WILD RICE DISEASE RESEARCH

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INTRODUCTION

The plant pathology research team's activities in 1990 focused on the following:

1. Completion of David Johnson's Ph.D. research on the development of a wild rice tissue culture system.

2. Continued research on the development of a defined hydroponic growth medium for future studies on the nutritional needs of wild rice (D. Malvick and J. Givens).

3. Successful Section 18 application for the systemic fungicide Tilt™ for fungal brown spot control on cultivated wild rice paddies in Minnesota (J. Percich).

4. Field evaluation of Tilt for fungal brown spot control (J. Percich and D. Malvick).

5. Field evaluation of wild rice varieties and germ plasm for fungal brown spot resistance (Dr. Ramey Porter and J. Percich).

6. Determination of abscisic acid levels in embryos of stored wild rice of different ages to identify those embryos most amenable to tissue culture (D. Johnson).

The project is hoping to identify a new graduate student to continue work on wild rice tissue culture by spring 1991. This individual will work closely with Dr. Ramey Porter, plant breeder, during the next three years.

HYDROPONIC WILD RICE GROWTH MEDIUM: A CHEMICALLY DEFINED GROWTH MEDIUM USEFUL FOR DETERMINING THE MINERAL NUTRIENT REQUIREMENTS OF WILD RICE (FUNDED RESEARCH FROM THE MINNESOTA PADDY WILD RICE COUNCIL).

Introduction:

Little was known concerning the growth of wild rice in hydroponic culture (defined water medium). Wild rice was grown hydroponically to flowering, for the first time to our knowledge, in our laboratory in May 1989. These plants, however, were stunted, did not set seed, had poor root and shoot growth, and were abnormal in structure.

The only proven and precise method to determine exact chemical nutritional requirements of wild rice is to grow the plant in a water solution containing known quantities of ultra-pure chemicals. This method is called "hydroponic culture"; has been used to grow and study the nutritional needs of many different crops.

During the past 15 months we have been working to improve plant growth in hydroponic culture. The effects of different nutrient solutions, pH levels, light quantity and quality, temperature, plant support containers, and growth chamber conditions were investigated.

The objectives of the hydroponic research program are as follows:

1. Perfect a chemically defined hydroponic growth medium for wild rice.

2. Determine minor element needs of wild rice by growing plants from seed to maturity in a defined hydroponic medium.

3. Investigate the role of silicon and minor elements on the severity of fungal brown spot, caused by Bipolaris oryzae.

4. Use hydroponic culture as a "nurse medium" to assist in the regeneration of wild rice plants from differentiated callus culture.

Methods and Materials:

Wild rice variety K-2 was used to evaluate the use of defined nutrient solutions for growing wild rice to maturity from seeds. Seed was germinated in distilled water and the resulting 14 to 21 day old seedlings were placed in small plastic pots with mesh bottoms containing nylon beads. The pots were than placed in 5.7 liter (1.5 gal) buckets, each containing a specific nutrient solution (Figure 1). Experiments were conducted in growth chambers having a 16.5 hr. photoperiod, 90 to 95% relative humidity, and a day and night temperatures of 22 and 17 C (72 and 62 F),

respectively. The nutrient solutions were changed weekly and pH was adjusted three times per week.

Five different defined hydroponic nutrient solutions were evaluated for the growing of wild rice. Nutrient media have been reported to be used to grow white rice, wild rice and other aquatic plants. The media investigated are as follows:

a. **Modified Hoagland's Solution (MHS).**

This was the only solution we could identify that had been previously reported for hydroponic culture of wild rice. Dr. Peter Lee, Lakehead University in Ontario, Canada used this medium but did not thoroughly explain the wild rice growth he obtained. We attempted to maintain the MHS at a pH of 5.5.

b. **Modified Shive's Nutrient Solution (pH 5.5).**

Has been used to grow barley.

c. **Modified Hoagland's Solution for Aquatic Plants (pH 5.5).**

Used in growth studies of several different aquatic angiosperm plants.

d. **International Rice Research Institute (IRRI) Nutrient Solution.**

This solution has been used successfully for the growth of domestic white rice at IRRI in the Phillipines. Experiments with this solution were done at pH 5.0 and 5.5.

e. **Hoagland's Original Solution.**

The ingredients and test results for this solution were originally reported in 1938 for growing vegetables and other plants. This nutrient solution has probably been used more frequently and widely than any other defined nutrient medium. Our selected pH's were 5.0 and 5.5.

Results and Conclusions:

Hoagland's solution (original) was the best overall solution for growth of wild rice in hydroponic culture. Plant grew to a height of 1.4 meters (4.6 ft) and were characterized as having good structure, seed set, color and root growth (Figure 2). Plant growth was greater at pH 5.0 than at pH 5.5.

The IRRI solution, at pH 5.0, also resulted in good wild rice growth and development. The plants in the IRRI solution have been grown to 1.4 meters (4.7 ft) in height and have set seed (Figure 3). These plants, however, had thinner stems and leaves and did not produce as many roots as plants in the original Hoagland's

solution.

The Shive's and modified Hoagland's solutions produced wild rice plants that were stunted and usually died prematurely. The modified Hoagland's solution for aquatic plants resulted in rapid wild rice seedling death.

We have demonstrated our ability to obtain mature seed from wild rice grown hydroponically. This seed will be used for experiments to determined the mineral nutrients required by wild rice. In addition, it will be of interest to determine whether wild rice seed produced in hydroponic culture will be more suited for growth in liquid culture than seeds produced in the field.

Hydroponic culture has been perfected to the point where we may be able to use this method to obtain mature wild rice plants from our tissue culture system. Tissue culture can be a source of plants with new and useful characteristics. Plants grown from tissue culture in our laboratory have not produced secondary root systems adequate to support plant growth. However, we believe tissue-cultured plants in hydroponic solution may produce healthy and normal root systems and, therefore, have the potential to grow to maturity.

Summary:

Progress has been made towards successful hydroponic culture of wild rice since 1989. Initially all plants in hydroponics were very small, abnormal in structure, did not set seed, and most died. Currently our laboratory is able to produce plants that are approaching the normal size and structure of plants grown in the field. Therefore, we are now in a position to begin experiments designed to answer more basic and applied questions concerning the effects of mineral nutrients on growth and fungal brown spot disease resistance in wild rice.

FIELD EVALUATION OF THE SYSTEMIC FUNGICIDE TILT FOR CONTROL OF FUNGAL BROWN SPOT.

Objective:

1. Evaluate the effectiveness of Tilt™ in controlling fungal brown spot (FBS), caused by Bipolaris oryzae under field conditions.

Methods:

1. Apply Tilt at 6 oz/A at the boot stage of development and

follow with an additional 6 oz/A application 14 to 17 days at early flowering.

2. Tilt to be applied to at least 4.1 ha (10 acre) area with an adjacent equal area not treated. Select at least two replicate study sites on each farm.

3. Evaluate FBS incidence and severity.

4. Determine wild rice treatment yields.

Results:

Research Site I. Clearwater Farms

A. Control (nontreated)

1. Disease (Fields 1 and 2 - untreated sections)

a. Average FBS incidence = 100%
b. Average FBS severity = 40/50/75% leaf area infected on the Flag, F-1 and F-2.
c. Plants were shorter by 20 to 30 cm (8 to 12 in) infection on flowers and flags,lower canopy was severely infected and remaining stems (stubble) after harvest were severely infected and discolored (brown).

2. Yield

a. Harvested 2.05 ha (5.7 acres) of test site. Green wt/ha was 602 kg (1328 lb/A). Finished wild rice was 218 kg (480 lb/A).

B. Treated (Tilt)

1. Disease

a. Average FBS incidence = 100 %
b. Average FBS severity; = 10/25/50% area infected on the Flag, F-1, and F-2.

2. Yield

a. Field 1: Harvested 2.05 ha (5.07 acres). Green wt was 1748 kg/ha (1542 lb/A). Finished wild rice was 679 kg/ha (599 lb/A), representing a 25% increase over the nontreated site.
b. Field 2: Harvested 2.2 kg/ha (5.45 acres) of treated site. Green wt/ha was 1748 kg (1552 lb/A). Finished wild rice was 626 kg/ha (619 lb/A), representing a 29% increase over the nontreated site.

c. Field 3: Harvested 1.2 ha (3.08 acres) of treated site. Green wt/ha was 1583 kg (1396 lb/A). Finished wild rice was 544 kg/ha (579 lb/A). The increase over farm average 544 kg/ha (480 lb/a) was 21 percent.

Note: Site 3 had only a single application of Tilt

C. Summary Fields 1 and 2:

1. Tilt reduced the size of FBS lesions and the percent leaf area affected by the pathogen.
2. Tilt resulted in increased plant vigor and height. Fungicide appeared to delay maturity by approximately 5 to 7 days.
3. Average yield was increased 146 kg/ha (129 lb/A) finished rice, which represents a 27% increase over the nontreated control.

Research Site II. Manomen Development Corporation

A. Control (Nontreated strips of fields 1, 2 and 3).

1. Disease

a. Incidence was 100% in all three fields
b. Average disease severity was 35/50/75% leaf area infected for the Flag/F-1/F-2.
c. Plants were shorter in height 15 - 30 cm (6 - 12 cm), greater disease throughout leaf canopy and on the flowers and stems.

2. Yield

a. Field 1: Finished wt was 537 kg/ha (474 lb/A).
b. Field 2: Finished wt was 548 kg/ha (484 lb/A).
c. Field 3: Finished wt was 544 kg/ha (480 lb/A).
d. Field 4: Finished wt was 323 kg/ha (285 lb/A).

B. Treated

1. Disease

a. Incidence was 100% in all treated areas
b. Average disease severity was 10/30/50% leaf area infected on the Flag/F-1/F-2.
c. Plants were taller than the nontreated and had greener leaves, stems and no FBS infection on the florets.

2. Yields

a. Field 1: Finished weight was 580 kg/ha (512 lb/A). Yield represents an increase of 43 kg/ha

(38 lbs/A), representing an increase of 8% over control.

b. Field 2: Finished weight was 674 kg/ha (595 lb/A). This is an increase of 126 kg/ha (111 lb/A), representing an increase of 23% over control.

c. Field 3: Finished weight was 591 kg/ha (552 lb/A). This was an increase of 82 kg/ha (72 lbs/A), representing a 15% increase in yield over control.

d. Field 4: Finished weight was 420 kg/ha (370 lb/A) This was an increase of 96 kg/ha (85 lb/A), representing a 30% increase in yield over control.

3. Summary of fields 1, 2 and 3: Tilt Treated

a. Plants on average had at least 15 -20% less disease.
b. Average yields were increased by 19% (8 - 30%).

Research Site III: University of Minnesota Research Paddies

A. Control (Field 1 and 2)

1. Disease

a. Incidence of FBS was 100 percent.
b. Severity of FBS was 25/50/100% leaf area infected on the Flag/F-1/F-2.
c. Plants were infected on florets, leaves and stems.

2. Yield

a. Field 1: Green weight was 527 kg/ha (465 lb/A). Finished weight was 316 kg/ha (279 lb/A).
b. Field 2: Green weight was 426 kg/ha (376 lb/A). Finished 255 kg/ha (225 lb/A).

B. Treated (Tilt)

1. Disease

a. Incidence of FBS was 100 percent
b. Severity of FBS was 15/30/55% leaf area infected on the Flag/F-1/F-2.
c. Plants were greener with no infection on the florets.

2. Yield

a. Field 1: Green weight was 649 kg/ha (572 lb/A).

Finished weight was 389 kg/ha (343 lb/A). This is an increase of 121 kg/ha (107 lbs/A) fresh wt, representing an increase of 23% over the control.

b. Field 2: Green weight was 536 kg/ha (473 lb/A). Finished weight was 322 kg/ha (284 lb/A). This represents and increase of 110 kg/ha (97 lbs/A) fresh weight. This represents an increase of 26% over the control.

ABSCISIC ACID LEVELS IN EMBRYOS AND WHOLE GRAINS OF WILD RICE

Introduction:

Cultivated varieties of wild rice (Zizania palustris L.) have retained the seed dormancy trait of plants from natural stands (Stucker et al. 1982). Wild rice seed normally shatters at maturity and remains dormant for 3-6 months (Simpson, 1966, Oelke et al., 1982). Dormancy is vital for the survival of Zizania spp. in nature, but it interferes with efforts towards plant improvement, such as greenhouse cultivation of varietal germplasm (Oelke and Albrecht, 1978) and initiation of tissue cultures (Percich et al., 1988). After the natural dormancy period, stored seed will germinate even in darkness at 1C, making storage of germplasm impossible.

Dormancy of wild rice seeds can be broken by chemical treatment (Oelke and Albrecht, 1980), ultrasonics (Halstead and Vicario, 1969) or mechanical scarification (Woods and Gutek, 1974, Oelke and Albrecht, 1978), but the resulting seedlings are often weak and fail to survive beyond the floating leaf stage (Campiranon and Koukkari, 1977). Thus, it is difficult to produce plants on demand from stored seed.

Dormancy also interferes with induction and maintenance of embryo-derived tissue cultures (Johnson, 1990). Dormancy is at least partially mediated by the level of endogenous abscisic acid (ABA) in the seed (Cardwell et al., 1977, Albrecht et al., 1979). This effect was partially reversed in some culture systems by amending the medium with the ABA synthesis inhibitor fluridone (1-methyl-3-phenyl 5-([trifluoromethyl]phenyl)-4-[1H] pyridinone), (CIBA-Geigy, Greensborough, NC) (Henson, 1984, Moore and Smith, 1984, Johnson, 1990). However, inhibition of ABA had no practical use in wild rice culture, since fluridone also inhibited morphogenesis and regeneration (Johnson, 1990).

It is hoped that seed dormancy can be overcome by embryo rescue techniques, and vigorous callus cultures can be obtained from embryo explants. Success in these efforts may depend on finding those embryos which are naturally lower in ABA. The purpose of

this study was to measure levels of ABA in embryos and de-hulled whole grains of stored wild rice of different ages to determine if ABA content is related to seed age.

Materials and Methods:

Extraction. ABA was extracted from 4- and 16-month-old de-hulled whole grains of wild rice and from embryos excised from these grains. Radiolabeled internal standard ^{3}H ABA (100 ul = 6089.8043 disintegrations per minute (DPM)) was added to each sample prior to extraction. Seeds or embryos were ground in 5 ml extraction buffer (methanol:H_2O (8:2 v/v) + 10 mg butylated hydroxytoluene) for 2 min at 4 C with a polytron grinder. The polytron head was rinsed into the suspension with an additional 2 ml of extraction buffer. The extraction buffer with suspended plant material was centrifuged at 9750 G for 20 min. Supernatants of each sample were decanted and retained. The pellets were re-extracted, centrifuged, and supernatants were combined with the previous corresponding supernatants. Extracts were decanted and evaporated to dryness in a Speedovac rotary evaporator (Savant Instruments Inc., Farmingdale, NY) (ca. 8 hrs). Dried extracts were re-suspended in 3 ml ddH_2O and filtered through a 5 um filter into a 6 cc syringe.

Liquid chromatography:

A two column preparative High Pressure Liquid Chromatography (HPLC) system (Waters Associates, Milford, MA) was used for separation and collection of the hormone fractions of each sample. Preparatory (PRP) and C-18 reverse phase columns and were used, and one strong and one weak solvent were used with each column (Table 1). Flow rates were 2.0 ml/min for the PRP column and 2.5 ml/min for the C-18 column. The PRP column was cleaned between each run by injection of dimethylsulfoxide (DMSO).

Retention time of ABA on the PRP column was determined by injection of a known quantity of ABA standard (10 ug/ml) and monitoring elution by UV absorbance at 254 nm. Sample extracts were injected into HPLC and fractions were collected during the determined proper elution time.

Gas chromatography:

Methylation of Abscisic Acid. An acetyl ferulic acid solution (1.5 ml of 1 ug/ml methanol) was added to each sample vial and mixed by sonication. Ethyl ether (1.5 ml) was then added to each vial. A 1 ug equivalent of Diazald (N-methyl-N nitroso-p-toluenesulfonamide) (Sigma, St Louis, MO) in 9 ml ethyl ether was reacted with conc. KOH (2 ml) and carbitol (2 ml) to produce di-azomethane gas. The ABA in samples was methylated by bubbling the gas through each of the sample solutions. Ether was evaporated under a stream of gaseous nitrogen and the remaining

solutions were transferred to GC vials. The methylated samples were evaporated to dryness in a rotory evaporator.

A standard curve for ABA was prepared by injection of 10-100 ng aliquots in 10 ng increments, plus 150, 200, 300, 400, 500, 750, and 1000 ng aliquots into the gas chromatograph (Hewlett-Packard 5985).

Gas chromatography. Samples were dissolved in ethyl acetate and 0.5 ml ethyl-abscisic acid (EABA) was added to each sample as an internal standard. The samples were injected into the gas chromatograph and peaks were obtained for ABA and EABA in each sample.

Scintillation counting. The sample vials were removed from the GC, and the ethyl acetate solution was evaporated to dryness in the rotary evaporator. Chloroform (1 ml) and Tris buffer (1 ml, pH 10.37) were added to the GC vials and mixed on a vortex mixer for ca. 1 min. Aliquots of 500 ul were taken from the chloroform layers of each sample and combined with 5 ml toluene scintillation cocktail in glass scintillation vials. The remainders of each sample were transferred by dropping the inverted GC vials into scintillation vials and adding 15 ml toluene scintillation cocktail. All samples were counted for 5 min. Methylation efficiency was determined by obtaining counts from the chloroform and chloroform/water layers and using them in the equation:

$$\text{efficiency} = \frac{\text{chloroform layer count} \times 2}{\text{chloroform layer count} + \text{chloroform/water layer count}}$$

which represents: $\frac{\text{MABA}}{\text{MABA} + \text{ABA}}$

Results and Discussion:

HPLC. Retention time of materials absorbing at 254 nm was 28-30 minutes. The window for collection was chosen as 27-31 min., and aliquots from each sample were collected.

GC Analysis. The regression equation for the GC standard curve for ABA was: $y = 7.6963 X + 159.92$. Peaks produced by the methylated samples were similar to that produced by the methylated ABA standard.

Calculation of ABA Concentration. The ABA concentration in the tissue samples was calculated as follows: The correction for internal EABA standard recovery was used in the standard curve equation ($Y = 7.6963 + 152.92(ABA/EABA)$) for the GC to determine actual ABA recovered. This value was multiplied by the recovery factor (1/percent ABA recovered as determined by scintillation

count values for the ^{3}H ABA internal standard, corrected for methylation efficiency) to obtain the total adjusted ABA in the sample. The total ABA/gfw for each tissue source is given in Table 2.

Although ABA is lost from the seed with age, the embryo retains a relatively higher percentage of ABA when compared with whole seed. Abscisic acid levels in 4-month-old embryos and seeds were higher than in 16-month-old embryos and seeds, respectively. Levels in 16-month-old embryos were 32% less than those in 4-month-old embryos, and levels in 16-month-old seed were 61% less than those in 4-month-old seed. The ABA content of embryos was always higher than that of whole seed, but the ABA concentration in 16-month-old embryos was 3.2 times higher than the concentration in whole 16-month-old seeds; while the concentration in 4-month-old embryos was only 1.8 times higher than in whole 4-month-old seeds. Thus, ABA in embryos does not decrease with age as rapidly as it does from the other seed components.

When wild rice is stored, 70 to 80% of seeds will have germinated by 16 months. The remaining seed either is non-viable or produces seedlings of poor vigor. This suggests that embryos from older seeds are not better for use as tissue culture explants when compared to relatively young embryos. It appears that wild rice seeds will germinate or lose viability long before age significantly reduces endogenous ABA levels.

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Table 1. Solvents used in two column preparative high performance liquid chromatography system for analysis of ABA.

Column	Solvent	Type	Formula
PRP[a]	A	weak	0.01 M NaH_2PO_4 in 20% EtOH
PRP	B	strong	0.01 M NaH_2PO_4 in 50% EtOH
C-18[b]	C	weak	0.1 N acetic acid in ddH_2O
C-18	D	strong	0.1 N acetic acid in 100% EtOH

a preparatory column
b C-18 column

Table 2. Total abscisic acid (ABA) per gram fresh weight in 16- and 4-month-old seeds and embryos of wild rice.

Age (months)	Tissue	Total ABA/fresh wt ng/g
16	Embryo	575.28
16	Seed	177.90
4	Embryo	848.87
4	Seed	454.56

LIST OF FIGURES:

FIGURE 1

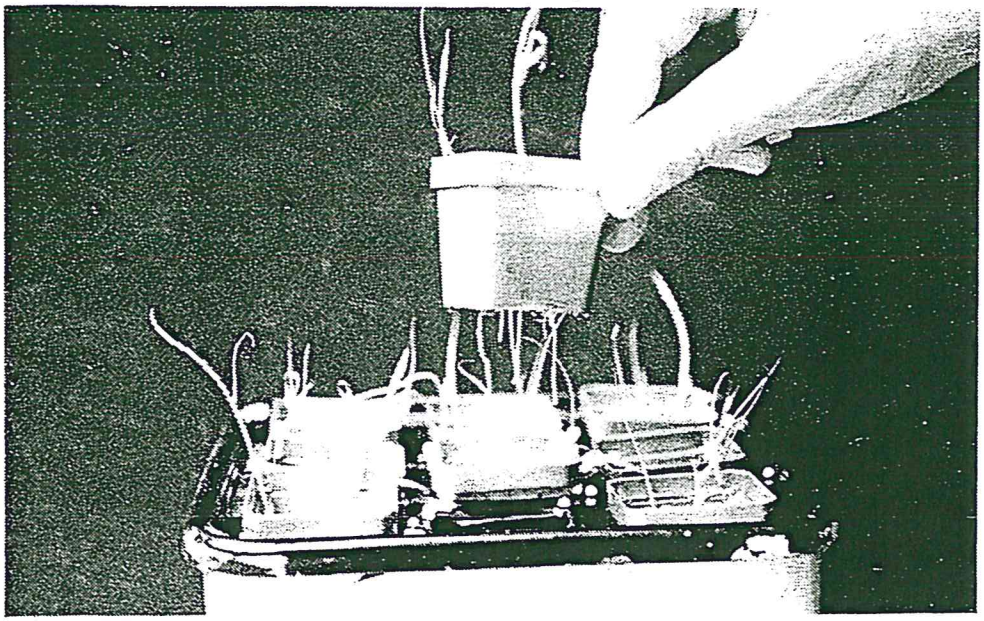

FIGURE 2

FIGURE 3

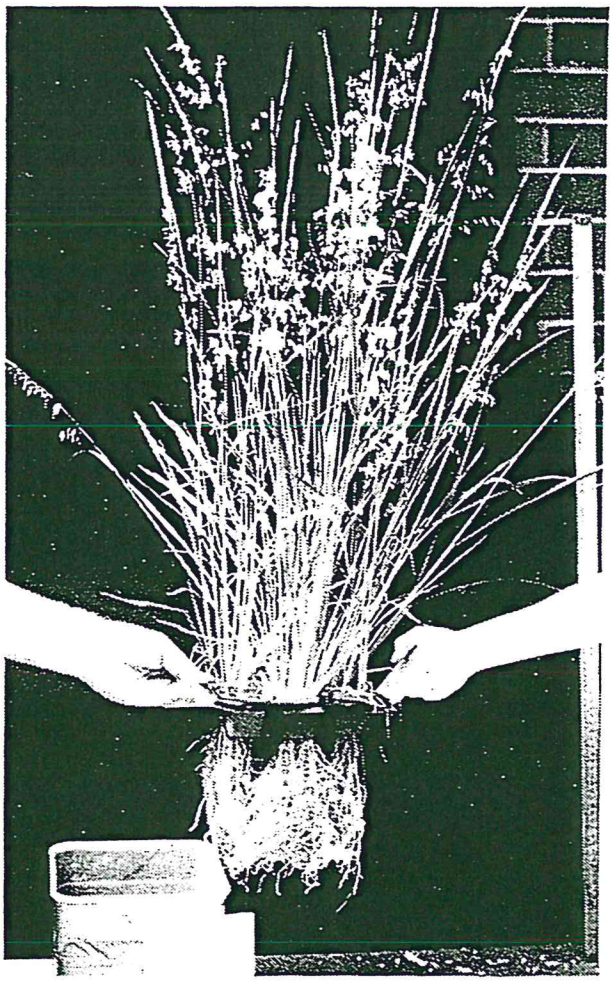

TABLE 1. Effect of TiltTM in controlling fungal brown spot, caused by Bipolaris oryzae and increasing yields on cultivated wild rice Minnesota in 1990.

Site	Yield kg/ha[1]	Mean Yield	Percent Control	Mean % Control	Mean Disease Severity[2]
I[3]					
	679		25		
	702		29		
	656		21		
		679		25	10/25/50
II[4]					
	581		8		
	614		23		
	626		15		
	420	560	30	19	10/30/50
III[5]					
	389		23		
	536		26		
		463		25	15/35/55

[1]Yield in pounds per acre of finished wild rice.

[2]Mean percent leaf area infected on the topmost leaves, Flag/ F-1/F-2.

[3]Yield (finished) and disease severity of untreated site were 480 lb/a and 40/50/75%, respectively.

[4]Average yield (finished) and disease severity at untreated sites were 431 lb/a and 35/50/75%, respectively.

[5]Mean yield (finished) and disease severity at the untreated sites were 421 lb/a and 25/50/100%.

Summary:

In 1990 when Tilt was applied twice at 6 oz/A (14 to 17 day interval) on three different wild rice operations the average resulting yield was increased by 23 %, when compared with the untreated controls (Table 1, above).