

## WILD RICE DISEASE RESEARCH

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The Saint Paul based wild rice disease research group will be focusing on the following areas of investigation in the future:

1. Continued development and use of wild rice hydroponic media for studying the effects of wild rice nutrition on the severity of fungal brown spot (FBS), caused by Bipolaris oryzae. Also, such a hydroponic medium may be useful in stimulating the growth and development of wild rice plants regenerated from callus culture (In cooperation with Drs. P. Bloom and R. Zeyen, Univ. of Minn. - Twin Cities Campus).
2. To study the use of toxins to screen current cultivars and advanced wild rice breeding material for FBS disease resistance (In cooperation with R. Porter, North Central Exp. Sta., Grand Rapids, MN).
3. To investigate the diversity of natural stands of wild rice in Minnesota. The ecology and genetic diversity of the two most important subspecies, palustris and interior.

Professor Robert Nyvall, the plant pathologist stationed at the Univ. of Minn. North Central Exp. Sta., in Grand Rapids, Minn. will now direct efforts for the continued field evaluation of Tilt™ and other fungicides for FBS control, field FBS screening and evaluation of wild rice introductions (in cooperation with Dr. R. Porter), and grower education for disease prevention and management. Also, Dr. Nyvall is currently developing a research program in the area of biological control of aquatic weeds.

### SUMMARY OF RESERACH ACTIVITIES:

In 1991 The plant pathology research team in Saint Paul focused on the continued investigation of hydroponic media for the culture of wild rice and the continuation of an emergency registration (a specific exemption under Section 18 of FIFRA) for Tilt™ as a foliar fungicide to control FBS on cultivated wild rice in Minnesota for 1992.

## HYDROPONIC CULTURE OF WILD RICE

### Introduction:

Wild rice was grown hydroponically in a chemically defined liquid growth medium to flowering for the first time in our laboratory in 1989. During the past two years we have been working to improve plant growth and development. The effects of different nutrient solutions, pH levels, and growth chamber conditions have been reported (1).

Wild rice have been grown to a height of 1.3 meters (4.3 ft) with flowers and seed heads approaching normal size and appearance. These plants had excellent root growth, tillering and produced mature seed. Experiments are continuing with the purpose of producing larger wild rice plants with normal structure and to determine the effects of silicon on growth, yield, and resistance to fungal brown spot disease.

The primary objectives of the hydroponic research project are as follows:

1. Determine if silicon nutrition plays an important role in resistance of wild rice to fungal brown spot disease, caused by Bipolaris oryzae.
2. Determine minor element needs of wild rice by growing plants hydroponically from seed to maturity.
3. Investigate the use of a hydroponic culture to promote regeneration of wild rice plants from differentiated callus culture.

### Materials and Methods:

**Hydroponic culture.** Wild rice 'variety' K2 was selected for all hydroponic experiments. Seedlings 2 - 3 weeks old were situated in small plastic pots containing plastic screen bottoms, and positioned in 6 liter (1.6 gal) buckets containing nutrient solution. Experiments were performed in growth chambers having a 16.5 hours photoperiod with light from a mix (60:40) of cool white fluorescent and Gro-lux. The relative humidity was maintained at 94 percent. The day and night temperatures were 22 C (72 F) and 17 C (62 F), respectively. Nutrient solutions were replaced weekly, and the pH was adjusted once each week between solution changes. The nutrient solution used most often was a modified Hoagland's at pH 5.0. This hydroponic solution was modified from the original recipe by reducing the concentration of micronutrients to one-quarter strength and increasing iron 1.4 fold. A nutrient solution (pH 5), developed at the International Rice Research Institute (IRRI) was used in some experiments.

**Silicon amendments.** Four different silicon (Si) compounds were added to nutrient solutions to determine their effect(s) on wild rice growth and fungal brown spot (FBS) resistance. These compounds were sodium silicate, meta soluble, technical grade (Fisher Co.); calcium metasilicate (Alpha Products); silicic acid (Fisher Co.); and potassium silicate, Kasil #1 (The PQ Corporation). Each silicon compound was added to obtain a Si concentration of approximately 1.5 mM in the nutrient solution.

**Inoculum preparation.** Bipolaris oryzae strain 8051 (isolated from wild rice in Aitkin Co.) was maintained on potato dextrose agar (PDA) in petri plates. The fungal cultures were grown 4-6 weeks at 24 C (75 F) to allow for the production of conidia (spores). The inoculum was prepared by flooding the culture plates with sterile water; spores were washed from the plates and suspended in water to a concentration of 10,000 spores per milliliter (.03 oz). Leaves of wild rice seedlings in the 4-6 leaf stage were inoculated by spraying leaves first with 95% ethanol; after evaporation of the ethanol the spore suspension was sprayed onto the plants. Inoculated plants were immediately moved to growth chamber at approximately 22 C (72 F) with 100% relative humidity where free water formed on the leaves within 1-2 hours.

#### **Results and Discussion:**

Wild rice plant growth, e.g., height, tillering, and total biomass has gradually increased as we have refined our techniques for hydroponic culture.

**Inoculation and infection of wild rice.** Obtaining reliable and consistent infection of wild rice with Bipolaris oryzae was a challenge; but progress was been made in the past year. We found treating wild rice leaves with ethanol before inoculation and the formation of free water on their surfaces after inoculation to be important factors for successful infection of wild rice.

Infection success under high relative humidity (100%) with and without the formation of free water on leaf surfaces was examined. It was found that infection was much more successful (approximately 100 - 200 fold increase) when free water was allowed to formed on the leaf surface.

The ethanol seems to disrupt the hydrophobic waxy layer on the surface of leaves thus allowing for increased spread and adhesion of the spore suspension to the leaf surface. As a result there was a much higher rate of infection by the pathogen.

An oil (Soltrol 170) for preparation and spraying of spores suspensions was also evaluated. This treatment resulted in poor infection.

However, in spite of these improved techniques for inoculation of B. oryzae on wild rice; the results were highly inconsistent among

K-2 plants of the same age even when inoculated at the same time. Lesion numbers and to some degree lesion size were extremely variable between plants and between leaves on the same plant. For example, in one inoculation experiment with seven plants (23 total leaves), all leaves were sprayed to runoff with a spore suspension prepared as described above. Fungal brown spot lesions formed on only 8 of 23 inoculated leaves after 5 days of incubation. The number of lesions per leaf ranged from 2 to 21. This inconsistency of infection success is a significant problem that must be overcome if we are to make progress in studies on fungus-plant interactions and in identifying and understanding FBS resistance in wild rice.

**Effect of silicon on plant growth and FBS resistance.** Results from experiments to determine the effect of amendments on growth, development and disease resistance of wild rice in hydroponic culture are inconclusive at this time. In three experiments, silicon amendments had no clear effect on plant size, total biomass development, and number or size of brown spot lesions on inoculated plants. Silicic acid appeared, in some cases, to promote growth more than the other silicon amendments. Whereas potassium silicate seemed to inhibit growth compared to unamended Hoagland's solution.

Much of the difficulty in obtaining conclusive results in these kinds of experiments is due to the genetic variability among individual wild rice plants. These experiments are labor-intensive and expensive in terms of time and space required to complete them; therefore large numbers of plants cannot be used to 'smooth out' variations due to individual plant variability. Therefore, the development of one or more wild rice varieties that are genetically and/or phenotypically consistent is critical if we are to make rapid progress in understanding the role (s) of plant nutrition, on fungal brown spot resistance.

#### **TILT™ REGISTRATION FOR MINNESOTA WILD RICE GROWERS:**

The systemic fungicide Tilt (CIBI-GEY Corp) was approved for use on cultivated wild rice in Minnesota for the 1990 and 1991 seasons. The approval for Tilt's use on Minnesota paddy wild rice was the result of a Section 18 request by Dr. J. Percich, plant pathologist, Univ. of Minn. The request was actively supported by the Minnesota Paddy Wild Rice Council and the Minnesota Department of Agriculture (MDA). The Section 18 was subsequently approved and granted by the Environmental Protection (EPA) Agency (Registration Support Branch, Regulatory Division).

A Section 18 request for Tilt's use on Minnesota paddy wild rice for the 1992 growing season was assembled by Dr. J. Percich in mid January. The request package contained published research and additional field evaluation data on Tilt supplied by Drs. Percich and Nyvall from the 1990 and 1991 growing seasons, respectively. The package as sent was approved by the MDA in late January.

However, the EPA notified MDA and Dr. Percich that additional current (last 5 years) economic information concerning wild rice production costs and other information was necessary. On February 13, 1992 a letter from The Minnesota Paddy Wild Rice Council was sent to EPA supplying the requested information.

Minnesota paddy wild rice industry desperately needs a fungicide to control fungal brown spot, caused by Bipolaris oryzae. Since the voluntary withdraw of Dithane M-45<sup>TM</sup> (Rhom & Haas), Tilt has been the only fungicide available. Since there has been some re-registration of many ethylene bisdithiocarbamate (EBDC) labels, perhaps, re-examination of the lost wild rice use permit may be in order.

Tilt has proven itself to be an excellent systemic fungicide to control FBS. It is hoped that CIBA-GEIGY Corporation is still vigorously supporting and moving forward on their attempts to achieve additional Tilt labels for wild rice and other crops.

**LITERATURE CITED:**

Percich, J., Malvick, D., Givens, J., and Zeyen, R. 1991. Wild rice disease research. pp. 35-50. Minnesota Wild Rice Research - 1990. in: Minnesota Wild Rice Research - 1990. Minnesota Agric. Esp. Sta. Misc. Publ. 70-1991. pp. 87.

