INTEGRATED MANAGEMENT OF FUNGAL BROWN SPOT

University Of Minnesota
Department Of Plant Pathology
Saint Paul

James A. Percich, Dean K. Malvick, and Jason R. Brantner
Professor, Research Fellow and Research Assistant, respectively

Introduction. The plant pathology research staff at the University of Minnesota in Saint Paul has continued to center their research activities on *Bipolaris oryzae*, the causal organism of fungal brown spot (FBS) of cultivated wild rice in Minnesota. The individuals responsible for the various research areas summarized (Table 1).

Table 1. Plant pathology staff and their areas of wild rice research interest at the University of Minnesota, Saint Paul.

Staff	Research areas of interest
James Percich	
	* Germ plasm preservation
	* Wild rice callus culture
	* Disease screening of non-cultivated wild rice germ plasm
	* Toxin screening for FBS resistance
	* Propiconazole (Tilt) Registration (In cooperation with Dr. R. Nyvall)
Dean Malvick	
	* Hydroponic culture of wild rice
	* Plant nutrition and disease
	* Germ plasm evaluation for FBS resistance
Jason Brantner	
	* Survival, early infection and spread of *Bipolaris oryzae*

Germ plasm preservation and disesase resistance screening. The wild rice varieties used in commercial production are heterogeneous mixtures exhibiting considerable intervarietal variation for morphological traits, but not for reaction to fungal brown spot (FBS) disease. The genetic base of these varieties may be quite narrow because all were selected from a

relatively small collection of non-shattering phenotypes. There may be no useful FBS resistance in the currently available varieties.

The most efficient method of improving disease resistance in other cereals has been the introgression of resistance genes from wild relatives. Resistance to FBS has not been reported in any *Zizania* species, but little screening of germ plasm has been reported (Percich, et al., 1989). The greatest diversity for genetic traits is thought to occur at the plant's center of origin (Wahl, et al., 1984). Thus, variation for reaction to FBS may exit in the wild populations of *Zizania palustris* and related species indigenous to the lakes and rivers of the United States and Canada (Atkin, 1986; Duvall and Biesborer, 1988). Short intervals of pollen viability (Stucker, et al., 1987) and lack of insect pollinators (Terrel and Batra, 1982) probably have resulted in genetic isolation of geographically isolated lake populations. There may be numerous genetically discrete populations which should be surveyed in the quest for desirable genetic traits.

Wild rice germ plasm from various lake and river sources were collected in 1972 and evaluated for FBS resistance (Elliott, 1974). However, this important work did not continue beyond 1975. The loss of this original wild rice germ plasm may have been a serious blow to the FBS disease screening effort. Developing a germ plasm bank without first identifying and establishing a germ plasm preserve is unsound. The first step must be germ plasm preservation. Natural stands of *Zizania aquatica* and *Z. palustris* are disappearing rapidly (Biesborer, *personal communication*). Preserving selected germ plasm sites will ensure they will be available for future study and evaluation. Simply collecting from ecologically challenged sites is not enough. The loss past "banked" wild rice germ plasm should be a fair warning, if we are willing to learn from history.

Wild rice callus culture. Seed dormancy traits make it difficult to produce plants on demand and maintain germ plasm. Seed dormancy can be overcome using *in vitro* embryo rescue of tissue culture medium (Johnson and Percich, 1988), but the method is not practical for large scale FBS screening.

Dormancy has also interfered with induction and maintenance of embryo-derived callus cultures. Callus initiated from immature or mature embryos of wild rice grows slowly and is much more prone to browning than embryo-derived callus of other cereals. Seed dormancy and poor callus growth are both partially mediated by the level of endogenous abscisic acid (ABA) in the seed (Percich et al., 1989). Methods to reverse the effects of ABA in wild rice culture were unsuccessful (Johnson, 1991). It is hoped that vigorous callus cultures can be obtained from embryos, since no other plant parts are useable as explants. Future success in

this effort may depend on using embryos from non-cultivated wild rice sources having reduced seed dormancy, such as *Zizania aquatica* and/or, perhaps, *Z. aquatica x Z. palustris* crosses. Such crosses have been made in Saint Paul and at the North Central Exp. Sta in Grand Rapids, Minnesota (R. Porter, *personal communication*).

Disease screening of non-cultivated wild rice. Cultivated wild rice varieties are heterogeneous mixtures which exhibit considerable intervarietal variation for morphological traits Stucker et al., 1987) but uniformly susceptible to FBS in commercial fields (Kohls and Percich, 1987). Varietal germ plasm had been evaluated in FBS disease nurseries since 1975, and greenhouse screening of commercial varieties for FBS resistance had been reported (Percich et al., 1980). However, little sustainted effort has been directed towards the screening of natural populations of *Zizania palustris* or *Zizania* species (Johnson, 1991).

Non-cultivated wild rice species (*Zizania aquatica, Z. latifolia, Z. palustris,* and *Z. texana*), interspecific *Zizania* hybrids, commercially cultivated varieties of *Z. palustris* ('Johnson','K-2', 'Meter', 'Minnesota' -1, -3, 'Netum' and 'Voyager') and one species of a related genus, *Zizaniopsis milaceae,* were tested for reaction to infection by *Bipolaris oryzae*, the causal organism of fungal brown spot (FBS)(Johnson, 1991). Plants of *Z. texana* and the interspecific hybrids were produced via in vitro embryo rescue and inoculated in the culture dish by streaking an aqueous suspension of conidia on leaves. This method was used to improve germination and survival when limited amounts of seed was available. Plants from more plentiful seed were greenhouse-grown to the floating leaf stage and inoculated again at the second aerial leaf and boot stage of development. All inoculated plants were evaluated 10 days and classified as resistant, intermediate or susceptible.

The reactions of *Zizania* species, and interspecific hybrids are shown (Table 2).

Apparent "resistance" was observed in a few plants of cultivated *Z. palustris* 'Meter', non-cultivated *Z. palustris* var. *interior*, *Z. palustris* var *palustris*. However, the "resistance" became less effective when inoculated at the second aerial leaf and was indistinguishable from the susceptible when inoculated at the boot stage of development (Table 2).

This preliminary screening for FBS was limited in all cases by the availability of germ plasm and/or physical limitations of the experimental environment (greenhouse, growth chamber or laboratory). The results obtained should in no way be a reliable guide as to the true status of FBS resistance in non-cultivated wild rice. But they serve to illustrate the possibilities these germ plasm sources may have. There appeared to be no exceptional

resistance to FBS in the collection screened. However, as previously stated, additional wild rice collections should be obtained and evaluated. Attempts to cross cultivated wild rice to *Z. milaceae* were unsuccessful. The immunity of *Z. milace*

Table 2. Number and reaction class of *Zizania* species and interspecific hybrids 10 days after inoculation with *Bipolaris oryzae*.

Wild rice species or interspecific hybrid	Growth stage at inoculation Number of plants and reaction class[a]		
	floating	2nd aerial	boot
Zizania aquatica var. *aquatica*	11 S	4 S	4 S
Z. aquatica var. *subbrevis*	4 S, 2 I	2 S	-[b]
Z. aquatica var. *aquatica* x *Z. palustris* var *interior*	1 S, 1 I	2 S	-
Z. palustris var. *interior*	9 S, 2 I, 1 R	1 R, 1 I	-
Z. palustris var *palustris*	11 S, 1 R	-	-
Z. palustris var. *interior*			
'Johnson'	200 S	200 S	200
'K-2'	200 S	200 S	200
'Netum'	200 S	200 S	200
'Meter'	230 S 4 R, 12 I	230 S	230
'M-1'	200 S	200 S	200
'M-3'	200 S	200 S	200
'Voyager'	145 S, 3 I	143 S, 2 R	14
Z. latifolia	-[c]	10	
Z. texana	1 S	1 S	

[a] Reaction types; R = resistant, I = immune, S = susceptible
[b] Plants did not survive
[c] No floating leaves
[d] Plants did not flower, no flag leaves were produced.

may represent a non-host reaction which cannot be transferred to *Zizania* species (Johnson, 1991). Since FBS does not appear to exert severe selective pressure in natural stands of *Zizania* species in the Great Lakes region (Percich et al., 1980), effective resistance may be very difficult to identify in these populations.

Toxin screening for fungal brown spot resistance. Screening wild rice (*Zizania palustris* L) germ plasm for resistance to fungal brown spot (FBS) is complicated and time-consuming. Greenhouse studies are limited by difficulty in artificially reproducing the aquatic habitat of wild rice; greenhouse grown plants typically respond poorly to lighting, nutrients and limited space (Aiken, 1988; Percich, 1988). In commercial fields the severity of naturally occurring epidemics can obscure differences in resistance, if it exits, among individuals (Johnson and Percich, unpublished data). Development of a simple assay for FBS resistance would facilitate wild rice resistance breeding programs.

The toxin ophiobolin was described in extracts from isolates of *B. oryzae* causing brown spot of rice (*Oryzae sativa* L.) (Nakamura, 1958) and has been successfully used in a tissue culture selection scheme for resistance to brown spot (Ling). In whole rice seedlings, ophiobolin caused greater growth inhibition in roots than in shoots. The site of action for the toxin is the calcium-modulating protein calmodulin (Nejidat, 1987). The ophiobolin is a non-specific toxin (it produces symptoms on plants which are not hosts of *B. oryzae*).

The methodology concerning the detection and possible use of ophiobolin to screen wild rice seedlings for resistance to FBS has been previously published (Johnson and Percich, 1988; Johnson, 1991).

A brief summary of ophiobolin's presence in pathogenic isolates of *B. oryzae* and its use on wild rice seedlings to screen for FBS is as follows:

1. Ophiobolin was confirmed in extracts of all 24 pathogenic isolates of *B. oryzae* by 2-dimensional thin layer chromatography (2-D TLC). Ophiobolin was not detected in extracts of three non-sporulating isolates.

2. Significant inhibition of primary root growth occurred in wild rice seedlings incubated in ophiobolin concentrations of 10^{-6} M or higher (Table 3). Seedlings grown in the toxin at 10^{-4} M did not survive for the duration of the experiment. Some seedlings survived in lower concentrations and in sterile distilled water.

3. Variation for seedling response to the toxin was evident within but not between cultivars. This is consistent with

the facts that wild rice varieties are actually heterogeneous mixtures with certain agronomic traits in common (Stucker, 1987). Also, there is considerable intervarietal hybridization because wild rice is oblig[illegible]e open pollinated and varieties are often grown in close proximity to each other.

4. There may be considerable useful variation for toxin sensitivity in existing varieties, and *in vitro* screening seedlings with ophiobolin may produce individuals which a[illegible] resistant to fungal brown spot (Johnson, 1991).

Table 3. Average lengths of primary roots of wild rice seedli[illegible] grown in different concentrations of ophiobolin or water.

Ophiobolin molar concentration	Average primary root growth length ([illegible]			
	Netum	M-3	Meter	K-[illegible]
10^{-4}	2 Da[x]	1 Da	1 Da	2 [illegible]
10^{-5}	8 Ca	5 Ca	1 Ca	2 [illegible]
10^{-6}	21 Ba	22 Ba	22 Ba	[illegible]
10^{-7}	26 Aa	27 Aa	24 Ab	27 [illegible]
Water	28 Aa	26 Aa	25 Aa	29 [illegible]

[x] Means in columns followed by the same upper case letter and [illegible] rows followed by the same lower case letter are not significantly different at the P=0.05 level according to Duncan's New Multiple Range Test.

Hydroponic culture of wild rice. A three year investigation o[illegible] hydroponic culture of wild rice and its application to studies silicon nutrition and FBS was completed in 1992. The methodol[illegible] involved in this study has been previously published (Percich [illegible] al. 1991; Malvick, et al., 1992). The following is a brief summary of some of the important findings of this study:

1. Five different hydroponic media [Shives, International Ri[illegible] Research Institute Medium, and three modified Hoagland's solutions (HS-1, -2, and -3)] were each tested and evaluated for their ability to support wild rice growth a[illegible] development.

2. First report of the hydroponic culturing of wild rice (*Zizania palustris* var. *interior* L.) from seed to maturity.

   a. Wild rice root development was normal and abundant. Hydroponic nutrient solutions may be a useful "nurse" media for plants produced from callus culture.

   b. Plant height (1.4 meters) was approximately 80% of plants grown in soil.

   c. Hydroponic wild rice biomass was 40% of plants grown in paddies or natural stands; but could be increased by an additional 47% when cultured in larger hydroponic containers.

   d. Hydroponic wild rice plants flowered and produced viable seed.

3. Wild rice biomass did not significantly increase in silicon-amended hydroponic medium (HS-1) when compared with non-silicon amended HS-1 medium.

4. Fungal brown spot disease resistance, as measured by leaf chlorosis and lesion numbers and size, apparently was not increased in wild rice plants growth in HS-1 medium amended with silicon when compared with those grown in HS-1 alone.

   a. Previous reports (Bloom and Meyer, 1988; Percich et al., 1988) of silicon increasing wild rice biomass, enhancing growth and development and FBS disease resistance in peat soil demonstrated in hydroponically grown plants. The field and greenhouse studies used calcium silicate slag in peat soil. The slag may have contained other essential micro and/macro elements and enhanced plant nutrition by interacting with the biological, chemical and/or physical soil environment. These factors alone or in combination may have caused the observed increase in wild rice growth and FBS disease resistance.

Survival, early infection and dissemination of *Bipolaris oryzae*. Little is known concerning the survival, primary inoculum, early infection and dissemination of *Bipolaris oryzae*, the causal organism of fungal brown spot (FBS) of cultivated wild rice in Minnesota. A potential research project was initiated by Mr. Jason Brantner to investigate these aspects of the pathogen's biology during the summer of 1992.

The project research goals are the following:

1. How does the pathogen survive over winter?

2. What is the form and source of the pathogen's primary

inoculum?

3. How is the primary inoculum disseminated? What part(s) of the wild rice plant is infected?

The answers to the above questions will result in a more successful integrated FBS management program. For example, knowing the source of primary inoculum may enable growers to reduce the amount of inoculum before it comes in contact with t crop. Understanding when and at what stage of plant developmen the patogen infects may result in more timely and successful fungicide applications.

Propiconazole (Tilt™) use on cultivated wild rice in Minnesota. An emergency registration (a specific exemption under Section 1 of FIFRA) for Tilt as a foliar fungicide to control FBS on cultivated wild rice in Minnesota was submitted (J. Percich) to the Minnesota Department of Agriculture (MDA) on December 17, 1992. The new registration packet contained additional field information (Dr. R. Nyvall, Univ. of Minn. North Central Exp. Stn, Grand Rapids) concerning Tilt's ability to control FBS as well as additional wild rice economic statistics. If the Secti 18 is approved by MDA and the Environmental Protection Agency, the fungicide would be available to Minnnesota cultivated wild rice growers during 1993.

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