Minnesota Wild Rice Research 1990

Miscellaneous Publication 70–1991
Minnesota Agricultural Experiment Station
University of Minnesota

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St. Paul, Minnesota

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WILD RICE PRODUCTION AND SEED RESEARCH - 1990

E.A. Oelke and M.J. McClellan[1]

Department of Agronomy and Plant Genetics

The 1990 growing season was cooler in all wild rice growing areas than 1989. The total growing degree days (GDD) averaged 239 less than for 1989 (Table 1), however 1990 averaged 32 GDD more than the 50-year averages ("normal"). The temperatures were cool during the early part of the growing season (April and May), warm in June, and cool again during July and August.

Table 1. Growing degree days[a] comparisons for 1989, 1990 and normal.

	Aitkin			Grand Rapids			Crookston		
Month	1989	1990	Normal	1989	1990	Normal	1989	1990	Normal
	GDD								
April	94	64	114	83	30	107	136	82	132
May	424	331	414	454	335	381	574	468	438
June	928	726	677	654	719	634	741	818	710
July	928	808	871	953	831	817	1069	907	900
August	830	766	785	811	810	733	965	963	850
Total	2934	2695	2861	2955	2725	2672	3485	3238	3030

[a] $\frac{\text{Maximum temp.} + \text{Minimum temp.}}{2}$ - 40°F; data from Mark Seeley, Soil Science Dept., Univ. of Minn.

Total precipitation was lower compared to both 1989 and normal (Table 2). It was especially dry in May in all areas and in July at Crookston. August also was drier compared to 1989 and normal. Generally it was a more ideal climate for wild rice than 1989 resulting in higher per acre yields even though the plant populations were low in some fields due to seed germination loss during the winter.

[1]Professor and Senior Research Plot Technician, respectively.

Table 2. Precipitation comparisons for 1989, 1990 and normal[a].

Month	Aitkin			Grand Rapids			Crookston		
	1989	1990	Normal	1989	1990	Normal	1989	1990	Normal
	---------- GDD ----------								
April	2.32	2.94	2.27	2.32	2.12	1.99	0.39	2.22	1.39
May	3.90	1.39	3.39	3.19	.96	3.16	4.56	0.71	2.20
June	5.66	5.25	3.83	4.64	4.51	3.79	2.71	5.83	3.61
July	0.62	2.13	4.79	2.74	3.23	4.12	0.56	0.48	3.17
August	6.27	2.18	4.19	4.54	2.14	3.38	3.76	3.01	3.04
Total	18.77	13.87	18.47	17.43	12.76	16.44	11.98	11.25	13.41

[a]Data from Mark Seeley, Soil Science Dept., Univ. of Minn.

Total paddy wild rice production in Minnesota was more in 1990 compared to 1989 mostly because of higher per acre yields (Table 3). California production was also higher but only slightly compared to 1989.

Table 3. Minnesota and California paddy wild rice production[a] (1000 processed pounds).

Year	Production		Year	Production	
	Minnesota	California		Minnesota	California
1968	36	0	1980	2320	400
69	160	0	81	2274	500
70	364	0	82	2697	880
71	608	0	83	3200	2500
72	1496	0	84	3600	3800
73	1200	0	85	4200	7900
74	1036	0	86	5100	9000
75	1233	0	87	4200	4200
76	1809	0	88	4000	3500
77	1031	0	89	3978	4000
78	1761	100	90	4600	4200
79	2155	200			

[a]1968-1982 Minnesota values from Winchell and Dahl and 1983-1990 from Minnesota Department of Agriculture; California values from Marcum, Cooperative Extension Service, University of California.

The total value of the 1990 crop is estimated at $7.82 compared to $6.56 million for 1989. The increase is due to increased production and price. The highest value was in 1986 when production was the highest and prices $0.90 more per pound than in 1990 (Table 4).

Table 4. Processed wild rice harvested and value from cultivated fields in Minnesota

Year	Production	Price	Value
	1,000 lb	$/lb	$ Millions
1968	36	3.30	0.12
1969	160	2.55	0.41
1970	364	2.80	1.02
1971	608	2.70	1.64
1972	1,496	2.30	3.44
1973	1,200	2.05	2.46
1974	1,036	2.37	2.46
1975	1,233	2.50	3.08
1976	1,809	2.70	4.88
1977	1,031	4.35	4.48
1978	1,761	5.10	8.98
1979	2,155	5.01	10.80
1980	2,320	4.47	10.37
1981	2,274	3.79	8.62
1982	2,697	3.41	9.20
1983	3,200	3.35	10.72
1984	3,600	3.30	11.88
1985	4,200	2.97	12.47
1986	5,100	2.60	13.26
1987	4,200	1.50	6.30
1988	4,000	1.65	6.60
1989	3,978	1.65	6.56
1990	4,600	1.70 (est.)	7.82

Research

The 1990 research focused on weed control, crop rotation, shading effects on wild rice yield, and effects of drying wild rice seed before storage on seed viability and storeability. The research was conducted on plot land and in laboratories of the University of Minnesota, at Grand Rapids and St. Paul, and on specially designed research paddies on the Vornela Wild Rice Farms near Aitkin.

Weed Control Research

The weed control effort was concentrated on the attempt to control giant burreed with fall applications of herbicides. Giant burreed was established by planting rootstocks into a paddy at Grand Rapids in the spring of 1987. The paddy was flooded each year to allow the giant burreed to become well established. On August 21, 1990, which is the normal time to harvest wild rice, the giant burreed plants were trimmed with a hedge trimmer leaving a 30-inch stubble. This is the normal height of the stubble left after harvesting wild rice with combines. The upper part of the giant burreed leaf was beginning to die back; however, this was removed by trimming and the remainder or lower part of the leaf was still green. The plot size was 10 by 20 ft and the experimental design was a randomized complete block with three replications. The herbicides were applied with a hand CO_2 sprayer at 25 psi at a total volume of 30 gal/A.

The herbicides applied were glyphosate (Roundup) at 1/2, 1 and 2 lb ai/A and 2,4-D amine and MCPA with and without crop oil at 1 and 2 lb ai/A. Some visual injury was evident from the herbicides six weeks after treatment. We will be closely monitoring giant burreed growth this spring to see which treatments, if any, were effective in controlling giant burreed.

In another area of weed control, we were able, through the Minnesota Department of Agriculture, to obtain a Section 18 for use of 2,4-D amine in wild rice. We are pursuing this again for 1991. In addition, we have received approval and funding from IR-4 (Interregional Research Project No. 4, New Jersey) to pursue labeling of MCPA for use in wild rice. We will be collecting more residue data in 1991 and asked for clearance for the 1992 growing season.

Sustainable Agriculture

A Sustainable Agricultural Grant was obtained by George Shetka and the University of Minnesota from the Minnesota Department of Agriculture to initiate research for comparing continuous wild rice production to rotating wild rice with another crop or fallow every other year. The experiment was established in the six 2-acre wild rice research paddies on the Vomela Wild Rice Farm near Aitkin. All six paddies were in wild rice production during 1989. After wild rice harvest all six paddies were rotovated. Three of the paddies were fertilized in the fall for wild rice production in 1990. Two hundred fifty pounds of 1-5-40-5S were applied before rotovating; while 19 gallons of 22-7-0 were injected after tillage.

In the spring of 1990, the other three paddies were divided into three strips. One strip was left fallow all summer, one strip was planted to alfalfa, variety 'Nitro,' while the third strip was planted to two varieties of spring canola. One variety of canola was 'Legend,' medium maturing while the other was a very early variety, 'Parkland.' The Legend seed was supplied by Interstate Seed Company and Parkland seed by Dr. Downey of Canada. The canola and alfalfa strips were fertilized with 15 lb/A of P and 100 lb/A of K. In addition, the canola strips received 100 lb/A N and the alfalfa strips 20 lb/A N. The fertilizer was incorporated with a field cultivator before planting.

Seeding was delayed until May 21 because of wet fields. The canola was seeded at the rate of 11.5 lb/A with a 12-ft press-wheel grain drill with 6 inch spacing between rows.

Alfalfa was seeded at the rate of 19 lb/A with a Brillion seeder.

One of the three 2-acre wild rice paddies was lost to crayfish, thus yields were only obtained from two of the paddies. The wild rice was harvested with the grower's combine on August 22. Also, wild rice plant density was high in one of the other paddies resulting in lower yields. The average wild rice yield at 40% moisture was 595 lb/A which was lower than expected even with two applications of Tilt (Table 5). Part of the reason for low yields may have been due to lack of nitrogen which was evident in one of the paddies with the higher plant density, and to diseased plants.

We were successful in establishing both canola and alfalfa in the peat soil. Both grew well except for small areas that were too wet. We hand harvested small areas of both crops. The remainder of the crop was incorporated into the soil in the fall. The Parkland canola flowered very early resulting in short (30 in.) plants and low yield (Table 5). This variety is too early for the Aitkin area. The Legend canola variety was taller (40 in.), later and yielded more than Parkland. However, the yield was still not as much as needed for economical canola production. We feel, however, that earlier planting could result in better yield. We had to delay planting because of some seepage from the adjacent flooded paddies. Also smartweed was present in canola which might be reduced if canola were planted earlier.

We were able to establish a good stand of alfalfa; however, in one paddy smartweed reduced the growth of alfalfa. We were able to reduce the smartweed competition some by mowing the smartweed just above the alfalfa on July 20. We harvested 3 X 20 ft areas on August 28 to obtain an estimate of alfalfa yield. We obtained 0.65 ton per acre (Table 5) with good quality (Table 6). It might be possible to obtain one cutting of hay from the alfalfa and still allow for enough nitrogen to be added to the soil for next year's wild rice crop. The alfalfa was tilled into the soil on September 19.

Wild rice was seeded into the three paddies in the fall that had the canola, alfalfa and fallow strips. All six paddies will be in wild rice in 1991. No fungicide will be used on the three paddies that were out of wild rice in 1990. Data will be collected on wild rice growth, disease incidence and yield in 1991. Water quality measurements will also be made on each paddy. An economic analysis will be made at the conclusion of the experiment in 1992.

Table 5. Wild rice, alfalfa and canola yields in crop rotation experiment-Aitkin, 1990.

Wild rice[a]	Canola[b]		Alfalfa[c]
	Legend	Parkland	Nitro
	lb/A		
595	792	303	1294

[a]40% moisture.
[b]Plants/ft^2 for Legend = 19.6 and for Parkland = 12.1.
[c]Plants/ft^2 = 15.4; harvested on 8/28.

Table 6. Alfalfa quality when harvested on August 28.

CP	ADF	NDF	DM	P	CA	K	MG
			%				
14.3	42.3	54.5	93.5	.32	1.25	3.06	.35

Influence of Shade During Grain Fill on Yield of Wild Rice

The third year of a 3-year experiment was completed at St. Paul on the effect of reduced light during grain fill on yield of wild rice.

Growers have experienced lower yields when long periods of cloudy days occur during grain fill. This trial was conducted to see if reduced light during grain fill could result in lower yield. The trial was similar to the one conducted in 1987 and 1989. The study was conducted utilizing 4 ft x 4 ft boxes that were 1 ft deep. The boxes were lined with black plastic sheeting and filled with 8 inches of greenhouse soil mix. The soil was fertilized with 40 lb/A N (urea) plus 6 lb/A of Fe chelate. Four rows of wild rice were seeded on May 4, 1 ft apart, into each box after which the boxes were flooded to the top. After the plants were in the 3- to 4-leaf stage the rows of plants were thinned to one plant every 2 inches. On July 14, when the plants were in late boot to early flowering, black plastic mesh screening that reduced light by 47% was placed over all boxes except the controls. The center two rows were harvested for grain yield and plant measurements. Grain was hand stripped 3 times beginning on August 7 and ending on August 21. There were 7 replicates and the experimental design was a randomized complete block. There were 3 light regimes; one with no shading during grain fill, one with the mesh removed after 2 weeks and another with the mesh removed after 4 weeks.

During 2 of the 3 years, yield was reduced by shading the plants even for 2 weeks. In the other year yield was reduced by 4 weeks of shading. The results from this 3 year experiment indicates that yields can be reduced by long periods of reduced sunlight which could be the case during long periods of cloudy weather.

Table 7. The effects of reducing natural light by 47% during grain fill on wild rice yield and plant characteristics, St. Paul, 1990.

Weeks of light reduction after flowering	Plant height	Plant number	Dry wt/ plant	Stem/ plant	Grain yield[a]
	cm	no./ft^2	gm	no.	lb/A
0	89	2.8	11.6	5.6	1656
2	84	2.6	11.2	4.8	1178
4[b]	86	2.7	11.1	4.6	1078
LSD .05	NS	NS	NS	NS	370

[a]40% moisture. [b]Harvested on this date.

Seed Storage and Handling

The results from the seed storage experiment reported in the 1989 report indicated that wild rice seed could be dried down to 9% moisture and still germinate even after 18 months of dry storage at 28°F. However it was still necessary to store the seeds for an additional 3 months in water at 38°F to obtain germination. A similar experiment was conducted starting in the fall of 1989 except with fewer seed moisture levels and comparing dry storage at 28°F and water storage at 38°F.

Seeds of the K2 variety were air-dried on a laboratory bench at room temperature for 10 days starting on 9/11/89. The room temperature was 70-75°F and the relative humidity was 40%. Seed was divided into 24 lots (6 sampling dates and 4 replicates). Twenty-four subsamples (1-pint bottle of seed) were taken immediately and 24 every other day for a total of 6 sampling dates. The 1-pint jars were sealed with silicone and immediately placed in a chamber kept at 28°F. In addition, at each sampling date 4 similar-sized samples were placed in plastic bags filled with water and stored at 38°F. Seed moisture was determined at each sampling date and when samples were removed from dry storage. Seed moisture content was determined by drying for 7 days in a forced air oven at 150°F.

Every 6 months, one subsample from each replicate of each seed moisture was removed from dry storage. Germination measurements were made immediately and after 3 months of additional storage in 38°F water. Germination was also determined for the seeds stored in 38°F water for the entire storage period. Germination was measured by placing 100 seeds into a petri dish filled with water and kept at 72°F. Seeds were determined to be germinated when the coleoptile had grown longer than the length of the seed.

The germination results for the first 15 months of the experiment are presented in Table 8.

Table 8. Germination percentage after dry storage at 6 moisture levels for 6 and 12 months followed by 3 months of storage in water compared to storage in water for 9 and 15 months, St. Paul.

Drying time	Seed moisture	Months dry at 28°F[a]		Months in water at 38°F	
		6	12	9	15
hrs	%	germination %			
0	34.0	65.0	47.0	25.2	0.2
65	25.8	30.2	28.2	25.5	1.0
96	20.4	35.5	33.8	15.5	0.8
148	15.0	37.5	32.0	11.5	0.2
185	11.4	38.2	15.8	8.2	0.5
233	8.6	27.0	16.0	5.5	0.0
Mean		38.9	28.8	15.2	0.4
LSD .05	0.9	18.1	20.8	5.8	NS

[a]Germination obtained after an additional 3 months in water at 38°F; less than 0.5% germination immediately after 6 or 12 months dry storage averaged over the 6 moisture levels.

After 6 months of dry storage and then 3 months of wet storage germination was best at the highest seed moisture, however germination was similar for the other seed moisture levels. This was also true for 12 months of dry storage and then 3 months of wet storage. The average germination was lower after 12 months of dry storage compared to 6 months of dry storage. Storing the seeds dry for a period of time was better than comparable storage length in water. Very little (1% or less) germination was obtained immediately when seeds were removed from dry storage after 6 or 12 months. Cold 38°F water storage for a period of time appears necessary to release dormancy after dry storage even at the higher seed moisture. We will continue to remove samples every 6 months from storage for a period of 3 years.

Based on the 1990 results and those from 1987-89 it appears that wild rice seed could be stored dry for a period of time thus increasing the storage life of wild rice seeds. This would be beneficial in maintaining germplasm for longer periods than we presently can in water storage.

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We wish to thank Henry Schumer, plot coordinator at Grand Rapids, for his continued support. The help of Drs. Nyvall, Boedicker and Rabas at Grand Rapids is also appreciated. The help of George Shetka and Duane Kramer of Vomela Wild Rice is greatly appreciated. We thank the Minnesota Department of Agriculture for providing funding for the Sustainable Agricultural project on the Vomela farm.

FILLING IN THE GAPS: FALL CARRYOVER N AND IN-SEASON N DEPLETION BY WILD RICE

William Zanner and Paul R. Bloom[1]
Soil Science

Introduction

Growers all know that wild rice responds to N fertilization, yet researchers have not been able to consistently demonstrate statistically significant responses to fertilizer N. Fly-on nitrogen remains a puzzle: if, how much, and when. These difficulties in understanding the response of wild rice to basal and fly-on nitrogen can be better understood by use of a model. We have slightly modified the model of Stanford (1973), which can be expressed mathematically as:

$$N_c = N_i + N_m + N_f - N_l$$

where

N_c = the crop uptake of N associated with an attainable yield (120 lbs/ac for a top yielding wild rice crop)
N_i = the initial quantity of mineral N in the profile
N_m = the estimated N mineralized during the cropping season
N_f = the amount of N fertilizer needed
N_l = N losses due to volatilization and/or nitrification-denitrification reactions

Nitrogen losses (N_l) were studied by Meyer, Bloom, and Grava (1989) under laboratory conditions. They showed that it is important to use ammoniacal forms of nitrogen with deep placement to minimize losses. Their data also indicate that losses can be significant even with careful management but loss rates are lower than the rate of ammonium-N production by mineralization of soil organic matter. Their data for one soil suggest that, in the top fifteen inches, mineralization (N_m) produced 80 lbs/ac of N which is enough to supply two-thirds of the N needed by a high yielding crop. Unfortunately peat soils vary considerably in mineralization rates (most soils appear to be in the range of 50 to 140 lbs/ac) and it is not possible to easily predict mineralization rates from soil properties (Bloom and Meyer, 1988.)

Compared to the other components in the model, the least is known about the initial mineralized N (N_i), that is, the portion of N in the soil at the beginning of the season that was not added as fertilizer. Preliminary data in the Wild Rice Report of last year suggests that ammonium (NH_4^+) produced by mineralization in the soil after harvest may contribute significantly to nitrogen fertility in the following season. This N should be considered in making N fertilizer recommendations. However, prediction of fall carryover N is not possible given our current level of understanding. This is due to a combination of interactions that occur in paddy soils after draining. As the soil dries, portions become aerobic and the rate of mineralization increases, releasing more NH_4^+. Under aerobic conditions, ammonium (NH_4^+-N) is oxidized to nitrate (NO_3^--N), a form that is lost on flooding. The degree of drying, and thus the depth to which the soil becomes aerobic, is determined by temperature and drainage. The net result of these gains and losses is to leave a portion of the NH_4^+ in the soil that will be available to the plant in the next growing

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[1] Graduate Research Assistant and Professor

season. However, given the interactions involving N_m, N_l, and N_i, it is difficult to predict what the net effects are and it is not surprising that the response of wild rice to fertilizer N is inconsistent.

The following studies were conducted in 1990 in an attempt to better understand these variables in the above model. During the 1990 growing season, soil solution N (NH_4^+ and NO_3^-) was sampled and measured and plant samples were collected to measure uptake of N. Soil solution N was measured in areas with and without rice plants. After harvest, soil samples were collected until the end of October. These studies were located at three different sites - the University paddies at Aitkin (identified hereafter as "Aitkin"), Gunvalson and Imle's paddies at Gully ("Gully"), and Clearwater Rice at Clearbrook ("Clearwater"). N management of these sites was as shown in Table 1. At Aitkin, a data logger was used to record air, water and soil temperatures from early June until mid-October.

The objectives were (1) to determine the relationship between soil NH_4^+ levels and plant uptake, (2) to relate these levels to N management, and (3) to determine amounts and persistence of soil NH_4^+ after harvest. The data collected will be used in determining methods for predicting the need for fly-on N and for developing soil test methods to determine fall carryover NH_4^+. In addition this information will provide background information for further laboratory studies of mineralization.

Table 1: N MANAGEMENT

	Aitkin	**Gully**	**Clearwater**
N Source, Fall Injected	45 lbs/acre urea-N	30 lbs/acre urea-N	45 lbs/acre NH_3
Fly-on N	None	30 lbs/acre urea-N	None
Residue	Chopped, incorporated	Chopped, burned	Chopped, incorporated

In-season plant N use

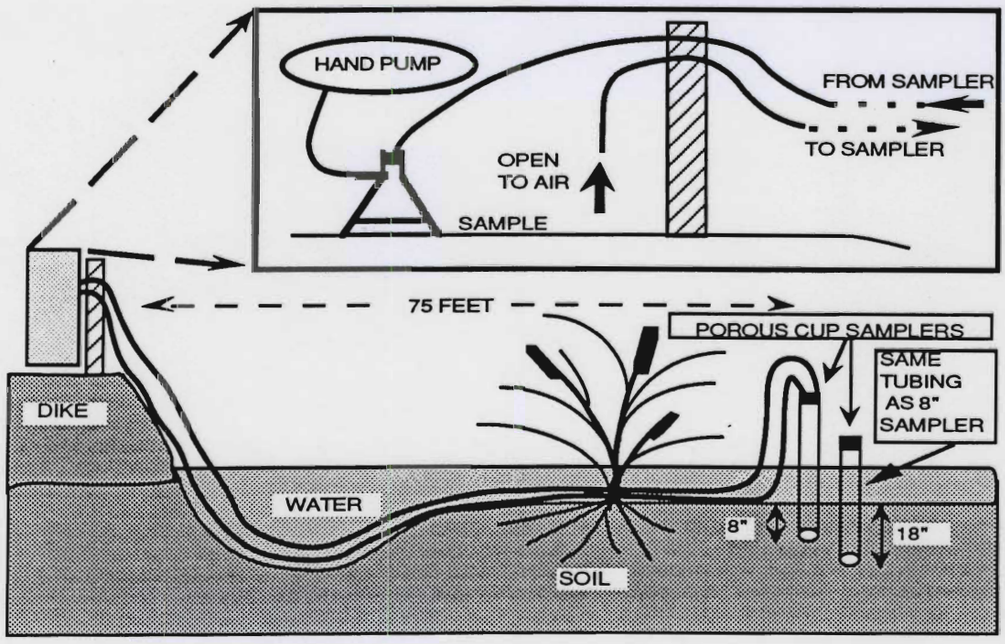
We chose to use porous cup samplers to collect soil solution samplers to look at NH_4^+ and NO_3^- fluxes. This method allows for simple sample collection once the samplers are installed (Figure 1). Sampling the soil solution measures only a portion of the NH_4^+ present, the portion of the NH_4^+ not held by cation exchange sites in the soil. However, Elder (1981) showed that NH_4^+ present in the soil solution represents a significant portion of that held in the soil. The NH_4^+ on the cation exchange sites is available to plants because as NH_4+ ions are taken up from soil solution by the plants, NH_4+ from cation exchange sites rapidly moves into solution. Solution sampling is thus an indicator of the N supplying capacity of a soil. As will be seen, plant demand is sometimes greater than the capacity of a soil to supply N from the soil solution and these cation exchange sites

The total N that is available to plant roots at any one time is a combination of the initial amount present (N_i), the amount mineralized (N_m), and the amount applied as fertilizer

(N_f). This N pool is in constant flux. Removals from the pool include plant take-up of N and nitrification-denitrification losses (N_l). The initial amount present (N_i) and the amount applied as fertilizer (N_f) are removed by plants and microbial activity. Additions to the system are supplied to the pool by mineralization (N_m) and more fertilizer can of course be applied. However, we are concerned about net mineralization, not just mineralization. Soil microorganisms are competing with the plants for N from the pool, and the N removed by microorganisms from the active pool is not available to the plant.

Net mineralization is then the difference between the total amount of NH_4^+ mineralized and that portion removed by microorganisms. As indicated above, net mineralization is capable of supplying much, if not all in some cases, of the N needed for a high-yielding crop, but the NH_4^+ may not be available at the right time for optimal growth. Grava and Raisanen (1978) found that wild rice accumulates 70% of its total N from flowering to grain fill. This suggests that there may be crucial stages of plant growth where the plant potential could be limited if mineralization is supplying NH_4^+ more slowly than the plant can take it up. Identification of these critical periods of shortfall would enable better judgments to be made about the need for additional fly-on N.

Figure 1: EXPERIMENTAL SET-UP FOR SOIL SOLUTION COLLECTION

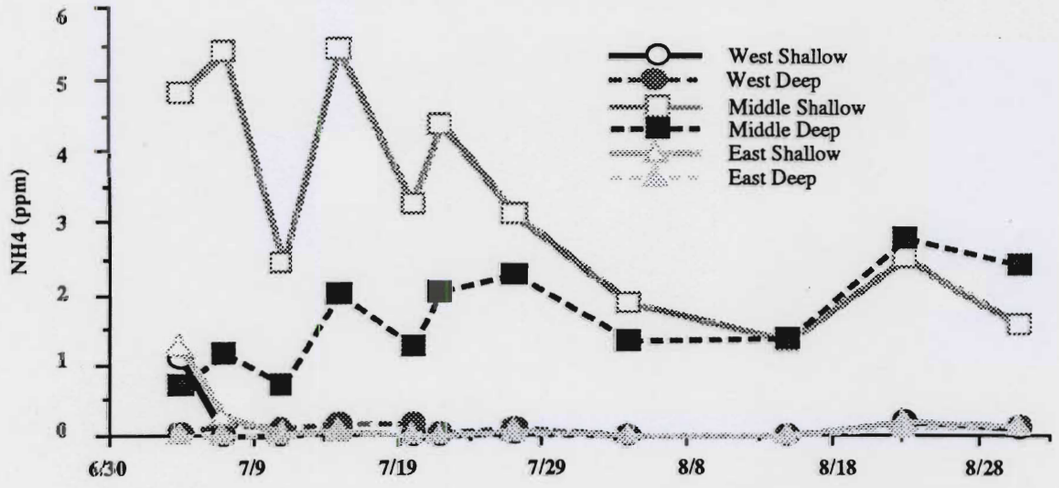


Soil solution samplers were installed in the three sites in June. The samplers were put into place from a boat so as to minimize disruption of the sampling site. The set-up (see Figure 1) enabled frequent collection of fresh samples without the problems of disturbance and inconvenience that would be experienced if the sample collector had to walk out into the paddy at each collection time. Each site had six samplers in a paddy. They were situated at three spots (described as north, middle and south or east, middle and west) and at two depths (shallow ≈ 8"; deep ≈ 18"). At each site, all plants were removed from an area about ten feet in diameter around the middle sampler. At Gully, fly-on N was applied in

the area of the middle and north samplers only. No fly-on was applied at the other two sites, as indicated in Table 1.

Results of the analyses of the soil solution samples are shown for each site in Figures 2, 3 and 4. Note that, due to poor growth in the area of the south samplers at Clearwater, only data from the north and middle samplers were used. Figure 5 shows a comparison of the cropped areas for the three sites. It should be noted that the amount of NH_4^+ found at the shallow sampling depth decreases to zero at all three sites, although at different rates. This indicates that the plants have used up the carryover N (N_i) and the fertilizer N (N_f), and that the plants are taking up mineralized N (N_m) as fast as it becomes available. At this stage, plant growth outstrips the soil's ability to supply NH_4^+.

Figure 2: AITKIN SOIL SOLUTION NH4



The problems experienced at Aitkin with plant density and water control are reflected in Figure 4 which shows that the plants had taken up most of the excess NH_4^+ in the soil solution at the start of sample collection on June 30. The area at Aitkin without plants remained lower in measurable NH_4^+ than did the no-plant samplers at the other two sites, indicating a general N-fertility problem in this paddy. Fallow paddies were on each side of the paddy where the samplers were located, and it was difficult to maintain water at consistent depths due to leakage into the adjacent paddies. Fluctuating water levels would tend to speed nitrification-denitrification reactions and thus reduce plant available NH_4^+, and NH_4^+ may also have been lost with the water seeping out of the paddy. The interaction of NH_4^+ and NO_3^- found in the middle shallow samples suggests that changing water levels caused N losses (see Figure 6): when NH_4^+ went down, NO_3^- went up and when NH_4^+ went back up, NO_3^- went down. Drainage adds oxygen to the system and increases the transformation of NH_4^+ to NO_3^-, which is then lost when water is put back on the paddy. The changing water levels resulted in high N losses. In contrast, note that NO_3^- was not detected at the other sites before the paddies were drained. Plant density was also too high at Aitkin which would have resulted in earlier depletion of the NH_4^+ available to the plants. The low NH_4^+ found in samples collected from the middle deep sampler increased from the time of installation because the plants in that area were removed and mineralization resupplied NH_4^+ to the soil solution.

Figure 3: GULLY SOIL SOLUTION NH4

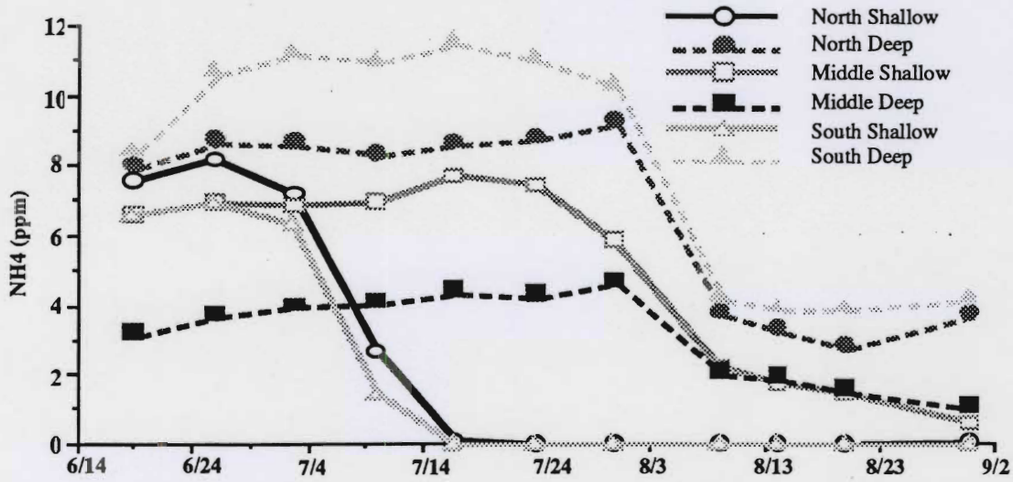


Figure 4: CLEARWATER SOIL SOLUTION NH4

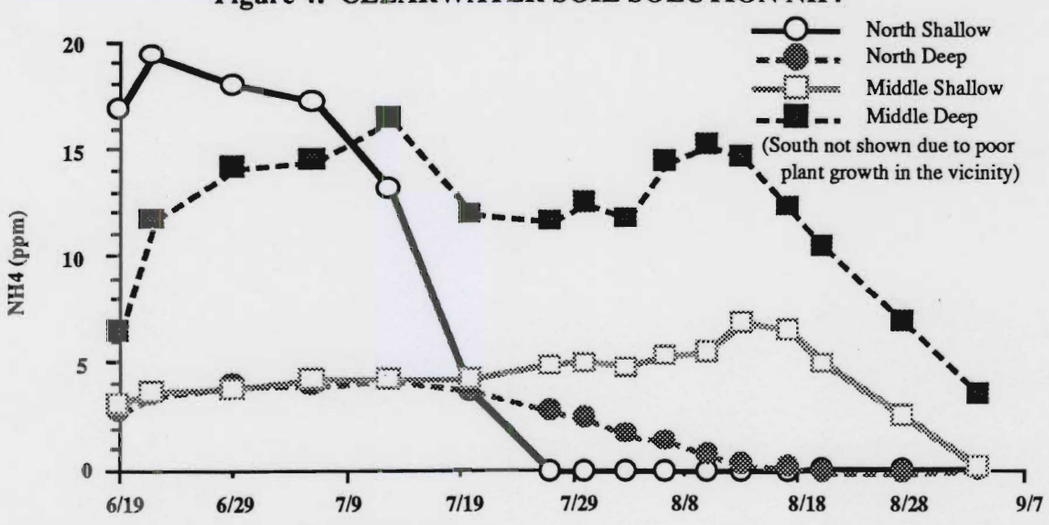


Figure 3 shows there were similar responses at Gully, but that the plants did not deplete NH_4^+ in the shallow sampler until July 14, and we still found NH_4^+ in the deeper sampler right up until harvest. Urea was flown on this paddy the week that the shallow sampler went to zero. Strips that included the middle and north samplers received urea. The south end of the field received no fly-on. No difference between the south and north samplers was found because any NH_4^+ applied at this stage would be utilized by plants before it diffused down to the area of the sampler. Note the steep decline in the amounts of NH_4^+ found by the deep samplers when water levels are lowered. The water was at two feet below field level on August 1. This decline is seen in the areas of crop and no-crop indicating that it may caused by lowering the water level. This decline is consistent with what was seen at Aitkin when water levels went down.

Figure 5: SOIL SOLUTION NH4 DURING GROWING SEASON AT AITKIN, GULLY, CLEARWATER

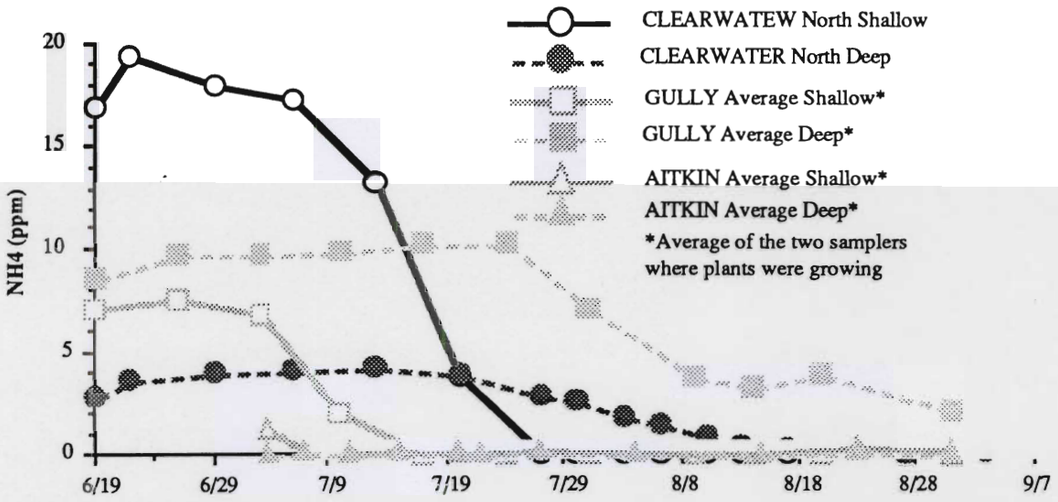
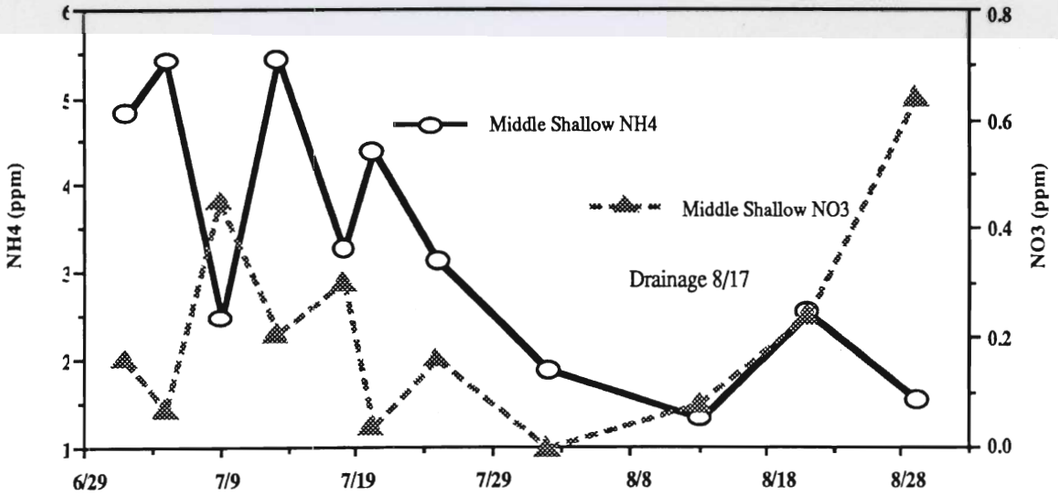


Figure 6: AITKIN SOIL SOLUTION NH4 VS. NO3



Clearwater (Figure 4) showed a similar pattern to Gully. (As mentioned above, the south samplers were in an area of poor growth so data from only the north sampler is presented. Installation of the samplers in a flooded paddy when the rice was still at the floating leaf stage during a rain storm resulted in less than optimal placement of the south sampler.) However, NH_4^+ detected in the shallower sampler reached zero two weeks later than at Gully, and the deep sampler still had detectable although very low levels of NH_4^+ through the end of August. Water levels were maintained at field level later than at Gully, but at drainage a similar sharp decline in NH_4^+ is seen.

This portion of our work leads to three preliminary conclusions:

The declines in NH_4^+ seen at both Gully and Aitkin at the time of drainage suggests that there is a relationship between drainage and NH_4^+ supply and that timing of drawdown may be critical. As mentioned above, wild rice takes up 70% of its N from early flower to the end of grain fill. Early drainage may deprive plants of needed N.

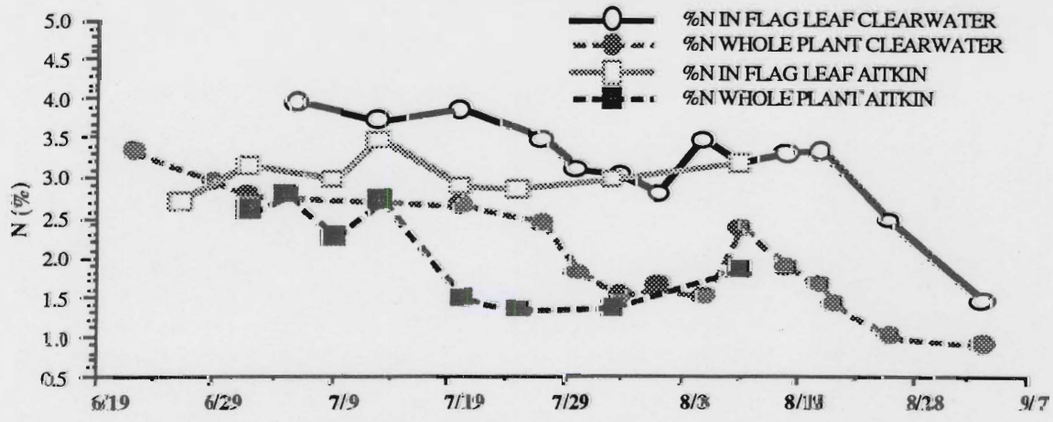
Our experience at Aitkin, and the rapid decline of NH_4^+ upon drainage seen at Gully and Clearwater, suggests that if management of water levels is a problem, the plants may benefit from (additional) fly-on nitrogen.

At Clearwater, adequate levels of NH_4^+ were found at all times suggesting that fly-on N would not have provided economic benefits. Yields of approximately 1400 lbs/acre green rice support this conclusion. The levels at Gully in the shallow samplers were lower than at Clearwater and went to zero earlier, suggesting that the plants may have been somewhat stressed for N and probably benefited from fly-on N. Yield was similar to that harvested at Clearwater. Aitkin was deficient early and the plants were also badly affected by fungal brown spot resulting in poor yields. Figure 5 compares NH_4^+ levels at the three sites. Measuring and monitoring these trends in NH_4^+ depletion in a particular paddy would be very helpful in deciding whether plants might benefit from additional fly-on N. However, soil sampling in a wet paddy is not easy.

Plant Data

As can be seen in Figure 7, the numbers obtained from the solution samplers are in agreement with the plant samples. The crucial level for N in wild rice plant leaves at early flower is about 3.25-3.5%. N levels at Aitkin are low compared to Clearwater. The graph lines for whole plant and flag leaves are in parallel through the season for Clearwater. For Aitkin, however, it can be seen that the two lines for the Aitkin plants diverge through the growing season. As noted in last year's report, this indicates that the plants were under stress and were reallocating N from older portions of the plant to maintain the most photosynthetically active tissue. Note again that low N tissue levels were present from the start, indicating that deficiencies occurred early on.

Figure 7: COMPARISON OF PLANT N STATUS, AITKIN AND CLEARWATER



Temperature Data

A data logger was used to record soil, air, and water temperatures at Aitkin. Thermistors were placed in the paddy where the samplers were located, at depths of 8" and 24", twenty feet into the paddy. Water temperature was measured in the ditch at a height approximately level with the soil. Air temperature was measured in the field box where the data logger was situated. The data logger samples temperature every minute, and then records an hourly average of the temperatures measured. Temperatures were recorded from mid-June until mid-October. Figures 8, 9 and 10 present a small portion of the information collected. Peak air temperature of 93° F occurred on July 3, and the soil 8" below the surface reached its maximum of 69° F on July 5. Relatively cool air temperatures from August 17-August 20 depressed soil temperatures just before harvest and apparently prevented soil temperatures from reaching that maximum of 69° again despite the removal of cover. In many years, we would predict that peak soil temperatures would occur when the soil is directly exposed to the sun after harvest. The lowest temperature recorded, 22° F, came on October 9. Note that in October the surface soil is cooler than the deeper soil which is the inverse of most of the season. The crossover occurred in late September.

Warmer temperatures below the surface have implications for deep placement of NH_4^+. As is shown in Figure 10, peat is a good insulator, and temperatures at the 8" depth did not drop below 50° F until October 8. The standard recommendation calls for waiting until soil temperatures are below 50° F before applying fertilizer. It is thus important to measure the temperature in the zone of application to ensure that potential N losses are minimized.

Figure 8: AITKIN TEMPERATURE DATA, JULY2-JULY9

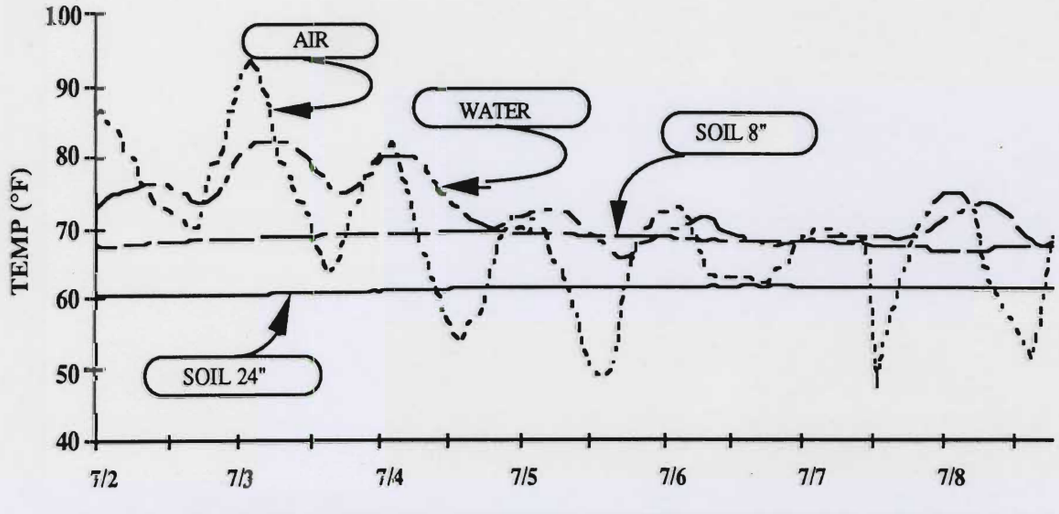


Figure 9: AITKIN TEMPERATURE DATA, AUGUST 13-AUGUST 29

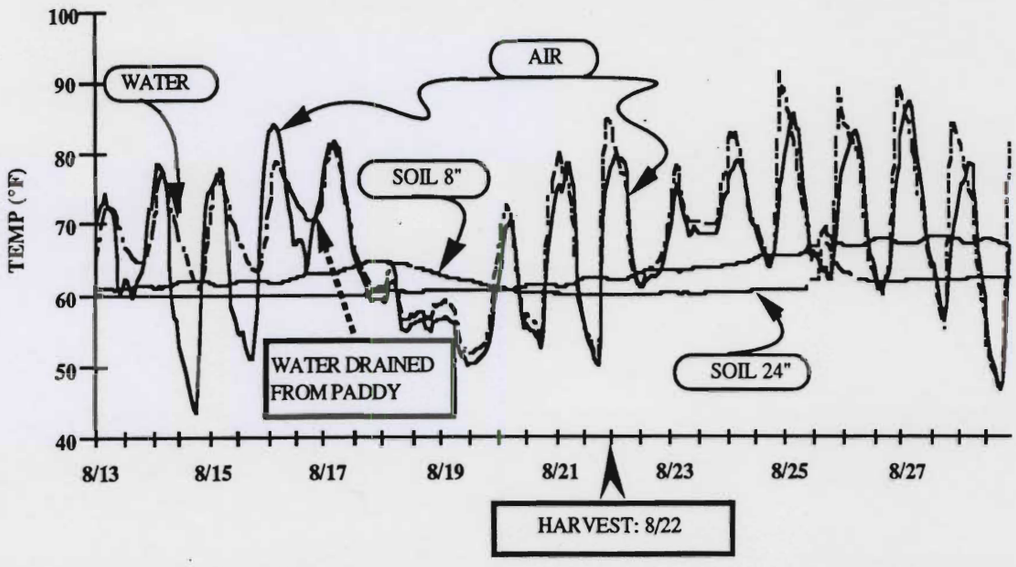
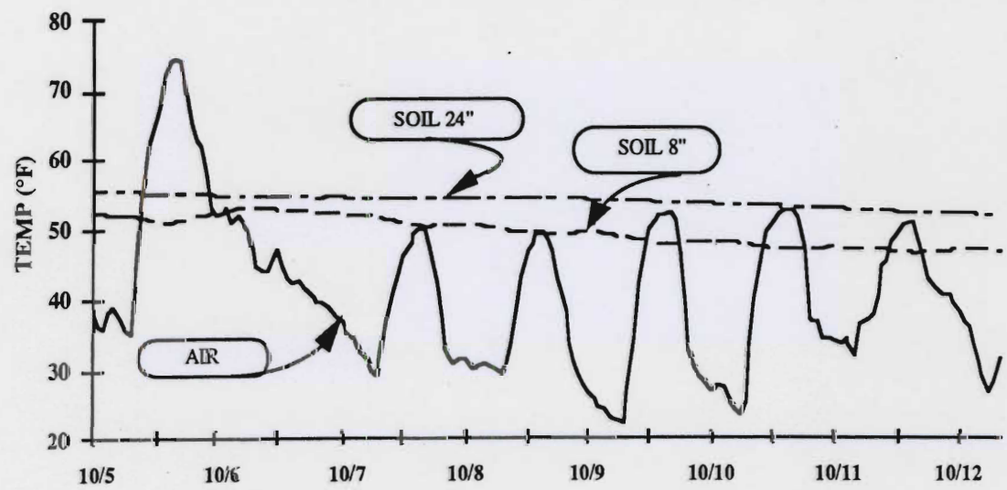


Figure 10: AITKIN TEMPERATURE DATA, OCTOBER 5-OCTOBER 12



Evaluation of Fall Carryover N

The first step in looking at fall ammonium carryover (N_i in the above model) was to develop a procedure for soil sampling. Typical procedures call for collecting, mixing, and air-drying samples before sending them to a lab for analysis. As indicated in last year's report, this method results in lowering ammonium levels and raising nitrate values. The conditions of drying will affect the values determined. Frozen soil samples will result in less change from field to lab, but are hard to handle, both for the sampler and for the lab.

The following procedure, which is more practical, was developed and used for handling samples.

Table 2. PROCEDURE FOR COLLECTION OF SOIL SAMPLES FOR NH_4^+ and NO_3^- ANALYSIS

1) Collect two representative soil samples, from 0-6" and 6"-24", for each paddy of interest.
2) For each soil depth, put a subsample into two 250 ml plastic bottles using a small beaker of known volume (eliminate air pockets, plant material, mineral sub-soil and large chunks of soil).
3) Tightly cap one bottle; this subsample is used to determine moisture content and to see if the bulk density of the disturbed sample differs from expected ranges seen in the field (important in Step 6).
4) Add 200 ml 2M KCl solution to the second bottle to extract and preserve NH_4^+ and NO_3^-.
5) Send to lab for analysis.
6) Analysis of the solution in the second bottle along with the determination of soil moisture and weight per volume of the sample in the first bottle enables calculation of soil NH_4^+ and NO_3^-.

Samples were collected through the fall at Aitkin and Clearwater from the area where the samplers had been in place to look at time trends for NH_4^+ and NO_3^- production and transformations. To determine the range of expected values, 58 samples were collected at Gully in early September, representing two depths from 20 different paddies or areas in paddies . These samples were collected once from each area of interest. The results of these tests are shown for Gully (Table 3), Aitkin (Figure 11), and Clearwater (Figure 12). These are soil samples from which NH_4^+ and NO_3^- have been extracted and the determined values are reported as parts per million of oven dry soil. For mineral soils, one ppm will equal 2 lbs/acre-6", but with peat the low bulk density must be corrected for. One ppm in peat is approximately 1/2 lb/acre for each 6" of peat.

Table 3: GULLY POST-HARVEST TEST RESULTS, NH_4^+ AND NO_3^-, SEPTEMBER 5-20, PRESENTED AS PPM OF DRY SOIL

	NH_4^+, ppm	NO_3^-, ppm
Average	57	3
Maximum	127	25
Minimum	4	0
Average 0-6"	56	4
Average 6"-24"	58	1

Figures 11 and 12 show that there is some NH_4^+ in the soil at Aitkin and substantial amounts of NH_4^+ at Clearwater in September. At both sites NH_4^+ in the 0-8" depth declines to a steady state of 18-20 ppm. This value was similar to that found in last year's preliminary study. The 8"-24" samples from Clearwater, however, maintain high levels. The N levels at Clearwater were higher than Aitkin through the growing season, and this

trend unsurprisingly continued into the fall. Little or no NO_3^- was detected at Clearwater until the 0-8" sample on November 1. (There was no significant NO_3^- detected in the 8"-24" samples, so this line was not plotted on Figure 12.) This suggests that for this past fall at least, the NH_4^+ mineralized after harvest should carry over to next year's crop.

Figure 11: AITKIN FALL NH4 & NO3 CHANGES

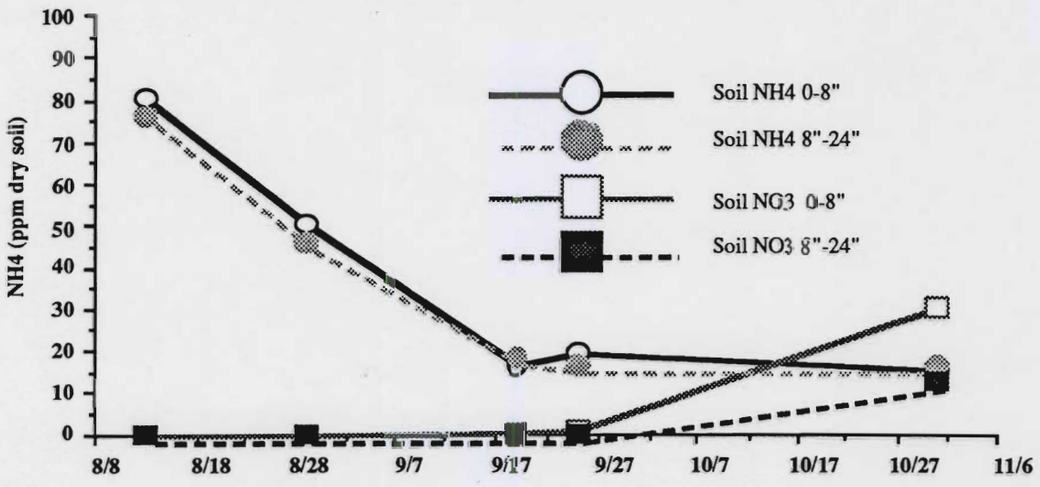
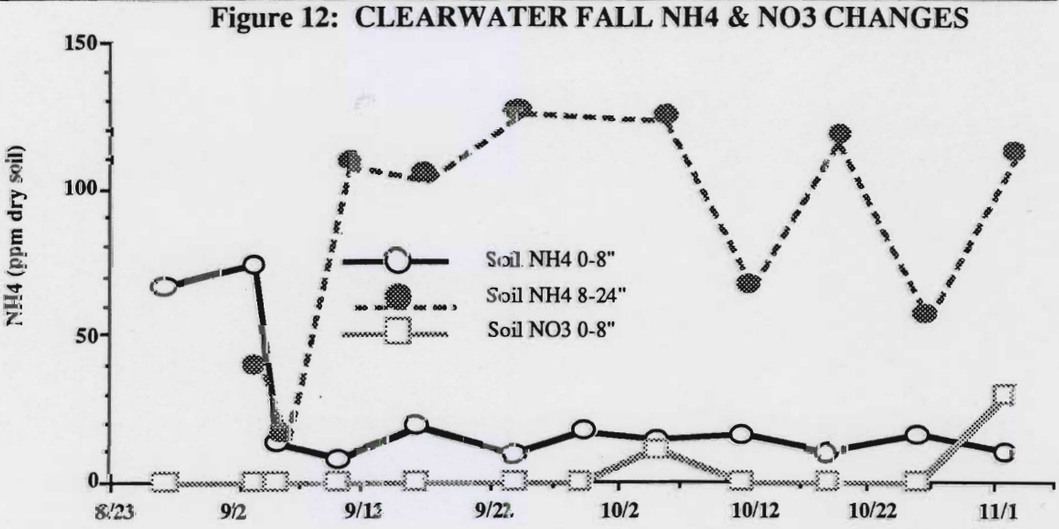


Figure 12: CLEARWATER FALL NH4 & NO3 CHANGES



The NH_4^+ depletion data in Figure 4 suggest that wild rice can remove nearly all of the NH_4^+ from as deep as 18" and may remove some NH_4^+ from greater depths. Using the assumption of total removal from 0-18" and no removal from greater depths we can make rough estimates of the carryover nitrogen (N_i). For Clearwater we estimate N_i to be approximately 110 lb/ac. This means that little or no fertilizer may be needed because N_{fi} should be able to supply the remainder of the needed N. At Aitkin N_i is approximately 20

lb/ac and fertilizer is definitely needed. Past experience also indicates that these paddies would benefit from fly-on N during the growing season.

The values from Gully would be expected to decline as the fall goes on as they did at the other two sites. NO_3^- levels are lower than what was found in the few samples that were run last year. Some variation is to be expected as the processes of mineralization and nitrification are dependent on temperature and moisture conditions. This variation may also represent differences in sampling time and technique. The differences in results among the three sites and among the different paddies at Gully certainly indicate that different paddies will have different mineralization rates and will differ in their capacity to supply N to the plant. The appearance of NO_3^- in the late Aitkin and Clearwater samples suggests that nitrification is still going on. As shown by the soil temperatures from Aitkin, the soil was at 50° F by October 8. The data suggest that some nitrification occurs under these low temperature conditions. In our current research, we are investigating the effects of soil temperature and moisture on mineralization and nitrification rates.

Conclusions

As stated in the introduction, our research this past summer and fall was undertaken to better understand the parts of the model ($N_C = N_i + N_m + N_f - N_l$) about which little was known. The next step in this process would be to use some of these findings about fall carryover and in-season plant uptake to study N management: paddies with various levels of N_i might receive strips of fall applied N and/or fly-on N and these strips would be compared to non-fertilized strips at harvest. A high-medium-low scale of NH_4^+ carryover levels could then be developed that could be used to modify fertilizer rates. Water management could also be investigated. The figures that show the relationship between nitrification and drainage suggest that fall flooding where and when possible might be an effective N management tool. Even raising the water level to just below field level might be beneficial. The laboratory experiments currently under way could be used in conjunction with some of these findings to suggest modifications in management based on temperature and moisture conditions in the fall. The uncertainties in the model we used are by no means solved, but this approach furnishes a starting point that allows for a more complete understanding of this wetland agricultural system, and should enable better recommendations for N management to be made in the future.

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Paul Imle and Don Barron volunteered to help with these experiments and for this, we are very grateful. We would have accomplished little without their help and interest. We hope that when these investigations are finished, we will have learned something that will repay their efforts. Thank you to Doug Stark and Calvin Trostle for help with organizing and setting up the experiments, and for lab assistance. Erv Oelke, Jim Percich, Raymie Porter, Mike McClellan, and Dean Malvick have been as usual most helpful. We are also grateful to our colleagues in Soil Science for suggestions and assistance with these investigations.

WILD RICE BREEDING

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In 1990, experiments were conducted at two locations: the research paddies at the North Central Experiment Station (NCES) in Grand Rapids and the research paddies on the Vomela farm near Aitkin. Discussion will emphasize three yield trials, each with a different objective. However, other activities to be discussed include selection for shattering resistance, selection for disease resistance, and hybridization.

Variety Trial

As in 1989, the primary purpose of the 1990 variety trial was to compare varieties and experimental populations for yield and shattering. In addition, harvest index (proportion of harvested grain to total biomass) and incidence of wild rice worm were estimated for the first time in a wild rice variety trial. The effects of delaying harvest for one week after the optimum were also examined.

Materials and Methods. Eighteen entries were fall-planted in six blocks in Grand Rapids and flooded in the early spring of 1990. Five entries represented currently used varieties, including Petrowske's variety (originally selected for vigorous plants of the bottlebrush phenotype), a shattering-type variety (originally selected from a lake but grown in a paddy for 20 years), M3, and two sources of K2. The rest were experimental populations under improvement. One population ('Frosty') originated from K2 and had been selected for shattering resistance for three cycles by delaying harvest until after frost. Two populations selected from K2 for increased shattering resistance each represented two entries: the most recent cycle of selection and the previous one. These populations are designated K2(1)C3, K2(1)C4, K2(2)C2, and K2(2)C3. Another population was selected simultaneously for yield and shattering resistance. The cycle 1 version of this population is K2(G)C1; the cycle 2 version with medium maturity is K2(GHYM)C2, while the late-maturing cycle 2 version is K2(GHYL)C2. Two cycles of an M3 population are represented by M3(M)C1, and M3(M)C2. A four-way population cross, using (MeterXJohnson) crossed as the male onto (M3XNetum), was carried out in 1989 and is designated (M3XNe)X(MeXJn). Finally, the germplasm release Pistillate M3, with 50% pistillate plant-types, was included along with a version which had been reselected for the normal plant type, Pist. M3 (Normal).

For each plot, we recorded the date at which 50% of the plants' mainstems were heading. Averaged over all reps, the heading date was used to make decisions about harvest. When a variety had approximately 50% mature seed on the mainstems, plots in the 3 blocks designated for "optimum" harvest were harvested. The other 3 plots were harvested 7 days later; for these plots, designated "delayed", plants were thoroughly agitated to permit full expression of shattering. Thus the design of the experiment at harvest was split-plot, with main plot treatments being 2 harvest dates and subplot treatments the entries planted in 4 ten-foot rows. The center two rows

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[1]Postdoctoral associate and research plot coordinator

were harvested. Stems were counted, threshed green seed was weighed, and wild rice worms were counted. After drying, seed was weighed and percent moisture was calculated. After harvest, shattered seed was collected from 3 pans placed between the center rows of each plot. After drying and cleaning this seed, the weight was used to calculate shattered seed on a per-acre basis. This was added to the harvested seed to calculate potential yield. Percent shattering could then be calculated. Finally, at harvest, all the above ground biomass was collected, dried and weighed. The weight of dry stems and leaves was added to dry seed weight to get total biomass. Harvest index was then calculated.

The Aitkin variety trial, planted in the spring, included the same entries as the one in Grand Rapids, except for K2(GHYM)C2 and K2(GHYL)C2, which failed to emerge. The same data were taken as in the Grand Rapids trial.

Results and Discussion. The data for the variety trial at Grand Rapids are shown in Tables 1 and 2. As expected, delay of harvest and application of the shattering treatment resulted in much lower harvested yields overall, due to much higher shattering losses. Most of the experimental populations had yields similar to or higher than the varieties at optimum harvest. The highest-yielding entries, K2(GHYL)C2 and Pistillate M3, were also among the last harvested, and also had the highest potential yields. K2(GHYL)C2 was actually later maturing than the indicated harvest date, but had to be taken several days early due to the approach of frost. In Pistillate M3 plots, pistillate and normal stem types were harvested separately. The pistillate fraction yielded 16% more grain on the average, in spite of having fewer stems. In this entry the average grain yield per pistillate stem was 1.55 times the yield per normal stem.

After the delay, several experimental populations were still relatively high-yielding, especially K2(2)C3, and Pistillate M3; however, the higher moisture of the latter suggests it was not as mature as K2(2)C3. Each K2 latest-cycle population had higher yield and less shattering than its previous-cycle counterpart, indicating selection progress. However, this was not true for M3. The two earliest entries, Lake (Figliuzzi) and (M3XNe)X(MeXJn) , were probably harvested too early, as indicated by their high moisture contents. This probably reduced their shattering and increased their yield relative to the other entries.The best entry overall is K2(2)C3. It shows high yield, even after harvest delay, low shattering (8% at optimum), and high harvest index.

Phenotypic correlations between optimum and delayed harvest means were 0.62 for harvested yield, 0.64 for potential yield, and 0.68 for shattering. Shattering and harvested yield were strongly correlated at delayed harvest ($r = -0.87$), indicating as expected that more shattering means less harvested grain.

Harvest index decreased due to the harvest delay. Even at optimum, it was much lower overall than harvest index of other cereals. For instance, semi-dwarf wheat can have a harvest index of 40 to 50%. This is another indication that the domestication of wild rice is far behind that of other cereal grains. Increasing harvest index of wild rice might occur if plant height is reduced, but reducing shattering losses will probably help more in the short term.

Delaying harvest decreased rice worm presence in harvested seed. Differences among varieties seemed related to maturity. The decline in worm count with harvest delay was probably due to either migration of worms out of the heads, or shaking some of them out during agitation, or both. We actually observed more damaged heads in the delayed harvest, but potential yields were still high, probably due to maturation of additional tillers and escape from damage by shattered seed.

In Aitkin (Table 3), yield, shattering, and harvest index were much lower than in Grand Rapids, due to a severe disease outbreak. Potential yield was much lower at delayed harvest, possibly because delayed harvest blocks had higher disease severity. Rice worm incidence was similar to that of the Grand Rapids trial, averaging about 30 at both harvests (data not shown). At optimum harvest, the highest yielding entries by far were K2(1)C4 and Pistillate M3. But in the delayed harvest, K2(2)C3 had the highest yield and lowest shattering, with K2(1)C4 slightly lower and not significantly different.

The phenotypic correlations between optimum yield and delayed yield were 0.66 for harvested yield, 0.52 for potential yield, and 0.59 for shattering. Once again, there was a strong correlation between shattering and delayed harvested yield ($r = -0.85$).

In both locations, then, the two best entries are K2(2)C3 and K2(1)C4. Pistillate M3 also looks promising. It outyielded its normal counterpart by 30-40% at Grand Rapids and 60% at Aitkin, but the pistillate heads showed a strong tendency to lodge. Hand harvesting individual stems compensated for this in our experimental plots, whereas much grain would probably be lost when machine harvested.

K2 Shattering Trial

When a production paddy is allowed to reseed itself from year to year, the plants which grow from the shattered seed have a greater probability of coming from the less shattering-resistant plants of the previous year. Natural selection under such conditions should therefore favor increased shattering. Conversely, any selection, artificial or natural, favoring increased seed retention by minimizing the contribution of more shattering susceptible plants to the next generation, should result in a population with decreased shattering. The objectives of this experiment were to test whether shattering is higher in populations grown for increasing periods under continuous production, and to compare the yield and shattering of these populations with experimental populations selected for increased seed retention.

Materials and Methods. Eleven entries were fall-planted in 1989 in six blocks in Grand Rapids and flooded in the spring of 1990. Four of the entries were K2 coming from growers' paddies under continuous cultivation for 15, 10, 4, and 2 years. The two-year-old K2 had been under "combine selection" for six consecutive years. That is, the harvested seed had been used to seed a virgin paddy each year except the last. The paddy was allowed to reseed itself, resulting in a "second year paddy" from which the 2-year entry was derived. Also in the last year, seed from the virgin paddy was sown over a deep-plowed paddy of M3, resulting in "K2/M3". Seed was harvested from that paddy to form a fifth population. Although most of the M3 seed was probably buried, some of it may have contaminated this population, in an unknown proportion.

Table 1. Yield and shattering--Grand Rapids variety trial (fall-planted 1989)

Entry	Green seed yield[b] Harvested Opt.[c]	Harvested Del.[c]	Potential Opt.	Potential Del.	Shattering[d] Opt.	Shattering[d] Del.	Harv. index[e] Opt.	Harv. index[e] Del.
	lb/A		lb/A		%		%	
Frosty[a]	1610	930	1960	1760	18	47	23	12
K2(1)C3[a]	1150	470	1380	1540	16	70	24	8
K2(1)C4[a]	1310	660	1770	1640	26	59	25	9
K2(2)C2[a]	1130	940	1580	1800	34	49	22	14
K2(2)C3[a]	1510	1100	1640	2000	8	45	25	16
K2(G)C1[a]	940	600	1470	1510	34	62	19	9
K2(GHYL)C2[a]	1800	890	2450	2070	27	57	25	11
K2(GHYM)C2[a]	1580	750	2060	1930	24	60	23	8
Petrowske (NCES)	1360	600	1980	1610	31	62	22	10
K2 (Kosbau)	790	490	1480	1730	47	75	18	8
K2 (Vomela)	1330	570	2080	2180	38	77	23	7
Lake (Figliuzzi)	890	690	1250	1570	29	56	16	9
M3 (Manomin)	1060	380	1820	1620	41	77	18	5
M3(M)C1[a]	1430	960	1610	1930	11	50	25	13
M3(M)C2[a]	1210	620	1880	2060	35	70	16	8
(M3XNe)X(MeXJn)[a]	1150	990	1410	1870	18	31	23	17
Pistillate M3[a]	1700	1200	2320	2260	27	48	23	15
Pist. M3 (Normal)[a]	1210	670	1800	1750	32	62	16	8
Mean	1290	750	1770	1820	28	59	21	10
$LSD_{.05}$ among entries	530	330	550	550	10	10	4	4
$LSD_{.05}$ betw. harvests[f]	NS		NS		11		4	

NS=not significant.

[a] Experimental; all others are cultivars--seed source given in parentheses (see text).
[b] Adjusted to 40% moisture; potential yield is harvested yield plus shattered seed.
[c] "Opt." = first harvest, at optimum maturity or approx. 6 weeks after 50% flowering; "Del." = second harvest, delayed one week past optimum maturity.
[d] Shattered seed ÷ potential yield; shattered seed collected in pans within plots.
[e] Harvested seed (dry wt.) ÷ total above-ground biomass, including seed (dry wt.).
[f] If error variances of Opt. and Del. were homogeneous, harvest dates were combined for analysis of variance; then, if interaction between harvest date and entry were significant, this LSD was calculated to compare harvest dates within an entry.

Table 2. Maturity and rice worm count--Grand Rapids variety trial (fall-planted 1989)

Entry	Flowering date (50%)	Opt. date of harvest	Seed moisture Opt.[b]	Seed moisture Del.[b]	Rice worms Opt.	Rice worms Del.
			-------% -------		per 100 hds	
Frosty[a]	July 22	Aug. 31	34	31	47	19
K2(1)C3[a]	July 21	Aug. 30	33	30	30	12
K2(1)C4[a]	July 22	Aug. 31	32	31	42	19
K2(2)C2[a]	July 22	Aug. 31	32	31	30	22
K2(2)C3[a]	July 21	Aug. 30	32	28	20	27
K2(G)C1[a]	July 25	Sep. 4	31	30	33	12
K2(GHYL)C2[a]	Aug. 4	Sep. 7	33	33	64	17
K2(GHYM)C2[a]	July 31	Sep. 7	32	30	53	9
Petrowske (NCES)	July 22	Aug. 31	31	30	34	15
K2 (Kosbau)	July 26	Sep. 8	30	29	32	16
K2 (Vomela)	Aug. 2	Sep. 7	32	31	26	9
Lake (Figliuzzi)	July 18	Aug. 21	39	38	6	14
M3 (Manomin)	July 24	Sep. 4	32	29	33	5
M3(M)C1[a]	July 21	Aug. 30	33	31	15	14
M3(M)C2[a]	July 26	Sep. 5	31	28	22	8
(M3XNe)X(MeXJn)[a]	July 15	Aug. 21	36	33	4	13
Pistillate M3[a]	July 27	Sep. 5	35	29	25	10
Pist. M3 (Normal)[a]	July 26	Sep. 5	34	30	23	10
Mean	July 24	Sep. 5	33	31	30	14
$LSD_{.05}$ among entries			2	3	NS	NS
$LSD_{.05}$ betw. harvests[c]			NS		NS	

NS=not significant.

[a] Experimental; all others are cultivars--seed source given in parentheses (see text).

[b] "Opt." = first harvest, at optimum maturity or approx. 6 weeks after 50% flowering; "Del." = second harvest, delayed one week past optimum maturity.

[c] If error variances of Opt. and Del. were homogeneous, harvest dates were combined for analysis of variance; then, if interaction between harvest date and entry were significant, this LSD was calculated to compare harvest dates within an entry.

Table 3. Yield and shattering--Aitkin yield trial (spring-planted 1990)

Entry	Green seed yield[b] Harvested Opt.[c]	Harvested Del.[c]	Potential Opt.	Potential Del.	Shattering[d] Opt.	Shattering[d] Del.	Harv. index[e] Opt.	Harv. index[e] Del.
	lb/A		lb/A		%		%	
Frosty[a]	660	200	790	400	15	49	12	5
K2(1)C3[a]	980	280	1170	360	18	23	14	6
K2(1)C4[a]	930	420	1450	560	0	18	14	9
K2(2)C2[a]	800	300	920	380	8	22	14	8
K2(2)C3[a]	800	500	910	570	10	17	13	8
K2(G)C1[a]	570	250	640	380	14	33	11	5
Petrowske (NCES)	410	190	520	280	13	34	9	7
K2 (Kosbau)	400	80	650	210	39	63	10	4
K2 (Vomela)	420	140	630	210	33	35	10	4
Lake (Figliuzzi)	250	100	500	240	53	61	5	3
M3 (Manomin)	660	130	730	210	14	43	12	4
M3(M)C1[a]	720	150	810	210	14	39	12	4
M3(M)C2[a]	580	190	630	330	7	48	11	3
(M3XNe)X(MeXJn)[a]	390	150	440	300	15	54	8	6
Pistillate M3[a]	1020	150	1110	310	9	45	14	3
Pist. M3 (Normal)[a]	620	200	690	360	11	44	12	3
Mean	610	250	760	316	18	39	11	5
$LSD_{.05}$ among entries	430	180	NS	NS	12	20	NS	3
$LSD_{.05}$ betw. harvests[f]	440		NS		NS		NS	

NS=not significant.

[a] Experimental; all others are cultivars--seed source given in parentheses (see text).
[b] Adjusted to 40% moisture; potential yield is harvested yield plus shattered seed.
[c] "Opt." = first harvest, at optimum maturity or approx. 6 weeks after 50% flowering; "Del." = second harvest, delayed one week past optimum maturity.
[d] Shattered seed ÷ potential yield; shattered seed collected in pans within plots.
[e] Harvested seed (dry wt.) ÷ total above-ground biomass, including seed (dry wt.).
[f] If error variances of Opt. and Del. were homogeneous, harvest dates were combined for analysis of variance; then, if interaction between harvest date and entry were significant, this LSD was calculated to compare harvest dates within an entry.

M1 and M3 were included as checks, as well as a population originally planted from shattering lake seed, then kept in production for 20 years--the same entry in the variety trial designated "Lake (Figliuzzi)". Three experimental populations which had been selected for shattering resistance were also added: K2(1)C4, K2(2)C3, and "Sturdy", which had been selected primarily for stiff, upright stems and to a lesser extent for shattering resistance.

The number of plants which were of the shattering phenotype was counted in each plot prior to harvest to calculate the proportion of shattering plants. These plants are characterized by the complete loss of staminate florets just after pollen shed. As in the Variety Trial, harvested yield, potential yield, and percent shattering were measured at two harvest dates.

Results and Discussion. Harvested seed yield tended to decrease markedly in proportion to the increase in number of years in production, particularly at the optimum harvest date (Table 4). The correlation between number of years of production and harvested yield for the five K2 varieties from growers was -0.93 and -0.79 for optimum and delayed harvest, respectively. Potential yield was very similar for these five K2 populations, except for K2/M3, which was intermediate between K2 and M3 at optimal harvest. M3 was significantly higher than the other K2 entries. Shattering, expressed as either seed loss or proportion of shattering plants, tended to increase with increasing stand age, supporting the hypothesis that natural selection favors shattering. The level of shattering in K2/M3 was intermediate between the best K2 and M3.

For all eleven entries, there was a very strong correlation between seed shattering at optimum harvest and proportion of shattering plants (r=0.95). Thus, a large proportion of the shattering variation ($r^2 = 0.91$) can be attributed to differences in the proportion of shattering plants. Also, delayed harvested yield was strongly correlated with both delayed seed shattering (r = -0.84) and percent shattering plants (r = -0.83). Therefore, any increase in the frequenty of shattering plants in a population is associated with greater shattering losses and lower yields. Frequency of shattering plants in turn increases with the number of years of continuous production (r = 0.96 for 5 K2 entries from growers). Delayed harvested yield was therefore inversely proportional to number of years of production for those 5 entries (r = -0.79).

The highest yielding entries were the experimental populations, headed by K2(1)C4; these were also the least shattering. Delayed harvest favored K2(2)C3 and Sturdy, which had the highest harvested yields. Although the two-year-old combine-selected population had almost as much shattering resistance as the experimental populations, it did not yield nearly as well, even at optimal harvest .

Table 4. Yield and shattering for Grand Rapids K2 shattering trial (fall-planted 1989)

Entry	Green seed yield[b] Harvested Opt.[c]	Harvested Del.[c]	Potential Opt.	Potential Del.	Shattering[d] Opt.	Shattering[d] Del.	Shattering plants[e]
	----- lb/A ------		------ lb/A -----		------- % -------		%
Lake (Figliuzzi, 20 yrs)	1050	540	1350	1020	22	46	66
K2 (Figliuzzi, 15 yrs)	960	570	1360	1000	21	30	68
K2 (Godward, 10 yrs)	1010	420	1260	940	22	55	53
K2 (Kosbau, 4 yrs)	1170	890	1310	1180	11	25	33
K2 (Godward, 2 yrs)	1240	870	1370	1290	9	32	9
K2/M3 (Godward 1 yr)	1410	800	1610	1230	9	36	19
M1 (Manomin, 1 yr)	1490	750	1650	1070	9	45	21
M3 (Manomin, 1 yr)	1410	640	1770	1290	16	52	46
K2(1)C4[a]	1770	1140	1850	1310	4	17	7
K2(2)C3[a]	1530	1390	1580	1640	3	15	9
Sturdy (C2)[a]	1410	1330	1520	1790	7	25	4
Mean	1310	850	1510	1250	12	34	31
$LSD_{.05}$ among entries	340	340	400	400	4	12	8
$LSD_{.05}$ betw. harvests[f]	NS		NS		NS		

NS=not significant.

[a] Experimental; all others are cultivars--seed source and years of continuous production given in parentheses (see text).

[b] Adjusted to 40% moisture; potential yield is harvested yield plus shattered seed.

[c] "Opt." = first harvest, at optimum maturity or approx. 6 weeks after 50% flowering; "Del." = second harvest, delayed one week past optimum maturity.

[d] Shattered seed ÷ potential yield; shattered seed collected in pans within plots.

[e] Number of shattering plants ÷ total number of plants (per plot); all plants which completely lost male florets just after pollen shed were considered shattering plants.

[f] If error variances of Opt. and Del. were homogeneous, harvest dates were combined for analysis of variance; then, if interaction between harvest date and entry were significant, this LSD was calculated to compare harvest dates within an entry.

Bottlebrush Trial

The bottlebrush phenotype consists of staminate branches that adhere to the rachis, and is usually associated with male sterility and a dark red color of the staminate florets. The merits of the bottlebrush trait of wild rice have been debated for some time. Growers have already selected or modified bottlebrush versions of K2 and Netum. Yields of these modified varieties are higher in some growers' paddies, not much different in others'. Kelly Petrowske developed the first Bottlebrush variety by selecting the best (most vigorous) K2 plants with the bottlebrush phenotype. However, bottlebrush plants with low vigor can be found. The effect of selection for vigor may not be separable from any advantage of the bottlebrush trait itself, unless selection for the bottlebrush trait can be done without regard to the vigor of the plant. The purpose of this experiment was to compare bottlebrush and normal populations for yield and shattering, and to ascertain the effectiveness of selecting for the bottlebrush trait.

Materials and Methods. Nine entries were fall-planted in Grand Rapids in six blocks. A former wild rice Research Assistant, Nat Page, extracted two populations from each of several growers' paddies by selecting 100 neighboring bottlebrush and normal plants at random in each paddy, then interplanting the normal seed from the different fields separate from the bottlebrush seed from those fields. In the second cycle, the normal population was reselected for normal plant type and the bottlebrush population for the bottlebrush trait, resulting in two sister populations.

The original Petrowske bottlebrush variety was reselected by Imle and Gunvalson near Gully for high-yielding bottlebrush by mass selection (individual plant selection). Seed from the first cycle of reselection resulted in a population with about 24% bottlebrush in four random samples of 100 ft^2 each. Seed from this cycle was bulked to form Petrowske C1 (Imle). Also, random bottlebrush plants were selected from the same paddy; this seed formed Petrowske C2 (Imle). Unselected K2 (Kosbau) was also included as a check. Bottlebrush which had not been reselected was included as a check. Also, K2 taken from a grower's field was used as a check.

Netum was similarly subjected to a cycle of selection for the bottlebrush trait and for yield at the Manomin Development Corp. farm near Aitkin. However, in the second cycle, two separate populations were selected: one from 36 small bottlebrush plants with small heads, Netum LY (C2), and the other from 36 vigorous bottlebrush plants with large heads, Netum HY (C2). A bulk of Netum from the Manomin seed field was included as a check.

In each plot of each entry, the numbers of bottlebrush and normal plants were counted. As in the Variety Trial, harvested yield, potential yield, and percent shattering were measured at two harvest dates.

Results and Discussion. The Petrowske variety apparently responded to selection for yield (Table 5). Cycle 2 was the highest yielding entry for both harvested and potential yield at the optimal harvest date. At delayed harvest, shattering of this entry was lower than any other entry. However, the yield was half what it was at the optimum harvest. Many entries had significantly lower potential yield at delayed harvest than at optimal harvest. The reason for this is unknown.

Netum also appeared to respond to selection for high yield at optimal harvest, and both the bottlebrush populations were higher than the check. However, none of the differences was significant except for the potential yield of Netum and NetumHY(C2) at optimal harvest. When harvest was delayed, though, the check had a slight advantage, but the difference was not significant. The Netum check also had the lowest shattering loss early, and the second lowest after the delay.

Page's Bottlebrush population was not significantly different from the Normal population, although the mean was slightly higher at optimal harvest. Shattering was not significantly different, either.

There was a good correlation between delayed harvested yield and optimum harvested yield ($r = 0.76$). The proportion of bottlebrush plants had a good correlation only with optimum potential yield ($r = 0.76$). This may indicate the strong relationship between these two variables among the Petrowske entries.

In those populations which had been selected for two cycles for bottlebrush, the proportion of bottlebrush was around half. This supports the idea that bottlebrush may be controlled by a single recessive gene for male sterility, similar to the pistillate trait. If so, continued selection should not increase the proportion beyond 50%.

Overall, the results are mixed. On the one hand, Petrowske's variety yields higher when more bottlebrush plants are present. On the other hand, Page's populations did not yield very differently, even though the proportion of bottlebrush plants was very different. One might speculate that selecting for high-yielding bottlebrush plants results in a population with high-yielding bottlebrush plants and average-yielding normal plants. When selection for bottlebrush is relaxed, the population reverts to more fertile plants, which also may yield less. The bottlebrush phenotype may allow more energy to be diverted from pollen production to seed production, but that advantage may only be realized in plants selected to exploit that advantage. These speculations can only be confirmed by further research.

Selection for Shattering Resistance

Selection for shattering resistance continued in several populations and was initiated in several others. Using color coded tape to indicate maturity, heads were tagged when the pistillate portion of the head had fully emerged from the boot and staminate branches first appeared. (Previously, heads were tagged only when the entire panicle had emerged.) In this way, any plant-to-plant differences in rate of head emergence should be reduced. Furthermore, since seed maturity should be related to the time of pollination of pistillate florets, it seemed logical that maturity classification be based primarily on the availability of that portion of the head for pollination. As a result plants in a given maturity range should show less variability in seed maturity, and comparisons for seed retention within a maturity group should be more closely related to genetic shattering potential, versus seed maturity effects. Tagging was carried out on Monday, Wednesday, and Friday of each week, resulting in maturity groups 2-3 days apart.

Table 5. Yield and shattering--Grand Rapids bottlebrush trial (fall-planted 1989)

Entry	Green seed yield[b] Harvested Opt.[c]	Harvested Del.[c]	Potential Opt.	Potential Del.	Shattering[d] Opt.	Shattering[d] Del.	Bottlebrush plants[e]
	----- lb/A ------		------ lb/A -----		------- % -------		%
Bottlebrush C2 (Page)	1630	780	1930	1670	16	53	47
Normal C2 (Page)	1600	910	1760	1700	9	47	13
Petrowske C2 (Imle)	2120	1060	2400	1650	12	34	52
Petrowske C1 (Imle)	1750	700	2240	1770	22	60	36
Petrowske (unsel.)	1430	700	1550	1430	21	51	23
K2 (Kosbau)	1210	590	1390	1080	13	50	11
Netum(HY)C2	1520	610	2000	1130	17	45	50
Netum(LY)C2	1360	500	1740	1030	22	53	49
Netum (Manomin)	1230	770	1320	1330	7	42	12
Mean	1540	730	1810	1420	16	48	32
$LSD_{.05}$ among entries	600	230	690	250	8	18	12
$LSD_{.05}$ betw. harvests[f]	NS		NS		NS		

NS=not significant.

[a] Experimental; all others are cultivars with seed source given in parentheses.
[b] Adjusted to 40% moisture; potential yield is harvested yield plus shattered seed.
[c] "Opt." = first harvest, at optimum maturity or approx. 6 weeks after 50% flowering; "Del." = second harvest, delayed one week past optimum maturity.
[d] Shattered seed ÷ potential yield; shattered seed collected in pans within plots.
[e] Number of plants showing the bottlebrush phenotype ÷ total number of plants.
[f] If error variances of Opt. and Del. were homogeneous, harvest dates were combined for analysis of variance; then, if interaction between harvest date and entry were significant, this LSD was calculated to compare harvest dates within an entry.

A fifth cycle of selection was carried out in this way on K2(1)C4. Two tagging-maturity groups at the peak of flowering were targeted for the most intensive selection in order to narrow the maturity range of the population. Of about 3000 plants, 350 and 240 were tagged in these two groups. Therefore, these two groups together accounted for the middle 20% in the range of maturity. In the two groups, 17 and 16 plants were selected based on seed retention of the tagged head, so selection intensity was 5 and 7%, respectively, for seed retention. In addition, a single round of selection was carried out within several medium to late-maturity groups combined, emphasizing visual selection for high-yielding plants (large heads, many tillers, healthy plants) while also selecting for good seed retention. Finally, a block of plants was left unselected for a bulk harvest, the seed of which will be used for next year's experiments and for possible increase.

A fourth cycle of selection was carried out in a similar fashion on K2(2)C3. Two maturity-specific selections of plants were carried out. Selected plants in these two groups appeared exceptionally shattering resistant; the results of the Variety Trial and the K2 Shattering Trial show that this population is the most promising of all those currently undergoing selection. From a total population of 2500, about 240 plants were tagged in each of these groups, accounting for the middle 20% of the maturity range. Within these groups, of 23 and 18 plants were selected using seed retention as the selection criterion, resulting in a selection intensity at the second level of 10 and 8%, respectively, for seed retention. In addition, a selection for high-yielding shattering resistant plants was carried out in several medium to late-maturing groups combined. A bulk selection was also taken.

Plants with "sturdy" stems are being selected in the 'Sturdy' population. These plants are characterized by stiff stalks and stiff, upright heads at maturity when most heads begin to nod over under the weight of maturing seed. In addition to being putatively more lodging resistant, these plants appear to be moved less by wind and may consequently shatter less. Three separate selections in Sturdy were made in the third cycle. First, 8 tall plants were selected which showed the most sturdiness and seed retention, then 8 short plants with sturdiness and shattering resistance. The last selection emphasized high-yielding sturdy plants with good seed retention and average to short plant height.

Pistillate M3 was planted in isolation and selected for stem sturdiness, seed retention, and medium maturity in the normal phenotype. Also, selection for increased seed retention and medium maturity was carried out among the pistillate plants. Currently, Pistillate M3 is one of the latest-maturing populations.

Petrowske's bottlebrush variety, reselected twice for the bottlebrush phenotype, was planted in Grand Rapids and selected for seed retention based on hand-stripping bottlebrush plants. Also, K2(4)C3 and M3(M)C2 were subjected to another cycle of selection for seed retention. In addition, Frosty was selected again for seed retention after frost. The two sister populations K2(GHYM)C2 and K2(GHYL)C2 were combined in a single selection at each of the two sites for high yielding, shattering-resistant plants, and also separately bulk-harvested without selection.

Selection for Disease Resistance

Families of K2(GHYM)C2 and K2(GHYL)C2 were planted specifically for selection for disease resistance at Aitkin. However, the incidence of disease in these families was not high enough to carry out a reliable selection, and individual plants were selected for yield, vigor, and shattering resistance (see above). However, when a severe epidemic was manifested in the Petrowske bottlebrush population in a separate paddy at Aitkin, 112 plants were selected from about 500-1000 plants tagged for medium maturity. The selection pressure appeared to be optimum for differentiating resistant plants, and tagging ensured that selected plants were not just escapes due to their maturity. In the four-way cross (M3XNe)X(MeXJn) planted at Grand Rapids, heavy disease pressure due to its earliness may have resulted in intense natural selection for disease resistance. Only a small amount of seed was recovered from that stand.

Hybridization Activities

Hybridization, or crossing of plants, families, or populations, creates new genetic variability. In 1990, plants of Pistillate M3 were transplanted among plants of several populations, then later rogued to remove normal plants, leaving only male sterile pistillate plants to receive pollen from the donor population. In this way, Sturdy was crossed to Pistillate M3 in hopes that a high-yielding pistillate population more lodging-resistant than Pistillate M3 would result. Pistillate M3 was also crossed to K2(1)C4 and K2(2)C3 to combine the pistillate trait with shattering resistance.

K2 was crossed as the pollen parent to extremely tall and robust plants of a related species, Zizania aquatica, obtained from the Suwannee River Florida. Seven seedlings of this cross are currently growing on hydroponic medium in Dr. Percich's lab, and an eighth plant is growing in the greenhouse. The seed of this population apparently lacks dormancy, or has greatly reduced dormancy--seed germinated within one week after harvest. The objective of this cross is primarily to transfer this non-dormancy, if possible, to cultivated varieties. This would allow shattered seed to germinate in the fall and die, permitting annual reseeding of paddies from harvested seed. Such a system, if feasible, would actually favor "natural selection" (really combine selection) for shattering resistance in production paddies. The current system in Minnesota favors natural selection for increased shattering for as long as continual production is allowed. In parts of California, shattered seed can be killed off by drying the paddies after harvest, favoring selection for shattering resistance. Therefore, obtaining non-dormancy in Minnesota varieties may help Minnesota growers harvest more seed and be more competitive with California in the long-term.

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WILD RICE DISEASE RESEARCH

Department of Plant Pathology
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Associate Professor, Research Associate, Laboratory Assistant, Research Assistant, and Professor, respectively.

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:

FIGURES 1, 2 and 3. Illustrate wild rice plants grown in hydroponic nutrient solutions in a growth chamber with controlled light, temperature and humidity.

FIGURE 1. Wild rice seedlings approximately seven days after being placed in hydroponic solution. The pots have bottoms made of plastic mesh through which the roots have grown.

FIGURE 2. Wild rice plants grown approximately 10 weeks in originial Hoagland's solution. Note tillering, pollen bearing flowers, and mature seed heads. The measuring stick in the center of the photograph is 1 meter (39.4) in height.

FIGURE 3. Wild rice plants grown for approximately ten weeks in IRRI nutrient solution. Note the developed roots, shoots, and flowers. The measuring stick is 1 meter (39.4) inches in height.

FIGURE 1

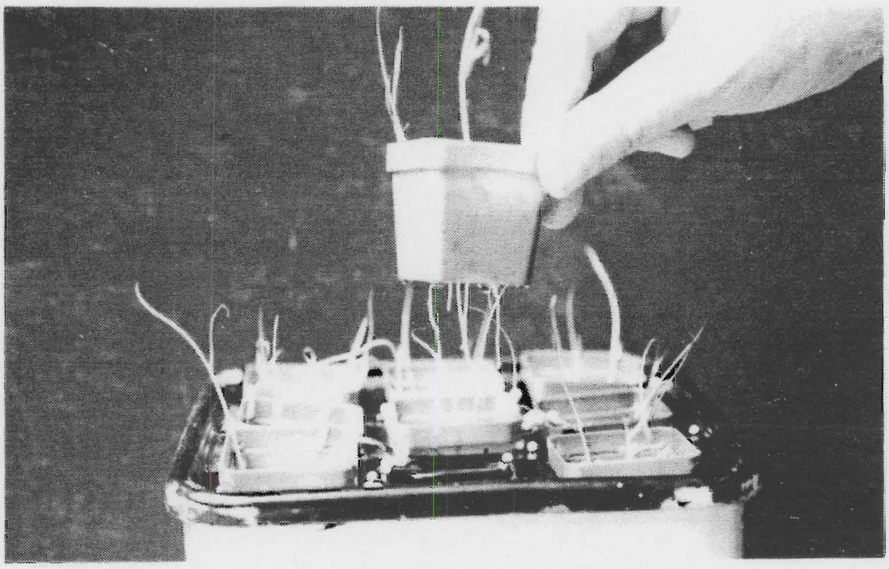

FIGURE 2

FIGURE 3

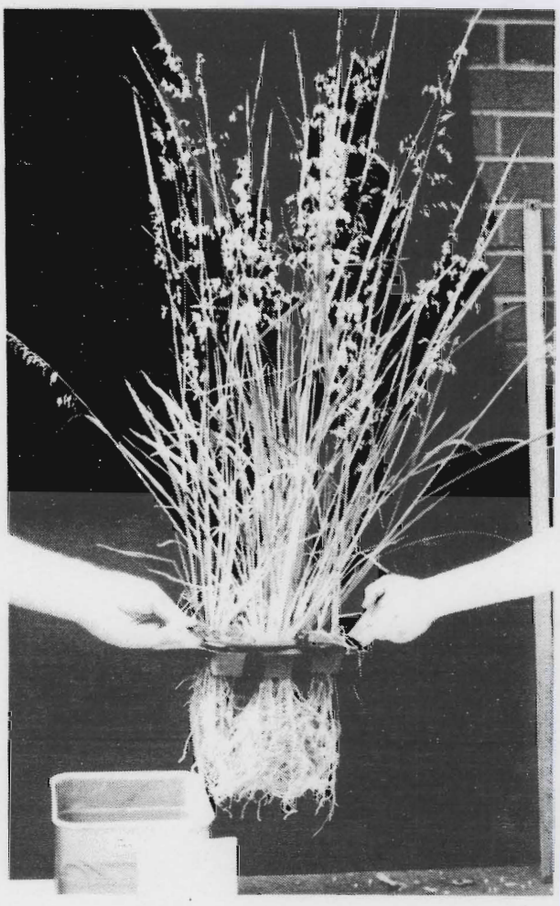

TABLE 1. Effect of TiltTM in controlling fungal brown spot, caused by Bipolaris oryzae and increasing yields on cultivated wild rice Minnesota in 1990.

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).

EVALUATION OF CONCEPTS FOR INDICATING MOISTURE CONTENT OF WILD RICE DURING PARCHING

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KERNEL LENGTH EVALUATION BY PHOTO SENSOR

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INTRODUCTION

The topics investigated by agricultural engineering in 1990 were:

1) Evaluation of two different concepts for their capability to indicate moisture content of wild rice during parching; viz., the use of:
   a) Commercially available moisture testers
   b) Grain temperature versus moisture content relationships
   and
2) Development of a system employing a photo sensor to indicate length of kernels for use in analyzing samples of processed wild rice for kernel length distribution.

EVALUATION OF MOISTURE TESTER AND GRAIN TEMPERATURE CONCEPTS FOR INDICATING MOISTURE CONTENT DURING PARCHING

Two electronic moisture testers, a Dickey-john Forage Moisture Tester (DJFMT) and a Datatec Moisture Analyzer (DMA) were evaluated for their capability for indicating moisture content of wild rice during the parching process. The DJFMT is the same unit tested previously with green wild rice (see 1989 report). The DMA is designed specifically for grain. Also evaluated was relationship between grain temperature and moisture content over the course of the parching process to determine if temperature might serve as an indicator of moisture content during parching. Of primary interest in the evaluations was the capability for accurately indicating moisture content at below the 10 percent level. This work was undertaken in response to a need expressed by processors for a practical and accurate method for determining when to terminate the parching process to guarantee sufficient drying while preventing needless overdrying and the concomitant loss of mass of finished product.

Data Collection

Data for evaluating moisture tester performance and grain temperature versus moisture content relationships were obtained over a two-day period at a wild rice processing plant while normal parching operations were in progress. The plant is equipped with batch-type, rotary drum parchers similar to those used

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at most other Minnesota plants. Evaluations were performed with the use of samples of wild rice taken from different parchers and at different times over the course of the parching process. All batches sampled were parched in accordance with standard procedures used at the plant. Decisions on when to terminate parching were made by the parcher operator.

The first day at the plant was partially spent developing procedures for obtaining grain samples from parchers, taking moisture tester readings and taking sample temperatures. A sample collector had been fabricated beforehand and was found satisfactory. It consisted of a 12 in. long x 8 in. wide x 6 in. high metal container, fastened to the end of a 10 ft. length of metal tubing. To obtain a sample, the sampling container was carefully inserted as far as practical into the rotating parcher drum, held in position to catch grain falling from vanes on the inside surface of the drum and, when filled, carefully retracted from the drum. Then, while still in the sampling container, a temperature measurement was quickly taken of the sample with a thermocouple inserted to near the center of the sample mass. The thermocouple was left in the sample until the readout on an accompanying monitor stabilized. Following temperature measurement, the sample was transferred into a container with a screened bottom, cooled with forced ambient air to about 100 degrees F and then transferred into a tub and stirred in preparation for use in moisture tester performance evaluations. Both the DJFMT and the DMA were each filled three times from each grain sample. Only one reading was taken per fill with the DMA while two were taken with the DJFMT, one without temperature compensation and the other with compensation. After obtaining each reading or pair of readings, grain in the tester was returned to and mixed with the remainder of the sample. With the completion of readings, subsamples were taken from each sample for moisture content determination by oven dry methods.

The above procedures having been established, two parcher loads of wild rice (Batches 1 & 2), begun 10 minutes apart, were each sampled at 20 minute intervals beginning at the 30 minute mark for Batch 1 when a cover was removed from the parcher drum and at the 50 minute mark for Batch 2. The last samples were taken when parching was pronounced completed. The last sample for Batch 2 was taken directly from the parcher as planned; for Batch 1, it was taken from a cart into which the parcher had just been emptied.

Two more parcher loads of wild rice (Batches 3 & 4) were sampled the following day but with a slight change in procedures. To minimize sample cooling during temperature measurement, an insulated mold was fabricated, conforming to the shape of the sampling container, into which it was placed immediately after retrieval of each sample. With the sampling container in the mold and the thermocouple inserted into the sample, an insulated cover was placed over the sample where it remained until the temperature reading stabilized. Another "change" was to ensure that no samples be taken except directly from parchers. (The grain in Batch 1 is believed to have cooled substantially upon transfer to the cart from which the last sample for that batch was taken.) A final change was to sample only one parcher batch at a time with samples being taken at 10 minute intervals.

Moisture Tester Performance Results

Performance evaluations with the DJFMT and the DMA showed both instruments to have limited capability for accurately indicating moisture content of wild rice during parching, particularly at moisture levels below 10 percent. Figure 1 shows mean readings obtained with both instruments for all parcher grain samples taken at the processing plant. Figure 2 shows all readings obtained with the instruments from those grain samples that had less than 10 percent moisture content. As expected, mean readings from both instruments varied directly with grain moisture content. For the DJFMT, mean readings with temperature compensation were slightly lower, and below 10 percent moisture content, more variable than mean readings without temperature compensation. Variation was low for all sets of corresponding readings as evidenced by the close groupings of data points in Figure 2. (The largest coefficient of variation for corresponding readings was less than 3.8 percent.) Overall, however, variations in response of both instruments were too large in relation to underlying instrument sensitivities to grain moisture content to expect that either one would be capable of providing a sufficiently precise indication of grain moisture content during parching, particualrly at low moisture levels.

Grain Temperature and Moisture Content Results

Figures 3a and 3b show grain temperature and moisture content, respectively, versus time in the parcher for all four sampled parcher batches. Grain temperatures taken almost immediately after removal of a cover from the end of the drum at the 30-minute mark were only a few degrees below the boiling point of water (212 degrees F). Over the next 30 minutes, grain temperature dropped to the 185-190 degree F range before rising again and at an increasing rate as parching neared completion. Grain moisture content, on the other hand, dropped at a generally constant and uniform rate for all batches except near the end of the parching period when the rate of decline decreased slightly. As parching approached completion, grain was losing nearly 1 percentage point every three minutes, thus quantifying how sensitive final moisture content can be to a difference of only a few minutes in the time parching is judged complete and the parcher emptied. Interestingly, final moisture content for the four batches sampled varied over a range of only 1.5 percentage points, thus demonstrating that subjective assessment of grain conditions by the parcher operator can produce final moisture levels that fall within a reasonably narrow range, irrespective of what the preferred final moisture content may be.

Figure 4 shows grain temperature versus moisture content for all parcher samples that were less than 13 percent moisture content. Dispite some differences in parching times among the sampled batches, relationships between grain temperature and moisture content were highly consistant for all four batches. These findings agree with those reported by Lund et. al (1977) in research conducted in Wisconsin in the early to mid-1970s. Their studies showed the existance of a consistant relationship between temperature of the wild rice "bed" and moisture content for any given set of parching conditions. Some factors that would serve to define "parching conditions" include parcher design, firing rate, method of heating control if any, batch size and grain quality characteristics, as well as perhaps ambient air temperature and maybe others.

Our limited studies in 1990, together with those of Lund et. al, suggest that grain temperature might indeed be a satisfactory indicator of grain moisture content during parching, at least in the important final stages where we found temperature increasing about 14 degrees F for every one percentage point drop in moisture content. Presently, the extent to which grain temperature and moisture content relationships differ for different parching conditions typical of Minnesota plants is not known. Before attempting to use grain temperature to accurately indicate moisture content for a particular set of parching conditions, it would seem necessary first to define the relationship for those conditions and then to try to maintain those conditions reasonably closely for all batches. Needed also is a practical method for accurately measuring grain temperature in the parcher. We hope to be able to work on that problem in 1991 and also to investigate temperature/moisture content relationships for other parching conditions.

EVALUATING KERNEL LENGTH BY PHOTO SENSOR

Kernel length distribution is frequently an important factor in characterizing particular lots of wild rice grain. Lot descriptions generally contain information on kernel length including an indication of the grain mass percentages within specified length categories. This information is frequently based on analysis of samples taken from the lot.

A system has been developed to assess the length of individual kernels for use in evaluating the kernel length distribution in samples of wild rice. This system involves passing individual kernels of the sample single file across a sensor consisting of an array of photodiodes. The length of each kernel is determined by computer which assesses the number of photodiodes being shaded from a light source by the kernel at a particular point in time and converts this number to kernel length. Present software simply counts the kernels that are in selected length categories. Future plans are for the software and hardware to also sort the kernels by directing them into different containers according to specified length categories for weighing to determine the mass relationships.

Description of the System

The system at the present stage of development consists of the following four sequential processes:

1) Singulating the kernels,
2) Spacing each kernel from other kernels,
3) Evaluating the kernel for length and
4) Tabulating the kernel length.

Figure 5 is a schematic of the system showing a vibratory bowl feeder for singulating the kernels, a slide for accelerating and spacing the kernels, and the photo array sensor.

The process of singulating the kernels into single file makes use of a vibratory bowl feeder. Vibratory bowl feeders are typically used to feed parts to an assembly operation or to count items that are placed in it. The bowl feeder has a spiral inclined ramp with increasing diameter from the

bottom of the bowl to the crest of the ramp at the top. With a sample of wild rice placed in the bowl feeder, the vibratory action of the bowl causes the kenels to move up the ramp to the crest. As the kernels move, they tend to form a single file pattern. Use of a narrow ramp section and a height strikeoff help ensure that kernels progressing to the crest of the ramp are in single file only.

The process of spacing the kernels is accomplished as the kernels, traveling over the crest of the vibratory feeder, are directed down a slide to the sensor. A glass tube is used for the slide to maintain kernel alignment over the sensor. Upon entering the slide the kernels accelerate and a space develops between successive kernels. The space between kernels is needed so that the photodiodes are covered by only distinct individual kernels. Without a space between kernels, two or more kernels would appear as a single extra-long kernel.

The process of evaluating the length is accomplished as the individual kernels slide down the glass tube and over the sensor, an array of photodiodes. The photodiodes are spaced at 0.039 in. intervals. The instrumentation is arranged to monitor for one of two conditions of the photocells: 1) receiving more than the set threshold of light and 2) receiving less than the set threshold of light (being shaded by a kernel). The first photodiode is used as the trigger cell. As a kernel slides through the tube, the sequence in the instrumentation process starts with the kernel obstructing the first photodiode from the light source. Then, when the trailing end of the kernel passes the trigger cell and the threshold of light on that cell is regained, the system evaluates the condition of all the cells in the sensor and calculates the kernel length based on the number of photodiodes shaded and the photodiode spacing.

The process of tabulating is accomplished with computer software designed to count the number of kernels within selected length categories. This system, at the present stage of development, simply provides information on the number (not mass/weight) of kernels within each length category. Figure 6 shows a sample computer printout of results obtained by the system, indicating the kernel count in specified length categories.

Anticipated Future Plans

The process of sorting the kernels according to length category has not been developed. However, it is anticipated that the system will be developed so that the individual kernels are sorted into the selected length categories as they travel on a conveyor. Sorting will permit weighing to determine the mass of kernels in each length category. For this process, the kernels will be received onto the conveyor from the inclined tube. The instrumentation will track each kernel as it moves from the point of length evaluation over the sensor, down the incline and onto the conveyor and will direct its discharge into a container corresponding to the respective length category.

ACKNOWLEDGEMENT

The authors wish to thank those individuals who permitted us to use their equipment and facilities to conduct the investigations described in this report.

REFERENCE

Lund, D., R. Lindsay, E. Marth and D. Striber. 1977. Methods to Extend the Storage Life of Green, Wet Wild Rice. Department of Food science, University of Wisconsin, Madison, Wisconsin 53706.

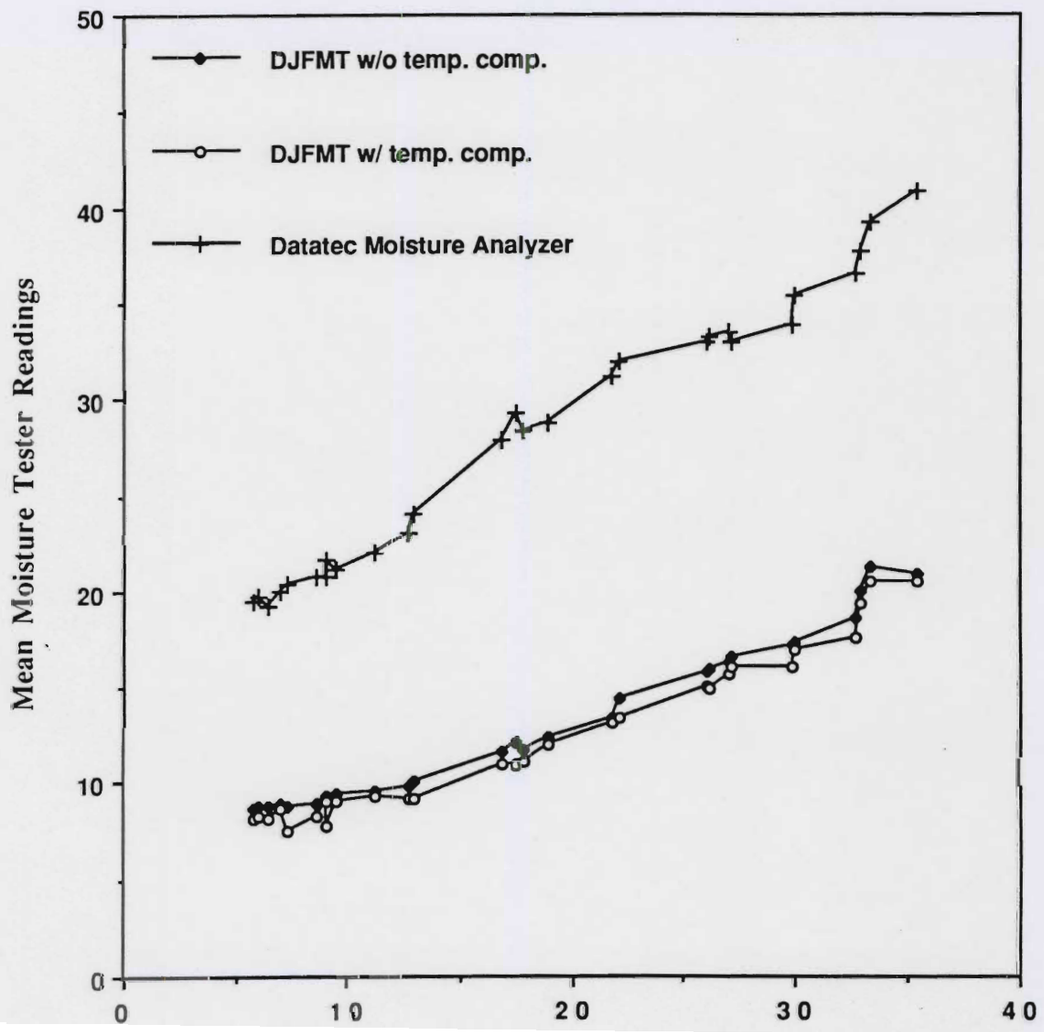

Figure 1. Mean moisture tester readings for Dickey-john and Datatec instruments vs. grain moisture content during the parching process.

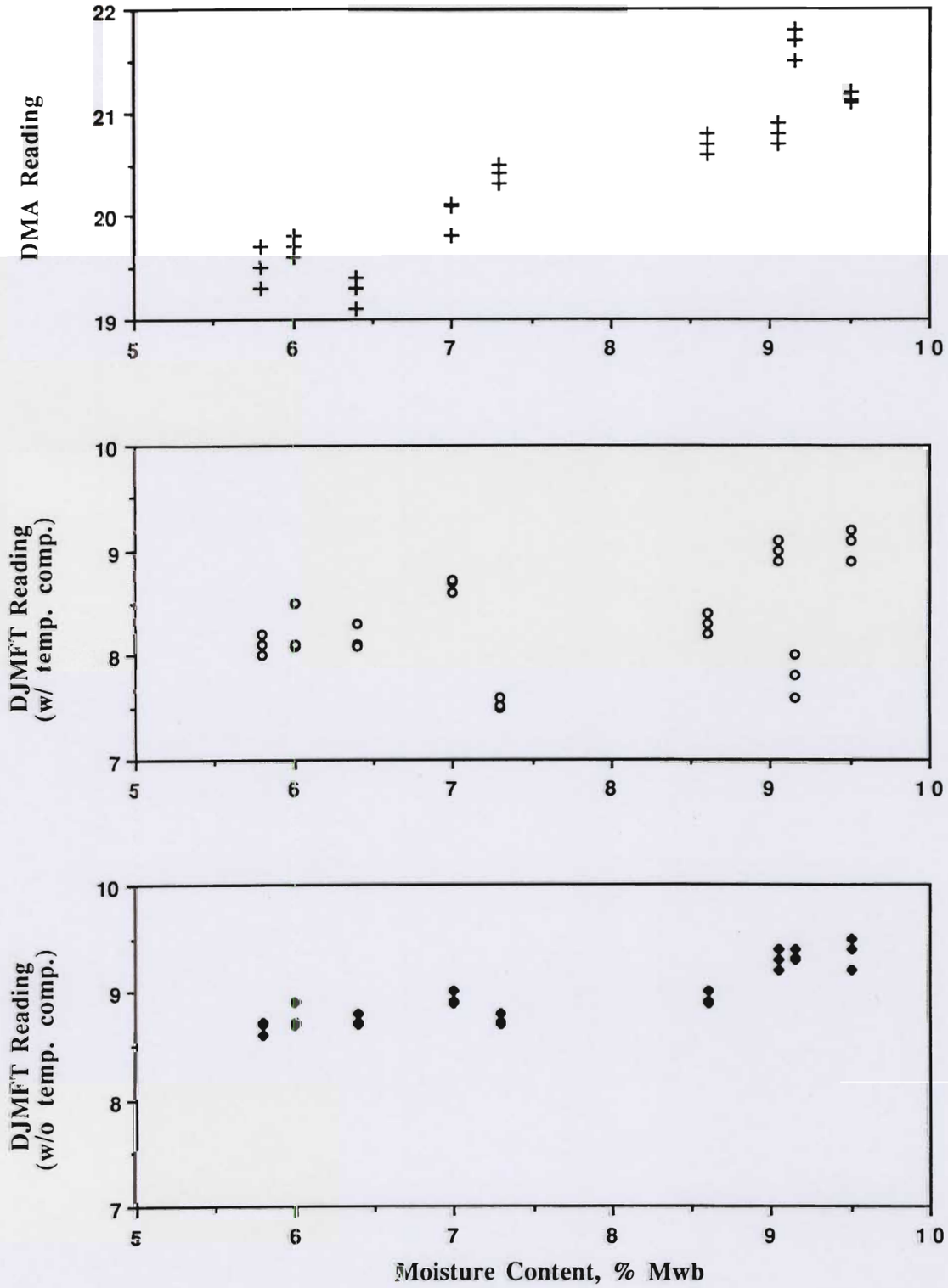

Figure 2. All moisture tester readings vs. grain moisture content for parcher samples below 10 percent moisture.

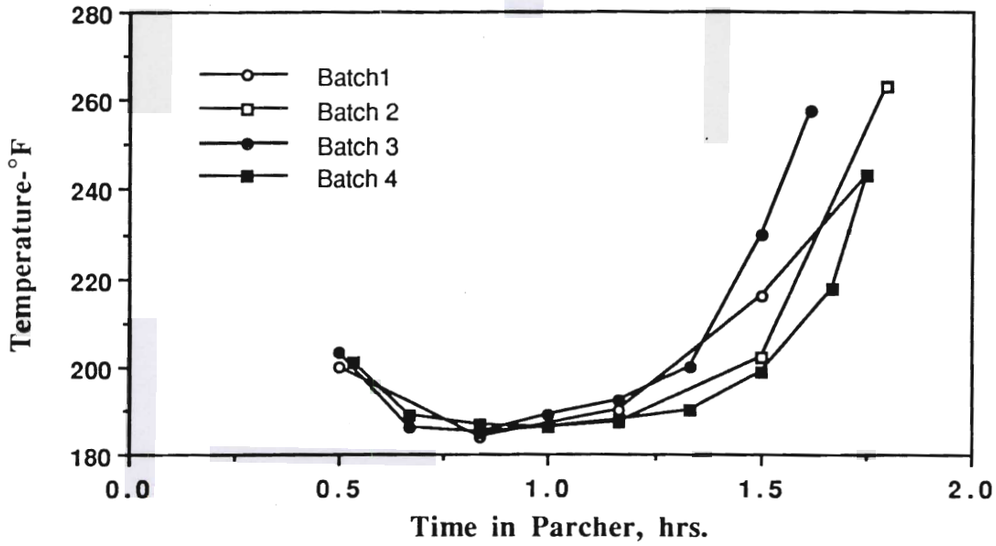

Figure 3a. Grain temperature vs. time in parcher for four batches of wild rice.

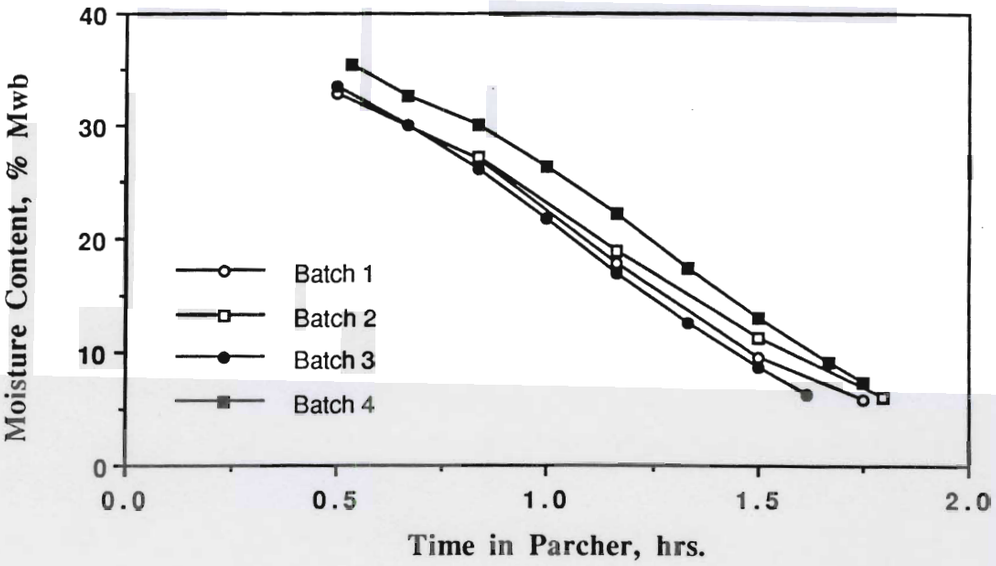

Figure 3b. Grain moisture content vs. time in parcher for four batches of wild rice.

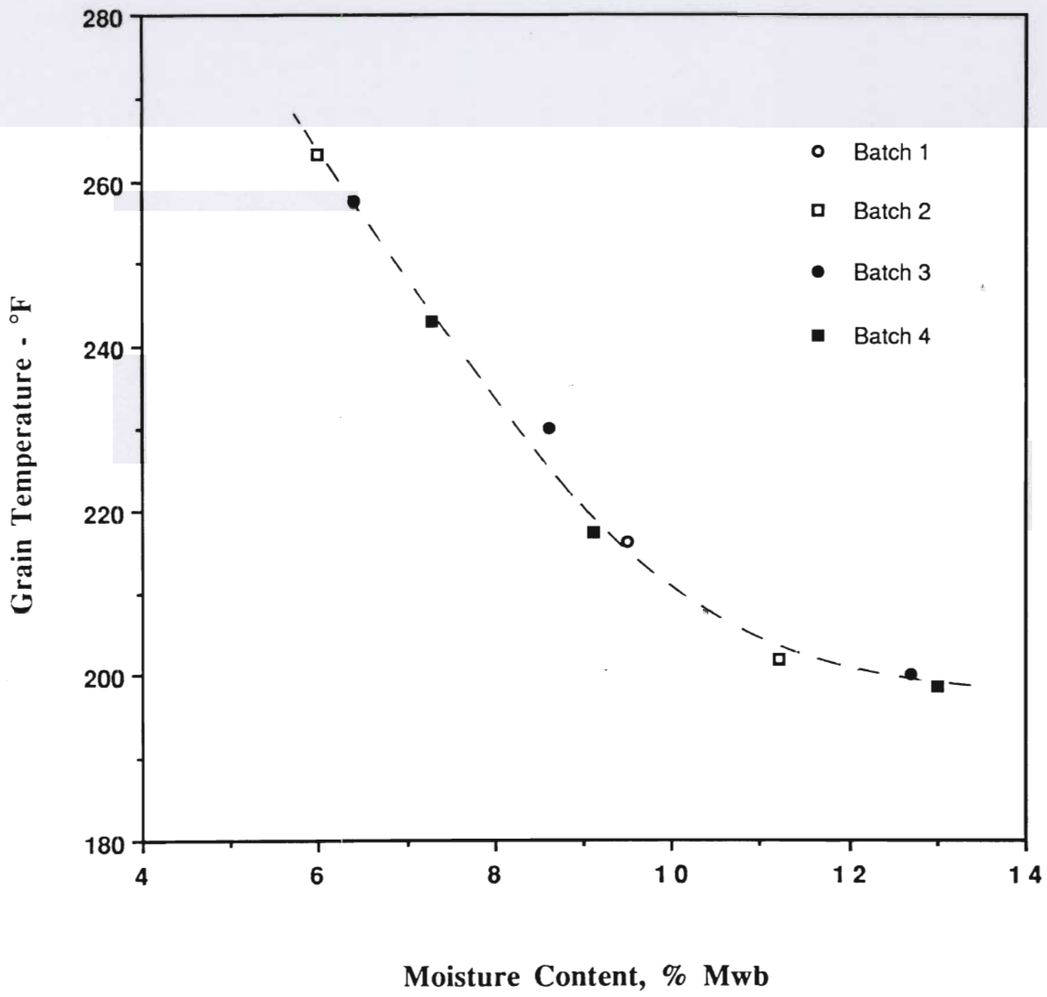

Figure 4. Grain temperature vs. moisture content for four batches of wild rice at below 10 percent moisture.

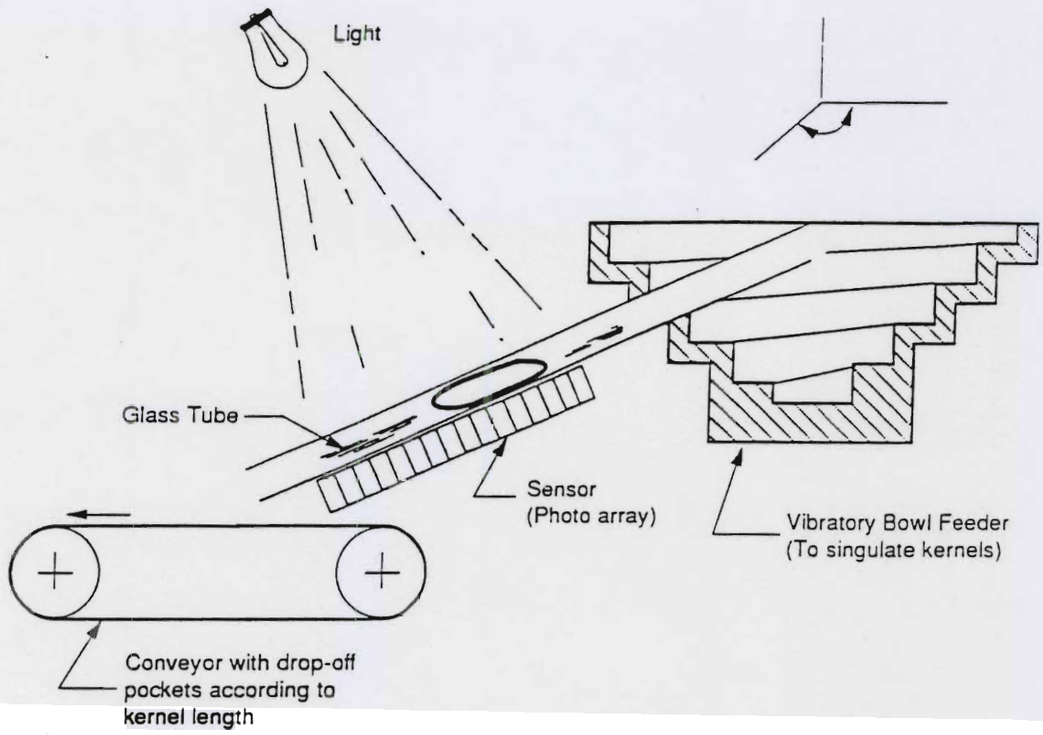

Figure 5. Schematic of equipment used for length evaluation of individual kernels by photo sensor.

```
57 KERNELS GREATER THAN 20/64"
67 KERNELS GREATER THAN 12/64" AND LESS THAN 20/64"
20 KERNELS GREATER THAN 6/64" AND LESS THAN 12/64"
2 KERNELS LESS THAN 6/64"
```

Figure 6. An example printout of tabulated results of kernel length evaluations by length category.

FIRST QUARTERLY PROGRESS REPORT
ON
WILD RICE ANTIOXIDANT

November 22, 1990

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SUMMARY OF PROGRESS

The results from preliminary studies were confirmed. The addition of 15% of hydrated wild rice resulted in 50% reduction in the TBA values of the ground beef stored at 4 ^{o}C, which strongly indicated the presence of antioxidant components in wild rice.

Three different solvents, i.e. methanol, ethanol, ethyl acetate, were used to extract the antioxidant components from ground wide rice. The yields of the extraction based on the weight of the starting wild rice were 3.1%, 1.9%, and 1.0%, respectively. As all three extracts had an dark green color, activated carbon was used to treat the extracts and it effectively removed the undesirable color, which was presumably caused by oxidation-promoting chlorophylls. However, the yield of the extract was decreased to a various extent depending on the amount of the activated carbon used.

The antioxidant efficiency of the extracts were evaluated in ground beef by TBA test. All three extracts, i.e. methanol, ethanol, and ethyl acetate extracts, exhibited certain activity when added at 0.02% and the effect was more pronounced at level of 0.05% based on the weight of the beef. The methanol extract was equally effective as the ethanol extract, while the ethyl acetate extract was the least effective. The methanol extract treated with activated carbon was more effective than the untreated extract and ..... The antioxidant activity of the extracts were also evaluated in lard by the peroxide value test, which is still in progress. The results obtained so far indicates .....

Effort in optimizing the extraction procedure to obtain maximal yield and antioxidant efficiency is under way. Preliminary studies on the purification and identification of the extracts will proceed soon.

RESULTS AND DISCUSSION

Preliminary studies conducted in this laboratory showed that the incorporation of hydrated wild rice into ground beef could retard the development of rancidity and warmed-over flavor from beef. The objective of the present project is to isolate and identify the antioxidant components from wild rice.

1. The Effect of Wild Rice on the Shelf-life(TBARS) of Ground Beef

In order to confirm the results from the preliminary study, the previous experiment was repeated. The hydrated wild rice and the water fraction were prepared according to the procedure described in the experimental section. The antioxidant activity of the hydrated wild rice and the water fraction was evaluated in ground beef. As shown in Table 1, the TBARS value of the cooked beef was twice as high as the uncooked (samples#1 and #2). BHA and TBHQ effectively inhibited the TBARS value (samples #3 and #4). Compared samples #6 and #7 with the control (sample #2), the addition of the hydrated wild rice at 15% significantly decreased the TBARS value, while the water fraction was ineffective. Note that the TBARS values of samples #5-7 were converted from the measured values by dividing the percentage (85%) of the beef in the mixture and thereby the dilution effect was excluded. The dilution effect was also eliminated by comparing sample #6 with sample #5, which contained 85% beef and 15% plain water and had the same TBARS value as the control.

A modified procedure was used to prepare hydrated wild rice and water fraction under refluxing. As shown in Table 2, the antioxidant activity of the hydrated wild rice and the water fraction so obtained were similar to the results from the previous experiment discussed above. The addition of 15% of the hydrated wild rice resulted in 50% reduction in TBARS value compared to the control.

This data strongly indicates the presence of antioxidant components in wild rice.

2. Isolation of Antioxidant Components

Solvent extractions were undertaken to isolate the antioxidant components. Three different solvents, i.e. methanol, ethanol, and ethyl acetate, were used in order to find the best solvent which would result in a product with optimal yield and antioxidant efficiency. The final yields of the extraction with methanol, ethanol, and ethyl acetate were 3.1%, 1.9% and 1.0%, respectively. It appears that the yield decreases as the polarity of the solvent decreased. All three products were semi-solid substances with an intense dark green color and roast-type aroma.

The dark green color of the extracts was most likely caused by chlorophylls, which not only are undesirable for the color *per se* , but also have been reported to act as photosensitizers promoting lipid oxidation. Thus, active carbon was used to treat the extract in attempt to remove the chlorophylls. Also it is was found that the extracts obtained using the above discussed extraction procedure were very difficult to dissolve in a single solvent due to their heterogeneous chemical composition. Therefore another change of the extraction procedure was that instead of completely removing the solvent from the filtrate as did in the previous extraction scheme, the filtrate was concentrated down to certain volume and the precipitate formed in the concentrated filtrate was separated through filtration. Thus, two fractions, i.e. the methanol-insoluble and the methanol soluble fractions, were obtained. The yields of the methanol insoluble fraction and the methanol soluble fraction was 0.9% and 0.04%, respectively, when 100g (20% the starting wild rice) activated carbon was used. The color of the extract was greatly improved and final product was a yellowish solid. The yield, however, is lower than the extraction without the treatment with activated carbon. Obviously, the decrease of yield was caused by the absorption of the activated carbon. In order to compare the effect of the amount of the activated carbon used on the yield and the antioxidant efficiency of the extract, a reduced amount (50g, or 20% of the starting wild rice) of activated carbon was used. The yields of the methanol insoluble and methanol soluble fraction were 1.3% and 0.06%, respectively. The methanol soluble fraction had a brown color, which is darker than that treated with 20% activated carbon, but far better than the untrated. The yields and the physical properties of the various extracts are summarized in Table 3.

3. Antioxidant Efficiency of Wild Rice Extracts in Beef

The antioxidant efficiency of various wild rice extracts were evaluated in ground beef by the TBA test. As shown in Table 4, all three extracts, i.e. methanol, ethanol, and ethyl acetate extracts, exhibited certain antioxidant activity at the level of 0.02% and their effect was more pronounced at 0.05%. The methanol extract was equally effective as the ethanol extract, while the ethyl acetate extract was least effective. It is not surprising that both BHA and TBHQ were more effective than the extracts, since they are of synthetic origin and of high purity, while the extracts may still contain a wide spectrum of compounds. One advantage of using natural antioxidants is that it can be added at any level of "good manufacturing practice", while the synthetic antioxidant, such as BHA and TBHQ, can only be added under the legal limit, which is 0.02%, or 200ppm.

The antioxidant activity of the extracts treated with activated carbon was also evaluated in ground beef by TBA test.....

POV test.....

FUTURE RESEARCH PLANING

The task prescribed in the milestone for the second quarter of the project was to fractionate and identify the antioxidant components. The extracts or fractions with high antioxidant activity and reasonable yield will be selected to be used as the starting material. First they will be fractionated using liquid chromatography into various fractions. The antioxidant activity will be assessed by the thiocyanate method as described by Osawa and Namiki (*Agric. Biol. Chem.* 1981. 45:735-739), which requires much less amount of the antioxidant than the TBA or peroxide value tests. Those fractions with significant antioxidant activity will be further fractionated on HPLC (high performance liquid chromatography) until chromatographically pure subfractions are obtained. Those subfractions with demonstrated antioxidant activity will then be analyzed using MS (mass spectrum), NMR (nuclear megnetic resonance), IR (infrared spectrum), and if necessary, elemental analysis, to identify their chemical structures.

EXPERIMENTAL

1. Preparation of Hydrated Wild Rice

30 grams of wild rice was boiled with 300 ml water for 1 hour. Part of the water was absorbed by the wild rice as a result of cooking. The remainder of the water(the water fraction) was then separated from the rice. The hydrated wild rice and the water fraction weighed 131g and 65g, respectively. Obviously, 134g of the water was lost through evaporation during cooking.

2. Preparation of Hydrated Wild Rice under Refluxing

20 grams of wild rice was cooked with 100 ml of water. The conditions were the same as discussed above except that the proportion of water used was smaller and reflux was used in order to prevent loss of water through evaporation. The weight of the hydrated wild rice and the water fraction was 90 grams and 30 grams, respectively.

3. Evaluation of Antioxidant Activity of the Hydrated Wild Rice in Beef

Freshly ground extra lean beef was mixed with 15% of the hydrated wild rice and the water fraction, using a household blender. The samples were cooked at 78±2 °C for 3 hours and then stored at 4 °C. The TBARS (thiobarbituric acid reactive substances) value, a commonly used indicator of rancidity in meat products, was determined after a period of storage, according to the method described by Rethwill et al. (Rethwill, C. E., Bruin, T. K., Wailbel, P. E., and Addis, P. B. 1981. Influence of Dietary fat source and vitamin E on market stability of turkey. *Poultry Sci.* 60:2466)

4. Solvent Extraction

The procedure for the extraction is shown in Fig. 1. The wild rice was first ground to fine particles approximately 0.5mm in diameter. 500g of the ground wild rice was then mixed with 3 liters(6X) of the solvent, i.e. methanol, ethanol, or ethyl acetate, and extracted in a 5 liter round bottom flask at 60-65 C for 2 hours under vigorous agitation. The vapor of the solvent was refluxed with a glass condenser cooled with running water. Upon completion of the extraction, the mixture was filtered through a Whatman No. 1 filter paper under reduced pressure. The residue was extracted again at the same conditions as for the first extraction, except that 2 liters(4X) of the solvent was used. The filtrates from the two extractions were combined and subjected to vacuum evaporation to remove the solvent.

The methanol and ethyl acetate used in the extraction were reagent

grade. The ethanol used was 95% and therefore the combined filtrates were treated with sufficient amount of anhydrous sodium sulfate to remove the moisture.

5. Solvent Extraction and Treatment with Activate Carbon

The procedure is shown in Fig. 2. The conditions for the extraction was the same as in Fig. 2. The combined filtrates were treated with activated carbon at two levels, i.e 100g (20% of wild rice) and 50g (10% of wild rice). The treated filtrate was filtered through a Whatman No. 1 filter paper aided with Celite^{R} 545. The filtrate was then concentrated to 300ml and the precipitate formed was filtered out, resulting the methanol insoluble and methanol soluble fraction.

6. Evaluation of Antioxidant Efficiency of Wild Rice Extracts in Meat Products

In order to ensure uniform dispersion, all the additives were pre-dissolved in a appropriate solvent at concentration of 10mg/ml. The solution was then mixed with ground beef using a household blender at moderate speed for 20 minutes. Unless otherwise stated, extra lean ground beef was used as a testing substrate. The mixed meat was then cooked, using a water bath, at 78±2, for 2 hours. The samples were stored at 4C in a refrigerator. TBA test were performed after a period of storage, according to the method described by Rethwill et al. (Rethwill, C. E., Bruin, T. K., Wailbel, P. E., and Addis, P. B. 1981. Influence of Dietary fat source and vitamin E on market stability of turkey. *Poultry Sci.* 60:2466)

7. Evaluation of Antioxidant Efficiency of Wild Rice Extracts in Edible Oils

For the same reason, all the additives were pre-dissolved in a appropriate solvent at concentration of 10mg/ml. Lard used (Geo A. Hormel & Co., Corporate Office. Austin, MN 55912) was unrefined and contained no antioxidant. After mixing with lard, the solvent was removed through rotary evaporation at 80-90C for 30 minutes.

The samples were place in wide mouth glass jars without caps and aged at 60±2C in an unlighted oven. Peroxide value was determined very 7 days according to the official method of the American Oil Chemists' Society, cd-53

8. Specification of the Other Additives

BHA and TBHQ: Food-grade antioxidants manufactured by Eastman Chemical Products, Inc.

d-α-Tocopherol: A mixture of natural tocopherols from vegetable oil, containing 670mg d-α-tocopherol per gram and 0.5-2% non-alpha-tocopherols. The remainder is soybean oil. Sigma Chemical Co. Cat. #T-3634

Herbalox^{R} seasoning type O: A natural antioxidant from Rosemary, marketed by Kalsec, Inc. Code: 41-19-01, Lot #8102-I

Table 1. The Effect of Wild Rice on the TBA Values of Ground Beef Stored at 4 °C

Sample #	Treatment	TBARS(ppm) after 10 days
1	Beef(100%, uncooked)	1.7
2	Beef(100%, control)	3.5
3	Beef + BHA(200 ppm)	0.3
4	Beef + BHT(200 ppm)	0.5
5	Beef(85%) + Water(15%)	3.5*
6	Beef(85%) + Hydrated wild rice(15%)	1.8*
7	Beef(85%) + Water fraction(15%)	3.9*

* Values given were converted from the measured values by dividing the % of beef in the mixture so as to eliminate the dilution effect.

Table 2. The Effect of Wild Rice on the TBA Values of Ground Beef Stored at 4 °C

Sample #	Treatment	TBARS(ppm) after 7 days
1	Beef(100%, control)	2.7
2	Beef(85%) + Water(15%)	2.3*
3	Beef(85%) + Hydrated wild rice(15%)	1.3*
4	Beef(85%) + Water fraction(15%)	2.4*

* Values given were converted from the measured values by dividing the % of beef in the mixture so as to eliminate the dilution effect.

Table 3. Yields and Physical Properties of Various Wild Rice Extracts

Extract	Yield(%)	Physical properties
MeOH-ext	3.1	Semi-solid, dark green, roast-type aroma
EtOH-ext	1.9	(same as above)
EtoAc-ext	1.0	(same as above)
MeOH-ext/AC(20%)-MI	0.9	white powder, water soluble, odorless
MeOH-ext/AC(20%)-MS	0.04	Solid, yellowish, roast-type aroma
MeOH-ext/AC(10%)-MI	1.3	White powder, water soluble, odorless
MeOH-ext/AC(10%)-MS	0.06	Solid, brown, roast-type aroma

Abbreviations:
MeOH-ext: Methanol extract
EtOH-ext: Ethanol extract
EtoAc-ext: Ethyl acetate extract
MeOH-ext/AC(20%)-MI: Methanol extract treated with activated carbon(20% of wild rice)-methanol insoluble fraction
MeOH-ext/AC(20%)-MS: Methanol extract treated with activated carbon(20% of wild rice)-methanol soluble fraction

Table 4. The Effect of Wild Rice Extracts on the TBA Values of Ground Beef Stored at 4 ^{o}C

Sample #	Additive(%)	TBARS(ppm) after 6 days
1	Control(no additive)	3.8
2	BHA(0.02)	0.3
3	TBHQ(0.02)	0.3
4	MeOH-ext(0.02)	3.5
5	MeOH-ext(0.05)	2.7
6	EtOH-ext(0.02)	3.3
7	EtOH-ext(0.05)	3.0
8	EtoAc-ext(0.02)	3.6
9	EtoAC-ext(0.05)	3.5

SD = 0.1 ppm

Table 5. The Effect of Wild Rice Extracts on the TBA Values of Ground Beef Stored at 4 °C

Sample #	Additive(%)	TBARS(ppm) after 6 days
1	Control(No additive)	3.4
2	BHA(0.02)	0.5
3	TBHQ(0.02)	0.5
4	d-α-toc.(0.02)	1.8
5	d-α-toc.(0.05)	0.7
6	Herbalox^{R} type O(0.02)	2.1
7	Herbalox^{R} type O(0.05)	0.7
8	MeOH-Ext/AC(20%)-MI(0.02)	2.9
9	MeOH-Ext/AC(20%)-MI(0.05)	2.5
10	MeOH-Ext/AC(20%)-MS(0.02)	3.0
11	MeOH-Ext/AC(20%)-MS(0.05)	2.1
12	MeOH-Ext/AC(10%)-MI(0.02)	3.0
13	MeOH-Ext/AC(10%)-MI(0.05)	2.7
14	MeOH-Ext/AC(10%)-MS(0.02)	1.9
15	MeOH-Ext/AC(10%)-MS(0.05)	1.4

SD = 0.1 ppm

Table 6. The Effect of Wild Rice Extracts on the Peroxide Values of Lard Stored at 60 °C

Sample #	Additive(%)	POV(meq/kg) after X days 7	10
1	Control(No additive)	40.3	86.7
2	BHA(0.02)	4.28	6.38
3	TBHQ(0.02)	1.61	1.50
4	d-α-toc.(0.02)	10.1	15.4
5	d-α-toc.(0.05)	13.8	23.4
6	Herbalox^R type O(0.02)	2.22	3.30
7	Herbalox^R type O(0.05)	2.41	2.22
8	MeOH-ext(0.02)	34.0	75.8
9	MeOH-ext(0.05)	28.2	72.7
10	EtOH-ext(0.02)	43.4	86.1
11	EtOH-ext(0.05)	29.9	75.5
12	EtoAc-ext(0.02)	45.9	86.9
13	EtoAc-ext(0.05)	57.6	91.0
14	MeOH-Ext/AC(20%)-MI(0.02)	31.0	69.6
15	MeOH-Ext/AC(20%)-MI(0.05)	35.3	70.5
16	MeOH-Ext/AC(20%)-MS(0.02)	25.0	64.5
17	MeOH-Ext/AC(20%)-MS(0.05)	24.5	66.9
18	MeOH-Ext/AC(10%)-MI(0.02)	33.0	84.7
19	MeOH-Ext/AC(10%)-MI(0.05)	40.0	82.7
20	MeOH-Ext/AC(10%)-MS(0.02)	35.7	74.0
21	MeOH-Ext/AC(10%)-MS(0.05)	18.4	65.2

SD = 0.2

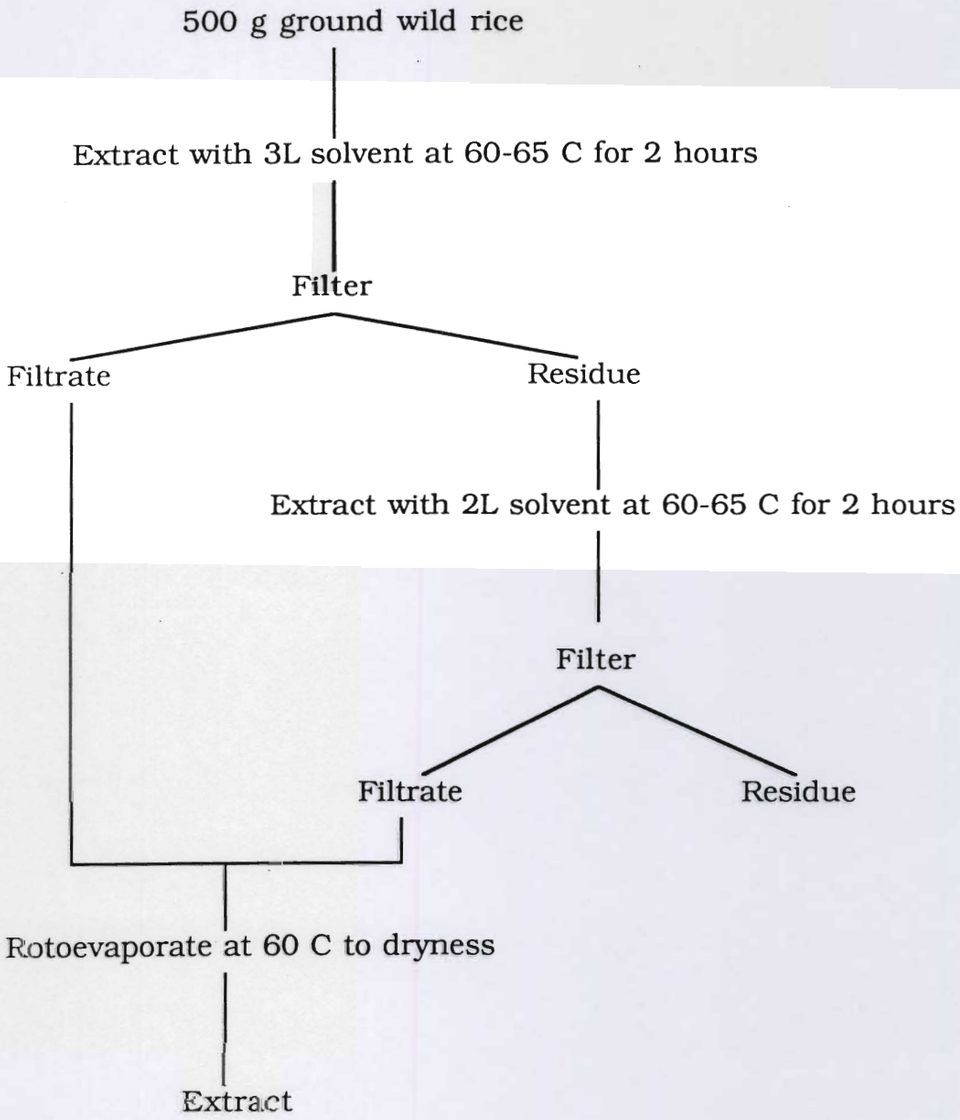

Fig. 1. Procedure for the Solvent Extraction

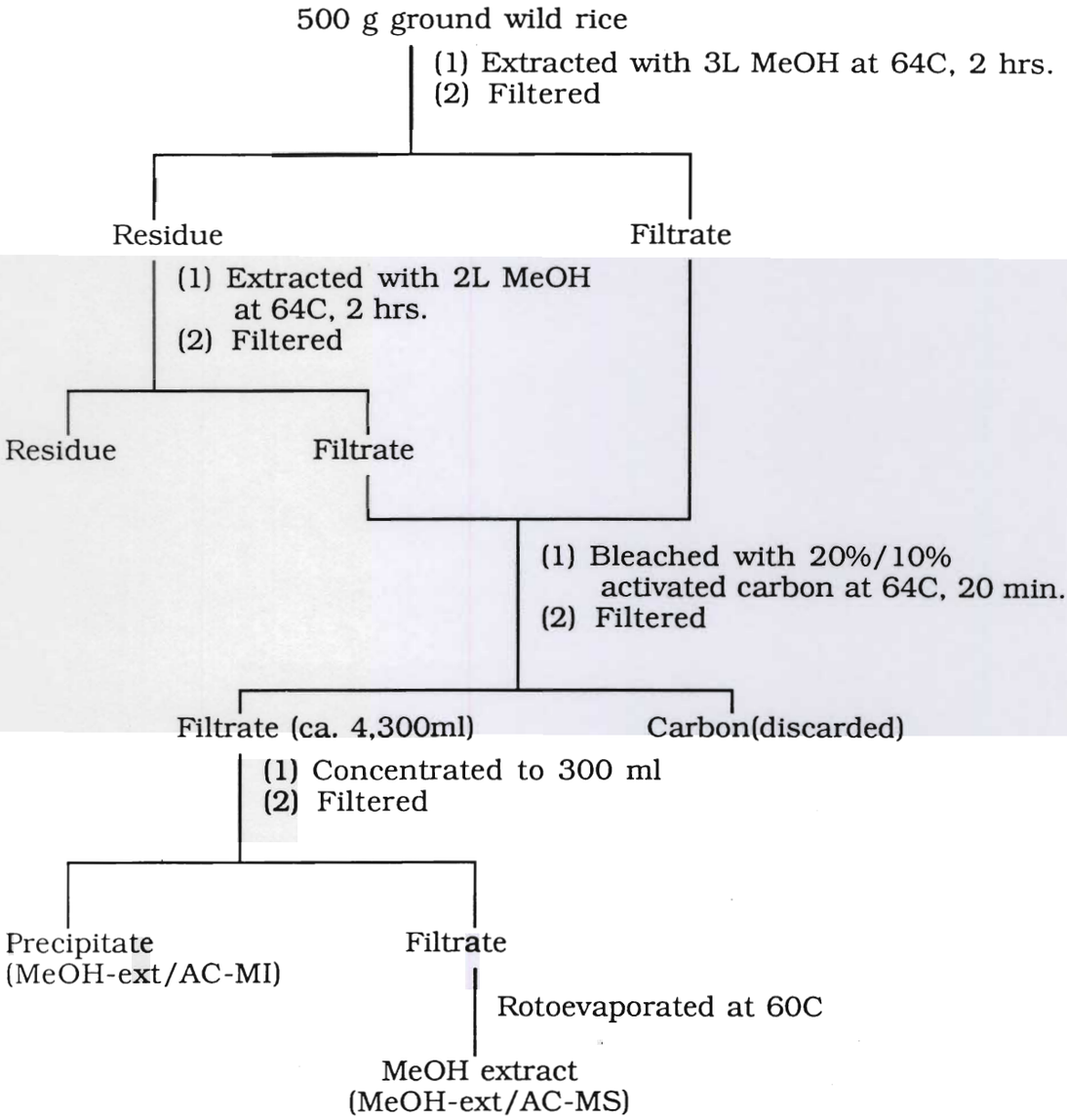

Fig. 2. Procedure for the Solvent Extraction and Treatment with Activated Carbon

SECOND QUARTERLY PROGRESS REPORT ON TG276-ISOLATION/CHARACTERIZATION/ APPLICATION OF ANTIOXIDANT IN WILD RICE

November 1, 1990 to January 31, 1991

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Animal Science

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SUMMARY OF PROGRESS

We have accomplished the milestones for the second quarter as outlined in the first quarterly report.

Several different schemes of solvent extractions have been carried out in order to maximize the yield and antioxidant activity of the extracts. The chlorophylls in the extract were effectively removed by treating the extract with 5-20% activated carbon based on the weight of the wild rice. The color and the antioxidant activity of the extract were improved as a result of the removal of the chlorophylls, at the expense of a reduction in yield. The extract treated with 5% of activated carbon showed strong antioxidant activity in ground beef at 0.3%. The extraction with a mixture of methanol and water at 50:50 resulted in an extract of fairly good yield, antioxidant activity and color.

An HPLC method for the fractionation and analysis of the extracts was successfully developed. Preparative fractionation is under way in order to identify the antioxidant components in the extracts.

RESULTS AND DISCUSSION

1. Isolation of Antioxidant Components

We reported earlier in the first quarterly report that solvent extraction was used for the isolation of antioxidant components from wild rice. Several modifications have been made in order to maximize the yield and antioxidant activity of the extract.

a. Removal of Chlorophylls by Treating with Activated Carbon

The previously reported extracts of wild rice with methanol, ethanol and ethyl acetate contained chlorophylls, which have been shown to promote lipid oxidation and are also undesirable because of their dark green color. Thus, the methanol extract solution was treated with activated carbon, using the procedure developed by Wu et al. (1982) as shown in Figure 1. Three levels of activated carbon, i.e. 5%, 10%, and 20% based on the weight of the starting ground wild rice, were used in order to study the effect of the amount of the activated carbon on the color, yield, and antioxidant activity of the extract. As shown in Table 1, two fractions, i.e. methanol soluble(fraction 1) and methanol insoluble(fraction 2), were obtained from each extraction. The

color of the extract was greatly improved. The higher the amount of activated carbon, the lighter the color of the extract. However, the yield decreased as the amount of activated carbon increased, obviously as a result of absorption. The yields of fraction 1 treated with 5%, 10%, and 20% activated carbon were 1.5%, 1.3%, and 0.9%, respectively. The yields of the fraction 2 were very low.

b. Removal of Chlorophylls by Extraction with Hexane

An attempt was also made to remove chlorophylls from the extract by pre-extracting the ground wild rice with hexane before extracting with methanol. The extraction scheme is shown in Figure 2. The yield of the extract, as shown in Table 1, was 1.6% based on the weight of the starting ground wild rice. The extract still appeared dark.

c. Extraction with Water/MeOH

Ramarathan et al. (1988) reported that extraction of rice hull with a mixture of water and methanol results in a better antioxidant activity than 100% methanol. Thus, a mixture of water and methanol at 50:50(v/v) was used for the extraction as shown in Figure 3. As shown in Table 1, the yield was 2.8% and the color was light even without the treatment of activated carbon.

2. Antioxidant Activity of Various Extracts

The antioxidant activity of the extracts were determined by thiobarbituric acid(TBA) test in ground beef (see first quarterly report for procedure) and peroxide value(POV) test(Official method of the American Oil Chemists' Society, Cd 8-53) in lard. As shown in Table 2, both fractions of the methanol extracts treated with activated carbon showed antioxidant activity as determined by TBA test in ground beef. It appeared that fraction 1 is more effective than fraction 2. The results from POV test(Table 3) showed the same trend.

As can be seen in Table 3, the methanol extracts treated with activated carbon showed higher antioxidant activity than the untreated (comparing samples # 7, #8 and #14, #15, #18, #19). The ethanol extract was slightly less effective than the methanol extract. The ethyl acetate extract (Ref. first quarterly report) exhibited prooxidant activity, which is probably caused by chlorophylls, since chlorophylls are more easily extracted by ethyl acetate than methanol and ethanol. These results are, in general, consistent with those discussed in the first quarterly report. It should be pointed out that none of the extracts are soluble well in lard even with the aid of a solvent and precipitate formed upon the removal of the solvent.

These results indicate that although the extracts invariably showed antioxidant activity, the efficiency was only low to moderate as compared to the commercial antioxidants. One obvious reason is that the extracts are not so pure and they contain some inactive substances with respect to antioxidant activity. One way to improve the activity is to obtain a purer extract either by trying different extraction procedures or purifying the existing extracts. Another approach is simply to increase the concentration. As shown in Table 4, the extracts at 0.1% are equally active as or more active than Herbalox^{R} type O at 0.02%. Strong activity was observed at 0.3% or higher. The extract pre-extracted with hexane and the extract with water/Methanol(50:50) were also quite effective.

3. Fractionation of Wild Rice Extract

High performance Liquid Chromatography (HPLC) was used to fractionate the wild rice extract. The conditions are shown in Table 5. Figure 4 shows the chromatogram of MeOH-AC(20%) fr. 1, which indicates that there are 5 major components in the extract at 4.93, 6.06, 6.72, 8.42, and 10.64 minutes. Preparative fractionation using HPLC is in progress. The major fractions will be collected and tested for their antioxidant activity. Those fractions possessing significant antioxidant activity will have their chemical structures identified.

References

Ramaratham, N., Osawa, T., Namiki, Mitsuo, and Kawakishi, S. 1988. Chemical studies on the novel rice hull antioxidants. 1. Isolation, fractionation and partial characterization. *J. Agric. Food Chem.* 36:732-737.

Wu, J. W, Lee, M. H., Ho, C. T., Chang, S. S. 1982. Elucidation of the chemical structures of natural antioxidants isolated from Rosemary. *JAOCS* 59(8):339-345.

Fig. 1. Procedure for the Extraction and Treatment with Activated Carbon

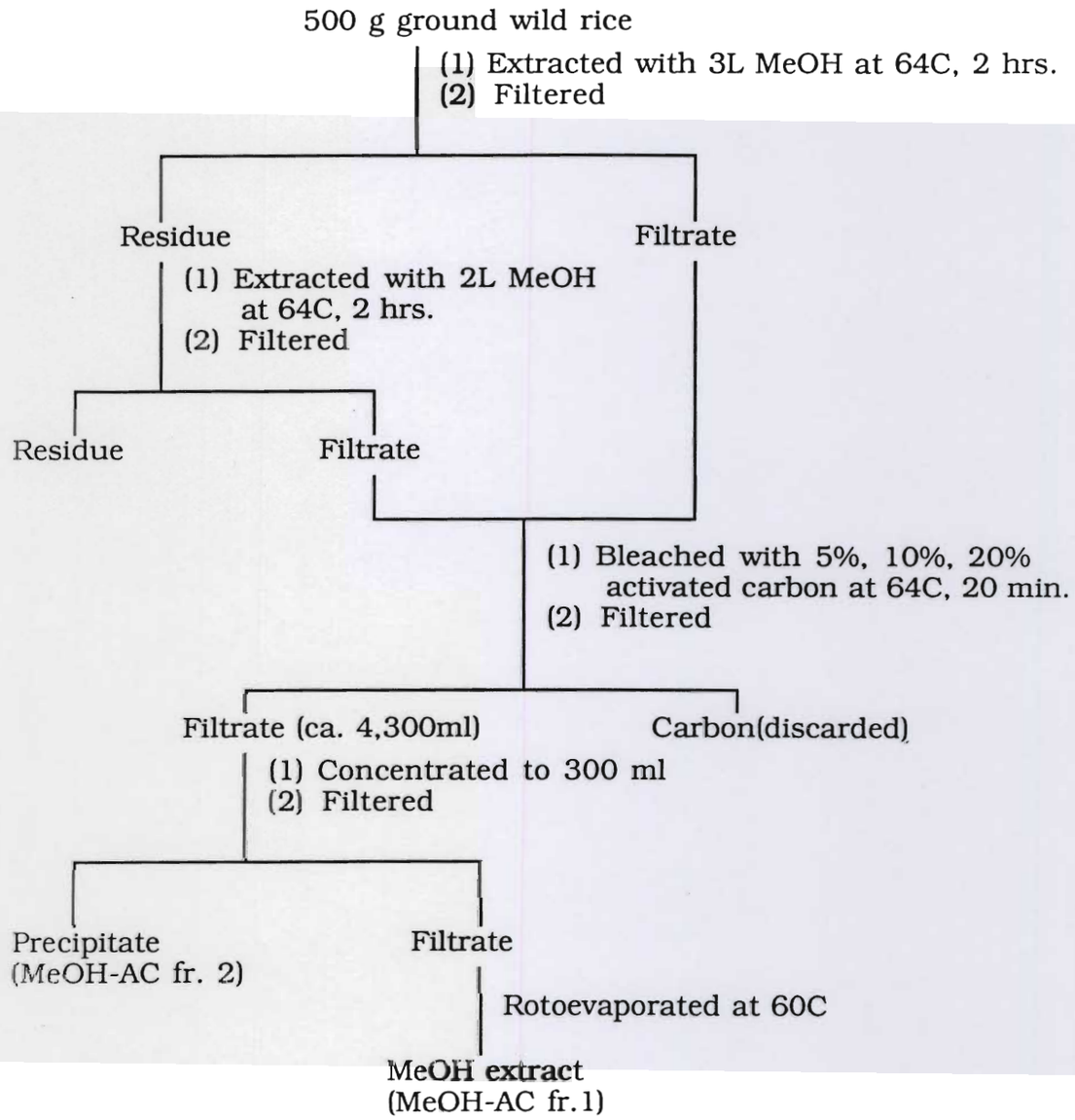


Fig. 2. Procedure for the Extraction with Hexane and Methanol

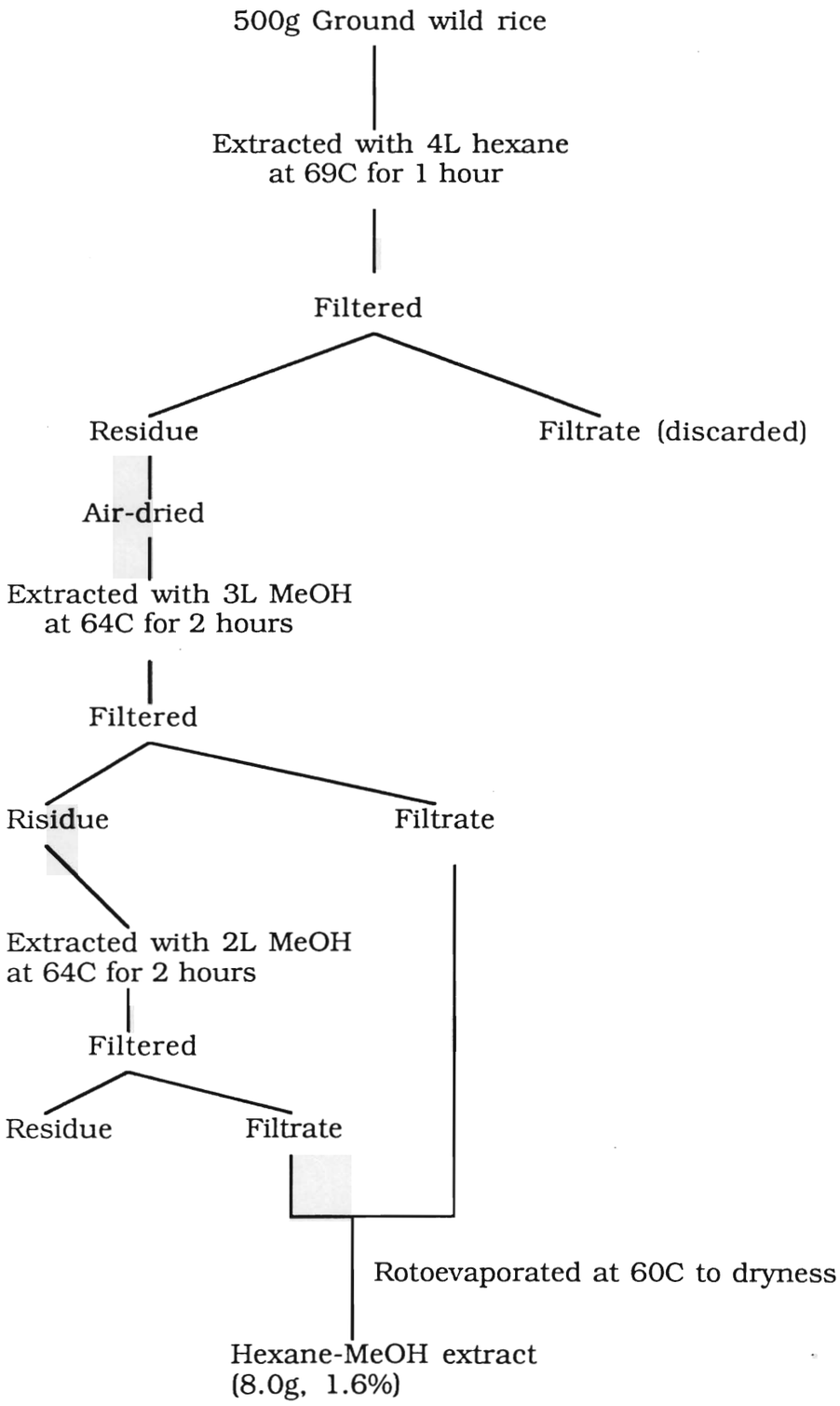


Fig. 3. Procedure for the Solvent Extraction

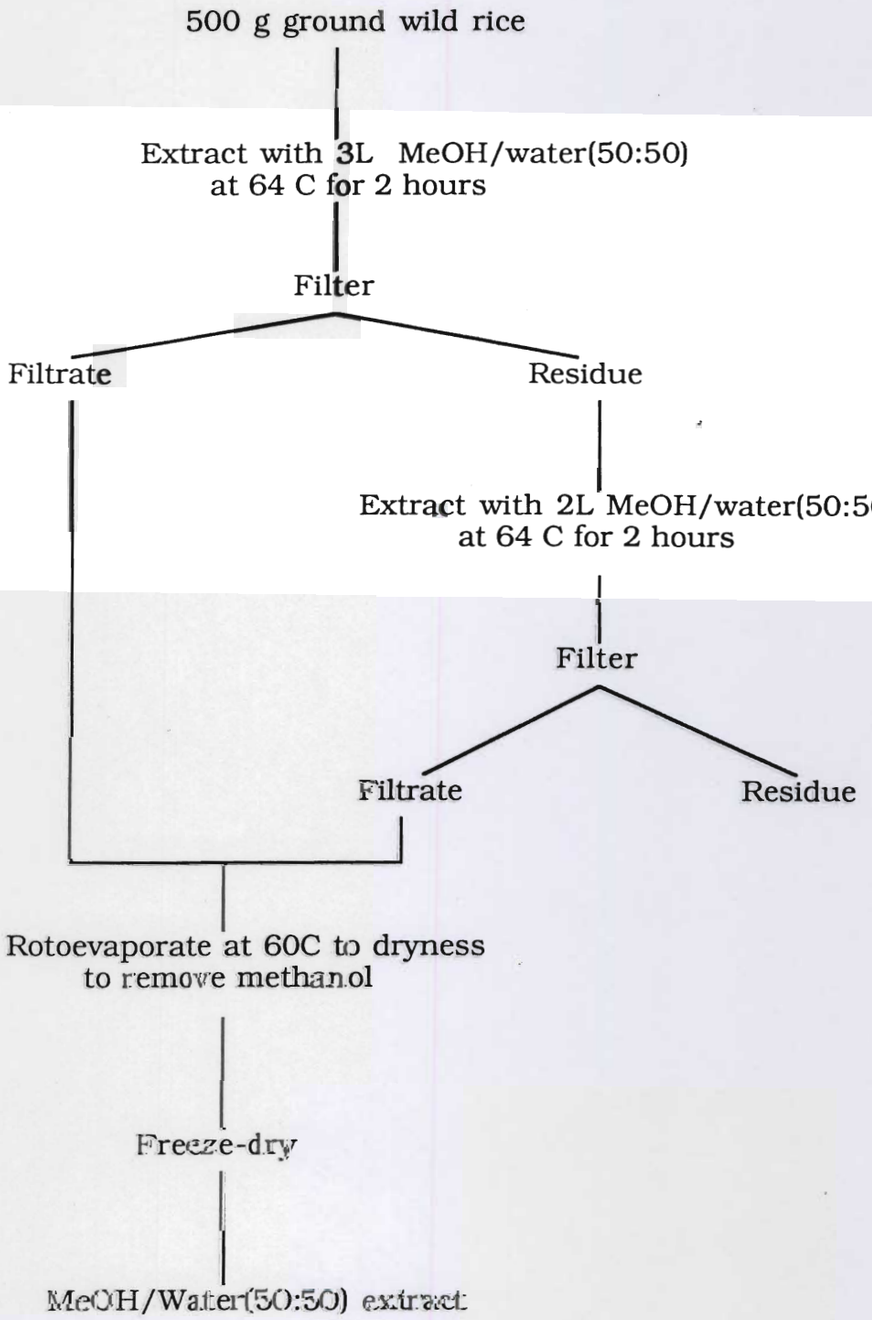


Table 1. Yield and Physical Properties of Various Wild Rice Extracts

Extract	Yield(%)*	Physical properties
MeOH-AC(5%) fr. 1	1.5	Brown, solid, wild rice flavor
MeOH-AC(10%) fr. 1	1.3	Brown, solid, wild rice flavor
MeOH-AC(10%) fr. 2	0.06	White, powder, water-soluble, odorless
MeOH-AC(20%) fr. 1	0.9	Yellowish, solid, wild rice flavor
MeOH-AC(20%) fr. 2	0.04	White, powder, water-soluble, odorless
Hexane-MeOH	1.6	Dark green, solid
MeOH/Water(50:50)	2.8	Brown, powder

* Based on the weight of the starting ground wild rice.

Note: MeOH- AC(%): Methanol extract treated with % of activated carbon.
Hexane-MeOH: Methanol extract pre-extracted with hexane.
MeOH/Water(50:50): Extract with a mixture of methanol and water at 50:50 by volume.

Table 2. Effect of Extracts Treated with Activated Carbon on the TBA Values of Ground Beef Stored at 4 °C

Sample #	Additive(%)	TBARS(ppm) after 6 days
1	Control(No additive)	3.4
2	BHA(0.02)	0.5
3	TBHQ(0.02)	0.5
4	D-α-tocopherol(0.02)	1.8
5	(0.05)	0.7
6	Herbalox^{R} type O(0.02)	2.1
7	(0.05)	0.7
8	MeOH-AC(10%) fr. 1(0.02)	1.9
9	(0.05)	1.4
10	MeOH-AC(10%) fr. 2(0.02)	3.0
11	(0.05)	2.7
12	MeOH-AC(20%) fr. 1(0.02)	3.0
13	(0.05)	2.1
14	MeOH-AC(20%) fr. 2(0.02)	2.9
15	(0.05)	2.5

SD = ±0.1 ppm

Table 3. The Effect of Wild Rice Extracts on the Peroxide Values of Lard Stored at 60 °C

Sample #	Additive(%)	POV(meq/kg) after X days 7	10
1	Control(No additive)	40.3	86.7
2	BHA(0.02)	4.28	6.38
3	TBHQ(0.02)	1.61	1.50
4	D-α-tocopherol(0.02)	10.1	15.4
5	(0.05)	13.8	23.4
6	Herbalox^{R} type O(0.02)	2.22	3.30
7	(0.05)	2.41	2.22
8	MeOH(0.02)	34.0	75.8
9	(0.05)	28.2	72.7
10	EtOH(0.02)	43.4	86.1
11	(0.05)	29.9	75.5
12	EtoAc(0.02)	45.9	86.9
13	(0.05)	57.6	91.0
14	MeOH-AC(10%) fr. 1(0.02)	35.7	74.0
15	(0.05)	18.4	65.2
16	MeOH-AC(10%) fr. 2(0.02)	33.0	84.7
17	(0.05)	40.0	82.7
18	MeOH-AC(20%) fr. 1(0.02)	25.0	64.5
19	(0.05)	24.5	66.9
20	MeOH-AC(20%) fr. 2(0.02)	31.0	69.6
21	(0.05)	35.3	70.5

SD = $\pm$0.2

Table 4. Effect of Various Wild Rice Extracts on the TBA Values of Ground Beef Stored at 4 °C

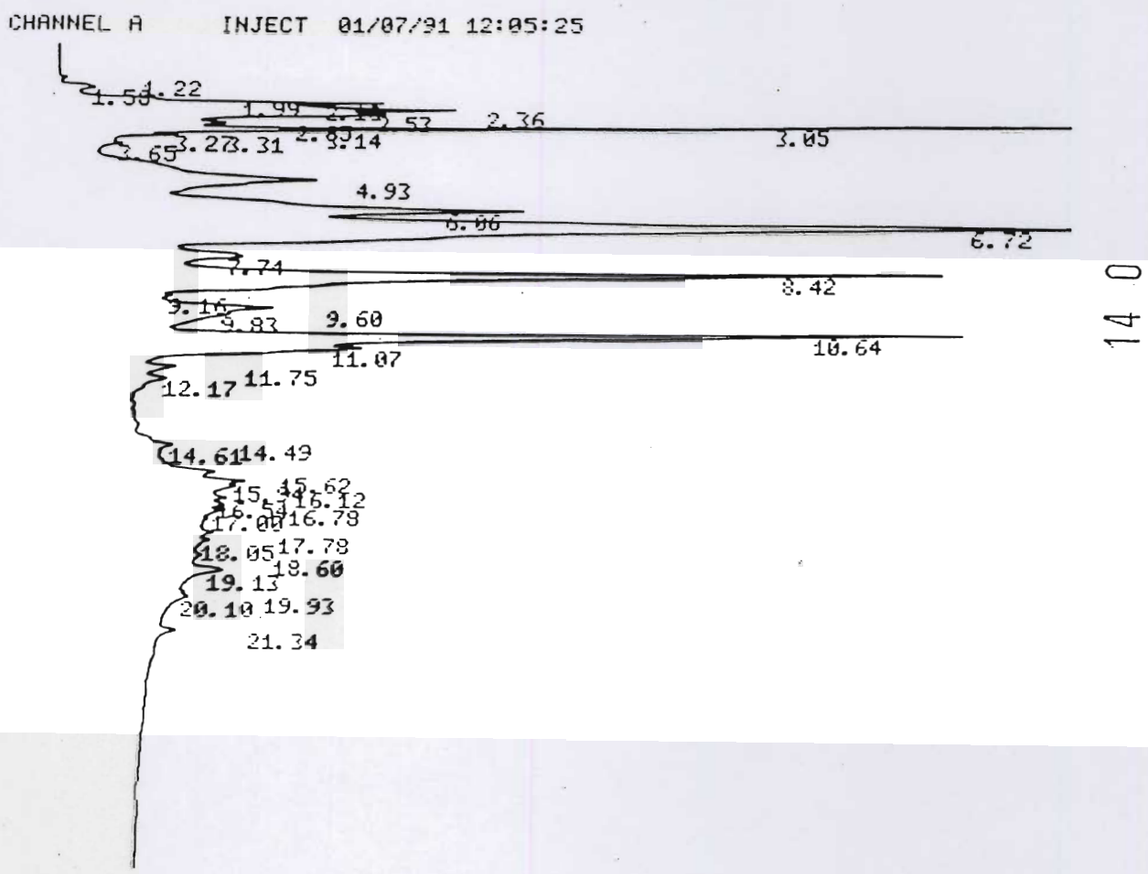
Sample #	Additive(%)	TBARS(ppm) after 6 days	9 days
1	Control(No additive)	2.72	3.23
2	Herbalox^{R} type O(0.02)	1.68	1.97
3	MeOH-AC(5%) fr. 1(0.10)	1.48	1.90
4	(0.30)	0.60	0.86
5	(0.50)	0.67	0.71
6	MeOH-AC(10%) fr. 1(0.10)	1.61	1.92
7	MeOH-AC(20%) fr. 1(0.10)	1.60	1.78
8	Hexane-MeOH(0.10)	1.59	1.87
9	Water/MeOH(50:50)(0.13)	1.05	1.56

SD = ±0.1 ppm

Table 5. HPLC Chromatographic Conditions for the Analisis of Wild Rice Extract

Chromatography	110B Solvent Delivery Module equipped with a Model 421A Controller and Model 210 sample injector (Bechman Instruments, Inc.)
Column	RP-C_{18}, 5 micron, 4mm i.d. X 25cm (IBM Instruments, Inc.)
Solvent gradient	A = water; B = Methanol t = 0-10 min., B = 0-30% t = 10-15 min., B = 30-60% t = 30-33 min., B = 60-0%
Sample	10 mg/ml in methanol
Injection volume	10 micron liter
Detector	Model 160 Absorbance Detector(Beckman Instruments, Inc.) λ = 254 nm Range = 0.1 Time constant = 0.5 sec.
Integrator	Model 427 integrator (Beckman Instruments, Inc.) Attenuation = 8 Chart speed = 0.5 cm/min. Peak threshold = 50

Fig. 4. HPLC Chromatogram of MeOH-AC(20%) fr. 1



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FILE 1. METHOD 0. RUN 26 INDEX 26

PEAK#	AREA%	RT	AREA	BC
1	0.021	1.22	402	01
2	0.264	1.58	4941	02
3	0.416	1.99	7788	02
4	1.638	2.1	30686	02
5	3.913	2.36	73306	02
6	2.973	2.53	55692	02
7	1.014	2.85	18995	02
8	4.21	3.05	78873	02
9	0.71	3.14	13298	02
10	0.13	3.27	2428	02
11	0.194	3.31	3639	03
12	0.027	3.65	509	01
13	6.071	4.93	113734	02
14	8.477	6.06	158808	02
15	25.119	6.72	470937	08
16	0.778	7.74	14575	06
17	16.375	8.42	306751	08
18	0.034	9.16	643	06
19	1.264	9.6	23676	06
20	0.828	9.83	15502	06
21	10.083	10.64	188880	02
22	3.509	11.07	65742	02
23	0.596	11.75	11170	02
24	0.581	12.17	10887	03
25	0.076	14.49	1426	02