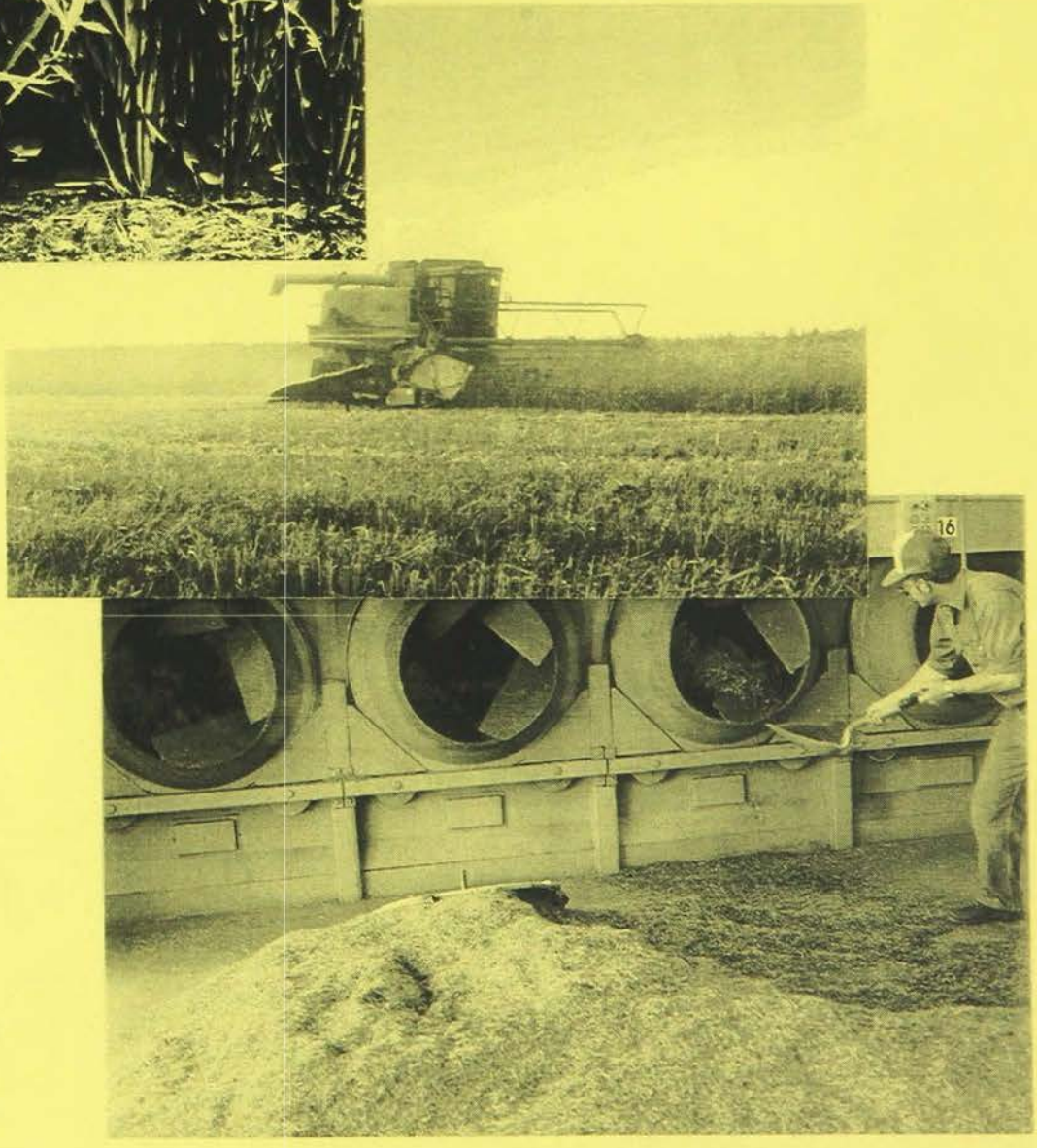
Minnesota Wild Rice Research - 1996



University of Minnesota

Agricultural Experiment Station

Extension Service

February 1997

Minnesota Wild Rice Research 1996

University of Minnesota
Agricultural Experiment Station
Extension Service

St. Paul, Minnesota

ACKNOWLEDGEMENTS

The wild rice team acknowledges the assistance provided by many people. Greatly appreciated was the cooperation of Dave Rabas, head of the North Central Experiment Station, Grand Rapids. We are also grateful for the daily supervision of the research plots and laborers at Grand Rapids by research plot coordinator Henry Schumer.

The wild rice team is also extremely grateful to the growers for providing seed, land area and facilities for research. For some of the research, funding from the Minnesota Cultivated Wild Rice Research and Promotion Council, Minnesota Department of Agriculture, USDA Farm Service Agency, Minnesota Sea Grant, Minnesota Crop Improvement Association, Franklin Kosbau Fund and USDA-North Central Biotechnical Program was very helpful.

We are also indebted to the Wild Rice Council for obtaining federal funds (USDA-ARS) for wild rice breeding and are grateful to USDA-ARS for providing the funds. We also appreciate the continued support of the Minnesota Agricultural Experiment Station for wild rice research.

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WILD RICE PRODUCTION RESEARCH - 1996

E.A. Oelke, D.G. LeGare and H.J. Schumer

It was generally cooler in 1996 compared to 1995 at all four locations. The average number of growing degree days over the 4 locations was fewer in 1996 by 212 (Tables 1 and 2). The season, April and May, started cool as it did in 1995 and continued cool through June which was much cooler than in 1995. July was also cooler in 1996 compared to 1995 at the four locations and so was August except at Waskish. At Waskish August was warmer in 1996 compared to 1995.

Compared to the long term average, the growing season at the locations of Aitkin, Grand Rapids and Crookston were cooler (Tables 1 and 2). The total seasonal growing degrees at Waskish was greater for 1996 compared to the average. At all four locations, April and May were cooler than the average. June was warmer at all four locations than the long term average. July 1996 was cooler than the long term average at Aitkin, Grand Rapids and Crookston but not at Waskish. August was warmer in 1996 at all four locations than the long term average.

Table 1. Growing degree days[a] comparisons for 1995, 1996, and normal (61-90).

	Aitkin			Grand Rapids		
Month	1995	1996	Normal	1995	1996	Normal
	GDD					
April	30	38	127	30	34	130
May	370	335	417	420	359	434
June	818	690	646	874	712	674
July	796	769	779	824	789	858
August	824	800	683	890	856	768
Total	2838	2632	2652	3038	2750	2864

[a] $\frac{\text{Maximum + minimum temp.}}{2}$ - 40°F; data from Mark Seeley, Department of Soil, Water and Climate, U of MN.

Table 2. Growing degree days[a] comparisons for 1995, 1996, and normal (61-90).

	Waskish			Crookston		
Month	1994	1995	Normal	1995	1996	Normal
	GDD					
April	10	20	103	35	33	151
May	326	300	369	394	393	488
June	810	652	518	907	803	743
July	764	714	642	852	828	926
August	794	818	563	904	883	867
Total	2704	2504	2195	3092	2940	3175

[a]$\frac{\text{Maximum + minimum temp.}}{2}$ - 40°F; data from Mark Seeley, Department of Soil, Water and Climate, U of MN.

Total precipitation for the growing season was less at all four locations in 1996 compared to 1995 (Tables 3 and 4). At Aitkin and Grand Rapids precipitation was less for all months of the 1996 growing season compared to 1995 except for June which was wetter (Table 3). At Waskish, April, May and June were wetter in 1996 than 1995 while July and August were drier (Table 4). At Crookston April and May were wetter in 1996 than in 1995 while the other 3 months were drier especially August (Table 4).

Compared to the long term precipitation averages over the growing season the totals were less in 1996 (Tables 3 and 4). At Aitkin all months in 1996 compared to the long term average were drier except July; this was also true for Waskish. At Grand Rapids, April, May and August were drier in 1996 while June and July were wetter than the long term average. At Crookston, April, June and August were drier in 1996 compared to the long term average while May and July were wetter.

The weather during 1996 was generally favorable for wild rice even though it started out cool. There were no severe storms or continuous rain during harvest resulting in good yields.

Table 3. Precipitation comparisons for 1995, 1996, and normal (61-90)[a].

	Aitkin			Grand Rapids		
Month	1995	1996	Normal	1995	1996	Normal
	inches					
April	1.93	1.37	2.30	1.46	1.81	2.10
May	3.07	1.69	2.88	2.55	1.43	3.04
June	1.42	3.15	4.09	1.53	5.17	4.11
July	6.28	5.54	4.14	8.55	6.13	3.89
August	3.18	1.21	3.83	6.25	1.29	3.59
Total	15.88	12.96	17.24	20.34	15.83	16.73

[a] Data from Mark Seeley, Department of Soil, Water, and Climate, U of MN.

Table 4. Precipitation comparisons for 1995, 1996, and normal (61-90)[a].

	Waskish			Crookston		
Month	1995	1996	Normal	1995	1996	Normal
	inches					
April	0.64	1.28	1.70	0.33	0.42	1.45
May	1.33	1.90	2.33	1.78	3.17	2.45
June	1.81	3.47	4.25	2.05	1.73	3.44
July	7.64	4.84	3.42	7.56	5.57	2.77
August	2.89	2.50	3.32	3.27	0.33	2.88
Total	14.31	13.99	15.02	14.99	11.22	12.99

[a] Data from Mark Seeley, Department of Soil, Water, and Climate, U of MN.

Total cultivated production in Minnesota was about 40% greater in 1996 compared to 1995 while in California it was about 18% greater (Table 5). The greater 1996 production in Minnesota compared to 1995 was partly due to good weather in 1996, particularly during harvest as compared to the storms that occurred during 1995. The total cultivated production for the two states was about 24% higher in 1996 compared to 1995.

Table 5. Minnesota and California paddy wild rice production[a] (1000 processed pounds).

Year	Production		Year	Production	
	Minnesota	California		Minnesota	California
1968	36	0	82	2697	880
69	160	0	83	3200	2500
70	364	0	84	3600	2500
71	608	0	85	4200	7900
72	1496	0	86	5100	9000
73	1200	0	87	4200	4200
74	1036	0	88	4000	3500
75	1233	0	89	3978	4000
76	1809	0	90	4800	4200
77	1031	0	91	5500	5500
78	1761	100	92	6100	7500
79	2155	200	93	5300	7500
80	2320	400	94	5300	5000
81	2274	500	95	4500	6440
			96[b]	6000	7600

[a] 1968-1982 Minnesota values from Winchell and Dahl and 1983-1995 from Minnesota Department of Agriculture; California values from Marcum, Cooperative Extension Service, University of California.
[b]Estimated value for 1996.

The estimated value of the Minnesota production was $9,00,000 which is more than it was for the previous 3 years (Table 6). The higher value in 1996 is due to the higher production compared to 1995.

Table 6. 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	4,800	1.70	8.16
1991	5,300	1.70	9.01
1992	6,100	1.70	10.37
1993	5,300	1.65	8.74
1994	5,300	1.65	8.74
1995	4,300	1.50	6.45
1996[a]	6,000	1.50	9.00

[a]Estimated values for 1996.

Research

Simulated Hail on Wild Rice

Introduction: The previous research on simulated hail was summarized in the 1995 Minnesota Wild Rice Research publication. Regression lines were developed to help determine yield losses based on percent leaf blade removal at the early stages and a combination of percent leaf blade removal and stem bending at the later growth stages. Even though there seemed to be a good relationship of percent leaf and stem damage and yield loss, there was enough variability in results that a similar trial was needed in 1996.

Materials and Methods: Wild rice, variety 'Franklin,' was planted with a cone planter on May 29, 1996, at the University of Minnesota, North Central Experiment Station. After planting, the paddy was immediately flooded to a depth of 6 inches. Individual plots consisted of 4 rows, 1 foot apart and 10 feet long with each treatment replicated 4 times. Before planting the plot area was fertilized on May 28, 1996 with 75 lbs/A of N and 40 lbs/A of K. The paddy was treated with one lb a.i./A malathion on June 19 to control midge. Stand establishment was poor partly due to low seed germination. Thus, the fourth replication was sacrificed and on July 2 and 3, wild rice seedlings from the fourth replication were transplanted into the center two rows of each plot in the remaining three replications. In addition, the first leaf blade removal date was omitted from the three replications since seedlings from this treatment were also used for transplanting. The intended plant population was 4 plants/ft^2.

To simulate hail damage, 33, 67 and 100% of each leaf blade in a plot was cut off with a scissors at six plant growth stages. Leaf blade tissue was removed at the aerial leaf, tillering, flowering, milk, soft dough, and 30% dark seed growth stages. The same percentages of stems were also bent to a 90 degree angle just below the panicle (not broken off) at the last 4 growth stages. Thus, at these four stages, the plots had both the leaves and stems injured. At these last four growth stages an additional set of plots was "beaten" until reaching approximately 50% leaf defoliation. A green willow branch with the leaves removed was used to "beat" the plants. There were 23 treatments including the control. The treatment dates for the six growth stages were: aerial leaf, 7-10; tillering, 7-26; flowering, 8-7; milk, 8-22; soft dough, 8-29; and 30% dark seed, 9-9. The treatments were made approximately every 2 weeks except between the milk and soft dough stages and the last one was made 3 days before harvest which was on 9-12.

Results and Discussion: Table 7 presents the results from the 1996 trial. Yield (dehulled grain) was not reduced significantly (5% level of significance) when leaf blade removal occurred at the aerial leaf stage of growth. However the yield trend was less as the percent of leaf blade removal increased. When leaf blade removal occurred at the tillering stage of growth, significant yield reduction was noted when 100% of the leaf blades were removed. Again, the trend was for lower yield as more leaf removal was done. At the flowering and milk growth stages, significant yield reductions occurred when a combination of leaf blade removal and stem bending was done at the 67 and 100% level but not at the 33% level. As in the previous growth stages the trend was for lower yield as the percentage of injury increased. At the soft dough growth stage only the 100% treatment significantly reduced yield but the trend again was that yield was reduced as percentage increased. At the 30% dark growth stage no statistically significant yield loss occurred when leaf blade removal and stem bending occurred, however the trend again was for lower yield as percent injury increased.

The beating of plants with a willow branch at the last 4 stages of growth resulted in statistically significant yield losses at all 4 stages of growth (Table 7). The attempt was to strip away about 50% of the leaves and to bend the stems. This was particularly true at the soft dough and 30% dark stages of growth, thus the low yields due to this treatment.

Figures 1a and 1b depict the regression lines for the 1996 dehulled grain yield in relation to percent leaf blade removal or a combination of percent leaf removal and stem bending. The combination was done only at the last 4 stages of growth. In figure 1a the lines slope downward more the later the treatments were made indicating injury at flowering is more detrimental than when injury is done at tillering and aerial stages of growth. In figure 1b the milk treatment line slopes the most indicating the plants are more susceptible to injury at this stage of growth compared to the soft dough and 30% dark stages of growth. This is to be expected since the seed has had more time to fill before injury occurred. As in previous trials, leaf blade removal and stem bending at the 30% dark stage of growth did not influence yield.

Table 7. Influence of removing 33, 67, and 100% of leaf blades on wild rice plants at 6 stages of growth plus 33, 67, and 100% of stems bent at last 4 growth stages. Plants in independent plots were beaten with a willow branch at the last 4 stages of growth. - Grand Rapids, MN -1996

Growth Stage	Leaf removal	Plant number	Stem number	Panicle number	Plant height	Straw dry weight	Green grain weight	Dehulled grain yield	Recov -ery	Hulls
	%	/ft^2	/ft^2	/plant	cm	lbs/A	lbs/A[b]	lbs/A	%	%
Aerial leaf	33	2.1	13.4	6.5	158	3990	2340	985	42.1	29.8
	67	2.0	9.4	5.2	148	3255	1650	658	41.0	31.7
	100	2.1	9.7	5.2	145	2663	1624	650	38.9	33.3
Tillering	33	2.0	11.0	6.1	140	3479	1726	708	41.4	31.6
	67	2.0	10.1	5.8	150	2531	1386	590	42.2	29.0
	100	1.8	7.9	4.9	127	1977	808	309	37.8	36.2
Flowering	33	2.2	10.0	5.8	145	2883	1630	657	40.3	32.8
	67	2.1	8.8	5.1	128	1865	1097	453	41.6	31.2
	100	1.6	6.9	5.5	105	1160	454	168	37.5	38.3
	Beat[c]	2.0	8.3	4.6	123	2051	925	369	39.3	33.5
Milk	33	2.1	9.5	5.6	153	3291	1477	610	41.4	31.2
	67	2.2	11.2	5.8	148	3047	1067	424	39.2	34.0
	100	2.3	8.3	3.5	110	2025	583	191	32.8	45.4
	Beat[c]	2.1	10.1	4.0	128	2981	722	275	41.1	36.5
Soft dough	33	2.1	9.8	5.5	145	3127	1652	678	41.0	31.6
	67	2.3	11.2	5.3	143	3121	1503	588	38.9	34.8
	100	2.3	8.9	4.0	113	1911	920	326	34.9	40.9
	Beat[c]	2.2	10.3	4.0	130	3639	795	320	39.6	32.9

Table 7. (continued)

Growth Stage	Leaf removal	Plant number	Stem number	Panicle number	Plant height	Straw dry weight	Grain weight	Dehulled grain yield	Recov -ery	Hulls
	%	/ft^2	/ft^2	/plant	cm	lbs/A	lbs/A[b]	lbs/A	%	%
Dark	33	2.3	11.6	6.5	157	3659	2654	1120	42.3	29.7
30%	67	2.0	10.6	6.1	145	2941	1981	839	42.4	29.4
	100	2.3	10.5	4.7	135	2749	1896	799	42.2	29.8
	Beat[c]	2.2	9.7	3.8	125	3101	554	228	40.1	31.4
Control	0	2.2	10.8	5.5	153	3265	1720	717	41.8	30.5
LSD (0.05)		0.5	2.9	1.5	21	1198	457	187	6.4	---
Means		2.1	9.9	5.2	137	2813	1353	551	40.0	---
C.V.		15.3	17.7	17.1	9.5	25.9	20.5	20.6	9.7	---

[a] Days after planting.
[b] Corrected to 40% moisture.
[c] Plants beat with a willow branch to a point of approximately 50% leaf defoliation.

Table 8. Influence on yield of removing 33, 67, and 100% of leaf blades on wild rice plants at 7 stages of growth plus 33, 67, and 100% of stems bent at last 4 growth stages. Plants in independent plots were beat with a willow branch at the last four stages of growth. Grand Rapids, 1980-81, 1994-96.

Growth Stage	Leaf removal	Grain yield at harvest						Grain yield reduction compared to control					
		1996	1995	1994	1981	1980	Ave.	1996	1995	1994	1981	1980	Ave.
	%	lbs/A[a]						%					
Floating leaf	33	---	3245	1700	848	1169	1740	---	16	(21)[b]	19	20	8
	67	---	3015	1611	712	1216	1638	---	22	(15)	32	17	14
	100	---	3592	1750	657	558	1639	---	7	(25)	37	62	20
Aerial leaf	33	2340	3399	1600	752	1216	1861	(36)	12	(14)	28	17	1
	67	1605	3411	1641	888	1423	1793	7	12	(17)	15	3	4
	100	1624	3829	1738	497	1335	1805	6	1	(24)	52	9	9
Tillering	33	1726	3386	1846	872	1415	1849	0	13	(32)	17	3	0
	67	1386	3597	1545	783	1508	1764	19	7	(10)	25	(3)	8
	100	808	2329	1159	648	867	1162	53	40	17	38	41	38
Flower 50%	33	1630	3461	1520	800	1482	1779	5	11	(9)	23	(1)	6
	67	1097	2788	1176	897	1116	1415	36	28	16	14	24	24
	100	454	928	420	272	288	472	74	76	70	74	80	75
	Beat[c]	925	2377	1093	--	--	1465	46	39	22	--	--	36
Milk	33	1477	3237	1117	640	1415	1577	14	16	20	39	3	19
	67	1067	2353	1090	672	994	1235	38	39	22	36	32	33
	100	583	1464	575	328	831	756	66	62	59	69	43	60
	Beat[c]	722	1506	695	--	--	974	58	61	50	--	--	57
Soft dough	33	1652	3025	1509	880	--	1766	4	22	(8)	16	--	8
	67	1503	2778	1216	912	--	1602	13	28	13	13	--	17
	100	920	2088	772	440	--	1055	47	46	45	58	--	49
	Beat[c]	795	1574	250	--	--	873	54	59	82	--	--	65

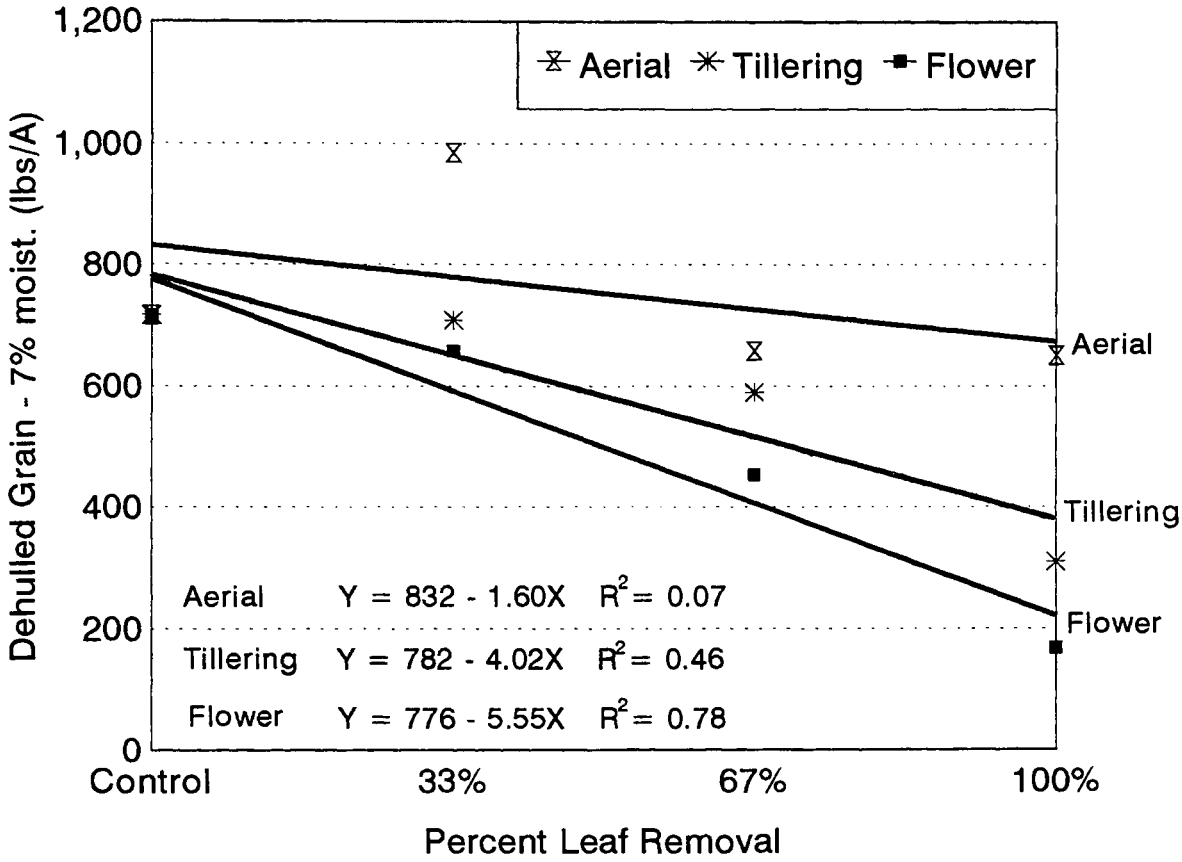

Figure 1a. The regression for percent leaf blade removal and dehulled grain yield per acre when leaf blade removal occurred at the aerial, tillering and flowering stages of growth. The regression line at the flowering stage includes leaf blade removal plus stem bending at this stage. 1996 data.

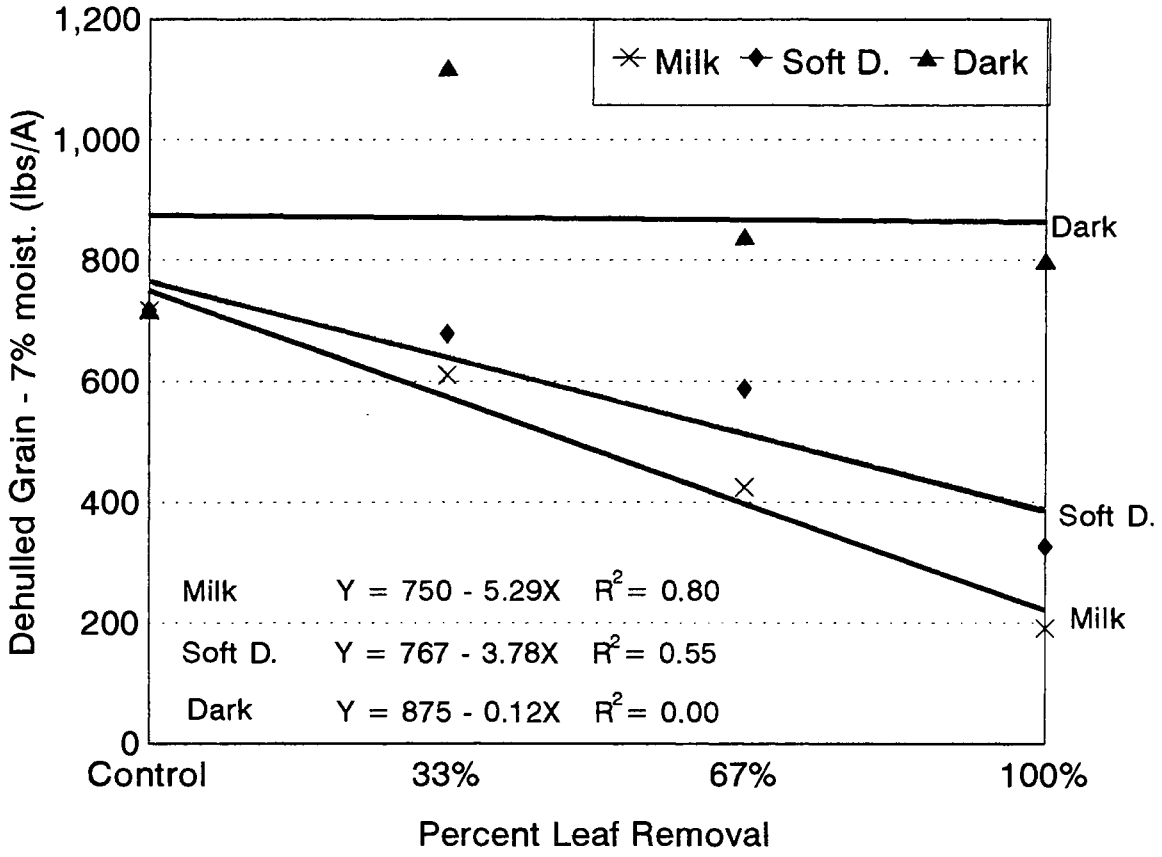

Figure 1b. The regression for percent leaf blade removal and stem bending and dehulled grain yield per acre when leaf blade removal and stem bending occurred at the milk, soft dough and 30% dark seed growth stages of wild rice in 1996.

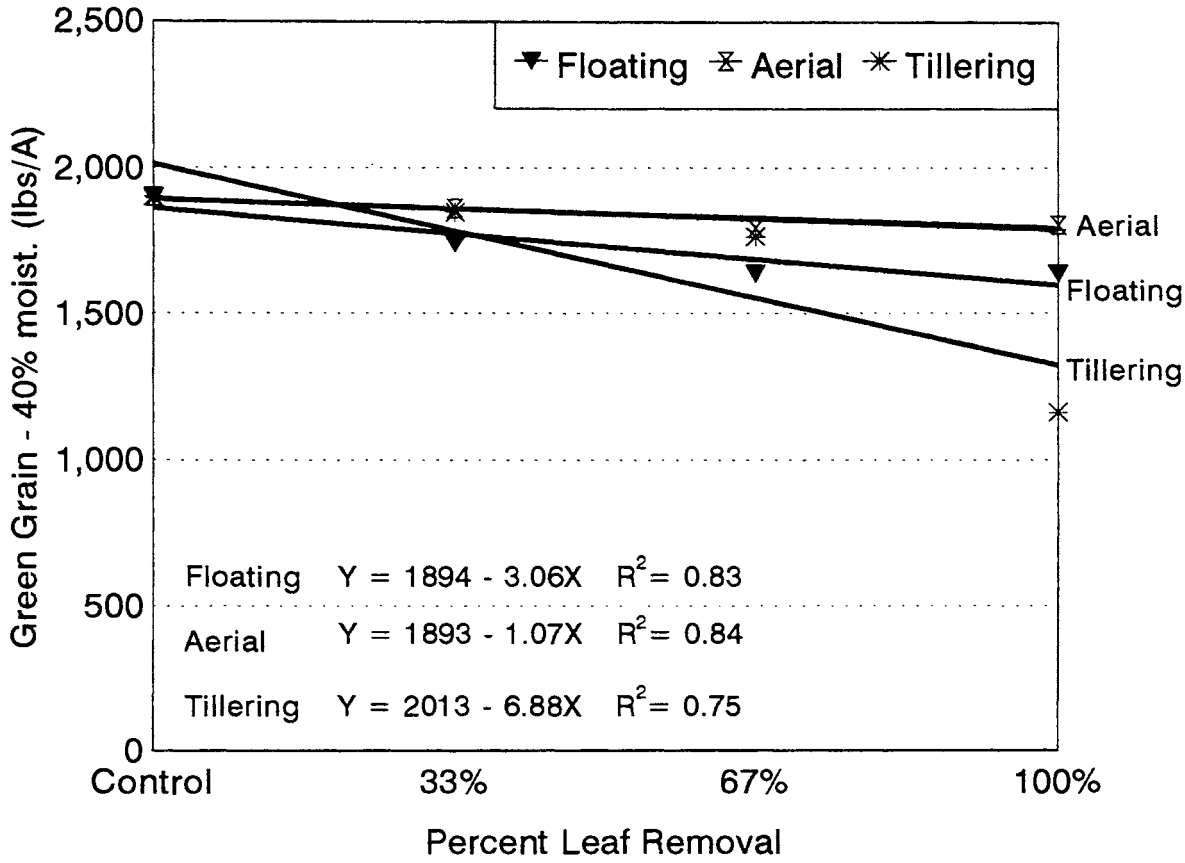

Figure 2a. The regression for percent leaf blade removal and harvested grain (40% moisture) yield per acre when leaf blade removal occurred at the floating leaf, aerial leaf, or tillering growth stages of wild rice. The regression was done on the averages for the years of 1980-81 and 1994-96.

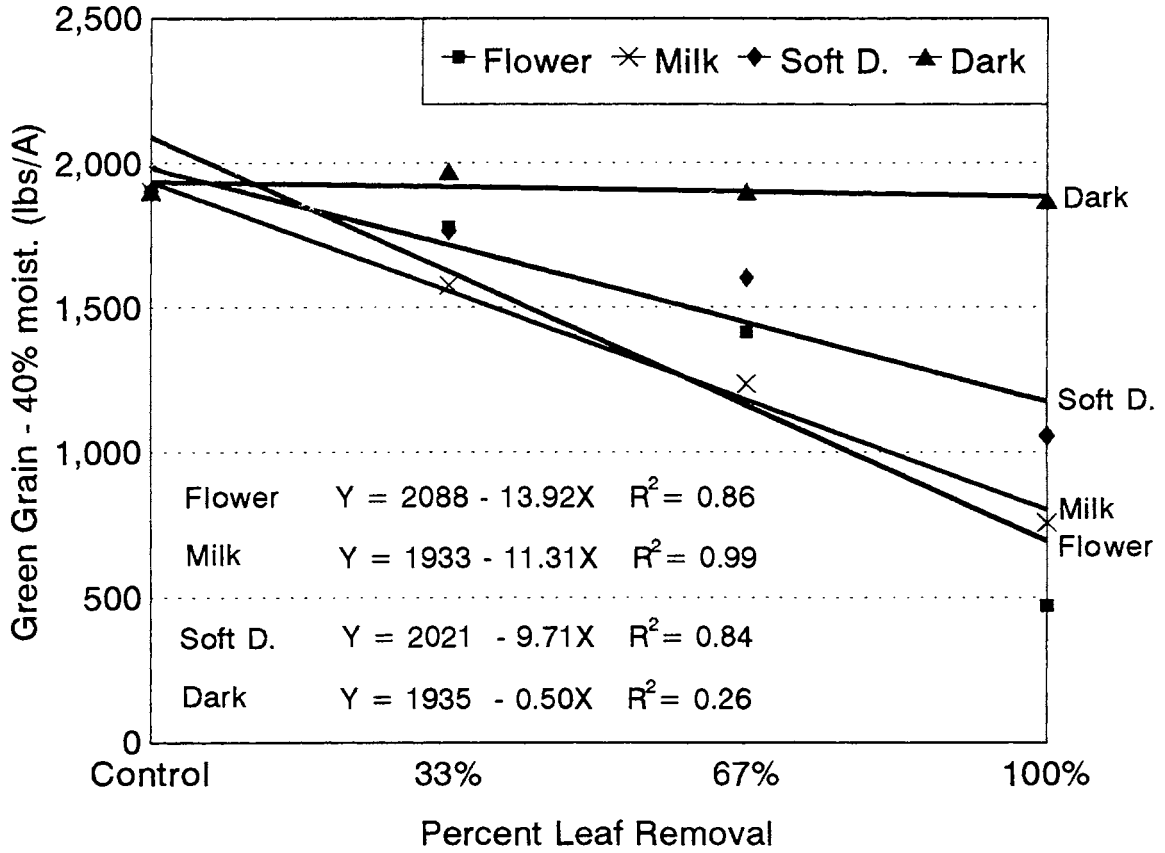

Figure 2b. The regression for percent leaf blade removal and stem bending and harvested grain (40% moisture) yield per acre when leaf blade removal and stem bending occurred at the flowering, milk, soft dough, and dark seed growth stages of wild rice. The regression was done on the averages for the years 1980-81 and 1994-96.

Table 8 summarizes the 1980-81 and 1994-96 yield data from the simulated hail treatments. The actual grain yields (40% moisture) and the percent yield reductions are given. The yield losses varied from year to year but when the average percent yield losses are calculated it is apparent that the most critical time to not have hail is during the flowering, milk and soft dough stages of growth. The highest yield loss of 75% was obtained when 100% of the leaf blades were removed plus 100% of the stems bent at flowering. Grain fill is just beginning at this stage and any injury reduces the production and movement of sugars needed from grain fill. Figure 2a and 2b show the regression lines for the average yield in relation to percent leaf blade removal and the combination of leaf blade removal and stem bending at the last 4 stages of growth. These regression equations can be used to develop yield loss charts for wild rice such as in Table 9.

Table 9. Estimated grain yield loss at various wild rice stages of growth due to plant injury from simulated hail research - 1980-81, 1994-95.

Plant growth stage	Percent defoliation									
	10	20	30	40	50	60	70	80	90	100
	Percent yield reduction									
Floating leaf[a]	1.6	3.2	4.9	6.5	8.1	9.7	11.3	12.9	14.6	16.2
Aerial leaf[a]	0.3	0.6	0.9	1.2	1.5	1.8	2.1	2.4	2.7	3.0
Tillering[a]	3.1	6.0	9.2	12.3	15.4	18.5	21.5	24.6	23.7	30.2
Flowering[b]	6.6	13.2	19.8	26.4	33.1	39.7	46.3	52.9	59.5	66.1
Milk[b]	7.5	11.4	17.2	22.9	28.6	34.3	40.0	45.3	51.5	57.2
Soft dough[b]	4.0	8.0	12.0	16.0	25.0	24.0	28.0	32.0	36.0	40.0
Dark seed[b]	0.2	0.4	0.6	1.3	1.1	1.0	1.4	1.6	1.8	2.0

[a] Leaf blade removal.
[b] Leaf blade removal plus stem bending.

In summary, it is very evident that leaf removal, combined with stem bending will result in yield losses at the critical flowering, milk and soft dough stages of growth. Leaf blade removal at the floating and aerial stages caused limited yield reductions as is true for early growth in other small grains.

Seed Storage

Introduction: Obtaining good wild rice seed germination can be difficult even after only 6 months of storage in cold (38°F) water. Previous seed storage experiments have shown that wild rice seed can be air dried at room temperature to about 20% moisture and then stored dry at 30°F for about 1 year. However, to release dormancy the seed still has to be stored for 3 months in cold (38°F) water. Based on these early experiments, we designed an experiment to see if storing seed even for a 6 to 7 month period could be dried and stored dry for 3 months and then placed in cold water for 3 months would maintain good germination.

Materials and Methods: Mature dark seeds of wild rice, variety 'Franklin', were hand harvested from a field on the Kosbau farm near Aitkin on August 27. The seed was transported the same day in a covered container to St. Paul. The containers with the seeds were put into a

cooler (38°F). Two days later only good plump seeds were sorted into lots of 50 seeds and each lot put into squares of mesh cloth and tied. Some seed lots at harvest moisture were placed into peat soil at field moisture capacity, flooded peat soil, water or dry. All lots were in sealed plastic freezer bags. These treatments were placed either in a freezer (28°F) or cooler (38°F). Four replicates were placed into one small insulated container and 4 into another. This was repeated making a total of 4 containers, 2 were placed into the 28°F larger freezer and 2 into the 38°F cooler. Two other treatments were imposed on the initial seed lots of 50 seeds. Some seed lots were dried at room air temperature (72°F) for 4 days to a seed moisture of about 20%. Some seed lots were dried to 20% moisture over a salt solution (75% relative humidity) which took 40 days at a temperature of 85°F. After the appropriate drying days, seed lots of these 2 drying treatments were also placed into field moisture capacity peat and at the two storage temperatures as described for the other earlier 4 treatments.

Rehydration of the dry seeds will be in water at 50°F for 30 days followed by 90 days in water at 38°F. The seeds initially stored in water or flooded peat will be placed at 38°F for 90 days to release dormancy. Gemination percentage of all seeds in all the treatments will be ascertained after the 90 days in water at 38° F which will be April, 1997.

Acknowledgment

The financial support from the Cultivated Wild Rice Research and Promotion Council and the National Crop Insurance Services for conducting the simulated hail study was greatly appreciated. The continued support for this research by the Minnesota Agricultural Experiment Station, the Department of Agronomy and Plant Genetics and the North Central Experiment Station is appreciated. Dr. Raymie Porter's and Henry Schumer's support at the North Central Experiment Station was very helpful as was the able assistance of Dan Brooten. The help of Heather Kroupa, undergraduate student, and Salina Amey, graduate student, was very valuable.

Wild Rice Fertility Research in Peat Soils, 1996

Paul R. Bloom and Deepa S. A. de Alwis[1]

In 1996 we completed a laboratory study of N mineralization rates in a flooded peat and a growth chamber study of N topdress efficiency. The N mineralization study has given us a much better understanding of the factors that control the rate and magnitude of organic N mineralization in wild rice paddies and of some of the factors that result in variation in the contribution of soil N mineralization to wild rice production. The topdress efficiency study demonstrated that under certain conditions topdress can be a very efficient way to delivery N to wild rice plants. This is contrary to our previous assumption that topdressing is not very efficient.

During the past year we continued to monitor the N and K status of soils in growers paddies. We also obtained plant tissue samples for nutrient analysis at the boot stage, a stage we have used in the past for determination of fertility status. We also continued our assessment of P in floodwaters in paddies to assess the potential for algal blooms and the potential for contribution of significant quantities of P to surface waters during drainage. This year we combined this with total soil P and soil test P analysis. These studies show that soil N in early June, before plant uptake, varies in a predictable manner according to the such factors as the quantity of fertilizer applied and the timeliness of fall flooding. Potassium is much less variable, and in most growers paddies, tends to range from slightly lower than recommended to very high. The floodwater P concentration in most paddies is low enough that algal blooms have no potential to form, but in some paddies the concentration is in the range where algal blooms are possible given a high enough water temperature. Paddy water P is not correlated in any simple way with total soil P or soil test P. The concentration of P in paddy water is not in the range that should be problematic for release to surface waters.

NITROGEN MINERALIZATION IN A FLOODED PEAT: EFFECT OF LIMING RATE AND FREEZING

In the past year we expanded our laboratory mineralization study to determine the effect of different rates of liming, and freezing on nitrogen mineralization rates in a flooded acid peat from the Aitkin area. We also fitted the data to an empirical formula which consists of two pools of mineralizable organic nitrogen and one pool for immobilization of ammonium. The experimental design was described in detail in our previous publication (Bloom and de Alwis, 1996) and will not be presented here.

The Mathematical Model

Modeling of nitrogen dynamics in cropping systems has attracted much interest in recent years for many upland crops and rice (*Oryza sativa*). We utilized the data presented in our 1996 report regarding mineralization of nitrogen in a wild rice/fallow rotation, and fitted it to a simple equation. The first order empirical formula used in the model is a modified version of the equation used by Michael Meyer in his M.S. thesis (1985).

[1] Professor and graduate research assistant. Department of Soil, Water and Climate, University of Minnesota, St. Paul, Minnesota.

$$NH_4 - N_{net} = N_1(1-e^{-K_1 t}) + N_2(1-e^{-K_2 t}) - N_3(1-e^{-K_3 t})$$

N_{net} = Net nitrogen mineralized (mg NH4-N kg^{-1} of oven dry soil)
t = Time (days)
N_1 = Total N in rapidly mineralized pool. (mg NH_4-N kg^{-1} of oven dry soil)
N_2 = Total N in slowly mineralized pool
N_3 = Total N in immobilization pool
K_1 = Rate coefficient for rapidly mineralized N pool (mg NH_4^+-N day^{-1})
K_2 = Rate coefficient for slowly mineralized N pool
K_3 = Rate coefficient for immobilization

The N_1 and N_2 constants represent the size of pools of organic N that can be mineralized at rates, K_1 and K_2 respectively. The lines for rapidly mineralized and slowly mineralized N in Figure 1 show the rates of release of N for a flooded peat at 54 °F. The N_3 pool is the potential amount of nitrogen that can be immobilized by soil microbes. The N_1 pool is completely mineralized by 45 days. The N_2 pool is slower to mineralize but is a much larger pool, (Table 1). As nitrogen is mineralized from the N_1 and N_2 pools, a parallel but opposite process occurs with immobilization. The combined effect of these opposing processes is the net mineralization, which follows the experimental data closely (Figure 1).

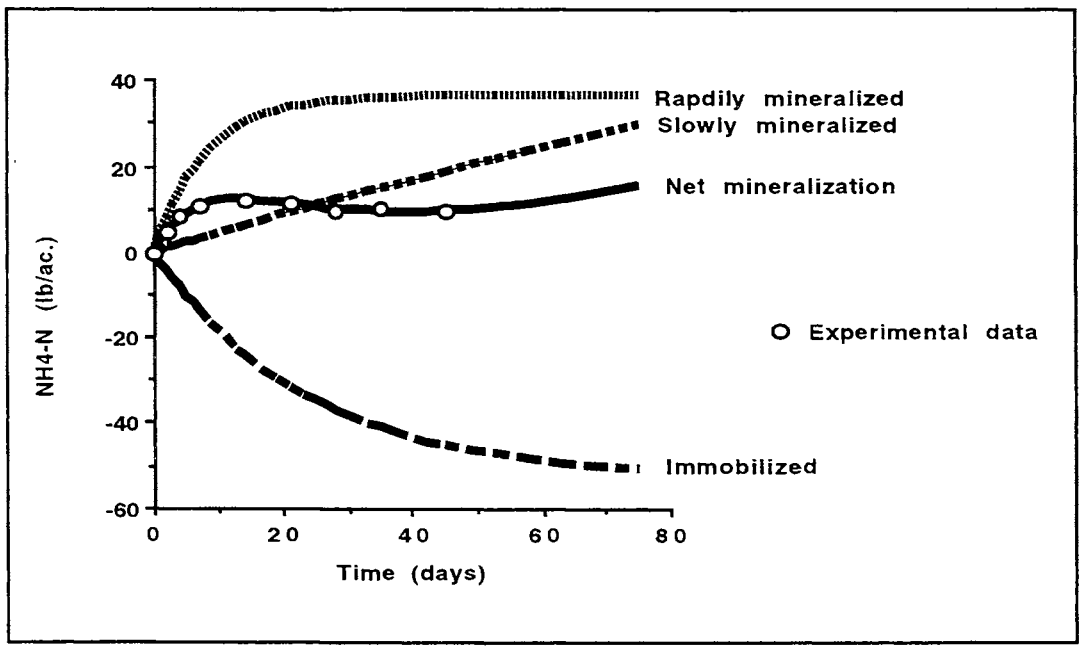

Figure 1. The contribution of the three N pools to the net nitrogen mineralization in a flooded peat as calculated. Black fallow treatment without liming at 54 °F.

Table 1. The mineralizable N pools and immobilization potential for the fallowed and wild rice straw incorporated peats.

Treatment	N_1 (lb/ac) Rapidly mineralized	N_2 (lb/ac) Slowly mineralized	N_3 (lb/ac) Immobilized
Black fallow	36	132	53
With wild rice straw	53	182	68

The data were analyzed using a mathematical software program that determines rate constants (K values) which provide the best fit. The K values increased with temperature showing that the rates increase with temperature. Furthermore, the size of three nitrogen pools (Table 1) were similar to the values obtained by Meyer using ^{15}N techniques for a similar peat soil.

Effect of Rate of Liming

This experiment was conducted to determine the effect of different rates of liming on the rate of nitrogen mineralization. In the previous study we had used only one high rate, 4.8 tons/ac. Different rates of lime from zero to 4.8 tons/ac were added to 5.0 g dry weight equivalent of peat from the wild rice treatment, in triplicate. The peat was placed in glass tubes, capped tightly and incubated in a dark incubator at 75 °F for 42 days. The most effective increment of lime was 1.2 tons/ac (Figure 2). These data suggest that for acid peats liming may be an effective way of increasing the plant availability of organic N.

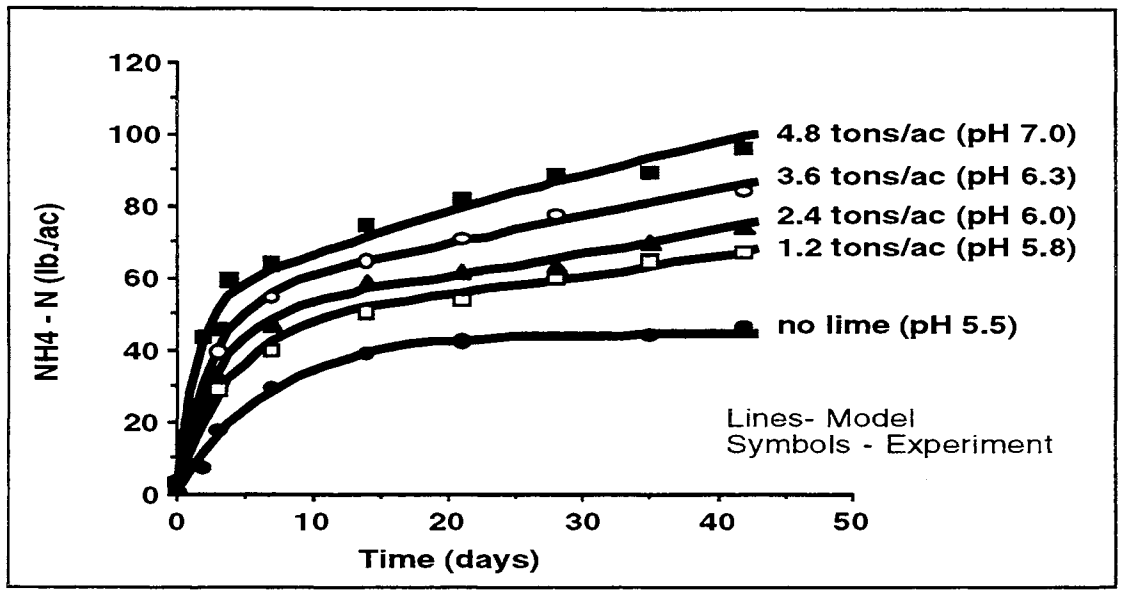

Figure 2. Effect of liming rate on nitrogen mineralization in a flooded acid peat with wild rice straw incorporation

Effect of Freezing on Nitrogen Mineralization.

Environmental factors such as drying, heating and freezing can effect the availability of organic nitrogen in soil. In all the experiments conducted earlier, the peat was frozen for several weeks following 8 weeks of aerobic incubation. Freezing breaks cell membranes of the soil microbes and releases cell materials which are rich in nitrogen, and are available for mineralization. Upon thawing and flooding the soil, dead microbes form a readily available carbon and nitrogen pool, (N_1) that can be mineralized rapidly.

We conducted an experiment to determine the effect of freezing on the mineralization rate. The temperature used in this experiment was 70 °F during day and 64 °F during night, but the soil was kept in dark to prevent algae from growing. These were the conditions used for the topdress nitrogen experiment discussed below. The soil used was obtained from a pH 6.0 paddy on the Godward farm near Aitkin in late September after harvest and tillage. The plant matter was incorporated into the soil but not nearly as well in previously discussed laboratory experiments. The mineralization data were fitted to the same mathematical formula described earlier (Figure 3).

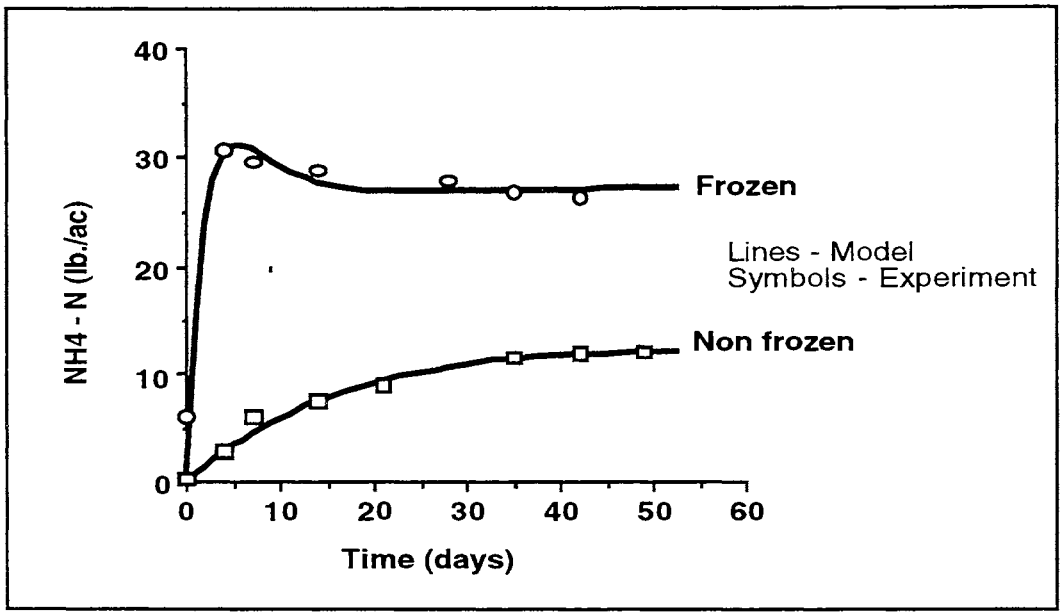

Figure 3. Effect of freezing on the mineralization of nitrogen.

The results we obtained for both the frozen and non frozen samples were somewhat different from our earlier experiments. This is likely due to the differences in handling of the two soils. In the previous experiment the straw was chopped to small pieces (0.25 - 0 .5 in) and incubated aerobically for 8 weeks in a darkened room. The plant matter was not incorporated as well in the field and the aerobic incubation period was shorter. Freezing increased the rapid initial release of ammonium N (Figure 3) which results from a much larger N_1 pool after freezing (Table 2). This is consistent with our hypothesis that N_1 pool largely consists of cell matter released by freezing.

Table 2. Nitrogen pools determined after flooding a pH 6.0 peat with and without freezing.

Treatment	N_1 (lb/ac) Rapidly mineralized	N_2 (lb/ac) Slowly mineralized	N_3 (lb/ac) Immobilized
Frozen	85	130	60
Non frozen	20	200	60

EFFICIENCY OF TOPDRESS NITROGEN IN WILD RICE

Nitrogen topdressing is a widely used practice among wild rice growers in Minnesota. Two or three topdressings of 30-50 lb N/ac are recommended during boot to flower stages, depending on the soil test values earlier in the season. Although the growers have experienced yield enhancements with these topdress treatments, there had been no experiments conducted to determine the efficiency of topdressing. The current experiment was aimed at determining the efficiency of topdress nitrogen, and the rate at which ammonium is removed from the floodwater after topdress.

Experimental Design

A pH 6.0 peat was collected from Godward farm near Aitkin in late September 1996. Peat was packed eight inches deep in five gallon buckets (bulk density 0.25 g cm^{-3}). Nitrogen and potassium were added at 40 lb/ac each. Phosphorus was not added because soil test results showed that there was adequate phosphorus in the soil. The fertilizer was added to the soil before packing the soil in the buckets to ensure uniform mixing.

Suction samplers were inserted into the buckets from the side walls at 0.75 and 4 inch depths and glued in place. The buckets were moved to a growth chamber and flooded. Six days after flooding wild rice seedlings were transplanted at a rate of 6 per bucket and thinned to 3 per bucket at 10 days after transplant (10 DAT). Growth chamber conditions (Table 3) were maintained to closely follow the conditions in Minnesota during late June. Floodwater and soil water at 0.75 and 4 in. was monitored weekly.

Table 3. Growth chamber conditions for ^{15}N topdress experiment

Day length	18 hours
Temperature - day	70 °F
night	63 °F
Humidity	95 %
Light intensity	250-280 µmol m^{-2} s^{-1}

When the soil water ammonium (obtained from the suction samplers) was less than 1 ppm, topdressing with ^{15}N enriched ammonium chloride (NH_4Cl) was initiated according to the experimental design shown in Table 4. The heavier isotope, ^{15}N is a non radioactive isotope of nitrogen, representing about 0.36% of nitrogen on earth. Fertilizers enriched in ^{15}N are routinely used in research to determine the fate of applied nitrogen. By using ammonium chloride with 5% ^{15}N, we were able to calculate the efficiency of topdress nitrogen. Growers use urea as the topdress nitrogen source, which is quickly transformed into ammonium by soil microbes.

The depletion of ammonium in soil to 1 ppm occurred at 33 DAT, at the early boot stage. The ^{15}N enriched NH_4Cl was dissolved in water and added to the floodwater and mixed carefully. After topdressing, floodwater and 0.75 in depth soil water from suction samplers were obtained and analyzed for ammonium every day.

Table 4. Treatments applied to wild rice 33 days after transplanting

Initial	Harvested at 33 DAT
Control	No nitrogen added
Topdress 1	40 lb/ac ^{15}N enriched NH_4Cl
Topdress 2	80 lb/ac ^{15}N enriched NH_4Cl

The initial treatment was harvested at the time of topdress to determine the nutritional status of the plants and the biomass at the time of topdress. The control treatment was used to assess the benefits of topdressing. The recommended single topdress is about 40 lb/ac, and we used this as one of the treatments. We doubled the dosage in the second topdress treatment to determine the capability of the plant to take up large quantity of nitrogen in a short period of time. If plants are able to take up 80 lb/ac N, then the growers will be able save the time and money spent on applying a second topdress shortly after the first during reproductive growth stages.

Chlorophyll readings were taken with a SPAD meter every day after topdress until harvest. The plants were harvested when the ammonium concentration in floodwater was less than 5 ppm, which occurred nine days after topdress (early flower stage). Shoots and roots from each of the buckets were harvested separately and dried at 140 °F in an oven for a week and weighed. The

plant matter was then ground and analyzed for total N, ^{15}N and other macro and micro nutrients. The macro and micro nutrients in the shoots were within the range typical for healthy wild rice.

Results

Floodwater ammonium was less than 1 ppm throughout the period before topdress application (Figure 4). Ammonium concentration at 4 in. declined slowly until 21 DAT, from 12 ppm to 8.5 ppm over 21 days. There was a sharp decline between 21 and 28 DAT, a drop of 7.5 ppm in 7 days (Figure 4). This corresponds to panicle initiation/early boot stage in plants, when nitrogen demand is very high due to rapid plant growth. The ammonium concentration was near zero at 33 DAT, when topdress N was applied. This was similar to what Bill Zanner observed in his suction samplers in wild rice paddies. (Zanner, M. S. thesis 1992)

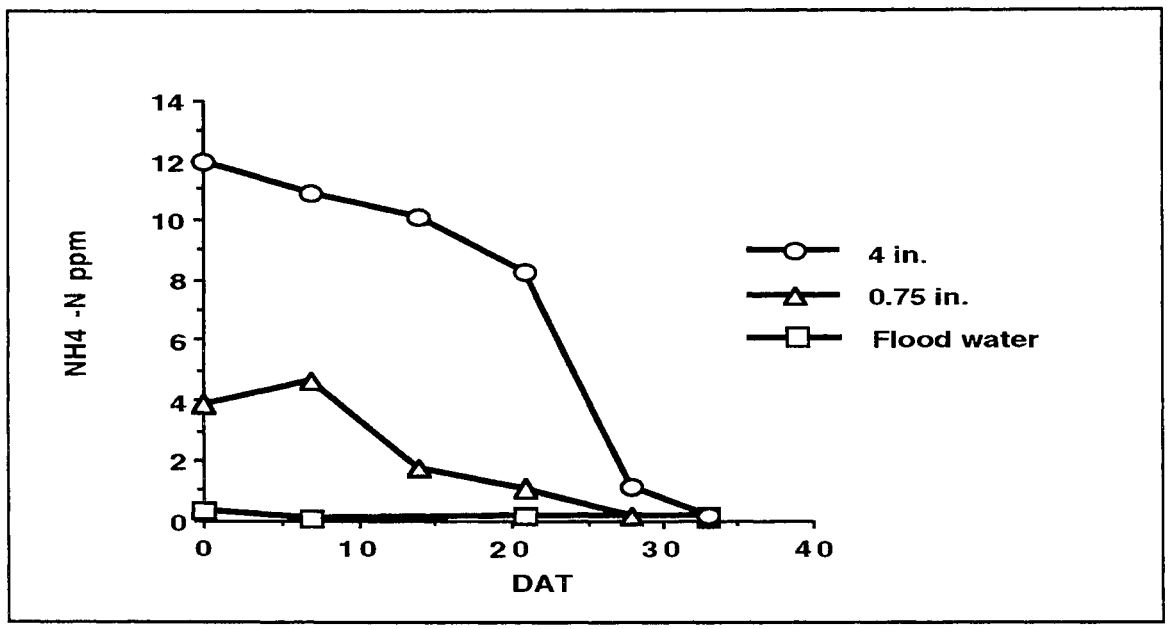

Figure 4. Soil water ammonium obtained from suction samplers and floodwater ammonium after transplanting, in the period before topdressing.

Floodwater ammonium decreased rapidly after topdressing (Figure 5). The plants were able to remove floodwater ammonium within nine days of topdress because they were growing very rapidly. The plants were N deficient at the time of topdress having lower N content than typically seen on growers fields (Table 5) at this growth stage. Because of the deficiency and the rapid growth at this stage, the plants were able to remove floodwater ammonium within nine days of topdress. The beneficial effects of the topdress is quite evident from the differences in SPAD readings and the increase in tissue N content (Figure 6 and Table 5). The control treatment plants were severely deficient at the end of the experiment and lower leaves were dying.

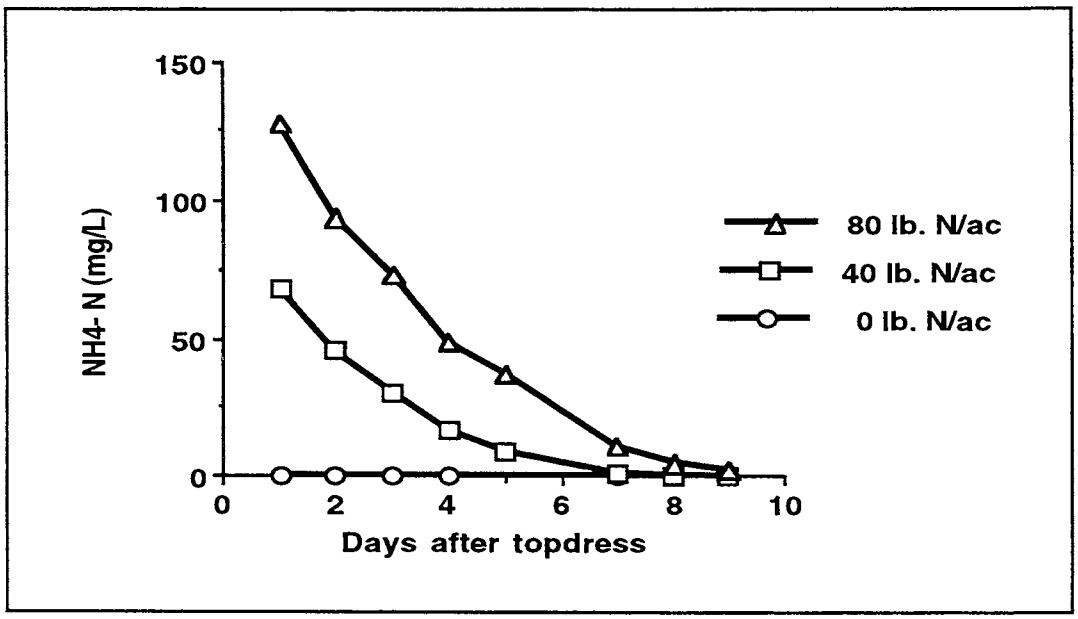

Figure 5. Ammonium nitrogen in floodwater after topdress.

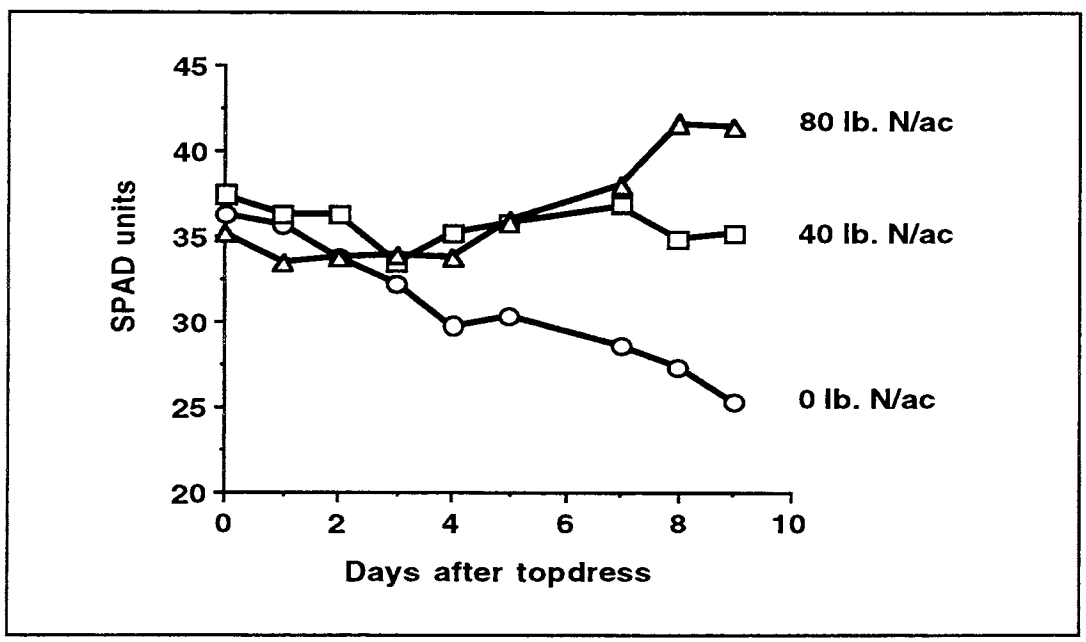

Figure 6. SPAD reading after topdress

The 80 lb/ac topdress treatment contained a high concentration of N for the early flower growth stage (Tables 5 and 6). The biomass for the shoots was in the range typical for this growth stage (Table 6) and represents more than two thirds of the 6000 lb/ac of biomass typical for high yielding wild rice at maturity. The quantity of N taken up in the shoots, 94 lb/ac is sufficient for a good yield. At this stage the plants may have sufficient nitrogen to utilize for grain fill.

Table 5. Nitrogen in wild rice plants following topdress at 33 days after transplant.

Treatment		Shoot N %	Root N %	Shoot N from topdress %	Root N from topdress %
Initial	33 DAT	1.24	1.14	0	0
Control	42 DAT	1.03	1.33	0	0
40 lb N/ac	42 DAT	1.53	1.61	36	14
80 lb N/ac	42 DAT	2.32	1.65	55	26

Table 6. Biomass and nitrogen in wild rice following topdress at 33 days after transplant (DAT).

Treatment	Shoot biomass	Root biomass	Shoot N lb/ac	Root N lb/ac	Total N lb/ac	N from soil* lb/ac
Initial 33 DAT	2000	1000	34	14	47	7
Control 42 DAT	3500	1800	36	24	60	20
40 lb N/ac 42 DAT	3900	2600	59	50	109	32
80 lb N/ac 42 DAT	4200	2200	94	34	129	37

* Assumption: no basal N loss.

Efficiency of topdress nitrogen was high, (Table 7). High calculated efficiency was partly due the fact that we considered root N as well as shoot N. Topdressed roots had an average of 1.6% nitrogen (Table 5), and the biomass of roots can be quite high (Table 6). The quantity of N in the roots accounted for more than 1/4 of the plant N and this must be considered in determining nitrogen needs of wild rice.

Table 7. Efficiency of topdress nitrogen in wild rice following topdress at 33 days after transplanting. Each replicated is shown separately to illustrate experimental variability. The third replicate of the 80 lb/ac topdress was unusable.

Treatment	**Replicate**	**Topdress efficiency %**
40 lb N/ac	1	65
	2	70
	3	68
80 lb N/ac	1	79
	2	78

The results of this experiment show that if the soil nitrogen is depleted at early boot stage or earlier, plants are able to take up high levels of topdressed nitrogen, with high efficiency rates (Table 6). In this experiment the mineralization of soil organic nitrogen is somewhat larger than predicted for the same soil in the freezing experiment. The soil used in this experiment was not frozen, and therefore we only expected about 15 lb N/ac from the soil but, our calculations show that the soil contribution was about 23 lb N/ac. This amount however, is not large compared to the plant requirements, which can be greater than 130 lb/ac.

A very large concentration of roots on the surface of the soil was observed after topdress, and some were floating in the floodwater. The root biomass increased by 2.2 and 2.6 times

compared to the initial biomass at 33 DAT (Table 6), and most of this increase appears to be on the top 1-2 inches of the soil. Because of this large concentration of roots on the surface of the soil, little of the topdressed nitrogen was detected by the suction samples at 0.75 in.

SOIL AND PLANT N, P AND K IN GROWERS' FIELDS

This year we continued to monitor extractable soil N and K using the 1 M NaCl extraction method we developed last year which allows us to sample wet soils for K and N using one extraction. Other researchers have shown that in mineral soils NaCl is not quite as good as NH_4Cl for the extraction of K but in organic soils K is not strongly retained and NaCl easily extracts K. The N data obtained for soils on June 19, before the plants are large enough to take up much N, show that the N available from fall fertilization is greatly influenced by whether or not paddies were flooded soon after N fertilization (Table 8). The results for fall flooding are inconsistent on the Clearwater farm because it is difficult to get timely flooding on all of the fields (Table 8). Some of the Clearwater paddies are below the recommended 40 lb/ac of extractable N.

Because of wet conditions during the fall of 1995 growers had difficulty applying N to some paddies. Where this was the case, the June soil N was < 20 lb/ac, even where Godward and Rennemo applied N over the ice in the spring. The one exception was the Imle-Gunvalson 8-1 paddy which was so wet the fall that no tillage was possible and this paddy can be considered to have been flooded in the fall. In June this paddy soil became gassy and the paddy had algae. Even without addition of fall N, fall flooding appears to be effective in producing > 20 lb/ac N, probably from natural mineralization. In the Imle Gunvalson paddy 9-5W where only 12 lb/ac of N was added as ammonium phosphate 27 lb/ac were found in June and the very wet paddy, 8-1, the extractable N in June was 32 lb/ac.

Although the wet soil sampling method for paddy soils is not recommended for mineral soils where N is injected or banded we did sample a mineral soil that was injected with anhydrous N. We sampled this soil because of the unusual application method that was used. The NH_3 was injected into wet soil. Under these conditions nitrification will not occur and loses during injection are prevented. Our sampling found a little more than the 100 lb/ac injected (Table 8). This method of injection appears to be a good way to apply N fertilizer to mineral soil.

In addition to our previous statements about the importance of timely fall flooding we can now say that application of N over ice in the spring is not an effective method of N fertilization. The data also suggest that under some conditions it may be possible to have about 30 lb/ac extractable N in soils after fall flooding, without addition of fertilizer, and that this N can contribute to crop production in the following season.

Table 8. Extractable ammonium N and K in wild rice paddies on growers' fields in mid June 1996.

Farm and field	Nitrogen	Potassium	Comments on management FF=fall flood, SF=spring flood
Imle Gunvalson 8-1	32	180	very wet in fall, no fertilizer
Imle Gunvalson 9-5 W	62	186	12-40-80 plus 70 anhydrous N, FF
Imle Gunvalson 9-5 E	27	235	12-40-80, no anhydrous, FF
Clearwater 6-W	69	192	65-35-80 after potatoes, FF
Clearwater 2-E	45	393	50-35-80 3rd yr, FF
Clearwater 7-E	18	297	50-35-80 4th yr, FF
Clearwater 13-E	22	191	50-35-80, FF
Rennemo D-6	51	171	76 N, 90 K, FF
Rennemo D-8	7	113	no fertilizer N, SF
Rennemo D-11	34	112	60 N, 90 K, FF
Rennemo D-18	12	192	0 N in fall, 40 in the spring, 90K, SF
Rennemo D-18	18	155	55 N fall and 40 over the ice, 90 K, FF
Godward H-6	18	378	no fertilizer N, 150 K,SF
Godward T-6	19	590	70 N, 150 K over the ice, SF
Godward T49	40	327	70 N, plowed down 150 K, FF
Mhos	127	177	Mineral soil, fall injection of 100 lb/ac anhydrous in wet soil.

Of the 16 paddies sampled in mid June for K, all but 2 had greater than 180 lb/ac (Table 8). Thus, most of the soils had concentrations that were close to or greater than the minimum value of 200 we previously suggested. The highest value measured was 590 lb/ac, much in excess of that required by wild rice. Perhaps this is the result of a history of high K fertilization. The mineral soil on the Mhos farm contained 177 lb/ac K which should be more than needed by wild rice. Mineral soil contains quantities of K bound by clay that are plant available, but would not be extracted by NaCl.

Plant samples were obtained on July 8 for tissue analysis. The plants were generally in the early boot stage, a stage used previous for comparison of tissue nutrient status. Of the nutrients analyzed, N was one of the most variable, with values ranging 1.77 t o 3.85 % (Table 9). At the time of sampling all of the paddies had been topdressed at least once which influences the tissue N content. Another influence is slight differences in growth stage. More advanced plants have greater content of low N stems and hence have lower N concentrations. The growth at Aitkin, site of the Godward farm, is generally behind that for the other farms. Many of the samples had N contents less than the limit 3.3 % for deficiency at boot we suggested in the 1991 report. One interesting observation made by Imle and Gunvalson is that the when fall fertilized and non fertilized portions of field SS-5 were compared, the side without fall N had shorter plants but less lodging and better yield. This is correlated with lower N on the side without fall fertilizer, even after 2 top dressings of 40 lb/ac N (Table 9). This suggests that there can be an advantage in having the plants a bit deficient at the boot stage when the stems are beginning to elongate. High N inputs at later growth stages should not cause the stems to elongate excessively. Perhaps a concentration of 2.75 to 3.00 % N at early boot might be better than a 3.3% limit.

Tissue concentrations of K were all in excess of the 2.5% deficiency limit that we reported in the 1993 report (Table 9). In only one paddy was the concentration even as low as 3.0%. The concentrations also were higher than we observed in 1992 . Most growers apply K well in excess of that needed for plant growth because high K is thought to provide some protection against some diseases.

Plant P ranged from 0.47 to 0.67 %, much in excess of the deficiency limit 0.25 % suggested in the 1993 report. High P is typical in wild rice, and indicates that, in general, wild rice paddies are over fertilized with P.

The concentrations of all the other nutrient elements except zinc and copper were within range of that is considered normal for wild rice. The value of 10.8 ppm for Zn in the Imle Gunvalson paddy 9-5 W is one of the lowest we have ever measured. Our recent hydroponic plant growth data (see below) suggests that this may be a concentration associated with reduced yield. More work is need to verify this suggestion. A copper concentration of 2.5 ppm, in the same paddy, is not the lowest value we have measured but comparison with other small grains including white rice suggests it is low enough to warrant further study.

Table 9. Plant N and K and readily available soil N and K in wild rice plants sampled from paddies at boot stage (about July 8)

Farm and Field	Plant N %	Plant K %	Soil N lb/ac	Soil K lb/ac	Comments on management
Imle Gunvalson 9-5 W	3.35	4.67	14	177	Fall N, 2-40 lb/ac topdresses
Imle Gunvalson 9-5 E	3.02	4.32	61	172	No fall N, 2-40 lb/ac topdresses
Imle Gunvalson SS-5 E	3.35	4.98	32	120	Fall N, 2-40 lb/ac topdresses
Imle Gunvalson SS-5 W	2.89	4.67	7	39	No fall N, 2-40 lb/ac topdresses
Q-8	2.36	4.76	8	124	
Clearwater 6W	1.96	3.91	----	----	65-35-80 after potatoes, FF
Clear water 2W or E??	2.55	4.00	----	----	
Godward H-6	3.66	4.49	----	----	no fertilizer N, 150 K,SF
Godward T-6	3.85	3.42	----	----	70 N, 150 K over the ice, SF
Godward T49	---	----	----	----	70 N, plowed down 150 K, FF
Rennemo D-11	1.77	3.04	2	37	N-K brown spot study area

Table 10. Mineral nutrient concentrations in wild rice plants sampled at boot stage from growers' paddies (about July 8). All concentrations are ppm except for P.

Farm and Field	P,%	Ca	Mg	Fe	Mn	B	Zn	Cu
Imle Gunvalson 9-5 W	0.67	3198	1555	114	161	8.1	10.8	2.5
Imle Gunvalson 9-5 E	0.61.	3191	1679	99	90	10.1	13.0	2.5
Imle Gunvalson SS-5 E	0.72	3550	1702	84	84	9.2	17.2	4.1
Imle Gunvalson SS-5 W	0.64	3626	1563	73	86	8.1	25.2	3.9
Q-8	0.54	3733	1388	71	91	7.9	26.9	3.7
Clearwater 6W	0.53	3632	1217	92	150	7.0	28.9	2.6
Clear water 2E	0.49	3944	1251	78	130	7.3	26.8	3.5
Godward H-6	0.55	4285	1170	126	155	9.1	53.6	6.5
Godward T-6	0.54	5273	1454	291	144	12.7	49.7	7.0
Rennemo D-11	0.47	4620	1601	165	184	7.6	46.6	3.2

SOIL WATER AND FLOODWATR PHOSPHORUS

Samples were taken on June 19 for soil and floodwater P analysis. A few floodwater samples were taken on July 23. Two types of floodwater analyses were conducted: 1) total P, which is done after an acid digestion of the non filtered floodwater and includes the P in suspended solids, and 2) molybdate reactive P, which is the P in water filtered through a membrane filter that reacts with analytical solution. The total P is the quantity of P that concerns regulators with respect to the quantity of P transported into surface waters during discharge of water to lakes and streams. The molybdate reactive P is the fraction of P readily available for the growth of algae. Of the 16 paddies sampled in June, 5 had reactive floodwater P greater the 0.05 ppm limit necessary for algae production (Table 11). This means that with sufficient warming of the paddy water an algal bloom could occur. The only mineral soil sampled had a reactive P of only 0.01 ppm (see Mhos farm, Table 11). This a value we expected for a mineral soil because mineral soils have a high capacity to bind P and unless fertilizer P is applied on the soil surface or into the water, little P is expected in the floodwaters of mineral soils. None of the paddy waters had total P in the water greater than 1 ppm, a value that might concern regulators when water is drained from the paddies (Table 11).

Wet soil samples taken from paddies in June were air dried and analyzed for soil test P using the Bray test and for total phosphorus content. Most soils had soil test P (Bray P) in the high range, > 15 ppm, a few were in the medium range (7 to 15), and one in the low range. The available data for plant tissue at boot shows that when soil test P was in the medium or high range the plants all had an abundant content of P (see Tables 10 and 11). This suggest that our soil test recommendations are too high for P. The test recommendations were adopted from small grain recommendations for upland crops and no research has been conducted to determine how P soil testing functions for wild rice in peat soils. Total soil P varied from 244 to 770 lb/ac, values that would be considered low in mineral soils but peat soils to not bind P like mineral soils and during soil formation P is not accumulated to the same level in peats. High values of total P are not needed to supply the approximately 20 lb/ac P needed for a wild rice crop. In the crop cycle most of this 20 lb/ac is recycled back into the soil even if the straw is burned. We thought we might be able to find a correlation between soil test or total soil P and P in the paddy water. The data, however, show no correlation.

Table 11. Total soil P, Bray P and floodwater total and reactive P for June and July 1996. Soil was collected on June 19 for soil P.

Farm and field	Soil		Water		
	Total P lb/ac	Bray P ppm	June 19 Total P ppm	June 19 Reactive P, ppm	July 22 Total P ppm
Imle Gunvalson 8-1	506	26	0.17	0.27	0.12
Imle Gunvalson 9-5 E	455	19	0.11	0.01	0.16
Imle Gunvalson 9-5 W	382	14	0.05	0.01	--
Clearwater 6-W	408	13	0.02	0.02	--
Clearwater 2-E	770	15	0.28	0.16	--
Clearwater 7-E	465	3	0.88	0.14	--
Clearwater 13-E	647	17	0.98	0.08	--
Clearwater River			0.03	0.02	---
Rennemo D-6	322	10	0.05	0.03	---
Rennemo D-8	325	15	0.10	0.12	---
Rennemo D-18	244	16	0.74	0.69	---
Tamarack River			0.07	0.04	---
Godward H-6	259	24	0.02	0.01	0.07
Godward T-6	553	11	0.03	0.01	0.04
Godward T49	358	26	0.13	0.04	---
Mhos			0.03	0.01	----

PRELIMINARY HYDROPONIC STUDY OF ZINC NUTRITION IN WILD RICE

Wild rice plants were grown in tubs filled with nutrient solutions having different Zn^{2+} concentrations using a method developed at the University of Minnesota for the study of Zn deficiency in white rice (*Oryza sativa*). The nutrient solutions contained chelators and a pH buffer to control concentration of plant available uncomplexed Zn^{2+}.

Plants were grown for 26 days after transplanting (late tillering stage), harvested and analyzed for Zn and other nutrient elements. All plants grown without zinc died within a few weeks of transplant, and all other treatments showed tremendous variability, due in part to our

difficulty in getting uniform seedlings. The only visible symptom of zinc deficiency observed was decreased growth, but there was too much variability to determine at what Zn^{2+} concentration wild rice plants were zinc deficient. In our lowest concentration, a treatment containing zinc 0.25 ppm solution zinc, plant concentration was 19 ppm in plants that were likely reduced in growth. At our highest concentration of solution zinc the plants contained 51 ppm zinc. All other nutrients were within the normal range for wild rice. In white rice, less than 25 ppm is considered deficient. In the wild rice paddies we have sometimes see tissue zinc in the range of 10 to 25 ppm.

The results of this study show that wild rice is able to accumulate Zn more effectively at low solution Zn than the white rice varieties we have studied. The results also suggest that, unlike white rice, reduced growth due to low Zn is not associated with distinctive tissue symptoms. We now know that we will have to adjust the method developed for white rice to include solutions with available Zn at lower levels than in the solution we used which contained 0.25 ppm total Zn.

CONCLUSIONS AND RECOMMENDATIONS

- Mineralization of organic N contributes only a small fraction of N nutrition in acid peats. This is at least part of the reason it is more difficult to get good yields in the Aitkin area. These soils could be limed or growers can add more topdress N to ensure the plants are not N deficient during flowering and grain fill.

- Managing wild rice straw to increase its contribution to the subsequent wild rice crop has potential.

- Environmental factors such as drying, freezing, and the length of aerobic period before flooding affect the contribution of mineralized N to wild rice production. Further research is needed to see which factors can be managed to increase mineralized N.

- The removal of topdress nitrogen is rapid and fertilizer efficiency can be very high when topdressing is done at early to mid boot if the soil is depleted of N.

- Accumulation of N in roots is significant and must be considered in the determination of N needs of wild rice.

- The very high plant N contents at boot recommended in the past may be too high. A slight N deficiency at this stage may be useful to minimize the potential for lodging. With effective fall flooding 30 - 40 lb/ac of N may be sufficient to get the plants to early boot. At that time a large addition of 50 to 80 lb/ac of topdress N may then be needed. This could be followed by a topdress at very early flower of 30 to 40 lb/ac.

- Application of fertilizer N in the spring before thawing is not an effective way to fertilize wild rice.

- Data from growers paddies suggests that growers are fertilizing with K well in excess of that needed to avoid K deficiencies. No research information exist to show whether or not high K fertilization is effective in helping protect wild rice against diseases.

- In the wild rice farms in the Clearwater River area low tissue Zn continues to be seen in some paddies. This may or may not be a problem but it warrants further investigation.

- The P testing recommendations currently used call for more P than is needed, and growers are applying more P than is needed for high yields of wild rice. More research is needed to determine the fate of P in flooded peats and develop better soil test methods.

- Total P in paddy waters continues to occur at less than 1.0 ppm (much less than 1 ppm in most paddies) and should not be a concern for discharge into most lakes and streams.

- Reactive soluble P in some paddies is higher than required to induce algal blooms and more effort is needed in control reactive P. Modifications in P fertilization recommendations may lower the incidence of high reactive P.

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Host Range and Survival of *Bipolaris oryzae*, Causal Organism of Fungal Brown Spot on Cultivated Wild Rice

A.M. Moffatt, R.F. Nyvall, J.A. Percich
Department of Plant Pathology
University of Minnesota

Introduction

Fungal brown spot (FBS) disease on wild rice (*Zizania palustris*) is caused by *Bipolaris oryzae*. Various aspects of *B. oryzae* infection and survival are known. However, the primary inoculum source for this disease is not well understood. Identifying the primary source of inoculum may be very important for developing improved methods for controlling this damaging disease of cultivated wild rice.

Previous research indicated wild rice residue did not act as a source of inoculum when fields were fall flooded and/or when residue was incorporated into the soil (1). Also, infected seed did not act as a source of inoculum and airborne spores (conidia) of the pathogen were rarely found using spore traps placed in wild rice fields. Because of these results, further research into pathogen ecology is necessary to understand of the origin of the fungus prior to wild rice infection.

It had been suggested that grasses growing on the dikes were a possible source of initial inoculum for FBS (4). Therefore, the objective of this study was to investigate the role these grasses played in the development of FBS. My studies determined the host range for *B. oryzae* on various grasses and sampled plants for the presence of FBS along wild rice paddies.

Results and Conclusions

I. Defining a Host Range for *B. oryzae*

Grasses typically found in Minnesota (Table 1) were grown in the greenhouse and inoculated with *B. oryzae*. Host range was measured by percentage of plants infected. The grasses displaying the highest percentage of infection

were barnyard grass (*Enchinochloa crus-galli*) at 100%, green foxtail (*Setaria viridis*) at 96%, wild oat (*Avena fatua*) at 88%, downy brome (*Bromus tectorum*) at 75%, and yellow foxtail (*Setaria lutescens*) at 70%. However, in all cases the lesions were small and did not spread (Table 1). Lesion size was determined using a scale where 1 is approximately 0.005 cm^2, 5 is 0.12 cm^2, and 9 is 3.5 cm^2 (3). Sparse sporulation was observed on yellow and green foxtail, and downy brome prior to reisolation of the fungus. All of the infected leaves showed less than one percent leaf area covered with lesions except for wild rice which showed 10-15 % leaf area covered (Table 1). Percent leaf area covered was determined by comparing leaves against templates of known percentages (2). These results indicate that these grasses are not good candidates for a potential inoculum source.

II. Sampling Grasses Along Dikes for the Presence of *B. oryzae*

Grass samples from dikes in each of three wild rice paddies were taken from Aitkin, Waskish, and Clearwater, Minnesota during the summer of 1996. Two representative sites at each location were chosen for sampling. The main grasses identified and sampled were reed canary (*Phalaris arundinacea*), timothy (*Phleum pratense*), and quack grass (*Agropyron repens*), though other less abundant grasses were also examined. *B. oryzae* was detected only on reed canary grass (RCG) and therefore only RCG data is reported here. In order to determine percent infection of the grasses, lesions were cut from the leaves, washed, and placed onto moist, sterile filter paper. After ten days, these lesions were examined for sporulation and the number of lesions caused by *B. oryzae* versus the total number of lesions was determined (reported as percent infection). Table 2 indicates the detection of FBS infection on dike grasses was rare and when infection did occur, it was at the same time or after wild rice infection. Although some infection was detected when grasses were inoculated in the greenhouse, the detection of *B. oryzae* in the field was uncommon. To date, these results suggest that dike grasses play a very minimal role, if any, in the development of FBS in cultivated wild rice fields.

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Future Studies

Future work will continue to look at reed canary, timothy, and quack grass to determine if developmental stage or plant stress affects susceptibilty to FBS. Also to be determined is whether *B. oryzae* is more saprophytic than parasitic (i.e. lives better on dead than living tissue) on wild rice and other grasses.

Table 1 : Evaluation of various grasses as possible hosts for *B. oryzae*, causal organism of FBS of cultivated wild rice.

Grass	% plants infected	lesion size	% leaf coverage
Avena fatua	88	1-2	less than 1%
Avena sativa	9	1-2	less than 1%
Agropyron repens	*	*	*
Andropogon gerardii	14	2	less than 1%
Agropyron smithii	5	1	less than 1%
Bouteloua curtipendula	20	1	less than 1%
Bromus tectorum	75	1-2	less than 1%
Bromus inermis	*	*	*
Dactylis glomerata	*	*	*
Digitaria sanguinalis	6	*	*
Enchinochloa crus-galli	100	0-1	less than 1%
Elymus canadensis	55	1-2	less than 1%
Festuca arundinacea	0	none	none
Hordeum vulgare	5	1	less than 1%
Lolium perenne	28	0-1	less than 1%
Panicum virgatum	11	1	less than 1%
Phalaris arundinacea	48	1-2	less than 1%
Poa pratensis	*	*	*
Phleum pratense	0	none	none
Poa trivialis	0	none	none
Panicum dichotomoflorum	*	*	*
Setaria viridis	96	0-1	less than 1%
Setaria lutescens	70	0-1	less than 1%
Sorghum sudanense	62	1-2	1%
Sorghastrum nutans	*	*	*
Schizachyrium scoparium	9	1	less than 1%
Triticum aestivum	0	none	none
Zea mays	*	*	*
Zizania palustris	100	1-5	10-15%

* to be evaluated

Table 2 : Date of initial detection and percent infection of *B. oryzae* on cultivated wild rice and reed canary grass in 1996.

Site	Date: Wild rice	Percent infection	Date: RCG	Percent infection
Aitkin 1	July 25	36.4	July 25	0.8
Aitkin 2	July 25	31.2	August 7	4.9
Waskish 1	August 7	2.9	no infection detected	0
Waskish 2	July 9	12.5	no infection detected	0
Clearwater 1	July 25	6.1	no infection detected	0
Clearwater 2	August 7	6.7	no infection detected	0

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Genetics and Utilization of Seed Nondormancy in Wild Rice

Raymond Porter, Wayne Kennard, Ronald Phillips, Bruce MacGregor

Nondormancy is a trait that will aid the domestication of wild rice, as it has been key in the domestication of other cereals (Harlan 1992). Seed dormancy in wild rice is typically overcome by a three-month cold treatment but may last as long as seven years. Due to dormancy of fallen seed, growers have difficulty switching varieties in a paddy unless it has been fallow for at least three years or otherwise treated to kill the seed. Selection for nondormancy is therefore necessary to allow complete seed replacement by the grower each year. In addition, since dormant seeds which fall into paddy soil are the result of shattering, nondormant cultivars should reverse the natural selection for shattering which currently exists under continuous cultivation.

Because seed nondormancy is a key trait in domestication, selection for seed nondormancy within cultivars of *Z. palustris* has been attempted on several occasions. Stucker et al. (1987 and 1989) describe two previous unsuccessful attempts to initiate a selection program for reduced seed dormancy. These populations were not maintained, but the apparent progress from selection is an indication that there is some genetic variability for dormancy. Still, additive genetic variability, heritability, and expected response to selection were not estimated. Estimates of parameters would be useful to a breeder in weighing the potential for success of a recurrent selection program for reduced dormancy. Furthermore, since dormancy involves plant growth regulators (phytohormones), and shattering is a result of abscission, which may also involve some of the same plant growth regulators, there could be some correlation between shattering and dormancy. Such a correlation, if it exists, should be known by the breeder planning to select for both reduced dormancy and shattering resistance.

In 1990, Porter and Schumer (1991) obtained seeds from a Z. aquatica population in Florida which germinated within 1 week after harvest. Since Duvall and Biesboer (1988b) reported that Z. aquatica and Z. palustris crosses produced viable seeds if Z. palustris were the pollen parent, they crossed the Florida population with K2 and obtained F_1 seeds, which germinated within 1 week after harvest. That initial hybrid has since been backcrossed to Z. palustris cultivars available in the greenhouse (Porter and Schumer 1992; Porter et al. 1993 and 1994). Five generations of backcrossing have suggested that this nondormancy is highly heritable and not recessive. These results were similar to studies on dormancy in Oryza sativa, where 1 or 2 dominant genes have been shown to control the trait (Seshu and Sorrells 1985). Therefore, both quantitative and qualitative nondormancy need to be investigated. RFLP analysis of these independently derived BC_4 or BC_5 isolines should allow the identification of linked markers. Rice (Oryza sativa) probes linked to nondormancy also can be used on the backcross lines.

A nondormant cultivar might be expected to germinate soon after harvest, compromising its ability to survive the winter. Ideally, none of the seeds would survive and a new cultivar (or harvested seeds of the same cultivar) could be planted for the following season. If conditions were unfavorable for germination in late fall, this nondormant cultivar might be fall planted, survive until spring, and germinate when the soil is warm enough and saturated. Alternatively, it could be spring-planted, but fall-planting is more reliable for most growers. In order to determine the usefulness of a nondormant cultivar, research must address the survival of nondormant seeds from one season to the next.

Our objectives in this study of nondormancy were to: 1) quantify genetic variability for dormancy in Zizania palustris populations and estimate the correlation between dormancy and quantitative shattering resistance; 2) understand the inheritance of qualitative nondormancy using Z. aquatica x Z. palustris backcrosses to find RFLP markers for the trait; 3) determine the effectiveness of nondormancy in reducing or eliminating seed survival from one season to the next.

PROGRESS

Objective 1: Quantifying dormancy in Z. palustris

We addressed the potential for reducing dormancy levels within cultivated *Z. palustris* by estimating heritability of dormancy and gain from selection. Seeds from 345 half-sib families of 'Franklin' and K-2Pi (a pistillate breeding population derived from Franklin) were tested last winter and spring, removing them from cold storage and germinating them at 3 one-month intervals. The number of seeds germinating after one month in storage was used as the measure of dormancy. (Other measurements involving the ratio of germination in the first month to the other two were less informative.) Heritability was calculated to be 66% (although any GxE variability was not separated from genetic variability). The average germination after 1 month of storage was 26 seeds per 5 g (150 seeds), or 17%. Gain from selection (assuming half-sib family selection, no pollen control, 10% selection intensity) was calculated to be 10.8% per year (equivalent to a gain of 3 seeds per year). At this rate, 80% germination would be reached in 34 cycles of selection, making this approach less desirable than the qualitative gains likely from the *Z. aquatica* nondormancy. Because of the time required to prepare the seed and carry out the germination tests, these families could not planted in the field to estimate correlation with shattering resistance.

Objective 2: Understanding the inheritance of qualitative nondormancy

Nondormant *Z. aquatica* collected from the Suwannee River in Florida was originally crossed in 1990 as the donor parent to the dormant *Z. palustris* cv. K2. Backcrosses to *Z. palustris* were carried out for four to five generations. Individuals were deemed nondormant if they came from seeds in which germination occurred within two weeks of cold storage. Three backcross lines are being used for RFLP mapping: two BC5S1 lines 95G-74-1, 95G-220-11, and the sister BC4S2 lines 95G-19-1 and 95G-21-1. These lines are segregating for nondormancy, except for 95G-19-1, which appears to be fixed for nondormancy. They were tested for germination at two week intervals by removing from cold storage (4°C) and placement at room temperature (20°C).

RFLPs linked to genes controlling nondormancy are being identified by comparing 4 nondormant individuals from each backcross line, 5 individuals of *Z. aquatica*, and representatives of the recurrent-parent *Z. palustris* cultivars. DNA were isolated from the tissue of each plant; four enzymes *Eco*RI, *Eco*RV, *Dra*I, and *Hind*III were used to digest DNA and screen for polymorphisms. We have currently screened the backcross lines using 31 probes of known location throughout the rice genome (*Oryza sativa*). The majority of these probes (21 of 31) detect a unique polymorphism between *Z. palustris* and *Z. aquatica* with at least one of the four restriction enzyme digests. Thus, RFLP probes are useful for the detection of donor parent *Z. aquatica* DNA in the *Z. palustris* background. Of the 21 probes that detected unique RFLPs, 4 probes detected unique *Z. aquatica* restriction fragments in one of the three BC lines and one probe detected unique *Z. aquatica* restriction fragments in two of the three BC lines. Using binomial probabilities and assuming the donor parent genome is reduced by 0.5 each backcross generation, the probability that *Z. aquatica* introgression occurred by chance in one of the three BC lines is $P = 0.186$ and in two of the three BC lines is $P = 0.003$. Thus, one probe (RZ698) has a strong likelihood to be linked to the gene controlling nondormancy. A test of this marker linkage will proceed with the analysis of dormant BC5 individuals as they should not retain the donor *Z. aquatica* RFLP allele. Further confirmation will proceed by testing other probes that are linked to this marker. We will use the existing Oryza *sativa* linkage map and the emerging wild rice map for candidate markers that may be more tightly linked to the gene controlling nondormancy .

Objective 3: Effectiveness of nondormancy in the field

In moving the backcross lines from last winter's greenhouse generation to the field this spring, there was a high mortality rate early in the season, probably due to a combination of transplant stress and midge larval feeding on the seedlings. As a result, we did not achieve the seed numbers needed to carry out the field testing stage this fall. However, we are now increasing and selecting open-pollinated progeny in the greenhouse this winter for testing next fall.

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Acknowledgments

The Wild Rice Breeding and Germplasm Improvement project is funded through Cooperative Agreement number 58-3640-4-123 with the USDA-ARS. The research presented here was also made possible by two grants, one from the Minnesota Crop Improvement Association and the other from the Minnesota Cultivated Wild Rice Council.

Combine Yield Trial

Raymond Porter, Bruce MacGregor, and Henry Schumer

OBJECTIVES

1. To compare the yields of three populations, including a pistillate population (K-2Pi) when harvested by a combine.

2. To compare combine-harvested yield with binder-harvested yield.

PROCEDURES

At each of two on-farm locations, Waskish and Gully, 9 plots were planted in a Latin-square design. At Waskish, 30 ft by 40 ft plots were fall-planted with K-2 Pi and two sources of Franklin as the treatments, replicated three times. At Gully, 40 ft by 50 ft plots were planted with K-2Pi, Franklin, and GIB-C7, a bottlebrush population selected on the Gunvalson-Imle farms. These were fertilized and managed according to the growers' standard practices. Two topdresses of urea (approx. 50 lb/A of N) were applied during flowering. At Waskish, there was a high proportion of volunteer plants from prior years, mostly of the shattering type.

At harvest, a walk-behind binder was used to cut borders in one direction. This allowed plots to be harvested with a combine perpendicular to the border through the middle of each plot. Grain was collected into an 18 gallon container as it augured into the hopper, allowing the combine to clean out for 2 minutes each time before proceeding to the next plot. After combine cuts, the length and width of each cut was measured. After combine harvest was completed, two swaths were cut out on each side of each plot with the binder, 8 ft apart and perpendicular to the combine swath edge. A binder strip was cut between the two swaths (8 ft. x 22 in.), and the bundles were threshed as is normally done for a variety trial. At Gully, one of the two binder strips was chosen as visually representative, or "typical," of the state of the rest of the large plot (shattering, lodging), and the other was chosen to be a "high" yielding portion of the plot. For both the combine and binder cuts, yield per acre was calculated for each plot, and combine-harvested samples were sent to Northern Rice Labs for analysis of percent recovery. Yield, percent recovery, and processed yield were analyzed for each location by Analysis of Variance.

RESULTS

The table summarizes the yields of the combine and binder harvests for each location. At Waskish, the entries were not significantly different because variability was high, for both combine harvest and binder harvest, although the binder yield had a lower measure of variability (LSD). The high variability was probably due to the variability introduced by the high proportion of shattering volunteer plants in all plots.

At Gully, GIB-C7 had the highest combine-harvested yield, followed by K-2Pi, then Franklin, although the differences were not statistically significant for yield, recovery, or processed yield. K-2Pi outyielded GIB-C7 only in the "high" binder cuts, but variability was very high, probably due to lodging. In general, K-2Pi lodged quite severely compared to the other entries, most likely reducing its yield potential.

Although binder-harvested yield did not prove to be less variable than combine-harvested yield in this experiment, variety trials with smaller binder-harvested plots can accommodate larger numbers of entries and reps, giving more degrees of freedom and greater statistical power for detecting real differences.

Summary of combine harvested plots, Waskish and Gully

WASKISH	Combine harvest			Binder yld (2 cuts/plot)
	Yield (lb/A)	Recovery (%)[1]	Proc. yld (lb/A)	(lb/A)
Franklin (GR)	615	45.2	278	535
Franklin (Ren)	653	45.5	297	495
K-2Pi	639	45.8	293	601
LSD (0.05)	*460*	*1.7*	*218*	*337*
P value	*NS*	*NS*	*NS*	*NS*

GULLY	Combine harvest			Binder green yield		
	Yield (lb/A)	Recovery (%)[1]	Proc. yld(lb/A)	Typical cut (lb/A)	High cut (lb/A)	Mean (lb/A)
Franklin	1306	42.1	549	841	1045	943
GIB-C7	1485	44.1	654	1292	1413	1353
K-2Pi	1430	40.2	575	1011	1579	1295
LSD (0.05)	*275*	*2.8*	*87*	*1030*	*992*	*725*
P value	*NS*	*NS (0.054)*	*NS (0.065)*	*NS*	*NS*	*NS*

[1] Estimated by Northern Rice Labs, B. Schwob

Seed Tensile Strength Variability in Wild Rice (Year 2)

Raymond A. Porter and Robert A. Shaner

INTRODUCTION

A major breeding objective for wild rice is seed retention or shattering resistance. Variability within varieties appears to be high (Fig. 1). One way of measuring seed retention is by means of a force gauge, which measures the strength necessary to pull a seed off a panicle (Fig. 2). Wild rice seeds tend to detach more easily as the seeds mature, even in cultivars which are considered "nonshattering". To obtain accurate comparisons of seed tensile strength between lines, we need to better understand how tensile strength changes over time and the amount of variability between vs. within populations.

The overall objectives were to:
1) quantify changes in seed retention over the time from flowering to seed maturity;
2) quantify and compare seed retention among nonshattering and shattering populations;
3) estimate variability in retention among florets or seeds within panicles, and among panicles of a population, in order to improve experimental design.

In year 2 of the study (presented herein), we sought to obtain more information on the changes in tensile strength over time in fewer varieties, although both shattering and nonshattering were represented.

METHODS

In the first year we had observed seed tensile strength in 25 populations (18 nonshattering breeding populations and 7 shattering wild accessions). By contrast, this year we studied one shattering (Little Rice Lake) and two nonshattering populations (Franklin and NACH-B). Instead of tagging and measuring 5 panicles per plot at each of two times, we tagged 10 or more panicles at three dates as they emerged from the boot, two days apart, and measured 8 panicles in each tagging group. Tensile strength measurements were taken on 1 seed per panicle, every day (except Sunday) beginning one day after panicle emergence (tagging date). The measurements were taken by attaching a force gauge to an individual female floret or seed, pulling it off, then transferring the measurement to an electronic data recorder.

In order to compensate for bias due to the loss of shattered seed, we estimated shattering on each panicle beginning about 10 days after panicle emergence. The rating scale used corresponded to quartiles of seed lost (0=0%, 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100%). The tensile strength was then adjusted by converting the rating to the midrange of the equivalent proportion (e.g., 1=0.125, 2=0.375, etc.), subtracting that from 1 and multiplying the remaining proportion by the measured tensile strength.

RESULTS

When tensile strength was averaged over all three tagging groups for each entry, all three varied somewhat initially, not showing any consistent difference among them, except that Franklin was generally slightly lower than the other two (Fig. 1). However, after 14 days, the differences between Franklin and NACH-B all but vanished. The shattering entry, however, declined more steeply after 12 days, until rising on day 17.

But the differences in estimated shattering loss became readily apparent after 14 days (Fig. 2). Little Rice Lake, quickly exceeded 50% loss, while the two nonshattering entries gradually increased but never exceeded 30% during the 4-week measurement period. Once again,

although Franklin shattered consistently less than NACH-B at each date, the difference was very small.

Once tensile strength was adjusted for shattering loss, the difference between shattering and nonshattering entries became even more clear (Fig. 3). The shattering population dropped off and continued to decline at a consistent rate of -0.025 kg/day from day 10 to day 17. During the same period, the NACH-B declined at a rate of -0.007 kg/day, and Franklin at -0.005 kg/day. After a slight increase at 19 days, the nonshattering entries dropped a little more sharply until day 28 at a rate of -0.011 kg/day. This period of time coincided with the appearance and steady increase of visible shattering losses in these two entries (Fig. 2).

When the individual tagging groups are compared for each calendar day, there is some consistency in the rise and decline of adjusted tensile strength (Fig. 4). July 20, 23, and 27 are all dates where all tensile strengths drop unexpectedly. This may be an indication of weather-related effects. Neither temperature change nor precipitation corresponds with these valleys. However, the sudden rise in tensile strength on September 3 and 4 (34 and 35 days after July 31), corresponded to a shift in tensile strength measuring time from the first half of the morning to mid-afternoon. This may be a temperature-related phenomenon, and indicates that time of day of the measurements as well as the conditions for a given day must be accounted for. Alternatively, comparisons may be best made when the measurements are taken during a narrow time frame.

CONCLUSIONS

- Upward bias in tensile strength due to measuring unshattered seeds can be compensated for by estimating percent shattering.
- Tensile strength changes over time may be subject to weather-related effects, but after 10 days show a general decline which is sharper in shattering genotypes.
- After 23 days, the nonshattering varieties showed a steeper decline than the one seen between 10 and 23 days.
- The two nonshattering varieties selected, Franklin and NACH-B, were nearly identical in their tensile strength profile.
- The best time to distinguish nonshattering from shattering lines is on or after 14 days from emergence. At that time, easily observable shattering will have occurred in shattering panicles.

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Acknowledgments

The Wild Rice Breeding and Germplasm Improvement project is funded through Cooperative Agreement number 58-3640-4-123 with the USDA-ARS. The research presented here was also made possible by a grant from the Parker Sanders Fund of the University of Minnesota. This fund provided the stipend for Mr. Shaner through the Minnesota Department of Agriculture's "Ag in the Classroom" Assistant Scientist program, allowing Mr. Shaner participate in this study. The Franklin Kosbau Fund provided the force gauge and data recorder used to measure tensile strength.

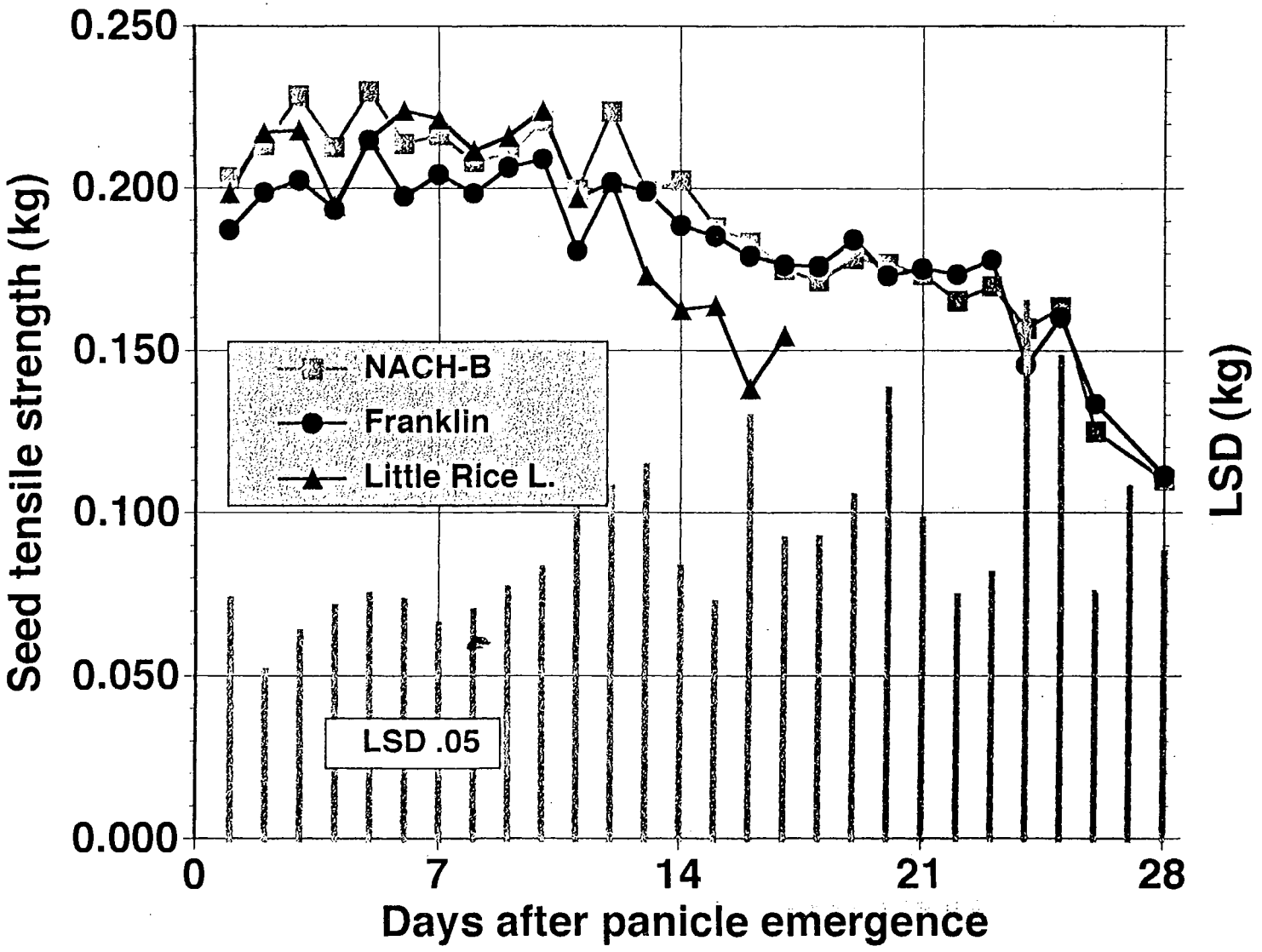

Fig. 1 TS averaged over standardized tagging groups showed little difference between entries. Shat TS dropped off, but was later biased upward. 1996.

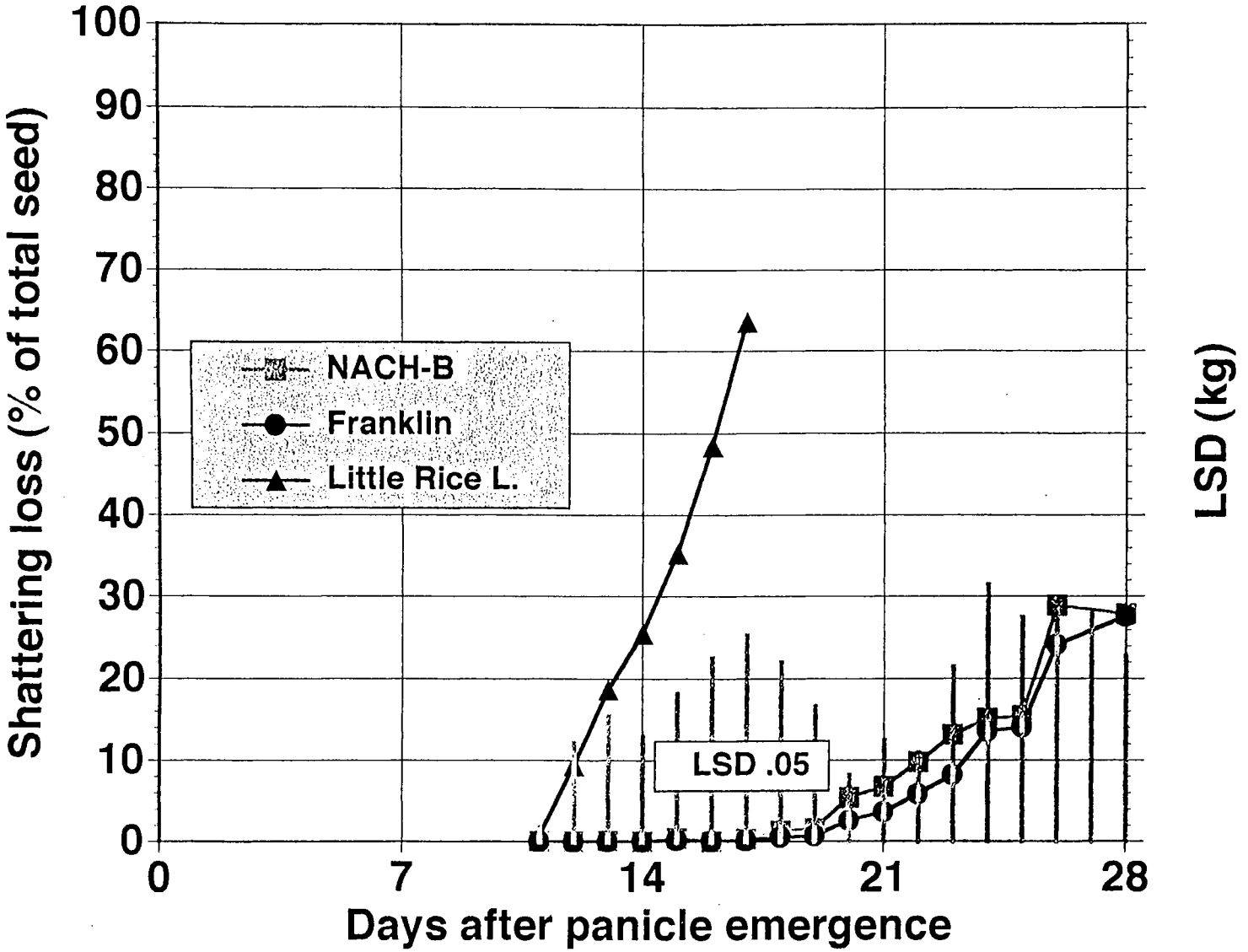

Fig. 2 Shattering loss (estimated from 1-4 score) increased dramatically in shat entry, and later increased gradually in two non entries. 1996.

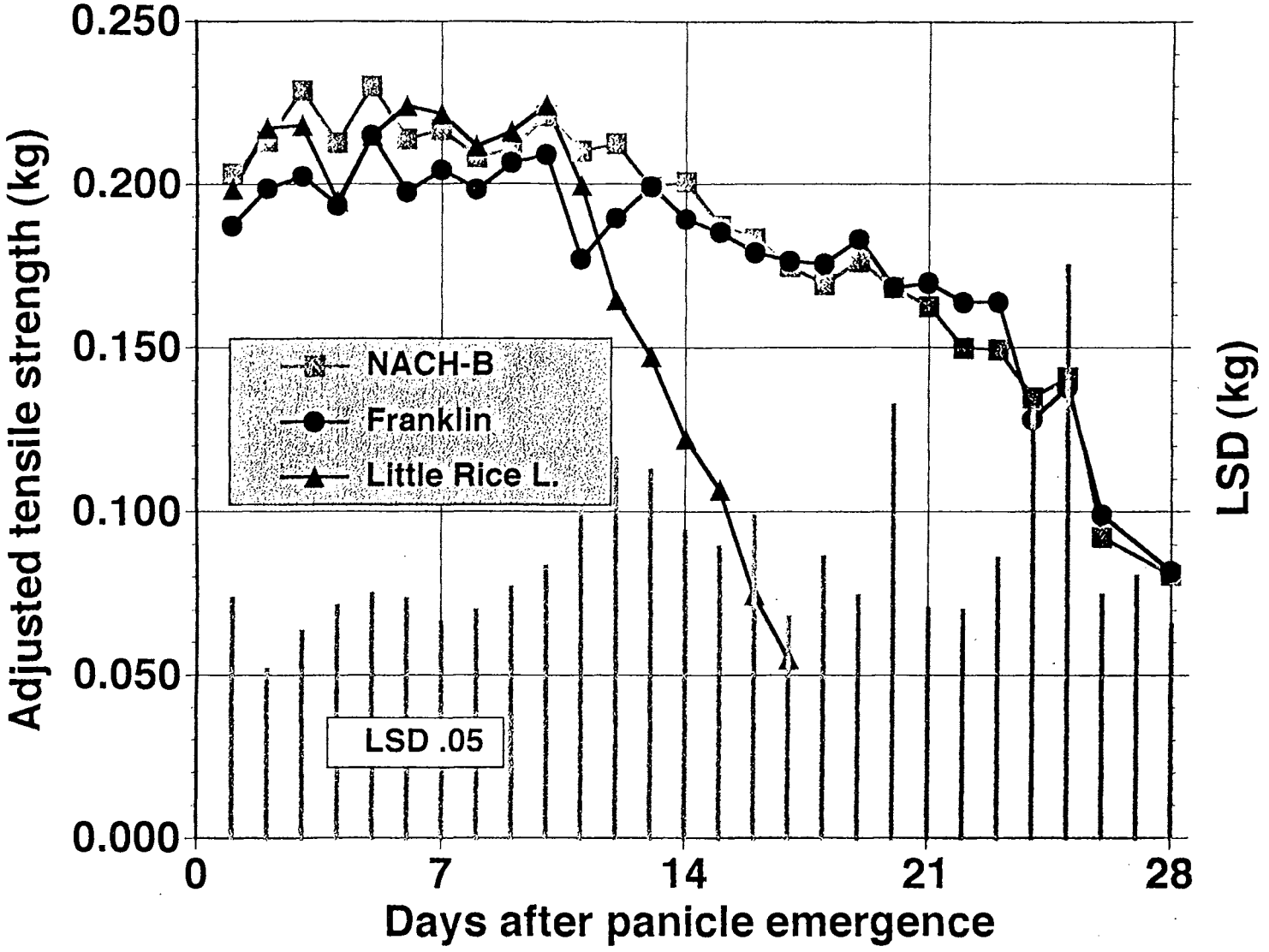

Fig. 3 TS (adjusted for shattered seed) of shat entry becomes significantly lower than non entries after 11 days. 1996. $TS_{adj.} = TS \times (100\text{-}\%shat) \div 100$

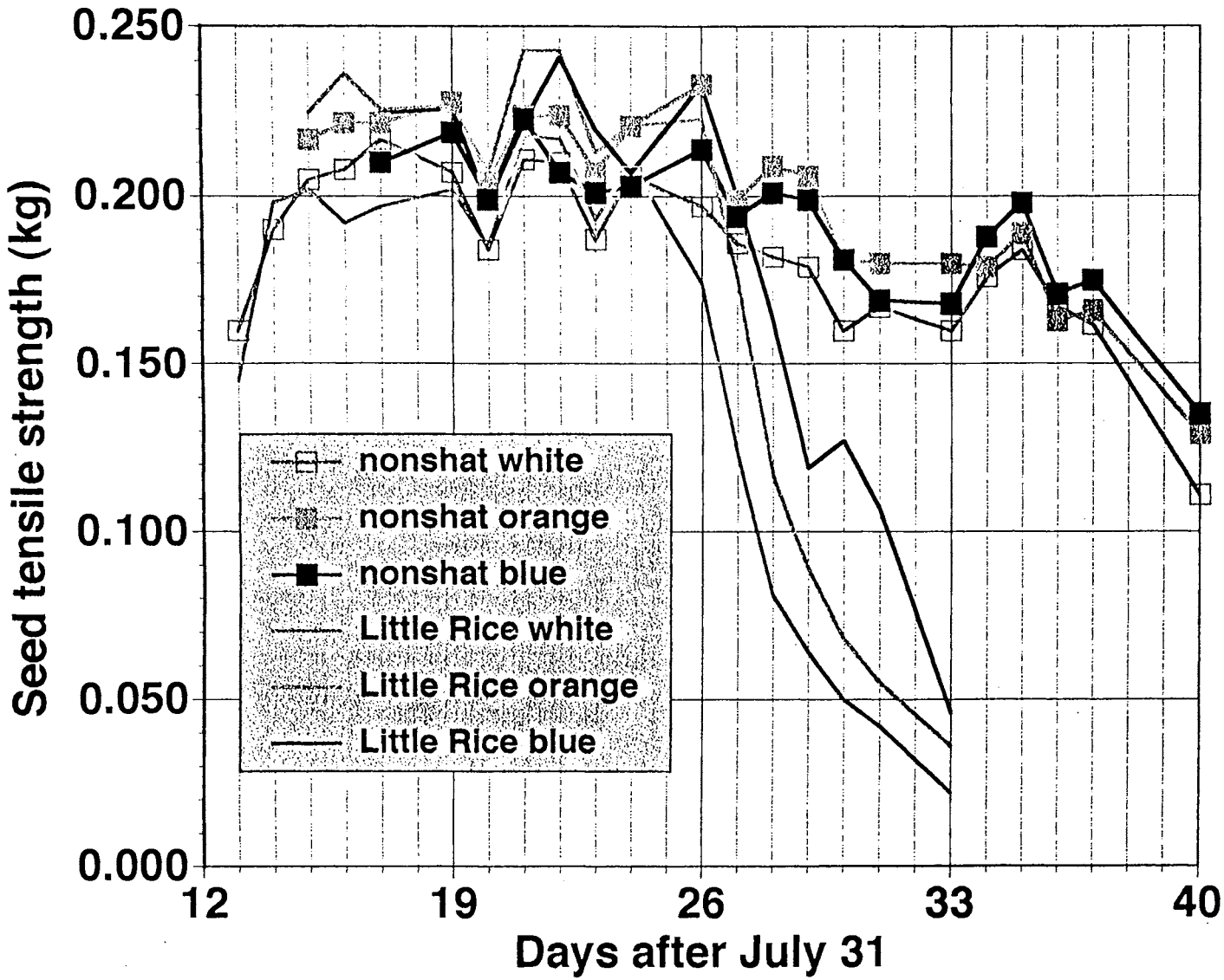

Fig. 4 TS for all three tagging groups fluctuate together, perhaps due to weather effects. Little Rice L. curves differ by only 1 day during decline. 1996.

Progress in Mapping Shattering and Nondormancy Genes.

Wayne C. Kennard, Raymie A. Porter, Ronald L. Phillips

Introduction and objectives.

The primary objective of the molecular genetics project is to increase the understanding of wild rice genetics and facilitate breeding objectives through the application of genetic markers. Our primary research areas involve: i. the development of a saturated genetic linkage map of wild rice for breeding applications, ii. comparative mapping to understand the relatedness of the wild rice genome to white rice and other cereals, iii. identification of markers linked to genes controlling shattering, nondormancy, and the pistillate trait, and iv. the development of lines via marker-assisted selection.

Development of a genetic linkage map.

Currently the wild rice map is unfolding. To develop a mapping population we have generated several F_2 populations from several controlled crosses of nonshattering by shattering plants. Three F_2 populations with greater than 150 individuals were evaluated for shattering, Johnson X Dora Lake (1 population) and K2-Vomela X Dora Lake(2 populations)and segregation ratios for shattering versus nonshattering were 125:63, 143:61, and 122:49 respectively. The K2-Vomela X Dora segregations fit a 3:1 ratio ($P > 0.05$) and these populations likely contain one segregating shattering gene. The Johnson X Dora does not fit a 3:1 segregation ratio ($P < 0.05$). This population is now being emphasized for mapping as it likely harbors more than one shattering gene.

We are in the process of restriction-fragment-length-polymorphism (RFLP) map construction with probes used as markers in white rice (*Oryzeae sativa*). By comparing wild rice to white rice we can build upon an extensive knowledge base and more quickly approach the resolution of existing high density maps of white rice (Causse et a1.,1994; Kurata et al., 1994; McCouch et al.,1988). It has been demonstrated that many genomic regions among grass genomes are conserved for RFLP markers and genes conditioning similar traits (Ahn and Tanksely 1993; Paterson et al. 1995). Sets of probes currently being used are of rice, oat, and barley origin that have been previously mapped in white rice (Kurata et al. 1994, National Institute of Agrobiological Resources, Tskuba, Japan; Causse et al. 1994, Cornell University). RFLP

evaluation consists of radioactively labelling probe DNA, and subsequent hybridization to wild rice DNA. The hybridization is based on the complementary base-pairing nature of double-stranded DNA. The wild rice DNA has been cut with restriction enzymes, which allow us to detect DNA variation. Restriction enzymes cut DNA at specific sites which may be unique (polymorphic) among individuals. The probe allows us to target small fragments of DNA which can be visualized as bands on gels. Thus a useful probe will hybridize to wild rice DNA, detect discrete bands, and detect size variation (polymorphism). The majority of our mapped white rice probes hybridize to wild rice DNA. cDNA probes (DNA encoding genes) have been found to be generally more useful in wild rice while genomic probes (DNA providing chromosome structure) have not (Table1).

Table 1. Probe hybridization to *Z. palustris* mapping population.

Type of probe	Number	Detected signal	Distinct band(s)	Non-distinct bands	Polymorphism (four enzymes)
cDNA					
Rice	94	87(92%)	80(85%)	7(9%)	51(63%)
Oat	57	43(75%)	37(65%)	6(14%)	28(75%)
Barley	8	6(75%)	4(50%)	2(33%)	2(50%)
Genomic					
Rice	26	15(57%)	8(31%)	7(47%)	3(38%)

In general, the number of restriction fragments hybridizing in wild rice is greater than white rice. It has been reported that wild rice has twice the genome size of white rice (2.2 pg/cell as opposed to 1.0 pg/cell, Bennett et al. 1982). The number of restriction fragments detected in wild rice by single copy rice probes is approximately two times that of white rice (2.2+/- 0.8, adjusting for heterozygous allelic fragments). The greater amount of total DNA and greater number of restriction fragments may reflect partial or global duplication events of the genome of wild rice chromosome with respect to white rice.

Construction of the wild rice map is an ongoing process. The polymorphic probes detected have been used to initiate wild rice map construction. A polymorphic probe can be used to obtain segregation data. Once segregation data is obtained, probes become markers (or loci) to which relative locations among other markers can be determined. These relative positions are illustrated by groups of co-segregating loci or linkage groups. Thus far we have obtained genetic segregation data for 64 loci. Expected 1:2:1 segregation ratios are found with 57 of 64 loci. Expected ratios indicate the Johnson X Dora population is useful for genetic analysis. Linkage analysis using computer

software LINKAGE-1 (Suiter et al., 1983) with 64 loci generated 13 linkage groups with 49 loci(Fig.1). Fifteen markers remain unlinked. Since we have fewer linkage groups than chromosome pairs as well as unlinked markers, we do not have a saturated map. Obtaining a saturated map is simply a matter of time and obtaining more markers. Continued effort will be made to increase map saturation as this is directly related to its value as a breeding tool. Of the linkage groups we have constructed, we have found colinear regions with white rice. This is an anticipated result based on comparative maps among other grass genomes. Other linkages are not colinear. Noncolinearity of wild rice and white rice markers may be due to rearrangements of wild rice with respect white rice chromosomes. Alternatively, the duplicated nature of wild rice with respect to white rice may complicate our ability to establish colinear relationships. Even though establishment of colinear linkages may be complicated, laying the comparative mapping foundation is a priority, as it will allow us the most efficient use the highly resolved white rice genetic map in wild rice.

Detection of genes for shattering resistance.

With our partial map we are tempted to use the markers therein to test associations to the shattering trait. We scored F2 individuals as shattering or nonshattering approximately 30 days after pollination (Elliot and Perlinger 1977). Marker genotypes were tested against shattering versus nonshattering phenotypes in single-factor analysis of variance tests using SAS statistical software (SAS Institute, Cary NC). Three (CD01387, CD0244, and RZ590) of the 64 markers tested were significantly ($P< 0.05$) associated to the trait (Fig.1). Of these three markers, two (CD0244 and RZ590) are linked and likely detect the same gene while the other (CD01387) is segregating independently and likely detects a different gene. Thus two independent regions have been found that appear linked to genes controlling shattering. These markers combine to explain 19.5% of the variation for the trait. The unaccounted variation for the trait is likely attributable to incomplete marker saturation. That is, the association may be diminished by genetic recombination between the marker and the shattering gene, or there may be one or more other shattering gene(s) that have as yet gone undetected. Another reason for undescribed variation may be that environmental factors may confound the genetic analysis. We will

evaluate F3 progenies derived from mapping F2 individuals in a replicated paddy trial to reduce possible confounding environmental variation.

Genes controlling traits, as well as RFLPs, are candidates for comparative mapping analysis. Two shattering genes in white rice have been mapped, *sh-1* and *Sh-3*. Interestingly, the dominant *Sh-3* gene in white rice maps in the region of chromosome four in which the markers CD0244 and RZ590 are located. Thus, the shattering gene in wild rice may be a similar (homeologous) gene in white rice. The use of the white rice map will help us increase marker saturation in this region.

We will begin to use the map and markers to develop a nonshattering population. Our initial strategy will be to identify lines from the mapping population itself that are fixed for nonshattering alleles. To do this we need to increase map saturation to identify markers flanking all nonshattering genes. We also need to propagate the mapping population via selfing until we can reliably identify all fixed nonshattering lines. Toward this end we will self F3 lines to generate F4 lines this spring. As fixed nonshattering lines become identified we will perform crosses among them (to avoid inbreeding depression). Markers associated to nonshattering genes may also be used in development of other nonshattering populations as genetic locations of genes should be maintained. RFLPs detected by the same probe or another tightly linked marker would be used for selection of homozygous nonshattering individuals.

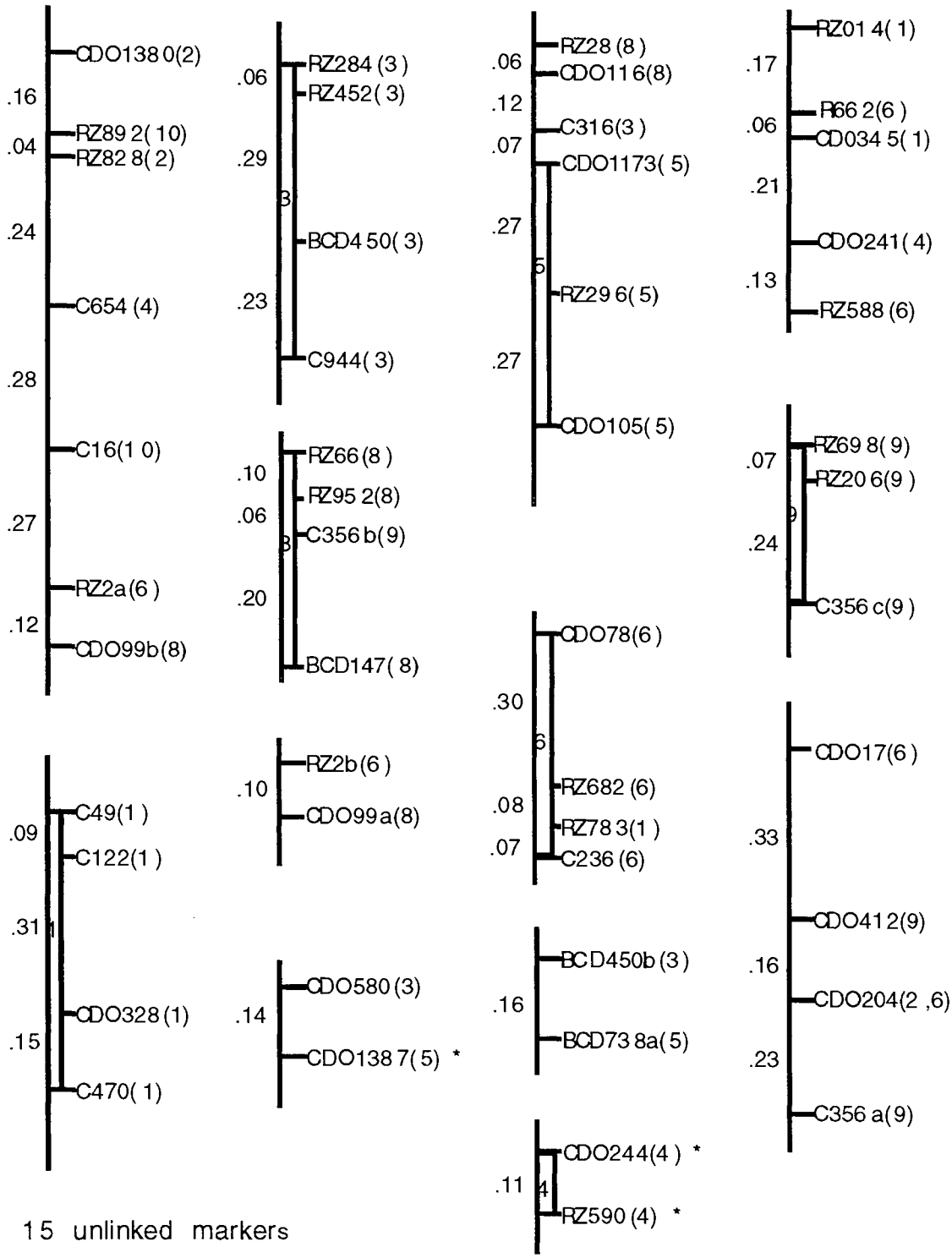

Fig1. Wild rice RFLP map. Vertical lines indicate linkage groups. Marker designations are on the right with the chromosome to which the marker was mapped in white rice in parenthesis(). Adjacent boxes indicate regions of colinearity with white rice. Recombination distance are on the left. * indicate markers were significantly ($P<$ 0.05) associated to the trait, shattering versus nonshattering.

Detection of markers linked to genes controlling nondormancy.

We are using a different approach to identify linked markers to genes controlling nondormancy. In this study, we are using probabilities associated with donor parent allele elimination through recurrent backcrossing to detect markers linked to genes controlling nondormancy (e.g., it is unlikely that donor parent germplasm remains in backcross lines except that which is linked to genes under selection; Kaeppler et al., 1993). We evaluated three different backcross families generated by crossing nondormant *Z. aquatica* to dormant *Z. palustris* with repeated backcrossing to *Z. palustris* and selection for nondormancy for 4 to 5 generations. We evaluated 30 probes and unique DNA fragments were readily observed between *Z. aquatica* and *Z. palustris* (16 of 30 probes). Evaluation of backcrosses indicated *Z. aquatica* DNA introgression in one of the three families (P = 0.093 by chance) and one probe (RZ698) indicated *Z. aquatica* DNA introgression in two of the three families (P = 0.003 by chance). There is a strong likelihood this latter marker is linked to a gene controlling nondormancy. We will test other markers that are linked to this probe on the basis of the recently determined linkages in wild rice.

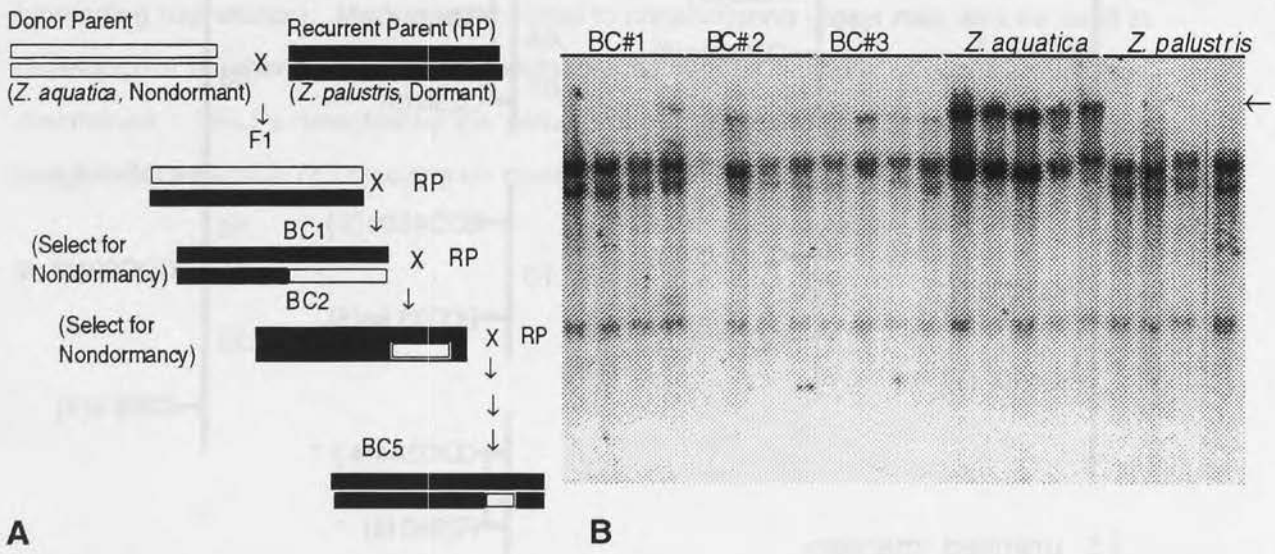

Fig 2. A.) Backcrossing scheme for introgression of nondormant genes. B.)Hybridization of rice cDNA probe RZ698 to nondormant backcross lines of wild rice. Note the unique *Z. aquatica* restriction fragment (absent in *Z. palustris*) but present in 2 of 3 backcross lines.

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Quantitative Analysis of Dynamic Development of Transition Zone in Wild Rice Panicle

Qinqin Liu, Ryan Reuter, R. A. Porter

Introduction:

The transition zone of wild rice (Zizania spp.) has been identified as the formation of hermaphroditic spikelets in the branch at the junction between the staminate and pistillate portions of the panicle (Troska and Liu 1996). Only limited information has been reported on the formation of hermaphroditic spikelets, which were found in some plants at the transition zone. These spikelets may be capable of self fertilization (Aiken, et al., 1988; Goldman, 1990; Weir and Dale, 1960). In this research, different populations with a relative large sample sizes have been investigated to gain some quantitative information on the development of hermaphroditic spikelets of the transition zone. By combined field and laboratory research, the frequency of transition zone formation was investigated by sampling wild rice plants in the field and dynamic development of the transition zone was characterized in the laboratory using dissecting microscope.

Materials and Methods:

Samples from the Mixed, Pistillate, Moose, Rice and Bow, and White Elk populations of wild rice were collected from the North Central Experiment Station of the University of Minnesota in Grand Rapids. The samples collected were at the developmental stage where the panicle had not grown out of the sheath. The panicles were fixed in ethanol: acetic acid (3:1) for 24 hours at 4°C, transferred to 70% ethanol and stored in plastic boxes in a cold room (4°C) for laboratory analysis (Troska and Liu 1995).

In the laboratory, transition zone branches were isolated and characterized using a Nikon dissecting microscope. The data was analyzed with Microsoft Excel. location of each floret on the transition zone branch, sex of the floret, and the length of each awn were recorded and analyzed. Perfect and female florets were also analyzed by measuring the awn length and stigma length of each floret.

Field data for analysis of transition zone was collected from eight different populations at the North Central Experimental Station, Grand Rapids. The Long and Short populations are from a strain that has been selected for their female inflorescence length. The pistillate population was segregated for plants with "pistillate" type panicles with only a female inflorescence and normal panicles with unisexual florets. Data from the Pistillate population was collected from the plants that appear normal. Data was not taken from plants with only a female inflorescence since no transition zone exists in these plants. The Mixed population

has a percentage of plants with a random distribution of female and perfect florets on the branches of the male inflorescence. The PiB-C2, Peterson Pond (Z. *aquatics*), and WLNS populations represent plants similar to wild type plants in different ecological environments. Field data was collected in the rice paddies at the developmental stage where the panicle had grown out of the sheath. Panicles were randomly sampled and analyzed by determining if a transition zone exists in each plant. Sample sizes ranged from 13 to 40 plants. If a transition zone was found, the number of branches in the transition zone and the number of male, perfect, and female florets were recorded. Also, for each plant, the length of the female inflorescence and the length of the male inflorescence were recorded.

Crosses were made between the Long and Short populations to define the genetic effect on male or female floret development. Long females were crossed with Short males and Long males were crossed with Short females. Both populations were also selfed. The process of making the crosses began by isolating the female inflorescence with a paper bag and paper clip before any stigmas had grown out of the floret. To make the crosses, the male inflorescence of another plant was also isolated with a paper bag and paper clip. The pollen was then collected 24 hours later with a plastic cup from the male inflorescence. The collected pollen was then sprinkled into the bag containing the appropriate female inflorescence at sexual maturity. The pollinated female inflorescence was then protected with a paper bag for seed production until harvesting.

Results and discussion:

Transition zones in wild rice panicles were observed in all populations. The frequencies of transition zones ranged from 25% in the Pistillate population to 69.9% in the Peterson Pond population (see fig. 1). The high frequency in Peterson Pond population may related to the larger size of panicales in Z. *aquatics*, which may produce more florets per branch. The repeat experiment with the large sample size in this population will confirm this result. Most plants had transition zones consisting of one branch, but every population had some plants with transition zones consisting of two branches (See Figure 2). There were no transition zones observed that consisted of more than two branches. Results from this study confirmed the formation of transition zone in different population from the previous study by Troska and Liu in 1996.

The arrangement of floret sex in a transition zone is variable. The transition zones all had a variable number of florets, and variable combinations of floret. Table 1 shows the different combinations of floret sexes and the frequency of each combination as well as the average number of male, perfect, and female florets in a transition zone from eight

populations. The average number of male and female florets in a transition zone (the total number of 140 transition zone branch observed) was 6.35 and 6.77, respectively. The average number of perfect florets in a transition zone branch was 3.67. Among the total of the transition zone branches, 50% had male, perfect, and female florets, 19% had no female florets and 19% had no perfect florets, 11% had no male florets, and 2% had only perfect florets. The pattern of arrangement of floret sex in individual population need to be compared for future analysis.

Floret sex distribution in transition zones was also analyzed. Most transition zones had male florets towards the bottom of the branch, female florets towards the top of the branch, and perfect florets in the middle of the branch. However, the location of each floret sex in the transition zone was variable. Figure 3 shows the distribution of male, perfect, and female florets along the transition zone branch. The transition zone branch was split into thirds for easier representation of location. Data was combined from three populations (Moose, Rice and Bow, and White Elk) because transition zones varied individually but not by population. Male florets were dispersed throughout the bottom 2/3s of the transition zone but none were found on the top 1/3 of the branch. The dispersal of perfect florets was almost equal throughout all of the transition zone branch. Female florets were observed in all three locations on the branch but, they were heavily concentrated on the top 1/3 of the branch.

Lengths of the male and female inflorescence were recorded to see if a significant difference exists between plants with transition zones and plants without transition zones. Each population measured showed no significant difference between plants with transition zones and plants without transition zones for the average length of the male and female inflorescence (See Table 2).

The awn lengths of transition zone florets from twelve transition zone branches from Moose, Rice and Bow, and White Elk populations were measured to see if there was a difference in awn length for each floret sex according to its location on the transition zone branch. If there was a significant difference in awn length at different locations it could show a developmental pattern as the indicator for different floret formation. Table 4 shows the average awn lengths of each sex relative to the floret location on the transition zone branch. Average awn lengths among different floret sexes appear to be significantly different. Male florets on the bottom 1/3 and middle 1/3 had average awn lengths of 2.52 mm and 3.82 mm, respectively. Perfect florets on the bottom 1/3, middle 1/3, and top 1/3 of the transition zone branch had average awn lengths of 16.24 mm, 15.67 mm, and 17.2 mm respectively. Female florets on the bottom 1/3, middle 1/3, and top 1/3 of the transition zone branch had average awn lengths of 27.8 mm, 34.24 mm, and 34.5 mm respectively. However, the average awn length for each floret sex was similar whether the floret was on the bottom

1/3, middle 1/3, or top 1/3 of the transition zone branch. This data suggests that the awn length is correlated with floret sex in the development.

The determination of sex in transition zone florets can be ambiguous in the rice paddies. The sex of female florets can be determined easily by noticing the absence of anther in the floret. The female floret also has an awn that is significantly longer than male floret awns. Male florets have anther in the floret and a very short awn. A problem in determining the sex of perfect florets arose while taking transition zone data in the field. A perfect floret has anther visible in the floret but the stigma is not visible with the naked eye. It was observed in the laboratory with a dissecting microscope that transition zone florets with awn lengths less than 6 mm and containing visible anthers were all male. Table 3 shows the frequency of awn lengths from twelve different transition zone branches. Perfect and female awn lengths are usually larger than 6 mm but male awn lengths were all 6 mm or less. Transition zone florets with awn lengths greater than 6 mm and containing visible anthers, were perfect. Transition zone florets with awn lengths of 6 mm were variable, some were male and some were perfect. The awn length can serve as an indicator of male or perfect floret sex in the field, but for an accurate determination of sex, the floret should be dissected for microscopic analysis.

More data was taken to see if the awn lengths of perfect and female florets could be correlated to the development of the stigma. No correlation could be made from this data analysis (figure 4). The awn lengths ranged from 61 mm to 6 mm and the stigma lengths varied from 2.5 mm to 1.0 mm. It was noted that stigmas of equal length displayed signs of unequal development. Weir and Dale in 1960 noted that the arms of the stigma, although presumably functional, seemed stunted and weaker in transition zone florets than stigmas in pistillate florets. Similar observations were made in this study.

Future research should investigate the contribution of perfect and female florets in transition zones for seed production to understand their biological function. The future genetic experiments will be followed to test genetic effects on the dynamic development of flowers for wild rice seed production.

Acknowledgments:

We are grateful for research support by the North Central Experimental Station of the University of Minnesota at Grand Rapids. Help with sample preparation from Mike Sedgwick was also greatly appreciated. This research was supported by funding from the Minnesota Cultivated Wild Rice Council and Minnesota Sea Grant.

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Sex Distribution in the Transition Zone

# of TZs	Male in TZ	Perfect in TZ	Female in TZ	Frequency,%
70	YES	YES	YES	50%
26	YES	YES	NO	18.60%
15	NO	YES	YES	10.70%
26	YES	NO	YES	18.60%
3	NO	YES	NO	2.10%
# TZs observed=140				
Ave. # of florets	6.35	3.67	6.77	
Standard Deviation	5.5	3.04	4.99	
Total # of florets	122	114	111	

YES- indicates the presense of a male, perfect, or female floret in the transition zone.

NO- indicates the absence of a male, perfect, or female floret in the transition zone.

TZ- transition zone

Table 1: This table shows the percentage of each different combination of sexes in the TZ and the average # of each sex in the TZ. Data was taken from plants with TZs from eight populations.

MALE AND FEMALE INFLORESCENCE LENGTH

	Normal	PiB-N Long	PiB-N Short	Pistillate	Mixed	PiB-N C2	Peterson Pond	WLNS
Ave. F ln. -**TZ**	20.73	25.81	14.54	21.2	20	19.85	27.22	23.8
Std. dev.	2.81	2.34	3.57	3.94	3.14	4.02	2.64	2.53
# of Samples	22	21	13	10	13	13	9	10
Ave. F ln. -**NTZ**	19.22	25.06	14.41	23.03	20.8	22.26	26	22.56
Std. dev.	2.34	2.6	2.94	3.39	2.25	3.62	2.94	2.34
# of Samples	18	18	17	30	10	27	4	16
Ave. M ln. -**TZ**	19.45	25.57	23.15	22.2	20.38	23	29.11	25
Std. dev.	3.67	3.34	3.24	3.74	3.2	3.94	4.14	3.77
# of Samples	22	21	13	10	13	13	9	10
Ave. M ln. -**NTZ**	20.17	23.44	21.56	23.43	19.1	21.44	28	24.63
Std. dev.	4.14	2.95	4.6	4.05	2.69	3.67	0.82	3.56
# of Samples	18	18	16	30	10	27	4	16

Ave. F ln.= Average length, in cm, of the female segment of the panicle

Ave. M ln.= Average length, in cm, of the male segment of the panicle

TZ= Plants with transition zones

NTZ= Plants with no transition zones

Std. dev.= Standard deviation

Table 2: This table compares some descriptive statistics of plants with no transition zone and plants with transition zones for each population.

Critical Awn Length of the Transition Zone

Length of Awn	# of Males	# of Perfect	# of Female
< 6 mm	33	0	2
6 mm	3	3	0
> 6 mm	0	36	79
total	36	39	81

Table 3: This table shows the frequency of awn lengths from twelve transition zone branches from Moose, Rice Bow, and White Elk populations.

Average Awn Lengths at Different Locations of the Transition Zone Branch

	Male Awn Length (mm)		Perfect Awn Length (mm)		Female Awn Length (mm)	
Top 1/3						
Mean	0		17.2		34.5	
Std. Dev.	0		5.84		12.74	
# of Samples	0		10		42	
Middle 1/3						
Mean	3.82		15.67		34.24	
Std. Dev.	1.25		6.66		14.15	
# of Samples	11		12		29	
Bottom 1/3						
Mean	2.52		16.24		27.8	
Std. Dev.	1.36		10.21		17.3	
# of Samples	25		17		10	

Table 4: This table shows the average awn length of each sex of florets according to position on the TZ branch. Data was taken from twelve transition zone branches from Moose, Rice Bow, and White Elk populations.

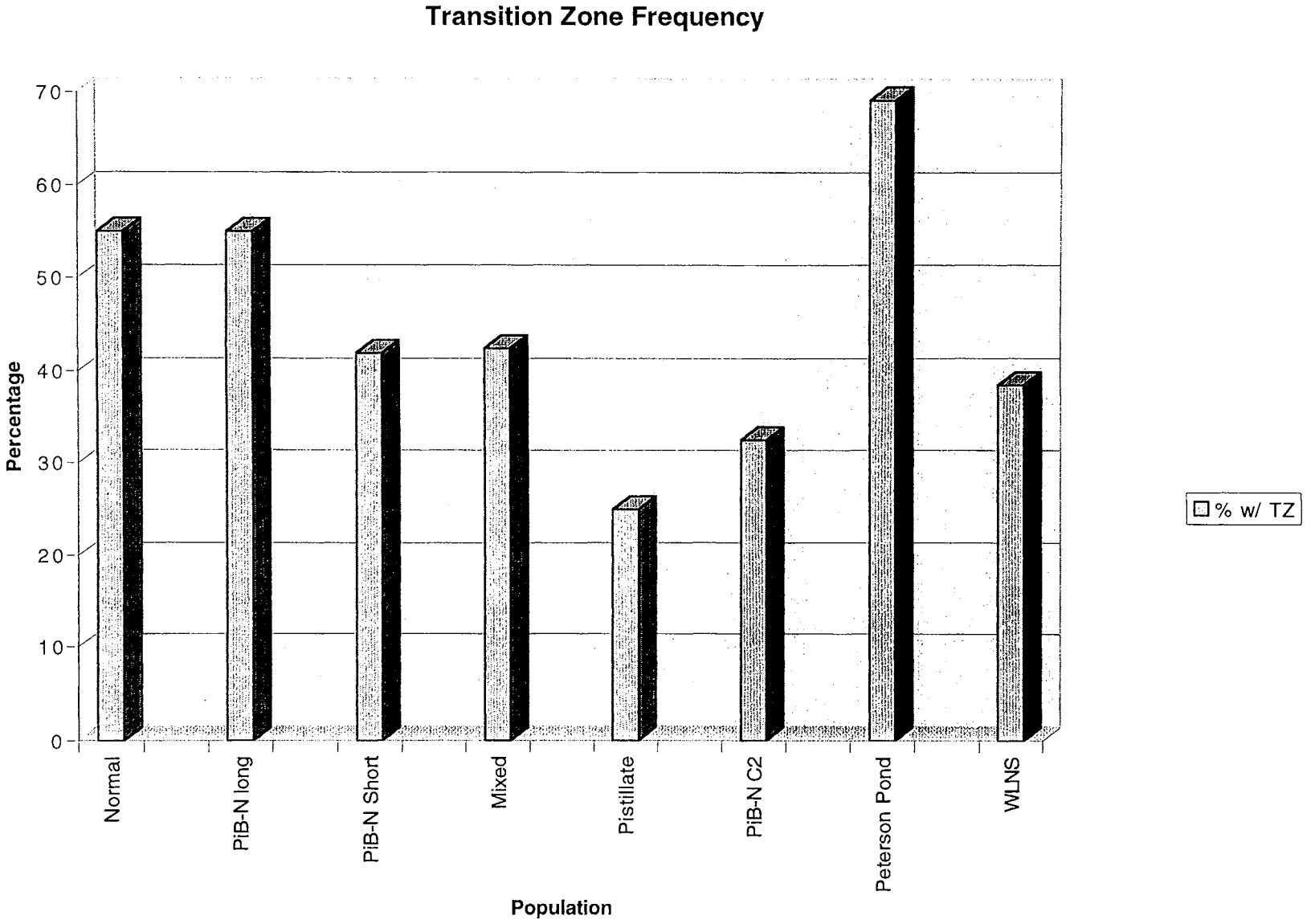

Figure 1: This figure shows the percentage of plants from each population that had a transition zone. The number of samples used in Peterson Pond=13, Short=31, WLNS=26, all others=40.

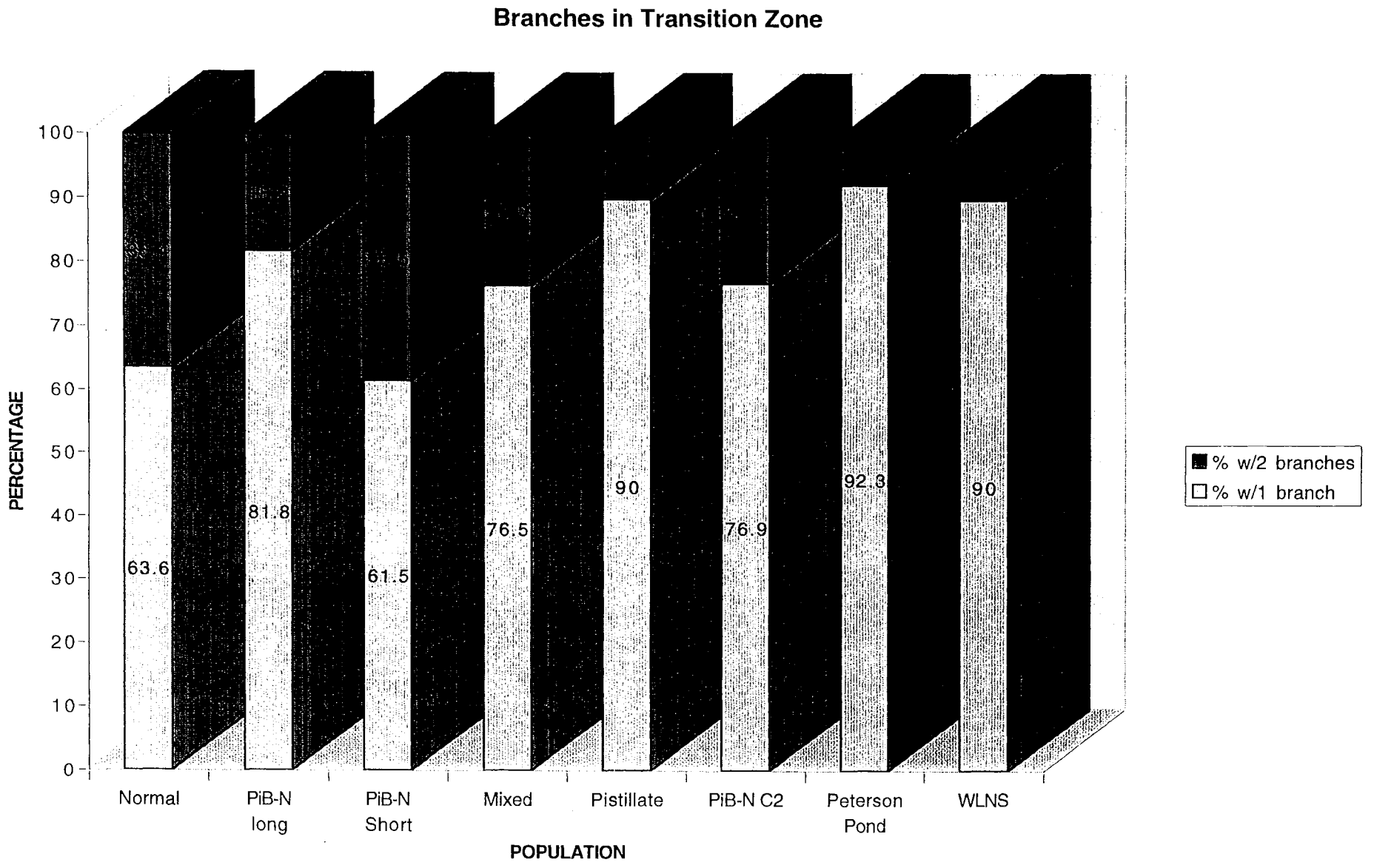

Figure 2: The transition zones of each population observed all had either 1 or 2 branches in the transition zone. This figure shows the percentage of TZs consisting of 1 branch and the percentage of TZs consisting of 2 branches, for each population.

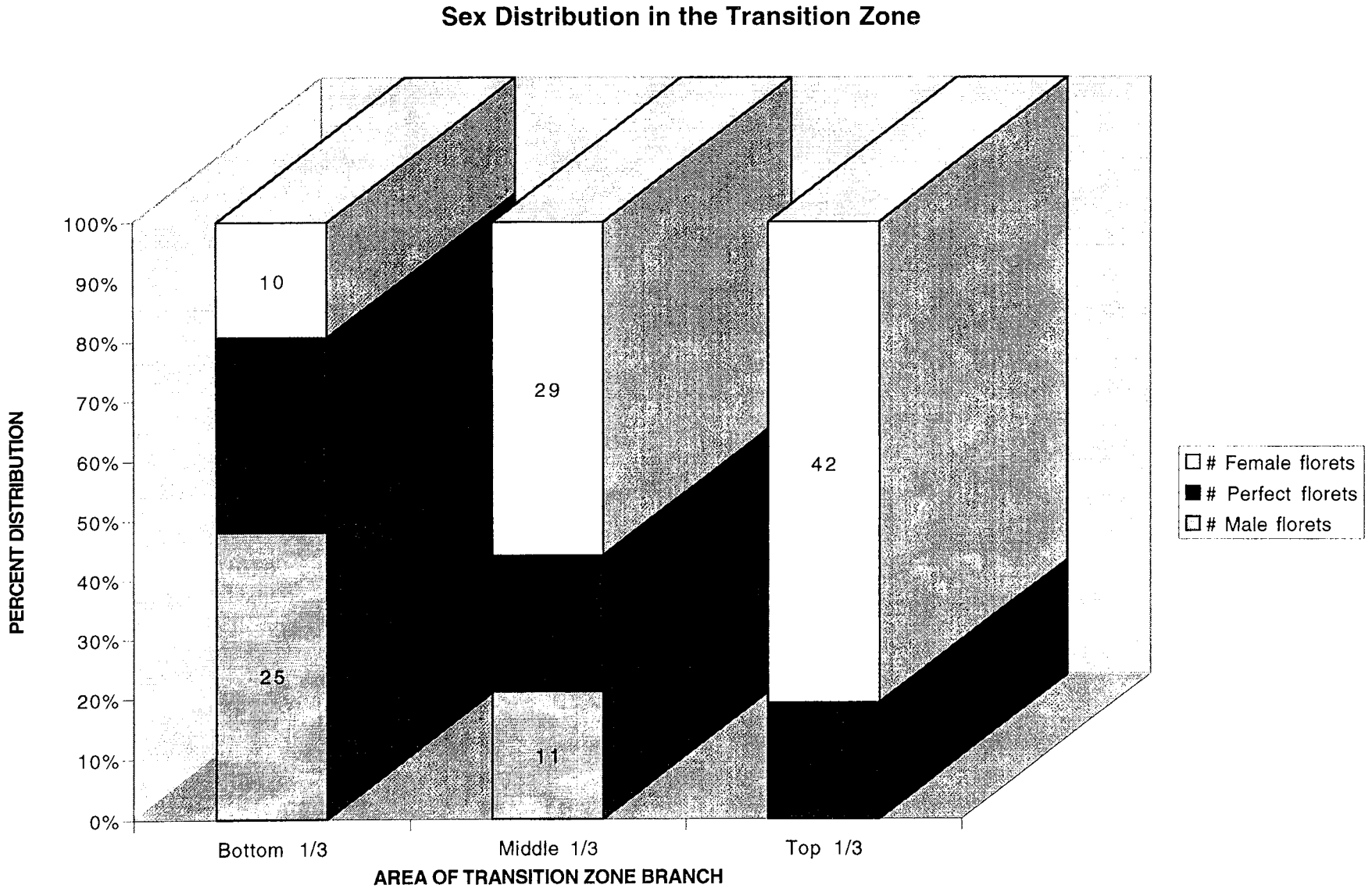

Figure 3: This figure shows the distribution of male, perfect, and female florets along a transition zone branch. Data was taken from twelve TZ branches from Moose, Rice Bow, and White Elk populations.

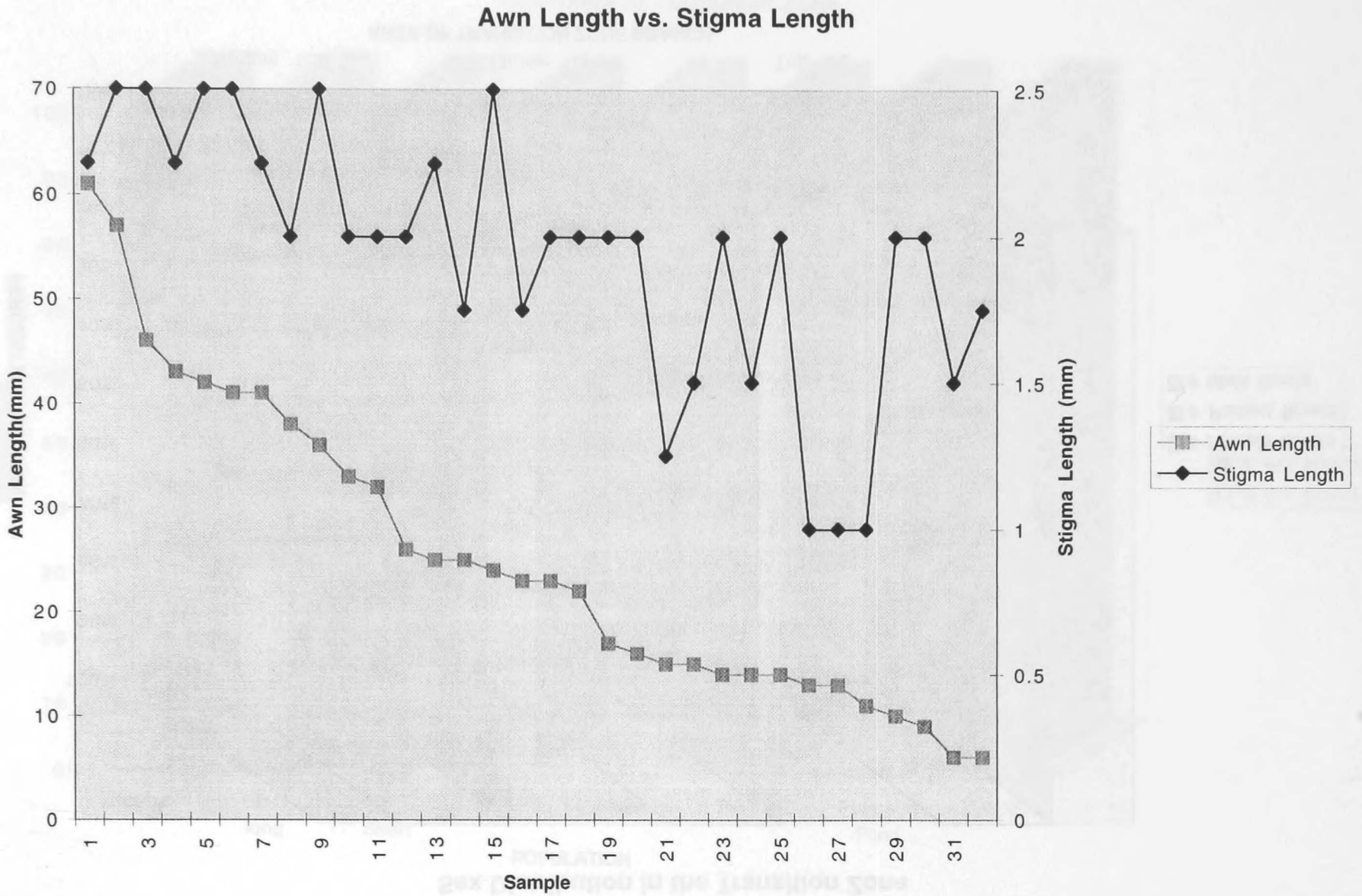

Figure 4: This figure shows the relationship between awn length and stigma length. Data was collected from perfect and female florets from Moose, Rice Bow, and White Elk populations.