isolates collected from johnsongrass within the research station were as simple as the isolates collected from wild swarms of johnsongrass. These observations support Vanderplank's theory of stabilizing selection (9). It appears that the individuals within the population that possess additional virulence capabilities were not selected on the wild grass.

It is noteworthy that isolates selected randomly from roadside johnsongrass over a 150-mile linear distance were either the same pathotype or had minimal differences in virulence characteristics.

Until it is known whether complex isolates persist in a population and whether they are a threat outside of research stations, care should be exercised. Seed transmission of *C. graminicola* is possible when care is not taken to eliminate seed showing anthracnose acervuli. Risk of introducing virulent isolates of sorghum anthracnose can be minimized by selection of clean seed for transport. Furthermore, the possibility of chance introduction would be eliminated if the clean seed is treated with a systemic fungicide effective against *C. graminicola*, such as benomyl, in addition to the normal topical treatment with captan (4).

Further research should be conducted to determine if complex isolates would be selected against in natural and or agricultural systems. The isolates of anthracnose from johnsongrass that infect sorghum and cause little damage might be manipulated as a useful biological control agent by possibly preempting more virulent isolates by niche possession.

There is a need for researchers who are studying the variability of *C. graminicola* to agree on a standard set of host-differential cultivars. The selected cultivars must exhibit low genotype × environment variance if they are to be adequate indicators of pathogen variability. Low host genotype × environment interaction appears in the study by Ali and Warren (1), in which plants at different growth stages produce the same reaction type in the field and greenhouse. Until there is some concordance among researchers it will not be possible to combine data to analyze population structures of this pathogen around the world.

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Comparison of Propiconazole and Mancozeb Applied Individually or Sequentially for Management of Fungal Brown Spot of Wild Rice

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ABSTRACT

Percich, J. A., and Huot, C. M. 1989. Comparison of propiconazole and mancozeb applied individually or sequentially for management of fungal brown spot of wild rice. Plant Disease 73:257-259.

Propiconazole and mancozeb applied at 0.24 and 1.12 kg a.i./ha, respectively, to wild rice (*Zizania palustris*) plants inoculated with *Bipolaris oryzae* resulted in lower yields and higher disease severity ratings than fungicide-treated noninoculated controls. Inoculated plants receiving one application of propiconazole followed by two of mancozeb (at boot and heading) had higher yields than other inoculated plants, but the yields did not differ significantly ($P = 0.05$) from those of noninoculated plants receiving five applications of mancozeb (at 7-day intervals, beginning at boot). Inoculated plants receiving one application of propiconazole plus two of mancozeb averaged 24% higher yields than inoculated plants receiving one application of propiconazole at either boot or heading. Propiconazole and mancozeb, individually or sequentially, resulted in significant ($P = 0.05$) increases in yield (38–120%) compared with the inoculated but nontreated controls.

Additional keywords: *Cochliobolus miyabeanus*

Wild rice (*Zizania palustris* L.) is grown commercially on 14,752 ha in Minnesota and California (13). Fungal brown spot, caused by *Bipolaris oryzae* (Breda de Haan) Schoem. (= *Cochliobolus miyabeanus* (Ito & Kurbayashi) ex Dastur), has resulted in widespread, recurring, severe losses in cultivated fields of wild rice in Minnesota (1,9) but not in California. Yield reductions associated with epidemics of fungal brown spot have been quantified (10,11).

Mancozeb (Dithane M-45) is currently the only registered fungicide for managing fungal brown spot on cultivated wild rice in Minnesota, where it has been applied as often as five times in a single season to many wild rice fields for 6 yr or more. Although *B. oryzae* has not shown resistance to mancozeb in the field, resistant strains of the fungus have been selected in the laboratory (8).

Propiconazole is a systemic fungicide having ergosterol-biosynthesis inhibiting (EBI) properties similar to other related compounds (12). The fungicide is broad-spectrum and has shown efficacy against many representative species in the Ascomycetes, Deutromycetes, and Basidiomycetes (4).

In 1985, 1986, and 1987, studies were done at the University of Minnesota North Central Experiment Station in Grand Rapids, MN, to determine the efficacy of propiconazole and mancozeb when used individually or sequentially at two critical stages of plant development to control fungal brown spot on cultivated wild rice.

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MATERIALS AND METHODS

Seed of the wild rice cultivar K-2, obtained from a commercial grower, was stored submerged in water at 2 C for 6 mo, then sown during the first week of May and June in 1984 and 1986. The experimental paddy was prepared by rototilling while incorporating 33.7 kg/ha of nitrogen, applied as urea. A second application of urea at 3.4 kg/ha was made during the boot stage of plant development. Seed was broadcast by hand in 1.5 × 2.1 m plots in a randomized complete block design with six replicates. Mean yield data were statistically analyzed (3). Plant density was adjusted to 23 plants per square meter. Most plants produced approximately three tillers. The plots were sprayed with the insecticide diazinon at 1.12 kg/ha 30 days after planting to control leafminer (*Eribolus longulus* (Loew)), then sprayed with malathion at 1.12 kg/ha at the early stage of grain filling (1, 4) to control riceworm (*Apamea apamiformis* (Guenee)).

Inoculation. Isolates of *B. oryzae* were collected from lesions on cultivated wild rice plants in Minnesota, maintained on potato-dextrose agar slants (PDA) at 24 C and cycled through wild rice plants in the greenhouse to ensure pathogenicity. Cultures were reisolated from lesions on infected plants, single-spored, and subsequently maintained on PDA in the dark. A culture medium consisting of 3:14:16 (w/w/v) of cornmeal, rinsed perlite, and 3% PDA, respectively, was placed in aluminum foil-lined galvanized trays (30 × 20 × 10 cm), covered with two layers of aluminum foil, and autoclaved for 1 hr at 121 C. After cooling for 4 hr, two PDA plates each containing 1- to 2-wk-old cultures of *B. oryzae* were diced into 1-cm squares and mixed into the medium. After 3 wk of incubation at 24 C, the inoculum was air-dried at 24 C, then stored in brown paper bags at 35 C until used. Approximately 1 L of bulked cornmeal-perlite inoculum, containing five to seven different isolates of *B. oryzae*, was mixed with 3 L of water immediately before inoculation. The resulting conidial suspension was sieved through a 300-μm screen and adjusted with water to a concentration of 1×10^6 conidia per milliliter. Each plot was inoculated by means of a backpack sprayer (Hudson Stainless Steel Syprema 67376, H. D. Hudson Mfg. Co., Chicago, IL) at 413.7×10^3 n/m^2 30–40 cm above the plants. Inoculations were usually made by 2000 hours on a day without rain and with expected night temperatures of 18–23 C.

An overhead misting system consisting of Maxijet nozzles (0.15-cm diameter orifice) (Thayer Industries, Inc., Dundee, FL) was timer-activated for 12 hr after inoculation to provide intermittent moisture (12 3-min periods per hour) uniformly over the plots. An electric motor and piston pump provided up to 206.9×10^3 n/m^2 to move the water from a large self-filling reservoir to the nozzles. Each nozzle delivered 1.9 L per cycle of water at 2.03×15 N/m^2. The distance between the nozzles was 6.95 m diagonally and 8.23 m front and back. The height of the nozzles was adjusted with plant growth.

Fungicide application. Mancozeb (Dithane M-45WP) and propiconazole (Tilt 3.6EC) were applied at 1.12 and 0.24 kg a.i./ha, respectively. The fungicides were applied with a sprayer pressurized by carbon dioxide and delivering 300 ml of material at 1.7×15 N/m^2 per plot, equivalent to a rate of 331 L/ha. The initial application of the chemicals was made during the boot and early flowering stages of plant development, approximately 72 days after the plants emerged from the water. Mancozeb and propiconazole applications were made at 10- and 14-day intervals, respectively (Table 1). Treatments were coded with descriptors to indicate the pattern of fungicide use. The first letter was a C (control) or an I (inoculated). M represented an application of mancozeb and P, an application of propiconazole; these letters were used to indicate which fungicide was used in what sequence (Table 1).

Disease assessment. Disease severity ratings were recorded periodically for each plot. Standard area diagrams (6) for Septoria leaf blotch were used to determine the percentage of diseased leaf area. Disease ratings were recorded for the individual leaves, and the amount of disease was expressed as the percentage of leaf area covered by lesions on the flag leaf and the second and third leaves down from the flag leaf.

Table 1. Schedule for inoculation of wild rice cultivar K-2 with *Bipolaris oryzae* and for application of mancozeb and/or propiconazole to control fungal brown spot, 1986 and 1987[z]

Growth stage	Noninoculated					Inoculated				
	C	CMM	CPP	CPM	CMP	I	IPP	IP	IPM	IMP
Boot + 0 days	...	...	...	...	...	I	I	I	I	I
Boot + 1 day	...	M	P	P	M	...	P	P	P	M
Boot + 8 days	...	M	...	...	M	...	...	...	M	P
Heading + 0 days	...	M	P	M	P	...	P	...	M	...
Heading + 7 days	...	M	...	M	...	...	...	...	...	...
Heading + 14 days	...	M	...	...	...	...	...	...	...	...

[z] C = control, M = mancozeb, P = propiconazole, I = inoculated.

Table 2. Effects of mancozeb (1.12 kg a.i./ha) and propiconazole (0.24 kg a.i./ha), used individually or sequentially, on yield and disease severity of wild rice cultivar K-2 inoculated with *Bipolaris oryzae* or not inoculated, 1986 and 1987

Treatment[x]	Yield (kg/ha)		Disease severity (%)	
	1986	1987	1986	1987
CMP	625 a[y]	558 a	<1/<1/<1[z]	<1/<1/<1
CMM	559 b	499 b	<1/<1/<1	<1/<1/<1
IPM	540 b	482 b	9/11/13	10/10/10
CPP	497 c	444 c	<1/1/1	<1/1/5
CPM	476 c	425 c	<1/1/1	2/5/5
IMP	430 d	384 d	5/7/10	5/10/15
C	447 d	386 d	7/14/15	5/10/15
IPP	360 e	321 e	2/3/5	1/5/5
IP	391 de	349 f	3/6/20	5/10/25
I	284 f	253 g	10/15/60	10/20/65

[x] C = control, M = mancozeb, P = propiconazole, I = inoculated (see Table 1).
[y] Means in a column followed by the same letter are not significantly different at the $P = 0.05$ level according to Duncan's new multiple range test.
[z] Percentage of the flag/second/third leaf area infected at harvest.

RESULTS AND DISCUSSION

Yields were significantly different among treatments that were artificially or naturally infected (Table 2). Average yields of all treatments during 1986 were greater than those during 1987; this may have been due to earlier sowing and plant emergence in 1986. Plants that were misted but not inoculated or treated with fungicides (C) resulted in average yield reductions of 18 and 22% because of natural infection during 1986 and 1987, respectively, when compared with plants receiving five applications of mancozeb (CMM). Average yield losses approaching 20% in commercial fields not treated with mancozeb are not unusual (10). All treatments that were not artificially inoculated with *B. oryzae* had significantly greater yields than inoculated treatments, with the exception of those given one application of propiconazole followed by two of mancozeb (IPM). The CMM and IPM treatment yields did not differ significantly from each other (Table 2), although the IPM treatment resulted in a greater amount of leaf infection because the artificial inoculation.

Noninoculated plants receiving two applications of mancozeb followed by one of propiconazole (CMP) resulted in yields significantly higher than those with any other treatment (Table 2). Less than [illegible] of the leaf area was infected in this treatment during both 1986 and 1987. When compared with the CMP treatment, one application of propiconazole followed by two of mancozeb (CPM) resulted in lower yields and higher percentage of leaf area infected. Natural disease pressure, as indicated by disease severity ratings during late fertilization through early grain filling stages of plant development, may have resulted in greater yield loss in the CPM treatment. Also, the lack of systemic activity by mancozeb coupled with possible washing of the fungicide from the sprayed leaf and stem surfaces may have contributed to the lower yields. Noninoculated plants receiving two applications of propiconazole (CPP) or the CPM treatment resulted in yields that did not differ significantly from each other in both years (Table 2). Apparently, a single application of propiconazole was as effective as two applications of mancozeb in controlling naturally occurring disease late in the season.

Inoculated plants receiving one application of propiconazole followed by two of mancozeb (IPM) had significantly higher average yields than all other inoculated and fungicide treatments (Table 2). This may have been due to residual systemic activity of propiconazole early in the developing epidemic coupled with the protectant activity of mancozeb later in the season during grain elongation (11). Inoculated plants receiving two applications of propiconazole (IPP) had significantly lower yields than those receiving either one application of mancozeb followed by one of propiconazole (IMP) or IPM treatments (Table 2). Even though there were no visible phytotoxic effects (leaf and/or stem damage, incomplete grain filling, and/or grain size differences) (10), there may have been some effect(s) on the resulting IPP yields (7).

Inoculated plants receiving a single application of propiconazole (IP) resulted in the lowest yields of any treatment except the inoculated control (I) (Table 2). This may have been due to sustained disease pressure resulting from both natural and artificial sources of inoculum.

In diseased plants, propiconazole followed by mancozeb should be considered the most effective use of propiconazole in fungal brown spot management. In the absence of artificial inoculation, the CMP treatment performed the best. Therefore, the use of propiconazole alone or in conjunction with mancozeb is effective in the management of fungal brown spot in cultivated wild rice. When mancozeb, either alone or in sequence with propiconazole, is applied to cultivated wild rice when trace amounts of disease (one to 10 lesions per leaf) are first found, excellent yields and disease control can result.

Fungal mutants resistant to EBI fungicides can be induced readily and are frequently cross-resistant to other EBI fungicides (2,4). The development of resistance to EBI fungicides in the field appears to be unlikely because of reduced fitness and pathogenicity of laboratory-selected resistant fungi (5). More complete information is needed on the biochemical mechanisms for increased resistance as well as on the reduced fitness of these mutants (4). Because it has been demonstrated that *B. oryzae* can become tolerant of mancozeb in the laboratory (8), its continued use alone to manage fungal brown spot of wild rice may be unwise. An evaluation of the judicious use of propiconazole and mancozeb coupled with their long-term effects on field populations of *B. oryzae* is in progress.

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