Comparison of Fungal Brown Spot Severity to Incidence of Seedborne *Bipolaris oryzae* and *B. sorokiniana* and Infected Floral Sites on Cultivated Wild Rice

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ABSTRACT

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The incidence of *Bipolaris oryzae* and *B. sorokiniana* in cultivated wild rice seed collected from two fields in Minnesota during 1991 and 1992 was related to disease severity on leaves. The site of *Bipolaris* spp. on the seed was primarily the awns. Caryopses were not infected under the conditions of this study. This is the first evidence for *B. oryzae* and *B. sorokiniana* being seedborne on cultivated wild rice and located primarily in the external tissues of the seed.

Additional keyword: *Helminthosporium* spp.

Cultivated wild rice (*Zizania palustris* L.) is grown on approximately 6,882 ha in Minnesota, contributing over $41.6 million to the state's economy (5). Fungal brown spot caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker and *B. sorokiniana* (Sacc.) Shoemaker, is the most important disease of cultivated wild rice in Minnesota with an estimated annual loss of $2.5 million. Depending upon disease severity, losses may vary from slight to 75% (1,3,4). Anecdotal evidence ascribes losses of 100% in fields where disease is especially severe. Current control of fungal brown spot can be achieved by an application of propiconazole (212 ml/ha) at the boot stage of plant development followed by a second application 14–17 days later (6,7).

Overwintering of the causal fungi and dissemination of primary inoculum to the host plants are poorly understood. Some researchers have proposed that seedborne infections may be important in overwintering and dissemination of these *Bipolaris* spp. (P. Imle and M. Kernkamp, *unpublished*). After the first year of the production cycle, the primary mode of sowing for the succeeding crop is by seeds that fall from panicles to the soil surface before harvest. Cultivated wild rice paddies are then flooded either in the autumn following harvest or early the following spring.

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Imle and Kernkamp (P. Imle and M. Kernkamp, *unpublished*) in 1971 stated that *Helminthosporium* spp. (= *Bipolaris* spp.) were frequently isolated from cultivated wild rice seed. They stated that a certain percentage of plants at the tillering stage would have a leaf blight caused from a systemic infection by *Bipolaris* spp. while most of the other plants in the vicinity would appear to be disease free. They hypothesized that infected plants somehow survived systemic infection long enough to infect other plants in the paddy. However, this study was never repeated nor was it published; additionally, *Bipolaris* spp. are not known to be systemic in rice plants. The objective of our research was to determine the incidence and origin of infection on seed by *Bipolaris* spp. This research is part of a larger study on the relative importance of seedborne *Bipolaris* spp. as a means of overwintering and source of primary inoculum to infect cultivated wild rice.

MATERIALS AND METHODS

Severity of fungal brown spot on plants in two cultivated wild rice fields each in 1991 and 1992 was measured by percentage of leaf area covered by lesions on the uppermost leaf of 50 plants per field using a modified Septoria disease assessment key (2). Plants from the same fields were rated in 1991 and 1992. Ratings of 1F to 50 were utilized corresponding to percentage of diseased leaf area (1F = 0.5 or flecks and 50 = 50 +% of leaf area diseased, respectively). One plant was rated for disease every 3.1 m in a "U" pattern beginning 17 m from the corner of the field.

To measure the incidence of wild rice seeds infected with *Bipolaris* spp., 500 seeds were randomly selected from each field after harvest in 1991 and 1992 for a total of 2,000 seeds. Seeds were not dried and were kept at 0 C for 7 days when isolations were made. Whole seeds were washed for 2 hr in running tap water (7 C, untreated with chlorine), placed in a 1:1 solution (v/v) of 1% NaOCl and 75% ethanol for 3 min, rinsed in sterile distilled water,and placed on moistened autoclaved filter paper (Whatman Number 1 or Fisher P5) in 9-cm-diameter glass petri dishes. Seeds were incubated utilizing available laboratory light at 22–26 C and examined at 5–7 days for growth of *Bipolaris* spp. with a stereo microscope (X30–60) utilizing a fiberoptic illuminator for light.

To determine which seed structures were infected by *Bipolaris* spp., an additional 100 seeds were randomly selected after harvest from each field in 1991 and 1992 for a total of 400 seeds. Seed storage, surface treatment, fungal growth, and examination were similar to those used for whole seeds except seeds were separated into awns, palea or lemma, and caryopsis after 2 hr in running tap water. The seed structures then were placed in the 1:1 solution of 1% NaOCl and 75% for 1 min.

Bipolaris spp. were identified in situ on the seed structures by the following conidial characteristics. *Bipolaris oryzae* conidia are light to golden brown, fusoid, obclavate to almost cylindrical, generally curved with six- to 14-septate and 14–22 × 63–153 μm. *Bipolaris sorokiniana* conidia are olive to dark brown, ovate to oblong with tapered ends, three- to 10-septate and 15–20 × 60–120 μm.

RESULTS AND DISCUSSION

Severity of leaf infection was greater in field A than B in 1991 but approximately the same in 1992 with ratings of 52.3 and 1.7; and 3.6 and 2.4, respectively (Table 1). Generally, the number of seeds infected by *Bipolaris* spp. corresponded to the degree of disease severity. The number of infected seeds was higher from the field with the highest disease rating and was statistically significant ($P = 0.05$). *Bipolaris oryzae* was isolated in higher frequency than *B. sorokiniana* from both fields regardless of the year but differences were significant only in field A in 1991 ($P = 0.05$).

There was a significant difference in

Table 1. Comparison of number of wild rice seeds infected with *Bipolaris oryzae* and *B. sorokiniana* to disease rating in 1991 and 1992[w]

Year[x] (field)	No. seeds infected[y]		Disease rating[z]
	B. oryzae	*B. sorokiniana*	
1991			
A	35 a	15 b	52.3 a
B	15 b	5 b	1.7 b
1992			
A	13 b	9 b	3.6 b
B	11 b	9 b	2.4 b

[w] Numbers followed by same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[x] Fields A and B in 1991 same as fields A and B in 1992.
[y] Isolation from 500 seeds per field per year.
[z] Disease ratings from 50 plants. Severity of fungal brown spot measured by percentage of leaf area covered by lesions on uppermost leaf.

Table 2. Number of *Bipolaris oryzae* and *B. sorokiniana* isolates from 400 cultivated wild rice seeds in 1991 and 1992

Seed structure	*B. oryzae*[z]	*B. sorokiniana*
Awns	70 a	34 a
Palea or Lemma	4 b	4 b
Caryopsis	0 b	0 b

[z] Numbers followed by same letter are not significantly different ($P = 0.05$).

frequency of infection of seed structures ($P = 0.05$). *Bipolaris* spp. were isolated primarily from the awns and infrequently from the palea or lemma (Table 2). No *Bipolaris* spp. were observed to originate from the caryopsis.

Awns are likely to be the primary site of infection. This is due to the dense pubescence on the awn compared with reduced pubescence or lack of it on the palea and lemma. The pubescence apparently acts as a spore trap and also retains moisture from dew or other sources longer than other parts of the seed. The trapped spores and retained moisture would allow the awn to become infected more rapidly than the rest of the seed.

This is the first evidence for *B. oryzae* and *B. sorokiniana* being seedborne on cultivated wild rice and located primarily in the external tissues of the seed. We conclude that the incidence of seedborne *Bipolaris* spp. is related to disease severity. However, it is uncertain if seedborne *Bipolaris* spp. influence future disease development. Cultivated wild rice paddies are flooded either in the autumn or early in the spring. Because the primary method of sowing for succeeding crops is the falling of seed from panicles to the soil surface, it is uncertain if *Bipolaris* spp. survive in or on seed submerged under water and eventually produce conidia that serve as inoculum for infection.

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