Minnesota Wild Rice Research – 1995

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St. Paul, Minnesota

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WILD RICE PRODUCTION RESEARCH - 1995

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

The total number of growing degree days in 1995 for the wild rice growing season was greater than for 1994 at all four locations, Aitkin, Grand Rapids, Waskish and Crookston (Tables 1 and 2). The average number of growing degree days in 1995 was 2918 across all locations compared to 2829 in 1994, thus the 1995 growing season was warmer than 1994. However, the 1995 growing season started out cooler than 1994 with April being much cooler and May also cooler than in 1994 and the long term average (normal) at all four locations. June was considerably warmer than 1994 and the normal average for all four locations. At Aitkin and Waskish, July was warmer than in 1994 and the normal average. At Grand Rapids and Crookston, July was warmer than 1994 but not warmer than the normal average. August was very warm and warmer than 1994 and the normal average at all four locations.

Table 1. Growing degree days[a] comparisons for 1994, 1995, and normal (61-90).

Month	Aitkin			Grand Rapids		
	1994	1995	Normal	1994	1995	Normal
			GDD			
April	114	30	127	114	30	130
May	494	370	417	496	420	434
June	726	818	646	726	874	674
July	762	796	779	800	824	858
August	673	824	683	720	890	768
Total	2769	2838	2652	2856	3038	2864

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

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Table 2. Growing degree days[a] comparisons for 1994, 1995, and normal (61-90).

Month	Waskish			Crookston		
	1994	1995	Normal	1994	1995	Normal
	- GDD -					
April	83	10	103	106	35	151
May	442	326	369	531	394	488
June	724	810	518	782	907	743
July	756	764	642	802	852	926
August	692	794	563	774	904	867
Total	2697	2704	2195	2995	3092	3175

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

Total precipitation was less than in 1994 at all four locations (Tables 3 and 4). It was also less than normal at Aitkin and Waskish but more than normal at Crookston and Grand Rapids. July and August were the wettest months. Based on long term averages, June is usually the wettest month at all locations except Aitkin where July is normally the wettest.

The weather during the 1995 growing season can be characterized as starting out cool, then very hot during the grain fill period, and wet and stormy just before and during harvest. A series of storms occurred in late July and early August, greatly reducing yields due to seed shattering and lodging from wind and rain.

Table 3. Precipitation comparisons for 1994, 1995, and normal (61-90)[a].

Month	Aitkin			Grand Rapids		
	1994	1995	Normal	1994	1995	Normal
	- GDD -					
April	4.69	1.93	2.30	2.91	1.46	2.10
May	3.11	3.07	2.88	2.20	2.55	3.04
June	4.82	1.42	4.09	10.66	1.53	4.11
July	2.27[b]	6.28	4.14	4.04	8.55	3.89
August	1.14	3.18	3.83	1.84	6.25	3.59
Total	16.03	15.88	17.24	21.65	20.34	16.73

[a] Data from Mark Seeley, Department of Soil, Water, and Climate, U of MN. [b] Precipitation for July taken from nearest DNR rain gauge in Aitkin County, township 47, range 27, section 26.

Table 4. Precipitation comparisons for 1994, 1995, and normal (61-90)[a].

Month	Waskish			Crookston		
	1994	1995	Normal	1994	1995	Normal
	- GDD -					
April	1.07	0.64	1.70	1.76	0.33	1.45
May	1.76	1.33	2.33	1.87	1.78	2.45
June	6.36	1.81	4.25	7.11	2.05	3.44
July	6.92	7.64	3.42	5.73	7.56	2.77
August	3.34	2.89	3.32	1.71·	3.27	2.88
Total	19.45	14.31	15.02	18.18	14.99	12.99

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

Total cultivated production in Minnesota was about 19% less in 1995 than in 1994 (Table 5). Much of this decreased production was due to the wind and rain from the storms that went through the wild rice production areas in late July and early August. California production in 1995 increased by about 29% over their 1994 production. Thus, total cultivated production in the two states was slightly more in 1995 than for 1994. However, total cultivated production in 1994 and 1995 was still less than for the previous two years.

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[b]	4300	6440

[a] 1968-1982 Minnesota values from Winchell and Dahl and 1983-1994 from Minnesota Department of Agriculture; California values from Marcum, Cooperative Extension Service, University of California. [b]Estimated value for 1995.

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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, 1981, 1994-95 (last 2 in 1980). Plants in independent plots were beat with a beating tool at the last 4 stages of growth, 1994-95. Grand Rapids, MN.

Growth Stage	Leaf re-moval	Grain yield at harvest					Grain yield reduction compared to control				
		1995	1994	1981	1980	Ave.	1995	1994	1981	1980	Ave.
	%	---------------------- lbs/A[a] ----------------------					----------------------%----------------------				
Floating	33	3245	1700	848	1169	1740	16	(21)[b]	19	20	8
leaf	67	3015	1611	712	1216	1638	22	(15)	32	17	14
	100	3592	1750	657	558	1639	7	(25)	37	62	20
Aerial	33	3399	1600	752	1216	1742	12	(14)	29	17	11
leaf	67	3411	1641	888	1423	1841	12	(17)	15	3	3
	100	3829	1738	497	1335	1850	1	(24)	52	9	10
Tillering	33	3386	1846	872	1415	1880	13	(32)	17	3	0
	67	3597	1545	783	1508	1858	7	(10)	25	(3)	5
	100	2329	1159	648	867	1251	40	17	38	41	34
Flowering	33	3461	1520	800	1482	1816	11	(9)	23	(1)	6
	67	2788	1176	897	1116	1494	28	16	14	24	20
	100	928	420	272	288	477	76	70	74	80	75
	Beat[c]	2377	1093	--	--	1735	39	22	--	--	30
Milk	33	3237	1117	640	1415	1602	16	20	39	3	20
	67	2353	1090	672	994	1277	39	22	36	32	32
	100	1464	575	328	831	800	62	59	69	43	58
	Beat[c]	1506	695	--	--	1100	61	50	--	--	56
Soft	33	3025	1509	880	--	1805	22	(8)	16	--	10
dough	67	2778	1216	912	--	1635	28	13	13	--	18
	100	2088	772	440	--	1100	46	45	58	--	50
	Beat[c]	1574	250	--	--	912	59	82	--	--	71
First	33	2947	1472	808	--	1742	24	(5)	23	--	14
dark[d]	67	3530	1434	657	--	1874	9	(2)	37	--	14
	100	3572	1427	583	--	1861	8	(2)	44	--	17
	Beat[c]	3084	555	--	--	1920	20	60	--	--	40
Control		3876	1400	1045	1463	1946	0	0	0	0	0
LSD 0.05		1003	263	256	585	--	--	--	--	--	

[a] Corrected to 40% moisture.   [b] Grain yield INCREASES compared to control.

[c] Plots beat with weeping willow tree branches to a point of approximately 50% leaf defoliation.

[d] Data from 1994 and 1995 were taken at 30% Dark.

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Simulated Hail on Wild Rice

Introduction: Two years of previous research in 1980 and 1981 indicated that leaf removal before flowering substantially reduced grain yield, whereas in 1994, these yield reductions were not observed. In all three years leaf removal in combination with stem bending during flowering and grain fill substantially reduced grain yield. Yields were considerably reduced even when leaves were removed at the floating leaf growth stage in 1980 and 1981. The 1994 trial was conducted to update the 1980 and 1981 information. The addition of the "beating" treatment to the 1994 trial was done to more accurately simulate the damage which would result from a hail storm because hail usually not only defoliates the plants but also damages stems. In addition, hail during grain fill can potentially strip grain from the panicles. The 1995 trial was a repeat of the 1994 trial.

Materials and Methods: Wild rice, variety 'Franklin', was planted with a cone plot planter on May 24, 1995, 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 8, 1995 with 75 lbs/A of N, 40 lbs/A of K, 3 lbs/A of boron, and 20 lbs/A of sulfur. On May 22, the plots were fertilized with an additional 75 lbs/A of nitrogen due to heavy rains experienced after the May 8 application. A June 13 soil test showed over 100 lbs/A of available nitrogen. Plots were not top dressed with nitrogen. Plant population was approximately 1.5 plants per square foot. Initial stands were good, but the heat of June and July resulted in infestations of algae which caused reduced stands observed later in about half the plots.

To simulate hail damage, 33, 67 and 100% of each leaf blade in a plot was cut off with a scissors at seven plant growth stages. Leaf blade tissue was removed at the floating leaf, 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 were "beaten" until reaching approximately 50% leaf defoliation. The beating devise consisted of a set of 5 - 34 inch strands of 14/2 electrical wiring with ground wire spread across a "T" handle with a 7 inch head and a 21 inch handle. There were a total of 26 treatments including the control. The treatment dates of the seven growth stages were: floating leaf, 6-16; aerial leaf, 6-26; tillering, 7-12; flowering, 8-2; milk, 8-18; soft dough, 8-28; and 30% dark seed, 9-6. The treatments were made approximately every 2 weeks with the last one made the day before harvest.

Results and Discussion: Table 7 presents the results from the 1995 trial. Yield (dehulled grain) was not reduced significantly (5% level) when leaf blade removal occurred at the first 3 growth stages except when 100% of the leaf blades were removed at the tillering stage of growth. During the last four growth stages when treatments (leaf blade removal and stem bending) were made at flowering, milk or soft dough growth stages, significant yield reductions occurred with 67 and 100% of the leaf blades removed, plus a similar percentage of stems were bent. No significant yield reductions occurred at 33% or when the treatments

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were made during the 30% dark seed stage of growth (one day before harvest). Beating the plants at last 4 stages of growth resulted in significant yield losses when done at all of the last 4 stages of growth.

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Table 7. Influence 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 beaten with a beating tool at the last 4 stages of growth. - Grand Rapids, MN -1995

Growth Stage	Leaf removal	Plant number	Stem number	Panicle number	Plant height	50% flower date	Straw dry weight	Green grain weight	Dehulled grain yield	Recov -ery	Hulls
	%	/ft²	/ft²	/plant	cm	DAP[a]	lbs/A	lbs/A[b]	lbs/A	%	%
Floating	33	1.4	10.8	7.9	169	70	4351	3245	1413	43.0	33.4
leaf	67	1.4	10.0	7.7	173	70	4131	3015	1248	40.9	36.7
	100	1.1	10.5	9.6	166	70	4037	3592	1579	44.0	31.9
Aerial	33	1.3	10.9	8.6	174	70	4727	3399	1434	42.3	34.5
leaf	67	1.5	11.7	8.4	170	70	4835	3411	1487	43.0	33.4
	100	1.4	13.8	10.0	164	70	4791	3829	1639	42.8	33.6
Tillering	33	1.2	11.5	9.1	175	69	5002	3386	1472	43.2	33.1
	67	1.2	12.8	10.4	154	70	4324	3597	1576	43.8	32.1
	100	1.2	12.1	9.6	149	70	3978	2329	939	40.0	38.1
Flowering	33	1.5	12.7	8.5	174	70	5225	3461	1320	38.3	40.7
	67	1.5	12.5	8.1	149	69	3942	2788	1141	40.9	36.6
	100	1.4	6.6	4.1	121	70	2236	928	282	30.1	53.5
	Beat[c]	1.5	NA	NA	153	68	3586	2377	839	34.2	47.0
Milk	33	1.5	12.2	8.3	173	69	5378	3237	1324	40.8	36.8
	67	1.5	11.7	8.0	169	70	4908	2353	808	34.2	47.1
	100	1.4	11.8	7.5	143	70	4641	1464	403	26.2	59.4
	Beat[c]	1.6	NA	NA	125	69	4610	1506	343	21.3	67.0

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Table 7. (continued)

Growth Stage	Leaf removal	Plant number	Stem number	Panicle number	Plant height	50% flower date	Straw dry weight	Grain weight	Dehulled grain yield	Recov -ery	Hulls
	%	/ft²	/ft²	/plant	cm	DAP[a]	lbs/A	lbs/A[b]	lbs/A	%	%
Soft	33	1.3	11.2	7.8	180	70	5256	3025	1263	40.9	36.6
dough	67	1.5	11.6	7.2	158	69	4502	2778	1107	39.7	38.4
	100	1.5	10.1	6.8	153	69	3879	2088	714	34.2	47.0
	Beat[c]	1.4	NA	NA	110	69	4690	1574	342	21.5	66.7
30% dark	33	1.5	10.1	6.7	181	70	4900	2947	1259	41.5	35.7
seed	67	1.5	11.4	7.8	176	69	4631	3530	1550	43.8	32.2
	100	1.5	11.0	7.6	185	69	4599	3572	1544	43.3	32.9
	Beat[c]	1.6	NA	NA	129	69	5779	3084	1065	34.6	46.5
Control	0	1.6	11.9	7.6	180	70	5599	3876	1626	41.4	35.9
LSD (0.05)		0.4	3.1	2.1	20	1	1301	1003	476	4.8	7.4

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

Figure 1a depicts the regression lines for the relationship of dehulled grain yield (7% moisture) to percent leaf blade removal at the first 3 stages of growth. The lines are nearly straight when different percentages of leaf blades were removed, meaning leaf removal at these early dates did not influence yield. The plants recovered from these early treatments. At tillering however, the line slopes down to 100% leaf blade removal; this indicates yields were reduced as more leaf blades were removed.

Figure 1b depicts the regression lines for the last 4 stages of growth. The lines relating yield to percent leaf blade removal and stem bending at flower and milk stages of growth declined the most, indicating the greatest effect on yield. The regression line did not decline as much when treatments were made at the soft dough stage. Leaf blade removal and stem bending at the 30% dark seed stage of growth (1 day before harvest) did not influence yield.

The actual grain yield (40% moisture) and percentage reduction in yield for the 4 years of simulated hail trials are presented in Table 8. The yields differed from year to year which can be typical for wild rice because the crop is still very much influenced by the climatic conditions during the growing season. Percentage yield reduction or increase compared to the control fluctuated considerably when leaf blade removal occurred during the first 3 stages of growth and it is difficult to see trends in yield reductions. However, when treatments were made during the last 4 stages of growth, trends in yield reductions were evident. The highest percentage yield reduction occurred when treatments were made during the flowering, milk and soft dough stages of growth. On the average, the highest yield reduction of 75% occurred when 100% of the leaf blades were removed and 100% of the stems bent at flowering.

Figure 2 (a and b) presents the average 1980-81, 1994-95 trend for yield (40% moisture) loss due to leaf blade removal and stem bending. Regression lines are shown in Figure 2a for leaf blade removal during the first three growth stages. Yield was not influenced by leaf blade removal at the aerial leaf stage since the regression line is almost without a slope. Yield was influenced by the amount of leaf blade removal at the floating and tillering growth stages since both of these lines have a downward slope with a high R^2 value (0.83 and 0.70). The relationship of amount of leaf blade removal to yield reduction was not expected at the floating leaf stage because of the 1994 and 1995 results in which this relationship was not evident. The strong relationship of 1980 and 1981 was apparently negated by the 1994 and 1995 results (Table 8).

Figure 2b presents the regression lines for percent leaf blade removal plus stem bending and grain yield. The slope is the greatest for flower, then milk followed by soft dough and dark seed stages of growth. This means that leaf blade removal and stem bending causes more yield reduction as the percentage of leaf blade removal and stem bending increases during the flower stage of development. This is not the situation when leaf blade removal and stem bending occurs close to harvest (dark seed growth stage).

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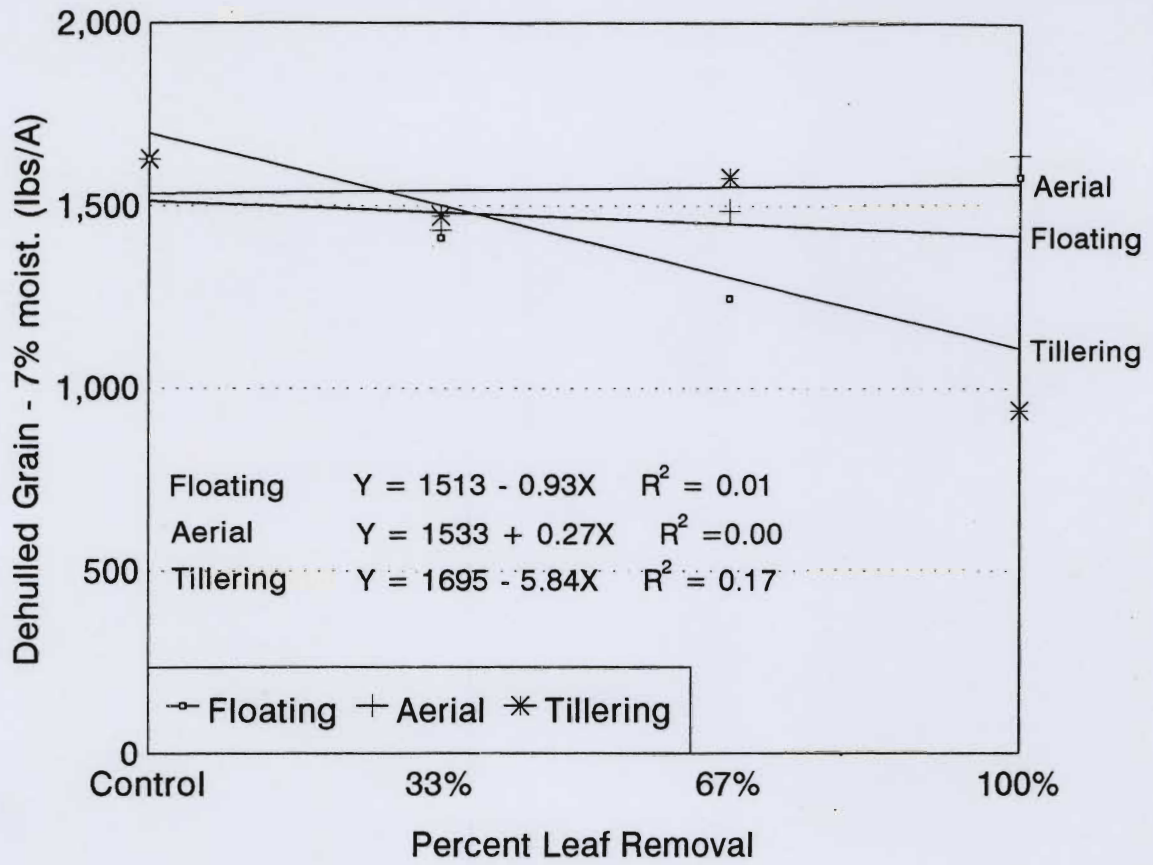Figure 1a. The regression for percent leaf blade removal and dehulled grain yield per acre when leaf blade removal occurred at the floating, aerial or tillering growth stages of wild rice in 1995.
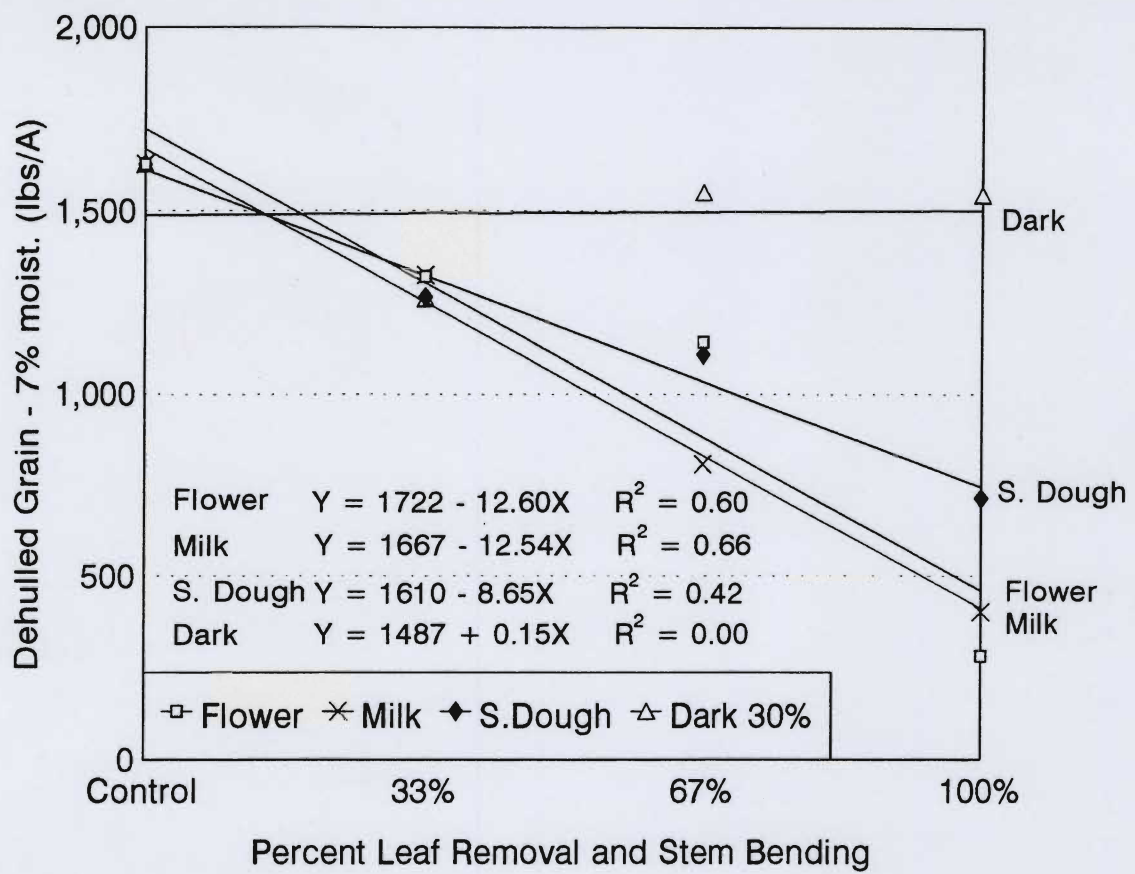
Figure 1b. The regression for percent leaf blade removal and stem bending and dehulled grain yield per acre when leaf blade removal occurred at the flower, milk, soft dough and dark seed growth stages of wild rice in 1995.

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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, 1981, 1994-95 (last 2 in 1980). Plants in independent plots were beat with a beating tool at the last 4 stages of growth, 1994-95. Grand Rapids, MN.

Growth Stage	Leaf re-moval	Grain yield at harvest					Grain yield reduction compared to control				
		1995	1994	1981	1980	Ave.	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	3399	1600	752	1216	1742	12	(14)	29	17	11
	67	3411	1641	888	1423	1841	12	(17)	15	3	3
	100	3829	1738	497	1335	1850	1	(24)	52	9	10
Tillering	33	3386	1846	872	1415	1880	13	(32)	17	3	0
	67	3597	1545	783	1508	1858	7	(10)	25	0	5
	100	2329	1159	648	867	1251	40	17	38	41	34
Flowering	33	3461	1520	800	1482	1816	11	(9)	23	0	6
	67	2788	1176	897	1116	1494	28	16	14	24	20
	100	928	420	272	288	477	76	70	74	80	75
	Beat[c]	2377	1093	--	--	1735	39	22	--	--	30
Milk	33	3237	1117	640	1415	1602	16	20	39	3	20
	67	2353	1090	672	994	1277	39	22	36	32	32
	100	1464	575	328	831	800	62	59	69	43	58
	Beat[c]	1506	695	--	--	1100	61	50	--	--	56
Soft dough	33	3025	1509	880	--	1805	22	(8)	16	--	10
	67	2778	1216	912	--	1635	28	13	13	--	18
	100	2088	772	440	--	1100	46	45	58	--	50
	Beat[c]	1574	250	--	--	912	59	82	--	--	71
First dark[d]	33	2947	1472	808	--	1742	24	(5)	23	--	14
	67	3530	1434	657	--	1874	9	(2)	37	--	14
	100	3572	1427	583	--	1861	8	(2)	44	--	17
	Beat[c]	3084	555	--	--	1920	20	60	--	--	40
Control		3876	1400	1045	1463	--	0	0	0	0	0
LSD 0.05		1003	263	256	585	--	--	--	--	--	

[a] Corrected to 40% moisture.   [b] Grain yield INCREASES compared to control.

[c] Plots beat with weeping willow tree branches to a point of approximately 50% leaf defoliation.

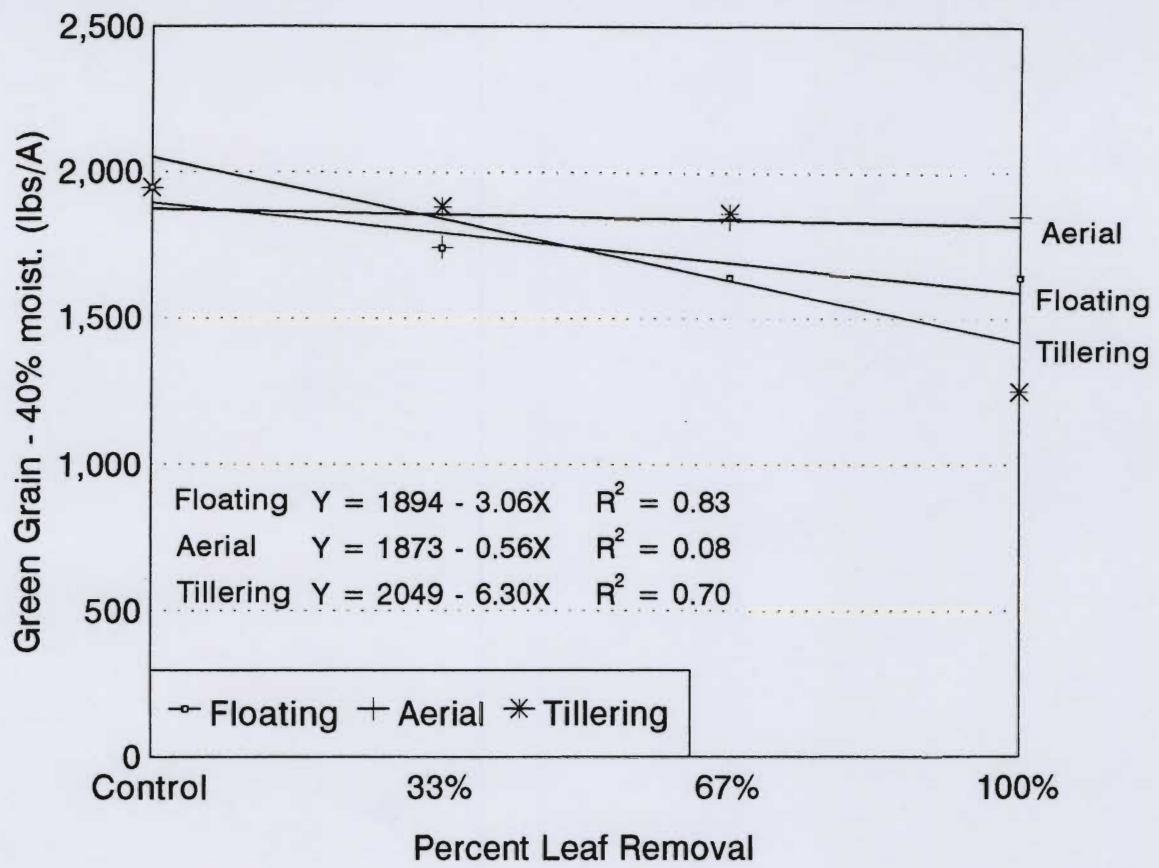[d] Data from 1994 and 1995 were taken at 30% Dark.

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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 average of grain yields for the years 1980-81, 1994-95.
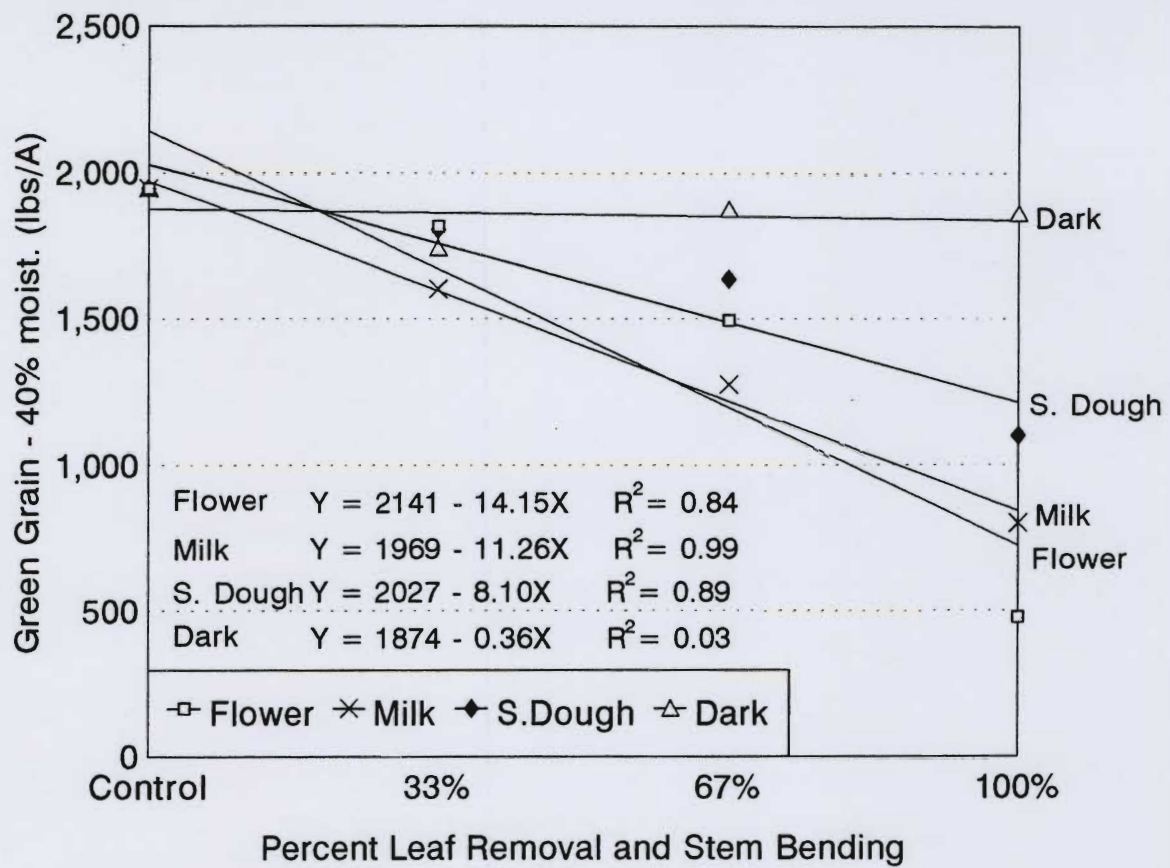
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Figure 2b. The regression for percent leaf blade removal and stem bending and harvested grain (40% moisture) yield per acre when leaf blade removal and stem bending occurred at the flowering, milk, soft dough, and dark seed growth stages of wild rice. The regression was done on the average grain yields for the years 1980-81, 1994-95.

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The "beating" treatments at the last 4 stages were added treatments in 1994. The attempt with this treatment was to more accurately simulate hail injury. In 1995 we used a wood "T" shaped handle which was equipped with wires instead of using a willow branch as in 1994. The 1995 "beater" was developed to have a uniform beating tool for each treatment period, since fresh willow branches were required for each treatment. Significant yield losses occurred at all four growth stages with the highest losses at milk and soft dough growth stages (Table 7). The last three growth stages showed more yield reduction from beating than from the 100% leaf blade removal. One possible reason for this increased effect on yield reduction is that the beating not only removed leaf blade material but it also damaged or stripped the panicles from the stems. The 100% leaf blade removal and stem bending took away much of the ability of the plant to produce photosynthate and deliver it to the panicles, but the panicles themselves were not damaged, leaving the developed grain intact. At the flowering stage, the beating treatment had a lesser effect on yield loss than the 100% leaf blade removal. This agrees with the 1994 results (Table 8) and is likely due to a greater loss of photosynthate production in the 100% leaf blade removal than in the beating treatment. At 30% dark seed, the beating treatment reduced yield significantly more than the 100% leaf blade removal, but not as much as in 1994 (Table 8). It is likely that the largest kernels were dislodged in the beating treatment as seen by the lower recovery rates (Table 7). A possible reason for the difference between 1994 and 1995 is the design of the 1995 beating tool, which delivered more of a focused beating effect by breaking stems and shaking the heads rather than stripping heads of the seed like the 1994 willow branch beating treatment did. Another possibility is since this treatment occurring right before harvest, the stems that were beat down were still picked up by hand harvesting and didn't have a chance to deteriorate before harvest. This may also explain why the beating treatment reduced yield more at the soft dough stage than at the 30% dark seed stage in both 1994 and 1995.

In 1995 stems per square foot and panicles per plant were significantly reduced only at the 100% leaf blade removal at flowering (Table 7). Panicles per plant were significantly higher with 100% leaf blade removal at the aerial leaf stage and at 67% leaf blade removal at the tillering stage. We do not know why these treatments would have higher panicles numbers than the check. Stem number and panicle number were not done on the beating treatments because we determined the plots were too damaged to get accurate numbers from those plots. Plant height was reduced, understandably, for all the 100% leaf removal and stem bending plots after aerial leaf, and for all the beating treatments. Flowering date was not affected by any of the treatments. Percent recovery was not affected until the flowering stage when the 100% leaf removal and stem bending and the beating treatment reduced recovery. At the milk stage, percent recovery was significantly reduced by all treatments except 33% leaf removal and stem bending. At the soft dough stage, only the 100% leaf removal and stem bending and beating treatments reduced recovery. Only the beating treatment reduced the percent recovery when treated just before harvest at the 30% dark seed stage of growth. The lower percent recoveries at these later growth stages from the higher percentages of leaf removal are likely due to the reduced photosynthate which did not allow the grain to fully develop.

In summary, it is very evident from the 4 years that leaf removal, combined with stem bending, even 33% of each leaf blade and 33% of the stems bent, will result in yield loss at the flowering, milk and soft dough stages of growth. The beating treatments with the 1995 beater showed similar losses as when the willow branch was used in 1994, with the

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exception of the 30% dark stage. The willow branches used in 1994 appeared to cause damage and yield losses that reflect that of a hail storm more so than the beating tool used in 1995. During the critical grain filling period, maintaining good healthy leaf tissue and stems is very important in the production and movement of photosynthate (sugars) to the grain.

The data is not as conclusive when leaves were removed before flowering. In 3 of the 4 years yield losses did occur, but in 1994 this was not true. In most small grains (wheat, barley, oats etc.) the plants usually recover from the early leaf losses.

With the 4 years of data we have developed regression equations that should help determine losses from hail. It would be wise to expand this effort to simulate wind losses at the later stages of growth. The Federal Crop Insurance Program will be changing during the next few years, thus having comprehensive information on plant injury and yield loss will be valuable.

Simulated Wind Damage

Introduction: The cultivated wild rice crop is now eligible for all peril federal crop insurance. Thus it is imperative to assess the amount of grain loss that can occur from winds without hail.

Materials and Methods: Wild rice, variety 'Franklin', was planted with a cone plot planter on May 24, 1995, 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 6 times. Before planting, the plot area was fertilized with 75 lbs/A of N, 40 lbs/A of K, 3 lbs/A of boron, and 20 lbs/A of sulfur on May 8, 1995. On May 22, the plots were fertilized with an additional 75 lbs/A of nitrogen due to heavy rains experienced after the May 8 application. A June 13 soil test showed over 100 lbs/A of available nitrogen. Plots were not top dressed with nitrogen. Plant population was approximately 1.5 plants per square foot. The plots lodged some from the storms of July and August.

To simulate wind damage, a backpack leaf blower (Toro - 40cc Power Blower) was used at full throttle to blow on the plots for a five minute period. At full throttle, the wind speed of the blower varied from 75 mph at 1 foot, to 44 mph at 2 feet, to 28 mph at 3 feet from the end of the hose with the plots receiving an average speed of roughly 30 mph. Since the plots were oriented in an East-West direction and a south wind prevailed each treatment day, we approached each plot with the blower on the south side of the plot. We blew half of the plot at a time, ie., first the east half and then the west half. A waving motion of the blower hose from side to side was used to direct the wind across each 5 foot portion of the plot. This waving motion caused the panicles to move back and forth and beat against each other, causing shattering. A "deflector", made from a 4 x 8 foot piece of plywood attached to stilts, was used to protect the adjacent plots and to catch dislodged seeds. This deflector was positioned on the opposite side of the plot from the blower. The deflector was built so the top of the plywood was about a foot higher than the plants. It was placed next to the outside row and angled away from the plot so the wind created by the blower would pass over the top. The deflector was fitted with a seven inch band of window screening material around the edge of the plywood to keep seed from being blown past the side. Screening was also placed from the bottom of the plywood down to a 4 inch by 10 foot trough which was used

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to catch seed that flew off during the treatment. The trough was positioned about 10 inches above the water. To determine natural losses, a 4 inch by 8 foot trough was placed in the center of the plot from flowering to harvest. The treatment dates were at the soft dough, 8-29; and 30% dark seed stages of growth, 9-7.

Results and Discussion: Harvested yield (dehulled grain) when the plants were subjected to the wind treatment at 30% dark growth stage was reduced 24% compared to the control (Table 9). Harvested yield from wind treatment at the soft dough growth stage was similar to the control showing no reduction in yield even though grain losses recovered during the wind treatments were significant from the control on both treatment dates. Loss from the 30% dark treatment date was over four times greater than the loss from the soft dough treatment date. Natural loss, which was the portion caught in the 4 inch by 8 foot trough before treatments were made, was similar. The estimated total yield was significantly lower from the 30% dark treatment date compared to soft dough treatment dates, yet neither treatment differed from the control.

The actual wind (natural) was measured during the grain fill duration. A high wind speed of 47 mph was recorded on July 13 (Figure 3). During August (Figure 4) high wind speeds up to 23 mph were recorded, while in September high wind speeds of 20 mph were recorded (Figure 5). The natural losses probably occurred from these winds.

As a preliminary study, we conclude that the simulated wind treatment done at the 30% dark growth stage did cause significant losses, probably since the abscission layer was not disintegrating yet on the immature seeds. However, the wind treatment at the soft dough stage did not result in losses. Further study is necessary to determine losses from different wind speeds and different durations of wind. It is likely that most losses will occur in the first five minutes, but this needs to be established. The techniques used in this preliminary study could be improved by perhaps utilizing a wind tunnel. We are encouraged that we can determine wind losses by artificially subjecting plants to wind.

Table 9. Influence on yield of a five minute 30 mph wind treatment on wild rice at the soft dough and 30% dark seed growth stages -Grand Rapids, MN - 1995.

| Treatment	Green Grain - 40% moisture				Dehulled Grain - 7% moisture				Percent Recoveries		
	Hrvst yield	Treat. losses	Natrl losses	Est. Total	Hrvst yield	Treat. losses	Natrl losses	Est. Total	Hrvst yield	Treat. losses	Natrl losses
	lbs./A	lbs./A	lbs./A	lbs./A	lbs./A	lbs./A	lbs./A	lbs./A	%	%	%
S. Dough	2974	132	324	3430	1464	57	102	1623	49.2	42.9	30.4
30% Dark	2114	609	366	3089	1019	292	116	1427	48.2	47.9	31.8
Control	2731	0	409	3140	1341	0	123	1464	49.1	---	29.4
LSD (0.05)	360	92	112	337	182	44	46	171	0.6	---	5.5
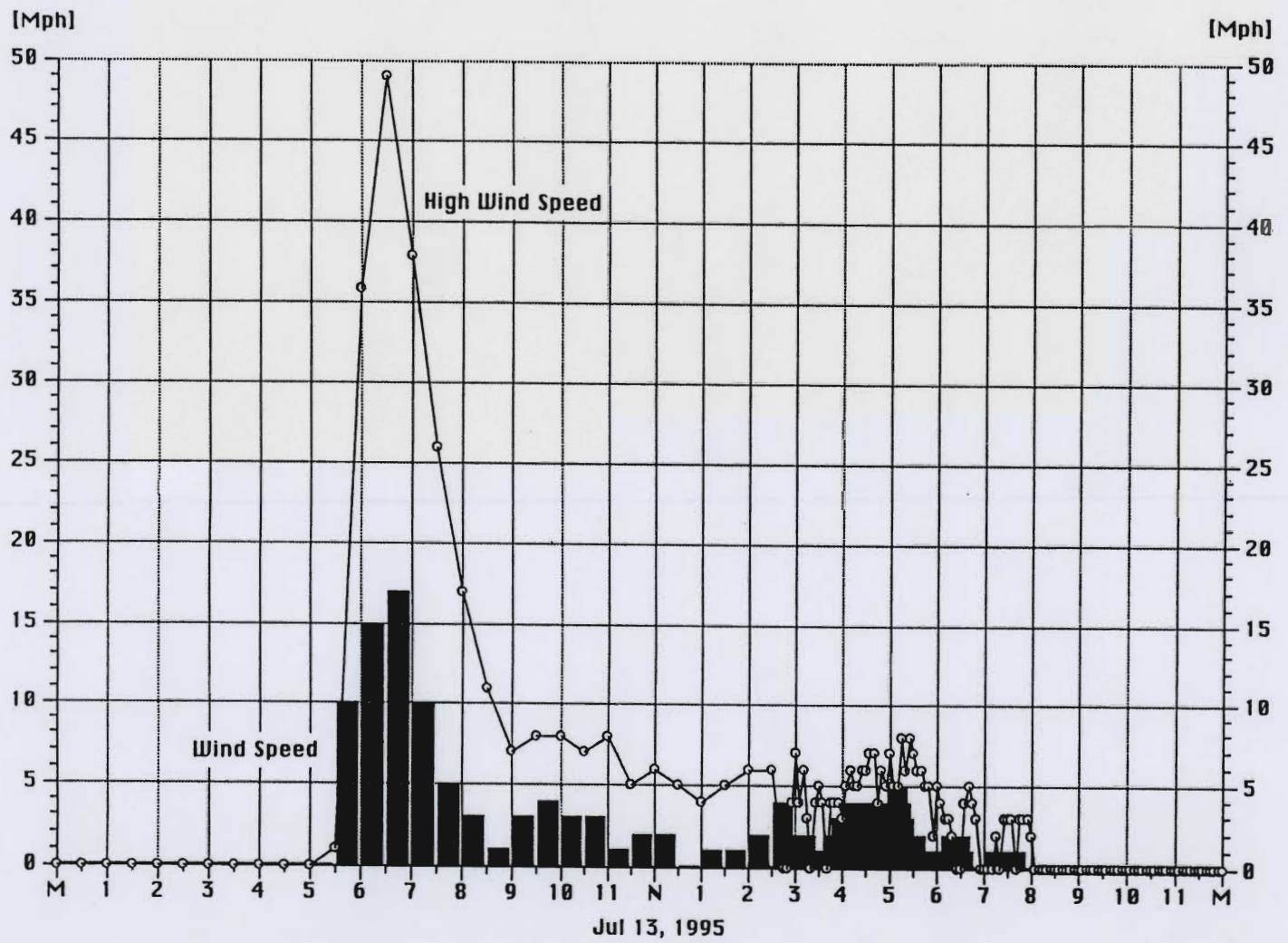
Figure 3. The highest wind speed and the average wind speed for each half hour on July 13, 1995 at the Grand Rapids, MN, wind study plots.
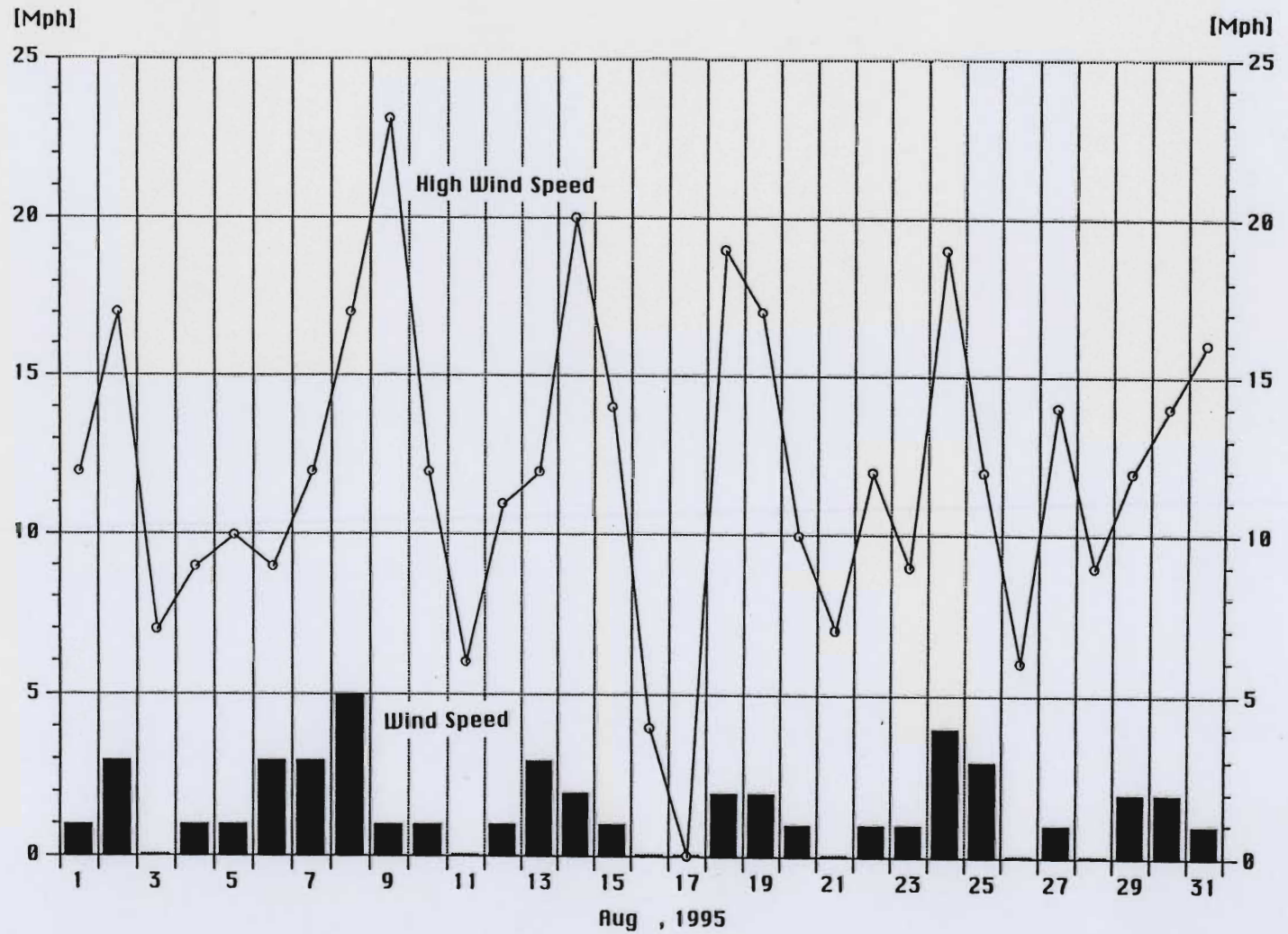
Figure 4. The highest wind speed and the average wind speed for each day during August, 1995 at the Grand Rapids, MN, wind study plots.

[Mph]   [Mph]

25

20   High Wind Speed

15

10

5

Wind Speed

0

1   3   5   7   9   11   13   15   17   19   21   23   25   27   29   31
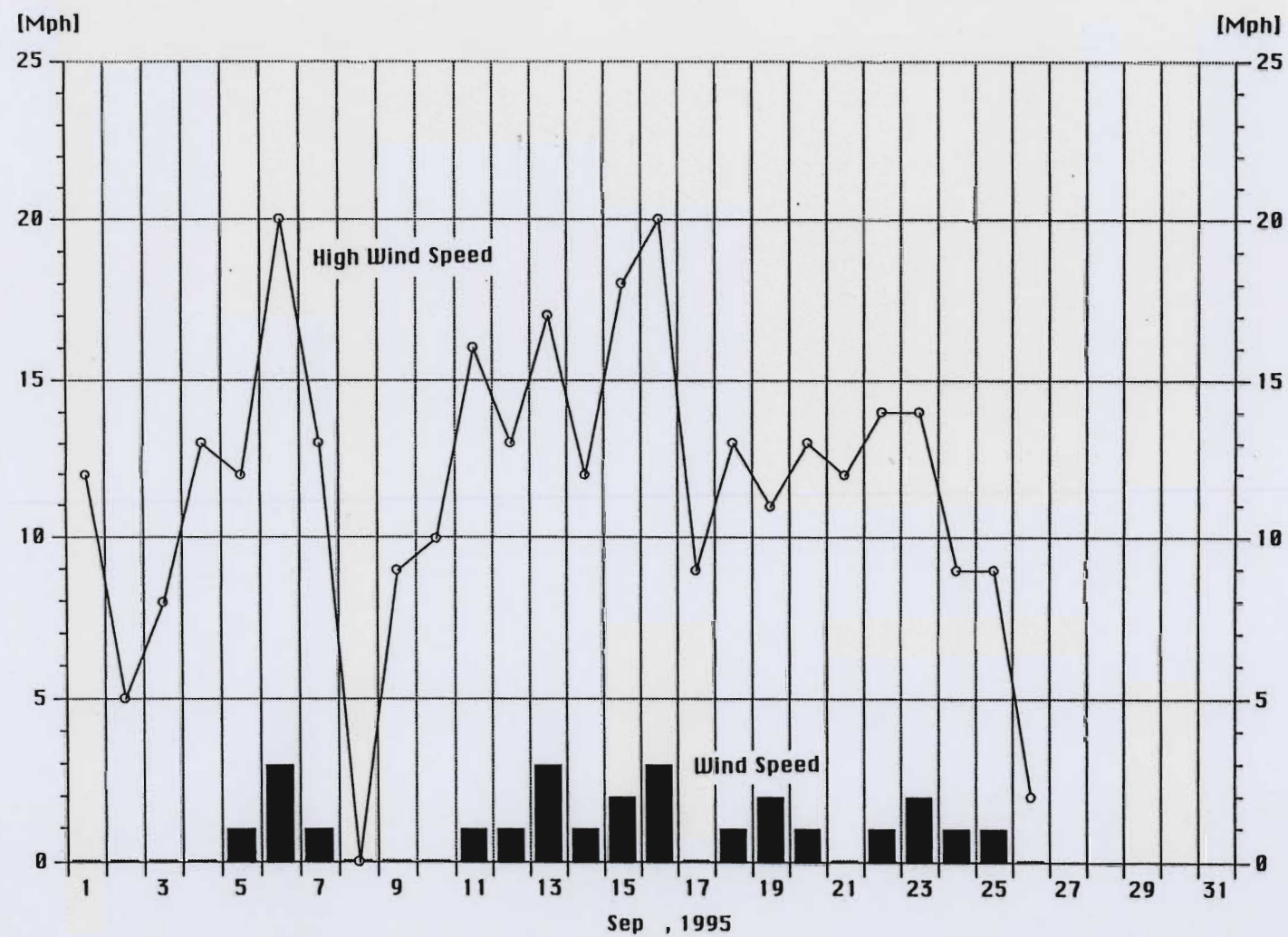
Sep   , 1995

Figure 5. The highest wind speed and the average wind speed for each day from September 1
through September 26, 1995, at the Grand Rapids, MN, wind study plots.

Acknowledgement

Nitrogen Mineralization and Availability in Flooded Peats

Paul R. Bloom and Deepa de Alwis[1]

The nitrogen taken up by wild rice plants consists both of fertilizer N and N mineralized from plant residues and other organic components in the soil. In previous studies we have generated information about the factors governing the loss of fertilizer N and information concerning the quantities of N taken up by plants. If we are to develop better predictions of the response of wild rice in peat to different alternatives of N fertilizer management we need to have a better understanding of the rates of mineralization of soil N and the contribution of plant residues to mineralization. Also we need to know if there are management techniques that can be used to increase the contribution of mineralized N to crop production. In 1995 most of our effort was concentrated on developing a laboratory and greenhouse method to evaluate N mineralization rates in flooded peats. However, we have continued our program of monitoring of N status in production paddies, including the monitoring of ammonium N in soils (readily available N) as well as leaf color using the Minolta 502 chlorophyll meter. We also evaluated a color book to aid in the visual estimation of leaf color. In addition we began a program of monitoring total and soluble phosphate P in paddies and drainage ditch water. The P monitoring is part of an effort to better understand the factors that result in algal blooms in paddies and to get a better idea of the contribution of paddy drainage water to P in lakes and streams.

Effects of Crop Residue Incorporation, Liming, and Temperature on Nitrogen Mineralization in Peat Soils.

A laboratory and greenhouse study of the influence of crop residue and liming on N mineralization rates in flooded soil at different temperatures is currently being conducted. An acid peat, pH 5.5, was collected from a paddy near Aitkin (Vomela Wild Rice) in the fall, after harvest and tillage; then frozen. Crops of wild rice, barley, lana vetch, and berseem clover were grown in 5 gallon buckets in the greenhouse to simulate the growth of wild rice or crops that can be grown in rotation with wild rice. Two additional treatments, black fallow (drained) and flooded fallow, were maintained during the "growing season" in the greenhouse. The wild rice was top dressed twice with N fertilizer (30 lb/ac) during the growing period. The wild rice grew well, although the plants were not quite as vigorous as a top production crop in a paddy. The barley also grew well but the biomass production by the clover and the vetch was disappointing. The water was drained from the wild rice during late grain fill and the grain was harvested. The barley grain was also harvested and for all of the crops, the remaining biomass was dried and chopped into 1/4-1/2 in. lengths and incorporated into the soil. The soil was then incubated for 6 weeks to simulate fall conditions. After a period of freezing, samples were taken for the determination of mineralization

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rates.

Small samples (containing 5.0 g dry wt of soil) were placed glass tubes, water was added to flood the soil, and the tubes were sealed air tight. Lime was added, as calcium oxide, to some of the treatments to raise the pH to 7. The quantity lime (0.080 g per tube) is equivalent to 5 tons per acre of ground limestone (calcium carbonate) added to the plow layer. The oxide form was used in the experiment rather than the carbonate form because the oxide form dissolves more readily. In one of the treatments Milorganite equivalent to 1000 lb/ac (0.015g per tube) was added to evaluate its potential as a slow release fertilizer. Milorganite is a product of the sewage treatment plant in Milwaukee, Wisconsin and is used in upland crops as a slow release fertilizer. The mineralization rates for the black fallow and wild rice treatments , with and without lime, were determined at 43, 54, 64, 75, and 86 °F over 42 days (only 35 days of data available at the time of this report). All the other treatments were incubated only at 75 °F. Periodically samples are taken and the ammonium (NH_4)was extracted using KCl.

The NH_4 mineralization data are presented in Figures 1 and 2 in units of mg/kg of soil (ppm). The release in terms of lb/ac for the plow layer can be estimated by multiplying by 0.4. All treatments showed an a rapid initial period of NH_4 production that was essentially complete in less than 10 days. This was followed by much slower release of N. As expected the at higher temperature the release of NH_4 was more rapid. What was not expected was the dramatic response to liming, and very significant release of NH_4 for wild rice straw treated soils. The lime generally caused about a doubling of the quantity of NH_4 released with and without wild rice straw incorporation. This is contrary to the statements in the rice literature concerning the minimal effect of pH on N mineralization. The incorporation of wild rice straw in the soil resulted in a great increase in available N compared to the black fallow treatment. This is contrary to the experience of growers which suggests greater N supply to a crop following a fallow period compared to continuously cropping. One of the main differences between our procedure and the practice of growers is that the wild rice straw was cut into very small pieces and evenly distributed on the soil. Wild rice straw has a low C/N ratio compared to other small grains, like barley, and incorporated wild rice straw should mineralize N more rapidly than straw from other grains.

Of the vetch, clover, and barley, the barley contributed to most to N mineralization because of the much greater biomass added to the soil. The vetch and clover did not produce much biomass. The barley biomass was high but because of the low C/N ratio it contributed less that 1/2 the N mineralized by wild rice straw after 35 days at 75 degrees.

The wild rice treatment with Milorganite mineralized about 35 mg/kg (14 lb/ac) more N than the wild rice treatment after 35 days at 75 degrees. This is much less that we were hoping for from the Milorganite which added 60 lb/ac of N. We were also surprised to see that the flooded fallow treatment mineralized about twice as much N as the black fallow treatment. This needs to be investigated further to see if management of a fallow that involving a period of flooding might result in an increased N supply to the following wild rice crop.

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Figure 1. Ammonium N mineralization in a flooded peat, with and
without incorporation of wild rice straw. Without lime the pH
of the peat was about 5.5 and with lime the pH was 7.0.
(WR = Wild Rice straw incorporated; BF = Black Fallow)

Temperature 43 F

Temperature 54 F

Figure 1. continued

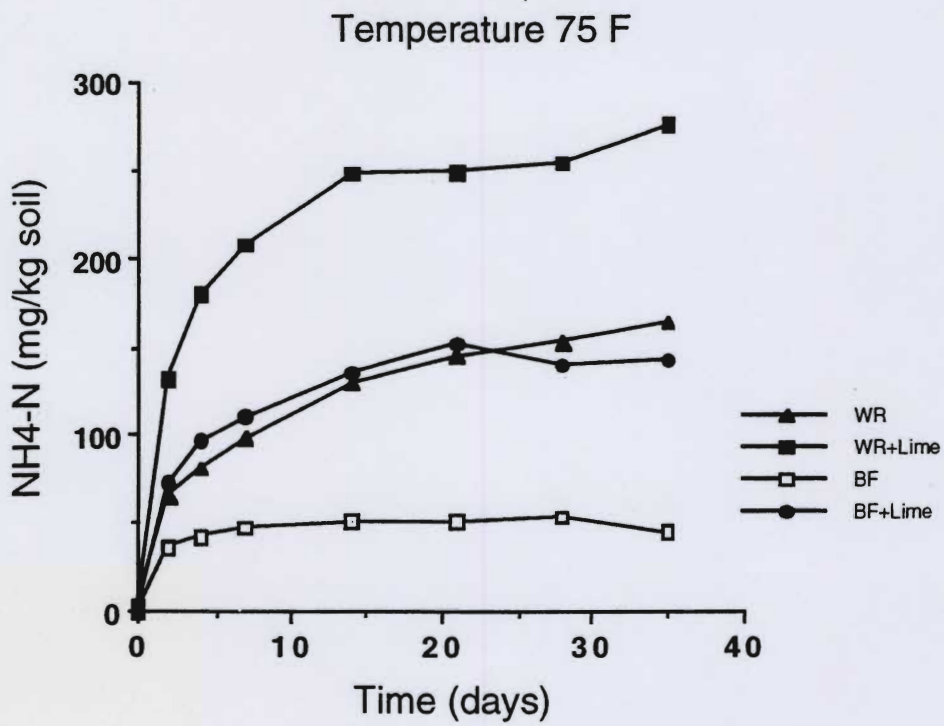## Temperature 64 F

Temperature 75 F

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Figure 1. continued

Temperature 86 F
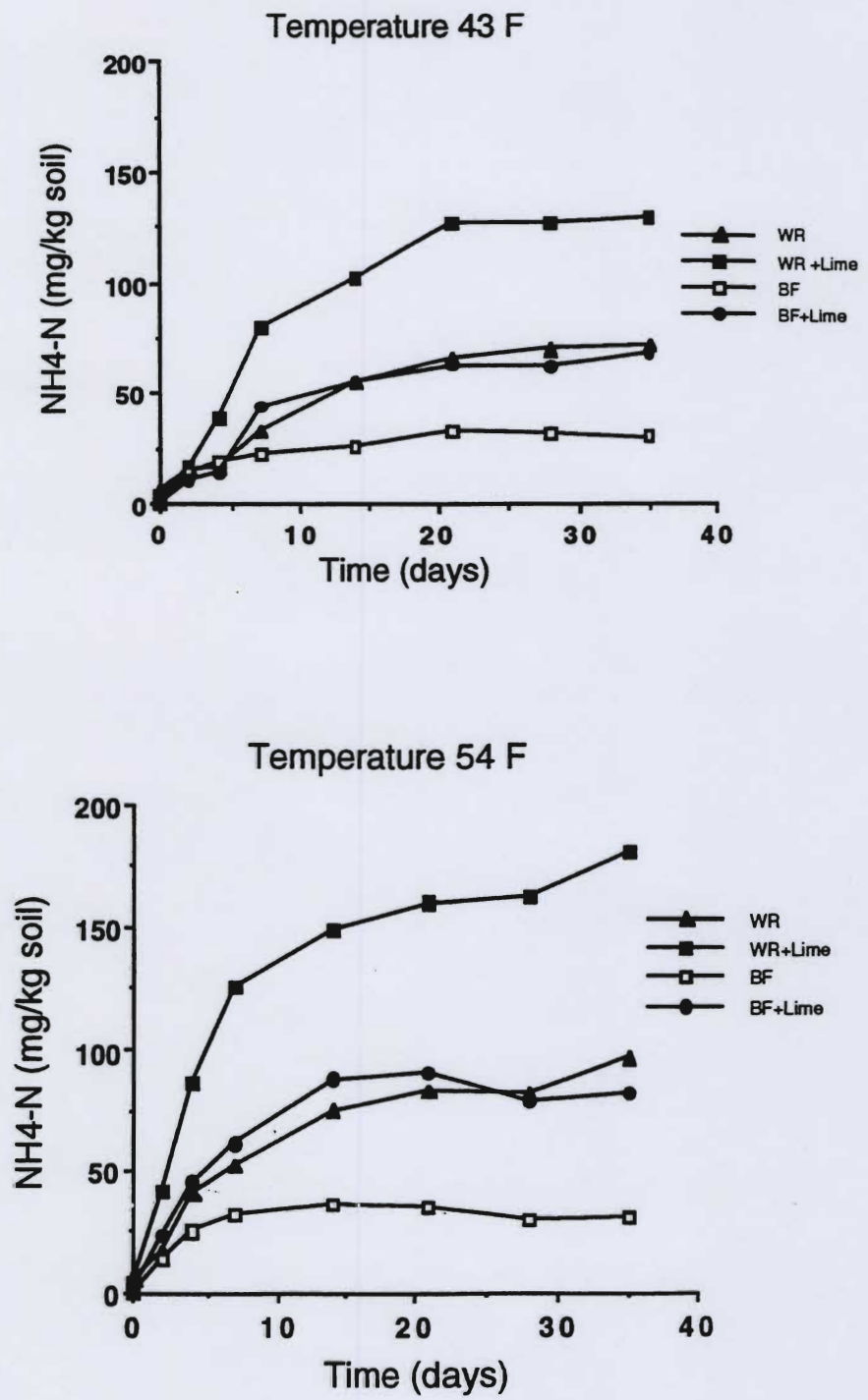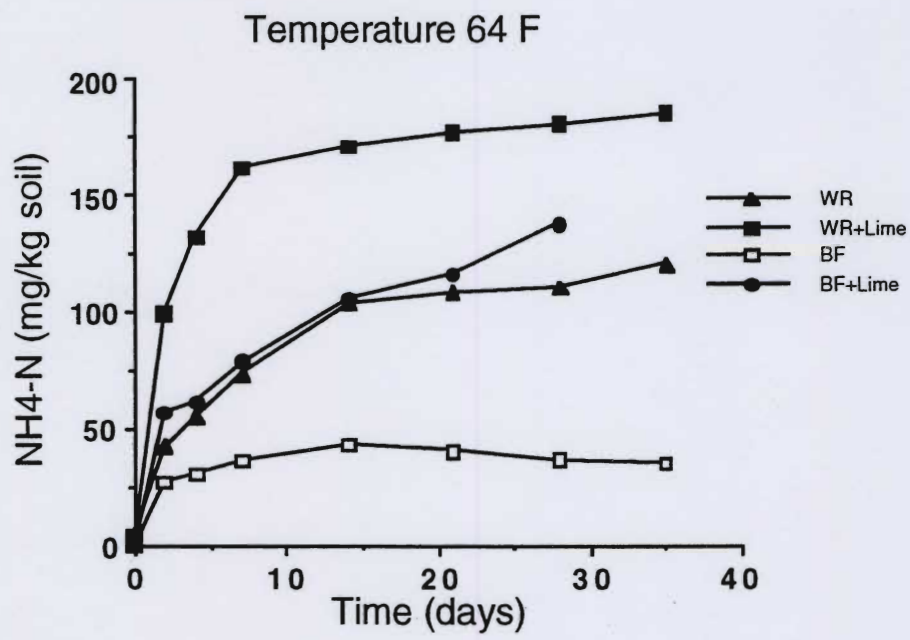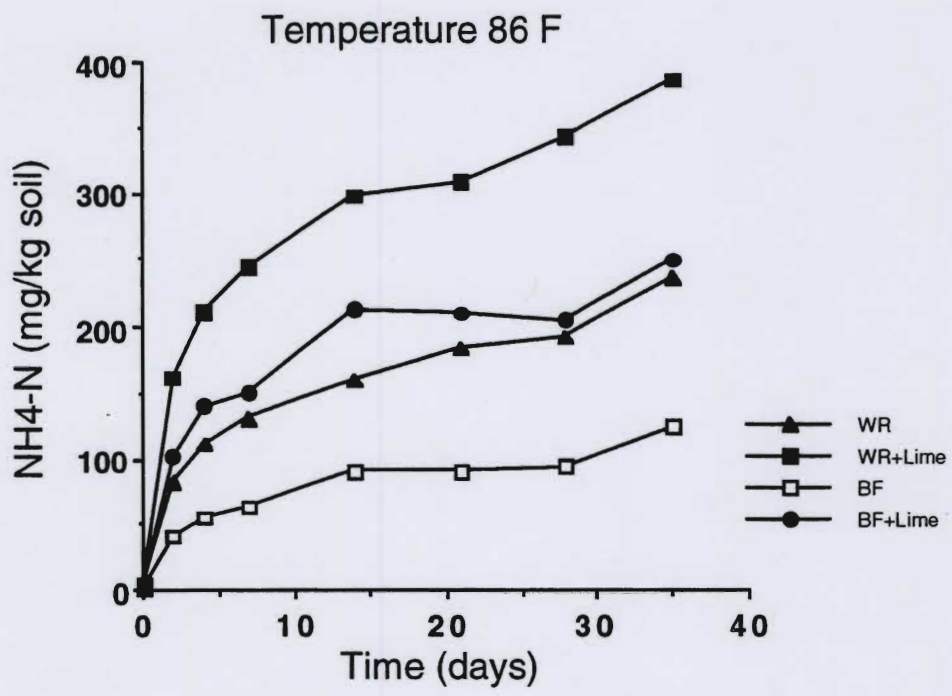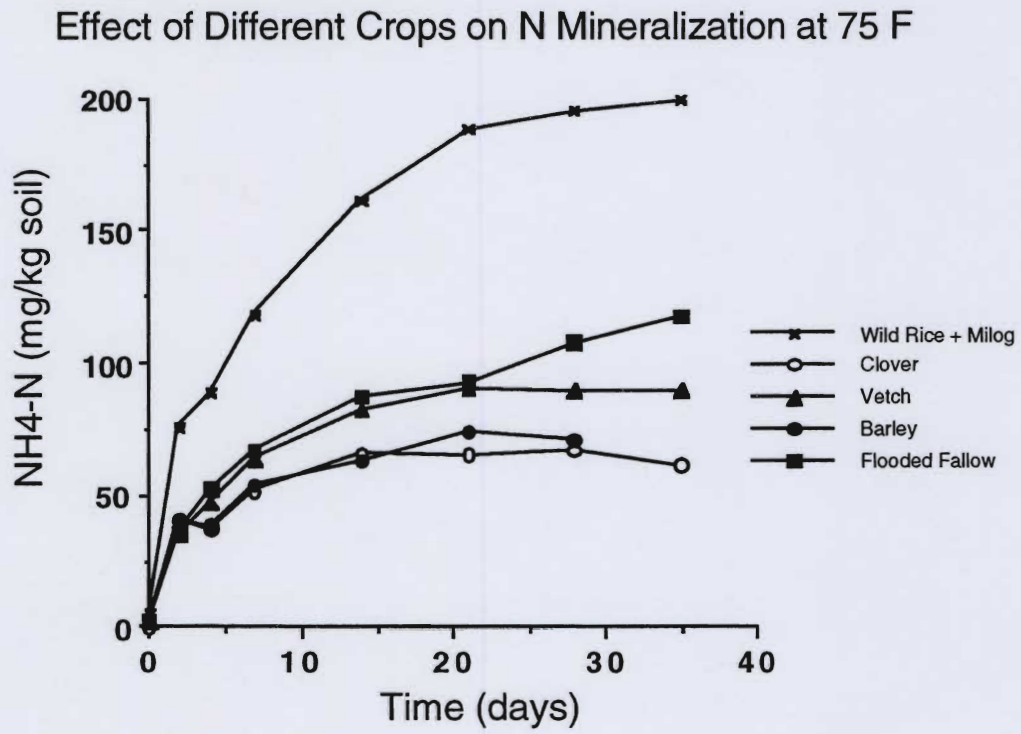
Figure 2. Ammonium N mineralization in flooded peat (pH =5.5) with crop residues added.   Also included are treatments with milorganite added to wild rice residue and a flooded fallow.

Effect of Different Crops on N Mineralization at 75 F

Comparison of N mineralized after 35 days after converting to approximate values in lb/ac is useful for gaining an understanding of the relation of the quantities of mineralized N to crop requirements. At 75 degrees the quantity mineralized by the black fallow treatment amounts to about 20 lb/ac, with almost all of the mineralization during the first 5 days. With liming this increases to 55 lb/ac. The flooded fallow treatment under the same conditions mineralized about 45 lb/ac, without liming. The wild rice treatment in the acid peat mineralized 65 lb/ac which increased to 115 lb/ac with liming.

The preliminary analysis of the data suggest that the liming of acid peats may contribute to N availability. The low rate of mineralization of in acid peats may be one reason for the continued difficulty in getting high production from some of the paddies in the Aitkin area where acid peats are common. In the past we have been hesitant to recommend liming because plant growth is not directly affected by acidity and because in peats that are not well decomposed liming can lead to gas production and "floating peat". However, in peats that have been cropped for many years there should much less danger of excess gas production. The quantity of lime used in the current study, which corresponds to 6 tons/ac, may be more than is needed to get the response we observed. More experimental studies will be needed to determine how high the pH has to be to get maximal mineralization of N.

The contribution of wild rice straw to mineralization in these experiments suggests we need to look more closely on how to utilize this N in wild rice production. Possibly with finer field chopping and better incorporation more of this N might be made available for wild rice growth.

Monitoring of Soil N Status and Plant Color in Production Paddies

Soil ammonium-N and SPAD readings of plants were determined in peat paddies in the Aitkin (Manomin Development Co.), Waskish (Rennemo) and Clearbrook/Gully areas (Clearwater Wild Rice and Imle-Gunvalson). We also sampled experimental paddies on mineral soils on the North Central Experiment Station at Grand Rapids (Table 1). In addition the new color book developed by Don Barron for assessing plant color was evaluated. All the production paddies were fertilized in the fall, but the experimental paddies were fertilized in the spring. The first measurements were made in mid June at a time when the plants were in the early stages of growth, varying from the floating leaf stage to a few leaves out of the water. At this stage little N has been removed from the soil by plant growth and the quantity of N measured is representative of the N carryover from the fall application of fertilizer and the quantity of N mineralized early in the season.

Generally, we found that the quantity of N in the soil is higher than we have seen in earlier years for early season N (Table 1). This is because many of the paddies were flooded in the fall. The effect of fall flooding was especially apparent on the Rennemo farm where all of the paddies sampled were flooded within 2 days of application of the N fertilizer. As we have seen in previous seasons, the best preservation of fall applied N is when the flooding is done within a few days of fertilizer application. If fall flooding is delayed, especially if the soil is warm, as was the case in the fall of 1994, considerable losses can occur.

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Table 1. Potassium Chloride extractable soil N, SPAD meter readings and color book readings on growers paddies.

Farm	June 13-15 N lb/ac	SPAD	July 13 - 14 N lb/ac	SPAD	Color Bk	July 29 N lb/ac	SPAD	Treatment
Manomin								
AF	82		0	52		9	48	60 lb. Urea + urease inhibitor
AM	41		14	53		20	44	1000 lb. milorganite
BF	35		1	49		8	43	
CM 1	31		13	48	43	4	42	1000 lb. milorganite
EF	40		4	53	43	5	48	30 lb. urea
Rennemo								
V7	105							60 lb. urea plow down, FF
V8	89							55 lb. NH$_3$, FF
V11	80		6		30			60 lb. NN$_3$, 1st yr. paddy FF
D3			2	38	30			60 lb. NH$_3$, FF
D4			1	42				60 lb. NH$_3$, FF
Clearwater								
7	44	30						Urea, FF
14	35	35	1	36	28			FF
22	51	33	1					Following Potatoes, SF
25	20	36			46			FF
Imle-Gunvalson								
4-6	82	35			46			Urea, FF
9-5	19	39			43			Fall plow, SF
16-1	55	33			46			NH$_3$, FF

Table 1. Potassium Chloride extractable soil N, SPAD meter readings and color book readings on growers paddies.

| Farm | June 13-15 | | July 13 - 14 | | | July 29 | | Treatment |
	N lb/ac	SPAD	N lb/ac	SPAD	Color Bk	N lb/ac	SPAD	
Grand Rapids (Mineral Soil)								
2-1	66							
2-2	78							
4-1	113							
4-2	162							
4-3	64							
5-1	25		17	42	40			
5-2	209		4	43				
5-3			17	48				
9	69							
169-1	215		12	46	44			
169-2	172							

FF = Fall Flooded, SF = Spring Flooded, NH_3 = Anhydrous ammonia

We had some difficulty sampling the mineral soils on the North Central Experiment Station. A strong plow pan has been developed in these soils at less than the 6 in. depth. The sampling (and probably the fertilizer application) was to only 3 in or less for some samples. Our calculations of available N from the NH_4 extraction data relies on an assumption of a 6 in. depth for N application and sampling. The shallower depths resulted in measured values for some samples of very much more than the 75 lb/ac applied (e.g. samples 4-1, 4-2, and 5-2). The very high values determined in 169-1 and 169-2 were not because of shallow sampling depths. The soil at that paddy is quite sandy and easy to sample. A few years ago this soil was treated with a very heavy application of manure which may have contributed to high mineralized N.

The low value for sample 5-1 was because this part of the paddy is higher than the other parts of the paddy and early in the season was not well flooded. Because of the dry conditions the NH_4-N was oxidized to nitrate and lost when the paddy was flooded to a greater depth.

The early SPAD measurements taken mid June did not appear to be very useful. We did measure the most mature leaves but at this early growth stage the readings varied widely and did not appear to correlate with the available soil N.

The July sampling on growers fields was done after one top dress had already been applied. Even with the addition of fly-on N the soil NH_4 was low, demonstrating the effectiveness of plant uptake and loss mechanisms in drawing down the soil NH_4. The highest soil N was on the Milorganite treated fields for which the only fall applied N was in the form of Milorganite. This may reflect the mineralization of Milorganite NH_4. All of the soil N measurements suggested at least one additional top dress of N was needed to get a top yield. The very high N in the 169 paddy at Grand Rapids was reduced by the great vegetative growth of these pistillate plants. This paddy is the first we have seen in our N monitoring studies in the last 4 years where there was excessive vegetative growth. When the winds came in August this paddy had severe blow down.

The SPAD readings in July (flag leaves) were made on plants in the early flower growth stage, except at Grand Rapids where the plants were in late boot. The readings were higher on the Manomin farm than at the other sites. We have seen this before and think it reflects varietal or environmental deference that results in thicker leaves on the Manomin farm. Most of the measurements suggest that N deficiencies were not common. The only readings that suggested deficiency were the few values less than 40 (Table 1).

The yields on the Manomin farm were much less than suggested by the good growth throughout most of the season. In the last few weeks before harvest there was a rapid decline in plant health. Tom Godward reported similar observations on his nearby farm. Part of the problem seems to be related to low plant K and insufficient N. Tom Godward reported on some of his mineral paddies the paddies where rice followed 3 years of clover yielded well. This suggests the N mineralized from the clover residue contributed to the good yield. Art Hedstrom reports low K analyses for plant tissue from some of the Manomin paddies. Another problem may have been the high infestation of stem borers common to the Aitkin area in 1995.

Plant N Status using a Color Book and Visual Assessment

An alternative to the SPAD meter was proposed by Don Barron of Clearwater Wild Rice. He reasoned that as, with the determination of soil color by soil surveyors, the green color of wild rice leaves might be determined by matching the color of a wild rice paddy to the color of paint chips. If the paint chips are calibrated to readings determined by a SPAD meter the color readings would be similar to SPAD readings. Because the color chips available at paint stores cannot match the subtleties of differences in green seen in wild rice plants Don had to painstakingly mix paints to match the colors of a set of wild rice leaves with known SPAD readings. The paint was applied to pages of a small loose-leaf book for handy use in the field. We searched for color standards to use instead having to mix paints and found that the subtleties of green in wild rice leaves is not even reflected in complete color systems (e.g. Munsell system) published by the color experts.

The method proposed by Don is to hold the book in line with the vision of a paddy and to match a page to the color of the paddy. One advantage of this method is that the color of the surface of a leaf is assessed rather than the quantity of green light that can be penetrate though a leaf, as for the SPAD meter. The problem with the SPAD meter method is that readings are different for leaves of the same color but of different thicknesses. We have found that year after year the SPAD readings at the Manomin farm are higher than at other farms for plants of the same N status. Apparently, because of varietal differences or environmental differences, the leaves of the plants on the Manomin farm are thicker. Another advantage for the color book method is that the assessment is for a large section of a paddy rather than plant by plant, and the measurement can be made from the dike. A disadvantage is that the readings can be only made to plus or minus of about two SPAD units

We tried comparison of the of the SPAD meter and the color book in mid July (Table 1). We found it is best to assess a paddy with he sun at the back of the observer. With some practice readings are quite reproducible with some differences between individual observers but the greater the experience the less the differences between individuals. As expected the readings with the book at the Manomin farm were lower than the SPAD readings. Also, we found that for plants with SPAD readings of 38 or less, color book readings are lower than the SPAD reading. When we tried to use the color book in late July we found that we had difficulty getting good readings because of the color of the flowers interfered with an attempt to read the color of the leaves.

We conclude that the color book method is a very useful aide to assess leaf greenness. The readings are much easier to get than SPAD readings and may be more comparable from farm to farm than SPAD readings, although the readings with the color book are more approximate. We did not assess the change in color book readings with growth stage but because the color book reading is not related to leaf thickness we expect the color book to vary less with growth stage. The use of the book takes some practice but it is not difficult. It is certainly a better method of assessment than using an unaided eye. As with the SPAD meter the reading should be compared to reading from a field with sufficient fertilizer. The rule of thumb from the SPAD literature is that paddies with readings less than 95% of that for an

adequately fertilized paddy are in need of N fertilization.

Phosphate in Paddy Water and Drainage Ditches

Phosphate (P) is of concern in wild rice because P in solution is important in the development of algae (algal blooms) and because of the environmental concern for P discharge into lakes and streams. We obtained water samples from paddies and drainage ditches in both mid July and late July (Table 2). Two types of P were determined; total P and soluble P. Total P is determined after acid digestion at high temperature to dissolve the P in suspended solids. Soluble P is determined after filtering with a 0.45 micrometer filter to remove suspended solids, without acid digestion. Soluble P is the P readily available for algal growth. The P represented by the difference between total P and soluble P represents the potentially available P. Soluble P data are only available for the Manomin farm in late July.

The highest value measured was for a drainage ditch mid July after several days of rain. The water in the ditch was quite high in suspended solids as might be expected when some erosion is occurring. This likely represents the a near maximum value for the sampled ditch.

The highest values of total P in paddies were about 0.4 ppm. Values of greater than about 0.1 or 0.2 ppm total P would normally be considered eutrophic (high nutrient status) and if the water is warm enough algal blooms can result. A better indication is the soluble P. Algal blooms can occur at >0.05 ppm P, especially at > 0.1 ppm. Because of the early warm water temperatures in the 1995 season many growers had more problems than usual with algae.

The fragmentary data suggest that in general the P status of paddy waters is near to or above the concentration that can result in algal blooms. If the temperature of the water is high early in the season before the canopy closes and blocks the light falling on the water, algal blooms can occur. One of the methods of minimizing algal blooms is to refrain from applying P fertilizer if it is not called for by a soil test.

The concentration of total P contributed to lakes and steams by agriculture is a growing concern. Limits on total P concentrations allowed in return water from paddies have not been set, but it is likely that the concentrations we measured in paddies of 0.4 or less will not concern regulators. However, the higher values that may occur in a ditch after rainfall may or may not be a concern. These concentrations of total P (>0.5 ppm) are at the level that has raised concern in water draining agricultural land along the Minnesota River. However, a concentration of 0.67 ppm is much less than that allowed for discharge from a sewage treatment plant. We will continue to monitor P concentrations in paddies and ditches.

Summary

1. Preliminary analysis of the laboratory N mineralization data suggest:
   • liming of acid peats will increase N mineralization rates,
   • wild rice straw has the potential for contributing very significant quantities of mineralized N,
   • flooding during the fallow period may increase N mineralization.

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Table 2. Total and soluble phosphorus concentrations in paddy and drainage ditch water.

Farm	July 13-14 Total P (ppm)	July 29 Total P (ppm)	Soluble P (ppm)
Manomin			
AM paddy	0.21	0.17	0.06
AF paddy	0.06	0.13	0.07
EF paddy	0.02	0.22	0.06
BF paddy	0.04	-	-
CM paddy	0.07	0.05	0.06
Rennemo			
Main drainage ditch	0.67		
Clear Water			
6 Drainage	0.39		
14 paddy	0.40		
25 drainage ditch	0.09		
Imle-Gunvalson			
16:1 drainage	0.21		

2. SPAD measurements very early in the growth do not appear to yield very useful information.
3. The color book is a useful tool for a quick easy survey of the N status of a paddy. It does not give exactly the same results as a SPAD meter and is not as precise but it is much better than assessing plant color using the unaided eye.
4. The water in the few paddies sampled contains sufficient P to be of concern for triggering algal blooms of when temperature is high enough.

Acknowledgments

SCAB OF CULTIVATED WILD RICE IN MINNESOTA

R. F. Nyvall, J. A. Percich, R. A. Porter, and C. J. Mirocha

Scab caused by Fusarium spp. is a widespread disease that affects most small grains; however, it has not been previously reported on cultivated wild rice. During l993, a severe epiphytotic of scab caused extensive damage to the wheat crop throughout northern Minnesota. A sample of wild rice seed that was stored dry (20-21% seed moisture) for several days on a laboratory bench after harvest was noticed to have pinkish to red mycelium growing over it. Most wild rice seed is immediately processed or stored by immersion in water after harvest. Other samples of seed that had been frozen immediately after harvest were examined and found to contain bleached or otherwise discolored seed. Fusarium spp. were isolated from both samples. Experiments were conducted during the 1994 and 1995 growing season to determine the importance of scab on cultivated wild rice.

Results

Scab symptoms on wild rice have not been readily noticed. Seed samples were examined for whole seeds that displayed typical scab symptoms common on other small grains, such as wheat. Light brown, bleached, or otherwise discolored and shrunken seed was examined and isolations made. Typical symptoms are a white to light brown color; however, infected seeds do not appear to be shrunken. Some seeds have a light pink discoloration that is apparently due to mycelial growth of Fusaria. Fusarium spp. were isolated from l00 % of seeds that displayed scab symptoms and from 1-26 % of seeds that did not display symptoms.

Fusarium spp. were most commonly isolated from seed at the milk stage and from shattered seed. No Fusarium spp. were isolated from processed seed. The most common species were F. athrophilum and F. graminearum (Table 1). There is no difference in incidence of scab between cultivated wild rice and seed from a natural wild rice stand (Table 2). Fusarium graminearum was commonly isolated from the whole seed, palea and lemma, and caryopsis. Other fungi were not commonly isolated from the caryopsis (Table 3). Fusarium spp. do not survive submersion in water at 4 C and their incidence is greatly reduced when submerged at -20 C (Table 4). There is no difference in incidence of Fusarium spp. between seed from a cultivated or natural source. Most fungi are isolated from the whole seed and palea and lemma at -20 C storage but not from caryopsis. However at 4 C, fungi are readily isolated from whole seed, palea and lemma, and caryopsis (Table 5). Fusarium graminearum is the fungus most commonly isolated from whole seed, palea and lemma, and the caryopsis of seed gathered from natural or cultivated stands. Other fungi are listed in table (Table 6). Koch's postulates demonstrated that F. graminearum is a cause of scab of wild rice seed (Table 7). Propiconazole does reduce the incidence of scab with two applications (Table 8). However the incidence of scab was too low to draw conclusions.

Nivalenol and dioxynivalenol were obtained from F. graminearum cultures but not from the seed sample from which the cultures were isolated.

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Discussion

Scab of wild rice is reported here for the first time. To date, no comparable disease has been reported from white rice (Oryzae sativa L.). The unusually high incidence of scab on wheat in northern Minnesota in 1993 may have accentuated the incidence of Fusarium spp. on wild rice. However, conditions of high humidity that favor the develop of scab on small grains are commonly found in wild rice stands regardless if they are located in a river, lake, or cultivated paddy. Anecdotal evidence suggest the presence of scab symptoms on wild rice from previous years. Therefore, it is likely that scab has been a common disease of wild rice in the past.

Scab may be more responsible for yield losses of cultivated wild rice than previously thought. The high incidence of scab infested seed that shatters suggests this disease may be a primary reason for shattering and a more important cause of yield loss than other diseases. The scab organisms do not survive submersion in water for a long period of time, therefore, inoculum likely comes from outside sources like grass along the dikes or other small grains or crops.

There is no difference in the incidence of scab between seed from cultivated or natural stands. Scab does not appear to be a problem in processed rice as the scabby seed either "shatters" and falls to the soil surface or the causal organisms do not survive the heat of the processing. However, the production of the toxins nivalenol and dioxynivalenol by F. graminearum cultures isolated from wild rice seed suggests similar cautions used in placing other small grains in the human food chain may have to be exercised with wild rice. In a preliminary study, no toxins were found in one wild rice sample. A more extensive survey is needed to determine the presence of toxins in wild rice seed; particularly processed wild rice and wild rice grown in environmental conditions conducive to development of scab.

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Table 1. Isolation of Fusarium spp. from cultivated wild rice seed at different growth stages in 1994 and 1995.

Fusarium spp.	Growth stage[1]				
	Milk	Dough	Ripe	Shattered	Processed
	%[2]	%	%	%	%
acuminatum	0.0	0.1	0.0	0.1	0.0
anthrophilum	14.5	2.0	0.6	21.2	0.0
camptoceras	0.0	0.0	0.0	0.1	0.0
culmorum	0.1	0.1	0.1	0.5	0.0
graminearum	21.8	4.5	0.5	34.2	0.0
semitectum	0.1	0.0	0.0	0.1	0.0
solani	0.0	0.0	0.1	0.8	0.0
subglutinans	3.4	0.0	0.4	6.7	0.0
Total	39.8	6.7	1.7	63.6	0.0

[1] Seeds were collected at approximately medium milk and soft dough. Ripe refers to the vascular strand collected on half of seeds. Shattered refers to seed fallen to ground and collected in plastic trays. Processed seed obtained from grocery stores.

[2] Percentage is based on 300 seeds from each of four locations per growth stage/ year for a total of 2,400 seeds per growth stage.

Table 2. Percentage of <u>Fusarium</u> spp. from cultivated or natural wild rice seed at different growth stages in 1994 and 1995.

	Growth stage [1]		
	milk	dough	ripe
	%[2]	%	%
Cultivated[3]	7.2	6.6	1.7
Natural	9.8	5.2	1.7

[1] Seeds were collected at approximately medium milk and soft dough. Ripe refers to the vascular strand collapsed on half of seeds.
[2] Percentage is based on 900 whole seeds, 300 palea and lemma, and caryopsis each year.
[3] Cultivated wild rice seed was gathered from a paddy near Clearwater, MN, natural wild rice seed was gathered from the Moose River near Hill City, MN.

Table 3. Comparison of seed structures of cultivated wild rice yielding <u>Fusarium</u> spp.[1]

	Seed structure		
<u>Fusarium</u> spp	Whole seed	palea and lemma	caryopsis
	%[2]	%	%
anthrophilum	26.9	15.6	3.0
culmorum	0.5	0.0	0.0
graminearum	34.1	18.1	22.3
moniliforme	1.5	1.0	0.5
sporotrichoides	0.5	0.0	0.0
subglutinans	1.4	1.0	0.0

[1] Ripe seed was gathered at harvest.
[2] Each percentage is based on a total of 1,800 whole seeds, 600 palea and lemma, and 600 caryopsis. Three hundred whole seeds, 100 palea and lemma, and 100 caryopsis were gathered from each of three wild rice fields per year and bulked together for a total of 900 whole seeds, 300 palea and lemma, and 300 caryopsis each year.

Table 4. Effect of source and storage conditions on isolation of <u>Fusarium</u> spp. from whole wild rice seed.

| Source[2] | Storage conditions[1] | | Proportion with Fusarium spp. |
	Temp.	Immersed (+) or not (-)	
	°C		%[3]
Natural	-20	-	5I.0 a
Natural	-20	+	13.5 b
Natural	4	-	63.5 a
Natural	4	+	0.0 c
Cultivated	-20	-	46.0 a
Cultivated	-20	+	11.9 b
Cultivated	4	-	80.5 a
Cultivated	4	+	0.0 c

[1] Seed was dried to 20-21% moisture for 2-3 days at 21-24 C then stored at either -20 or 4 C and immersed or not in water for 5 mo.

[2] Natural seed was obtained from Dora Lake in north central Minnesota. Cultivated seed was obtained from a cultivated wild rice paddy near Clearbrook in north central Minnesota.

[3] Based on 1000 seeds per sample. Ratings followed by the same letter are not significantly different (P = 0.05, LSD test).

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Table 5. Effect of source and storage temperature on isolation of <u>Fusarium</u> spp. from wild rice seed structures.[1]

		Seed structure		
Source[2]	Temperature	Whole seed	Palea & lemma	Caryopsis
	°C	%[3]	%	%
Natural	-20	51.1 a	28.0 b	4.0 c
Natural	4	63.5 a	35.5 b	28.0 b
Cultivated	-20	46.0 a	24.0 b	3.0 c
Cultivated	4	60.5 a	59.5 a	65.5 a

[1] Seed was dried to 20-2l % moisture for 2-3 days at 21-24 C then stored for 5 mo.
[2] Natural seed was obtained from Moose River in north central Minnesota. Cultivated seed was obtained from a cultivated wild rice paddy near Clearbrook in north central Minnesota.
[3] Percentage is based on isolations from 300 whole seeds, 100 palea and lemma, and 100 caryopsis. Ratings followed by the same letter are not significantly different (P=0.05, LSD test).

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Table 6. Comparison of Fusarium species isolated from structures of wild rice seed from natural or cultivated sources.

	Seed structure		
Fusarium spp.	Whole seed	Palea & lemma	Caryopsis
	%[1]	%	%
Cultivated			
anthrophilum	1 a	0 a	0 a
culmorum	1 a	0 a	0 a
graminearum	58 b	48 b	45 b
moniliforme	2 a	1 a	1 a
sporotrichioides	1 a	0 a	0 a
subglutinans	1 a	0 a	0 a
Natural			
anthrophilum	0 a	0 a	0 a
culmorum	0 a	0 a	0 a
graminearum	59 b	33 c	19 c
moniliforme	1 a	1 a	1 a
sporotrichioides	0 a	0 a	0 a
subglutinans	0 a	0 a	0 a

[1] Percentage is based on isolations from 900 whole seeds, 300 palea and lemma, and 300 caryopsis. Seed was stored at 0 C for 2 mo. Ratings followed by the same letter are not significantly different (P=0.05, LSD test).
[2] Natural seed was obtained from Moose River in north central Minnesota. Cultivated seed was obtained from a cultivated wild rice paddy near Clearbrook in north central Minnesota.

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Table 7. Recovery from wild rice seed inoculated or not with Fusarium graminearum

Treatment	Recovery of Fusarium
	%[1]
inoculated	81
not inoculated	0

[1] Percentage is based on 300 seeds.

Table 8. Effect of propiconazole on seedborne Fusarium spp. in cultivated wild rice.

Number of propiconazole applications	Percentage with Fusarium spp.				
	Whole seed[1]	Palea and lemma	Caryopsis	Chaff	Seed on soil surface
	%[2]	%	%	%	%
0	3.5 a	0.0 b	0.0 b	0.5 b	3.0 a
1	2.0 b	0.5 b	0.0 b	2.0 b	9.0 c
2	0.5 b	3.0 a	0.0 b	2.0 b	8.0 b

[1] Whole seed, palea and lemma and caryopsis were obtained from combine at harvest. Chaff consisted of plant debris ejected out of combine from which palea and lemma were collected. Caryopsis on soil surface gathered in plastic trays on soil surface prior to harvest.
[2] Based on 300 whole seeds, palea and lemma, caryopsis, chaff, and caryopsis on soil surface. Ratings followed by the same letter are not significantly different (P = 0.05, LSD test).

Wild Rice Breeding and Germplasm Improvement
Raymie Porter, Bruce MacGregor, Henry Schumer, and Wayne Kennard[1]

Controlled Pollinations

We planted approximately 2000 rows in our nursery this year, about 80% of which were progeny from controlled pollinations (crosses and selfs) made in the 1994 field season. Because of germination problems and high water temperatures during the early stages of plant development, only 320 nursery rows had surviving plants with which to continue line development through controlled pollinations (mostly selfing). Also a number of the plants which were pollinated this year lost their bags during unusually stormy weather in August, but we were able to obtain several hundred selfs and crosses from the nursery, and we are currently processing the seed of these for storage until spring planting. The improved pollinating bags and techniques we have developed over the last two years provide us with more consistent seed set than we had before. However, we are considering ways of protecting the nursery from wind and rain for next year's growing season to allow us to recover most of our successful pollinations.

Pollination methodology study

We continued looking at pollination meth   improvements by focusing on one aspect of field pollination--timing with respect to fema   ceptivity. Our objective was to estimate the optimum timing for pollination with respect to the time when most of the florets on a panicle were exserting stigmas. Understanding this optimum should help us to maximize our seed set from crosses.

Materials and Methods. During a one day period, we placed approximately 20 bags over tiller panicles which were just beginning to emerge from the boot, in each of two populations: NC3-C1, a selection from NorCal-3, and PiB-C1, a 50% pistillate population. If florets which had already emerged were exserting stigmas, that part of the panicle was clipped off with scissors. In the latter population, we bagged tillers of pistillate plants. For each tiller bagged, we tagged another tiller of the same plant which was at the same stage of development/emergence as the bagged tiller. The panicle was clipped if necessary to make it approximately the same size as the bagged panicle. Beginning two days later, we checked the panicles daily. If the pistillate portion had cleared the boot, the bags were clipped shut at the bottom, removing and sealing out male panicle branches in the case of the normal plants of NC3-C1 (the pistillate panicles of PiB-C1 were similarly sealed off to isolate the pistillate florets inside the bag from ambient pollen). If, as in a few cases, pollen shed had already begun inside the bag, the male branches were removed and the bag was labelled self-pollinated and dated. Each day the bags were checked, stigmas inside the bag were inspected to estimate the percentage of florets which had exserted their stigmas. Pollinations were made on some panicles on the day 80% exsertion was reached (day 0), and in other panicles 1, 2, or 5 days after exsertion. After sufficient time for seed maturation, seed set was counted in each bagged panicle and each open pollinated panicle.

Results. Because of high winds and stormy weather during the seed maturation period, many of the bags were lost, or panicles were broken over into the water. Therefore, the data for the two populations were combined. Figure 1 shows that seed set for open-pollinated panicles

declined slightly, but still averaged between 100 and 130 seeds per panicle for the 4 treatment groups. The open-pollinated panicles were expected to have higher seed set than the bagged controlled-cross panicles, since the former were continuously exposed to ambient pollen, while the latter were exposed to collected pollen of unknown viability during one point in time. If some of the stigmas had not yet been exserted inside bagged panicles at the time of pollination, those florets would not have been pollinated. Conversely, if the stigmas which had been exserted at an earlier time had lost their receptivity or dried out, pollination of those florets would not result in seed set.

Seed set of controlled crosses peaked 1 day after 80% exsertion and declined thereafter, whether seed set is expressed in absolute terms or relative to the open-pollinated panicle. This indicates that pollination should be made within one day after 80% stigma exsertion is reached. Since this preliminary experiment use a limited number of plants, we may repeat this preliminary experiment on a larger scale next season to confirm these findings, possibly adding another factor to the treatments: pollen age.
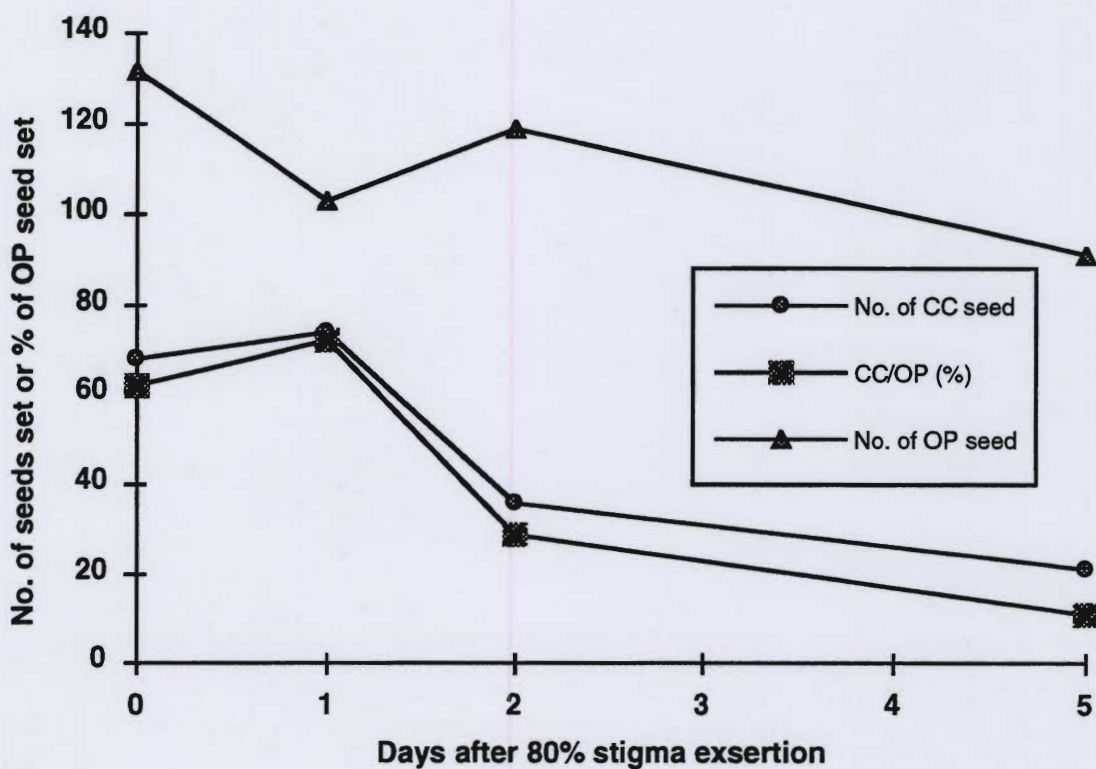

Fig. 1 Seed set of controlled crosses and open-pollinated panicles over a period of 0 to 5 days after 80% stigma exsertion.

Phenotypic recurrent selection

We continued to select for several traits through mass selection of open-pollinated materials. In 12 of the 17 open-pollinated populations grown, shattering resistance was a selection criterion, usually the primary one, including some elite populations which have come through 6 to 8 cycles. Disease resistance was the primary selection criterion in two populations inoculated with *Bipolaris oryzae*, the causal agent for fungal brown spot disease (done in collaboration with Dr. Robert Nyvall, NCES).

Pistillate trait and variant thereof

The pistillate panicle type has all female florets. A population selected to reach 50% pistillate would decline in frequency after release, but a 25-50% pistillate cultivar still has a greater yield potential than a normal one. We are increasing a 50% pistillate population (K-2 Pi) with hopes of releasing it, possibly in 1997. We will collect additional data on its performance in variety trials in 1996, and in larger plots which will be harvested with combines to asses the harvestability of these plants with heavier panicles.

Seed tensile strength study

Through a Parker Sanders grant, and in conjunction with the Minnesota Department of Agriculture, a high school science teacher was recruited to assist us for several weeks this summer. The teacher, Mr. Robert Shaner, took data on seed tensile strength in a number of populations over time. The results of that study are presented in a separate article in this report .

Nondormancy research

With supplemental funding from Minnesota Crop Improvement Association and the Minnesota Cultivated Wild Rice Council, we continued our investigation of seed nondormancy. We grew out BC_2 through BC_5 lines in which we have been transferring nondormancy from a Florida *Zizania aquatica* population. These lines were selfed in the field and nondormant seeds were germinated for a winter greenhouse generation. We also grew out half-sib families to study the heritability of dormancy in cultivated *Z. palustris*. This study is still in progress, as the harvested seed of these families is being evaluated for the length of storage time necessary to break dormancy.

Variety trials

Although we planted two variety trials on growers' paddies, early foraging by waterfowl and unexpectedly high competition from volunteer plants rendered these trials useless for obtaining data this year. We have taken steps to improve our chances of growing successful variety trials in 1996.

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Seed storage research

We have provided seed of 'Franklin' to Dr. Chris Vertucci (National Seed Storage Laboratory) to continue her research on optimum drying and freezing protocols for wild rice seed storage. We are initiating experiments in Grand Rapids to improve short-term seed storage methods. We also anticipate collaborating with Dr. Oelke in this research.

Molecular genetic mapping

The collaboration between the breeding project and Dr. Ron Phillips and Dr. Wayne Kennard continued, mainly in providing field space and assistance for growing and crossing lines, and in strategizing the best ways to develop useful mapping populations.

Germplasm exploration

This year we obtained seed samples from three lakes. One lake near Virginia, MN, had a declining stand according to DNR records. Breeding project personnel obtained a representative sample for genetic comparison to other lakes. In the process, we recovered seed from one plant which had very high tensile strength. Some of this seed has germinated and is being grown out in the greenhouse to develop a new nonshattering line. Dr. Wayne Kennard also assisted in collecting seed from "Pool 10" of Carlos Avery Wildlife Area. In addition, we found several unique populations of *Z. aquatica* on the Minnesota Valley National Wildlife Refuge, and collected seed with their permission. The plants resembled the tall Florida population of *Z. aquatica* from which we originally obtained nondormant seed. However, the Minnesota Valley *Z. aquatica* plants obtained their height of 10-15 feet during a much shorter (Minnesota) growing season.

Seed Tensile Strength Variability In Wild Rice
Raymie Porter and Robert Shaner[1]

A major breeding objective for wild rice is seed retention or shattering resistance. 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. Wild rice seeds tend to detach (shatter) more easily as the seeds mature, even in cultivars which are considered "nonshattering". However, the variability of seed tensile strength and its change during seed maturation is not well quantified. Variability within a variety, and even within an individual panicle appears to be high. In order to obtain accurate comparisons between varieties or populations for seed tensile strength, breeders need a better understanding of how seed tensile strength changes over time, and the amount of variability between vs. within populations. Also, the relationship between tensile strength of immature florets and that of mature seeds is still unclear. If a relationship exists, early measurements could aid the breeder.

Hanten (1975) has hypothesized that wild populations from lakes may have a potential for seed retention which could be assessed by measuring the seed tensile strength at a stage before abscission occurs. If so, populations or individuals with high tensile strength could be identified for incorporation of the major genes for nonshattering, possibly resulting in a nonshattering population or line with greater seed retention than other currently available cultivars.

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

Materials and Methods

Design of Experiment. An observation trial was planted in the fall of 1994 for the 1995 growing season. Although each of the three replicates was planted with 52 entries (genetically distinct populations), only 25 of these entries in two reps were chosen for this study, primarily on the basis of adequate plant numbers. Of these 25 entries, 7 were wild populations of shattering type plants, and 18 were breeding populations or cultivars with mostly nonshattering phenotypes (i.e., containing genes for substantially reduced shattering) Each plot consisted of two 6 foot rows, 10 inches apart, with 20 inches between plots and alleys of 4 feet between groups of plots. Plots were maintained using standard field practices, which included N-P-K applied prior to planting at a rate of 50-40-60 lb/A, and one nitrogen topdress of 30 lb/A applied as urea during flowering.

Measurements. When enough panicles were beginning to emerge in all plots, 5 panicles (per plot) which had already emerged were tagged with a colored tape using a Max Tapener. Panicles were chosen which were judged to have emerged less than a week earlier. Within 1-2 days, another 5 panicles per plot which were in the process of emerging were tagged with a different color. These two groups of panicles were considered distinct panicle ages in the analysis. As the developing seed matured, seed tensile strength was measured by individually pulling off 5 florets per panicle using a surgical clamp attached to a digital force gauge. Thus, for the 25 entries, measurements were taken at days 7, 14, and 21 on 5 seeds per panicle, 5 panicles per panicle age (tagging color), 2 ages per plot, and 2 plots (reps) per entry. In addition one plot of a shattering entry and one of a nonshattering entry were selected for additional more frequent measurements--at 7, 10, 14, 16, 18, 21, 23, and 26 days after tagging.

Analysis of Data. No statistical analysis could be done on the unreplicated data from the more frequently measured plots. Instead, patterns and differences in the tensile strength changes over time were observed and noted. For the replicated plot data, Analyses of Variance (ANOVAs) were computed. Data were first analyzed by the GLM procedure of SAS, individually analyzing data for each combination of measurement time (7, 14, and 21 days) and panicle age group (immature and mature). Heterogeneity of error variance was tested and found to be insignificant, so data were combined for the overall analysis. However, since too much memory was required by the GLM procedure to include the subsampling sources of variance (plant and seed), the combined ANOVA was calculated using the GLM procedure on plot means. Mean comparisons were done in both the individual analyses and the combined analysis. However, we used the separate ANOVAs to estimate sampling variances. The mean squares were used to calculate variance components, and were also pooled (averaged) to calculate the overall variance components.

Results and Conclusions

Changes Over Time. We graphed changes in seed tensile strength over time from flowering to seed maturity in the two frequently measured populations (Fig. 1 and 2). Because they were considered to be typical shattering and nonshattering populations, we expected to see differences in their tensile strengths. However, the changes in seed tensile strength for the shattering population parallels the changes seen in the nonshattering population for "immature" panicles, except at day 21, where the shattering population tensile strength drops below the nonshattering population (Fig. 1). For the more mature panicles, a difference appears beginning after day 21 as the tensile strength of the shattering population declines while that of the nonshattering population is relatively stable (Fig. 2). During most of the seed maturation period, the two populations stayed within approximately 0.010 kg of each other. However, at the most advanced maturity, the shattering population is 0.045 kg lower than the nonshattering population. The seed tensile strength of the immature panicles peaked at 23 days after tagging, 5 days after the peak for the mature panicles. This difference fits with the estimated difference in time of emergence from the boot, and therefore probably reflects a 5-day age difference between seeds of immature and mature panicles. Thus, by the end of the experiment, the two populations' immature panicles would not have been expressing the difference seen in the populations' mature panicles. Had the measurements continued for at least one more week, a difference would probably have been more evident in the less mature panicles.

For the immature panicles (Fig. 1), the shattering population's tensile strength dropped on day 21, then increased on day 23. During that time, the nonshattering population's tensile strength continued to *increase* at a consistent rate. Conversely, for the more mature panicles (Figure 2), the nonshattering population's tensile strength dropped on day 21, then increased. During that time, the shattering population continued to *decrease* at a consistent rate. This difference could be due to changes in the weather (a rain storm occurred prior to one of the measurements) reflected in a physiological change in the plant as part of the maturation process. On the other hand, the difference in response of the two populations seems to favor an explanation relating to the difference in the populations themselves. During the last few measuring dates, the number of seeds left on the panicle was generally less, due to shattering losses. For shattering populations, this loss begins earlier, but even in the nonshattering varieties, shattering eventually occurs. When it does, those seeds on a panicle which are more mature are lost first, leaving mostly the less mature seeds on the panicle for tensile strength measurements. Although the initial decline in tensile strength may have been detected at day 21, any seeds which had already shattered by day 23 would not have been included in the measurements. Instead, the less mature seeds of the panicle would be measured, biasing the average upward. In the case of shattering type plants, the real decline may begin to show up on day 21 after panicle emergence. For nonshattering panicles, a decline may be delayed, becoming evident at the equivalent of day 26 after emergence (day 21+5 for the mature panicle group). There also may have been a few shattering type plants in the nonshattering plot.

To more accurately assess tensile strength changes and differences in future studies, we would do three things differently. First, we would either disregard or account for shattering-type plants in nonshattering plots. Second, we would quantify the percentage of seed remaining on shattering type panicles at each measurement date and use that estimate as a covariate to adjust the tensile strength measurements. Third, to correct for weather effects, we would tag panicles that emerge from the boot at several successive initiation dates using different colored ribbons, thus averaging out weather-related influences.
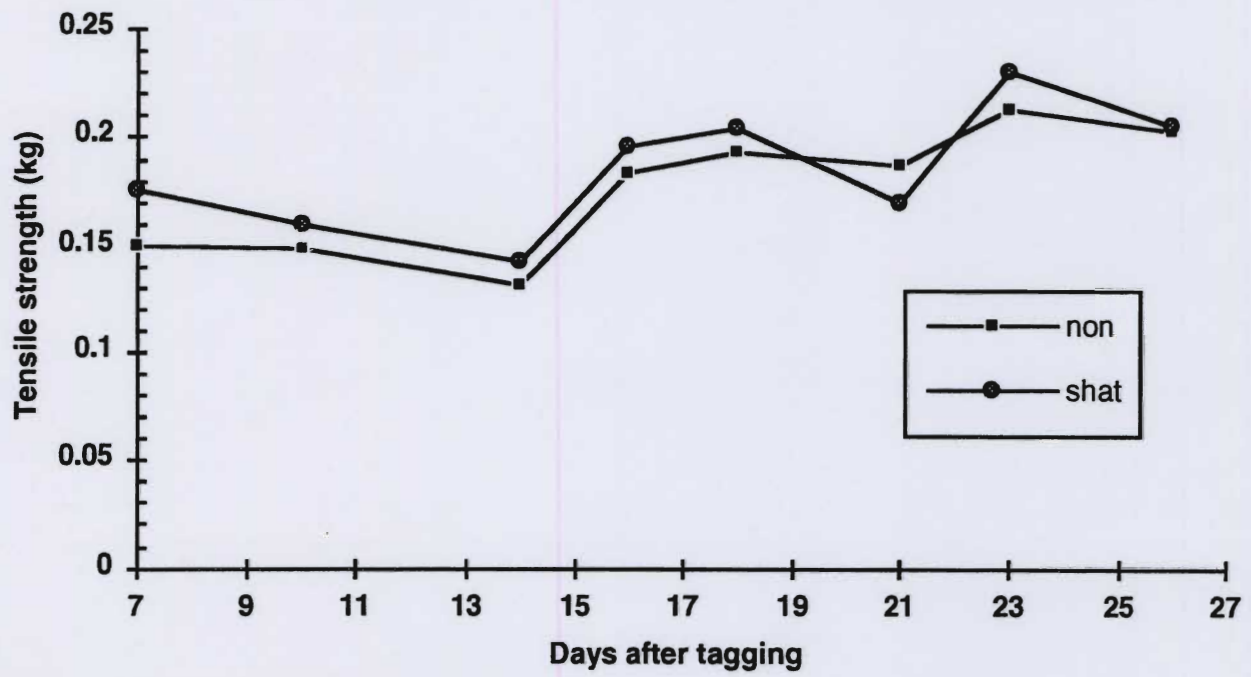
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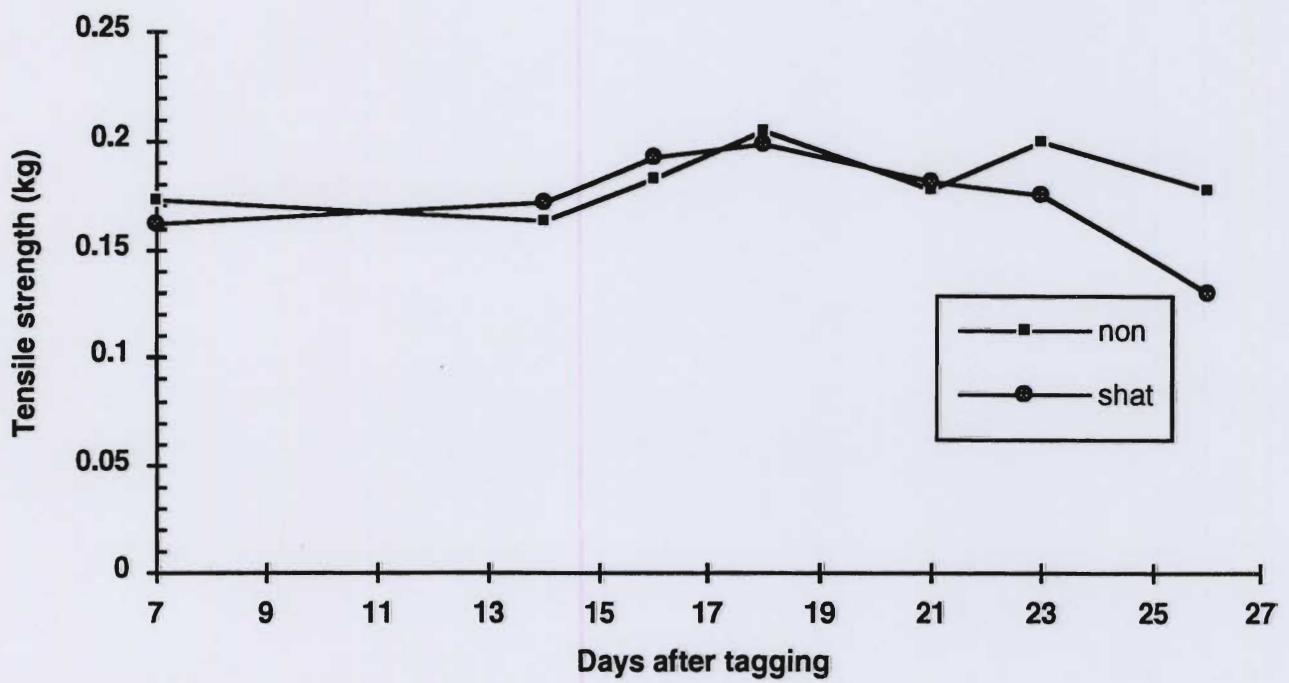Fig. 1   Tensile strength of immature panicles over time in two unreplicated plots.

Fig. 2   Tensile strength of mature panicles over time in two unreplicated.

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The mean tensile strength of 7 shattering and 18 nonshattering entries were calculated for each date. Assuming the difference in maturity between the "immature" and the "mature" panicles to be 5 days, we adjusted the latter by adding 5 days to the measurement date. The pattern of changes in tensile strength (Fig. 3) are similar to those seen in Fig. 1. The maximum tensile strength is reached at day 21, or perhaps slightly after, similar to the day 23 peak in Fig. 1. The shattering entries parallel the nonshattering entries, but as maturity progresses, the shattering entries become relatively lower than the nonshattering entries. The drop in tensile strength for shattering entries at the later dates is no greater than the drop for nonshattering entries. However, the peak tensile strength of the shattering entries is preceded by a drop between day 14 and day 19. As discussed above, that initial drop might signal the beginning of the real decline in tensile strength. Later averages may have been biased upward by the shattering loss of low tensile strength seeds, leaving less mature, higher tensile strength seeds on the panicles. This explanation would need confirmation by additional research. Refinements in methodology mentioned in the discussion of the frequently measured plots should help clarify the picture, especially if replication and perhaps a few more entries are added.
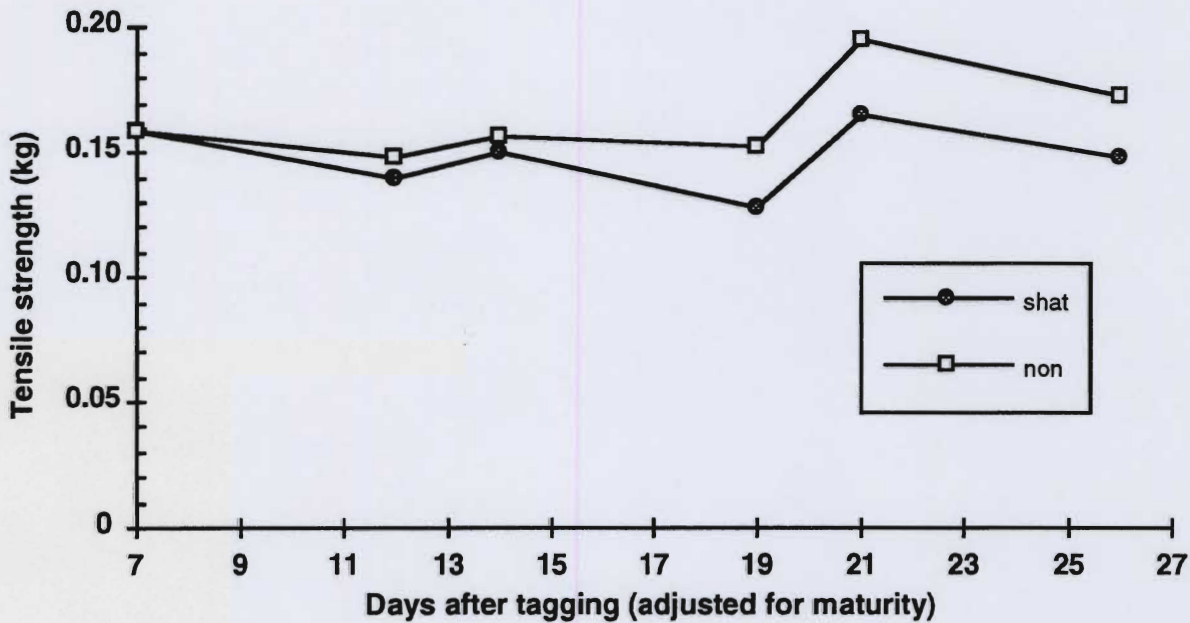

Fig. 3 Change in tensile strength over time for replicated data adjusted for panicle age--"mature" panicles were considered to be 5 days older than "immature panicles. Circles are means of 7 shattering entries and squares are means of 18 nonshattering entries averaged over two reps.

Differences Among Populations. The statistical analysis enabled us to quantify and compare seed retention between populations. An individual analysis at each Age and Time combination showed that there were significant differences in tensile strength among entries in each analysis, except for Day 7-mature and Day 21-immature. This lack of significance comes from a larger mean square for Rep X Entry, which is used as the error term for the F-test for Entries--it was over 50% higher at those two Age-Time levels than at the other four levels, while the mean squares for Entry remained relatively consistent at all six levels. Replicates were almost always a significant source of variation, indicating that blocking was effective. At day 21, Replicate variance was considerably greater, possibly due to different operators taking measurements in the two reps. Plant was always a significant source of variation, probably reflecting the heterogeneity of all the populations used.

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Table 1   Individual Analyses of Variances for each combination of measurement time and panicle age.

Source	df	Day 7 immature	Day 7 mature	Day 14 immature	Day 14 mature	Day 21 immature	Day 21 mature	Average
Rep	1	0.0436	0.0848*	0.0889*	0.0994**	1.1495**	0.6135**	0.3466
Entry	24	0.0278*	0.0244	0.0301*	0.0244*	0.0335	0.0274*	0.0279
Rep X Entry	24	0.0112**	0.0172**	0.0130**	0.0103**	0.0186**	0.0106**	0.0135
Panicle (R E)	200	0.0077**	0.0076**	0.0090**	0.0068**	0.0079**	0.0059**	0.0075
Seed (error)	1000	0.0015	0.0018	0.0018	0.0018	0.0022	0.0022	0.0019

* Significant at $\alpha = 0.05$
** Significant at $\alpha = 0.01$

For the combined analysis (Table 2), Entry and Age effects were statistically significant--in other words, the overall entry averages differed from each other, and the overall age averages differed. When shattering entries on the average were compared to nonshattering entries (Shatt vs non) the difference was not significant at $\alpha = 0.05$, but would be significant at $\alpha = 0.10$. There was no significant interaction between Entry and Age (entries were relatively consistent across ages).

Time, Time x Entry, and Time x Age were statistically significant. In other words, there were differences in time averages, the entries differed in their response over time, and the relative tensile strength for each panicle age changed over time. However, since Entry x Age x Time interaction was not significant, entries behaved similarly for different panicle ages and times.

Table 2   Combined Analysis of Variance for tensile strength.

Source	df	Mean square	F Value	Pr>F
Rep	1	0.7⬚⬚⬚4	16.33	0.0005
Entry	24	0.1⬚⬚⬚9	2.37	0.0194
Shatt vs non	1	0.13⬚⬚941	3.09	0.0918
Rep x Entry	24	0.04473913	15.92	0.0001
Age	1	0.47736010	21.22	0.0001
Entry x Age	24	0.02249296	1.60	0.1254
R x E x A	25	0.01406926	5.01	0.0001
Time	2	0.45433272	39.60	0.0001
Entry x Time	48	0.01147372	4.08	0.0001
Age x Time	2	0.04489847	15.98	0.0001
E x A x T	48	0.00641584	0.31	1.0000
R x E x A x T	100	0.02066643	7.35	0.0001

Since Entry is a significant source of variation, there is support for genetic differences in tensile strength. Comparisons can be observed in Table 3. The nonshattering varieties which appear to have the highest average tensile strength at all levels were FBB, Sunshine, and NACH-B. Of those three, FBB was the most consistent over time, but on day 21 NACH-B showed the greatest tensile strength of all shattering and nonshattering types. K-2 Pi, a pistillate population related to Franklin, was fourth overall, followed by PB(E)C3--a population selected for shattering resistance from Petrowske Bottlebrush--then Franklin. FBB is a half-sib family selected from Franklin, a cultivar released for superior shattering resistance. NACH-B is a cultivar which Manomin Development Corp. developed by selecting for seed retention and bottlebrush panicles from the cultivar Netum, the first U of M cultivar released. Sunshine

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was developed by Kelly Petrowske for shattering resistance, but has been observed to have a significant proportion of shattering-type plants. Because the calculated Least Significant Difference (LSD) was relatively high (0.034 kg), none of the top six entries can be declared significantly different from each other, but the highest (FBB) is significantly different from any entry averaging below 0.166 kg.

The shattering entries with the highest average tensile strength at 7 days, 14, days and 21 days after tagging for both mature and immature plants were Dora Lake, Big Rice Lake, and White Elk Lake. The data from the immature Rice Lake/Bowstring River plants (where Bowstring River and Rice Lake come together) is very high at the beginning. One plant within the population (in the immature group) is especially high at each date, sometimes showing tensile strengths well above 0.300 kg., which would bring up the average considerably. This may indicate a nonshattering plant which accidentally grew in the plot, or an unusually high-tensile-strength plant within the population. In case the latter is true, the population bears a second look.

Table 3   Tensile strength means for each entry for each panicle age and time.

Entry	Day 7 imm	Day 7 mat	Day 14 imm	Day 14 mat	Day 21 imm	Day 21 mat	Mean
				kg			
Shattering							
Big Rice L.	0.145	0.141	0.145	0.136	0.171	0.166	0.151
Bowstring R.	0.202	0.147	0.179	0.118	0.182	0.133	0.160
Dora L.	0.165	0.153	0.149	0.148	0.183	0.179	0.163
Moose L.	0.145	0.132	0.113	0.128	0.160	0.142	0.137
Rice L. / Bowstring R.	0.184	0.153	0.206	0.142	0.156	0.124	0.161
Wall L.	0.111	0.108	0.121	0.102	0.110	0.134	0.114
White Elk L.	0.161	0.140	0.143	0.122	0.195	0.162	0.154
Nonshattering							
Franklin	0.164	0.133	0.178	0.149	0.215	0.180	0.170
FBB	0.202	0.195	0.188	0.206	0.217	0.194	0.200
FSSR-C7	0.159	0.146	0.164	0.152	0.195	0.192	0.168
FY-C1	0.154	0.162	0.141	0.151	0.216	0.178	0.167
Johnson	0.106	0.113	0.108	0.113	0.161	0.162	0.127
K2	0.182	0.138	0.149	0.152	0.182	0.176	0.163
K-2 Pi	0.176	0.170	0.170	0.170	0.190	0.192	0.178
M3(M)C2	0.147	0.148	0.131	0.155	0.166	0.166	0.152
NACH-B	0.169	0.162	0.175	0.162	0.227	0.212	0.184
PBB-C1	0.160	0.157	0.160	0.150	0.204	0.141	0.162
PB(E)C3	0.145	0.158	0.153	0.167	0.217	0.195	0.172
PB(M2)C3	0.166	0.130	0.161	0.147	0.192	0.139	0.156
PBR-C1	0.168	0.158	0.159	0.158	0.174	0.188	0.168
Petrowske BB	0.127	0.113	0.143	0.125	0.176	0.153	0.139
PLAR-C1	0.159	0.167	0.162	0.141	0.181	0.129	0.156
PM3(E)C4	0.158	0.123	0.167	0.137	0.218	0.176	0.163
Sunshine	0.189	0.184	0.199	0.179	0.225	0.172	0.191
Voyager	0.176	0.181	0.176	0.162	0.184	0.121	0.167
Mean	0.161	0.149	0.157	0.147	0.189	0.165	0.161
LSD 5%	0.031	0.038	0.033	0.030	0.040	0.030	0.034

The nonshattering and shattering varieties with the least tensile strength were Johnson and Wall, respectively These are both selections brought in from outside Minnesota. If they are not well adapted to Minnesota conditions, their tensile strengths may be accordingly less.

As discussed above, data obtained after seed reached full maturity for both shattering and nonshattering plants could have been skewed due to the later maturity of seed remaining on the panicle. For shattering populations, their true potential may be better reflected by the earlier measurements. Once nonshattering genes can be incorporated into them, they may maintain a higher level of tensile strength than lower-tensile-strength wild populations, or perhaps even higher than current nonshattering populations.

When is the best time to make comparisons? If the objective is to identify the population which retains its seed best over time, then measurements taken at the most advanced seed maturity tested (Day 21-mat or equivalent to 26 days after emergence) are most revealing, and appear to have relatively low error (Rep x Entry) as shown in Tables 1 and 2. The low error term resulted in a low LSD for discerning the best entries, although there is still room for improvement in precision. If the objective is to identify potentially useful shattering populations or plants, the early measurements might be more revealing of their potential tissue strength once nonshattering genes are incorporated. In any event, when correlation coefficients were calculated, they indicated no strong relationship between early measurements and later measurements.

Sampling Variability. In order to design future experiments more efficiently, we need to estimate variability among seeds, variability among panicles (or plants), and variability among plots of an entry. We can then use these estimates to find ways to reduce the number of measurements, or reduce the error of the entry means, or both. From the separate ANOVAs for each age-time combination (see Table 1), we calculated variance components for Rep x Entry (among plots), Panicles within plots, and Seeds within panicles (Table 4). Experimental error variance includes Panicle variance divided by the number of panicles, and Seed variance divided by the product of the number of seeds and the number of panicles. These latter two estimates make up 43% and 15% of the experimental error. Thus, the panicle-to-panicle variance contributes much more to experimental error than the seed-to-seed variance in this design. These variance components were also used to calculate standard error of the mean (SE) and coefficient of variation (CV), or the SE expressed as a percentage of the mean. The CVs calculated average about 10%, which shows that variability was not as low as desirable (closer to 5%).

Table 4   Estimates of variance components, mean seed tensile strengths, standard errors of the mean, and coefficient of variation (CV) at each panicle age and date.

	Variance component estimate for seed tensile strength						
Variance component	Day 7 imm.	Day 7 mat.	Day 14 imm.	Day 14 mat.	Day 21 imm.	Day 21 mat.	Average
				kg^2			
Rep	0.00005	0.00011	0.00012	0.00014	0.00181	0.00096	0.00053
Entry	0.00033	0.00014	0.00034	0.00028	0.00030	0.00034	0.00029
RxE (plot)	0.00014	0.00039	0.00016	0.00014	0.00043	0.00019	0.00024
Panicle	0.00125	0.00114	0.00144	0.00098	0.00115	0.00074	0.00112
Seed	0.00150	0.00184	0.00181	0.00184	0.00217	0.00220	0.00189
Panicle / p	0.00025	0.00023	0.00029	0.00020	0.00023	0.00015	0.00022
Seed / sp	0.00006	0.00007	0.00007	0.00007	0.00009	0.00009	0.00008
Exp. error (entry mean)	0.00045	0.00069	0.00052	0.00041	0.00074	0.00043	0.00054

Parameter	Parameter estimate for seed tensile strength						
				kg			
mean tensile strength	0.161	0.149	0.157	0.147	0.189	0.165	0.161
std. error of mean	0.015	0.019	0.016	0.014	0.019	0.015	0.016
CV	9.3%	12.5%	10.3%	9.8%	10.2%	8.8%	10.1%

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In order to improve future experimental designs, the variance components were used in the following equation (adapted from the equation on page 535 of Gomez and Gomez 1984) to calculate the margin of error (M.E.) when reps, plants, and seeds were varied. This margin of error term is similar to the LSD, being approximately twice the standard error of the mean.

$$M.E. = \sqrt{\frac{Z_\alpha^2}{r}\left(\sigma_{re}^2 + \frac{\sigma_p^2}{p} + \frac{\sigma_s^2}{sp}\right)}$$

The three terms inside the parentheses were actually the third, sixth, and seventh lines of Table 4. The sum of these is the same as Exp. error (entry mean) in that table. The "Average" column was used to provide the estimates for the equation. The term Z_α^2 is equal to 1.96^2 for $\alpha = 0.05$. The results of calculations using several levels of reps (r), plants (p), and seeds (s) are shown in Table 5. It is easy to see why increasing the number of reps would have the greatest effect, since r is effectively a denominator of all three terms inside the parentheses--increasing r would decrease all three terms. Since σ_p^2 and σ_s^2 (lines 4 and 5 of Table 4) are similar in magnitude, it makes sense that increasing the number of plants would have more of an effect of reducing error by increasing the denominator of two of the terms on the right side of the equation. Increasing the number of seeds would have the least effect, even though variance among seeds is greater than variance among plants, since it is only a denominator of one term.

The initial line of table 5 reflects the parameters for the current experiment. Note that the margin of error is quite close to the LSD value given in Table 3 and is exactly twice the standard error of the mean in Table 4. By increasing the reps from 2 to 6, the margin of error can be reduced to 0.019 kg. A margin of error of 0.020 kg would be enough to distinguish the highest entry (FBB) from the fourth entry (K-2 Pi). With 6 reps, if the number of seeds measured per panicle were decreased to 2, that level of precision could be maintained, and even decreasing it to 1 would still reduce the error to 72% of the error of the current experimental design. Increasing the number of plants from 5 to 8 would reduce the error further to 0.020 kg, but the further decrease would come at a cost of additional measurements--48 total measurements per entry, which would be almost as many as the 50 measurements per entry at each time taken in this experiment. So by changing the number of reps to 6 (which is the usual number in each location of a variety trial) and reducing the number of seeds measured per panicle to 1, the number of measurements can be reduced by 40% without sacrificing precision--indeed, error would be reduced by 28%.

Table 5   Estimated margin of error for different combinations of numbers of reps, plants, and seeds.

No. reps	No. plants	No. seeds	Margin of error
			kg
2	5	5	0.032
4	5	5	0.023
6	5	5	0.019
6	5	4	0.019
6	5	3	0.019
6	5	2	0.020
6	5	1	0.023
6	6	1	0.022
6	8	1	0.020

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In summary, this experiment showed that: 1) tensile strength changes over time for both shattering and nonshattering populations, shattering populations reaching a peak and subsequently declining faster than nonshattering populations; 2) tensile strength differences between populations can be detected and useful comparisons can be made; 3) precision of tensile experiments can be improved by increasing reps and decreasing the number of seeds measured per plant. Further studies may give a more accurate picture of when tensile strength peaks and the relationship it has to actual shattering losses.

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Acknowledgments

Wild Rice Molecular Genetic Marker Mapping Progress Report

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Introduction and objectives.

The primary objective of the molecular genetics project is to increase the understanding of wild rice genetics and facilitate several breeding objectives through application of genetic markers. Specific projects include, i. the development of a saturated genetic linkage map and location of genes for shattering resistance, ii. comparative mapping and detection of markers linked to genes controlling the pistillate trait, and iii. identification of markers linked to genes controlling nondormancy.

Development of a genetic linkage map and location of genes for shattering resistance.

Development of a saturated genetic linkage map and detection of markers linked to genes for shattering resistance has commenced with development of appropriate populations. Towards the development of a mapping population we now have F_2 populations from several controlled crosses of nonshattering by shattering plants. The 20 F_2 candidate populations with greater than 500 seed include Johnson X Dora (3 populations), K2 (Vomela) X Dora (12 populations), K2(Pi) X Dora (5 populations). We will be growing these populations in the greenhouse and field to obtain a population with adequate numbers (150+) and to study the genetics of shattering loci. One population will initially be emphasized for constructing a saturated linkage map and finding linkages with genes controlling shattering versus nonshattering.

Comparative mapping and detection of markers linked to genes controlling pistillate.

Grass species have a high degree of similarity at the DNA level. Comparative mapping strategies employ probes detecting RFLPs markers in one species and use them for mapping another species (Ahn and Tanksley, 1993). This will allow for a genetic description of wild rice in terms of a reference species as rice or maize. A comparative map

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framework will provide a strong foundation for genetic studies as information from other species will be readily tested with common reference points. Genes conditioning similar (homoeologous) traits in other crops could include loci controlling pistillate or shattering (Blakey et al., 1994; Paterson et al., 1995).

Comparative mapping efforts have begun with the evaluation of probes used as markers in oat, rice, and maize. Evaluation consists of radio-labeling probes and successful detection of both signal (discrete bands) and size variation (polymorphism). Markers from maize, oat, and rice have been found to be generally useful in wild rice (Table1). In general, more bands were detected with a given probe in wild rice (3.5 +/- 1.7) than either an inbred maize line (B73; 2.0 +/-1.3) or rice variety (1.8 +/- 1.1). The greater number of bands detected with a given set of probes may be due to the open-pollinated nature of wild rice (greater heterozygosity) or duplication events in the evolution of the genome of wild rice.

Table 1. Evaluation of maize, oat, and rice probes as markers in wild rice.

Origin of Probe	Number	Discrete Bands	Polymorphism
Maize	15	8	6
Rice	24	21	17
Oat	18	9	5

Comparative mapping efforts with wild rice have proceeded with the testing of linked marker loci in maize and rice. We have initiated linkage analysis in a mapping population from a single open-pollinated maternal pistillate plant. From this plant, 90 progeny were grown and scored for the pistillate and color traits. DNA was isolated from each plant and scored for RFLP markers. RFLPs were scored presence/absence because parental genotypes were unknown and the chance that more than two alleles were segregating in the open-pollinated population. Markers were first evaluated for acceptable genetic segregation ratios (Table 2). Segregating markers in this open-pollinated population generally fit 3:1 or 1:1 segregation ratios as would be expected if the maternal plant was either homozygous or heterozygous and allele frequencies in the pollen pool were 0.5.

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Table 2. Segregation patterns for visible (A) and molecular genetic markers (B).

A. Morphological markers

Morphological Characters	Total Traits	3:1	1:1	Neither 3:1 or 1:1*
Sex Expression (*pi*)	1	0	1	0
Color	4	2	0	2

*p <0.05

A. Molecular genetic markers

Probe Origin	Total Probes	Total RFLPs	3:1	1:1	Neither 3:1 or 1:1*
Maize	3	- 5	1	3	1
Rice	9	22	14	3	5
Oat	2	7	5	1	1

*p <0.05

Of those probes that gave acceptable segregation ratios, linkage tests were performed with the aid of the LINKAGE-1 (Suiter et al., 1987) computer program. We found linkages (< 30 cM) among 8 markers of the 26 markers analyzed. As more markers are added, a greater proportion will likely be found linked.

Of the 8 combinations of colinear RFLP markers (under 25 cM) from either maize or rice, two were found linked. Two pairs of markers linked in maize (CSU33b and RZ912) and rice (RZ952 and RZ66) were found linked in the wild rice mapping population. Six other pairs of linked markers in maize and rice were found unlinked in wild rice. Lack of linkages in wild rice with linked reference markers from maize or rice may be due multiple segregating RFLPs, different recombination rates, or genetic rearrangements.

We have taken a comparative mapping approach to target marker linkages to the pistillate (*pi*) locus in wild rice. The *pi* gene (Schumer 1985) conditions feminization in wild rice whereas tassel seed (*ts*) genes condition feminization in maize. Probes detecting markers linked to tassel seed loci and the cloned *ts2* cDNA (DeLong

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et al. 1983) are being used to provide evidence for homoeologous gene function of *ts2* in maize and *pi* in wild rice.

Probes detecting RFLP markers linked to tassel seed loci in maize were evaluated for linkage to *pi* in wild rice. Linkage to *pi* was not found with 5 markers which mapped to maize chromosome #1 or with the cloned cDNA of *ts2*. However, a marker locus, RZ66, from linkage group 8 of rice was found linked to *pi*. RZ66 is mapped to a conserved region of rice that shows colinearity with maize linkage group 1. This region is flanked by *ts2* and *Ts3* loci (Figure 1).

Wild Rice   Maize Chromosome #1   Wild Rice

ts2 cDNA — ts2 cDNA, bnl7.21 Pericarp color   2   pericarp color
17
CDO99   35   32

CDO464   pi
40   27

CDO99   18 — — — RZ66
RZ66*
RZ952   25
28   RZ952

Bz2
Ts3
40

RZ912   18 — RZ912
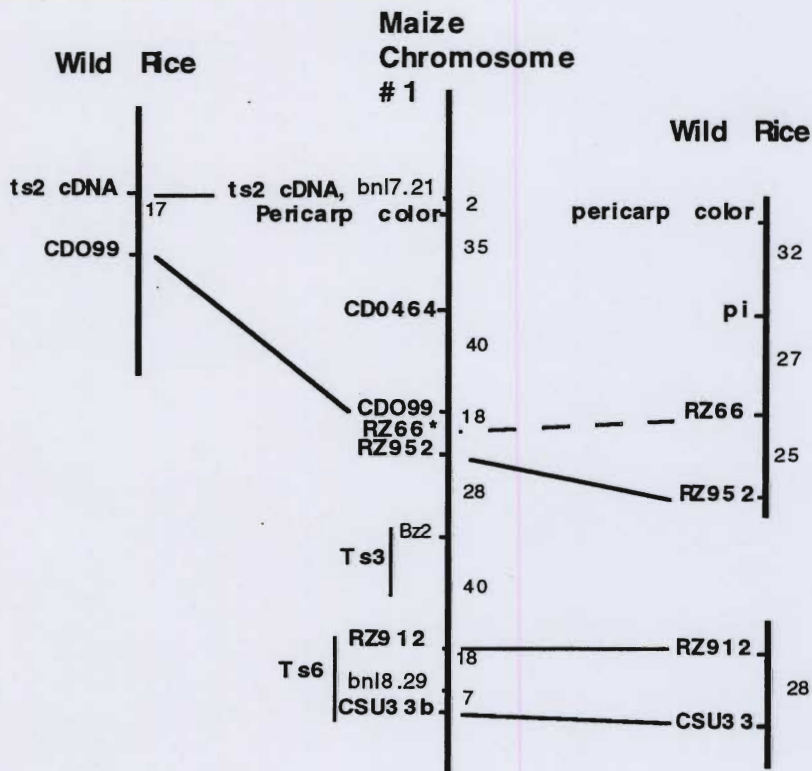Ts6   bnl8.29   7   28
CSU33b   CSU33

Figure 1. Comparative linkages in wild rice to maize chromosome #1. Marker names are listed on the left of vertical bars and linkage distances (cM) are on right. Maize linkages are reported by Ahn and Tanksley (1993) and the UMC Maize Genetics Cooperative (Coe, 1995). Rice (RZ), oat (CDO), maize (CSU), and the cloned *ts2* cDNA markers mapped in the wild rice population are shown in bold face type and reference markers relating genetic distance among maps are shown in plain type. The probe RZ66 has been mapped in rice to a region exhibiting colinearity with maize chromosome #1 between CDO99 and RZ952 (Causse et al. 1994). Approximate locations of

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Ts3 and *Ts6* loci are designated with vertical bars to the left of maize chromosome #1.

Our comparative mapping strategy has apparently been successful in targetting the *pi* locus. Comparisons among maps may not always be straight-forward due to multiple segregating RFLPs. Still, the comparative targetting approach appears more efficient than attempting to detect linkage with random markers. The population we are currently using is useful to generate preliminary linkages. Confirmation of the linkage to *pi* will entail finding multiple, linked flanking markers.

Detection of markers linked to genes controlling nondormancy.

The backcrosses generated by Dr. Porter should provide an efficient means to screen markers linked to genes controlling nondormancy. For a given backcross line, there is a high probability of recovering a RFLP donor allele if it is linked to a gene conditioning nondormancy (in the BC_5 at 10 cM, $P=0.59$), but not if the marker is unlinked (in the BC_5 at 50 cM $P=0.031$; Kaeppler et al.1994). Nondormant *Z. aquatica* collected from the Suwannee River in Florida was originally crossed as the donor parent to the dormant *Z. palustris* cv. K2. Backcrosses to *Z. palustris* were carried out for four to five generations selecting for nondormancy each generation (Figure2.). Individuals are deemed nondormant if they come from seeds in which germination occurred within two weeks of cold storage. Three lines of different pedigrees have been selected for RFLP tagging: two BC5S1 lines 95G-74-1, 95G-220-11, and the sister BC4S2 lines 95G-19-1 and 95G-21-1.
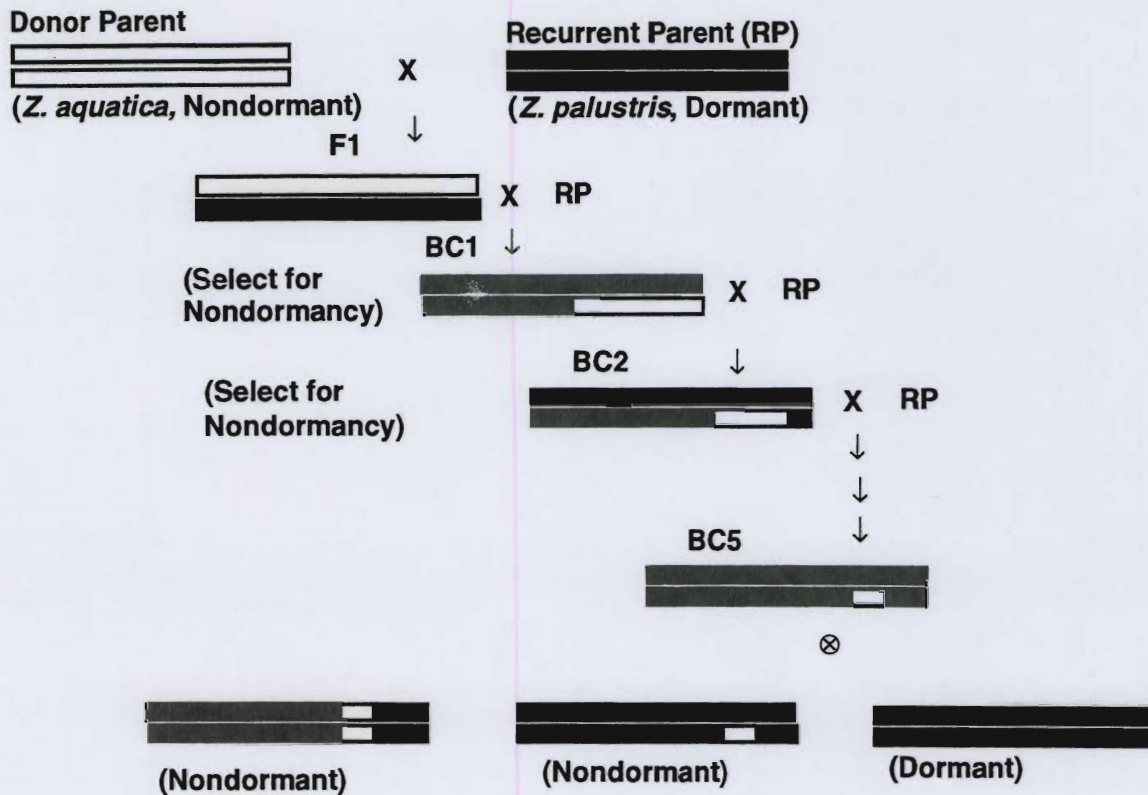
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Figure2. Backcross strategy of *Z. aquatica* to the reccurent parent *Z. palustris* is carried out for four to five generations.

RFLP analysis of *Z. aqautica* from Suwannee River and the *Z. palustris* lines used during the backcross has been initiated. Probes unique between *Z. palustris* varieties and *Z. aquatica* Suwannee River will be used to screen for RFLPs associated to genes controlling nondormancy.

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Characterization of Male Floret Developmental Sequence in both Normal and Mutant Wild Rice Plants

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Introduction:

The basic events of flower development have been extensively studied in maize and other plants. In maize, pollen development has been correlated with tassel formation in microsporogenesis to provide a development index for genetic breeding and molecular genetics (M. T. Chang and M. G. Neuffer, 1989). There was very limited information on the development of wild rice florets (C. E. Weir and H. M. Dale, 1960; D. A. Goldman, 1990, Aiken et al. 1988). The current research in my laboratory is to establish a developmental index for the formation of male florets. Such research involved defining developmental sequences of male floret formation and correlation of those sequences with nuclear changes in cell division events during pollen development.

The wild rice (Franklin) inflorescence samples were collected with the collaboration of Dr. R. A. Porter, and analyzed at the University of Minnesota, Duluth by using 4',6-diamidino-2-phenylin (DAPI) as a fluorescence probe. Our results indicate that a DAPI fluorescence probe is a useful marker to identify nuclear changes in each stage of microsporogenesis in male floret development of wild rice.

Materials and Methods:

Sample preparation:

Wild rice panicles (Franklin) at different stages of growth were collected from the Grand Rapids Experiment Station of the University of Minnesota. Samples were fixed in ethanol: acetic acid (3:1) for 24 hours at 4 $^{\circ}$C, transferred to 70% ethanol and stored at 4 $^{\circ}$C for characterization of developmental sequence in male florets (Liu, et al., 1993).

Morphological and cytological analysis of male floret development:

The morphology of anther development in each side branch of the panicle was investigated by following the main branch from bottom to top. Cytological features of microspore development were correlated with their morphology. Variable anther lengths were chosen to isolate microspores/pollen to study the cell division index during their development. Side branches from the bottom to the top of the main branch were selected, and anthers from the base, middle and tip of each side branch at each node were sampled to establish their cell

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division index. Relative positions of each sample location, anther size and cell division index were recorded for analysis of their developmental sequence.

The length of the panicle inflorescence was correlated with cytological features of microspore/pollen development. Five panicles between 20 and 35 cm in length were used to isolate the cells from the anthers, as they had the highest probability of containing all relevant developmental stages in a single plant.

Cytological features of microspores/pollen were used to identify the cellular stages corresponding to the male floret developmental sequence. The anthers were dissected and microspores/pollen were stained with DAPI for fluorescence microscopic analysis (Liu, et al., 1993). Stained cells were incubated in an aluminum foil covered moisture chamber for 45-60 minutes. The slides were then washed with PBS three times. This removes excess DAPI, reducing background staining. Permanent slides were made by sealing a coverslip to the slide with clear fingernail polish. A Nikon fluorescence microscope was used to identify cytological features of chromosomes and cell wall formation in the development of microspores/pollen.

Statistical pollen counts were obtained by dissecting a single anther to isolate microspores or pollen using a Nikon dissecting microscope. A Hausser Scientific Brightline Hemacytometer was used to count microspore/ pollen number per anther. Twelve Pollen counts were obtained from six individuals by multiplying the number of pollen grains in a single square by the number of squares on hemacytometer.

Mutant analysis:

Anthers of Bottlebrush with developmental defects were also collected from the Grand Rapids Experiment Station. Panicles with lengths between 25 and 35 cm were selected to identify initial cellular defects of the mutation during pollen development. Anther lengths ranging from 3.5 mm to 3.85 mm were dissected and cells were stained with DAPI to observe early developmental defects. Three different individuals were used to study cellular defects contributing to male sterility. Three samples from each of three individuals were also taken from Franklin at similar stages as a control.

Results and discussion:

Microsporogenesis and developmental sequence in male florets:

The developmental sequence of male florets in a panicle of wild rice was determined (Franklin) by correlation of cytological features with their morphology. Statistical pollen counts ranged from 3552 to 3992 per anther in twelve wild rice anther samples. Male floret development progressed from pollen mother cell to mature pollen from the base of the panicle to the top of the panicle, and suggested the direction of development (see fig. 1). Also the developmental sequence progressed from early stages to later stages of cell division from the base to the tip of the side branch in the male inflorescence

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(see fig. 1). This suggested that the side branch of the panicle developed in the direction from the base to the tip (see fig. 1).

Chromosomal changes in cell cycle progression from meiosis to pollen mitosis (from pollen mother cells to pollen formation) are defined as the cell division index during microsporogenesis (Fig. 2 and 3). Anther sizes from six individuals were correlated with a cell division index in Franklin. Table 1 shows a positive correlation between anther length and cell division index. As the anther size increased, cell division progressed further during development.

Developmental stages that overlap were also observed in branches adjacent to each other. In the region of overlap, a similar cell division index occurred during the panicle development of Franklin. It was noted that the internodes from plant to plant may vary in different individual plants in Franklin. This led to different amounts of overlap of the cell division index between plants.

Male sterile mutation:

Microspore development in three male sterile (ms) plants from Bottlebrush were analyzed. When comparing ms panicles with those in Franklin, the spikelets in male sterile plants were generally more brown than that in Franklin. This may be attributed to the cells death in the mutation. Anthers in Franklin and Bottlebrush with similar sizes had approximately the same cell division index at similar developmental stages.

Cell synchronization was highly conserved in Franklin as opposed to that in Bottlebrush. However, various cell stages such as Prophase I, Metaphase I, Anaphase I, and Telophase I were observed in a single anther of Bottlebrush (see table 2). This suggested that cell divisions were not synchronized in mutant plants. After the tetrad stage, Bottlebrush cell size appeared to be larger overall and the nucleus was also enlarged compared to that in Franklin. Furthermore, the cell wall formation was not observed in microspores of Bottlebrush and the chromatin stained less intensely. The degradation of the chromatin along with cytoplasm discoloration indicated cell death of these mutants.

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Table 1. Anther size in relation to cell division index in Franklin. This table represents a range of values obtained from 6 individuals.

Anther size (mm)	Cell Division Index
< 2.63	Before Pollen Mother Cell
2.68-3.68	Pollen Mother Cell
3.73-3.94	Dyads
3.99-4.99	Tetrads
> 5.04	Pollen

Table 2. Comparison of synchronization of cell divisions in Franklin vs.
Bottlebrush. All values except total cell number are expressed as percentages.

SAMPLE	DEVELOPMENTAL STAGE									
Franklin	A	B	C	D	E	F	G	H	I	Total # *
1	100	0	0	0	0	0	0	0	0	583
2	100	0	0	0	0	0	0	0	0	655
3	0	0	0	0	1	99	0	0	0	600
Bottlebrush										
1a	16	52	2.2	4.2	3.7	13	3.3	4.5	0.6	753
1b	72	23	3.7	0	1.4	0	0	0	0	642
1c	28	54	2.8	4.5	7.5	12	3.9	3.3	0	593
2a	25	47	1.8	3.7	7.0	9.7	3.1	2.2	0	667
2b	25	24	2.5	5.7	11	20	2.8	9.7	1.0	596
2c	47	32	0.9	2.2	4.4	11	0.7	0	0	610
3a	21	32	0.6	1.3	5.9	29	7.2	2.1	0.2	651
3b	16	26	1.0	2.7	11	32	3.0	7.5	0.7	660
3c	5.8	7.5	2.7	2.4	7.5	31	2.4	38	5.4	733

A= Prophase I
B= Metaphase I
C= Abnormal Metaphase I
D= Anaphase I
E= Telophase I
F= Dyads
G= Tetrads
H= Microspores
I= Abnormal microspores
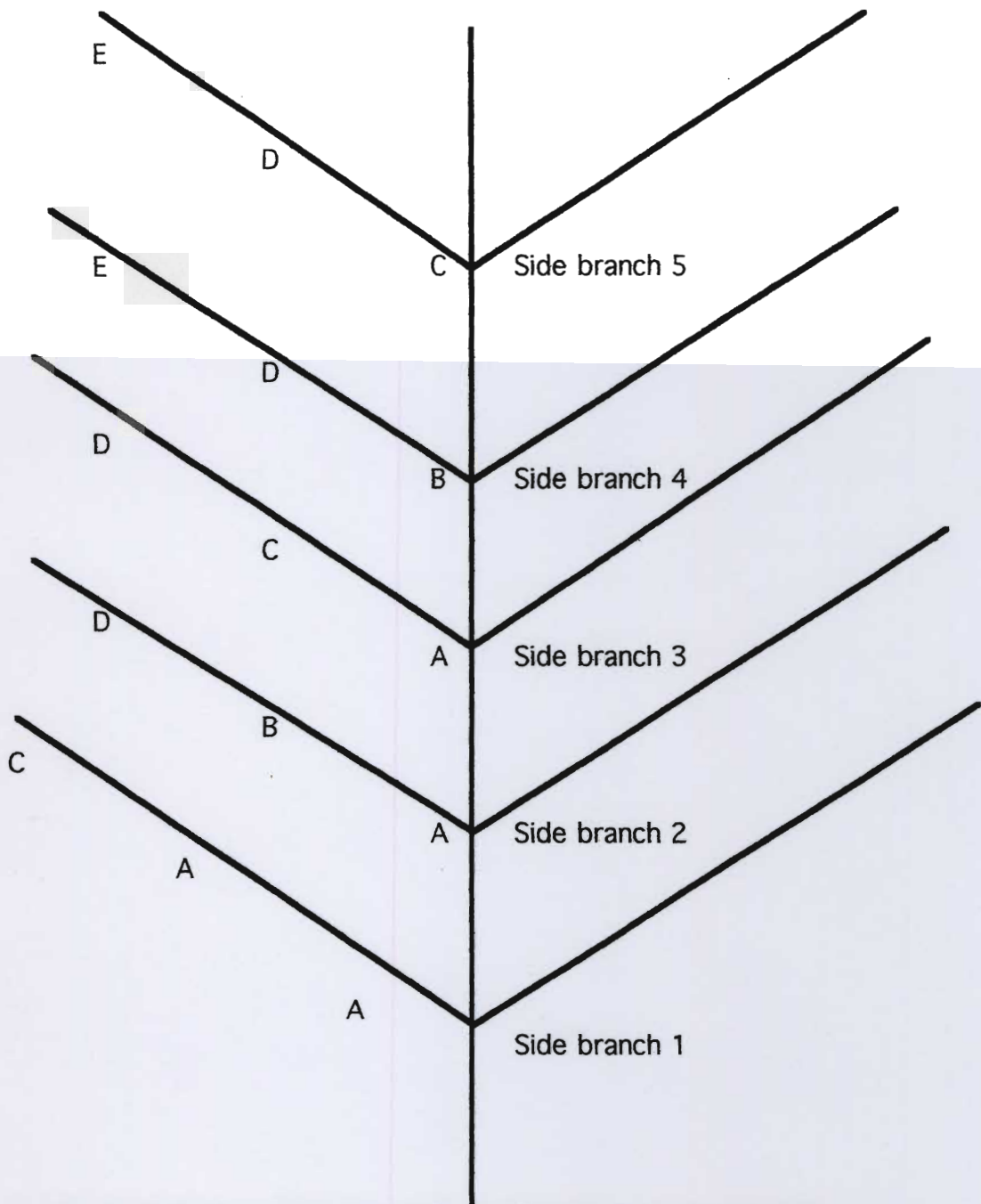* Total cell number counted by fluorescence microscopy

Figure 1. Representative model of panicle development correlated with cell division index. All nodes have three branches from each node, however only two are shown for simplicity. Side branch 1, cell division index (A) overlaps parts of side branches 2 (A) and 3 (A); side branch 2, cell division index (D) overlaps parts of side branches 3 (D) and 4 (D), etc. A= before PMC B= PMC C= dyad D= tetrad E= pollen
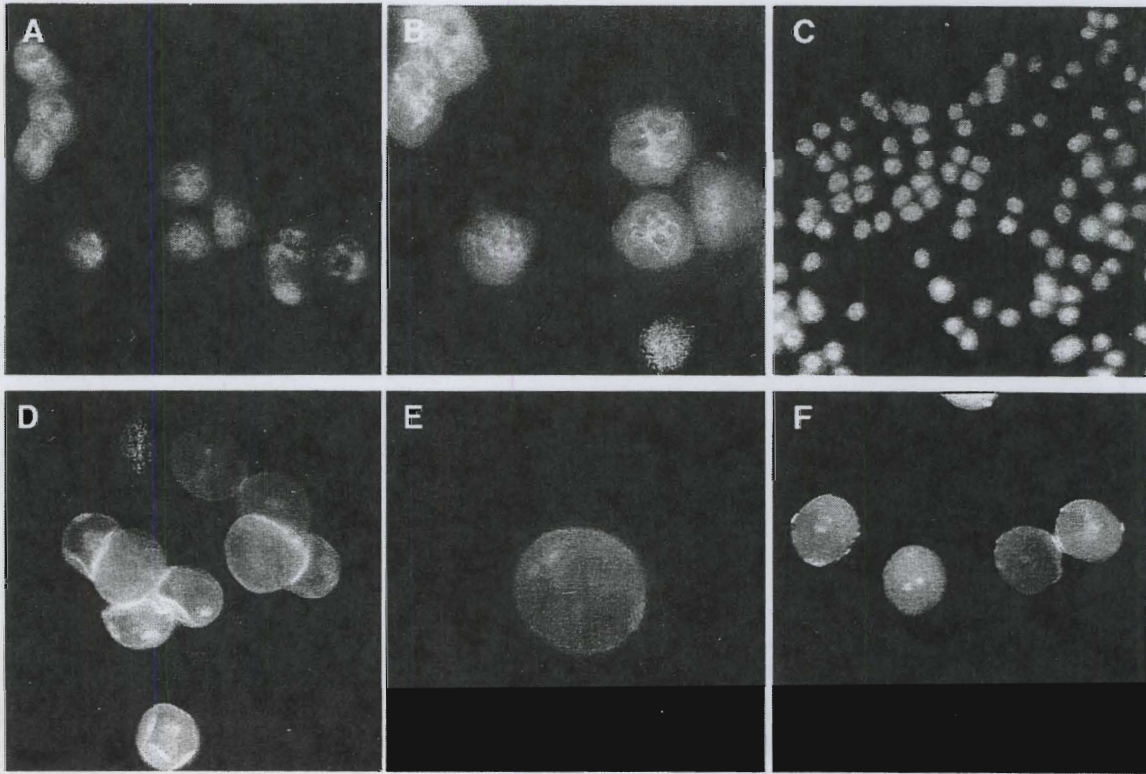
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Fig. 2 (A-F). Chromosomal morphology in pollen mother cells, meiocytes and pollen of wild rice as visualized by epi-fluorescence optics using a DAPI stain for chromatin. (A-B) Prophase chromatin in pollen mother cells; (C) Interphase nucleus of the tetrad; (D) Microspores with formation of the cell wall and nuclear migration during pollen development. (E-F) Two nuclear stage after microspore mitosis during pollen development.
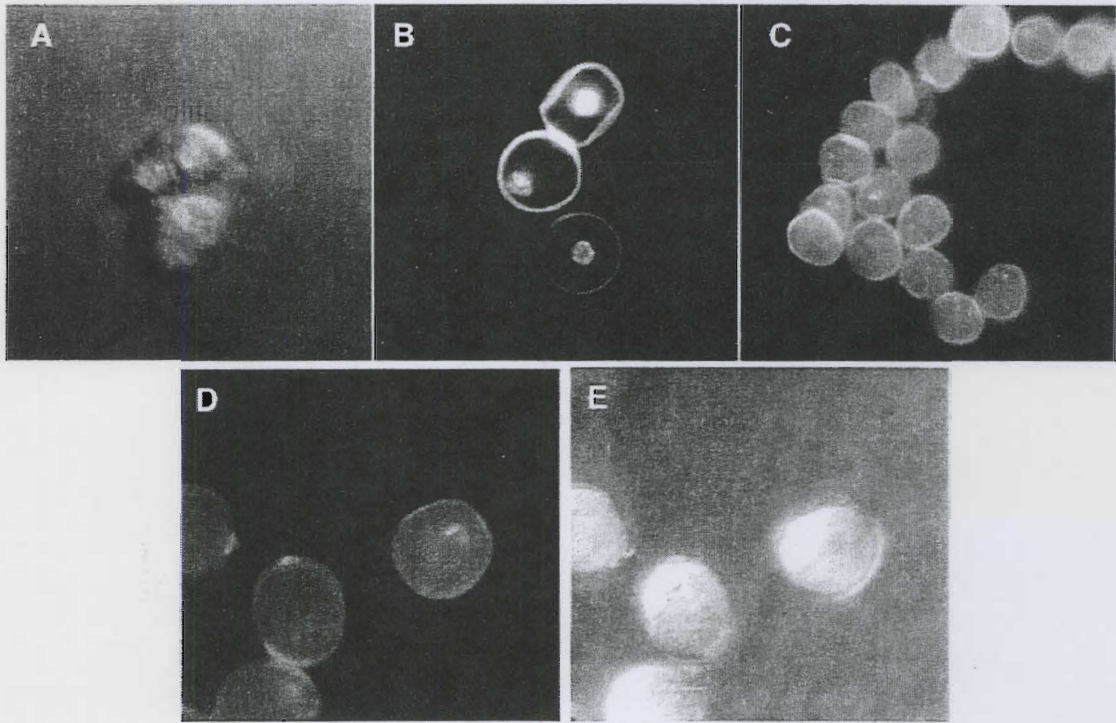
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Fig. 3 (A-E). Microspore and pollen development in wild rice. (A) Interphase nucleus in the tetrad; (B) Interphase nucleus in pollen cells; (C-E) Pollen development after the first microspore mitosis.

Acknowledgement

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Analysis of Transition Zone in Wild Rice Panicle Development

Craig J. Troska, Qinqin Liu*

Introduction:

Wild rice is an aquatic plant which has both male and female spikelets on a terminal panicle. The female inflorescence is located on stiff branches on the terminal end of the panicle. The male inflorescence is located below the female inflorescence on more flexible branches. This spatial relationship, coupled with an earlier maturation of female spikelets (Aiken, et al., 1988), promotes cross fertilization. The transition zone between male and female inflorescence is the branch at the junction between the staminate and pistillate portions of the panicle. 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). Detailed information on the development of hermaphroditic spikelets of the transition zone was investigated in this research. Morphology of hermaphroditic spikelets, cytology of microspore/pollen, and environmental effects on the formation of the transition zone are reported as the results of this research.

Materials and methods:

Identifying the transition zone in wild rice plants:
Wild rice plants originated from eight different lakes were grown at the Grand Rapids Experimental Station of the University of Minnesota. The panicles of these wild rice plants were collected and fixed in ethanol: acetic acid (3:1) for 24 hours at 4 °C, transferred to 70% ethanol and stored in covered plastic boxes in a cold room (4 °C) for future analysis (Liu, et al., 1993). Anther samples from the transition zone of male sterile plants (Bottlebrush) were also prepared to study microspore/pollen formation.

The morphological features of both male and female florets were investigated to identify the changes from male to female florets in the transition zone of these panicle samples. Panicles between 30 and 40 cm in length were selected to identify pollen cytology in the anthers of hermaphroditic spikelets of the transition zone. Using a Nikon dissecting microscope, anthers were dissected, and statistical pollen counts were obtained by multiplying the number of pollen grains in a single square by the number of squares on a Hausser Brightline Hemacytometer. Microspores/pollen were stained with 4',6-

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diamidino-2-phenylindole (DAPI) (Liu et al., 1993) and viewed under a Nikon fluorescence microscope to study their cytological features during anther development in the transition zone of the panicle.

Results and discussion:

A unique branch was observed in the transition zone in Franklin and other wild rice plants from different lakes, with staminate spikelets on the lower portion of the branch and pistillate spikelets on the upper portion of the branch. Two or three hermaphroditic spikelets were found in the middle of this branch. There was no significant difference in pollen counts in transition zone anthers compared to staminate anthers (see table 1). Chromatin morphology and other cytological features in transition zone anthers were normal, which indicated that the pollen cells were functional for self fertilization. According to Goldman (1990), the pistillate spikelets matured before the staminate spikelets. However, selfing may occur in transition zone spikelets. In transition zone spikelets, the stamens and ovaries were quite well developed (Weir and Dale, 1960). It was noted that the arms of the stigma, although presumably functional, seemed stunted and weaker than stigmas in pistillate spikelets (Weir and Dale, 1960).

Transition zone anthers were also found in male sterile Bottlebrush, but an accurate percentage could not be obtained as the sample size was too low. No transition zone was observed in pistillate mutations. Only microspores with degrading chromatin were observed in anthers from the transition zone in Bottlebrush.

Ecological lake effects on the frequency of transition zone formation were investigated by sampling wild rice plants that originated from eight different lakes (see table 2). Transition zone formation ranged from 11.1% to 57.1% in plants from different lakes. Such variable lake effects on transition zone frequency suggested an environmental regulation on wild rice development. As the sample sizes were not large enough to yield final data, it is impossible to present speculation on this matter at this time. Future studies on environmental regulation of wild rice development will be followed to address this important issue.

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Table 1. Comparison of pollen counts in transition zone anthers vs. staminate anthers in Franklin.

Franklin	A*	B*
1	3591	3800
2	3872	3800
3	3800	3718
4	3700	3860
5	3576	3552
6	3872	3992

A= Pollen count in transition zone anthers
B= Pollen count in staminate anthers
* Indicates normal cytology in chromosomal structure and cell wall formation in both A and B.

Table 2. Comparison of Transition zone frequencies in wild rice plants from eight different lakes.

Lake	N	Normal*	Transition Zone	Frequency, %
Big Rice	10	7	3	30
Bowstring	7	3	4	57.1
Dora	17	13	4	23.5
Laure	10	6	4	40
Moose	6	4	2	33
Prairie	9	8	1	11.1
Rice and Bow	12	9	3	25
White Elk	13	8	5	38.5

N= total sample number
* Indicates that there is no transition zone observed in these plants

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CULTIVATED WILD RICE PADDIES AND THEIR RELATIONSHIP TO

WATERFOWL IN NORTHWESTERN MINNESOTA - 1995 ANNUAL REPORT

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INTRODUCTION

Wild rice (*Zizania palustris*) has long been recognized as a preferred duck food. Martin and Uhler (1939) called it one of the best known of North American duck foods, and Rogosin (1951) classified wild rice as one of the important duck foods in the United States and Canada. Cultivated wild rice is produced in diked paddies that are flooded in the spring and drained in late summer. Water levels in paddies are maintained at 15 - 25 cm throughout most of the growing season (early May - mid June), providing large expanses of shallow, open water available for use by waterfowl and other waterbirds during spring and fall migration and the breeding season.

Paddy waters are attractive to waterfowl due to invertebrates; wild rice seeds; sago pondweed (*Potamogeton pectinatus*) seeds and tubers; and arrowhead (*Sagittaria latifolia*) tubers. Heitmeyer (1990) recognized the importance of harvested white rice (*Oryzon sativa*) fields to California's wintering waterfowl and stated that food sources such as harvested grain crops, especially rice, are critical to the survival and reproduction of many migratory water birds. The amount of food found on California's cultivated rice fields may provide as much as 70 percent of the food required to meet the waterfowl population objectives of the California Central Valley Joint Venture (CVHJV), a component of the North American Waterfowl Management Plan (Heitmeyer et al. 1989).

Sorenson (1973) and Johnson (1976) found large numbers of migrant waterfowl, and many breeding duck pairs, to use Minnesota's cultivated wild rice paddies during spring. We documented significant waterfowl breeding populations as well as high fall migrant densities using wild rice paddies in our northwest Minnesota study area. This study (spring 1993 - fall 1995) will provide a more complete understanding of the extent to which waterfowl use Minnesota's cultivated wild rice paddies and associated upland nesting cover during nesting, brood-rearing, and migratory periods.

STUDY AREA

Our study area was located in northeast Polk, northwest Clearwater, and southeast Pennington counties, about 19 km north of Gully, Minnesota. It is located along a major migration corridor of the prairie pothole and lake country of western Minnesota. Agassiz National Wildlife Refuge and the Thief Lake Wildlife Management Area are

located about 45 and 65 km to the northwest, respectively. The study area included 3600 ha (14 sections) of land which contained a variety of idle brushlands, pasturelands, small grain fields, woodlands, and Conservation Reserve Program (CRP) land, in addition to about 1300 ha of wild rice distributed in some 80 paddies. The Clearwater River bisects the study area and serves as the water source for wild rice production. The land is owned and operated by John, Jim, and Ken Gunvalson, Paul Imle, and Duane Erickson. This area is representative of the prairie - forest transition area, where about half of the cultivated wild rice is produced in Minnesota.

METHODS

Objective 1: Use of cultivated wild rice paddies by migrant waterfowl.

Spring and fall census routes

Species composition and density (number of birds per flooded paddy hectare) were recorded along a pre-selected, 8-km census route. The route included up to 17 paddies (depending on paddy flooding chronology) covering up to 316 ha. The route was chosen with regard to representative habitat and vehicle access, and included paddies of various sizes (2.18 - 101 ha), shape, and surrounding habitats. The census was conducted weekly, with counts alternating between mornings (0700 - 1000) and evenings (1700 - 2000) when waterfowl were most active. Due to variation in paddy size and the potential for ducks to be hidden by vegetation along paddy and island edges, data were recorded using a combination of the single point observation method and the circuit count (Hammond 1970). Researchers stopped at several observation points when observing large paddies to more accurately record bird numbers. Birds flushed from cover along paddy edges were recorded as researchers moved from one observation point to the next and were discounted if they landed in paddies farther along the route. Spring data collection began in early April, when open water began to appear on some of the paddies, and continued to late May, when the visibility of waterfowl became restricted by standing rice. Census routes were resumed in mid to late September, once flooding on some of the paddies had begun, and continued through October until paddy freeze-up.

Paddy food resources

To estimate food resources available to migrating waterfowl, soil core samples were collected from 6 paddies from 1-10 October 1993, following cultivation and prior to fall flooding. To evaluate effects of different cultivating practices on food availability, 3 moldboard and 3 chisel - plowed paddies were sampled. A soil bulk density sampler with a diameter of 10.16 cm. was used to collect 240 cores from the top 15 cm. of paddy substrate. Samples represented the peat - water interface where food items were accessible to feeding waterfowl when paddies are flooded. Beginning at the center of the paddy, 10 cores were collected at 10 m intervals in each of the 4 cardinal

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directions. Half of the samples from each paddy (20) were randomly chosen for analysis. Samples were placed in warm water to loosen the mostly peat substrate, screened through a 2 mm soil screen, then transferred to a clean separation tray where food items were separated and tallied. Plant seeds and other vegetative parts were identified using Martin and Barkley (1961). Differences in the occurrence of food items in moldboard versus chisel-plowed paddies were assessed.

Objective 2: Waterfowl production and habitat use of paddies and associated uplands.

Breeding population census

Local breeding populations were estimated using methods similar to those of the Wetlands Wildlife Research Unit, Minnesota Department of Natural Resources, Bemidji (Todd Eberhardt, pers. comm.) and the Agassiz National Wildlife Refuge (Gary Huschle, pers. comm.). Census routes were chosen where vehicle access on dikes or roads was good. All ducks within a 200 - meter strip from the vehicle into a paddy were identified to species and placed into 1 of 4 categories: lone drakes, flocked drakes (<5), pairs, and groups (>5). Only the length of the 200 - meter transects along paddy edges was used in calculating total area censused. Counts were conducted during the third week in May between 0600 and 0930.

Nest searching

Nest searching began during the second week in May when some upland - nesting hens had begun incubation. Searches were conducted in several ways. Open, upland cover (i.e., CRP land) was searched by dragging a 15.2 m length of cable or a 30.5 m length of chain between 2 all - terrain vehicles (ATV's) to flush females from nests (Klett et al. 1986). Paddy dikes were searched with ATV's equipped with a rack-mounted flushing bar, and nesting cover inaccessible to ATV's was systematically searched by researchers on foot, using sticks to disturb vegetation and flush nesting hens. Potential nesting cover associated with paddies (bordered by a paddy on at least 1 side) was searched at 2 - week intervals throughout the nesting season.

Nests were marked 3 m to the north with a 1.5 m willow stake and flagged with surveyor's tape. The following information was recorded for nests: date found, location on base map, 100% visual obstruction reading (VOR) using a Robel pole (Robel, et al. 1970), number of eggs present, and laying or incubation stage when found. Information was updated each time a nest was revisited.

Trapping and marking nesting hen mallards

After a mallard nest was located, it was revisited at an estimated incubation stage of 20 days and the hen was flushed. During the visit, nest data were updated and a walk - in

nest trap (Dietz et al. 1994) was placed over the bowl. If the hen's approach path to the nest could be located, the single funnel-like opening of the trap was positioned over it, allowing the hen unobstructed access to her nest. The trap was left in position for 3 hours following placement, allowing the hen time to return and resume incubation. Researchers rapidly approached the trap from the direction of the opening, and the hen was usually captured. Captured hens and the nest trap were immediately removed from the nest site to reduce the amount of disturbance to surrounding cover. Processing took place 50 to 100 m from the nest site where hens were radio-marked with anchor transmitters weighing about 3.8 grams (Source: Advanced Telemetry Systems, Esko, MN) and equipped with U.S. Fish and Wildlife Service leg bands. Transmitters were attached at shoulder level along the midline of the back where they were held in place with three polypropylene sutures and a stainless steel anchor-shaped wire that was inserted subcutaneously. Transmitter attachment procedures were similar to those described by Pietz et al. (1995). Average processing time was about 15 minutes per hen. After processing, some hens were mildly anesthetized using methoxyflurane and placed beside their nests; others were released from the site of processing. The anesthetic was used to allow researchers to leave the nest site without flushing the hen from the immediate area, reducing the risk of abandonment (Rotella and Ratti 1990).

Processing newly-hatched ducklings

Mallard nests were visited near the predicted date of hatch (24-25 days incubation). If the nesting hen had previously been equipped with a transmitter, this visit would occur while she was absent from the nest. Candling eggs at this stage of incubation allowed a more accurate estimate of hatch date. On the predicted date of hatch, the hen was flushed from the nest and the newly-hatched ducklings were processed. The entire brood, or those ducklings completely out of their eggs, were placed in a 5 - gallon container, covered with a towel and removed from the immediate nest area. All recovered ducklings were web-tagged, and up to 5 randomly chosen ducklings were equipped with 1.9 gram transmitters, using procedures similar to those described for hens. After processing, ducklings were returned to the nest bowl and covered with nesting material and/or a towel until their activity level dropped to a point where there was minimal risk of them leaving the nest bowl before return of the hen.

Monitoring movements and survival of radio-marked hens and broods

Once radioed, adult hens were located daily to monitor movements and nest status. Broods were tracked from the nest to their first wetland and then located twice daily. Broods and individual hens were monitored using truck-mounted, dual 5-element null detection systems, and locations were determined by triangulation from 2 or 3 points identifiable on base maps. Broods were tracked either by monitoring individual duckling frequencies, or in the case where only the hen had been radioed, by following her frequency. Radio-marked individuals were monitored throughout the summer until

fledging, mortality, or loss of radio signal. Visual observations of broods were obtained when possible to verify survival of non-radioed ducklings. Data collected on radioed hens and broods were used to determine movements, habitat use, and survival of broods.

RESULTS

Objective 1: Use of wild rice paddies by migrant waterfowl.

Spring and fall census routes

Census route data collection began the first week in April, when open pockets of water appeared on some of the paddies flooded the previous fall. Although open water was limited, what was available held large numbers of waterfowl: particularly mallards (*Anas platyrhynchos*), pintails (*Anas acuta*), Canada geese (*Branta canadensis*), common goldeneye (*Bucephala clangula*), and ring-necked ducks (*Aythya collaris*). These 5 species accounted for 100% of birds recorded on the first spring census visit in 1993, 91.5% in 1994, and 65.6% in 1995. By mid-April, most of the fall-flooded paddies had begun to thaw, containing some open water, and pumping had begun to fill other paddies. Spring use of paddies peaked between mid-April and the first week in May when densities reached 22.5, 24.5, and 14.8 birds per flooded paddy ha in 1993, 1994, and 1995, respectively (Fig. 1). Species richness was highest during the first 2 weeks in May, when between 16 and 18 different waterfowl species were recorded during counts (Fig. 2). Species represented by greatest numbers (% composition) were; tundra swans (*Cygnus columbianus*) (21.2 - 48.4%), ring-necked ducks (5.0 - 29.4%), lesser scaup (*Aythya affinis*) (7.0 - 26.0%), mallards (5.4 - 21.7%), northern pintails (0.4 - 8.9%), American wigeons (*Anas americana*) (2.6 - 6.3%), and blue-winged teal (*Anas discors*) (0.2 - 13.0%). Also present were gadwalls (*Anas strepera*), green-winged teal (*Anas crecca*), northern shovelers (*Anas clypeata*), wood ducks (*Aix sponsa*), canvasbacks (*Aythya valisineria*), redheads (*Aythya americana*), American coots (*Fulica americana*), snow geese (*Chen caerulescens*) and Canada geese.

By mid-May, many of the more northern breeders (northern pintails, tundra swans, and most of the divers) had departed, and waterfowl densities began to decrease, stabilizing at about 10% of peak spring density (Fig. 1). By the beginning of June, wild rice had passed the floating leaf stage of development and restricted visibility to the extent that routes were discontinued. Species present at that time were mallards, gadwalls, American wigeon, blue-winged teal, wood ducks, northern shovelers, redheads, lesser scaup, ring-necked ducks, and Canada geese. All species except redheads were confirmed to have nested on the area.

To estimate paddy use by fall migrants, the census was resumed during the third week in September, the onset of fall-flooding. Waterfowl began using paddies immediately after flooding. Flooding continued until freeze-up, and waterfowl use increased as

more paddies were flooded. A large influx of birds occurred just prior to freeze-up in all years, with densities in excess of 54 birds per flooded paddy ha recorded in 1993 and 1994 and over 30 birds per ha recorded in 1995. Although peak fall densities were considerably higher than those observed during spring, the greater numbers were represented by fewer species. Tundra swans, Canada and snow geese, mallards and ring-necked ducks accounted for between 89 and 99% of all birds recorded during peak fall use periods.

Spring use of paddies varied somewhat between years, with more species arriving on the paddies earlier and remaining longer in 1994 and 1995 as compared to 1993 (Fig. 2). Maximum use was similar during the 3 years, but peaked about 2 weeks later in 1994 and 1995. Peak fall use in 1994 and 1995 also occurred almost 2 weeks later than 1993. Species observed using paddies changed little between years, except for American coots, which were very abundant on paddies throughout the spring and summer of 1993, but were seldom observed in 1994 and 1995.

Paddy food resources

In addition to wild rice, seeds from sago pondweed, arrowhead, smartweed (*Polygonum* spp.), and water plantain (*Alisma plantago-aquatica*) were recovered from cores. Sago pondweed and arrowhead tubers comprised the remaining major food items in samples. Total weight for the most frequently observed food items (wild rice, sago pondweed seeds and tubers, and arrowhead tubers) was calculated on a per ha basis, with fresh (wet) weights multiplied by 0.13 to obtain dry weights (Kantrud 1990). Using averages for gross energy of these items (Reinecke and Owen 1980, Fredrickson and Reid 1988), and assuming an overall 70% conversion efficiency, nutritional value and availability were calculated (Table 1). Combining the 4 food items, our samples indicated that prior to fall-flooding, a single ha of paddy contains over 315 kg (dry weight) of quality duck food. Duck use days (DUD's), the number of ducks that could survive on a ha of wild rice paddy for 1 day based on food availability, was calculated following Prince (1979) and Reinecke et al. (1989) where:

$$DUD's = \frac{[food\ available\ (g\ dry\ mass)\ X\ metabolizeable\ energy\ (kcal\ /\ g\ dry\ mass)]}{[daily\ energy\ requirement\ (kcal\ /\ day)]}.$$

Based on the energy requirements of mallard ducks (Prince 1979), a single ha of wild rice paddy can provide over 3200 duck use days.

Analysis of food abundance suggested that method of cultivation may affect availability of some food items. We found that chisel-plowed paddies contained significantly more wild rice and sago pondweed seeds, while moldboard-plowed paddies contained significantly more arrowhead and smartweed seeds. No differences in abundance were

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found for water plantain seed or sago pondweed and arrowhead tubers. Due to the soft nature of the peat, cultivation method probably has little effect on the availability of food items to long-necked species, such as geese and swans.

Objective 2: waterfowl production and habitat use of paddies and associated uplands.

<u>Breeding population</u>

The breeding population of ducks was estimated from total lengths of 200-meter strips along paddy edges of 19.6 km in 1993, 22.2 km in 1994, and 23.5 km in 1995. Combining the 3 year's data, 13 species were recorded (Table 2), 11 of which nested on the area. Mallards, blue-winged teal, and northern shovelers accounted for the bulk of the estimated breeding population, comprising between 78 and 88% of the total breeding birds counted during the 3 years. Density of breeding ducks was 2.61, 1.87, and 1.43 birds per ha of paddy in 1993, 1994, and 1995, respectively. This translates into roughly 675, 480, and 370 breeding ducks per square mile of paddy. Improved water conditions on the western prairies of the Dakotas and Canada may have accounted for decreased breeding pairs recorded on paddies in 1994 and 1995.

<u>Nesting</u>

Throughout the study, 392 upland waterfowl nests were located while cable-dragging, dike dragging, searching potential nesting cover on foot, or incidental to other field work. The peak of nest initiation for upland nesting duck species (mallards, blue-winged teal, green-winged teal, northern shovelers, gadwalls, and northern pintails) occurred during the middle of May (Fig. 3). Over 38% of all upland duck nests were initiated from 8 May to 22 May, so the peak of hatching occurred during mid-June with ducklings fledging in early August. Mallard nest initiation was relatively constant from the first week in May through the third week in June with only 7 nests initiated outside of this period. Blue-winged teal nest initiation peaked during the second and third weeks of May, gradually tapering off in early July. Most of the northern shoveler nests (28 of 34) were initiated during weeks 2, 3, and 4 of May and week 1 of June with no distinct peak. Of the 12 gadwall, 10 American wigeon, 2 green-winged teal, and 2 northern pintail nests located, all were initiated between the last 2 weeks in May and the first 2 weeks in June.

On 29, 30, and 31 May of 1993, the emergent vegetation, [mainly cattail (*Typha* spp.)], along perimeter and interior ditches of about 315 h of paddies was searched via canoe. Overwater nests of 58 American coots, 1 pied-billed grebe (*Podilymbus podiceps*) and 1 canvasback were located. Due to time constraints of other field work, nest success was not determined except for the canvasback nest which was successful. During 1994 and 1995, no American coot nests were observed on paddies.

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Nest success was determined for all waterfowl found nesting on the study area during 1993, 1994, and 1995 (Table 3). Nine species nested in uplands, mainly blue-winged teal, mallards, and northern shovelers. A total of 392 nests were located and monitored, 155 of which hatched, yielding an apparent nesting success of 39.5%. Overall nesting success varied little between years with Mayfield estimates being 22.7%, 20.5%, and 21.9%, respectively. Northern shovelers had the highest success with an average Mayfield success of 30.4% for the 3 years while mallards showed the lowest success, averaging 9.1%. Because hens and broods were radioed at or near the nest site, mallard nests were visited almost twice as often as nests of other species and probably experienced lower nesting success because of it.

Analysis of nesting data from 1993 suggested nest site selection influenced success of a given nest. Hens nesting on islands, or in large blocks of cover had 41.5% apparent success, while those nesting on road sides, paddy dikes, or other strip cover showed only 7.1% success. Nest success was significantly dependent on nest site selection by upland-nesting hens (chi-square = 11.406, P<0.001). Investigator influences, in addition to nest site selection by hens, were also found to influence nest success or failure. Factors such as: whether or not a capture attempt was made on a nesting hen, how a nest was located, and the number of investigator visits to a nest all influenced the predictability of nest success. Analysis of 1994 and 1995 nesting data will provide a more complete understanding of factors affecting nesting success throughout the study.

Predation was the main cause of nest failure, accounting for 81% of unsuccessful nests in 1993, 85% in 1994, and 84% in 1995. Predators commonly recorded on the study area and suspected to have preyed on duck nests were: striped skunk (*Mephitis mephitis*), Franklin's ground squirrels (*Spermophilus franklinii*), mink (*Mustela vison*), red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), and coyote (*Canis latrans*). Abandonment, agricultural practices, and investigator disturbance contributed to remaining losses.

Capture, processing, and radio tracking mallard hens and broods

During the 1993, 1994, and 1995 nesting seasons, 32 mallard hens were captured on nests and tracked a total of 915 radio days. Average length of monitoring period for individual radioed hens was 28.6 days. Nineteen of these hens were successful in their nesting attempts and we radio tagged 17 of their broods at the nest. A total of 77 mallard duckling were equipped with radio transmitters, with 70 captured on the nest at hatching, representing 19 separate broods (16 with radioed hens and 3 with unradioed hens). Ducklings processed at the nest site were tracked for a total of 1,070 radio days and the average monitoring period per radioed duckling was 15.3 days (range: 1 - 65 days). In 1994, a brood of 14 - day old radio-marked ducklings was apparently abandoned by their radioed hen and adopted by a green-winged teal hen. In 1995, brood mixing occurred in a brood of 19 - day old ducklings. One radioed duckling remained with the radioed hen until fledging, and 3 radioed ducklings apparently joined

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a brood of 10 - 12 non-radioed mallard ducklings. All 3 of these radioed ducklings also fledged.

All 17 radio-marked broods were located on a wild rice paddy within 24 hours of being radioed. Distance traveled from the nest to the first wetland (paddy) varied from a few meters (island nesters) to over 1.5 km, with the average straight-line distance about 155 m. Over 78% of the radioed ducklings survived the period between radio attachment and arrival on first paddy, and about 34% survived to an age of 14 days. Survival beyond 28 days was 21.4% (15/70) and all but 1 of these ducklings were suspected to have fledged. Fledging success of radio-marked broods, using visual observations obtained late in brood rearing to verify survival of non-radioed ducklings, was 30%. In 1993, 7 additional ducklings were captured and radioed when they were incidentally encountered on paddies with unmarked hens. These ducklings were tracked for a total of 17 days, but none fledged.

Depredation accounted for about 59% of unsuccessful radioed ducklings with mink being the primary source of mortality for ducklings once they had reached paddies. Franklin's ground squirrels accounted for the loss of 7 radioed duckling in 1995 when they were killed in the nest bowl after processing and before the return of the radioed hen.

Broods reaching paddies prior to the emergence of aquatic vegetation (primarily wild rice) restricted habitat use to cover along paddy edges or interior ditches. Once developing rice passed the floating leaf stage, broods utilized the entire paddy, but still seemed to associate with the open water ditches along paddy edges. Broods reared on paddies remained relatively stationary, unless there had been a disturbance (suspected predation event or agricultural activity) or paddies being drawn down in preparation for harvest. Paddy drawdown began in late July, slowly draining paddy interiors. Broods became concentrated in paddy ditches during drawdown and increased movements and mortality were associated with this event.

SUMMARY

Substantial progress was made during the 3 years of this study (1993-1995) towards a better understanding of the relationship between wild rice farming and waterfowl. Wild rice paddies were found to serve as attractive staging areas for spring and fall migrating waterfowl. In addition to providing expanses of open water resting areas, the shallow water paddies contained a considerable food base to help meet the energetic demands of migration.

A considerable number of nests (392) were located and monitored during the study, despite the relatively limited amount of "high quality" nesting cover in the study area. The decline in breeding duck counts from 1993 to 1994 and from 1994 to 1995 was probably related to abundant precipitation farther west presenting more favorable

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nesting conditions; however, the number of nests located during each of the 3 years documented a substantial, local breeding population. High striped skunk and mink numbers in 1995 probably accounted for some of the reduction in number of nests located that year.

To provide a more complete picture of waterfowl production from wild rice paddies; home range, habitat use, and movement patterns will be related to the annual chronology of wild rice production practices. Preliminary findings suggest that around 40% of broods associated with paddies fledged prior to paddy drawdown but about 25% of observed nests had not hatched when paddy levels began to drop in mid-July.

The importance of natural or constructed wetlands to local waterfowl populations can be generally assessed in terms of use they receive during migration, nesting, and brood-rearing periods. Although the cultivated wild rice paddies of northwestern Minnesota are intensively managed, man-made "wetlands", they play an important role in fulfilling some of the needs of breeding and migrating waterfowl, and may act as "reserve" habitat during years of poor water conditions in natural wetlands.

Data collected during this study will form the basis for a doctoral dissertation at the University of North Dakota.

Acknowledgments

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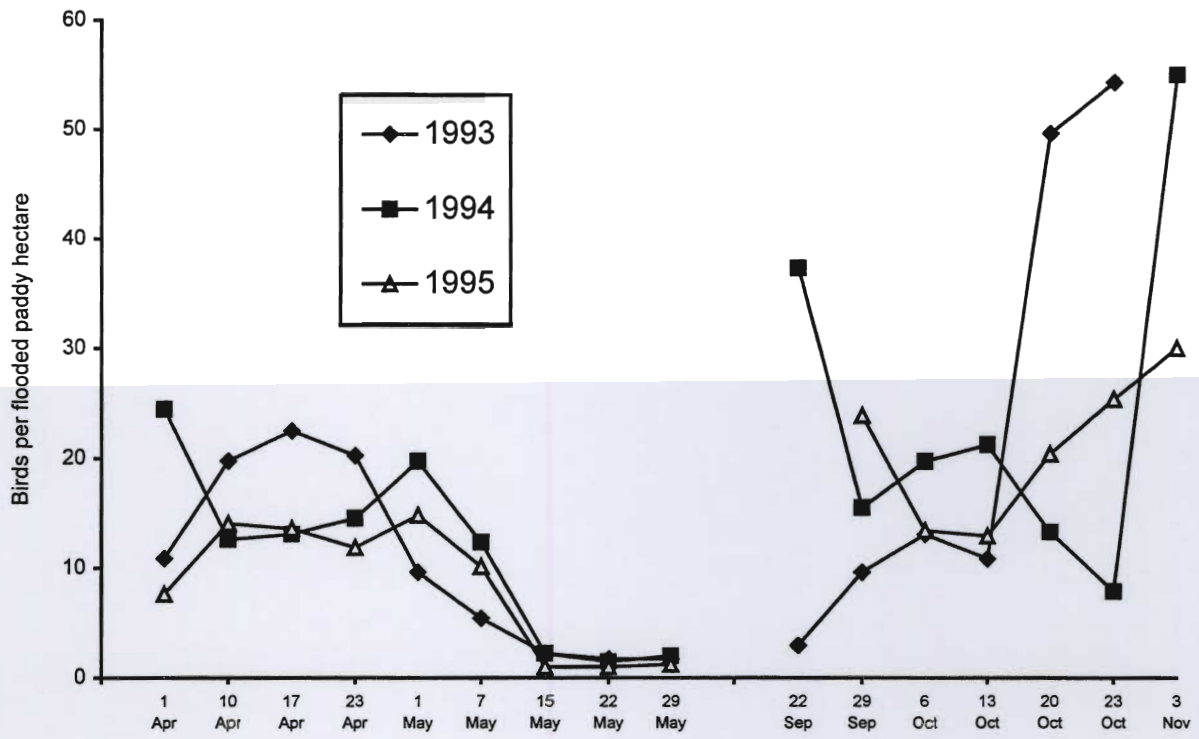
Figure 1. Density of migratory waterfowl using rice paddies in northwest Minnesota, 1993-95.
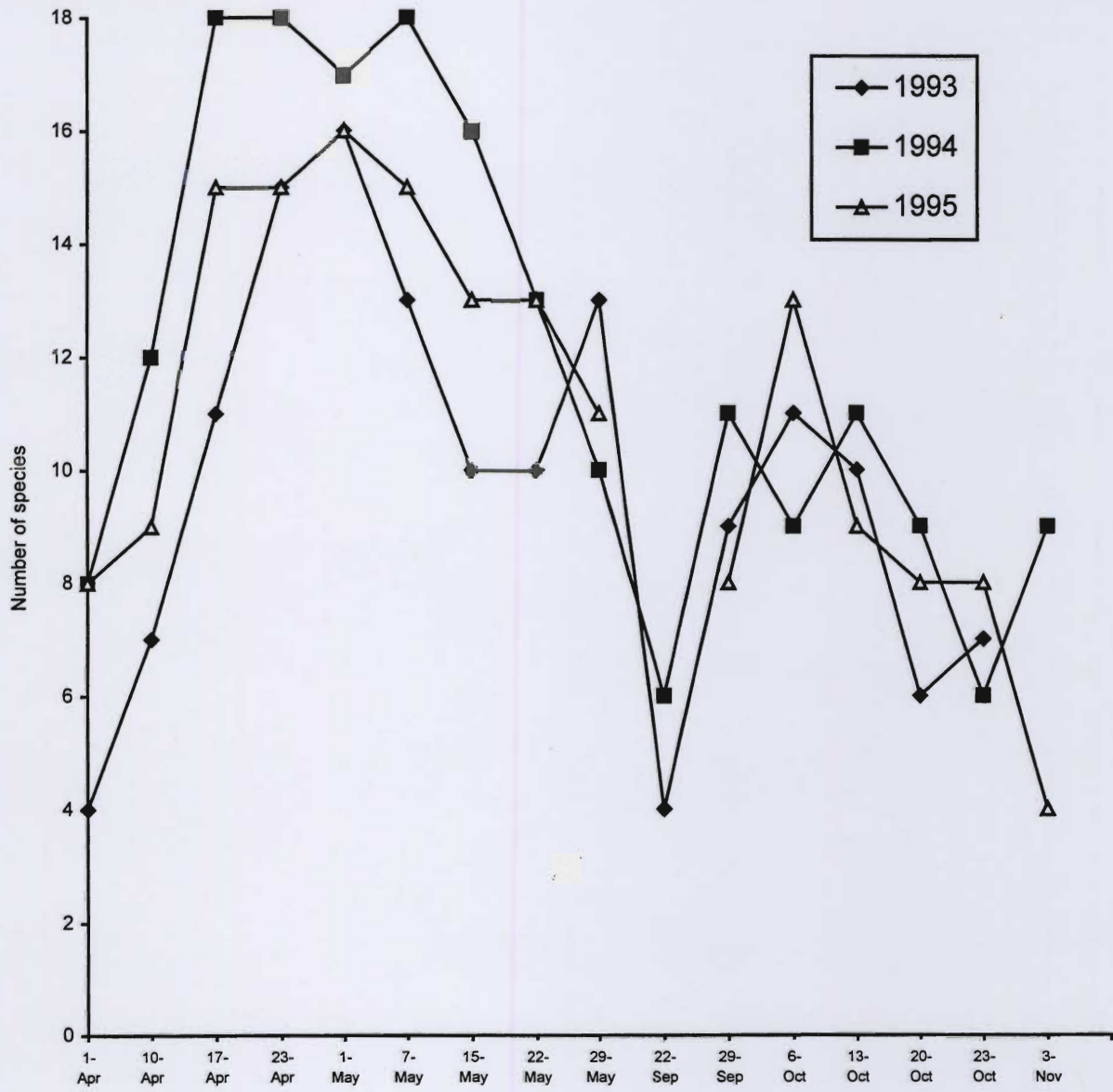
Figure 2. Species richness of migratory waterfowl using wild rice paddies in northwest Minnesota, 1993-95.
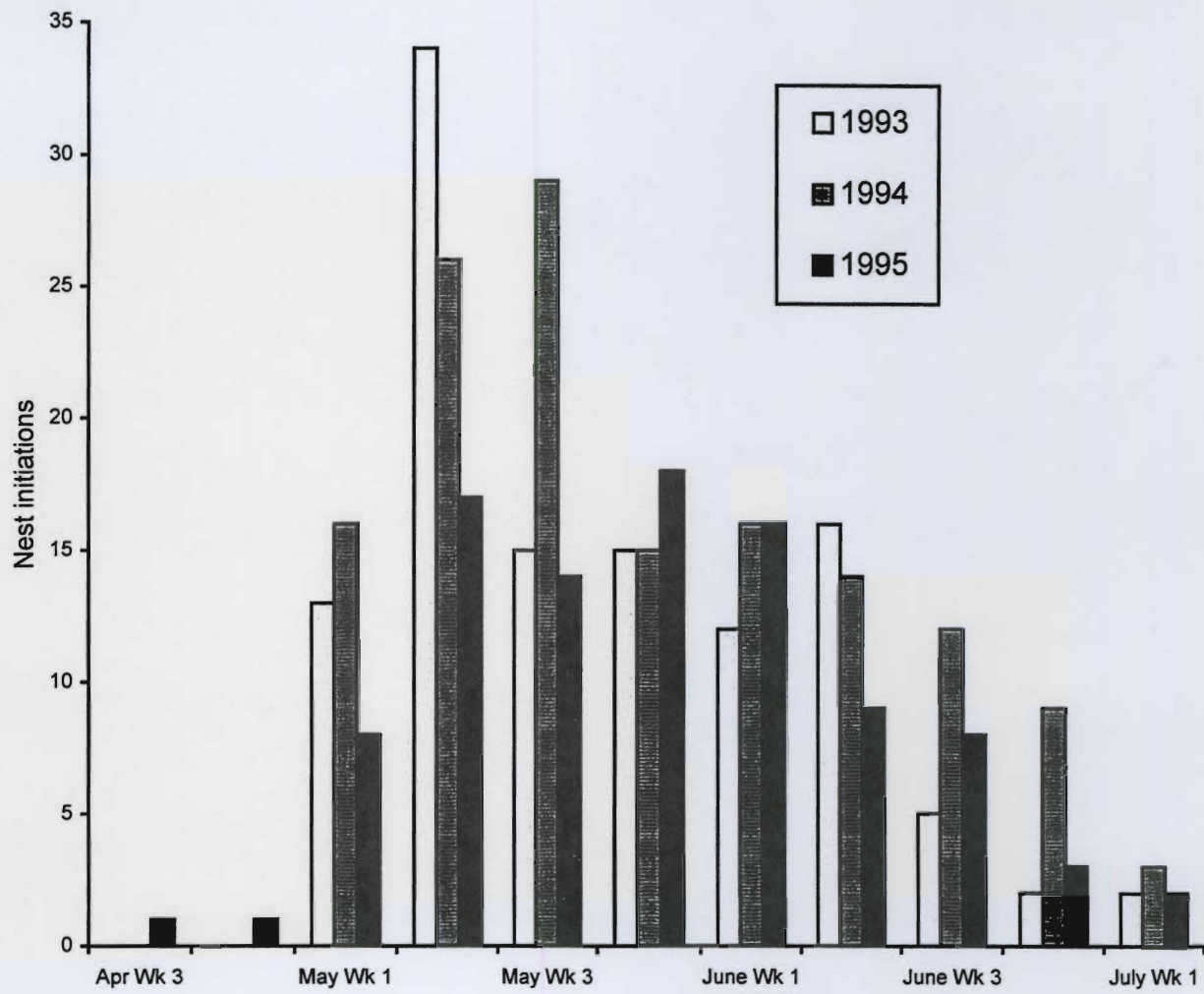
Figure 3. Waterfowl nest initiation chronology in northwest Minnesota, 1993-95.

Table 1.   Food resources available to migrant waterfowl in northwest Minnesota wild rice paddies.

Food item	kg/ha	Metab. energy (kcal/ha)
Wild rice (seeds)	205.59	610,947
Sago pondweed (tubers)	90.90	273,042
Sago pondweed (seeds)	12.77	43,022
Duck potato (tubers)	6.50	21,504
Total	315.76	948,515

Table 2.   Duck breeding populations associated with wild rice paddies in northwest Minnesota, 1993-95.

Species	Density (birds/paddy hectare)			Percent composition		
	1993	1994	1995	1993	1994	1995
Mallard	1.388	0.885	0.672	53.3	47.3	46.5
Blue-winged teal	0.647	0.605	0.294	24.8	32.3	20.3
Northern shoveler	0.259	0.128	0.168	10.0	6.9	11.6
Wood duck	0.077	0.178	0.052	2.9	9.5	3.6
Scaup	0.057	0.047	0.146	2.1	2.6	10.0
Gadwall	0.052	0.010	0.022	2.0	0.6	1.6
Green-winged teal	--	0.005	0.020	--	0.3	1.3
Redhead	0.047	--	--	1.8	--	--
Northern pintail	0.047	0.005	0.015	1.8	0.3	1.0
American wigeon	0.010	0.005	0.037	0.4	0.3	1.0
Ring-necked duck	0.010	--	--	0.4	--	--
Canvasback	0.010	--	--	0.4	--	--
Ruddy duck	0.005	--	--	0.2	--	--
Total	2.609	1.868	1.426			

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Table 3.   Waterfowl nesting in upland cover associated with wild rice paddies in northwest Minnesota, 1993-95.

	1993		1994		1995		
	No. hatched/ No. found	Mayfield success %	No. hatched/ No. found	Mayfield success %	No. hatched/ No. found	Mayfield success %	TOTAL
Blue-winged teal	23/63	18.15	42/94	27.53	18/41	30.05	83/198
Mallard	13/43	9.51	7/42	6.34	8/38	11.43	28/123
Northern shoveler	10/14	37.05	3/8	22.40	5/12	31.69	18/34
Gadwall	2/4	25.88	2/5	12.75	0/1	N.A.	4/10
American wigeon	3/4	N.A.	4/4	N.A.	0/2	N.A.	7/10
Green-winged teal	--	--	1/1	N.A.	1/1	N.A.	2/2
Northern pintail	--	--	--	--	1/2	N.A.	1/2
Ring-necked duck	--	--	1/1	N.A.	--	--	1/1
Canada goose	3/4	N.A.	4/4	N.A.	4/4	N.A.	11/12
Total	54/132 (40.9%)	22.7%	64/159 (40.25%)	20.5%	37/101 (36.63%)	21.98%	155/392 (39.54%)