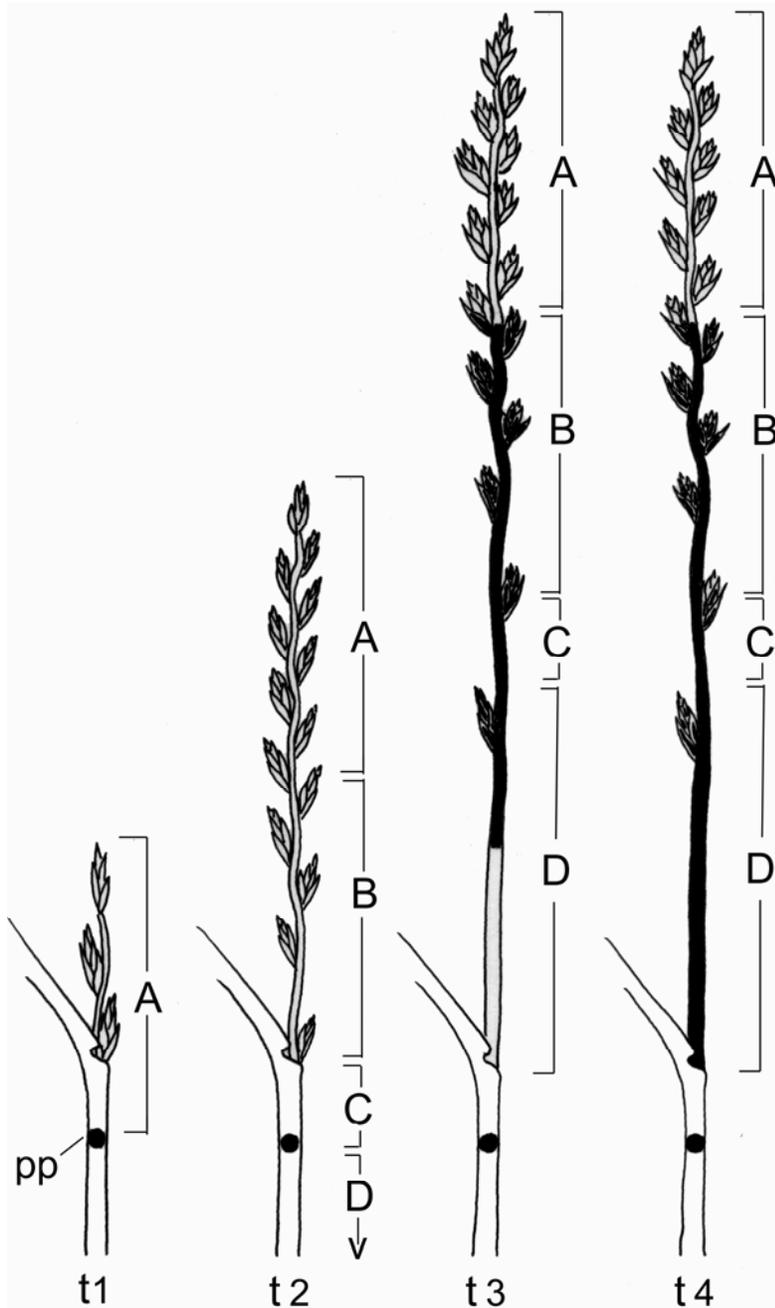


Fig. 1. Areas of secondary disease relative to fungicide application. Secondary, within-plant spread of disease begins at time t1, when a primary pustule (pp) from an infection on the outer surface of the leaf sheath first erupts on the plant surface. This pustule also releases urediniospores from the inner (adaxial) surface of the sheath, and these spores initiate secondary infections along the length of the enclosed inflorescence as it extends. Segment A of the inflorescence, distal to the primary infection site at time t1, does not receive secondary inoculum. At the time of fungicide application, shown here arbitrarily as time t2, four regions of the expanding inflorescence can be designated with respect to fungicide exposure. Segment B has been exposed to secondary inoculum prior to fungicide application and is now exposed to direct fungicide contact. Segment C has been exposed to secondary inoculum prior to fungicide application but is shielded from direct fungicide contact by the sheath distal to the primary infection site. Segment D has not been exposed to secondary inoculum at the time fungicide is applied; secondary infections on this part of the inflorescence proximal to the site of primary infection can only occur after time t2, as inflorescence extension continues. At time t3, the inflorescence has completely extended and all four regions are exposed. For purposes of illustration, all secondary infections (designated as solid black fill) have erupted in segments B, C, and part of D. The nonlesioned, lower part of segment D bears latent infections which become erumpent by the time another latent period has passed (time t4).

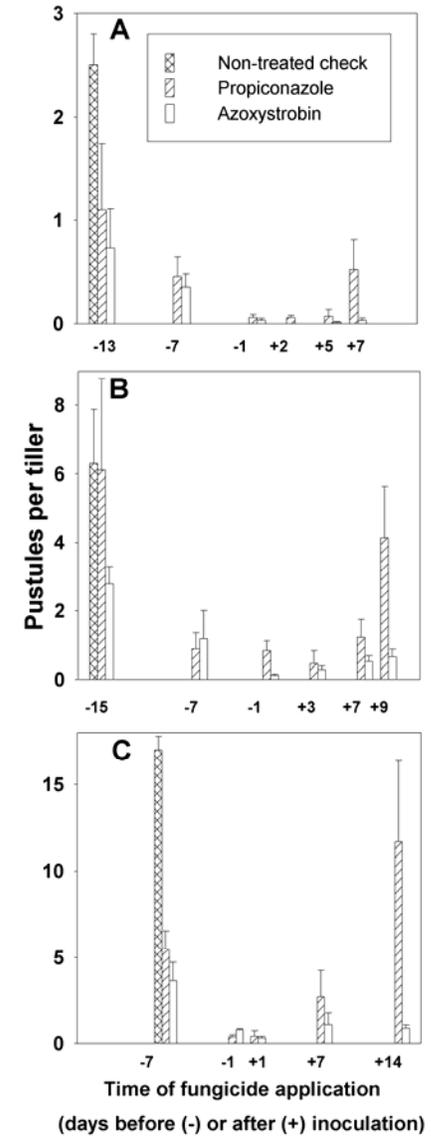


Fig. 2. Stem rust severity in field experiment of perennial ryegrass treated with fungicides before or after inoculation. Disease severity (pustules per reproductive tiller) of fieldgrown perennial ryegrass plants inoculated with *Puccinia graminis* subsp. *graminicola* and treated with propiconazole (189 mg a.i. per ha) and azoxystrobin (165 mg a.i. per ha) at various times before or after inoculation. Three different field trials are illustrated (A, B, and C), and severity in the inoculated, nontreated check is placed at the left of each graph for convenience. The data were collected at 1.6 latent periods after infection. Vertical lines above bars indicate standard error. There were four replicate plots per treatment in each trial of the experiment, and 50 tillers sampled per replicate.

When data from the three trials were combined in a regression analysis (Fig. 3), the effect of application timing on fungicide activity was significant ($P < 0.01$) for each fungicide. When applied near the time of infection, each fungicide reduced disease nearly to zero (Fig. 3). As the time between pre-infection (protective) application and infection increased, the degree of fungicide activity decreased, and the decrease was sharper for propiconazole than for azoxystrobin. In post-infection treatment, azoxystrobin showed more prolonged curative effects than did propiconazole (e.g., 90 versus 30% control, respectively, when applied 14 days after infection). Expressing time before and after infection in terms of heat units (LP units for the pathogen) rather than chronological time produced a slightly better correlation with fungicide effect ($r^2 = 0.90$ versus 0.83 for propiconazole and 0.91 versus 0.90 for azoxystrobin, respectively; Fig. 3C).

Production and germinability of spores from fungicide-treated plants. Urediniospores, produced between 2 and 4 days after pustules were sprayed with propiconazole at labeled rates, were unaffected in their viability (93%) compared with the spores from nontreated checks (98%) (Table 2). Azoxystrobin, in contrast, reduced germinability of spores significantly ($P < 0.05$) to 82%.

Between 2 and 4 days after plants were sprayed with propiconazole, the number of urediniospores produced per pustule was only 27% of the number produced by pustules on nontreated plants. Pustules on azoxystrobin-treated plants produced only 5% as many spores as pustules on the nontreated plants. All three treatments (azoxystrobin, propiconazole, and check) were significantly ($P < 0.05$) different from one another (Table 2).

Effect of fungicides on within-plant disease spread. When plants were inocu-

lated at a single site on the flag leaf sheath, the severity of secondary disease from within-plant spread was greatly affected by fungicide type and application timing (Fig. 4). Plants treated with propiconazole ultimately had approximately 80% as much disease as nontreated plants when the fungicide was applied early in the secondary-infection process (1.1 LP after primary infection, which is 0.1 LP after presumed start of secondary infections). As time of propiconazole treatment was delayed, final disease severity decreased to reach approximately 20% of the nontreated checks in plants treated 1.8 LP after primary infection. In contrast, azoxystrobin treatment during secondary, within-plant disease spread reduced final disease severity to between 15 and 20% of that in the check, whether treatment occurred at 1.1, 1.2, 1.6, or 1.8 LP after initial infection (Fig. 4).

A more detailed examination of this difference between fungicides revealed a pattern in the distribution of secondary infections along the inflorescence (Table 3). A *t* test was used to compare the two fungicides for proportion of diseased length of each tiller segment in each of four cases (two application times in each of two trials, with eight replicates per treatment in each case). In segment B, which is the portion of the inflorescence where secondary infections were exposed directly to applied fungicides (Fig. 1), propiconazole and azoxystrobin were similar in their effects in two of three cases (in the early-timed application of trial II there was no segment B, because the infected segment had not emerged from the enclosing sheaths when fungicides were applied). Azoxystrobin was significantly more effective against these infections than propiconazole was in one case. The fungicides usually did not differ ($P < 0.05$) in their effects on secondary infections that were still covered by the enclosing sheath at the

time of application (Fig. 1, segment C; Table 3). Again in the case where the difference was significant, azoxystrobin reduced disease more than propiconazole

Table 2. Production and germinability of urediniospores of *Puccinia graminis* subsp. *graminicola* from perennial ryegrass plants treated with fungicides

Fungicide ^x	Urediniospore assay ^w	
	Spores per pustule ^y	Germination (%) ^z
None (check)	4,074 a	98 a
Propiconazole	1,114 b	93 a
Azoxystrobin	184 c	82 b

^w Two days after fungicide sprays were applied, arbitrarily-selected pustules were gently vacuumed to remove any accumulated urediniospores. Two days later, spores were collected from each pustule for analysis of the spores produced between days 2 and 4 after fungicide application. Different pustules were used for production and germinability analyses.

^x Perennial ryegrass plants were inoculated with *P. graminis* subsp. *graminicola* and maintained in the greenhouse until erumpent pustules appeared. Two days after pustules erupted (11 days after inoculation), plants were sprayed with propiconazole (Tilt at a rate of 182 g a.i./ha) or azoxystrobin (Quadris at a rate of 150 g a.i./ha) or left nontreated. Plants were maintained in the greenhouse after treatment.

^y Each value represents the number of spores produced between 2 and 4 days after fungicide application. Values followed by the same letter do not differ ($P < 0.05$), by the Student-Newman-Keuls test.

^z Urediniospores were collected onto the surface of a plate of water agar on day 4 and incubated in darkness at 24°C for 24 h before assessing germination. A spore was considered germinated if the germ tube length exceeded the spore diameter. In all, 100 spores/replicate were counted, and there were five replicate pustules per treatment in each of two experiments. Values followed by the same letter do not differ ($P < 0.05$), by the Student-Newman-Keuls test.

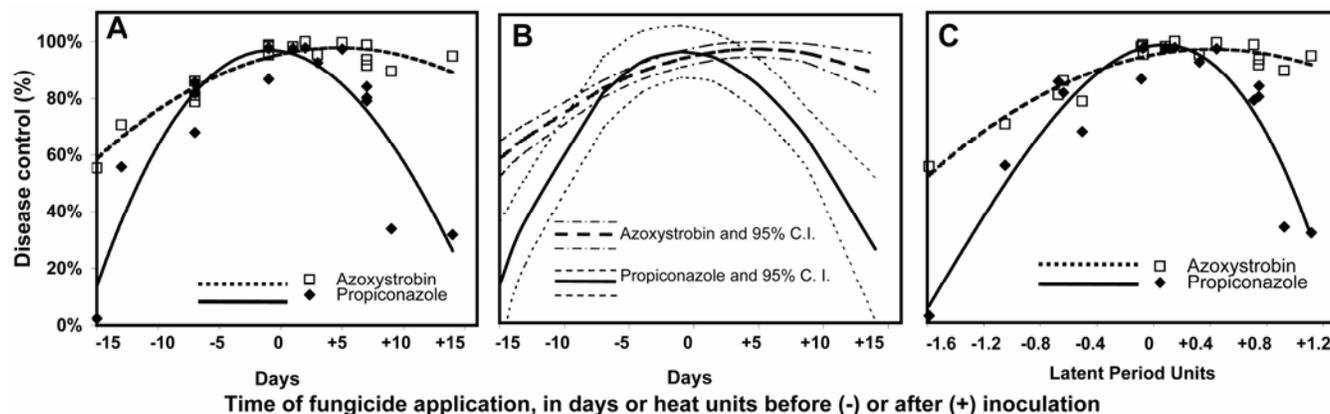


Fig. 3. Protective and curative activity of fungicides against stem rust of perennial ryegrass in field plots. Data from three field trials normalized to disease severity as a proportion of that in the respective inoculated check, and expressed as percent control (100 – percent relative severity). **A**, Data points and regression line for effectiveness of propiconazole ($y = 96.6 - 0.182x - 0.368x^2$, $r^2 = 0.83$) or azoxystrobin ($y = 95.6 - 0.968x - 0.10x^2$, $r^2 = 0.90$), where y is percent control and x is the number of days between fungicide application and inoculation. **B**, The regression lines of Figure 3A with associated boundaries for the 95% confidence interval of the regression. **C**, Data points and regression line for effectiveness of propiconazole or azoxystrobin as a function of latent period (LP) units between fungicide application and preceding or ensuing inoculation. Propiconazole: $y = 96.8 - 6.34x - 49.0x^2 - 10.1x^3$ ($r^2 = 0.90$). Azoxystrobin: $y = 95.3 - 9.46x - 11.1x^2$ ($r^2 = 0.91$), where y is percent control and x is LP units.

did. The major difference between the fungicides in their effect on within-plant spread of stem rust was seen in segment D (Fig. 1), which bears the secondary infections that are initiated after the time of fungicide application (Table 3). In three of

four cases, propiconazole permitted significantly ($P < 0.05$) more secondary disease development on this segment than azoxystrobin did. It is notable that, in the case where the difference was not significant, the segment length available for this

type of infection was relatively short due to the fungicide application being quite late in the process of secondary infection, when there is little stem extension remaining (Table 2). The weighted average disease severity (proportion rusted \times segment length) for segment D across the four application times was 33% for propiconazole and only 5% for azoxystrobin. An examination of the inner sheath surfaces directly under the initial inoculation sites at the end of the experiment showed that only 7% of the azoxystrobin-treated plants had urediniospores at these sites, significantly ($P < 0.05$) less than the 72% of the propiconazole-treated plants or the 90% of the nontreated check plants that had abundant spores there.

DISCUSSION

Azoxystrobin and propiconazole each provided significant protective and curative activity against stem rust of perennial ryegrass when tested under field conditions. The observed protective activity of azoxystrobin (significantly less disease than the check when applied 15 days prior to infection) is similar to that reported for three rust diseases of ornamentals (10), which were significantly reduced by an application 15 days before infection. Other reports (3,4) also show significant protective activity against rusts, although none tested as great a time period. We observed curative activity against stem rust from applications of azoxystrobin made as many as 14 days (1.1 LP) after infection. In other reports (3,4,10), curative activity of this fungicide against other rust diseases was significant

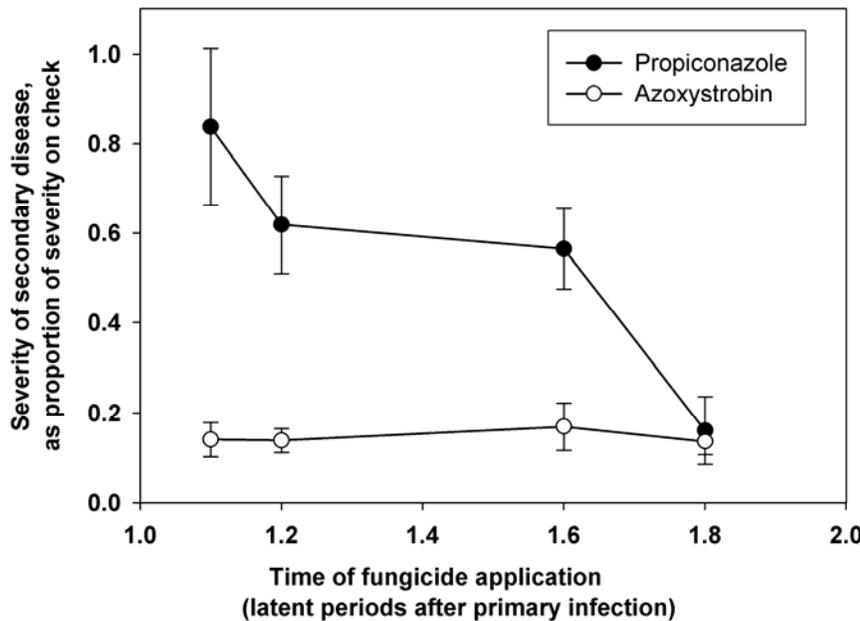


Fig. 4. Effect of fungicides on stem rust severity resulting from secondary, within-plant spread of disease. Each tiller was inoculated to produce a single primary pustule on the flag leaf sheath, then sprayed with propiconazole or azoxystrobin at one of the times indicated. Time is expressed as latent periods (LPs) after primary infection; 1 LP is equivalent to approximately 8 days under the conditions of this greenhouse experiment. Sprays were applied at 1.1 or 1.6 LP in one trial, and at 1.2 or 1.8 LP in a second trial. Length (cm) of the diseased portion of the inflorescence was measured 4 LP after primary infection. Severity is expressed as percentage of the diseased proportion of the inflorescence on nontreated (check) tillers in the same trial. The bars indicate \pm standard error.

Table 3. Comparison of secondary disease (proportion of vulnerable stem rusted) on tillers treated with propiconazole or azoxystrobin at indicated times after primary infection on the sheath

Fungicide application ^x				Disease severity on inflorescence exposed to secondary infection ^y							
				Segment B		Segment C		Segment D		Full-length	
Trial	Days	LP	Material	Length (cm)	Proportion rusted	Length (cm)	Proportion rusted	Length (cm)	Proportion rusted	Length (cm)	Proportion rusted
I	10	1.1	Propiconazole	0.88	0.01 ns	0.99	0.39 ns	28.56	0.31*	30.43	0.30
			Azoxystrobin	0.82	0.00	0.83	0.27	27.71	0.04	29.36	0.05
			None	36.52	0.36
II	11	1.2	Propiconazole	0.00 ^z	...	2.46	0.84 ns	19.03	0.44 *	21.49	0.48
			Azoxystrobin	0.00	...	1.94	0.73	20.39	0.04	22.33	0.11
			None	25.66	0.77
I	15	1.6	Propiconazole	14.36	0.11 *	1.23	0.54 *	14.90	0.25 *	30.49	0.20
			Azoxystrobin	12.76	0.01	1.06	0.18	14.87	0.10	28.68	0.06
			None	36.52	0.36
II	17	1.8	Propiconazole	11.29	0.08 ns	2.86	0.34 ns	5.74	0.12 ns	19.89	0.12
			Azoxystrobin	12.41	0.03	2.82	0.53	4.74	0.04	19.97	0.10
			None	25.66	0.77

^x Primary infection from applied inoculum resulted in a single pustule induced on the flag leaf sheath. Fungicides (propiconazole at 189 g a.i./ha and azoxystrobin at 165 g a.i./ha) were applied, as sprays simulating field application with complete coverage, at the indicated times after inoculation. Latent period (LP) is a heat-unit measure of the time needed to complete the pathogen's LP on this host species (12).

^y Severity of secondary disease due to spread from primary pustule (on sheath) to enclosed, extending inflorescence. Severity measured 30 days after primary infection, expressed as diseased length (in centimeters) of inflorescence. Values are averages of eight replicates per treatment, two tillers/replicate. Segments B, C, and D designate portions of the inflorescence according to their exposure to secondary infection and fungicide treatment. Segment B was exposed to secondary infection, then direct fungicide contact; segment C was exposed to secondary infection, but shielded from direct fungicide contact by overlying sheath; and segment D was exposed to secondary infection only after fungicide application had occurred. Full-length is the sum of B, C, and D, representing the entire length that is exposed to secondary infection. Segments B, C, and D are defined with respect to fungicide application times and, thus, are not defined for nontreated checks. Paired tests of proportion rusted (propiconazole versus azoxystrobin within a treatment time) was done by the Mann-Whitney procedure; * = difference significant at $P < 0.05$ and ns = not significantly different.

^z There was no segment B in this trial, because the site of secondary infection had not emerged above sheath at the time of fungicide application.

at the longest durations tested (5 to 8 days). Azoxystrobin had protective but no curative activity against grape downy mildew (18). In our study of stem rust, propiconazole had protective activity when applied at 13, but not at 15, days before infection; it had curative effects when applied at 7, but not at 9 or 14, days after infection. Protective activity of propiconazole against other rusts varies. Mueller et al. (10) observed no protective activity for propiconazole against geranium rust, but significant activity with a 15-day pre-inoculation treatment of daylily rust and sunflower rust. The experiments reported here were conducted under field conditions, including fungicide applications at labeled rates and typical spray volumes. The resultant data are somewhat variable, likely due to variability in infection levels and fungicide coverage, but probably are representative of protective and curative activity that would be experienced in actual agricultural use. It is clear that either fungicide is very effective when applied close to the time of infection, and that azoxystrobin has better protective and curative activity than propiconazole as the time interval between fungicide application and infection increases. It is possible that this difference in activity between the two fungicides reflects genetic selection due to the longer exposure history of the local stem rust fungus population to propiconazole than to azoxystrobin. However, it is noteworthy that qualitatively similar results with respect to differences in activity of these two fungicides have been reported for some other rust pathogens (10).

The observed effects of azoxystrobin and propiconazole on spore production and germinability are congruent with reports about other pathosystems. Azoxystrobin, applied post infection, effectively inhibited sporulation of the grape downy mildew fungus (18). We observed a significant reduction in urediniospore production (compared with the nontreated check) following post-symptom application of either azoxystrobin or propiconazole, although the effect was significantly greater on azoxystrobin-treated plants (Table 2). Pustules on azoxystrobin-treated plants not only produced fewer spores but the spores produced also had a reduced probability of germinating compared with those produced on propiconazole- or nontreated plants (Table 2). Previous reports have shown moderate (11) or no inhibition (2) of urediniospore germination by propiconazole, and irreversible toxicity by azoxystrobin, when spores are exposed directly to the fungicides (2,11). This difference in degree of inhibition was supported by preliminary experiments (W. F. Pfender, unpublished) in which urediniospores were transferred in bulk from pustules to water agar immediately after diseased plants were treated with fungicide. In the experiment reported in Table 2, the

combined effects of azoxystrobin on sporulation and germinability result in only 4% as many germinable spores per pustule as in the nontreated checks and 15% as many as in the propiconazole-treated plants. This reduction in germinable spores per pustule could produce a marked difference between azoxystrobin and propiconazole in their effects on plant-to-plant spread, and, thus, epidemic progress.

In addition to possible effects on plant-to-plant spread, differences between azoxystrobin and propiconazole on within-plant disease spread in this pathosystem could result in particularly stark differences in control of stem rust. As previously reported (14), most of the final disease severity in stem rust of perennial ryegrass grown for seed results from an adaptation of the pathogen to the host's growth habit, such that a single initial lesion on a leaf sheath can produce extensive secondary lesion areas on the extending stem. The contiguous stem lesions are the hallmark of stem rust disease, and they result in extensive damage and large numbers of urediniospores. The effect of a fungicide on this process is the combined outcome of its protective or curative activity and its activity against production of germinable spores. For infected stems that are exposed at the time of fungicide application (Fig. 1, segment B), the two fungicides are equivalent if applied early in the process. Azoxystrobin has a greater curative activity than propiconazole, however, so that, if the fungicides are applied when the infected sites of segment B are older than approximately 0.6 LP (Fig. 3C), azoxystrobin reduces disease severity more than propiconazole does. A more important difference is the effect of azoxystrobin on post-symptom spore production and germinability. The process of secondary infection occurring on the portion of the stem proximal to the initial sheath lesion at the time of fungicide application (Fig. 1, segment D) is largely unaffected by the application of propiconazole. However, azoxystrobin inhibits most of these secondary infections, presumably because of its post-symptom activity against spore production and germinability from the primary pustule. By interfering with the ability of the pathogen to produce germinable spores from the inner face of the pustule on the adaxial surface of the sheath, the contiguously infected length of extending stem or inflorescence is eliminated or greatly reduced. The difference in the capability of the two fungicides to affect the final disease severity depends greatly on the time of fungicide application relative to stem extension. Early in stem extension, segment D (secondary infections occurring after fungicide application; Fig. 1) represents a greater proportion of the final stem length than it does later in stem extension. Therefore, propiconazole is less effective

than azoxystrobin when applied early in this process (Fig. 4). The effects of the fungicides on this process also depend on the location of the sheath lesions; note that neither fungicide is greatly inhibitory to existing infections that are covered by the sheath at the time of application (Fig. 1, segment C; Table 3). It should also be noted that effectiveness of fungicide application depends upon adequate coverage of plant surfaces with fungicide. In our experiments on within-plant spread of disease, we measured the outcomes for infection sites we knew to be adequately exposed to fungicide. Under field conditions, some infection sites will escape fungicide coverage. Our field experiments (Fig. 3) provide an efficacy estimate that includes variability due to coverage; a model for fungicide activity that accounts for coverage, timing, and phenological effects could be constructed by combining results in Figure 3 with those in Figure 4.

Physical modes of action can be very important to the outcome of fungicide application in disease control. The grass stem rust pathosystem is particularly sensitive to fungicide physical modes of action, as illustrated in this research. Quantitative knowledge of the preventative or curative and sporulation effects of the two fungicides most commonly used to manage stem rust in perennial ryegrass seed fields will enable construction of an epidemic and management model that realistically and adequately incorporates fungicide effects on disease development.

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