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Effects of Propiconazole on Morphology, Development, and Epidemiology of Rhizoctonia Solani, Causal Agent of Rice Sheath Blight.

Elly M. Van eekhout  
Louisiana State University and Agricultural & Mechanical College

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Effects of propiconazole on morphology, development, and epidemiology of *Rhizoctonia solani*, causal agent of rice sheath blight

Van Eeckhout, Elly M., Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1991
EFFECTS OF PROPICONAZOLE ON MORPHOLOGY, DEVELOPMENT, AND EPIDEMIOLOGY OF \textit{RHIZOCTONIA SOLANI}, CAUSAL AGENT OF RICE SHEATH BLIGHT

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Botany

by

Elly M. Van Eeckhout
Agricultural Engineer
State University of Ghent, Belgium, 1985
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Finally, I would like to dedicate this dissertation to my parents who taught me, by example, that courage and hard, earnest work are never fruitless.
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ABSTRACT

Propiconazole was inhibitory to mycelial growth of *Rhizoctonia solani* in vitro. Sclerotial weight per colony was also reduced by propiconazole treatment but, in relation to total amount of mycelium, sclerotia formed earlier in 1 to 10 μg/ml plates than in control plates. Colony diameters from germinating sclerotia obtained from cultures growing at 0.5 to 10 μg/ml, were significantly greater than those of control colonies. Prolonged inhibitor activity resulted in increased branching, swollen or strongly tapered hyphal tips, formation of 'beaded' hyphae, and rupturing of hyphal walls when intrahyphal hyphae emerged. Abnormal fluorescent patches, representative of abnormal cell wall deposition, were present in propiconazole-treated hyphae. At the ultrastructural level, abnormal wall inclusions were observed after exposure to 1 μg/ml.

Infection cushions formed on glass coverslips from hyphae growing on propiconazole-amended medium at 0.25 to 5 μg/ml, were visibly more abundant than those formed from control hyphae. Although external morphology of infection structures was the same for all treatments, the cytoplasm showed signs of degeneration at 1 and 5 μg/ml. Often, penetration pegs underneath infection cushions on the inner surface of propiconazole-treated rice sheaths, did not penetrate the inner epidermis but developed into regular hyphae that continued to grow on the sheath surface. Collapsed hyphae occurred near deposits of propiconazole residue on the inner epidermis and, occasionally, directional growth away from a deposit was observed. At lower propiconazole concentrations, mycelial growth and infection cushion formation on the inner sheath surface were significantly greater than on control sheaths.
Single applications of propiconazole at the green ring stage of rice and sequential applications at the green ring and boot stage effectively reduced rice sheath blight incidence over time. Severity of sheath blight after green ring applications was significantly greater than in the nonsprayed control, whereas boot applications of propiconazole followed by benomyl, iprodione, or pencycuron at heading significantly reduced disease severity. Consistent positive yield responses compared with the control resulted from all boot and heading applications, whereas green ring applications generally resulted in a negative yield response.
INTRODUCTION

Sheath blight, caused by *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (Frank) Donk) anastomosis group 1 IA (AG 1 IA), is a major disease of rice in the southern United States. It has been estimated that states like Arkansas, Mississippi, and Missouri lose about 10 percent of their annual production to this disease. The losses run even higher in Texas and Louisiana. The disease also causes serious losses worldwide in both temperate and tropical rice-producing countries. Sheath blight depresses rice yield by reducing grain filling and by causing increased lodging. Control strategies for this potentially devastating disease have centered around the use of foliar fungicides due to the lack of monogenic resistance. In 1987, propiconazole (Tilt) became registered in the United States, except California, for disease control in rice. Propiconazole is a triazole compound belonging to a group of systemic fungicides that inhibit ergosterol biosynthesis. The ergosterol biosynthetic pathway in the targeted fungi is disrupted by blocking of C-14 demethylation of the precursor lanosterol.

Few biochemical studies on the mode of action of propiconazole have also examined morphological and ultrastructural responses to propiconazole treatment. Knowledge of how propiconazole interferes with growth and development of *R. solani* on rice is completely lacking. Yet, such information may contribute greatly to our understanding of sheath blight development under field conditions. Up to the present, control of sheath blight by propiconazole has mostly been evaluated based on determination of disease incidence and severity at the end of the growing season. However, disease assessment several times during the growing season is essential to understand
how disease progress is altered over time. Also, the effect of timing of application, an important factor in chemical control of sheath blight, can be assessed more precisely.

This dissertation describes several aspects of in vitro and in vivo activity of propiconazole against *R. solani* and the rice sheath blight disease. The principal objectives of the research were:
1) to determine, in vitro, the effects of propiconazole on mycelial growth, sclerotium formation and germination, and hyphal morphology and ultrastructure of *R. solani*.
2) to document, in vitro and in vivo, the qualitative and quantitative effects of propiconazole on infection structure formation by *R. solani*.
3) to study the effects of rate and timing of the application of propiconazole and selected comparison fungicides on sheath blight incidence and severity and the resultant grain yield of rice.

Light and fluorescence microscopy, scanning and transmission electron microscopy were employed to study aspects of the first and second objective. The results are presented in chapters 1 and 2, respectively. The third objective was accomplished by conducting an extensive 2-yr field study at the Rice Research Station in Crowley, LA. The results of this study are discussed in chapter 3.

The three chapters of this dissertation were written in manuscript form for submission to refereed journals. Chapters 1 and 2 will be submitted to *Mycologia* and *Phytopathology*, respectively. Chapter 3 is accepted for publication in *Plant Disease* and is scheduled to appear in the December, 1991 issue.
CHAPTER 1

IN VITRO EFFECTS OF PROPICONAZOLE ON GROWTH AND MORPHOLOGY OF RHIZOCTONIA SOLANI

INTRODUCTION

Propiconazole is a triazole compound belonging to a group of systemic fungicides that inhibit ergosterol biosynthesis (Kato, 1986). The ergosterol biosynthetic pathway in the targeted fungi is disrupted by blocking of C-14 demethylation of the precursor lanosterol (Buchenauer and Kemper, 1981; Weete et al., 1983).

Morphological responses of fungal cells to treatment with propiconazole have been described. Cultured yeast cells of Taphrina deformans became swollen and vacuolated within twelve to thirteen hours after treatment with 0.073 μg/ml propiconazole. They often formed linear and branched chains containing three to six cells (Sancholle et al., 1984). Propiconazole-treated sporidia of Ustilago avenae appeared swollen, multicellular and branched (Buchenauer and Kemper, 1981). Hyphae of Sclerotium rolfsii exhibited irregular growth and branching (Sancholle et al., 1984). Effects of propiconazole on fungal ultrastructure have been reported by Sancholle et al. (1988) for T. deformans and by Dahmen et al. (1988) for germ tubes of Puccinia graminis f. sp. tritici. Using video time-lapse light microscopy, Dahmen et al. (1988) also established a time course of early effects after fungicide treatment in relation to fungicide concentration.

The present study documents, in vitro, the activity of propiconazole against Rhizoctonia solani Kühn, the causal agent of rice sheath blight. Mycelial growth and sclerotium formation and germination were measured. Hyphal morphology
and wall deposition were investigated using fluorescence and light microscopy and scanning and transmission electron microscopy. Special emphasis was placed on responses occurring after prolonged fungicide exposure.

MATERIALS AND METHODS

Organism. Isolate LR 172 of *R. solani*, anastomosis group 1 IA (AG-1 IA), was originally isolated from naturally diseased Lebonnet rice from Acadia Parish, LA and was maintained on Difco potato dextrose agar (PDA, 39 g/L) at 25 C.

Fungicide and media preparation. Technical grade propiconazole ([2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole) was provided by Ciba-Geigy Corp., Greensboro, NC. Working stock solutions of propiconazole dissolved in 95% ethanol were prepared at concentrations ranging from 10 to 100,000 μg/ml. Appropriate amounts from the stock solutions were added to autoclaved PDA (50-60 C) to achieve concentrations of 0.01, 0.1, 0.5, 1, 5, 10, 25, 50, and 100 μg/ml. Control medium was prepared by adding 95% ethanol to PDA at an amount that ensured the same alcohol concentration (0.001%) as in the fungicide-amended medium.

Mycelial growth experiment. Fungicide-amended and control PDA was poured into 9-cm-diameter petri dishes and inoculated with mycelial plugs (4 mm diameter) obtained from the actively growing edge of 2-day old *R. solani* cultures on 2% water agar (WA). The inoculum plugs were inverted onto the center of three replicate plates per treatment, which were then incubated at 28 C in continuous dark. Maximum colony diameter was measured at 12-hr intervals up to 4 days after inoculation. The experiment was repeated three
times. Based on growth measurements recorded at 36 hr after inoculation, that is, when control colonies had grown about halfway across the plate, percent growth inhibition due to fungicide treatment was calculated and plotted as a function of log transformed concentrations. Linear regression was used to extrapolate concentrations that give 50% (EC$_{50}$) and 90% (EC$_{90}$) growth inhibition. Ten days after inoculation, mycelial plugs, exposed to propiconazole concentrations that caused 100% growth inhibition, were transferred to nonamended PDA to see if regrowth occurred.

**Sclerotium formation and germination experiment.** Sclerotia produced in control and fungicide-amended (0.01, 0.1, 1, 5, 10 µg/ml) plates were harvested 10 days after inoculation and dried in preweighed, loosely covered 5-cm diameter petri dishes for three weeks. For five plates per treatment, sclerotia from each plate were weighed and recorded. The experiment was repeated three times. In two experiments, fifteen sclerotia (2-3 mm diameter) per treatment were selected and placed on nonamended PDA (5 sclerotia per plate). The plates were kept at 28°C in continuous dark. After 24 hr incubation, the maximum colony diameters of mycelium from the germinating sclerotia were measured. Analysis of variance combined with Fisher's least significant difference (LSD) multiple comparison procedure was used to compare weight and colony diameter means. The analysis was performed using PROC GLM of the SAS computer package (SAS Institute, Inc., 1985).

**Scanning electron microscopy.** Mycelial plugs, exposed to propiconazole-amended PDA at 25, 50 and 100 µg/ml for 7 days, were removed and fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1.5 hr at room temperature. Samples were rinsed in 0.1 M phosphate buffer for 15 min and dehydrated in a graded ethanol series (50, 70, 80, 95, and 100%). Absolute
alcohol served as the intermediate fluid for the critical point drying procedure performed in a Denton DCP-1 apparatus. The dried specimens were mounted on aluminum stubs with double stick tape and coated with 30 nm (300 Å) of gold-palladium in a Hummer sputter coater. The mycelial plug side that was in contact with propiconazole-amended medium, was examined using a Hitachi S-500 or Cambridge S-260 scanning electron microscope. Hyphae growing from mycelial plugs on control PDA medium were prepared and observed in the same way. The experiment was repeated twice.

**Fluorescence and light microscopy.** For fluorescence microscopy, the procedure described by Roberson et al. (1989) was adapted with some modifications. Autoclaved dialysis membrane pieces (3-4 x 7-8 mm) were placed in a circle around a mycelial plug on low strength PDA medium (10 g/L Difco potato dextrose agar and 10 g/L Difco agar). This allowed sparser colony development with hyphae growing well separated over the membrane and generation of very few aerial hyphae. When the membrane pieces were just overgrown, hyphae on the sides were severed with a sharp razor blade. The colonized membrane pieces were picked up with forceps, placed on the agar surface near the side of the petri dish and left undisturbed for 1.5 hr. They were then transferred to control and propiconazole-amended PDA (1, 5, 10, 25, 50, 100 μg/ml) and removed 2, 7, 22, and 50 hr after inoculation. The colonized pieces were oriented vertically and lowered on a drop of distilled water on a microscope slide to let the hyphal mat float from the membrane. Removal of the membrane was necessary to avoid background reflective fluorescence. A 20 μl drop of the fluorescent brightener calcofluor white (Cellofluor, Polysciences Inc., Warrington, PA) at a 0.01% (w/v) concentration in water, was added to the drop with the hyphal mat. Calcofluor white binds to linear β-1,4-linked polymers
such as chitin, a major structural component of fungal cell walls (v. Sengbusch et al., 1983). A coverslip was placed over the mounted specimens which were then examined with a Leitz Ortholux II microscope, equipped with a Ploemopak fluorescence vertical illuminator. A filter block with excitation filter BP 355-425, dichroic mirror RKP 455, and barrier filter LP 460 was used for observation.

Hyphae from mycelial plugs exposed to 25, 50, and 100 µg/ml of propiconazole for 7 days, were mounted on microscope slides and stained with a drop of lactophenol cotton blue (1%) for 1 min. The staining solution was replaced with clear lactophenol for observation. The specimens were examined with a Leitz Ortholux II microscope using Nomarski interference-contrast optics.

Micrographs for both fluorescence and light microscopy were taken on Kodak T-Max film (100 ASA) with a Nikon automatic camera system connected to the microscope. Fluorescence micrographs of similar magnifications were printed under the same conditions.

**Transmission electron microscopy.** Autoclaved dialysis membrane strips (3-4 x 50-70 mm) were placed around a mycelial plug on the surface of propiconazole-amended (1 µg/ml) and control WA (2%). Five days after inoculation, colonized membrane strips were trimmed to 3-4 x 7-8 mm and left undisturbed on the agar surface for 1 hr. Freeze substitution, embedding, sectioning, and observation of the specimens were performed in the laboratory of Dr. Charles Mims, Department of Plant Pathology, University of Georgia. The procedures used were described in detail by Mims et al. (1988). Observations on two hyphal tips per treatment were recorded.
RESULTS

Effects on mycelial growth and sclerotium formation and germination.
Figures 1.1 to 1.4 illustrate effects of propiconazole on mycelial growth and sclerotium formation and germination of *R. solani*. Increasing concentrations of propiconazole resulted in increasing inhibition of mycelial growth compared with the nontreated control (Figs. 1.1 and 1.3). Increasing inhibition of growth was coupled with increasing branching and development of aerial hyphae (Fig. 1.3). Maximum colony diameter of control colonies and colonies grown on 0.01 \( \mu \text{g/ml} \) of propiconazole reached the border of the petri dish by 60 hr after inoculation (Fig. 1.1A). When exposed to 0.1 and 1 \( \mu \text{g/ml} \), colonies reached the border by 72 and 96 hr after inoculation, respectively. At 5 and 10 \( \mu \text{g/ml} \), colonies expanded very slowly and had not reached the edge of the petri dish by the end of the observation period (108 h). Colony growth at 5 and 10 \( \mu \text{g/ml} \) originated from aerial hyphae that developed from the inoculum plug, touched the medium, started branching profusely, and formed new aerial hyphae. Colonies expanded further by a repetition of this cycle. Mycelial plugs did not form colonies at propiconazole concentrations of 25 \( \mu \text{g/ml} \) and higher. When these plugs were transferred to nonamended PDA medium, only plugs that were exposed to 25 \( \mu \text{g/ml} \) were capable of forming a new colony. Figure 1.1B shows the inhibition of colony growth plotted against the log of propiconazole concentration, 36 hr after inoculation. Extrapolated EC\textsubscript{50} and EC\textsubscript{90} values were 0.093 and 4.4 \( \mu \text{g/ml} \), respectively.

Sclerotium formation occurred at propiconazole concentrations that permitted mycelial growth. In control, 0.01, and 0.1 \( \mu \text{g/ml} \) plates, sclerotia developed after the colony had reached the border of the petri dish. At 1, 5, and 10 \( \mu \text{g/ml} \),
Fig. 1.1. Effects of propiconazole on mycelial growth of *R. solani*. A. Maximum colony diameter in function of propiconazole concentration and time after inoculation. B. Linearized dosage-response curve, 36 hr after inoculation.
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Figs. 1.3-1.4. Effects of propiconazole on mycelial growth and sclerotium germination of *R. solani*. 1.3. Agar incorporation test showing increasing mycelial growth inhibition at increasing propiconazole concentrations (60 hr after inoculation). 1.4. Colony growth on nonamended PDA from germinating sclerotia obtained from cultures growing on control and propiconazole-amended PDA (70 hr after inoculation).
sclerotia formed before the colony reached the edge of the petri dish. Sclerotial weight per colony was significantly reduced ($P=0.05$) compared with the control by propiconazole treatment at 0.1, 1, 5, and 10 $\mu$g/ml (Table 1.1 and Fig. 1.2A). Sclerotia obtained from control and treated colonies all germinated when transferred to nonamended PDA. Twenty four hours after inoculation, colony diameter was positively correlated with propiconazole concentrations from which sclerotia were obtained (Fig. 1.2B). Average colony diameters were significantly greater ($P=0.1$) at 0.5, 1, 5, and 10 $\mu$g/ml than the average control diameter (Table 1.1 and Fig. 1.2B). Figure 1.4 shows representative colonies about 70 hr after inoculation. At that time, sclerotia from 10 $\mu$g/ml plates had formed colonies with an abundance of newly developing sclerotia, whereas, at lower propiconazole concentrations and in control plates, new sclerotia had not yet developed.

Effects on hyphal morphology and cell wall deposition. Scanning electron microscopic observation showed that control hyphae were mostly smooth and had fairly uniform diameters. At branch points, a constriction usually occurred (Fig. 1.5). Control hyphal tips were mostly tapered half-ellipsoidal, although, more globoid forms could also be observed. Seven-day exposure to propiconazole at 25, 50, and 100 $\mu$g/ml caused complete inhibition of colony growth from the mycelial plug but hyphae in the plug were excessively and irregularly branched with clearly shorter intervals between branches than for control hyphae (Figs. 1.6-1.8). Branches generally remained short. Often branch points originated near the apex of the parent hypha resulting in a dichotomous growth pattern (Figs. 1.6 and 1.8). Treated hyphae showed abnormal thickenings and constrictions (Fig. 1.8) and were often 'beaded', that is, shaped in the form of a series of short swollen segments (Figs. 1.7 and
Table 1.1. Analysis of variance of sclerotial weight per colony and colony diameter from germinating sclerotia obtained from control and propiconazole-amended PDA plates

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>Significance level</th>
<th>df</th>
<th>MS</th>
<th>Significance level</th>
</tr>
</thead>
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<tr>
<td>Model</td>
<td>17</td>
<td>0.0128</td>
<td>0.0001</td>
<td>13</td>
<td>90.559</td>
<td>0.0001</td>
</tr>
<tr>
<td>Experiment (Exp)</td>
<td>2</td>
<td>0.0023</td>
<td>0.3293</td>
<td>1</td>
<td>192.386</td>
<td>0.0692</td>
</tr>
<tr>
<td>Treatment (Tmt)</td>
<td>5</td>
<td>0.0389</td>
<td>0.0001</td>
<td>6</td>
<td>124.716</td>
<td>0.0935</td>
</tr>
<tr>
<td>Exp x Tmt (Error A)</td>
<td>10</td>
<td>0.0019</td>
<td>0.004</td>
<td>6</td>
<td>39.430</td>
<td>0.0067</td>
</tr>
<tr>
<td>Error B</td>
<td>72</td>
<td>0.0005</td>
<td></td>
<td>196</td>
<td>12.825</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>209</td>
<td></td>
<td>209</td>
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<td></td>
</tr>
</tbody>
</table>
Figs. 1.5-1.8. Scanning electron microscopy of *R. solani* control hyphae and hyphae exposed to propiconazole for 7 days. 1.5. Control hyphae with smooth surface, fairly uniform diameter and slight constrictions at branch points. x1740. 1.6. Propiconazole-treated (25 µg/ml) hyphae showing branches that arose near the apex of the parent hypha resulting in a dichotomous growth pattern. x840. 1.7. Propiconazole-treated (100 µg/ml) hyphae showing extremely short internodes between branches. Note tapered hyphal tips (arrowheads) and 'beaded' hypha (asterisk). x1050. 1.8. Propiconazole-treated (50 µg/ml) hyphae showing abnormal thickenings and constrictions. Note dichotomous branching pattern. x2160.
1.15). Figures 1.7 and 1.8 demonstrate the presence of strongly tapered hyphal tips. At all three propiconazole concentrations tested, ruptures, usually in inflated hyphal parts, could be observed (Fig. 1.9). Further observation indicated that the hyphal wall gradually ruptured when intrahyphal hyphae emerged (Figs. 1.10-1.15). Intrahyphal hyphae either emerged as branches in the form of small bulges (Figs. 1.10-1.12), or cracked open the old hypha by increasing in diameter (Fig. 1.11), or, occasionally, ruptured the old hyphal wall when several intrahyphal hypha had developed or met in the same compartment (Fig. 1.14). In many instances, bulging branches occurred close to a septum (Figs. 1.10-1.12). However, branches were capable of emerging from any location within a compartment. After breaching through the old hyphal wall, intrahyphal hyphae were able to form new branches (Fig. 1.15).

Fluorescence microscopy of calcofluor-stained hyphae 2, 7, and 22 hr after inoculation on control medium, showed mostly uniform cell wall deposition over the entire hypha, while septa, corners of branch points, newly developing and young branches, and areas of anastomosis, were strongly fluorescent (Figs. 1.16A, 1.17A, and 1.18A). Fluorescence of apical regions was variable. Hyphal apices were slightly brighter or not brighter in fluorescence than subapical regions of the hypha (Fig. 1.17A). Fifty hours after inoculation, the hyphal mat on the membrane was very dense due to abundant branching and anastomosis, but hyphal characteristics remained the same (not shown). Exposure to propiconazole for 2 hr at 1, 5, 10, 25, 50, and 100 μg/ml, resulted in brighter fluorescing hyphal tips that were often swollen and irregularly shaped (Fig. 1.16B and C). The zone of increased fluorescence intensity was usually sharply delimited (Fig. 1.16C). Burst hyphal tips were not observed. At 100 μg/ml, some bright fluorescing patches were scattered along the hyphae (Fig.
Figs. 1.9-1.15. Scanning electron microscopy of emerging intrahyphal hyphae of *R. solani* in response to 7-day-exposure to propiconazole at 25, 50, and 100 µg/ml. 1.9. Formation of a rupture. x4340. 1.10. Top view of an intrahyphal hypha emerging as a bulge, close to a septum (arrowhead). x8840. 1.11. Side view of an intrahyphal hypha emerging as a bulge, close to a septum (arrowhead). x5580. 1.12. Ruptured old hyphal wall with emergence of intrahyphal hyphae either as a bulge, or when increasing in diameter (arrowhead). x3780. 1.13. Branch of intrahyphal hypha breaching through old hyphal wall. Arrowhead points to a septum. x2480. 1.14. Multiple intrahyphal hyphae emerging from the same compartment. x2850. 1.15. Branch formation of intrahyphal hypha after emergence from old hypha. Arrowhead indicates point of emergence. Asterisks indicate 'beaded' hyphae. x1080.
Figs. 1.16-1.18. Fluorescence microscopy of calcofluor white-stained control hyphae of *R. solani* and hyphae grown on propiconazole-amended PDA. 1.16. After 2-hr incubation period. A. Control hyphae. Note fairly uniform fluorescence of hyphal walls and brightly fluorescent septa and corners of branch points. x 1730. B. Hyphae exposed to 25 μg/ml. Note irregularly shaped, swollen hyphal tips. x 1730. C. Hyphae exposed to 100 μg/ml. Note brightly fluorescent hyphal tips. Arrowheads point to patches of irregular wall deposition. Asterisk indicates empty hyphal compartment x 1730. 1.17. After 7-hr incubation period. A. Control hyphae. Arrowhead points to hyphal apex that is slightly more fluorescent than the subapical region. Arrow indicates a brightly fluorescent young branch. x 1730. B. Hyphae exposed to 5 μg/ml. Note numerous small fluorescent patches. x 1730. 1.18. After 22-hr incubation period. A. Control hyphae. Arrowhead points to brightly fluorescent newly developing branch. x 1730. B. Hyphae exposed to 1 μg/ml. All hyphae show numerous small fluorescent patches. Arrowheads indicate areas of anastomosis. x 1730.
Degenerating or empty hyphal compartments could be observed regularly at this concentration (Fig. 1.16C). Characteristic features for hyphae exposed to 1, 5, and 10 μg/ml, 7, 22, and 50 hr after inoculation, are shown in Figures 1.17B and 1.18B. Numerous small, randomly distributed, fluorescent patches were present in almost all hyphae on the dialysis membrane. They occurred in both the hyphal tip and other hyphal regions (Figs. 1.17B and 1.18B). Bright field microscopy of these hyphae showed them to be highly vacuolated (not shown). Some hyphae exposed to 25, 50 and 100 μg/ml for 7, 22, and 50 hr showed the same features. At these concentrations, however, larger but fewer patches could be observed (Figs. 1.19 and 1.24A). Brightly fluorescent, bulging branches were also more frequent (Fig. 1.20). The large irregular patches reacted strongly with cotton blue stain (Figs. 1.28-1.30). After 50-hr exposure to 25 and 50 μg/ml of propiconazole, branches with broad fluorescent bands were typically present (Figs. 1.21-1.23). They were occasionally observed at 100 μg/ml. These branches correspond with the 'beaded' branches observed with scanning electron microscopy (Figs. 1.7 and 1.15). Figure 1.21 shows the initiation of this type of branch. A faintly fluorescent hyphal apex extended from a subapical region that contained a more intensely fluorescing band. Repetition of this pattern apparently results in the formation of beaded branches. It is not clear whether a septum is present between two swellings. It may be obscured by the bright fluorescent band.

Fluorescence and light microscopy very well demonstrated the initiation and development of intrahyphal hyphae (Figs. 1.24-1.31). Based on numerous observations, a sequence of initiation could be compiled and is presented in Figure 1.24A-D. When the protoplasm in a hyphal compartment degenerated, the hemispherical area of the septal pore cap of the adjoining intact
Figs. 1.19-1.23. Fluorescence microscopy of calcofluor white-stained hyphae of *R. solani* grown on propiconazole-amended PDA. 1.19. Hyphae grown on 50 μg/ml for 50 hr. Note large bright fluorescent patches (arrowheads) in intensely fluorescing compartments. x1700. 1.20. Hyphae grown on 100 μg/ml for 22 hr. Note brightly fluorescing branches appearing as bulges close to a septum. x1700. 1.21. Hyphae grown on 50 μg/ml for 50 hr. Faintly fluorescing hyphal tips extend from brightly fluorescing subapical regions. x1700. 1.22. Hyphae grown on 25 μg/ml for 50 hr. 'Beaded' branches with intensely fluorescing bands. x1700. 1.23. Hyphae grown on 25 μg/ml for 50 hr. 'Beaded' intrahyphal branch with intensely fluorescing bands. x4000.
Figs. 1.24-1.27. Fluorescence microscopy of calcofluor white-stained hyphae and intrahyphal hyphae of *R. solani* grown on propiconazole-amended PDA. 1.24. Initiation of intrahyphal hyphae. A. Fluorescing septal pore cap (arrowhead) in intact compartment adjoining empty compartment. Asterisk denotes septum between two intact compartments. Also note brightly fluorescing patches in branch. x4060. B. Increased fluorescence of septal pore cap (arrowhead) and cross wall. x4060. C. Initiation of apex from the septal cross wall region, pushing aside the septal pore cap (arrowhead). x4060. D. Newly developed intrahyphal hypha. x4060. 1.25. Fluorescing septal pore caps adjoining empty compartments. x1675. 1.26. Intrahyphal hypha extending a branch through the old hyphal wall (arrowhead). Asterisk denotes a newly developed intrahyphal hypha. x1675. 1.27. Intrahyphal hypha and two outward branches developing from the same septal area (arrowhead). x1675.
Figs. 1.28-1.31. Light microscopy of lactophenol cotton blue-stained hyphae of *R. solani* exposed to 50 μg/ml of propiconazole for 7 days.

1.28. Newly developed intrahyphal hypha. Arrowhead indicates irregular wall deposition. x4500. 1.29. Intrahyphal hypha and branches developing from septal regions. Arrowheads indicate irregular wall depositions. x4500.

1.30. Intrahyphal hypha in empty apical compartment. Note short branches arising from septal regions. Arrowheads indicate irregular wall depositions. x1550. 1.31. Three intrahyphal hyphae developing in the same compartment. Arrowhead points to swollen septal pore cap. x4500.
compartment(s) started fluorescing intensely (Figs. 1.24A and 1.25). Then, coinciding with increasing fluorescence of this area, the septal cross wall appeared brightly fluorescent (Figs. 1.24B and 1.25). Later, a new apex was initiated from the septal cross wall region (Figs. 1.24C and D). It either pushed the septal pore cap aside (Fig. 1.24C), or grew constricted in between the pore cap and the lateral wall (Figs. 1.28 and 1.31). At times, the septal pore cap region appeared swollen (Figs. 1.19 and 1.31). Septal pore caps between two intact compartments were usually not visible with fluorescence microscopy (Fig. 1.24A). Figure 1.26 shows a further development of an intrahyphal hypha, forming a branch through the old hyphal wall. In Figure 1.27, an intrahyphal hypha together with two outward branches seemed to form from the same septal area. Branches arising from septal areas are also pictured in Figures 1.29 and 1.30. Two intrahyphal hyphae were able to develop from the same septal region (Fig. 1.31). This phenomenon, however, was observed only twice. Intrahyphal hyphae occurred at all propiconazole concentrations. Their frequency increased with increasing concentration because more compartments degenerated at higher concentrations. Occasionally, they were also observed in control hyphae.

Transmission electron microscopic observation of two hyphal tips exposed to 1 μg/ml of propiconazole for 5 days, demonstrated the presence of randomly scattered wall inclusions of various sizes and electron densities (Fig. 1.32A-E). Some inclusions were bound by a membraneous sheath (Fig. 1.32B and E). The plasma membrane subtending the inclusions was slightly invaginated (Fig. 1.32B-E). Walls of control hyphae did not reveal such inclusions (Fig. 1.32A).
Fig. 1.32. Transmission electron microscopy of the cell wall of apical and subapical regions of (A) control and (B-E) propiconazole-treated (1 μg/ml) hyphae of *R. solani*. A. Wall of control hyphal tip without inclusions. x90,100. B. Membrane-bound wall inclusions in subapical region. x105,000. C. Wall inclusions of different electron densities in apical region. Outer wall boundary is not resolved. x140,350. D. Irregularly shaped wall inclusions in apical region. x144,200. E. Vesicle (arrowhead) seemingly escaping through plasmamembrane in subapical region. Outer wall boundary is not resolved. x101,600. Bars = 0.1 μm.
DISCUSSION

Propiconazole was highly active against *R. solani* in vitro (Figs. 1.1 and 1.3). The EC$_{50}$ and EC$_{90}$ (0.093 and 4.4 μg/ml, respectively) determined in this study are in fair agreement with values reported by Jones et al. (1987) for 21 isolates of *R. solani* (EC$_{50}$=0.05 and EC$_{90}$=7.9 μg/ml). The EC$_{50}$ values for *R. solani* are within the lower range of concentrations obtained for propiconazole activity against other fungi (Sancholle et al., 1984; Whitson and Hine, 1986; Bowen and Pedersen, 1988; Dahmen et al., 1988).

Propiconazole did not prevent formation of sclerotia at those concentrations that permitted mycelial growth. The average sclerotial weight per culture plate was significantly reduced with increasing concentrations of propiconazole (Fig. 1.2A) but, in relation to total amount of mycelium, sclerotia formed earlier at 1 to 10 μg/ml plates than in control plates and at lower propiconazole concentrations. Because profuse branching of hyphae is a *sine qua non* of sclerotial primordium formation (Cooke, 1983), we postulate that sclerotia in plates with propiconazole at 1 to 10 μg/ml were induced by stimulated hyphal branching due to propiconazole treatment. Induction of primordia in colonies on control medium and at lower propiconazole concentrations occurred after mycelial growth was impeded by the edge of the petri dish. According to Cooke (1983), the presence of a mechanical barrier does not only stop hyphal extension, but also creates conditions in which metabolites rapidly accumulate within hyphae and/or the surrounding medium, all factors that may be involved in primordium induction.

Growth of germinating sclerotia harvested from propiconazole treatments was greater than growth of sclerotia harvested from the control (Figs. 1.2B and
The three week air drying period of sclerotia after harvesting may not be long enough to kill adhering hyphae. At increasing propiconazole concentrations, sclerotia developed deeper in the medium and seemed to have more adhering hyphae. Regrowth from these sclerotia might be faster if surrounding hyphae were not completely killed. Otherwise, a morphological and/or physiological change of sclerotia due to propiconazole treatment might be responsible for the observed effect.

The effects of propiconazole on hyphal morphology of *R. solani*, as demonstrated with scanning electron, fluorescence, and light microscopy, largely correspond to effects of propiconazole reported for *U. avenae* (Buchenauer and Kemper, 1981), *T. deformans* (Sancholle et al., 1984), and *S. rolfsii* (Sancholle et al., 1984). Similarly, various other compounds that inhibit sterol synthesis caused excessive and irregular branching, swollen or strongly tapered apices, and localized swellings of yeast cells, sporidia and filamentous fungi (Hippe, 1983; references in Kato, 1986; Roberson et al., 1989). The presence of fluorescent patches, representative of areas of abnormal cell wall deposition (Fuller et al., 1990; Roberson and Fuller, 1990), and abnormal wall inclusions, seen at the ultrastructural level, are again in accord with observations by numerous other investigators (Hippe and Grossmann, 1982; Kerkenaar and Barug, 1984; Richmond, 1984; Sancholle et al., 1988; Fuller et al., 1990; Roberson and Fuller, 1990).

In an excellent review by Vanden Bossche (1990) and in comprehensive discussions by Hippe (1984), Sancholle et al. (1988), Roberson et al. (1989), Steel et al. (1989), and Roberson and Fuller (1990), increased branching, abnormal wall depositions, and wall inclusions have been explained in view of the mode of action of sterol synthesis inhibitors. The lack of functional sterols
and/or accumulation of sterol intermediates, alters membrane structure (e.g., increased fluidity) and, directly or indirectly, membrane function (e.g., increased permeability, interference with activity of membrane-bound enzymes like chitin and glucan synthase). These effects likely trigger disruptions of and/or prevent a variety of interdependent cell functions, leading to growth inhibition and expression of abnormal morphological and ultrastructural features. The fact that responses such as excessive branching and abnormal wall deposition have also been described for various other growth inhibiting compounds with different modes of action, indicates that they may be general responses to inhibited growth (Roberson et al., 1989).

Effects of inhibitors of sterol synthesis differ with concentration (De Nollin and Borgers, 1975; Vanden Bossche et al., 1984). For propiconazole at fungistatic concentrations (1 and 10 µg/ml) there was no significant increase in electrolyte leakage from germinating uredospores of *P. graminis* f. sp. *tritici* (Dahmen et al., 1988). Many germ tube tips eventually ruptured. At increasing fungicidal concentrations (25, 50, 75, and 100 µg/ml), increasing electrolyte leakage was detected, accompanied by rapid cessation of germ tube elongation and cell death. Germ tube tips did not rupture. This phenomenon may explain why in the present study all *R. solani* hyphae exposed to 1, 5, and 10 µg/ml showed numerous sites of abnormal wall deposition starting at 7 hr after inoculation (Figs. 1.17 and 1.18), whereas, at the higher concentrations not all hyphae showed these patches. When such patches occurred, they were usually larger and less frequent (Figs. 1.16B, 1.19, and 1.28-1.30). Presumably, at these concentrations, many hyphal compartments quickly degenerated due to a direct disruption of the membrane systems, thereby largely destroying the mechanism(s) responsible for abnormal wall deposition. Burst hyphal tips at
lower concentrations were not observed. Osmotic pressure exerted by the propiconazole-amended PDA medium used in this study was likely lower than for the aqueous solutions used by Dahmen et al. (1988) for propiconazole treatment.

Interpretation of staining patterns obtained with calciofluor white is limited in the sense that the nature of its differential binding characteristics is unclear. This in turn limits interpretation of propiconazole activity. Calciofluor white binds to linear β-1,4-linked glucans such as cellulose, chitin, and chitosan (v. Sengbusch et al., 1983). *R. solani* and other Basidiomycetes do not contain cellulose or chitosan in their walls (Bartnicki-Garcia, 1968), so calciofluor staining here can be considered to be specific for chitin. However, as pointed out by Gull and Trinci (1974), high intensity fluorescence may be the result of increased availability of receptor sites, that is, limited or no chitin crystallinity, and/or reflection of a chemical composition that allows for abundant binding to chitin. Bright fluorescence of hyphal tips has been interpreted as indicative of active wall differentiation and hyphal tip extension (Gull and Trinci, 1974; v. Sengbusch et al., 1983; Roberson et al., 1989). This could be attributed to the presence of recently synthesized chitin in noncrystalline condition (Wessels et al., 1990), or, in view of the theory put forth by Bartnicki-Garcia (1973) and Gooday (1983), occurrence of higher lytic activity in the extending hyphal tip.

Apical regions of control hyphae usually did not fluoresce markedly brighter than subapical regions (Figs. 1.16A and 1.17A), contrary to previous reports for *Botrytis cinerea* (Gull and Trinci, 1974) and *S. rolfsii* (Roberson et al., 1989). It is not understood why this occurred. Fluorescence of many propiconazole-treated tips was a lot more intense than that of control tips, although, the apical zone of bright fluorescence was rather short and often sharply delimited. The
typically 'beaded' branches with bands of intense fluorescence observed in this study (Figs. 1.21-1.23), clearly present a challenge with respect to interpretation of intensely fluorescing areas. Figure 1.22 indicates that these branches are formed by alternating inhibited and resumed hyphal tip extension. It is also shown that the hyphal tips are swollen and intensely fluorescing by the time extension resumes. This condition seems to persist throughout formation of several more 'segments'. During inhibited hyphal tip extension, some degree of wall deposition may have continued, resulting in thicker walls with more chitin and, therefore, showing more intense fluorescence. This is in agreement with numerous observations of thickened cell walls after treatment with inhibitors of sterol synthesis (Hippe and Grossman, 1982; Hippe, 1984; Sanchoille et al., 1988; Fuller et al., 1990; Roberson and Fuller, 1990). On the other hand, the fact that the areas with a bright fluorescent band are slightly swollen, is perhaps indicative of a less rigid wall. We could refer here to Dahmen et al. (1988), who attributed the bursting of hyphal tips of *P. graminis* f. sp. *tritici* at low concentrations of propiconazole to a weakened physical strength of the germ tube wall. So, it cannot be excluded that chitin microfibril formation and/or cross-linking between chitin microfibrils and β-1,3-glucan (Wessels et al., 1990) was interfered with to some degree, or that the overall chemical composition of the wall was changed. These effects would result in an increased number of binding sites available for the fluorochrome marker and, thereby, result in intense fluorescence.

Upon prolonged exposure to propiconazole, *R. solani* developed intrahyphal hyphae, frequently observed at the higher propiconazole concentrations (25, 50, and 100 μg/ml) (Figs. 1.9-1.15 and 1.24-1.31). Formation of intrahyphal hyphae or even intra-intrahyphal hyphae has been documented by Butler and
Bracker (1970) in aged cultures of *R. solani*. In the present study, intrahyphal hyphae were occasionally observed in the control treatment. Thus, it seems that intrahyphal hyphae represent a response to senescence or injury. It should be pointed out, however, that intrahyphal hyphae formed in the presence of 50 and 100 μg/ml of propiconazole, were not vigorous enough to reestablish a colony after transfer to nonamended PDA.

The high frequency of occurrence of intrahyphal hyphae in propiconazole-treated hyphae made it possible to determine how they were initiated. The first visible event occurred at the dolipore septum of an intact compartment that adjoined a degenerating or empty, apical or intercalary, compartment with no apparent septal apparatus (Figs. 1.24A and 1.25). Fluorescence of the septal pore cap (Bracker and Butler, 1963) or parenthesome (Moore, 1984) enclosed region of the intact compartment seemed to increase gradually in intensity. This phenomenon may very well represent an event described by Todd and Aylmore (1984) based on ultrastructural examination of punctured hyphae of *Schizophyllum commune*. After degeneration of the septal apparatus of a punctured compartment of *S. commune*, the parenthesome of the intact compartment disappeared and the septal swellings deformed and fused over and around the pore plug to form a pad-like structure. This structure had staining properties similar to the rest of the cross-wall. Bracker and Butler (1963) and Butler and Bracker (1970) showed similar structures for *R. solani*. Although not much information is available on the composition of septal swellings, electron micrographs of dolipore septa of *R. solani* invariably show a continuation of the central chitin plate of the cross wall (Wessels and Sietsma, 1979) into the swellings (Bracker and Butler, 1963; Setliff et al., 1972; Tu et al., 1977). In *S. commune*, the chitin microfibrils are covered by a deposit of
R-glucan in the central part of the septal swelling. The periphery consists of highly hydrated material susceptible to β-glucanase (Wessels and Sietsma, 1979). Deformation of the septal swellings in the pad-like structure may cause exposure and/or differentiation of its chitin component. As a result, availability of binding sites for calcofluor would increase.

In the next step of the initiation process of intrahyphal hyphae, the entire cross wall became brightly fluorescent (Figs. 1.24B and 1.25). This probably coincides with the deposition of a new cross wall. Butler and Bracker (1970) reported walling off of intact hyphal compartments of *R. solani* by deposition of new wall material inside the existing lateral walls and across the septum. One of the micrographs in their report shows the pad-like structure described above, covered with a new wall and a membrane that is continuous with the plasma membrane along the cross wall. It would be most interesting to determine where this membrane originated. Perhaps the septal pore cap plays a functional role in its formation. The lateral walls near the septum did not show increased fluorescence in the present study. However, scanning electron microscope observations of small bulges breaching through the old hyphal wall close to a septum, indicate that new wall material was deposited there as well.

At some point, tip growth is initiated at either or both sides of the fluorescent pad-like structure (Figs. 1.24C and D). The new hypha either pushes the structure aside, or grows constricted in between the structure and the lateral wall. In a light and electron microscopy study, Calonge (1968) described a similar process of initiation of intrahyphal hyphae in *Sclerotinia fructigena*. In this fungus, the old, plugged, simple septum was pushed aside, with the plug disintegrating in front of the new growing tip. The studies by Calonge (1968) and Lowry and Sussman (1966), are, to our knowledge, the only studies on
ultrastructure of intrahyphal hypha initiation and development. The high frequency of intrahyphal hypha formation by *R. solani* when exposed to propiconazole, makes it an attractive system for detailed electron microscopic examination of this process.
LITERATURE CITED


CHAPTER 2

IN VITRO AND IN VIVO INFECTION STRUCTURE FORMATION BY RHIZOCTONIA SOLANI IN RESPONSE TO PROPICONAZOLE TREATMENT

INTRODUCTION

Propiconazole is one of the fungicides registered in the United States, except California, for control of rice sheath blight caused by Rhizoctonia solani Kühn (teleomorph: Thanatephorus cucumeris (Frank) Donk). The compound is highly inhibitory to mycelial growth of R. solani in vitro. Jones et al. (1987) reported average EC$_{50}$ and EC$_{90}$ values of 0.05 and 7.9 μg/ml, respectively, for 21 isolates of R. solani. Similar values were found in the present study (0.093 and 4.4 μg/ml, respectively) (Chapter 1). The biochemical mode of action of propiconazole against fungi has been identified as a disruption of ergosterol synthesis by blocking C-14 demethylation of the precursor lanosterol (Buchenauer and Kemper, 1981; Weete et al., 1983).

Initial symptoms of rice sheath blight occur as water-soaked greenish-grey lesions on the sheaths of lower leaves near the waterline (Lee and Rush, 1983). When microclimatic conditions are favorable, the pathogen spreads rapidly to upper plant parts, including leaf blades, by means of runner hyphae on the sheath surface. Secondary lesions are then formed (Lee and Rush, 1983). Most frequently, R. solani initiates lesion formation by penetration of the rice sheath by means of infection pegs that arise from the underside of specialized infection structures. Infection structures include lobate appressoria and infection cushions (Marshall and Rush, 1980a and b; Manian and Manibhushanrao, 1982; Matsuura, 1986). Entry of the host through stomata has also been
reported (Marshall and Rush, 1980a and b; Manian and Manibhushanrao, 1982).

The objectives of the present study were to determine the effects of propiconazole treatment on formation of infection structures by *R. solani*, in vitro, and to investigate protective activity of propiconazole against infection structure formation on rice sheaths. Qualitative and quantitative responses were investigated using light and scanning electron microscopy.

**MATERIALS AND METHODS**

**Organism.** Isolate LR 172 of *R. solani*, anastomosis group 1 IA (AG-1 IA), was originally isolated from naturally diseased Lebonnet rice from Acadia Parish, LA and was maintained on Difco potato dextrose agar (PDA, 39 g/L) at 25 C.

**Plant material.** Rice plants (*Oryza sativa* L. cv. Lemont) were grown in a mixture of steam-sterilized soil, sand and peat (2:1:1) in 15-cm-diameter plastic pots. The plants were kept on flooded benches in a greenhouse with natural light and day and night temperatures of 30±5 C and 26±3 C, respectively.

**In vitro study.** Technical grade propiconazole (1-\{(2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl\}1-H-1,2,4-triazole) was provided by Ciba-Geigy Corp., Greensboro, NC. Working stock solutions of propiconazole dissolved in 95% ethanol were prepared at concentrations ranging from 250 to 10,000 μg/ml. Appropriate amounts from the stock solutions were added to cooled (50-60 C), autoclaved full strength PDA (first experiment) or low strength PDA (10 g/L Difco potato dextrose agar and 10 g/L Difco agar) (second experiment) to obtain final concentrations of 0.25, 0.5, 1, and 5 μg/ml. Control medium was
prepared by adding 95% ethanol to PDA at an amount that ensured the same alcohol concentration (0.001%) as in the fungicide-amended medium.

Fungicide-amended and control PDA were poured into 9-cm diameter petri dishes and inoculated with mycelial plugs (4 mm diameter) obtained from the actively growing edge of 2-day old *R. solani* cultures on PDA. Four glass coverslip circles (10 mm diameter) were placed around the inoculum plug at a distance of 5 mm. The plates were incubated at 28 C in continuous dark. Seventy-two hr after inoculation, hyphae on the sides of the colonized glass coverslips were severed with a sharp razor blade. The coverslips were then removed and prepared for either light or scanning electron microscopy.

**Qualitative in vivo study.** Sheath pieces (7-8 mm long) were excised from the main tiller of 2-month old rice plants. The pieces were cut in half along the midrib and placed, outer surface up, on 60 μl sterile distilled water drops containing 0.25 and 1 μg/ml of propiconazole dissolved in 95% ethanol. The control treatment consisted of sterile distilled water drops containing the same amount of alcohol (0.001%) as the propiconazole drops. After 1 hr, the sheath pieces were removed from the drops and touched against filter paper to drain excess fluid. Four pieces per treatment were placed, outer surface up, around a mycelial plug of *R. solani* on PDA in 9-cm-diameter petri dishes. The plates were incubated at 28 C in continuous dark. Forty-eight hr (first experiment) and 60 hr (second experiment) after inoculation, the colonized sheath pieces were prepared for scanning electron microscopy.

**Quantitative in vivo study.** Forty days after seeding, rice plants were removed from the pots and most soil was removed from the root system by repeated submergence in water. Individual tillers were then transferred to plastic pots with nutrient solution prepared according to Yoshida et al. (1976).
Fifteen-cm-diameter styrofoam disks with 8-mm-diameter holes supported 6 tillers per pot. The culture solution was renewed twice a week. When abundant new roots had developed, about three weeks after transfer, the tillers were brought from the greenhouse to the laboratory for propiconazole treatment. Appropriate amounts of propiconazole stock solutions were added to 750 ml of nutrient solution in plastic pots to obtain concentrations of 5, 10, 25, and 50 μg/ml. Control nutrient solution contained the same ethanol concentration (0.001%) as the fungicide-amended solutions. The roots of 10 tillers per pot were submerged in the nutrient solution. After a 40-hr treatment period, 5-cm-long sheath pieces were excised from control and treated tillers and cut longitudinally into two strips. Twelve strips per treatment were inoculated with half of a 4-mm-diameter mycelial disk (1 mm thick) placed in the middle of the sheath strip on the inner epidermis. Another 12 strips per treatment were inoculated on the outer epidermis. The sheath strips were then placed, inoculated side up, on moist filter paper in petri dishes and incubated at 28 C. Hyphae were able to grow around the edges of the sheath towards the uninoculated side. Eight strips per treatment, 4 each from outer and inner surface inoculation, were cut in half after 1, 2, and 3-days of incubation. Half strips (25 mm long) were fixed and cleared in lactophenol-ethanol (1:2) at room temperature and prepared for light microscopic examination. The maximum length of mycelial growth (LMY), starting from the inoculum, and the length of areas with infection cushions (LIC) were measured on the outer (LMYO and LICO) and inner (LMYI and LICl) surface of all pieces. The experiment was repeated twice.

Statistical data analysis was performed using the procedures available in the SAS computer package (SAS Institute, Inc., 1985, 1986). Because observations
at each time were made on different sampling units, the data were analysed as a split-plot design with experiment (block), treatment, and inoculation assigned to the whole unit and time to the subunit. Fisher's least significant difference (Fisher's LSD) was calculated for multiple treatment comparisons when a main effect or interaction was significant.

**Light microscopy.** Colonized coverslips were stained with lactophenol cotton blue (1%) for 1 min, rinsed with and mounted in clear lactophenol. The specimens were examined with a Leitz Ortholux II microscope and a Wild-Heerbrugg Model M5A stereomicroscope using bright-field optics. Micrographs were taken on Kodak T-Max film (100 ASA) with a Nikon automatic camera system connected to the microscope. Rice sheath strips were stained with aniline blue (0.01%) in lactophenol for 1 min. After blotting off excess stain on filterpaper, the strips were rinsed in clear lactophenol and mounted in glycerol:distilled water (90:10). For observation and measurement of LMYs and LICs, a Nikon SMZ-10 stereomicroscope was used.

**Scanning electron microscopy.** In the first experiment, sheath pieces were fixed in FAA (37% formaldehyde:95% ethanol:glacial acetic acid:distilled water; 10:50:5:25) and kept overnight in the refrigerator. Colonized coverslips and sheath pieces from the second experiment were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1.5 hr at room temperature. The specimens were rinsed in 0.1 M phosphate buffer for 15 min and dehydrated in a graded ethanol series (50, 70, 80, 95, and 100%). Absolute alcohol served as the intermediate fluid for the critical point drying procedure performed in a Denton DCP-1 apparatus. The dried specimens were mounted on aluminum stubs with double stick tape and coated with 20 or 30 nm (200 or 300 Å) of gold-palladium
in a Hummer sputter coater. A Cambridge S-260 scanning electron microscope was used for observation.

RESULTS

**In vitro study.** Control hyphae and hyphae growing on propiconazole-amended medium at 0.25, 0.5, 1, and 5 μg/ml were all capable of forming lobate appressoria and infection cushions on glass coverslips after a 72-hr incubation period. Figure 2.1 shows representative coverslips colonized on control plates and plates containing 0.25, 1, and 5 μg/ml of propiconazole. Growth of control hyphae on the coverslips was sparse with some lobate appressoria and infection cushions beginning to develop (Fig. 2.1A). At 0.25 (Fig. 2.1B), 0.5 (not shown), and 1 μg/ml (Fig. 2.1C) of propiconazole, mycelial growth on the medium was increasingly inhibited but growth on the coverslips was increasingly denser. Lobate appressoria and infection cushions were also visibly more abundant. At 5 μg/ml (Fig. 2.1D), only about one fourth of each coverslip was overgrown because mycelial growth inhibition on the medium was high. However, abundant infection structures developed from the hyphal mat on the coverslip. At higher magnifications (Figs. 2.2 and 2.3), 5 μg/ml treated hyphae were highly vacuolated or granular. This effect also occurred at 1 μg/ml of propiconazole, but to a lesser extent (Fig. 2.4A). Control hyphae near the edge of the coverslip did not show signs of degeneration (Fig. 2.5). Closer to the center of the coverslip, both control and treated hyphae that were depleted of nutrients were empty or highly vacuolated (not shown). External morphology of lobate appressoria and infection cushions formed on coverslips from control and treated hyphae was the same. Light and scanning electron
Fig. 2.1A-D. Light microscopic view of glass coverslips overgrown with cotton blue-stained *R. solani* hyphae from colonies on control PDA (A) and PDA with propiconazole amended at 0.25 (B), 1 (C), and 5 (D) µg/ml. The mycelial inoculum plug was positioned to the right of the coverslips. x7.
Figs. 2.2-2.5. Light microscopy of *R. solani* hyphae and infection structures on glass coverslips overlaying control and propiconazole-amended PDA. 2.2. Portion of 5 μg/ml treated colony. Dense areas represent infection cushions. Arrowheads point to degenerating hyphae. X380. 2.3. Higher magnification of portion of 5 μg/ml treated colony showing vacuolated and granular hyphae. x1500. 2.4. Infection structures formed from 1 μg/ml treated hyphae. A. Focused on granular hyphae. Arrowhead points to lobate appressoria. B. Focused on underside of lobate appressoria and infection cushion (asterisk). x1500. 2.5. Control hyphae. x1500.
micrographs of top and bottom views of representative structures are shown in Figures 2.4B and 2.6A and B. Interestingly, penetration peg-like structures extended from the appressoria at the base of infection cushions (Fig. 2.6B).

**Qualitative in vivo study.** In the second experiment, 1-hr propiconazole treatment of the inner epidermis of detached sheath pieces at 0.25 and 1 μg/ml did not visibly alter the number of infection cushions formed on the inner surface compared with the control treatment. An example of the extent to which infection cushions developed on the inner surface of both control and treated pieces is given in Figure 2.7A. Infection cushions on both control and treated sheaths were seen to penetrate the inner epidermis. A view of the basal side of a detached infection cushion on 1 μg/ml treated sheaths (Fig. 2.7B) shows the scars where penetration pegs broke off of the appressoria. Figure 2.8 demonstrates an area of the inner epidermis of control sheaths where an infection cushion was removed. Numerous penetration pores and marks were visible. In some penetration pores, remains of broken penetration pegs were left behind. Penetration marks presumably represent sites where penetration was either not successful or not yet accomplished. Penetration pores at the junction of anticlinal walls of epidermal cells and stomatal guard cells were clearly larger. Also, the stomatal guard cells were remarkably free of penetration pores or even penetration marks.

Commonly on control and treated inner sheath surfaces, infection cushions were lifted up, revealing elongate, slender penetration pegs with constricted sites near the base of the appressoria (Fig. 2.9A). However, in many cases on propiconazole-treated sheaths, penetration pegs underneath lifted infection cushions did not penetrate the inner epidermis, but developed into regular hyphae that continued to grow on the sheath surface (Figs. 2.9B and C). Also
Figs. 2.6-2.9. Scanning electron microscopy of infection structure formation of control and propiconazole-exposed *R. solani* hyphae, in vitro on glass coverslips and in vivo on the inner epidermis of rice sheaths. 2.6. Top (A) and bottom (B) view of infection structures induced on glass coverslips. A. Control hyphae. Note lobate appressoria (arrowheads). Asterisks indicate infection cushions. x240. B. 5 μg/ml treated hyphae. Penetration pegs extend from appressoria at base of infection cushion (arrowheads). x700. 2.7. Top (A) and bottom (B) view of infection cushions on inner rice sheath surface. A. Infection cushions on 1 μg/ml treated sheath piece. x130. B. Infection cushion formed on 1 μg/ml treated sheath piece. Note scars where penetration pegs broke off of appressoria. Arrowhead indicates appressorium with two scars. x880. 2.8. Penetration pores and marks on control sheath where an infection cushion was removed. Note large penetration pores at junction of anticlinal walls of epidermal cells and stomatal guard cells (arrowheads). Note also absence of penetration marks on guard cells. x620. 2.9. Lifted infection cushions on control and treated sheath pieces. A. Control sheath. Elongate penetration pegs enter the epidermis. Arrowhead points to constricted region near base of appressorium. x840. B. 0.25 μg/ml treated sheath. Hyphae developed from within an infection cushion, seemingly lifting it up from the sheath surface. x 240. C. 1 μg/ml treated sheath. Hyphae developed from appressoria and continued to grow on the sheath surface (arrows). Asterisk denotes collapsed hyphae near residue deposit. Arrowhead points to directional hyphal growth away from the deposit. x250.
on treated sheaths, collapsed hyphae occurred near deposits on the epidermis that were probably areas of propiconazole residue. Figure 2.9C shows collapsed hyphae near a deposit and directional growth of a hypha away from the deposit. On another occasion, an infection cushion had developed up to the border of such a deposit and was tilted away from it (not shown).

In the first experiment, sheath pieces were rather closely touching the underlying agar medium so that few hyphae were able to grow on the inner surface. Only some lobate appressoria on treated and control sheaths were observed. On the outer sheath surface, 2 days after inoculation, infection cushions had not formed on control pieces and pieces treated with 0.25 μg/ml of propiconazole (Fig. 2.10A and B). They were abundantly present on the outer epidermis of 1 μg/ml treated sheath pieces (Fig. 2.10C). Mycelial growth on the treated pieces was visibly denser than on control pieces (Fig. 2.10). Observations from the second experiment, however, did not confirm these results. Infection cushions had developed on the outer surface of all sheath pieces and there were no apparent differences in number of infection cushions due to treatment.

Infection cushions and lobate appressoria developed on the outer epidermis of both vein ribs and intercostal regions (Fig. 2.11). Infection cushions on the outer intercostal regions were usually less sharply delimited than those formed on the inner surface (Figs. 2.7A and 2.11), partly because hyphae tended to grow erratically in between the papillae of the outer epidermis (Fig. 2.12). As shown in Figures 2.12 and 2.13, hyphae were capable of considerable constriction when making their way through closely positioned papillae. When encountering a leaf hair on the outer epidermis, hyphae formed appressoria and started growing parallel to the hair shaft (Fig. 2.14) or coiled around it.
Figs. 2.10-2.14. Scanning electron microscopy of control and propiconazole-exposed hyphae of *R. solani* on the outer epidermis of rice sheaths. 2.10. (A) Control, (B) 0.25 µg/ml, and (C) 1 µg/ml treated sheath pieces. Note denser mycelial growth on treated pieces and infection cushion formation at 1 µg/ml. x50. 2.11. Infection cushion formation on vein rib (arrowhead) and intercostal regions (asterisks) of 1 µg/ml treated sheath piece. x180. 2.12. Hyphal growth on 1 µg/ml treated sheath piece. Note wax-covered epidermis with papillae. Arrowheads indicate constricted growth in between papillae. Asterisk denotes base of leaf hair. x630. 2.13. Constricted growth in between papillae surrounding a stoma. x1120. 2.14. Colonization of a leaf hair. Wax crystals on epidermis were dissolved during preparation procedure. x930.
Collapse of the shaft was not necessarily due to fungal penetration because collapsed uncolonized leaf hairs were observed as well. Penetration sites beneath appressoria on the outer sheath surface were not detected.

**Quantitative in vivo study.** Analysis of variance of length of mycelial growth and length of areas with infection cushions on the inner and outer sheath surface, indicated no significant interactions ($P=0.05$) of the block (experiment) effect with the other main effects. For all variables the main effects behaved the same in the two experiments. The block interactions were, therefore, pooled in the sampling errors (Table 2.1).

Few infection cushions formed on the outer epidermis, even when inoculated on the outer sheath surface. Consequently, no significant main effects or interactions were detected for LICO (Table 2.1). The time by inoculation interactions for LICI and LMYO were significant ($P=0.01$) (Table 2.1). This means that for these variables the main effect inoculation behaved differently over time. Figure 2.15A shows that, 1 day after inoculation, inner surface inoculation resulted in significantly more infection cushions on the inner surface than outer surface inoculation. Likewise, length of mycelial growth on the outer surface, 1 day after inoculation, was significantly higher after outer inoculation than after inner inoculation (Fig. 2.15B). Although the same trend occurred for LMYI (Fig. 2.15C), the overall time by inoculation interaction was not statistically significant (Table 2.1).

Significant treatment by inoculation interactions were detected for both LMYI and LICI ($P=0.05$) (Table 2.1). Thus, for some treatment(s), mycelial growth and infection cushion formation behaved differently with outer or inner inoculation. Figure 2.16A shows that, at 5 and 25 μg/ml of propiconazole, average length of mycelial growth on the inner epidermis was significantly
Table 2.1. Analysis of variance source table for length of mycelial growth and length of areas with infection cushions on the inner and outer surface of control and propiconazole-containing rice sheaths after inoculation with *R. solani* on the inner and outer surface

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>LMYI (mm)</th>
<th>LiCl (mm)</th>
<th>LMYO (mm)</th>
<th>LICO (mm)</th>
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<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>Experiment (Exp)</td>
<td>1</td>
<td>1037.50</td>
<td>15.25**</td>
<td>161.70</td>
<td>6.18*</td>
</tr>
<tr>
<td>Treatment (Tmt)</td>
<td>4</td>
<td>382.55</td>
<td>5.62**</td>
<td>114.69</td>
<td>4.38**</td>
</tr>
<tr>
<td>Inoculation (Inoc)</td>
<td>1</td>
<td>1470.15</td>
<td>21.61**</td>
<td>5.40</td>
<td>0.21</td>
</tr>
<tr>
<td>Tmt x Inoc</td>
<td>4</td>
<td>176.70</td>
<td>2.60**</td>
<td>79.29</td>
<td>3.03*</td>
</tr>
<tr>
<td>Error A</td>
<td>69</td>
<td>68.03</td>
<td></td>
<td>26.17</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>1653.90</td>
<td>33.96**</td>
<td>336.61</td>
<td>20.35**</td>
</tr>
<tr>
<td>Time x Tmt</td>
<td>8</td>
<td>55.60</td>
<td>1.14</td>
<td>21.55</td>
<td>1.30</td>
</tr>
<tr>
<td>Time x Inoc</td>
<td>8</td>
<td>97.63</td>
<td>2.00</td>
<td>96.38</td>
<td>5.82**</td>
</tr>
<tr>
<td>Time x Tmt x Inoc</td>
<td>8</td>
<td>96.57</td>
<td>1.98</td>
<td>16.34</td>
<td>0.99</td>
</tr>
<tr>
<td>Error B</td>
<td>140</td>
<td>48.70</td>
<td></td>
<td>16.54</td>
<td></td>
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<tr>
<td>Total</td>
<td>239</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Length of mycelial growth (LMY) and length of areas with infection cushions (LiCl) on the inner (LMYI; LiClI) and outer (LMYO; LiCO) sheath surface.

** A main effect or interaction is significant at $P=0.05$ or $P=0.01$ when indicated with * and **, respectively.

x Propiconazole was added to nutrient solution at 5, 10, 25, and 50 μg/ml and taken up through the roots of ten tillers per treatment.

y Half a mycelial disk was placed on the inner or outer epidermis of 25-mm-long sheath strips.

z Measurements were taken 1, 2, and 3 days after inoculation.
Fig. 2.15. Length of areas with infection cushions on the inner sheath surface (A) and length of mycelial growth on the outer sheath surface (B) of rice as a function of inner (I) or outer (O) surface inoculation and incubation time. Values are means of 40 observations. Means within each time period indicated with the same letter are not significantly different according to Fisher's LSD ($P=0.05$).
Fig. 2.16. Length of mycelial growth on the inner (A) and outer (C) sheath surface and length of areas with infection cushions on the inner (B) sheath surface of rice as a function of inner (I) or outer (O) surface inoculation and propiconazole concentration. Values are means of 24 observations. Means within each inoculation indicated with the same letter are not significantly different according to Fisher’s LSD ($P=0.05$).
higher than the control when inoculated on the outer surface, but was not significantly different from the control when inoculated on the inner epidermis. The same effect was observed at 5 and 10 μg/ml for infection cushion formation (Fig. 2.16B). However, a significantly higher amount of infection cushions was also formed after inner inoculation of 25 μg/ml treated sheaths compared with control sheaths (Fig. 2.16B). Average length of mycelial growth in this case was also higher than the control, but not statistically significant (Fig. 2.16A). At 50 μg/ml, reduced mycelial growth and complete inhibition of infection cushion formation on the inner epidermis were observed after outer surface inoculation, whereas these variables were not significantly different from the control values after inner surface inoculation (Fig. 16A and B). The treatment by inoculation interaction was not significant for LMYO (P = 0.05). With increasing propiconazole concentration, mycelial growth on the outer surface decreased for both inner and outer inoculation (Fig. 2.16C). The overall treatment effect, however, was not significant (Table 2.1).

**DISCUSSION**

Lobate appressoria and infection cushions of *R. solani* were induced on glass coverslips. This has also been reported for another *R. solani* isolate from rice (Manian and Manibhushanrao, 1982). The ability to form infection structures on coverslips implies that *R. solani* isolates from rice are capable of forming infection structures in the absence of host exudates or host surface features. The external appearance of the infection cushions on coverslips was nearly identical to those formed on the inner rice sheath epidermis although the
developmental sequence of infection cushion formation in vitro (Armentrout and Downer, 1987) was not examined in detail in the present study.

At all propiconazole concentrations tested, hyphae growing on amended medium were capable of forming infection structures on a fungicide-free glass coverslip surface. Growth of hyphae on PDA was increasingly inhibited with increasing propiconazole concentration, but infection structure formation on the coverslips was visibly greater compared with the control (Fig. 2.1). This may reflect the fact that control hyphae produced fewer aerial branches than treated hyphae which appeared more aerial with increasing propiconazole concentrations. As a consequence, it was physically more difficult for control hyphae to overcome the rim of the coverslip and colonize it. Coverslips on control medium were colonized more in the first experiment where full-strength PDA was used than in the second experiment where low-strength PDA was employed.

On the other hand, propiconazole-treated hyphae exhibited a high degree of branching which is a formative step in the development of lobate appressoria and infection cushions (Dodman and Flentje, 1970; Armentrout et al., 1987). Promotion of branching may indirectly promote infection structure formation, provided that the stimulus necessary for their induction is present. Because propiconazole also causes significant growth inhibition, treated hyphae would not be expected to develop overall more infection structures than control hyphae, but rather to produce infection structures earlier. This agrees with observations from the qualitative in vivo study. Two days after inoculation, infection cushions had developed on the outer surface of 1 μg/ml treated sheath pieces and not on control pieces (Fig. 2.10). In this respect, it is important to keep in mind that the outer sheath surface did not come in contact
with propiconazole during treatment. Although the sheath pieces were blotted
on filter paper between propiconazole treatment and placement on agar
medium, some remains of fungicide had diffused to the medium. This was
discernable through slightly inhibited colony growth from the inoculum plug.
Hyphae were thus exposed to propiconazole before growing on the sheath
pieces. In a second experiment, where observations were made 2.5 days after
inoculation, there were no apparent differences in infection cushion formation
due to treatments. Presumably, any earlier differences were no longer visually
distinguishable.

Although penetration peg-like structures were observed on the underside of
in vitro induced infection cushions from treated hyphae, it is not clear that actual
penetration and/or colonization of a fungicide-free substrate would be
successful. At higher concentrations (1 and 5 μg/ml), hyphae and infection
structures showed signs of degeneration (Figs. 2.2-2.4). This may point to
partial or complete penetration and/or colonization inability. Infection cushions
produced on the inner surface of propiconazole-containing sheath pieces were
seen to penetrate the epidermis. Because degree of penetration and
colonization was not assessed, conclusions cannot be made in regard to
quantitative differences with the control treatment. However, signs of protective
activity of propiconazole may be present in the observation that penetration
pegs underneath lifted infection cushions did not penetrate the inner epidermis,
but developed into regular hyphae that continued growing on the sheath
surface (Fig. 2.9B and C). Lifting of infection cushions was probably the result of
this process. Lifted cushions were also observed on control inner sheaths
where the lifting may have occurred when more and more branches developed
within the cushion and thereby raised the whole cushion. Curative activity of
propiconazole was evidenced by collapsed hyphae near residue deposits and occasional directional growth away from such deposits (Fig. 2.9C).

In the quantitative in vivo study, propiconazole treatment at 25 μg/ml and lower concentrations resulted in significantly more mycelial growth and infection cushion formation on the inner sheath surface than the control treatment (Fig. 2.16). In contrast, in vitro mycelial growth at low propiconazole concentrations was inhibited compared with growth on control medium (Chapter 1). It is hypothesized that, when propiconazole is present in the sheath at concentrations high enough to prevent or inhibit penetration and/or colonization, this could result in increased mycelial growth on the sheath surface, either through penetration pegs that develop as regular hyphae and continue to grow on the surface (as shown in the qualitative in vivo experiment), or through formation of runner hyphae as it occurs in normal disease development. This assumes that, when penetration and/or colonization is limited, sufficient nutrients are available on the sheath surface to support hyphal growth. Secondly, increased branching in response to contact with propiconazole, either on the sheath surface (curative activity) or at the time of penetration or colonization (protective activity), could increase the potential for infection structure formation, as discussed for the in vitro study in this chapter. The degree to which these processes would occur, will very much depend on the balance of propiconazole concentration in and on the sheath. At sufficiently high concentrations, growth inhibition would be so strong that neither event would occur.

The fact that lower propiconazole concentrations caused significantly more mycelial growth and infection cushions on the inner surface compared with the control in combination with upper surface inoculation, but not in combination
with inner surface inoculation (Fig. 2.16), is puzzling. The aforementioned hypothesis cannot solely offer a plausible explanation for this observation, suggesting that other factors may be involved. For instance, host-related factors may play a role. Propiconazole could have some effect on the host, expressed at the outer surface, which would lead hyphae to grow towards the inner surface and develop profusely. Mycelial growth on the outer surface after outer inoculation indeed tended to decrease with increasing propiconazole concentration. Another explanation would be that propiconazole is somehow more available at the outer epidermis than at the inner epidermis, resulting in the observed effect. Information on how propiconazole is distributed in the plant after uptake through the roots or where it is located would be helpful in this respect. Also, differences in the nature of outer and inner epidermis may be of importance.

Comparison of the results of this study with published reports is limited because, as far as we are aware, only two accounts of fungicide effects on infection structure formation of *R. solani* are available. Hirooka et al. (1989) found inhibition of hyphal growth and infection cushion formation in similar experiments with flutolanil-treated rice sheaths. Likewise, Kataria and Grover (1975) reported inhibition of infection cushion formation on hypocotyls of bean seedlings when the roots were dipped in benomyl, thiophanate-methyl, chloroneb, and PCNB. These fungicides have different modes of action than propiconazole and this may account for the fact that increased mycelial growth and infection cushion formation did not occur. In the study by Hirooka et al. (1989), flutolanil concentrations that could cause increased growth and infection cushion formation may not have been tested. Similarly, the highest propiconazole concentration tested in the present study was apparently not
high enough to inhibit mycelial growth and infection structure formation on the inner epidermis after inner inoculation.

In rice field trials, it was observed that early applications of propiconazole caused increased sheath blight severity later in the season compared with the nonsprayed control (Van Eeckhout et al., 1991). This effect was interpreted as an indication of decreased microbial antagonism attributable to fungicidal activity. Because exclusion of phylloplane microorganisms was not attempted in the present study, this hypothesis cannot be rejected. The results merely point to other factors that may be responsible for the observed increased sheath blight severity, namely, concentration-dependent direct effects of propiconazole on *R. solani* and/or undetermined indirect effects through interference with a host component. Additional and independent experimentation based on the working hypotheses provided in this and the previous report, is strongly recommended. This will hopefully elucidate which factors are involved in this undoubtedly complex system.
LITERATURE CITED


CHAPTER 3

EFFECTS OF RATE AND TIMING OF PROPICONAZOLE APPLICATIONS ON INCIDENCE AND SEVERITY OF SHEATH BLIGHT AND GRAIN YIELD OF RICE

INTRODUCTION

Sheath blight, caused by Rhizoctonia solani Kühn (Thanatephorus cucumeris (Frank) Donk) anastomosis group 1 IA (AG-1 IA) (Ogoshi, 1987), is a major disease of rice (Oryza sativa L.) in the United States (Lee and Rush, 1983; Groth et al., 1990). It has been estimated that states like Arkansas, Mississippi, and Missouri lose about 10% of their annual production to this disease. The losses run even higher in Louisiana and Texas (Groth et al., 1988). The disease also causes serious losses worldwide in both temperate and tropical rice-producing countries (Manibhushanrao et al., 1979; Ou, 1985). Sheath blight depresses rice yield by reducing grain filling and by causing increased lodging (Groth et al., 1988). Major factors associated with the increased occurrence of sheath blight include the widespread cultivation of high-yielding but highly susceptible long-grain cultivars, the high tillering capacity of these mostly semi-dwarf cultivars that creates a favorable microclimate for disease development, and the tendency to apply higher rates of nitrogen fertilizers resulting in increased susceptibility (Lee and Rush, 1983). Another important contributing factor, relevant to the rice-producing areas of the southern United States, is the use of soybean (Glycine max (L.) Merr.) as a rotation crop with rice. Aerial blight of soybean, caused by the same intraspecific group of R. solani, sustains sheath blight epidemics in rice by increasing the numbers of sclerotia in field soil that serve as primary inoculum (O’Neill et al., 1977; Belmar et al., 1987).
In the United States, control strategies for this potentially devastating disease have centered around the use of foliar fungicides (Lee and Courtney, 1981; Whitney, 1983; Groth et al., 1990). Due to the absence of satisfactory levels of resistance in existing cultivars or the ineffectiveness and/or impracticality of recommended cultural control measures, rice growers are restricted to the use of chemicals for disease management (Groth et al., 1990).

Benomyl (Benlate), iprodione (Rovral), propiconazole (Tilt), thiabendazole (Folatec), and copper plus sulfur (Top-Cop) are currently registered for commercial use in the United States. Evaluation of these fungicides for sheath blight control performance (Lee and Courtney, 1981; Jones et al., 1987; Groth et al., 1990) are based on determination of disease incidence and estimation of visual canopy damage (0-9 scale) (International Rice Research Institute, 1975; Groth et al., 1990). These assessments are usually made once at the end of the growing season.

Although these measures are adequate and efficient for field screening of new products, disease assessment at several times during the growing season is essential to understand how fungicides impact disease progress over time. Disease progress curves can be used to approximate the length of residual fungicidal activity. Also, the effect of timing of application, an important factor in chemical control of sheath blight (Chin and Bhandhufalck, 1990), can be assessed more precisely when disease measurements are made throughout the season. Despite these benefits, no published accounts are available on how sheath blight epidemics are affected by fungicides used in the United States.

Time-course evaluations require a disease assessment method suitable for weekly assessments. Traditional sheath blight ratings, determined at the whole plot level, do not fulfill this requirement, so other methods must be adopted.
Results here are reported from a 2-yr study on the effects of rate and timing of application of propiconazole and other selected fungicides on sheath blight incidence over time, disease severity over time, and grain yield of rice. A sheath blight severity assessment method suitable for this type of study is described.

MATERIALS AND METHODS

The experiments were conducted at the Louisiana State University Rice Research Station, Crowley, LA, during the 1989 and 1990 growing seasons. Experimental units were plots 1.2 by 2.4 m (1989) and 1.2 by 4.9 m (1990) consisting of seven drill strips with 18-cm row spacings and 35-cm alleys. The plots were drill-seeded with rice cv. Lemont on 28 April 1989 and 24 April 1990 with 112 kg of seed per hectare. At planting, 670 kg/ha of 20-10-10 (1989) or 13-13-13 (1990) NPK fertilizer was drilled over the test area. Propanil (Prostar 4E) at 4.5 kg a.i./ha (1989) or propanil plus bentazone (Basagran) at 0.75 kg a.i./ha (1990) was applied for weed control immediately before applying the permanent flood. In 1989, the plots received a 112 kg/ha application by airplane of 21% ammonium sulfate fertilizer 1 wk after the green ring (GR) stage (first internode starting to elongate). In both years, carbofuran (Furadan 3G) at 0.6 kg a.i./ha was applied 1 wk after flooding to control the rice water weevil (Lissorhoptrus oryzophilus Kushel).

**Inoculation.** At maximum tillering stage (5 June 1989 and 11 June 1990), the plots were inoculated with *R. solani* isolate LR 172 grown for 20-30 days on an autoclaved rice grain/rice hull (1:2) mixture. Approximately 40 ml of inoculum
(13 ml/m²) was distributed evenly over each plot in 1989. In 1990, the amount of inoculum per square meter was doubled to increase the potential for a higher disease pressure.

**Fungicide application.** Propiconazole (Tilt 3.6 EC) was applied at 0.18, 0.32, and 0.48 kg a.i./ha at either GR stage (15 June, 1989 and 1990), early boot (B) stage (1- to 5-cm panicle in the boot) (2 July 1989 and 29 June 1990), or sequentially at GR and B. Propiconazole was also applied as a B spray (0.32 kg a.i./ha) followed by a heading (H) (70-80% of panicles emerged) (17 July 1989 and 18 July 1990) application of either benomyl (Benlate 50 DF) at 0.57 kg a.i./ha, iprodione (Rovral 50 WP) at 0.57 kg a.i./ha, or pencycuron (Monceren 50 WP) at 0.18 kg a.i./ha. Sequential sprays at B and H of both benomyl and pencycuron were also included (Table 1). The chemicals were applied in water at 189 L/ha with a backpack CO₂-sprayer equipped with a two-nozzle boom.

Nonsprayed, inoculated or noninoculated plots served as control treatments. The treatments were arranged in a randomized complete block design with six (1989) or five (1990) replications.

**Disease assessment and yield determination.** Twenty-five tillers were randomly chosen at each of four locations per plot and the number of diseased tillers was counted. Disease incidence was then expressed as the percentage of diseased tillers out of 100 tillers per plot. Incidence was assessed weekly from GR to maturity in control plots and plots that received a GR fungicide application. For treatments that did not include a GR application, weekly incidence assessments started at the B stage. These treatments were 'control' treatments up to the B stage; therefore, disease incidence progress before the B stage was assumed to be the same as that of the control treatment.
Tillers with early developing, watersoaked lesions on the lower first and/or second sheath near the waterline were flagged. In 1990, five tillers per plot were flagged at GR and monitored up to maturity for the control treatment and those treatments that included a GR propiconazole application. In addition, four (1989) or five (1990) tillers per plot were flagged at the B stage and monitored up to maturity for all treatments. For each flagged tiller, the height of the uppermost sheath lesion and the total sheath height were measured from the soil level. Disease severity at each assessment was determined by expressing lesion height as a percentage of the total sheath height.

Three days (1989) or 1 day (1990) before harvest, each plot was given a whole-plot 0-9 rating where 0 represented no disease and 9 indicated plots with most of the plants dead at maturity. A detailed description of this rating system is given by Groth et al. (1990).

On 21-22 August 1989 and 16 August 1990, the center four rows of each plot (1.5 and 3.5 m², respectively) were harvested with a small-plot combine. Yields were recorded after correction of rough grain weights to 12% moisture.

**Data analysis.** Disease progress curves for incidence and severity were plotted separately using the weekly assessment data. The area under the disease progress curves (AUDPC) was calculated by trapezoidal integration as described by Shaner and Finney (1977). AUDPC values were divided by the total duration of the assessment period and are referred to as relative AUDPC (rAUDPC). For the treatments for which disease incidence was not assessed before the B stage, the disease incidence values of the control treatment before the B stage were used to theoretically extend the incidence progress curves over the entire assessment period. Relative AUDPCs for incidence could then be directly compared statistically over all treatments.
Statistical data analysis was performed using the procedures available in the SAS computer package (SAS Institute, Inc., 1985, 1986). Analysis of variance (ANOVA) combined with Fisher's least significant difference (LSD) multiple comparison procedure were used to compare treatment rAUDPC and yield means. To compare treatment incidence, severity, and sheath height means at each assessment time within each year, the data were analyzed with repeated measures ANOVA. Incidence and sheath height data were normally distributed; severity data, however, were not. Transformation \([\text{arcsine}(y^{1/2}), \log(y), \log(y^{1/2})]\) of the severity values (Madden, 1986) failed to normalize the data distributions and stabilize variances. Therefore, nonparametric analyses were performed using PROC RANK and PROC GLM. However, because means obtained by these procedures have no biological meaning, raw means were calculated. Because variations in initial disease severity (disease severity of the tillers at the time of flagging) could be attributable to the variation in subsequent severity assessments or AUDPC values, initial disease severity was considered as a covariable. When the covariable was statistically significant in the overall models, adjusted treatment means were obtained by the LSMEANS statement in PROC GLM (SAS Institute, Inc., 1986).

The need for correction for serial correlation (Madden, 1986) was determined based on the sphericity tests obtained through the REPEATED statement in PROC GLM (SAS Institute, Inc., 1986). Where appropriate, Greenhouse-Geisser (G-G) adjusted probabilities were adopted (SAS Institute, Inc., 1986). Fisher's LSD was calculated for multiple treatment comparisons when a main effect or interaction was significant. PROC CORR was used to determine Pearson's correlation coefficients (SAS Institute, Inc., 1985).
RESULTS

Fungicide effects on disease progress measured as incidence. The treatment by time interaction in the overall repeated measures ANOVA model was significant in both years with G-G \( P=0.0018 \) in 1989 and G-G \( P=0.0001 \) in 1990. Fisher's LSD \( (P=0.05) \) was used to compare treatments at individual assessment times, as indicated in Figures 3.1 and 3.2.

In both test years, disease incidence was consistently lower in plots treated with propiconazole at the GR stage compared with nonsprayed plots (Fig. 3.1). Incidence was significantly reduced in plots receiving sequential applications at GR and B stages until maturity (Fig. 3.1C and D), except for the 0.18 kg a.i./ha treatment in 1989 when a rapid disease incidence increase occurred near maturity (Fig. 3.1C). Single propiconazole applications at GR also significantly reduced disease incidence over time, although the reductions were generally not as great as those observed in sequentially sprayed plots (Fig. 3.1A and B). Relative AUDPC values for the single and sequential applications were significantly different \( (P=0.05) \) from rAUDPC of the control curves in both years (Table 3.1).

Single boot applications of propiconazole, propiconazole at boot followed by a heading spray with benomyl, iprodione, or pencycuron, and sequential applications of benomyl at B and H failed to significantly reduce disease incidence over time in 1989 (Fig. 3.2A and C, Table 3.1). In 1990, these treatments tended to slow development of the epidemic (Fig. 3.2B and D), although rAUDPCs were only significantly reduced with single B applications of propiconazole at 0.32 and 0.48 kg a.i./ha and the combination of propiconazole and iprodione at B and H (Table 3.1). Pencycuron applied at B and H almost
Fig. 3.1. Effects of rate and timing of fungicide application on sheath blight incidence in Lemont rice in (A and C) 1989 and (B and D) 1990. Percentage of diseased tillers determined from counts of 25 tillers at each of four randomly chosen locations per experimental unit. Means are from six (1989) and five (1990) plots per treatment. Treatment codes: control = no fungicide application; pro = propiconazole at 0.18, 0.32, or 0.48 kg a.i./ha; GR = green ring growth stage; B = boot growth stage. Arrows indicate timing of application. Day 0 = inoculation day. Day 77-78 (1989) and 66 (1990) = harvest day. Vertical bars indicate Fisher's LSD (P=0.05); ns = nonsignificant.
Fig. 3.2. Effects of rate and timing of fungicide application on sheath blight incidence in Lemont rice in (A and C) 1989 and (B and D) 1990. Percentage of diseased tillers determined from counts of 25 tillers at each of four randomly chosen locations per experimental unit. Means are from six (1989) and five (1990) plots per treatment. Treatment codes: control = no fungicide application; pro = propiconazole at 0.18, 0.32, or 0.48 kg a.i./ha; ben = benomyl at 0.57 kg a.i./ha; ipr = iprodione at 0.57 kg a.i./ha; pen = pencycuron at 0.18 kg a.i./ha; B = boot growth stage, H = heading growth stage. Arrows indicate timing of application. Dotted lines represent theoretical disease incidence progress for the B and B+H treatments based on the incidence values of the control treatment. Day 0 = inoculation day. Day 77-78 (1989) and 66 (1990) = harvest day. Vertical bars indicate Fisher's LSD (P=0.05); ns = nonsignificant.
Table 3.1. Sheath blight incidence and severity in Lemont rice plots sprayed with foliar fungicides at different rates and at different plant growth stages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (kg a.i./ha)</th>
<th>Timing</th>
<th>Mean percent diseased tillers at maturity</th>
<th>Mean disease rating at maturity</th>
<th>rAUDPC for disease incidence (GR to maturity)</th>
<th>rAUDPC for disease severity (B to maturity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated control</td>
<td>5 f</td>
<td>12 f</td>
<td>0.5 e</td>
<td>1.2 e</td>
<td>68.5 ab</td>
<td>65.8 cd</td>
</tr>
<tr>
<td>Inoculated control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propiconazole</td>
<td>0.18 GR</td>
<td>85 a-d</td>
<td>92 a-c</td>
<td>4.3 a-c</td>
<td>46.1 b-d</td>
<td>75.0 ab</td>
</tr>
<tr>
<td></td>
<td>0.32 GR</td>
<td>76 cd</td>
<td>65 a-d</td>
<td>4.5 a-c</td>
<td>38.7 de</td>
<td>50.6 c-e</td>
</tr>
<tr>
<td></td>
<td>0.48 GR</td>
<td>78 b-d</td>
<td>80 a-e</td>
<td>5.2 a</td>
<td>43.8 e</td>
<td>42.9 e</td>
</tr>
<tr>
<td></td>
<td>0.18 B</td>
<td>92 ab</td>
<td>95 e</td>
<td>4.3 a-c</td>
<td>59.3 a</td>
<td>67.2 ab</td>
</tr>
<tr>
<td></td>
<td>0.32 B</td>
<td>90 a-c</td>
<td>85 a-d</td>
<td>3.8 c</td>
<td>52.6 a-c</td>
<td>56.7 b-d</td>
</tr>
<tr>
<td></td>
<td>0.48 B</td>
<td>92 ab</td>
<td>80 a-e</td>
<td>3.7 c</td>
<td>56.2 ab</td>
<td>55.1 b-e</td>
</tr>
<tr>
<td></td>
<td>0.18 GR+B</td>
<td>91 a-c</td>
<td>78 b-e</td>
<td>5.0 ab</td>
<td>38.2 de</td>
<td>47.4 de</td>
</tr>
<tr>
<td></td>
<td>0.32 GR+B</td>
<td>72 d</td>
<td>75 de</td>
<td>4.5 a-c</td>
<td>35.4 e</td>
<td>42.6 e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propiconazole</td>
<td>+ benomyl +0.57 B+H</td>
<td>83 a-d</td>
<td>83 a-d</td>
<td>2.5 d</td>
<td>50.2 de</td>
<td>52.9 e</td>
</tr>
<tr>
<td></td>
<td>+ iprodione +0.57 B+H</td>
<td>78 b-d</td>
<td>67 de</td>
<td>1.8 d</td>
<td>53.6 a-c</td>
<td>53.5 b-e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propiconazole</td>
<td>+ pencycuron +0.18 B+H</td>
<td>87 a-d</td>
<td>80 a-e</td>
<td>2.2 d</td>
<td>58.7 a</td>
<td>60.4 a-d</td>
</tr>
<tr>
<td></td>
<td>+ pencycuron +0.18 B+H</td>
<td>78 b-d</td>
<td>76 c-e</td>
<td>2.0 d</td>
<td>52.5 a-c</td>
<td>63.3 a-c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benomyl</td>
<td>0.57 B+H</td>
<td>76 b-d</td>
<td>76 c-e</td>
<td>1.7 d</td>
<td>37.9 de</td>
<td>61.8 a-d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pencycuron</td>
<td>0.18 B+H</td>
<td>52 e</td>
<td>63 e</td>
<td>1.5 d</td>
<td>39.3 e</td>
<td>44.5 f</td>
</tr>
</tbody>
</table>

u Fungicides were applied at the green ring (GR), boot (B), and/or heading (H) growth stage of rice.

v Percentage of diseased tillers determined from counts of 25 tillers at each of four randomly chosen locations per experimental unit. Means are from six and five plots per treatment in 1989 and 1990, respectively.

w Ratings determined at the whole-plot level based on a scale of 0 = no disease to 9 = severe disease (Groth et al., 1990). Means are from six and five plots per treatment in 1989 and 1990, respectively.

x Area under disease progress curve was calculated based on percentage of diseased tillers determined at weekly intervals from green ring (GR) stage to maturity. Calculated areas were divided by the total duration of the assessment period (58 days in both years), yielding relative values in the range of 0-100.

y Relative area under disease progress curve based on percent lesion height determined on 24 and 25 rice tillers per treatment in 1989 and 1990, respectively, at weekly intervals from boot (B) stage to maturity or from green ring (GR) stage to maturity in 1990. Total assessment duration was 41 and 44 days, respectively. Relative AUDPCs for tillers at B stage in both years were adjusted for initial percent lesion height according to analysis of covariance using PROC GLM and the LSMEANS statement available in the SAS computer package (SAS Institute, Inc., 1985, 1986).

z Means in columns followed by the same letter are not significantly different according to Fisher's LSD (P=0.05).
stopped disease incidence increase through maturity in 1989 (Fig. 3.2C). In 1990, this treatment also prevented an increase in disease incidence over time, but the rAUDPC was not significantly different from the control (Table 3.1). This can be attributed to the unusually high numbers of diseased tillers before the B application compared with the control disease incidence at that time (Fig. 3.2D).

**Fungicide effects on disease progress measured as severity.** Disease severities decreased over time when sheath height increased, whereas lesion height remained the same or increased at a lower rate (Figs. 3.3-3.5). When sheath heights had reached maximum size (about day 45 in 1989 and day 50 in 1990), disease severities either leveled off or increased. Figure 3.3C and D shows sheath growth of control and treated tillers flagged at GR in 1990. For the tillers flagged at B in both years, sheath growth curves were very similar. Repeated measures analysis of sheath heights over time indicated no significant differences for any of the treatments in both years. Therefore, treatment comparison using disease severities as proportions of the growing host was valid.

Because the treatment by time interaction was highly significant (G-G $P=0.0001$ for tillers flagged at B in both years; G-G $P=0.0043$ for tillers flagged at GR in 1990), multiple comparisons of disease severities at individual assessment times were performed using Fisher's LSD ($P=0.05$) (Figs. 3.3-3.5). Initial severity, entered in the repeated measures model as a covariable, was significant in all analyses with $P=0.0001$ for tillers flagged at B in both years and $P=0.0241$ for tillers flagged at GR in 1990. Analysis of covariance of rAUDPCs for the tillers flagged at B in both years also showed initial severity to be significant as a covariable ($P=0.0001$ in 1989 and $P=0.0267$ in 1990). Therefore, data in Figures 3.3-3.5 and rAUDPCs for severity (B to maturity) in Table 3.1
Fig. 3.3A and B. Effects of rate and timing of fungicide application on severity of sheath blight in Lemont rice in 1990. Lesion height is expressed as percentage of total sheath height and was determined on 25 rice tillers per treatment from green ring growth stage to maturity. Treatment means on each day were adjusted for initial disease severity according to analysis of covariance using PROC GLM and the LSMEANS statement available in the SAS computer package (SAS Institute, Inc., 1985, 1986). Vertical bars indicate Fisher's LSD (P=0.05); ns = nonsignificant. C and D. Sheath height of the rice tillers on which disease severity was monitored. There were no significant differences in sheath height over time for any of the treatments (P=0.05). Vertical bars indicate standard deviations. Treatment codes: control = no fungicide application; pro = propiconazole at 0.18, 0.32, or 0.48 kg a.i./ha; GR = green ring growth stage; B = boot growth stage. Arrows indicate timing of application. Day 0 = inoculation day. Day 66 = harvest day.
Fig. 3.4. Effects of rate and timing of fungicide application on severity of sheath blight in Lemont rice in (A and C) 1989 and (B and D) 1990. Lesion height is expressed as percentage of total sheath height and was determined on 24 (1989) and 25 (1990) rice tillers per treatment from boot growth stage to maturity. Treatment means on each day were adjusted for initial disease severity according to analysis of covariance using PROC GLM and the LSMEANS statement available in the SAS computer package (SAS Institute, Inc., 1985, 1986). Treatment codes: control = no fungicide application; pro = propiconazole at 0.18, 0.32, or 0.48 kg a.i./ha; GR = green ring growth stage; B = boot growth stage. Arrows indicate timing of application. Day 0 = inoculation day. Day 77-78 (1989) and 66 (1990) = harvest day. Vertical bars indicate Fisher's LSD (P=0.05); ns = nonsignificant.
Fig. 3.5. Effects of rate and timing of fungicide application on severity of sheath blight in Lemont rice in (A and C) 1989 and (B and D) 1990. Lesion height is expressed as percentage of total sheath height and was determined on 24 (1989) and 25 (1990) rice tillers per treatment from boot growth stage to maturity. Treatment means on each day were adjusted for initial disease severity according to analysis of covariance using PROC GLM and the LSMEANS statement available in the SAS computer package (SAS Institute, Inc., 1985, 1986). Treatment codes: control = no fungicide application; pro = propiconazole at 0.18, 0.32, or 0.48 kg a.i./ha; ben = benomyl at 0.57 kg a.i./ha; ipr = iprodione at 0.57 kg a.i./ha; pen = pencycuron at 0.18 kg a.i./ha; B = boot growth stage; H = heading growth stage. Arrows indicate timing of application. Day 0 = inoculation day. Day 77-78 (1989) and 66 (1990) = harvest day. Vertical bars indicate Fisher's LSD (P=0.05); ns = nonsignificant.
represent treatment means adjusted for initial severity, as obtained by the LSMEANS statement in PROC GLM (SAS Institute, Inc., 1986).

In 1990, monitoring of tillers flagged at GR showed that the application of propiconazole at this growth stage slowed disease progress up the tiller for about 2 wk (Fig. 3.3A). When B applications of propiconazole followed the GR applications (Fig. 3.3B), severities were only slightly reduced compared with no B application (Fig. 3.3A). In fact, around the H growth stage, lesions progressed upward at a faster rate on treated than on control tillers resulting in a significantly higher disease severity at maturity for the sequential 0.18 kg a.i./ha applications. This response also occurred after the single GR applications (Fig. 3.3A), but severities of control and treated tillers were not significantly different at maturity. None of the treatment rAUDPC values were significantly different from the control (Table 3.1). The initial decrease in severity compensated for the increase at the end of the growing season.

On tillers that were recently diseased when flagged at the B stage in 1990 and subjected to the same single or sequential propiconazole applications (Fig. 3.4B and D), the rapid increase in severity started earlier and reached significantly \(P=0.05\) higher levels than on control tillers. Relative AUDPCs for these treatments were significantly higher than for the control curve (Table 3.1). For tillers flagged at the B stage in 1989, the same general trends were observed, except that with increasing rates of single GR application, severities later in the season were increasingly higher (Fig. 3.4A). In 1990, there was no such differential response (Fig. 3.4B).

When the effects of sequential applications of propiconazole (Fig. 3.4C and D) are compared with the effects of single B applications (Fig. 3.5A and B), it shows that disease severity over time following a B application is affected by a
preceeding GR application. About 3 wk after the B spray (day 53 in 1989, day 39 in 1990), mean disease severity for tillers that were treated previously at GR was approximately equal to the mean control severity. In contrast, on tillers that were not previously sprayed at GR, mean disease severity 3 wk after B application was lower than on control tillers. This effect was significant for the 0.32 and 0.48 kg a.i./ha rate. The B applications were actually rendered less effective by a preceeding GR application. In 1989, single 0.32 and 0.48 kg a.i./ha B sprays of propiconazole significantly reduced disease severity up to maturity (Fig. 3.5A and Table 3.1). In 1990, fungicidal activity from single B applications did not restrict disease development up to maturity (Fig. 3.5B and Table 3.1). The single 0.18 kg a.i./ha B application resulted in the same adverse effect on disease progress as discussed for GR applications.

Fungicide applications at H after a B spray suppressed severities up to maturity (Table 3.1 and Fig. 3.5C and D). Relative AUDPCs for all B and H applications were significantly lower than the control value (Table 3.1). A boot spray of propiconazole followed by either benomyl, iprodione, or pencycuron successfully suppressed upward lesion progression in both years. Two applications of benomyl gave similar results. The decrease in sheath blight severity after a B application of pencycuron lasted about 1 wk longer than the decrease after the 0.32 kg a.i./ha rate of propiconazole applied at B.

Disease assessment at maturity. Disease ratings determined at the whole-plot level and percent diseased tillers at maturity are given in Table 3.1. They reflect the same general trends as discussed for the severity and incidence data over time. Percent diseased tillers at maturity was highly significantly correlated with rAUDPC for incidence (r = 0.54 in 1989; r = 0.65 in 1990) (Table 3.2). Disease ratings were highly significantly correlated with rAUDPC for severity on tillers.
Table 3.2. Correlation coefficients and probability levels for sheath blight assessments and yield of Lemont rice sprayed with foliar fungicides

<table>
<thead>
<tr>
<th>Factor</th>
<th>rAUDPC severity&lt;sup&gt;1&lt;/sup&gt; (B to M)</th>
<th>Yield</th>
<th>Percent diseased tillers&lt;sup&gt;u&lt;/sup&gt; at maturity</th>
<th>Disease rating at maturity&lt;sup&gt;v&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1989&lt;sup&gt;w&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAUDPC incidence&lt;sup&gt;x&lt;/sup&gt;</td>
<td>-0.18</td>
<td>0.07</td>
<td>0.54</td>
<td>-0.13</td>
</tr>
<tr>
<td>(GR to M)</td>
<td>0.1036</td>
<td>0.5323</td>
<td>0.0001</td>
<td>0.2584</td>
</tr>
<tr>
<td>rAUDPC severity</td>
<td>-0.20</td>
<td>0.15</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>(B to M)</td>
<td>0.0701</td>
<td>0.1729</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>-0.22</td>
<td></td>
<td>-0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0503</td>
<td></td>
<td>0.0585</td>
<td></td>
</tr>
<tr>
<td>Percent diseased tillers at maturity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td></td>
<td>0.0040</td>
<td></td>
</tr>
<tr>
<td><strong>1990&lt;sup&gt;y&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAUDPC incidence</td>
<td>-0.24</td>
<td>0.19</td>
<td>0.65</td>
<td>-0.06</td>
</tr>
<tr>
<td>(GR to M)</td>
<td>0.0522</td>
<td>0.1158</td>
<td>0.0001</td>
<td>0.6062</td>
</tr>
<tr>
<td>rAUDPC severity</td>
<td>-0.72</td>
<td>0.35</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>(B to M)</td>
<td>0.0001</td>
<td>0.0034</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>-0.29</td>
<td></td>
<td>-0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0189</td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Percent diseased tillers at maturity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td></td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td><strong>1990&lt;sup&gt;z&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAUDPC severity</td>
<td>0.13</td>
<td>0.03</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>(GR to M)</td>
<td>0.4889</td>
<td>0.8566</td>
<td>0.4367</td>
<td>0.3404</td>
</tr>
<tr>
<td>rAUDPC severity</td>
<td>-0.49</td>
<td>0.07</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>(B to M)</td>
<td>0.0075</td>
<td>0.7204</td>
<td>0.0266</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Relative area under disease progress curve based on percent lesion height determined on four and five tillers per plot in 1989 and 1990, respectively, at weekly intervals from boot (B) growth stage to maturity (M).

<sup>u</sup> Number of infected tillers out of 25 counted at each of four randomly chosen locations in each plot.

<sup>v</sup> Ratings determined at the whole-plot level based on a scale of 0 = no disease to 9 = severe disease (Groth et al., 1990).

<sup>w</sup> Correlation coefficients based on 81 observations (all treatments included).

<sup>x</sup> Relative area under disease progress curve based on percentage of tillers infected determined at weekly intervals from green ring (GR) growth stage to maturity (M).

<sup>y</sup> Correlation coefficients based on 66 observations (all treatments included).

<sup>z</sup> Correlation coefficients based on 29 observations (control treatment and treatments receiving GR application included).
flagged at B ($r=0.66$ in 1989; $r=0.81$ in 1990) (Table 3.2). In contrast, severity determined over time on tillers flagged at GR in 1990 was not significantly correlated with disease rating. Based on 29 observations, the correlation coefficient for rAUDPC for severity data from GR to maturity with disease rating was not significant, whereas rAUDPC for severity from B to maturity and disease rating were significantly correlated (Table 3.2). In both years, disease rating and percent diseased tillers at maturity were significantly positively correlated with $r=0.32$ in 1989 and $r=0.45$ in 1990. Relative AUDPCs for sheath blight incidence and severity were negatively correlated (Table 3.2).

**Yield response.** In 1989, none of the yields were significantly different (Table 3.3). Disease pressure was relatively low that year, and yield variation among plots within treatments was high. Variation coefficients ranged from 10 to 29%. Nevertheless, a positive yield increase was obtained for all B and H applications (Table 3.3). Yield was negatively correlated with rAUDPC for sheath blight severity, disease rating, and percent diseased tillers at maturity (Table 3.2).

In 1990, coefficients of variation within treatments were considerably lower (1.4 to 11%). Plots treated singly at GR, B, or GR plus B generally yielded less than the inoculated control plots (Table 3.3). This effect was significant only with the single 0.18 kg a.i./ha GR application of propiconazole. Significant yield increases over the control were obtained from plots receiving a B spray of propiconazole at 0.32 kg a.i./ha followed by either benomyl or pencycuron at H and by two applications of pencycuron (Table 3.3). Yield was highly significantly negatively correlated with rAUDPC for disease severity on tillers flagged at B and with disease rating ($r=-0.72$ and $-0.75$, respectively) (Table 3.2). Relative AUDPC for disease severity on tillers flagged at GR was not correlated with yield.

Correlation of yield with percent diseased tillers at maturity was significantly
Table 3.3. Plot yields of Lemont rice inoculated with *R. solani* and sprayed with foliar fungicides at different rates and at different plant growth stages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (kg a.i./ha)</th>
<th>Timingx</th>
<th>Yieldy (kg/ha)</th>
<th>Change from inoculated control (kg/ha)</th>
<th>Yieldy (kg/ha)</th>
<th>Change from inoculated control (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1989</td>
<td></td>
<td>1990</td>
<td></td>
</tr>
<tr>
<td>Noninoculated control</td>
<td></td>
<td></td>
<td>6149 a(^z)</td>
<td>+458</td>
<td>8215 bc</td>
<td>+698</td>
</tr>
<tr>
<td>Inoculated control</td>
<td></td>
<td></td>
<td>5691 a</td>
<td></td>
<td>7517 d-g</td>
<td></td>
</tr>
<tr>
<td>Propiconazole 0.18 GR</td>
<td>0.18</td>
<td>GR</td>
<td>6065 a</td>
<td>+374</td>
<td>6635 h</td>
<td>-882</td>
</tr>
<tr>
<td>Propiconazole 0.32 GR</td>
<td>0.32</td>
<td>GR</td>
<td>5474 a</td>
<td>-217</td>
<td>7378 e-g</td>
<td>-138</td>
</tr>
<tr>
<td>Propiconazole 0.48 GR</td>
<td>0.48</td>
<td>GR</td>
<td>5606 a</td>
<td>-85</td>
<td>7058 gh</td>
<td>-458</td>
</tr>
<tr>
<td>Propiconazole 0.18 B</td>
<td>0.18</td>
<td>B</td>
<td>5920 a</td>
<td>+229</td>
<td>7498 c-g</td>
<td>-19</td>
</tr>
<tr>
<td>Propiconazole 0.32 B</td>
<td>0.32</td>
<td>B</td>
<td>5366 a</td>
<td>-325</td>
<td>7450 e-g</td>
<td>-67</td>
</tr>
<tr>
<td>Propiconazole 0.48 B</td>
<td>0.48</td>
<td>B</td>
<td>5085 a</td>
<td>-606</td>
<td>7116 gh</td>
<td>-401</td>
</tr>
<tr>
<td>Propiconazole 0.18 GR+B</td>
<td>0.18</td>
<td>GR+B</td>
<td>5691 a</td>
<td>0</td>
<td>7271 f-h</td>
<td>-246</td>
</tr>
<tr>
<td>Propiconazole 0.32 GR+B</td>
<td>0.32</td>
<td>GR+B</td>
<td>5084 a</td>
<td>-627</td>
<td>7130 gh</td>
<td>-386</td>
</tr>
<tr>
<td>Propiconazole 0.32 + benomyl + 0.57 B+H</td>
<td>0.32</td>
<td>GR+B</td>
<td>6068 a</td>
<td>+377</td>
<td>8371 ab</td>
<td>+854</td>
</tr>
<tr>
<td>Propiconazole 0.32 + iprodione + 0.57 B+H</td>
<td>0.32</td>
<td>GR+B</td>
<td>6167 a</td>
<td>+476</td>
<td>8047 b-e</td>
<td>+530</td>
</tr>
<tr>
<td>Propiconazole 0.32 + pencycuron + 0.18 B+H</td>
<td>0.32</td>
<td>GR+B</td>
<td>6062 a</td>
<td>+371</td>
<td>8392 ab</td>
<td>+875</td>
</tr>
<tr>
<td>Benomyl 0.57 B+H</td>
<td>0.57</td>
<td>B+H</td>
<td>6870 a</td>
<td>+1179</td>
<td>8145 b-d</td>
<td>+628</td>
</tr>
<tr>
<td>Pencycuron 0.18 B+H</td>
<td>0.18</td>
<td>B+H</td>
<td>5905 a</td>
<td>+214</td>
<td>8467 ab</td>
<td>+950</td>
</tr>
</tbody>
</table>

\(x\) Fungicides were applied at the green ring (GR), boot (B), and/or heading (H) growth stage of rice.

\(y\) Yields are averages over six plots per treatment in 1989 and five plots per treatment in 1990.

The harvested area per plot was 1.5 m\(^2\) in 1989 and 3.5 m\(^2\) in 1990.

\(z\) Means in columns followed by the same letter are not significantly different according to Fisher's LSD (\(P=0.05\)).
negative with $r = -0.29$ (P=0.019). In both years, yield was not significantly correlated with rAUDPC for disease incidence (Table 3.2).

**DISCUSSION**

The assessment method used to monitor sheath blight severity over time proved suitable for demonstrating the effects of fungicide application on disease progress up the tiller. Significant correlations of rAUDPC for severity on tillers flagged at B with disease rating at maturity and with plot grain yield suggested that monitoring of lesion heights on four or five tillers per experimental unit from B stage to maturity represented disease development at the whole-plot level. In contrast, lack of such correlation for tillers flagged at GR indicated that monitoring of tillers that became diseased around GR stage was less representative for disease development at the whole-plot level. A possible explanation for this would be that the frequency of tillers that become diseased at GR is smaller than at B, as shown in the incidence progress curves, so that disease severity progress on tillers that became diseased around the B stage would be more representative for the whole plot.

Application of propiconazole at GR successfully delayed disease incidence increase. Without a GR spray, approximately 40% of the tillers were infected at the B stage. B applications of propiconazole could do little to prevent further disease progress. A boot application of propiconazole combined with a H application of benomyl, iprodione, or pencycuron suppressed disease incidence during the grain filling stages of rice in 1990 but not in 1989. The same happened for B and H applications of benomyl. B application of pencycuron in both years stopped disease progress almost completely, even though applied at
only two-thirds of the recommended rate, and a subsequent H spray kept incidence low through maturity. Pencycuron is a protectant fungicide, and the possibility for being redistributed downward may be higher than for the systemic fungicides propiconazole and benomyl. This would give greater protection of the lower sheaths at application times, such as B and H, when fungicide penetration into the canopy is limited by the upper foliage.

Although beneficial in reducing sheath blight incidence over time, GR applications of propiconazole resulted in increased severity compared with the control. Disease severity increased more rapidly on treated tillers than on nontreated tillers following the loss of fungicidal activity. This response was consistent in both 1989 and 1990 and was also observed during a preliminary experiment in 1988 (E. Van Eeckhout, unpublished). We interpret these results as an indication of decreased microbial antagonism attributable to fungicidal activity. Propiconazole is a broad-spectrum fungicide (Ciba-Geigy Agricultural Division, 1987; Groth et al., 1990) that will probably affect many nontarget fungi. Riesen and Close (1987) demonstrated that significantly fewer isolates of the endophytic fungi Didymella phleina Punith and Årvoll, Alternaria spp., and Cladosporium spp. were obtained from barley leaf blades after a first propiconazole treatment. Significantly more isolations of Alternaria spp., Epicoccum purpurascens Ehrenb. and Stemphylium botryosum Wallr. after the second spraying was linked to a significant delay in decline of green leaf area in treated plots.

Differential sensitivity of phylloplane fungi to propiconazole is very likely because in vitro activity against fungal plant pathogens covers a broad range of genera. For example, reported EC_{50} values for 19 species ranged from less than 0.1 ppm to 6.0 ppm (Ciba-Geigy Agricultural Division, 1987). In vitro sensitivity of
R. solani was intermediate (Ciba-Geigy Agricultural Division, 1987), although differences among various isolates may occur (Jones et al., 1987). Antagonists of R. solani that are more sensitive to fungicide application may not recover as fast as the pathogen, which could then develop at a faster rate. Also, because of its high intrinsic growth rate, R. solani could possibly outcompete antagonists that are equally sensitive to fungicidal activity but have lower growth rates. Recent evidence that propiconazole can restrict growth of certain bacteria that exhibit antibiosis in vitro against R. solani (M. C. Rush and A. S. Prabhu, unpublished) suggests that reduction of bacterial phylloplane antagonists by fungicide application may also be involved.

Species composition and relative prevalence of antagonistic microorganisms on rice leaves can undoubtedly differ from field to field and from year to year. This could explain why in 1989 increasing propiconazole dosages, applied singly at GR, caused increasing sheath blight severities, while in 1990, all rates were equally detrimental. It could also explain why in 1990, single B applications resulted in increased disease severity earlier in the season and to a larger extent than the same applications in 1989. Occasional performance failures of the recommended GR plus B (0.18 + 0.18 kg a.i./ha) or single B (0.32 kg a.i./ha) application of propiconazole in the past were attributed to a low rate of application that could not withstand high disease pressure. Higher rates were thought to be necessary (Groth et al., 1990). Our time-course investigation, however, contradicts this and suggests that the cause of failure may lie elsewhere.

Fungicide trials conducted at the Rice Research Station in Crowley, LA, during 1981-1990 (Rush et al., 1981, 1982, 1985-1989; Rush and Groth, 1990) showed that early applications (GR + B) of seven of 22 fungicides tested did not
significantly control sheath blight development, whereas later (B + H) applications of the same compounds at the same rate significantly reduced disease levels compared with the control treatment. GR and B applications of Rovral in 1987 (Rush et al., 1987) and BAS 480 OOF in 1990 (Rush and Groth, 1990) resulted in a significantly higher percentage of tillers dead at maturity than in nonsprayed plots. In contrast, B and H applications of these compounds at the same rate significantly reduced sheath blight development. These results suggest that disease development after early fungicide application would have to change from an expected initial decrease to an increase at a higher rate for it to result in a disease level at rice maturity comparable to or higher than the disease level in nonsprayed plots. The disease progress curves presented in this paper support this assumption. Disease development after later applications is presumably interrupted by harvest before increased disease development can occur. The fact that fungicides with different modes of action and with mostly broad spectrum activity seem to be involved favors the hypothesis that nontarget antagonistic microorganisms may be affected.

Examples of fungicides that have caused a so-called 'boomerang' effect (Bollen, 1982) or 'iatrogenic' disease (Horsfall, 1979) were reviewed by Hislop (1976) and Bollen (1982). Fokkema et al. (1975) obtained substantial evidence that reduction of the saprophytic mycoflora of rye leaves caused by benomyl resulted in an increased susceptibility to Cochliobolus sativus (Ito & Kuribayashi) Drechs. ex Dastur). However, well-documented cases are scarce (Bollen, 1982). Hislop (1976) mentioned that any chemical applied to plants may alter the microflora either directly, by affecting its components, or indirectly by altering the physiology of the host. In addition, a boomerang effect could be brought about by changes in plant resistance mechanisms after fungicide application (Bollen,
Owen and Donzel (1986) reported that the overall rate of propiconazole metabolism in rice cell cultures was considerably slower than in wheat cells. They linked this observation to the greater phytotoxicity of propiconazole to rice when compared with wheat and other cereals. Thus, it cannot be ruled out that host physiology may be altered, resulting in increased rice plant susceptibility and/or changed antagonist populations.

Yield responses were significantly negatively correlated with rAUDPC for sheath blight severity and were not correlated with disease incidence over time. This implies that relatively high sheath blight incidences could be tolerated as long as infection remains in the lower part of the canopy. For very susceptible cultivars, this would not apply. Lemont, however, is a susceptible cultivar with some degree of resistance to penetration of the culm, and it can withstand infection on the lower sheaths without severe lodging. Lack of correlation between yield and incidence over time also implies that scouting procedures based solely on determination of disease incidence during the season may not be the most effective basis for recommending fungicide applications. Current recommendations for sheath blight in the United States are to apply a fungicide when 5-10% of the tillers of a susceptible cultivar are diseased at the early jointing stages of growth (Jones et al., 1987; Groth et al., 1990). The observations made during this study suggest that sheath blight thresholds that also incorporate disease severity indices might give a more accurate basis for decision making.

Based on this 2-yr investigation, it appears that fungicide application at both B and H stages were imperative for consistent control of sheath blight development and positive yield response. A single B application, however, might be effective enough to suppress disease severities up to maturity. This will
depend on the interplay of several components: host, pathogen, chemical, biotic, and abiotic environment. As long as the interactions among these components are not fully understood, attempts to develop a system that would forecast the need for an additional H application are likely to fail. The results of this study indicate that it would be of great interest to elucidate the impact of propiconazole and other fungicides on nontarget phylloplane antagonists on rice and to determine how this impact might be related to sheath blight development.
LITERATURE CITED


SUMMARY AND CONCLUSIONS

Propiconazole was highly active against *R. solani* in vitro. At a concentration of 0.093 and 4.4 μg/ml, propiconazole caused 50 and 90% colony growth inhibition, respectively. Sclerotial weight per colony was significantly reduced with increasing concentrations of propiconazole, but, in relation to total amount of mycelium, sclerotia were produced earlier in 1 to 10 μg/ml plates than in control plates and at lower propiconazole concentrations. Average colony diameters from germinating sclerotia obtained from cultures growing in the presence of 0.5, 1, 5, and 10 μg/ml of propiconazole, were significantly higher than those of control colonies.

Effects of propiconazole on hyphal morphology of *R. solani*, as demonstrated with scanning electron and light microscopy, included excessive and irregular branching, swollen or strongly tapered hyphal tips, formation of 'beaded hyphae', and rupturing of hyphal walls under the pressure of emerging intrahyphal hyphae. Fluorescence microscopy of calcofluor white-stained specimens revealed bright fluorescence of propiconazole-treated hyphal tips. Abnormal fluorescent patches, representative of abnormal cell wall deposition, were randomly distributed over treated hyphae. At the ultrastructural level, abnormal wall inclusions of various sizes and electron densities were observed after exposure to 1 μg/ml of propiconazole. The high frequency of intrahyphal hyphae in propiconazole-treated hyphae allowed observation of their initiation and development by means of fluorescence and light microscopy.

Hyphae growing on propiconazole-amended medium (0.25, 0.5, 1, and 5 μg/ml) were capable of forming lobate appressoria and infection cushions on a fungicide-free glass coverslip surface. Mycelial growth on the medium was
increasingly inhibited with increasing concentrations of propiconazole, but infection cushion formation on the coverslips was visibly higher than for the control treatment. Although external morphology of infection structures was the same for all treatments, the cytoplasm showed signs of degeneration at 1 and 5 μg/ml of propiconazole.

Infection cushions on the inner surface of propiconazole-containing rice sheaths were seen to penetrate the epidermis. However, signs of protective activity of propiconazole were present in the observation that penetration pegs underneath lifted infection cushions did not penetrate the inner epidermis but developed into regular hyphae that continued to grow on the sheath surface. Curative activity of propiconazole was evidenced by collapsed hyphae near residue deposits and occasional directional growth away from such deposits.

Two days after inoculation, infection cushions had developed on the outer surface of 1 μg/ml treated sheaths and not on control sheaths. In a quantitative in vivo study, it was found that, at low propiconazole concentrations, mycelial growth and infection cushion formation on the inner surface of sheath strips were significantly higher than on control strips. This effect was not only concentration-dependent but differed with mode of inoculation. After inoculation on the inner sheath surface, mycelial growth and infection cushion formation were generally not significantly different from control values, whereas after outer surface inoculation they were significantly higher. Mycelial growth on the outer sheath surface after both inner and outer inoculation tended to decrease with increasing propiconazole concentration.

In a 2-yr field study, incidence and severity of rice sheath blight were monitored from the green ring growth stage to maturity in nonsprayed, inoculated plots and inoculated plots sprayed with propiconazole and other
selected fungicides at different rates and growth stages. Single green ring applications of propiconazole at 0.18, 0.32, or 0.48 kg a.i./ha and sequential green ring and boot sprays (0.18 + 0.18 and 0.32 + 0.32 kg a.i./ha) effectively reduced disease incidence over time. Severity of sheath blight over time, expressed as percent lesion height to total sheath height, was increased by green ring applications. With the loss of fungicidal activity over time, lesions on treated tillers progressed upward at a faster rate and reached higher levels than on nonsprayed tillers. Single boot applications at 0.18, 0.32, or 0.48 kg a.i./ha; boot applications of propiconazole at 0.32 kg a.i./ha followed by heading applications of either benomyl, iprodione, or pencycuron; or boot and heading sprays of benomyl (0.57 + 0.57 kg a.i./ha) had little effect on disease incidence. In contrast, disease severity was significantly reduced by all boot and heading treatment combinations. Disease progress measured as incidence or severity was reduced most effectively by the application of pencycuron at boot and heading (0.18 + 0.18 kg a.i./ha). Consistent positive yield responses compared with the nonsprayed control resulted from all boot and heading applications, whereas green ring applications generally resulted in a negative yield response. Yields were significantly negatively correlated with areas under the disease progress curves (AUDPC) based on weekly severity measurements. Yields were not correlated with AUDPC for disease incidence over time.

Several hypotheses are proposed to interpret the observation that early applications of propiconazole caused increased sheath blight severity later in the season compared with the nonsprayed control. A first hypothesis is based on results from the in vitro and in vivo laboratory experiments. Earlier in vitro sclerotium and infection structure formation from propiconazole-treated hyphae
is probably related to increased hyphal branching caused by propiconazole treatment. Similarly, increased branching in response to contact with propiconazole, either on the sheath surface or at the time of penetration or colonization of the sheath, could be responsible for the observed increased number of infection cushions on the inner surface of treated sheath strips. At some concentrations of propiconazole, mycelial growth on the inner sheath surface was also significantly higher than on control sheaths. It is hypothesized that, when propiconazole is present in the sheath at concentrations high enough to prevent or inhibit penetration and/or colonization, this could result in increased mycelial growth on the sheath surface, either through penetration pegs that develop into regular hyphae and continue to grow on the sheath surface, or through the formation of runner hyphae. This assumes that, with limited penetration and/or colonization, sufficient nutrients are available on the sheath surface to support hyphal growth.

The combination of increased mycelial growth and increased infection structure formation in the presence of low concentrations of propiconazole may be responsible for the increased disease severity observed under field conditions. Immediately after application, propiconazole concentration would be high enough to cause strong growth inhibition, resulting in decreased disease severity. With loss of fungicidal activity over time, propiconazole concentration would decrease and reach a level at which increased mycelial growth and infection structure formation can be expressed, resulting in increased disease severity. Many factors will likely influence the degree to which these processes would occur. For example, the balance between propiconazole concentration in and on the sheath, the ease of reaching the inner sheath surface, and/or the availability of nutrients on the sheath surface, may play a role.
A second hypothesis is based on the knowledge that propiconazole is a broad-spectrum fungicide that will probably affect many nontarget phylloplane fungi. Propiconazole application could decrease populations of fungi that are antagonistic to *R. solani*. If these antagonists are more sensitive to fungicide application or grow slower than *R. solani*, the pathogen could develop at a faster rate following the loss of fungicidal activity. Sheath blight severity would, therefore, increase more rapidly on treated tillers than on nontreated tillers.

In addition to these hypotheses, undetermined indirect effects through interference of propiconazole with a host component may also be involved. None of the interpretation excludes the others. Aspects of all may act together to produce the observed effect. Additional and independent experimentation based on the working hypotheses provided here, is strongly recommended. This will hopefully further elucidate which factors are involved in this undoubtedly complex system.
VITA

Elly M. Van Eeckhout was born on January 11, 1962 in Oudenaarde, Belgium. In 1985, she obtained the degree of Agricultural Engineer (Plant Protection) from the State University of Ghent, Belgium. Following graduation, she was employed subsequently at the Laboratory of Phytochemistry and the Laboratory of Horticulture, State University of Ghent, Belgium. In August 1987, she obtained a teaching assistantship from the Department of Botany, Louisiana State University, to pursue graduate studies. In June 1988, she was awarded a three and a half-yr research grant from Ciba-Geigy Corp., Greensboro, NC. While at Louisiana State University, she was the recipient of the Best Student Presentation Award at the annual meeting of the Louisiana Plant Protection Association in 1989 and 1990. She is presently a candidate for the degree of Doctor of Philosophy.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Elly M. Van Eeckhout

Major Field: Botany

Title of Dissertation: Effects of Propiconazole on Morphology, Development, and Epidemiology of Rhizoctonia solani, Causal Agent of Rice Sheath Blight

Approved:

[Signatures]

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: 8 November 1991