Minnesota Wild Rice Research – 1993

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WILD RICE PRODUCTION AND PLANT DEVELOPMENT RESEARCH-1993

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The total number of growing degree days in 1993 for the wild rice growing season was greater than for 1992, at all four locations, Aitkin, Grand Rapids, Waskish and Crookston (Tables 1 and 2). The average number of growing degree days in 1993 across the locations was 2660 compared to 2522 for 1992, thus the 1993 season was slightly warmer than 1992. Waskish and Grand Rapids were the two locations that had 264 and 193 more growing degree days in 1993 compared to 1992. Much of this was due to the warmer July and August in 1993 compared to 1992 at both Waskish and Grand Rapids. Crookston and Aitkin averaged only slightly higher growing degree days in 1993 compared to 1992. This again was also due mainly to the warmer temperatures in July and August. Comparisons with long term averages (normal) Crookston and Grand Rapids were cooler in 1993 than normal, while Aitkin was similar and Waskish warmer.

Table 1. Growing degree days[a] comparisons for 1992, 1993 and normal (61-90).

	Aitkin			Grand Rapids		
Month	1992	1993	Normal	1992	1993	Normal
	---------- GDD ----------					
April	92	53	127	75	67	130
May	502	413	417	509	395	434
June	670	616	646	607	605	674
July	647	778	779	644	807	858
August	700	794	683	680	834	768
Total	2611	2654	2652	2515	2708	2864

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

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Table 2. Growing degree days[a] comparisons for 1992, 1993 and normal (61-90).

Month	Waskish			Crookston		
	1992	1993	Normal	1992	1993	Normal
	---------- GDD ----------					
April	66	78	103	91	92	151
May	493	366	369	584	465	488
June	625	563	518	642	612	743
July	656	731	642	669	767	926
August	415	781	563	720	824	867
Total	2255	2519	2195	2706	2760	3175

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

Total precipitation for the growing season at the four locations ranged from 0.75 inches at Crookston to 8.37 inches at Aitkin more rainfall in 1993 compared to 1992 (Tables 3 and 4); at Grand Rapids the difference was 3.65 inches while at Waskish it was 4.22 inches. 1993 was a wet year. The wettest month at Aitkin was May while at Grand Rapids and Crookston it was July, and at Waskish it was June. Compared to the long term average all locations were wetter in 1993. The largest difference was 6.12 inches more at Aitkin, 5.28 more at Waskish, 4.49 more at Grand Rapids and 0.49 more at Crookston.

Table 3. Precipitation comparisons for 1992, 1993 and normal (61-90)[a].

Month	Aitkin			Grand Rapids		
	1992	1993	Normal	1992	1993	Normal
	---------- Inches ----------					
April	2.41	2.63	2.30	1.13	2.82	2.10
May	1.30	6.47	2.88	1.19	3.07	3.04
June	2.66	5.43	4.09	4.78	3.83	4.11
July	3.93	5.45	4.14	4.78	7.63	3.89
August	4.69	3.38	3.83	5.69	3.87	3.59
Total	14.99	23.36	17.24	17.57	21.22	16.73

[a]Data from Mark Seeley, Soil Science Dept., U of MN.

Table 4. Precipitation comparisons for 1992, 1993 and normal (61-90)[a].

Month	Waskish			Crookston		
	1992	1993	Normal	1992	1993	Normal
	----------Inches----------					
April	1.45	1.36	1.70	0.98	0.32	1.45
May	1.58	1.90	2.33	1.97	1.58	2.45
June	2.32	6.94	4.25	4.30	3.71	3.44
July	4.13	5.05	3.42	1.86	4.77	2.77
August	6.05	5.05	3.32	3.58	3.06	2.88
Total	15.53	20.30	15.02	12.69	13.44	12.99

[a]Data from Mark Seeley, Soil Science Dept., U of MN.

Total cultivated wild rice production in Minnesota was less in 1993 compared to 1992 partly because of fewer acres (Table 5.) California production was similar to 1992.

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

Year	Production		Year	Production	
	Minnesota	California		Minnesota	California
1968	36	0	1981	2274	500
69	160	0	82	2697	880
70	364	0	83	3200	2500
71	608	0	84	3600	2500
72	1496	0	85	4200	7900
73	1200	0	86	5100	9000
74	1036	0	87	4200	4200
75	1233	0	88	4000	3500
76	1809	0	89	3978	4000
77	1031	0	90	4800	4200
78	1761	100	91	5500	5500
79	2155	200	92	6100	7500
80	2320	400	93[b]	5300	7500

[a]1968-1982 Minnesota values from Winchell and Dahl and 1983-1993 from Minnesota Department of Agriculture; California values from Marcum, Cooperative Extension Service, University of California. [b]Estimated value for 1993.

The total value of the 1993 crop is estimated at $8.74 M compared to $10.37 M for 1992. The decrease is due to decreased production and price. The highest value was in 1986 when production was the fourth highest and prices were more per pound than in 1993 (Table 6).

Table 6. Processed wild rice harvested and value from cultivated fields in Minnesota

Year	Production	Price	Value
	1,000 lb	$/lb	$ Millions
1968	36	3.30	0.12
1969	160	2.55	0.41
1970	364	2.80	1.02
1971	608	2.70	1.64
1972	1,496	2.30	3.44
1973	1,200	2.05	2.46
1974	1,036	2.37	2.46
1975	1,233	2.50	3.08
1976	1,809	2.70	4.88
1977	1,031	4.35	4.48
1978	1,761	5.10	8.98
1979	2,155	5.01	10.80
1980	2,320	4.47	10.37
1981	2,274	3.79	8.62
1982	2,697	3.41	9.20
1983	3,200	3.35	10.72
1984	3,600	3.30	11.88
1985	4,200	2.97	12.47
1986	5,100	2.60	13.26
1987	4,200	1.50	6.30
1988	4,000	1.65	6.60
1989	3,978	1.65	6.56
1990	4,800	1.70	8.16
1991	5,300	1.70	9.01
1992	6,100	1.70	10.37
1993[a]	5,300	1.65	8.74

[a]Estimated values for 1993.

Research

The 1993 research focused on the cooperative soil fertility experiments conducted by Dr. Paul Bloom and on the plant development experiments conducted by visiting scientist Dr. Il-doo Jin from Suncheon University, Korea. Dr. Bloom's report discusses the fertility research and will not be discussed in this section.

Photoperiod Response of Three species, *Z. palustris, Z. aquatica,* and *Z. texana.*

This study was a continuation of our efforts to more clearly establish the photoperiod requirements of the different species of wild rice and of the different cultivars or types within a species. The goal is to permit researchers to manipulate flowering of the different species or cultivars so crosses can readily be made to facilitate the breeding efforts to develop superior cultivars.

Materials and Method: Six seed sources of *Z. palustris* (`Franklin-1´,`Franklin-2´, `K2´, `NorCal 3´, Wisconsin-1 and Wisconsin-2), two of *Z. aquatica* (Iowa and Virginia) and one of *Z. texana* (Texas) were grown in three growth chambers. All seed sources were grown in 5 pots each containing 7 plants in all three chambers. All three chambers had 10 hours of full light with 1/3 lighting for 1 hour at the beginning and end of the 10 hours full light to simulate a 12-hour daylength.

One chamber in addition to the 12-hour simulated day had a 12-hour dark period to give a 12-hour photoperiod. A second chamber had in addition to the simulated 12- hour day, a 3-hour low light period before the beginning of the 12-hour day. This gave a simulated 15-hour photoperiod. The third chamber had in addition to the simulated 12-hour day and a 6-hour low light period before the beginning of the 12-hour day a 6-hour low light period before the beginning of the 12-hour day. This gave an 18-hour photoperiod. The lighting during the 12-hour day was supplied by cool-white fluorescent lamps with a high light level (photon flux density) of 390 μ mol^{m-2} sec^{-1}. The low light (23 μ mol m^{-2} sec^{-1}) during night interruption was supplied by incandescent lamps. All chambers were maintained at a constant 21°C (70°F).

Data collected were days from planting to panicle emergence, female flower emergence, and male flower emergence, and days to panicle emergence to pistillate emergence and pistillate emergence to anther emergence. Other data obtained were panicle emergence to anther emergence, panicle length, pistillate flower length, number of tillers, florets on main stem, and plant height.

Results: All three species and types within a species flowered (panicle and female flower emergence) earlier with a 12-hour photoperiod compared to a 15-hour photoperiod (Fig. 1 and 2). All cultivars or types of *Z. palustris* flowered later at the 18-hour photoperiod than at the 15-hour photoperiod. Flowering of *Z. aquatica* (Iowa) and *Z. texana* was not delayed by increasing the photoperiod to 18 hours compared to 15 hours. Male flower emergence followed the same pattern as panicle and female flower emergence (Fig. 3).

The response of photoperiod to number of days from panicle emergence to male anthesis, panicle emergence to female anthesis and female anthesis to male anthesis was variable and not consistent (Fig. 4, 5 and 6). Five of the eight cultivars or types took more days from panicle emergence to female anthesis under 15-hour photoperiod compared to 12-hour photoperiod, however the opposite was true for the other cultivars or types. All except *Z. aquatica* from Iowa took longer under 18-hour photoperiod compared to 15-hour photoperiod from panicle emergence to female anthesis.

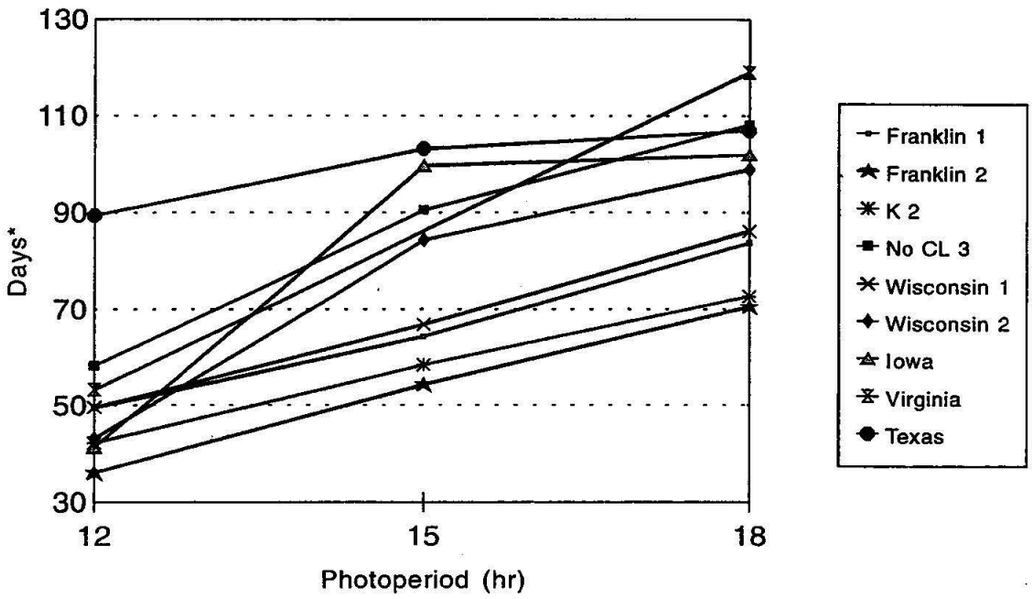
All but the two *Z. aquatica* types took longer from panicle emergence to male anthesis under 12-hour photoperiod than 15-hour photoperiod (Fig 4). The days from panicle emergence to male anthesis was similar under 15- and 18-hour photoperiods except for *Z. texana* and the Iowa type for *Z. aquatica*. The number of days from female anthesis to male anthesis were less under 15-hour photoperiod compared to 12-hour photoperiod except for Wisconsin-1 type of *Z. palustris* (Fig. 5). There was little response by increasing the photoperiod to 18 hours.

Panicle length increased as photoperiod increased for all types and cultivars of *Z. palustris* and *Z. aquatica* (Fig. 7). There was little response to photoperiod for *Z. texana*. The total length of male flower portion of the panicle increased from 12-hour to 15-hour photoperiod for all types and cultivars of *Z. palustris* (Fig. 8). There was little increase when these plants were grown under an 18-hour photoperiod. *Z. texana* did not respond to change in photoperiod. The length of female flower portion of the panicle responded similarly as the male portion (Fig. 9). The number of female spikelets on the main stem increased as photoperiod increased for all species (Fig. 10) however, *Z. texana* did not respond from a 15-hour to 18-hour photoperiod.

Main stem height (soil surface to upper node) increased as photoperiod increased for all species, cultivars and types (Fig. 11). Plant height (soil surface to tip of flag leaf) responded the same way to daylength as main stem height (Fig. 12). Tiller number increased under 12-hour compared to 15-hour photoperiod for all species, cultivars and types except for *Z. texana* (Fig. 13 and 14). The number did not increase much when the photoperiod was increased to 18-hours.

As in our previous research *Z. palustris* and *Z. aquatica* types flowered earlier when subjected to short days compared to long days. Plant size and flower number, however, are less under short days compared to long days. Thus, these two species could be easily manipulated in their flowering time to facilitate crossing. *Z. texana* however did not respond as much to daylength thus would be more difficult to manipulate.

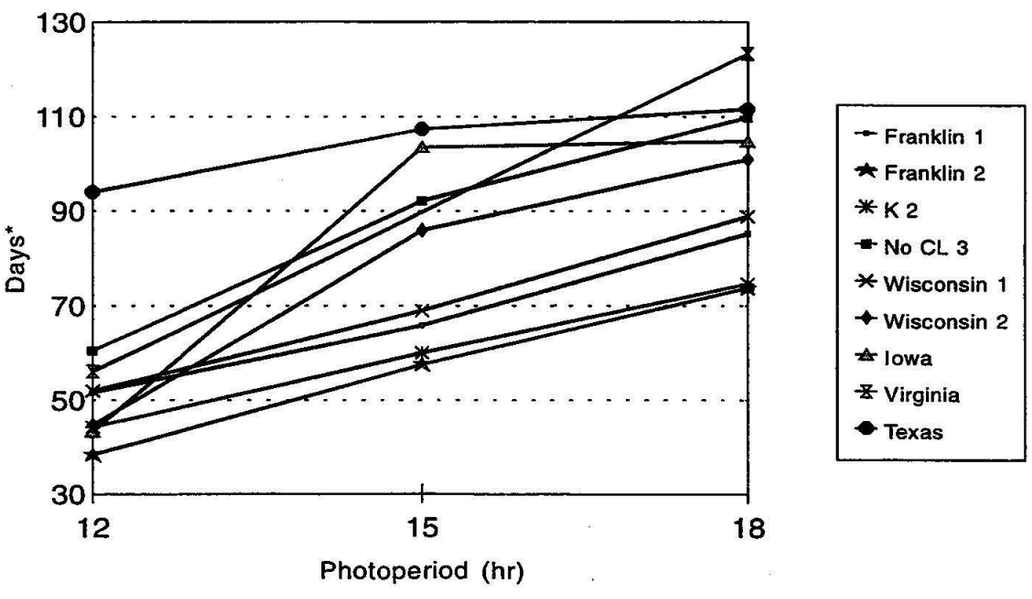
PANICLE EMERGENCE



*The number of days from seeding to the beginning of panicle emergence.

Figure 1

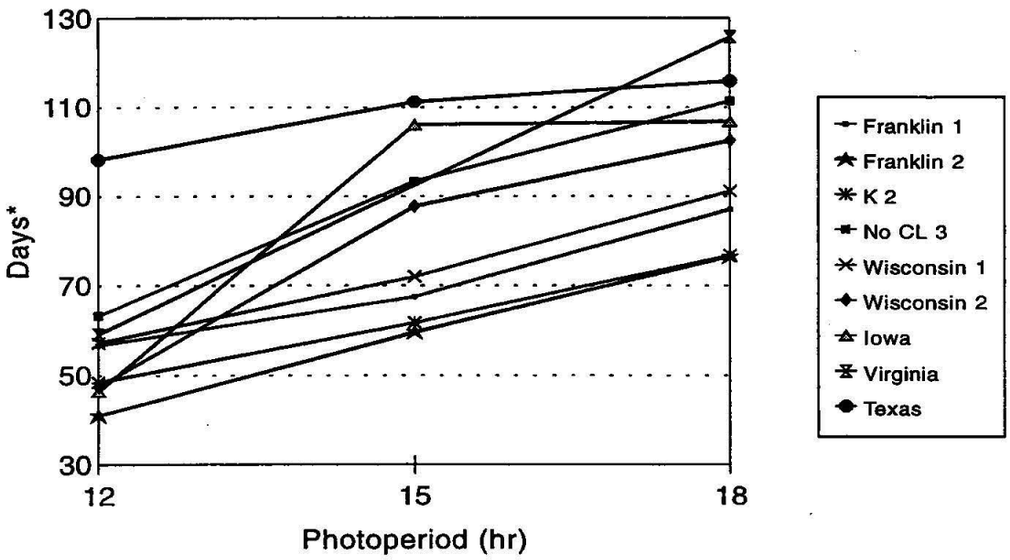
FEMALE FLOWER EMERGENCE



*The number of days from seeding to the beginning of female flower emergence.

Figure 2

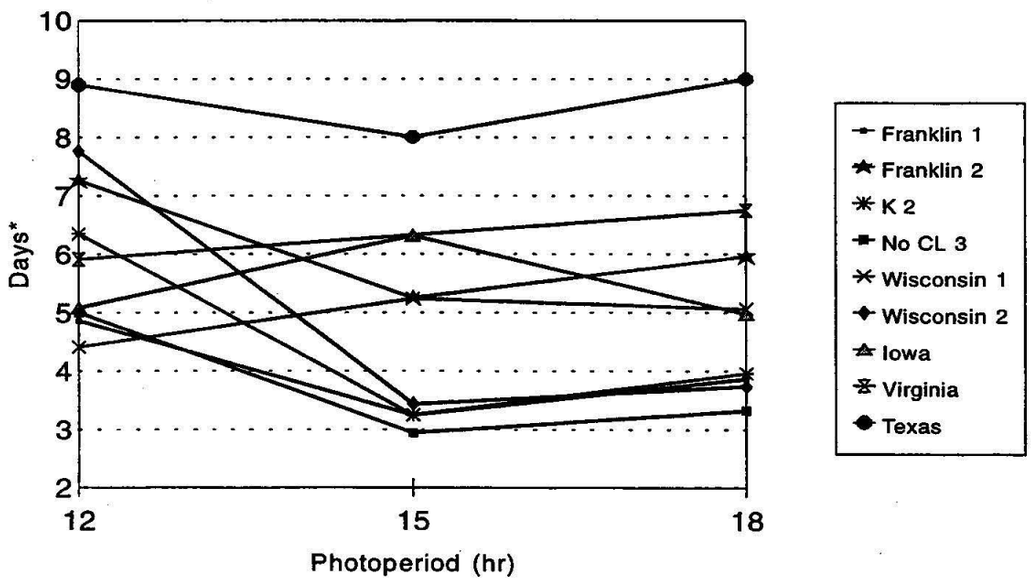
MALE FLOWER EMERGENCE



*The number of days from seeding to the beginning of panicle emergence.

Figure 3

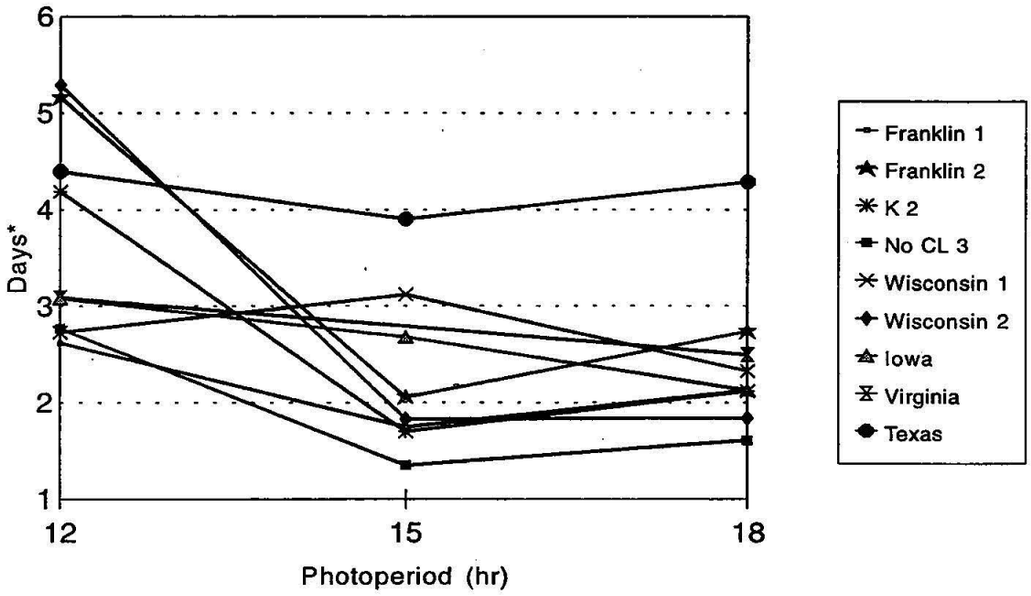
PANICLE EMERGENCE TO MALE ANTHESIS



*The number of days from panicle emergence to male anthesis.

Figure 4

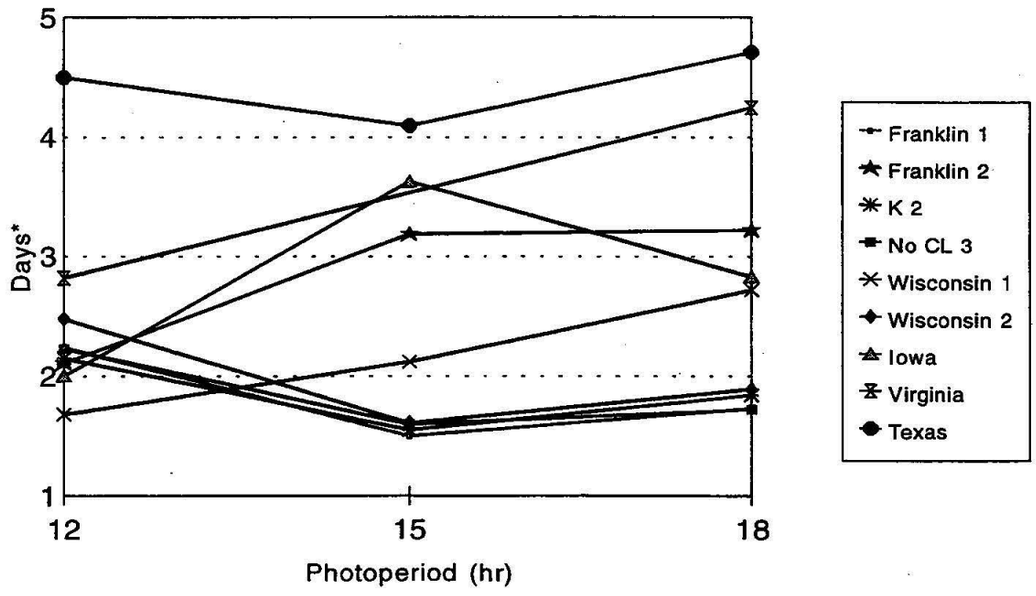
FEMALE ANTHESIS TO MALE ANTHESIS



*The number of days from female anthesis to male anthesis.

Figure 5

PANICLE EMERGENCE TO FEMALE ANTHESIS



*The number of days from panicle emergence to female anthesis.

Figure 6

PANICLE LENGTH

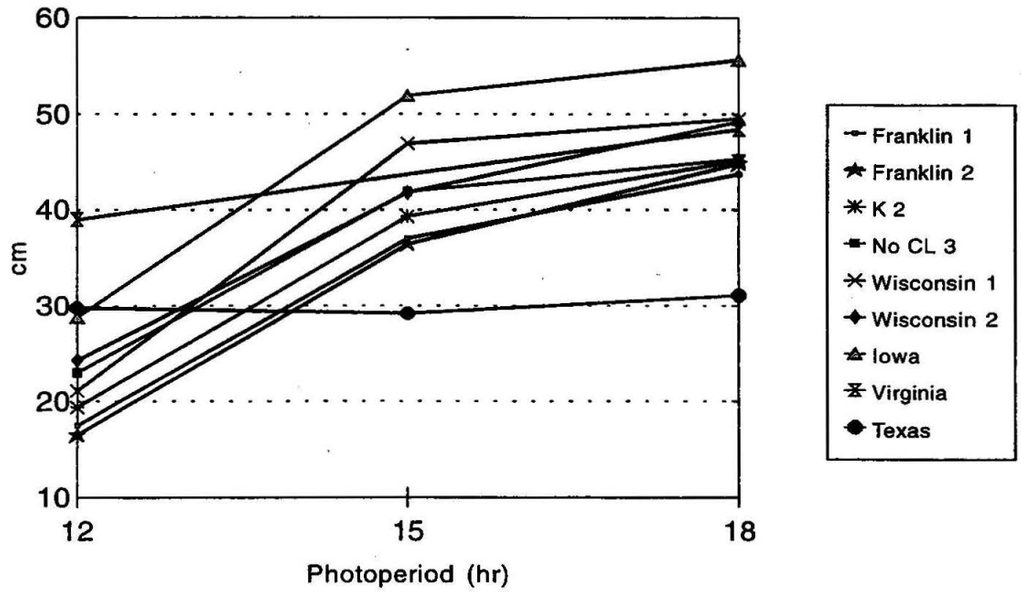


Figure 7

LENGTH OF MALE FLOWER AXIS

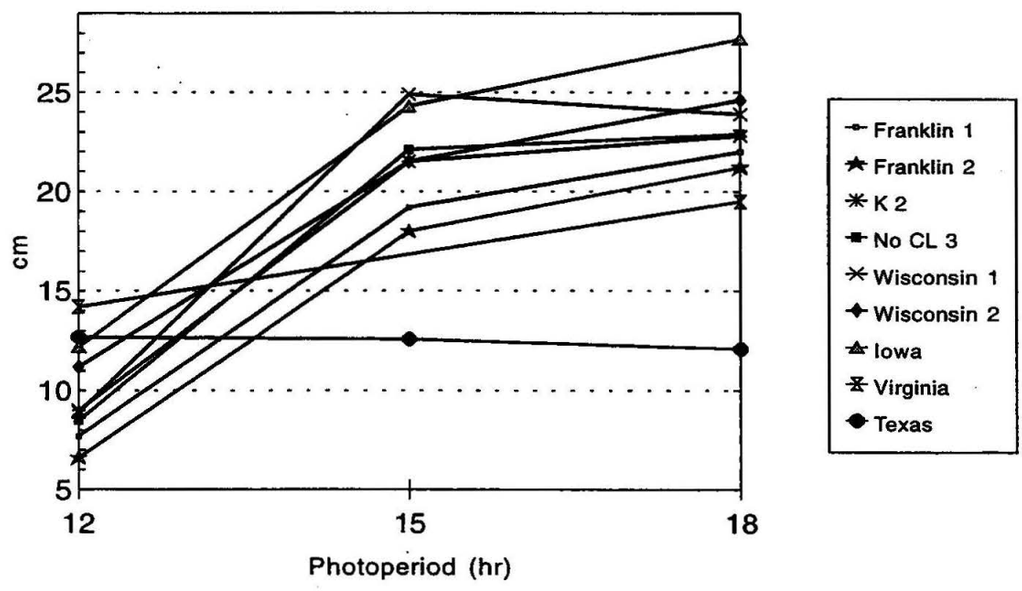


Figure 8

LENGTH OF FEMALE FLOWER AXIS

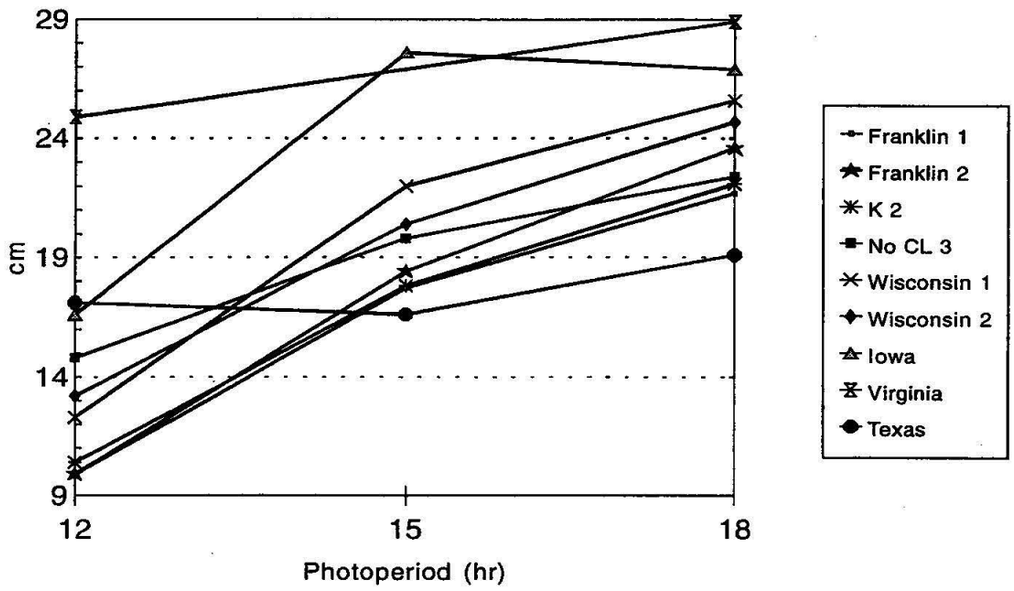


Figure 9

FEMALE SPIKELETS

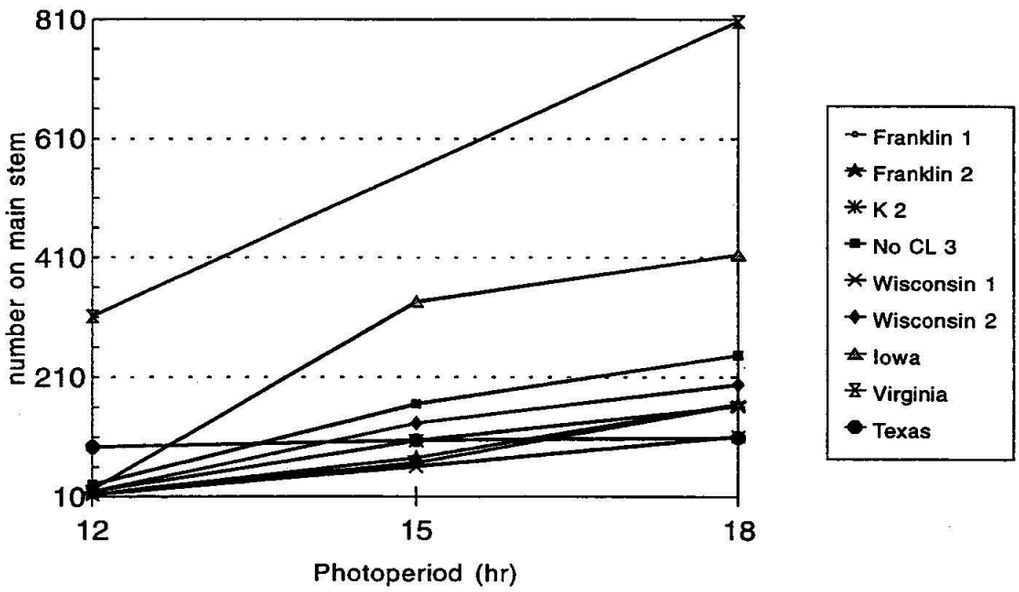
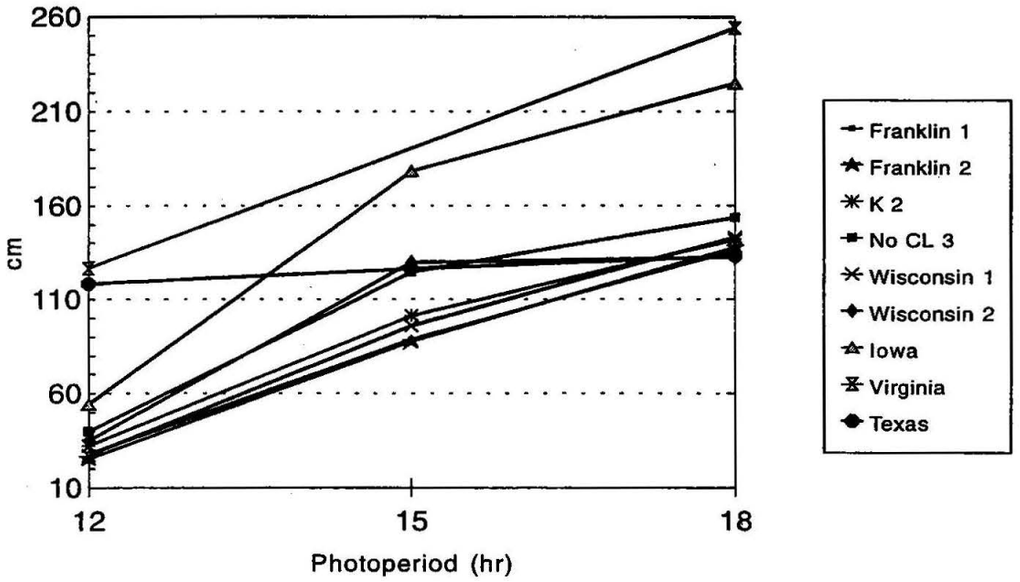


Figure 10

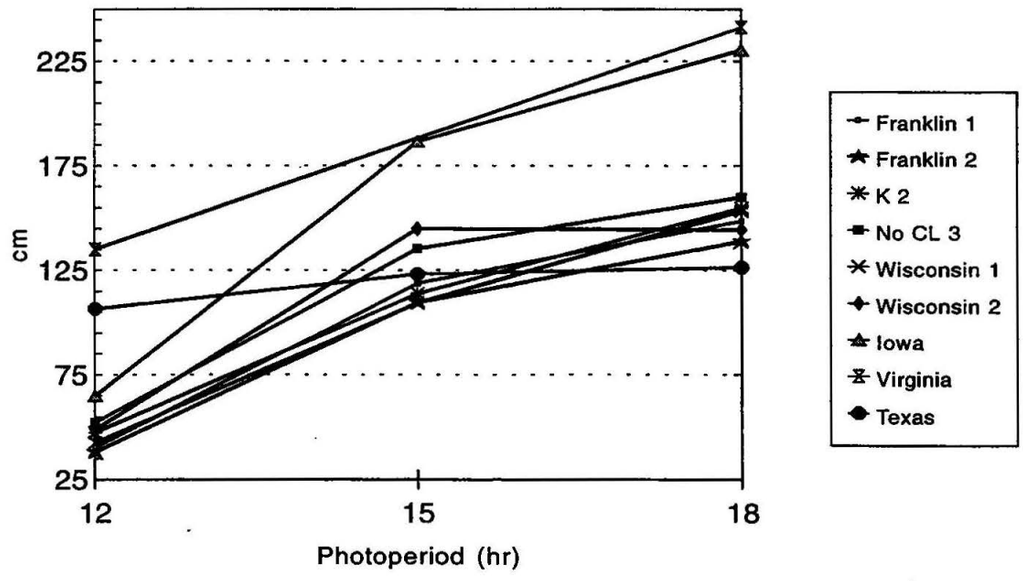
MAIN CULM HEIGHT*



*Soil surface to uppermost node

Figure 11

PLANT HEIGHT*



*From soil surface to top of flag leaf

Figure 12

TILLER NUMBER

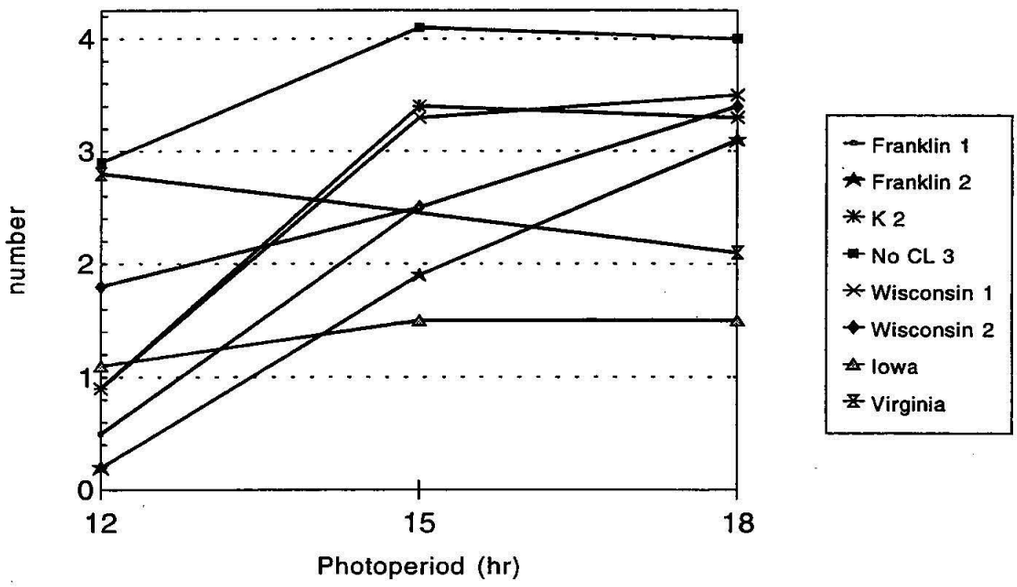


Figure 13

TILLER NUMBER

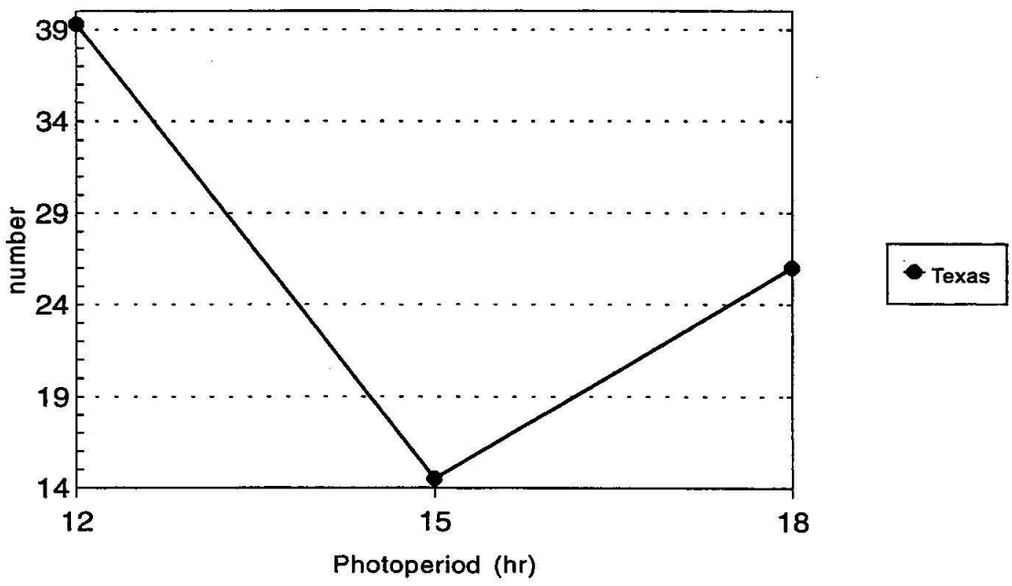


Figure 14

Acknowledgement

The continued support for this research by the Minnesota Agricultural Experiment Station and the Department of Agronomy and Plant Genetics Department is greatly appreciated. Dr. Raymie Porter's support of supplying some of the seed and consultations was greatly appreciated.

ANATOMICAL DIFFERENCES OF ABSCISSION REGIONS AMONG AND WITHIN *ZIZANIA* SPECIES AND IN RELATIONSHIPS TO SEED SHATTERING.

Il-Doo Jin, Ervin A. Oelke, David D. Biesboer and Raymond A. Porter[1]

Introduction

In the wild, seed shattering from plants is an important characteristic for dispersal and survival. However, when plants are grown specifically for their seeds (cultivated) this characteristic causes much seed loss before harvest. The seed from wild rice, *Zizania palustris* L. has long been utilized by Native North Americans for food, but the seed was only collected from wild stands. Wild rice is a recently developed cultivated crop and seed shattering remains in the crop and is a major problem in cultivation. Breeders have selected lines to reduce the tendency of seeds to shatter (abscise), however considerably more shattering resistance is needed to achieve comparable levels of other cultivated cereals such as wheat. Today the main focus in variety improvement continues to be improved seed retention (Hayes et. al, 1988).

The nonshattering characteristic in wild rice is controlled by a small number of recessive genes (Woods and Clark, 1976) and two complementary dominant genes were proposed to explain shattering habit of staminate florets (Elliott and Perlinger, 1977). Hanten et al. (1982) have reported the anatomical changes associated with morphology of the abscission zone of wild rice and correlates those changes with embryo development.

In *Oryza sativa*, the degree of seed shattering is related to the thickness of the cell zone which supports the seed. The zone consists of the vascular channel and the lignified cells around the channel at the separating zone in the variety that has a cracked (cell separation) abscission layer at harvest. In the genus *Oryza*, however, there are many types of abscission regions such as no abscission layer, cracked or noncracked abscission layer, complete or incomplete abscission layer, fully developed or partially developed abscission layer, and dome type or plate type abscission layer that have been observed and several types are closely related with the degree of the seed shattering. The formation of abscission layer is controlled by a dominant gene.

Zizania is a genus of four species and of those species only *Zizania palustris* has been cultivated. This study provides an anatomical description of the abscission zone in wild rice, and the differences among four species belonging to the genus *Zizania* and between shattering and non-shattering types of cultivated wild rice in relation to degree of seed shattering.

Materials and Methods

Germinated seed of *Z. palustris*, cultivar 'Netum', *Z. aquatica*, and crosses between *Z. palustris* and *Z. texana*, obtained from Duvall, National Museum, and *Z. latifolia* collected from Suncheon, Korea were transplanted into flooded soil contained in plastic containers. The plants

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were grown outside at Suncheon, Korea in 1992. In 1993, *Z. texana* obtained from Power, Southeast Texas State University, plants were grown in a growth chamber, 20°C and 12-hour photoperiod, at Minnesota. At panicle emergence and maturity, the pistillate florets of each species were collected.

To observe morphological features of the abscission region about 20 samples, the basal portion of seed and the upper portion of the pedicel, were excised from the upper portion of each panicle and preserved in formalin-acetic acid-alcohol (F.A.A.) for later processing for light microscopy using standard procedures (Sass 1958). Longitudinal serial sections of 10 μm were stained with safranin and fast green.

In this experiment, the strength in grams required to detach a seed from its pedicel or rachilla by a bending force (bending) or by a straight pull force (tensile) as an index of the degree of seed shattering, was measured by an unbonded gauge type transducer (UT: 1 kg) and automatic null balancing recorder, using the same panicle as in the above experiment.

Results and Discussion

Anatomical features of abscission zone observed in this study agrees essentially with the description of Hanten et al. (1980).

At panicle emergence, clearly distinguished abscission layers consisting of one or more layers of parenchyma cells with dense cytoplasm and dark staining nuclei are located in the junction of the spikelet and pedicel surrounding two or more layers of sclerenchyma cells above and below the abscission layer in all of examined lines and cultivars (Fig. 1A through 1F). Parenchyma cells in the abscission layers extend from epidermal cell to central vascular tissue (Fig. 1A through 1F). Therefore, any thicker walled lignified cells between abscission layer and central vascular tissue in the separating zone are not evident as they are in some cultivars of rice, *Oryza sativa*, which have some seed shattering resistance. In wild rice lines and cultivars, except *Z. latifolia*, most abscission layers consist of one or two layers of parenchyma cells and two or more layers adjacent to the vascular channel (Fig. 1A,C-F). In *Z. latifolia* parenchyma cells of the abscission layer are two or three layers at the epidermis, six or eight layers near the cortex and three or four layers adjacent to the vascular channel (Fig. 1B).

Parenchyma cells in the abscission layer are smallest in *Z. aquatica*, and in crosses (open pollinated) between *Z. palustris* and *Z. texana*. The size is similar in *Z. texana, Z. palustris* nonshattering type from Netum, *Z. latifolia* and *Z. palustris* shattering type of cultivar Netum. The number of rows of cells from epidermis to vascular channel is greater in *Z. palustris* both in the nonshattering and shattering types of Netum, and fewer in *Z. aquatica* and *Z. latifolia* (Table 1.).

In this study, kernels were mature approximately four weeks after panicle emergence as indicated by their dark aleurone layer. Parenchyma cells in the abscission layer were cracked completely at maturity in *Z. aquatica* (Fig. 2A), *Z. palustris* shattering type in Netum, (Fig. 2C), *Z. texana* (Fig. 2E) and crosses between *Z. palustris* and *Z. texana* (Fig. 2F). Cracking of the abscission layers was not evident in *Z. latifolia* or nonshattering type of cultivar Netum, *Z. palustris.* The mass of parenchyma cells in the abscission zone of the former were larger at maturity than at panicle emergence and had dense cytoplasm and enlarged dark staining nuclei (Fig. 1B, 2B and Table 1). However, parenchyma cells of the nonshattering types of *Z. palustris* were smaller at maturity compared to panicle emergence stage of development (Fig. 1D, Fig. 2D and Table 1).

Hanten et al. (1980) concluded that plasmolysis, first anatomical evidence of abscission, occurred prior to separation (cracking) of epidermal cells and parenchyma cells of abscission layer. But any evidence of separation of abscission layer could not be found in *Z. latifolia* or nonshattering types of *Z. palustris*, Netum (Fig 2.B. and D.) examined in this study.

In the abscission layers cracked at maturity, two or three rows of parenchyma cells adjacent to the vascular tissue did not collapse, (Fig. 2A,C,E and F). In *Oryza sativa*, mechanical strength of the lignified cells of the supporting zone and the vascular channel could contribute to the resistance to seed abscission in nonshattering cultivars. The thicker walled lignified cells are more effective for mechanical strength then the vascular channel which consists of thin walled phloem and parenchyma cells and xylem cells without fiber. Sclerenchyma cells between abscission layer and central vascular tissue could not be found in wild rice, genus *Zizania*, which is similar to easily shattered wild and weed type of *Oryza*.

Hanten et al. (1980) concluded that the separation of abscission layer in wild rice, *Z. palustris* is due to dissolution of middle lamella and at completion of seed abscission, wall material appears to be missing or fragmented on the separated surface of the abscission layer. In our study though some parenchyma cells of the abscission layer remained attached to proximal or distal sclerified cells, most of the parenchyma cells in the abscission layer were observed to be eroded with remnants of wall materials attached to proximal or distal surface in all plants having cracked abscission layers (Fig. 2A, C, E and F).

It has been reported that some histological peculiarities are associated with seed shattering in *Oryza*. Histological examination of wild rice in this study showed that histology varies by the species (Table 2.). The diameters of separating zone of *Z. palustris*, both shattering and nonshattering, are large and those of *Z. aquatica* are small among the species of *Zizania*. However, the diameter of the separating zone of the other species appear to be near that of *Z. aquatica*. The diameters of the crosses, between *Z. palustris* and *Z. texana* appear to be near that of *Z. texana*. Also, the diameters of upper most part of the pedicel had a similar tendency as above but not the diameter of vascular channel of the crosses appeared to be near that of *Z. palustris*. *Z. latifolia* has a thicker abscission layer with more layers of parenchyma cells among all the species investigated in this study (Table 2.). The thinnest layers were observed in both *Z. palustris* and *Z. texana* appears to be similar to that of *Z. palustris*.

The abscission zone from numerous wild rice plants from a number of crosses between nonshattering and shattering types of *Z. palustris* were also sampled and will be examined for differences. This will be done by Dr. Jin in Korea and the information should be helpful in the development of more shatter resistant wild rice varieties for Minnesota.

The amount of force necessary to detach a seed by bending or pulling (tensile) is shown in Table 3 for the three species and for shattering and nonshattering types of *Z. palustris*. At heading (panicle emergence) time both bending and tensile forces were greatest for *Z. palustris* than for the other three species and crosses between *Z. palustris* and *Z. texana*. At maturity this was still true except for *Z. latifolia* which had a higher tensile strength than the shattering type of *Z. palustris*. The tensile strength at maturity was highest for the nonshattering type of *Z. palustris* indicating that the degree of seed shattering has been improved in wild rice.

Table 1. Morphological features of parenchyma cells in the abscission layer of four *Zizania* species.

Species	Cell size (μm) at		No. of cells at		Amount of cracking at
	Heading	Maturity	Heading	Maturity	Maturity
Z. aquatica	6.1-6.5	—	14-15	—	Cracking
Z. latifolia	7.5-8.5	9.0-10.0	12-16	12-14	Non-cracking
*Z. palustris**					
shattering types	8.1-8.5	—	24-28	—	Cracking
nonshattering types	7.6-8.0	7.1-7.5	26-30	28-30	Non-cracking
Z. texana	7.5-8.0	—	16-20	—	Cracking
Z. palustris X *Z. texana* crosses**	6.5-7.0	—	20-22	—	Cracking

*Cultivar Netum
**Open pollinated

Table 2. Difference of some histological peculiarities of seed abscission region among four *Zizania* species.

Species	Diameter of abscission region	Diameter of pedicel	Diameter of vascular tissue	Abscission Layer: Middle cortex thickness	Abscission Layer: No. of cell layers at epidermis	Abscission Layer: No. of cell layers at middle cortex	Abscission Layer: No. of cell layers near vascular tissue
	μm						
Z. aquatica	222±17.5	194±14.5	44±2.2	13.3±1.8	1-2	1-2	2-3
Z. latifolia	304±11.4	264±14.6	76±5.3	42.1±6.0	2-3	6-8	3-4
*Z. palustris**							
shattering types	549±50.0	500±50.3	107±8.2	18.6±3.5	1-2	1-2	2-3
nonshattering types	510±39.2	470±40.2	102±11.2	20.1±3.7	1-2	1-2	2-3
Z. texana	305±12.9	295±14.0	66±1.7	11.1±1.0	1-2	1-2	2-3
Z. palustris X *Z. texana* crosses**	326±15.5	310±16.6	91±3.2	16.9±2.1	1-2	1-2	2-3

*Cultivar Netum
**Open pollinated

Table 3. Differences in bending and tensile force required to detach a seed from its pedicel at heading and maturity in four *Zizania* species.

Species	Heading time		Maturity	
	Bending	Tensile	Bending	Tensile
	g			
Z. aquatica	10.4±4.6	63.4±8.4	1.1±0.5	7.4±2.6
Z. latifolia	18.5±5.8	148.1±23.6	6.2±2.8	49.3±14.1
*Z. palustris**				
shattering types	49.8±8.4	244.3±22.2	3.7±1.2	28.9±4.4
nonshattering types	46.4±10.8	217.6±36.6	9.8±2.9	75.2±22.7
Z. texana	16.1±5.3	114.6±19.4	0.2±0.1	1.2±0.4
Z. palustris X *z. texana* crosses**	14.9±5.4	128.4±18.2	2.6±1.2	6.4±2.5

*Cultivar Netum
**Open pollinated

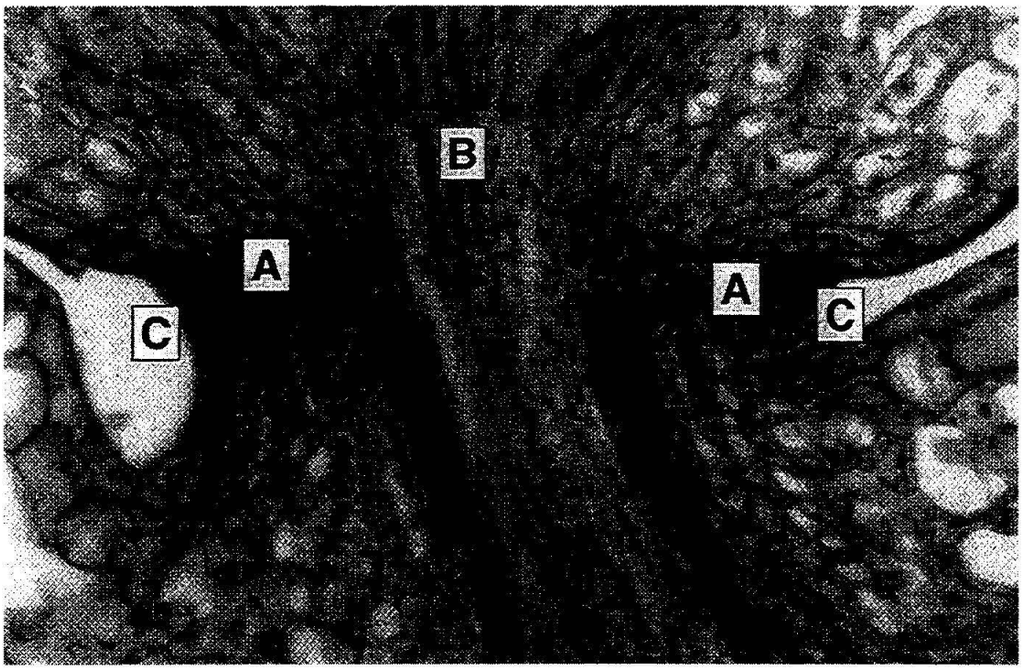

Figure 1A. *Z. aquatica* abscission zone, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

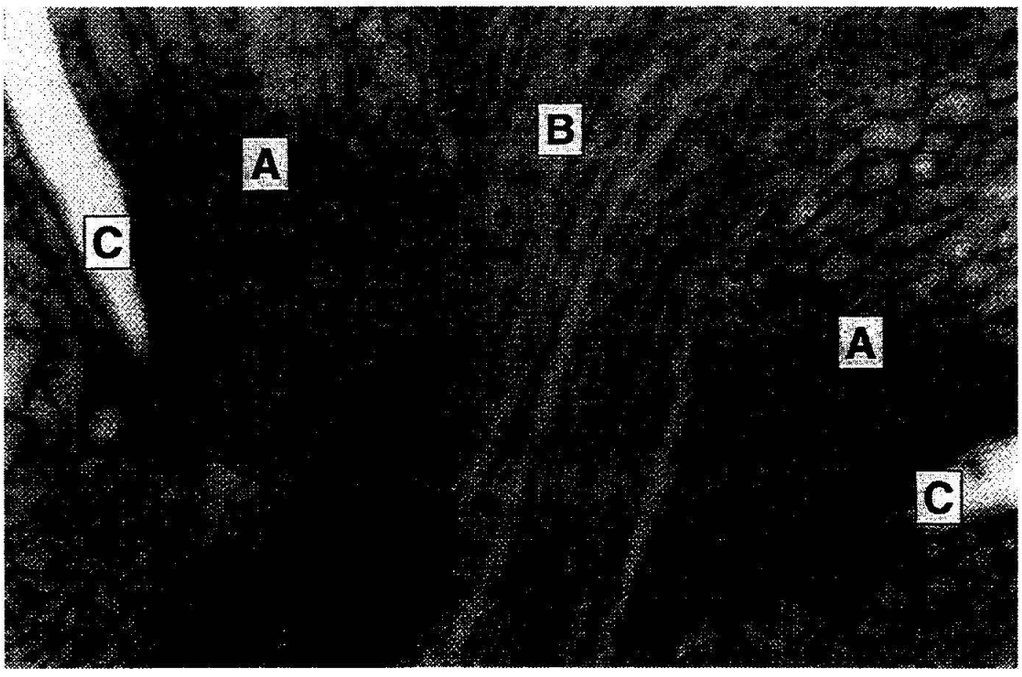

Figure 1B. *Z. latifolia* abscission zone, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

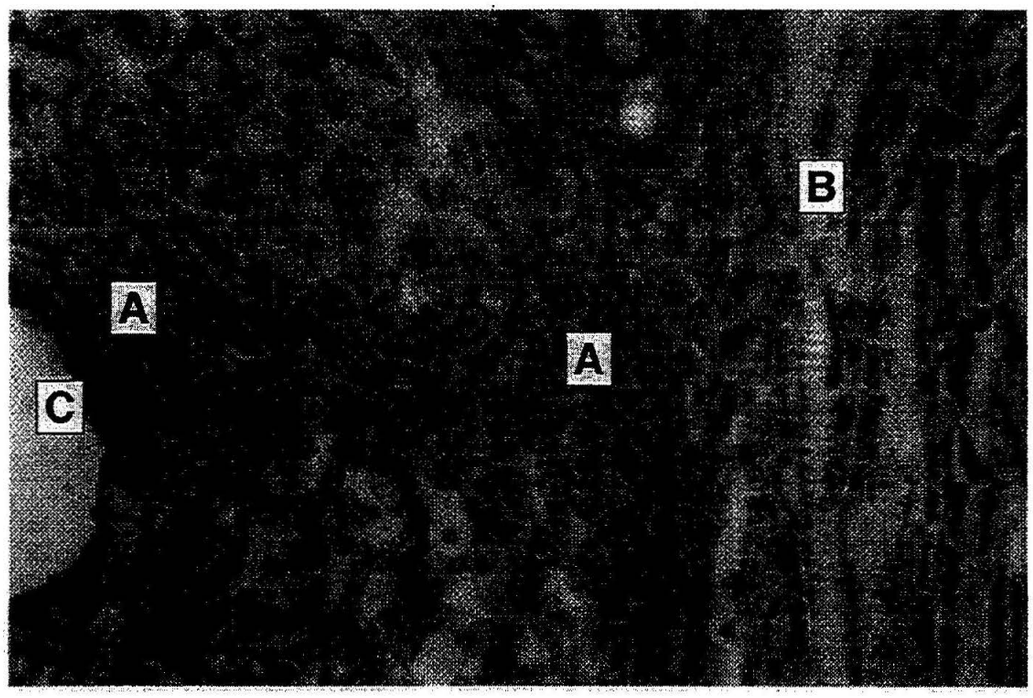

Figure 1C. *Z. alustris* left half of abscission zone, cultivar Netum, shattering type plant, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

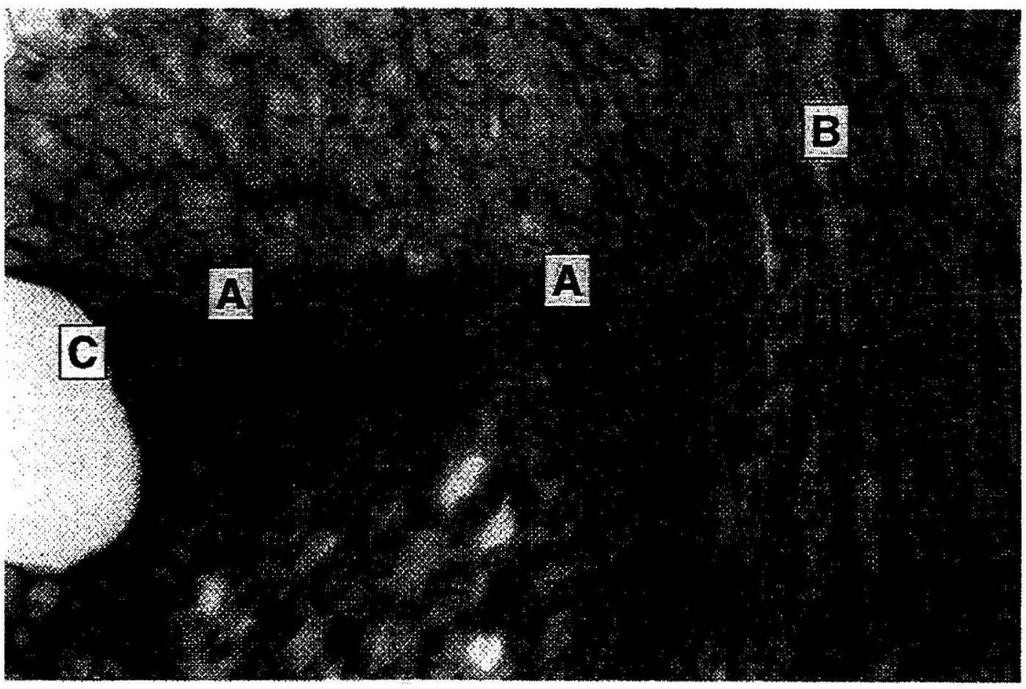

Figure 1D. *Z. palustris* left half of abscission zone, cultivar Netum, nonshattering type plant, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

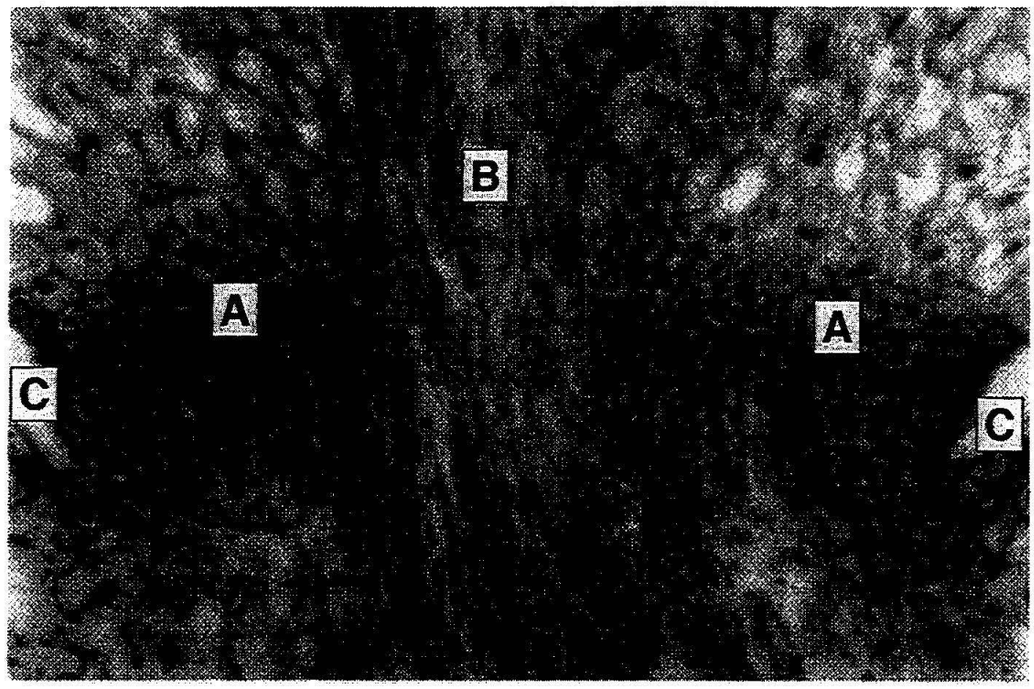

Figure 1E. *Z. texana* abscission zone, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

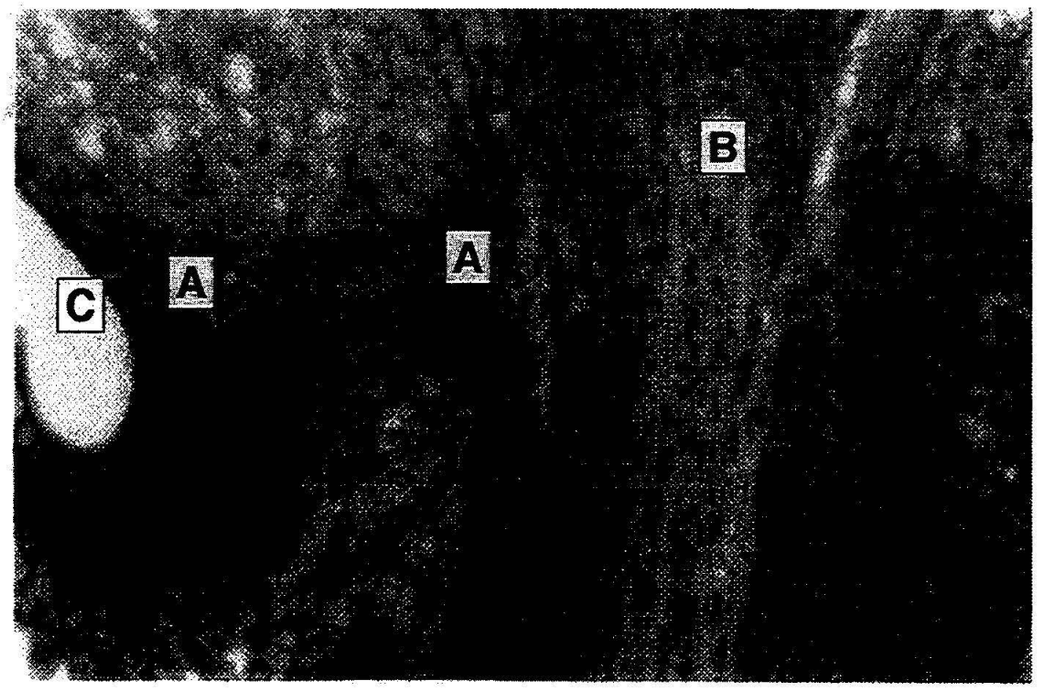

Figure 1F. Left and part of right half abscission zone in plant from cross of *Z. palustris* and *Z. texana*, immature kernal (A = abscission layer; B = central vascular tissue; C = epidermal cells)

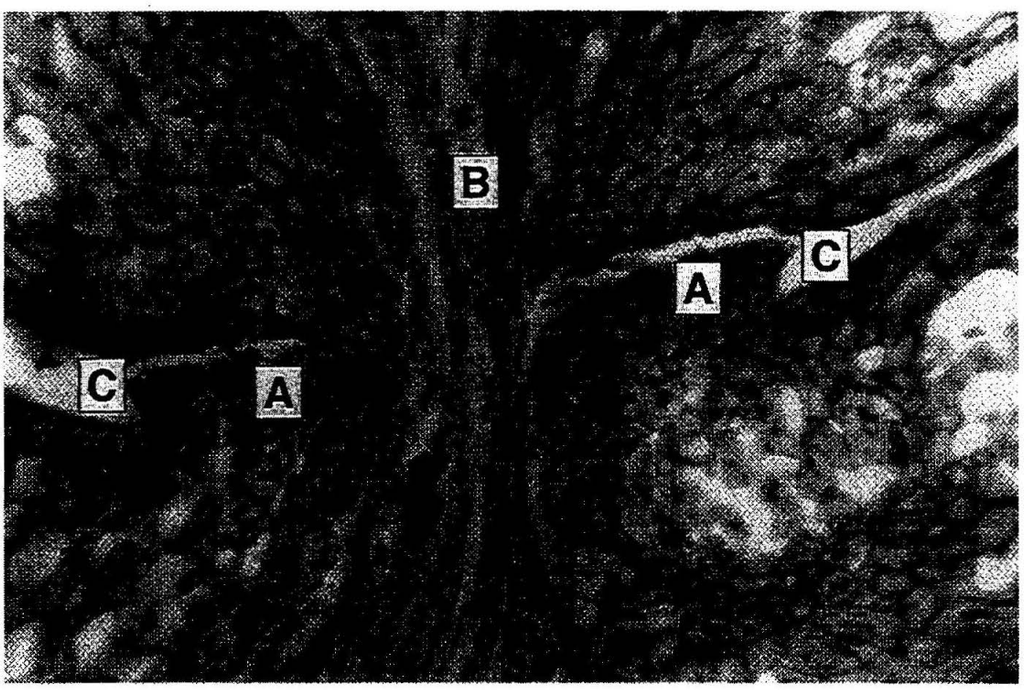

Figure 2A. *Z. aquatica* abscission zone, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

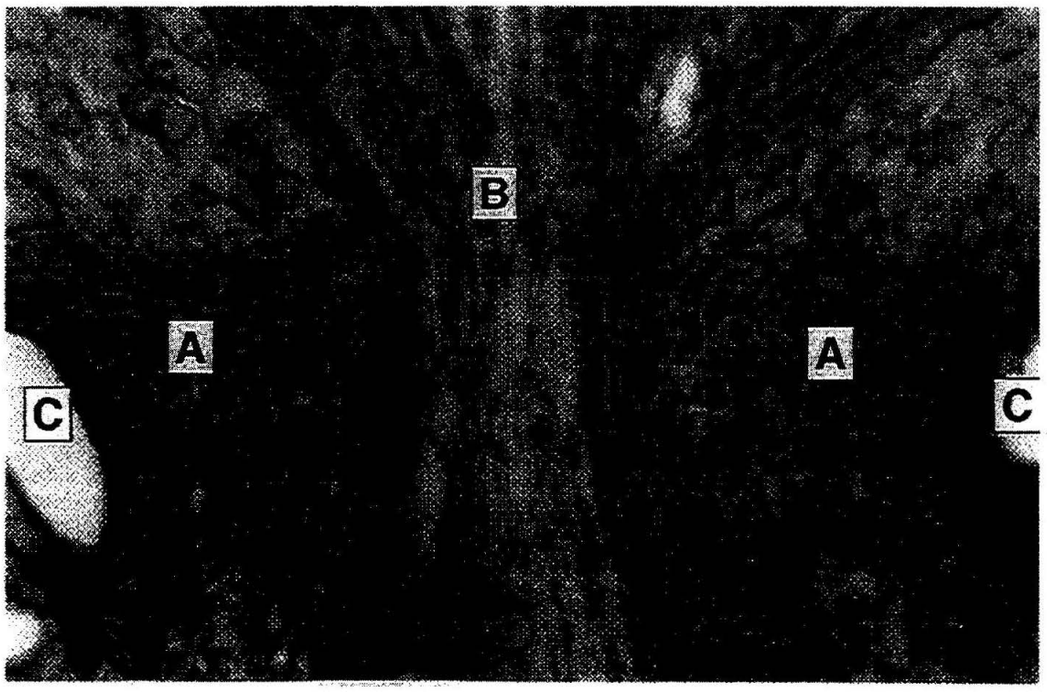

Figure 2B. *Z. latifolia* abscission zone, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

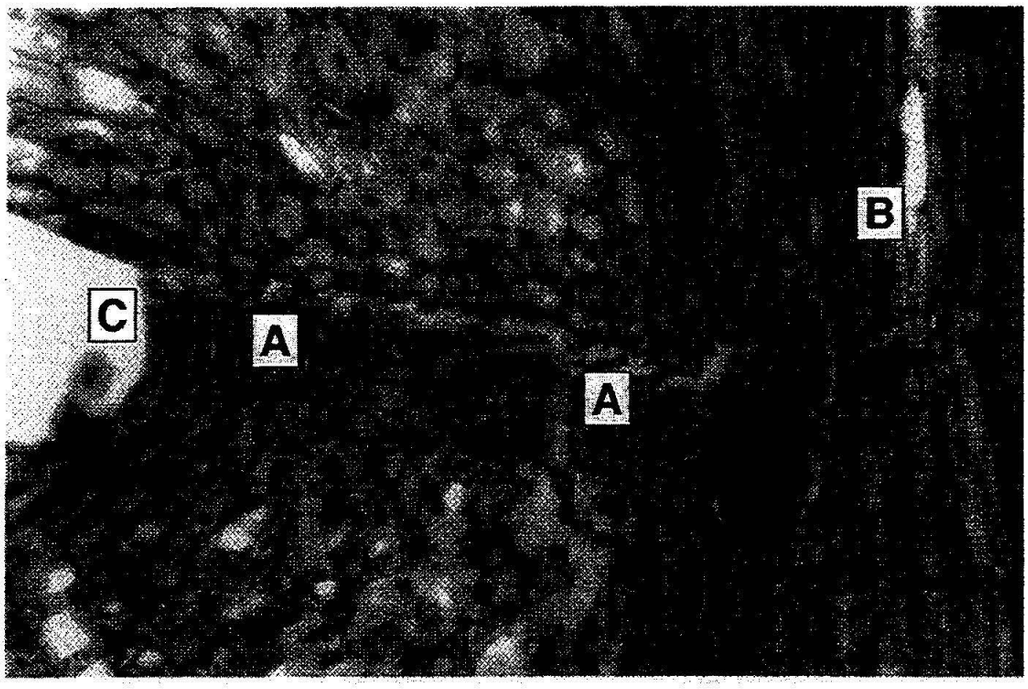

Figure 2C. *Z. palustris* left half of abscission zone, cultivar Netum, shattering type plant, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

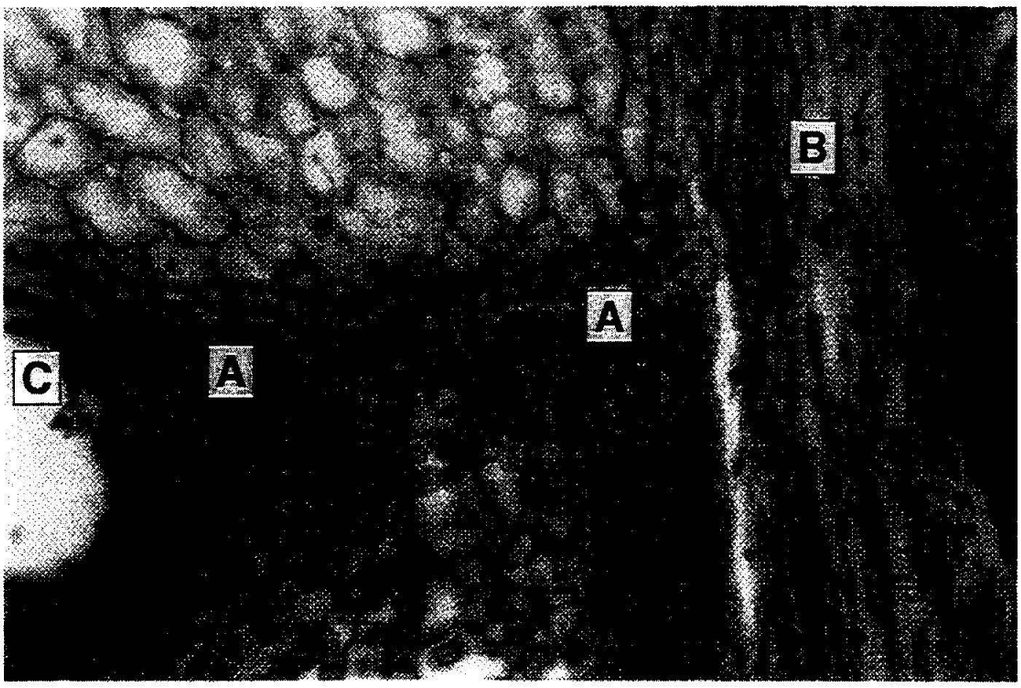

Figure 2D. *Z. palustris* left half of abscission zone, cultivar Netum, nonshattering type plant, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

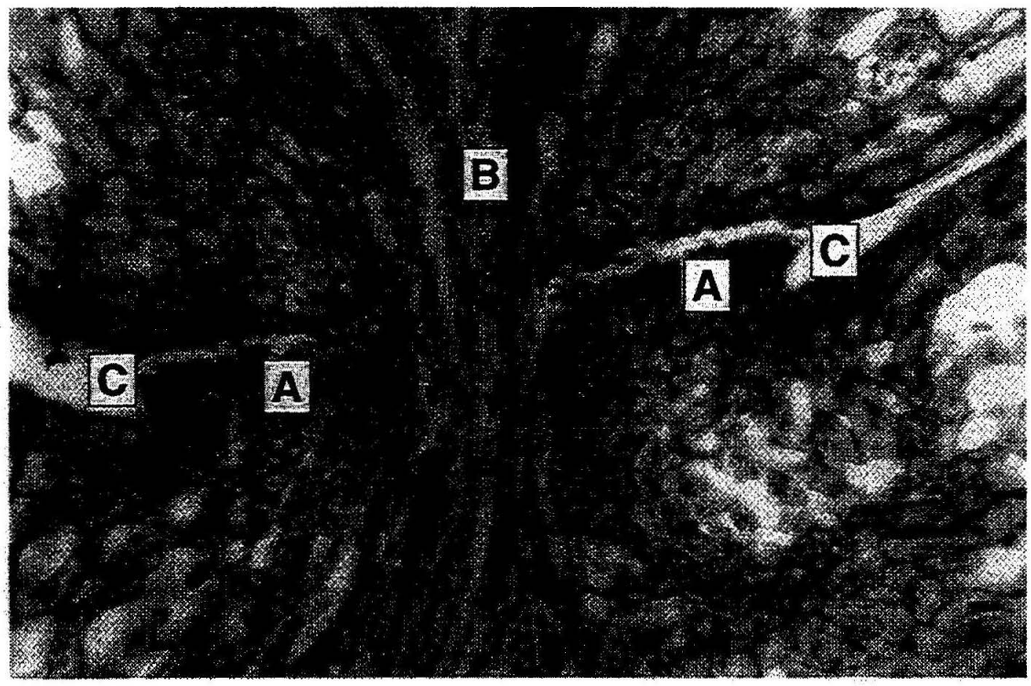

Figure 2E. *Z. texana* abscission zone, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

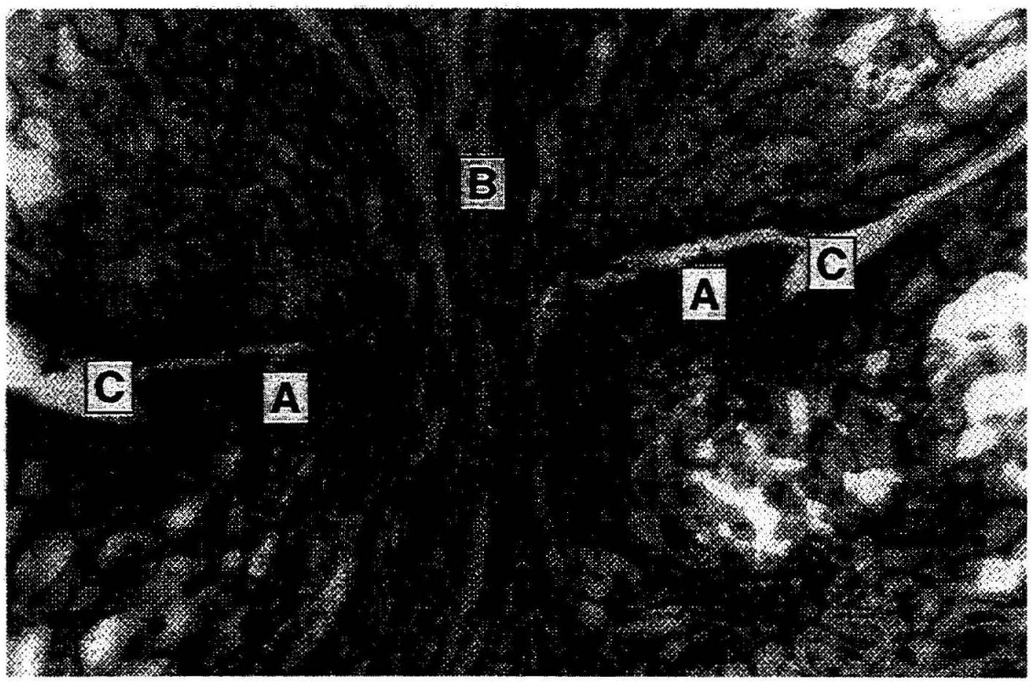

Figure 2F. Left and part of right half abscission zone in plant from cross of *Z. palustris* and *Z. texana*, mature kernal (A = abscission layer; B = central vascular tissue; C = epidermal cells)

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EVALUATION OF SPAD METER AND SOIL AMMONIUM DATA FOR TOPDRESS N RECOMMENDATIONS

Paul R. Bloom

Most of the fertility studies in 1993 were a continuation of the studies on utilizing soil testing of readily available ammonium (NH_4) N and the SPAD 502 chlorophyll meter for prediction of the need for topdress N. In addition, cooperative studies were carried out with Dr. R. Porter to assess the fertility status of plant breeding paddies to assure sufficient fertility in the breeding program and to determine the response of pistillate selections to N on a mineral soil. The results of this cooperative work are given in the wild rice breeding report.

The results in 1991 and 1992 reports suggest that the chlorophyll meter along with soil ammonium especially early in the season, are valuable tools for N management. They allow prediction of the necessity for topdress N and determination of whether or not several topdressings might be needed. The results of the previous studies demonstrate that SPAD readings of the first mature leaf below the emerging leaf (the minus one leaf) gives a good assessment of plant N. Also, the NH_4 extracted from soil samples taken from the plowlayer is strongly associated with plant N as well as being a good predictor of how much N the plants will take up. The problem with the soil NH_4 test was the lack of a good soil sampler for peat paddies and the inability to stabilize the nitrogen in a sample for shipment to a laboratory. Also, it was shown that the slow diffusion of NH_4-N in mineral soils makes it impossible to get a good average of the fertilizer N where fertilizer was applied in bands. The method, however, worked well on mineral soils where the fertilizer was tilled-in or on peats with or without banding. The research in 1993 was concentrated N in peats except for the studies on the breeding paddies at Grand Rapids.

Traditionally, plant analyses in wild rice have been done using whole plants. The problem with this approach is that after stem elongation occurs, much of what is analyzed is stem tissue which is low in nutrients and doesn't contribute much to photosynthesis. Also, it is difficult to sample many plants. Results from 1992 suggest that sampling of the minus one or minus two leaf might be better. The 1992 data showed that both minus one and minus two leaf N are highly correlated with total N but that minus two is slightly better. For 1993 we chose to analyze minus one leaves because these are the leaves that we have been using for SPAD readings. (This is similar to the method used by the white rice researchers.)

SAMPLING AND ANALYSIS OF AMMONIUM N IN SOIL

Peat samples were taken with a coring device that resembles a posthole digger. The sampler was made by Ross Renemo, Kelliher, MN, using electrical conduit pipe for handles and a split 3" PVC pipe for the sampling end. It was designed to take a 12" core but in many paddies it was only possible to sample to 10". In mineral soils, a 1" PVC pipe was used with a 3/4" pipe with end caps used to push out the core. Usually 6 cores were taken per sample. The more cores taken, the better the sample represents the area sampled. In peats where the N was applied in bands, 6 cores is a

bare minimum. The details of the sampling and field kit analysis are given in the appendix of this report.

The wet-soil cores were mixed in a bucket and a 50 milliliter (50 ml) sample of soil slurry was taken from the mixture for extraction with 50 ml of 2 molar (2M) potassium chloride (KCl). For laboratory analysis the KCl contained 0.025% merthiolate to slow mineralization of soil N and the samples were placed on ice. These samples were taken back to St. Paul for filtering within 48 hr of sampling and subsequent analyses for NH_4 or filtered on the form and then sent to St. Paul. Filtered samples are stable. The samples for field kit analysis were processed in the field by us or by growers. The field kit (Aquaquant 14423-1) requires a 5.0 ml sample. We used 2.0 ml of soil extract plus 3.0 ml of distilled water. The dilution was necessary to lower the chloride concentration to a level that does not interfere with the analysis and to get the concentration of NH_4 into the correct range for the best analysis. The value of NH_4 in ppm is multiplied by 15 to get pounds per acre of N.

A comparison of the kit data with laboratory data in Figure 1 suggests there is a very good correlation of the kit with the lab analysis but, of course, the kit is less precise especially when the NH_4-N exceeds 50 lb/ac. The decrease in precision at high NH_4 is not much of a problem because where NH_4-N exceeds 50 lb/ac no topdressing is needed and the only result needed from the analysis is that, indeed, NH_4-N is greater than 50 lb/ac.

MONITORING GROWERS FIELDS

Growers fields were monitored in June for extractable soil NH_4 at Aitkin, Washkish, Clearwater and Gully. Growers were to use the field kit to measure soil NH_4 when the crop was 1 to 2 feet out of the water - late panicle initiation to early boot - as well as take whole plant samples. This sampling can be done most easily with a boat. Soil samples can also be taken at thinning time if thinning is needed. Samples were filtered within a few hours after sampling and part of the filtrate was used for analysis with the kit and the rest sent to St. Paul for laboratory analysis.

Difficulties were encountered because the original KCl solution contained merthiolate which was shown to interfere with the analysis. Also, it was found that dilution of the samples was needed to reduce chloride interference and get the samples in a range that gives a more precise analysis with the kit. Where possible, SPAD readings were obtained.

Data from the Clearwater Rice Co. farm (Figures 2 and 3) at early boot (June 20) show that both laboratory soil NH_4 and SPAD readings were correlated with plant N content. The kit data for soil NH_4 were very similar to the laboratory data. This year at the Clearwater Co. farm carryover of fall fertilizer N was very poor and values of soil NH_4 for half of the paddies shown in Figures 2 and 3 were 7.0 lb/ac or less. A value of 5 is the minimum value that will be extracted from a peat (due to NH_4 from living soil microbial cells). Only one paddy had 30 or more lb/ac of NH_4. A value of 30 is a minimum in the early growth stages to give sufficient N for top production without using more than one topdress application. For the data shown, none of the plant samples had more than 3.0% N. In 1991 well fertilized paddies at a similar growth stage had a minimum of 4.0%. In other years we have seen that this minimum

Figure 1. Correlation of field kit NH4 with lab data

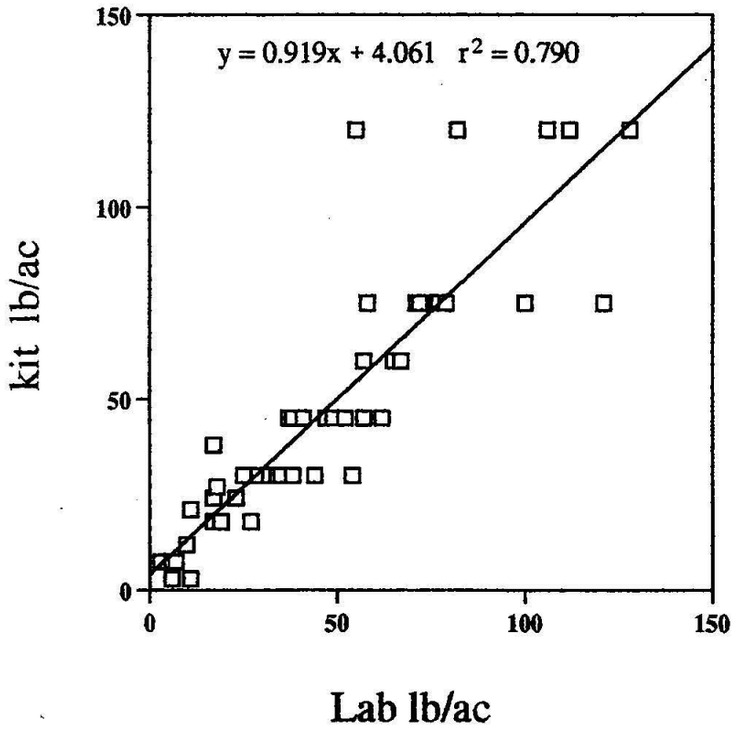


Figure 2 Variation in plant N with soil NH4 at Clearwater June

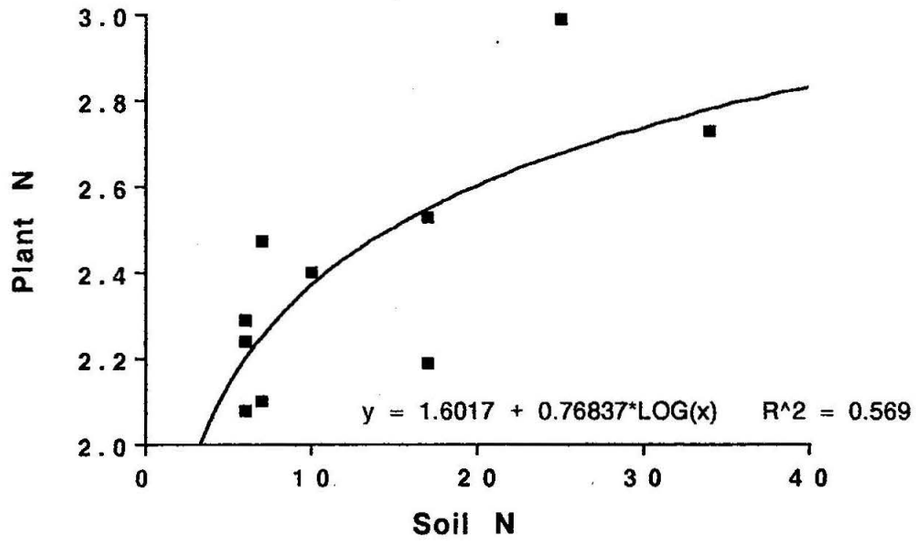
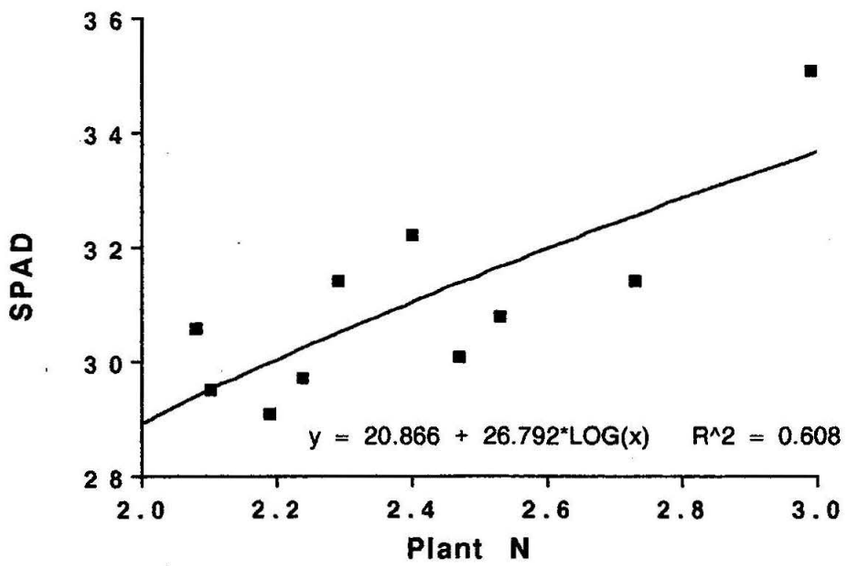


Figure 3. Variation in SPAD with soil NH4 at Clearwater June 20



varies with growing conditions and that the minimum values at this growth stage for good growth can be as low as 3.5%.

The highest SPAD readings measured at the Clearwater farm were 35. Normally 95% of the maximum value is considered to indicate deficiency. Thus the SPAD reading necessary to indicate no deficiency is 33.25. Although this value may really have been greater because the high value should be set by a paddy with known excess N fertilization. All of the points on Fig. 3 except the highest surely represent deficiency. The very low values of soil N on many paddies (<10) and very low SPAD readings suggested immediate application of topdress N was needed to minimize yield loss. On these paddies, three or four topdress applications of 30 lb/ac at about 2 week intervals were used to minimize yield loss due to insufficient N (SPAD readings verified the need for topdress). This produced immediate satisfactory yields in about 900 lb/ac of green grain for the very poor areas despite the very low N soil early in the season. Without these extra topdressings a yield of less than 500 lb/ac would have been expected.

RATE OF UPTAKE TOPDRESS N

Don Barron of the Clearwater Rice Co.. evaluated the rate of uptake of topdress N by analyzing the change in soil in NH_4 after topdressing using the field kit. The data in Figures 4 and 5 show that at the time of topdressing on July 15 (early flower) the soil contained 4.5 lb/ac NH_4. At 28 hr after topdressing with about 40 lb/ac of urea, about 50 lb/ac of N was found as NH_4; but by 96 hr little NH_4 was left. The SPAD readings also suggest that by 96 hr there was a 0.5 unit increase in the chlorophyll reading. This verifies the common assertion that greenup follows topdress by only a few days and shows that plant uptake and soil loss processes rapidly remove added N. Analysis of the NH_4 in the flood water showed that 28 hr after topdress the flood water contained only 0.2 ppm NH_4-N but after 96 hr the flood water contained 1.2 ppm NH_4^+-N. The increase from 28 hr to 96 hr after application may be due to the slower hydrolysis of urea to NH_4 in the water compared to the soil. At pH values less than 8, this quantity of NH_4 is not toxic to fish. (The pH was not measured but previous measurements suggest that pH should be in the range of 7-8.)

RESEARCH PADDIES AT AITKIN

An experiment was initiated on the University paddies at Aitkin to investigate the response to topdress N with and without spring applied basal N. The experiment was in paddies 1, 3, and 5 (1 is on the west end). These paddies were to have been in wild rice in 1992 but wild rice was destroyed by crayfish so the paddies had been, in fact, used for crayfish and weed control studies. Reseeding was necessary. Soil tests showed very low K, especially in paddy 3 where poor growth had been seen in the past, in the thin peat area in the center of the paddy. The P test values were medium to high. Phosphorus was applied at the rate of 50 lb/ac P_2O_5 and K at 250 lb/ac K_2O. Topdress urea was added by aircraft in 30 lb/ac additions in an east-west pattern across the paddies at the growth stages shown in Table 1.

Figure 4. SPAD after topdress

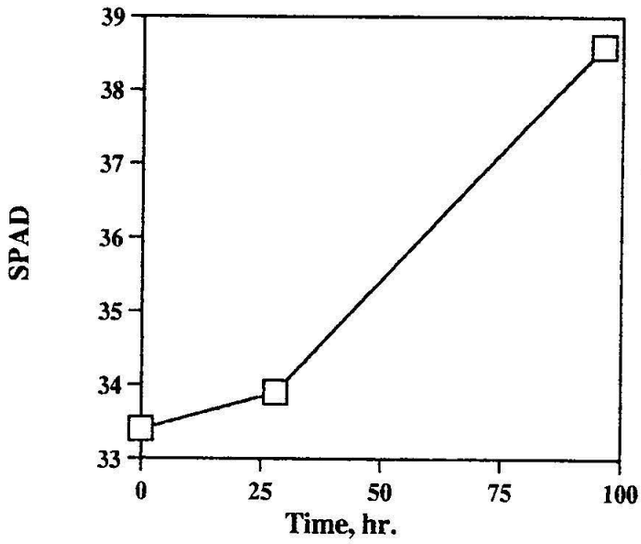


Figure 5 Soil NH4 vs. time after topdress

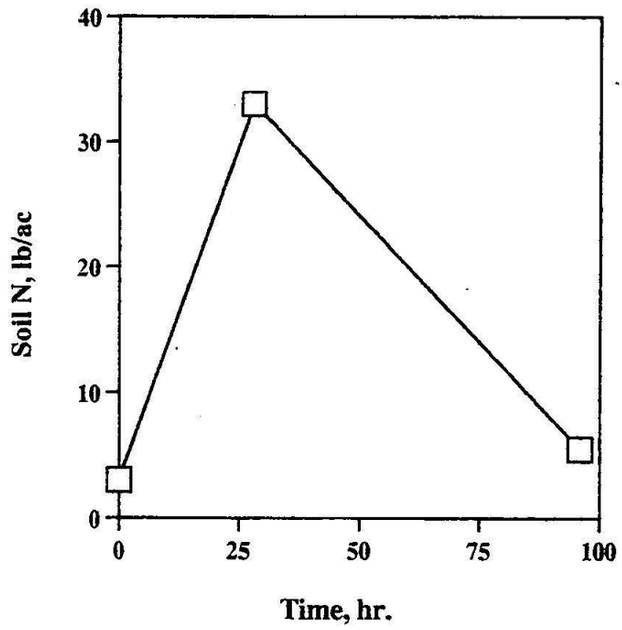


Table 1. Topdress treatments for topdress N response study at Aitkin.

Growth Stage	Topdress N, lb/ac			
Boot	-	-	-	30
Early to mid-flower	-	-	30	30
Late flower	-	30	30	30
Total	0	30	60	90

Table 2. Soil NH_4 at Aitkin experimental paddies in mid-June.

Paddy	+ Basal N		- Basal N	
	mean	S.D.	mean	S.D.
	lb/ac			
1	115	66	41	14
3	140	59	37	1.5
5	83	51	29	2.6
2			113	46
4			48	7.4
6			55	7.2

S.D.=standard deviation

Soil NH_4 was sampled on June 14 to determine the initial levels of NH_4 before the plants had removed significant N. Crop emergence was slow in these newly seeded paddies and at this date, the plants were in the floating leaf stage. The stand was thin (maximum of 2 plants/ft^2 but usually less) and uneven. Soil samples, whole plant samples, and SPAD readings were taken on July 6 at early boot, three days before the first topdressing occurred. Samples were also taken on July 20, before the second topdress, and on August 5, before the third topdress. On July 20 and August 5, the plants were sampled by taking minus one leaves. Harvest was September 3. Panicles and straw were taken from 4' x 4' squares, two squares to a plot.

The soil N values on June 14 were surprisingly high. The half of the paddies with no basal N in paddies 1, 3, and 5 ranged from 29 lb/ac in paddy 5 to 41 lb/ac in paddy 1 (Table 2). These values would normally indicate that only one late topdress would be needed to produce a top yield. The high NH_4 was not expected because our previous experience has shown that early season soil NH_4 on continuous wild rice paddies is very low where fertilizer was not applied. The flooded fallow that was induced by the crayfish damage may have been a factor, although any NH_4 that accumulated should have been lost by nitrification after fall drainage. Paddy 5 was used as a burreed weed control study in 1972 after the wild rice was eliminated. This may account for the lower N in paddy 5.

The soil N in the half of the paddies with basal N was very high, much greater than can be accounted for by the additions of planned 45 lb/ac. The high standard derivation means that these numbers are not known with great precision but they do suggest that more than 45 lb/ac may have been added. Paddies 2, 4 and 6, which in 1992 had strips upland crops - alfalfa, canola and black fallow - had higher N than the paddies that were flooded in 1992. Paddy 2 may have inadvertently been fertilized. We have found that rotating from upland commonly results in much higher soil NH_4. This is due to the death of soil microbes adapted to well drained soil when a soil is flooded. Apparently, death of the microbes releases significant quantities of NH_4. This very high N in the fertilized half of the paddies suggests no topdress was needed and we expected no response from topdress of the +basal N treatment and, indeed, we found that the topdressing had no effect on soil N, plant N, or SPAD reading.

Comparison of the +basal N, (Table 3) and -basal N for the zero topdress treatment shows the effect of plant uptake on soil N. The soil N in the -basal N was exhausted by the August 5 and addition of topdress N at this time would have been to help attain maximum yield.

Table 3. Variation in soil NH_4, plant N and SPAD reading over the growing season in the zero topdress treatments with and without spring applied basal N.

Treatment	Soil NH_4, lb/ac	Plant N %	SPAD
	---------- July 6 ----------		
+ Basal N	64 ± 14[†]	4.15 ± 0.16[†]	42.1 ± 0.6[†]
- Basal N	27 ± 7.3	3.79 ± 0.72	39.3 ± 1.4
	---------- July 20 ----------		
+ Basal N	56 ± 20	3.42 ± 0.21	40.9 ± 2.0
- Basal N	18 ± 7	2.90 ± 0.14	37.9 ± 4.3
	---------- August 5 ----------		
+ Basal N	19 ± 13	3.82 ± 0.38	45.7 ± 1.8
- Basal N	6.8 ± 2.4	3.40 ± 0.55	41.4 ± 4.2
	---------- September 3 Harvest ----------		
+ Basal N		1.37 ± 0.12	
- Basal N		1.23 ± 0.13	

[†] standard deviation

The SPAD and plant N data in Table 3 show the -basal N treatments were consistently lower but that the differences were not great. The mean SPAD readings for -basal N were only slightly below 95% of the +basal N treatments suggesting a slight deficiency. This is also suggested by the plant N on July 6 that was less than 4.0% but only slightly less. There was, however, a visual response to the basal N with the noticeable increase in vegetative growth in the +basal N treatment. This was especially true in the north end of paddy 5 where the -basal N treatment had only 19 lb/ac soil NH_4 on July 6 with a SPAD reading of 37.6. This area had only 5 lb/ac soil NH_4^+ on July 20 with a SPAD reading of 29.2. The fertilized part of the north end of paddy 5 had 38 lb/ac of soil NH_4 on July 20 with a SPAD of 39.0. There was also visible 2-4 D damage on the poor side but not on the good side. It appears the 2-4 D damage is more severe when the plants are N deficient.

Plant N was well correlated with soil N both on July 6 and August 8 (Fig. 6 and 7) and plant N was also well correlated with SPAD readings on the same dates (Figures 8 and 9). The degree of correlation of SPAD with the plant N on August 6 is remarkable. This is due to the use of the minus one leaf samples for both SPAD and N analysis.

Brown spot incidence was assessed on August 11th. The incidence was very low with 2% or less leaf coverage. Tilt had been applied but in the past even with Tilt we have had problems with brown spot. The high fertility (especially N and K) probably helped avoid brown spot problems.

Bird damage was severe even though we had a shot gunner on the paddies for the last 2 weeks before harvest. We expected we might be able to see differences in yield on the -basal N side of the paddies but because of the uneven stand and bird damage this was not possible.

The amount of biomass harvested varied from about 5,000 - 5,700 lb/ac with panicle yield weights (40% moisture) of about 1800 - 2000 lb/ac. We estimate that combine harvest may have been in the range of 1000 - 1500 lb/ac of green grain, if not for the bird damage.

The quantity of N in the harvested biomass was about 60 lb/ac. If we account for bird loss a more realistic value might be 70 lb/ac. Values of about 70 lb/ac are typical of N removal for a good wild rice crop.

OBSERVATIONS CONCERNING K AND P DEFICIENCIES AT AITKIN

We monitored soil NH_4 on the pistillate-N response study at Aitkin and found an area across the middle of the paddy where SPAD readings were low despite the high soil NH_4^+ (greater than 70 lb/ac). Plant analysis at the early boot stage showed plant content of only 0.21% P in the poor area and 0.41% P in the better areas. Our data 1993 data taken together with previous results suggest a deficiency limit of 0.25% with a practical lower limit of 0.30% P. This will assure no deficiencies appear. To maintain this level, soil P must be above the medium soil test.

Comparing our plant analysis results in paddies 1-6 with previous data suggests that 2.5% K in whole plants at boot is the deficiency limit with a practical limit of 3.0%. Both this limit and the limit above for P are much higher than white rice data

Figure 6. Variation of plant N with soil NH_4 at Aitkin on July 6.

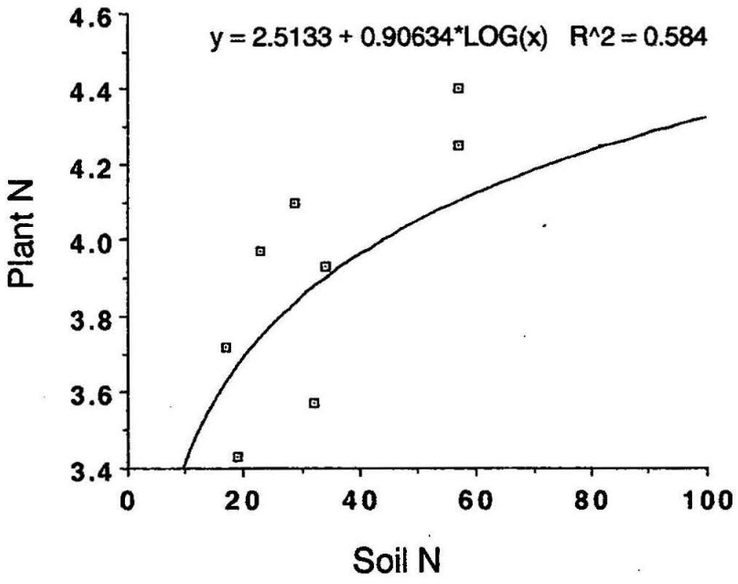


Figure 7. Variation of plant N with soil NH_4 at Aitkin on August 5.

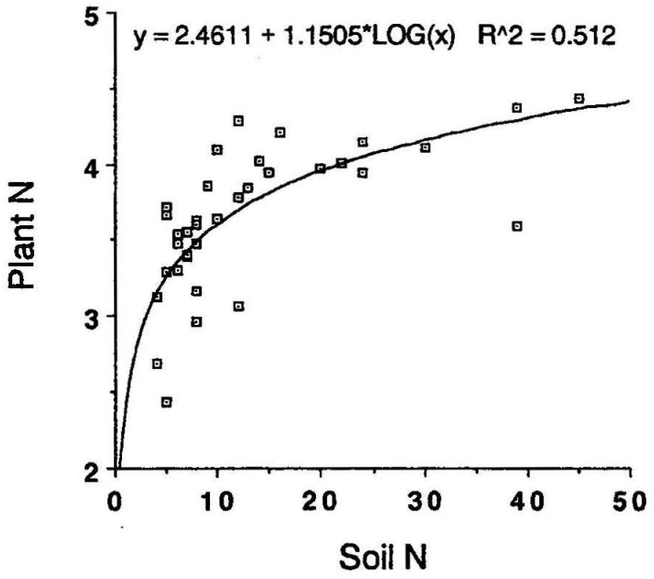


Figure 8. Variation in SPAD reading with plant N at Aitkin on August 6.

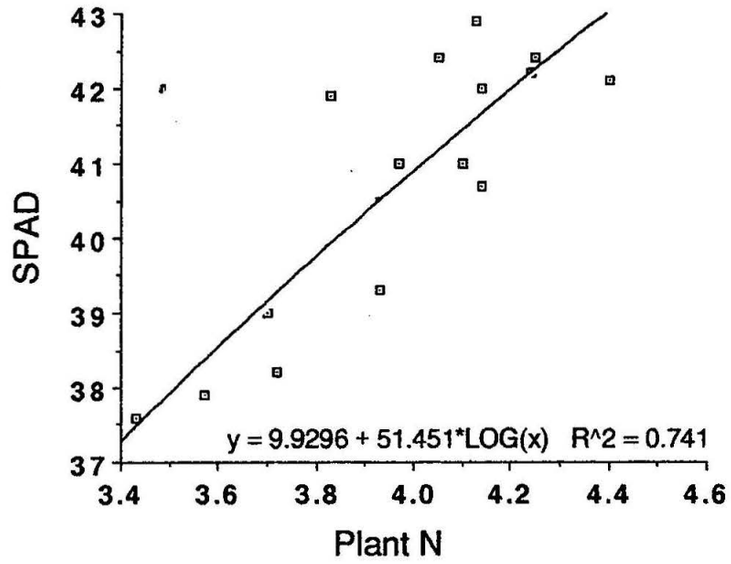
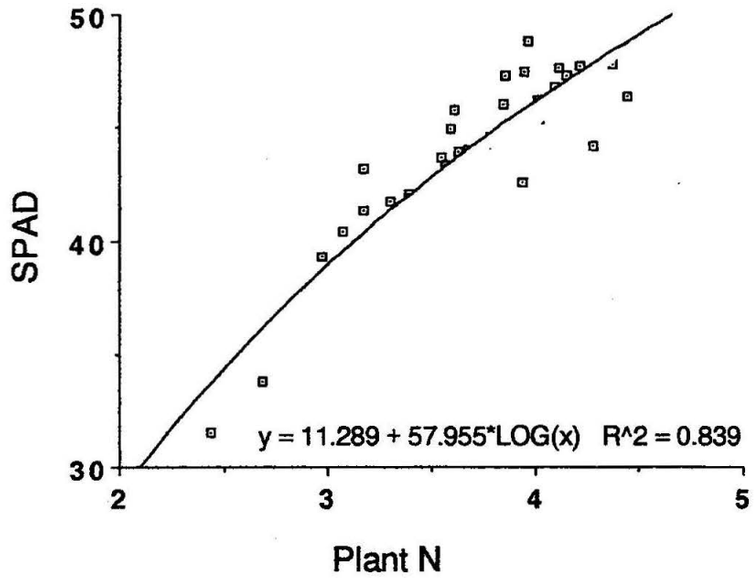


Figure 9. Variation in SPAD reading with plant N at Aitkin on August 15.



RECOMMENDATIONS

Soil NH_4

If soil NH_4-N is greater then 50 lb/ac than no topdress is needed. In the range of 30-50 lb/ac one topdress of about 30 to 40 lb of N at early to mid-flower is needed. In the range of 10-30 lb/ac two topdresses are needed at very early flowering and mid-flowering. In the range of 0-10 lb/ac three topdresses are needed, one as early as possible and the other two at very early and mid-flowering.

SPAD

The SPAD meter cannot tell if there is much reserve of NH_4 in the soil so without soil NH_4 data you probably end up applying more N than if soil NH_4 data are available. At 95 to 100% of the reading for an excessively fertilized plot one late topdress should be applied unless soil data shows a good reserve of NH_4. At 85 to 95% two topdresses are needed while at less than 85% three topdresses are needed.

P and K

At boot, plant tissue K should be greater than 3.0% and P should be greater than 0.30% to have 100% confidence of no deficiency. On peats this requires a soil test for K of 90 ppm or more and P soil test into the high range.

WILD RICE BREEDING

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Variety Trials

This year's variety trials were at the University research site on the Vomela farm near Aitkin and at Clearwater Wild Rice near Clearbrook, and provided good data, particularly at the Aitkin site. Crayfish were mostly excluded from plots, and stands were more uniform than in previous years, enabling a more fair comparison among varieties. Stands at the Clearbrook site were thin, probably due to floating peat early in the season, but after filling in plots with transplants from the sixth rep, the plots of the other five reps achieved an adequate stand density for evaluation. We had planted a variety trial at another site on a grower's paddy near Aitkin, but the stand was too thin to obtain reliable data and the plots were not harvested by the researchers. Six replicates were also spring-planted at the research paddies at Grand Rapids, but due to loss of seed viability in storage, only 13 of the entries had adequate stands. After some deliberation, we decided not to collect data from that site. Plants in some of these plots were used for crosses carried out by Dr. Grombacher to develop mapping populations.

Methods. At each of the two locations which yielded data, we planted 20 entries in six replicates in the fall of 1992. All plots were four 10-foot rows with 12 in. between rows and 30 in. between plots. In June, we thinned the plots and transplanted into already thin plots until they were relatively uniform. We sacrificed one rep at Clearbrook to fill in with transplants into the other five reps.

At Aitkin, we applied 50 lb/A of N as urea and 100 lb/A of K prior to planting. At the onset of flowering, we applied a topdressing of a blend of urea and K, amounting to 30 lb/A of N and 50 lb/A of K, and repeated the application 10 days later. During grain fill, we applied malathion once to control riceworm.

At Clearbrook, plot management was consistent with the grower's normal practices. They applied 60, 30, and 80 lb/A of N (anhydrous ammonia), P, and K, respectively, and one topdress, during flowering, of 30 lb/A of N as urea. They applied 6 oz./A of propiconizole (Tilt) twice, July 13 and Aug. 11 to control Fungal Brown Spot, and malathion at the recommended rate on Aug. 18 to control riceworm.

When heading began, we noted the date at which 50% of the mainstems in each plot had emerged from the boot (at Clearbrook, this data was taken by Don Barron of Clearwater Wild Rice). Prior to the onset of seed shattering, we placed PVC troughs between the center two rows to catch shattered seed. We estimated shattering losses

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per acre based on the area of these troughs, which was 1/6 of the harvested area of the plot. We collected seeds from the troughs and rated the plots for lodging immediately prior to harvest. At Clearbrook, we harvested the earliest 10 entries first (according to average of flowering dates over both locations), then the remaining 10 entries three days later. We harvested the center two rows with a two-row grain binder and threshed the bundles with a Vogel thresher. We removed most of the stem debris from the threshed samples using a shaker-sieve seed cleaner. The final sample included seeds and other material which passed through a 9/64 by 3/4 inch slotted sieve, but not through a 1/14 by 1/2 inch sieve. We weighed fresh samples, dried them, and weighed them again to determine dry weight and percent moisture.

Results and Discussion. The occurrence of pistillate and bottlebrush plants counted in 4 reps at Aitkin (Table 1) generally correspond to the entries which have been selected for either trait. Entries with at least 25% pistillate plants generally yielded highest, exceeding 2000 lb/A in several cases. Bottlebrush populations also yielded well. Overall, Aitkin yields were 40% higher than Clearbrook yields (Table 2), probably due to lower plant densities at the latter site. Also, birds were successfully excluded from the plots at Aitkin; however, before we could cover the plots with netting, birds had already damaged Meter, the earliest variety by several days.

Table 1 Proportion of pistillate and bottlebrush plants in selected variety trial entries (4 plots/reps per entry, approx. 100 plants each plot)

Entry	Percent pistillate plants	Percent bottle-brush plants
Franklin (MCIA)	1	7
K-1(Pi)F3	26	
K-2(Pi)F3	27	
NACH-B		21
PB(M)C2	1	35
PBB-C1		41
PBP-C1	14	25
Petrowske BB (G/I)	1	28
MK-1(Pi)F3	26	
MK-2(Pi)F3	30	
PLAR	32	
PM3(E)C3	42	

K-1(Pi)F3 and K-2(Pi)F3 are pistillate selections from K2-derived populations which were being selected for shattering resistance; the former yielded well at Aitkin, while the latter yielded better at Clearbrook. Another exceptional entry was PBB-C1, a population selected from Petrowske Bottlebrush. The highest yielding entry at Aitkin had the most pistillate plants and was also the latest, PM3(E)C3; however, it was only average at Clearbrook. This experimental population was much improved over its parent population, Pistillate M3. Topdressing twice with urea probably increased its grain fill more than other entries at Aitkin. The newly-released Franklin also yielded respectably,

and had the second lowest average shattering over both locations--FSSR-C6 (the frost-selected shatter-resistant population) was the least-shattering entry.

All the entries had a similar percent moisture at harvest, except for two earliest at Clearbrook--Meter and NR. We had waited to harvest these until the rest of the earlier group of 10 entries was ready.

Table 2 Yield and shattering losses of wild rice variety trial entries at 2 locations.

Entry	Aitkin(N=6)			Clearbrook(N=5)			Combined (N=11)		
	Yield	Shat	Mois	Yield	Shat	Mois	Yield	Shat	Mois
	lb/A[a]	%[b]	%[c]	lb/A[a]	%[b]	%[c]	lb/A[a]	%[b]	%[c]
Meter (Imle)	891	16	32	652	43	25	772	29	29
NR (Manomin)	1400	11	33	885	36	26	1142	23	30
Voyager (G/I)	1854	11	33	1266	23	28	1560	17	31
K-1 C5	1862	17	32	1340	18	28	1601	17	30
K2 (Godward)	1488	22	32	1414	25	29	1451	23	30
Franklin (MCIA)	1819	13	32	1371	15	29	1595	14	31
Petrowske BB	1862	14	33	1447	18	28	1654	16	31
FSSR-C6	1758	8	32	1303	13	29	1531	11	31
PLAR	2026	17	34	1594	18	30	1810	17	32
NACH-B	1777	13	32	1419	21	29	1598	17	31
K-2(Pi)F3	1665	19	33	1463	28	29	1564	23	31
PBB-C1	2040	12	31	1382	29	29	1711	21	30
PB(M)C2	1917	12	32	1292	34	28	1605	23	30
PBP-C1	1762	18	32	1175	35	28	1469	27	30
K-1(Pi)F3	2301	12	33	1098	32	29	1700	22	31
M1 (Manomin)	1359	27	33	956	38	29	1157	32	31
MK-1(Pi)F3	1895	16	33	1188	34	30	1541	25	31
PM3(E)C3	2599	19	33	1392	25	29	1996	22	31
MK-2(Pi)F3	1952	21	32	1194	30	29	1573	26	31
SS-C5 short	1562	20	32	1197	30	31	1380	25	31
mean	1790	16	32	1251	27	29	1520	22	31
LSD(5%)	363	6	1	273	7	1	230	6	1

[a] Adjusted to 40% moisture. [b] Shattering as % of harvested plus shattered grain weight.
[c] % moisture in grain at time of harvest, fresh weight basis.

Heading dates, shown in Table 2, indicate the relative maturity of the entries. In the same table, plant heights measured at Aitkin reveal the relative stature of the entries. The shortest entry was Meter, and the tallest was PBB-C1. The rest were not much different from each other. NR, Franklin, NACH-B, PBP-C1, and K-1(Pi)F3 had the longest non-pistillate head length, though not by much. SS-C5 had the most compact head, but it was not much shorter than Petrowske Bottlebrush, K2, FSSR-C6, or PB(M)C2. Seed tensile strength was so variable that even 25 measurements per plot was not enough to reveal significant differences. Nevertheless, we are encouraged that Franklin, a shattering-resistant variety, had the highest average tensile strength. Shattering was not correlated to tensile strength using this location's data ($r^2 = 0.14$).

Table 3 Other characteristics of wild rice variety trial entries at 2 locations.

	Aitkin (N=6)			Clearwater (N=5)		Combined (N=11)	
entry	Heading date	Plant height	Head length	Heading date	Tensile strength	Heading date	Lodging score
	DAF[a]	in.[b]	in.[c]	DAF[a]	g[d]	DAF[a]	units[e]
Meter (Imle)	88	61	6.6	95	134	91	1.1
NR (Manomin)	91	67	7.2	94	156	92	1.2
Voyager (G/I)	94	68	6.8	97	148	95	1.6
K-1 C5	95	69	6.9	98	154	96	1.5
K2 (Godward)	95	64	6.4	99	163	97	1.1
Franklin (MCIA)	96	69	7.1	100	187	97	1.6
Petrowske BB	97	67	6.3	98	158	97	1.3
FSSR-C6	97	66	6.4	99	165	98	1.6
PLAR	97	67	6.7	99	187	98	2.0
NACH-B	97	66	7.1	98	177	98	1.4
K-2(Pi)F3	98	64	6.3	99	181	98	2.3
PBB-C1	98	74	7.1	99	183	98	1.1
PB(M)C2	99	63	6.4	100	157	99	1.5
PBP-C1	99	66	6.5	100	170	99	1.6
K-1(Pi)F3	98	67	7.1	100	180	99	2.4
M1 (Manomin)	99	68	6.6	101	159	99	1.7
MK-1(Pi)F3	98	69	6.8	101	170	99	2.2
PM3(E)C3	100	69	6.8	100	166	99	2.4
MK-2(Pi)F3	100	67	6.8	100	172	100	2.1
SS-C5 short	100	66	6.2	101	161	100	1.1
mean	96.7	68.8	6.7	98.8	169	97.6	1.6
LSD(5%)	1.6	4.7	0.7	1.9	NS	1.2	0.4

[a] Number of Days after flooding at which 50% of mainstems had emerged. [b] Height from soil to tip of tallest panicle of plant (10 plants/plot). [c] Length of pistillate (grain-bearing) portion of normal panicles (up to 10 plants/plot). [d] Linear force required to detach seed (data taken on 25 seeds/plot in 3 of the 5 reps). [e] Rated on a 1-5 scale, 1=all stems upright, 5=all stems lodged.

Lodging tended to be highest in the pistillate entries, as expected. SS-C5, which had been selected for stem sturdiness, had one of the lowest average lodging scores.

When we examined the stand data more closely (Table 3), we found that there were some differences between entries in spite of our attempts to thin stands to a uniform plant density. Since data from past years usually shows a positive relationship between panicle density and yield, we need to consider any differences in stem density when interpreting yield data. Of most interest to us was Franklin, which had the lowest plant and stem density at both locations. It also had the highest yield per panicle of any entry, even more than the pistillate entries. This may have been an indication of greater seed retention, resulting in a decent yield in spite of the thinner plant density, or it may have been due in part to compensation by the plants for the lower density. So we reanalyzed the yield data using the panicle density as a covariate to adjust the yield. The adjusted yield per acre of Franklin was higher than any non-pistillate entry, save PBB-C1.

Table 4 Stand density and yield of wild rice variety trial entries at 2 locations.

	Clearbrook			Aitkin			Combined				
Entry	Plant dens.	Stem dens.	Panicle yield	Plant dens.	Stem dens.	Panicle yield	Plant dens.	Stem dens.	Panicle yield	Yield	Adj. yield
	ft.$^{-2}$	ft.$^{-2}$	g/hd[a]	ft.$^{-2}$	ft.$^{-2}$	g/hd[a]	ft.$^{-2}$	ft.$^{-2}$	g/hd[a]	lb/A[b]	lb/A[c]
Meter (Imle)	2.1	6.6	0.6	2.2	8.6	0.7	2.2	7.6	0.6	772	713
NR (Manomin)	1.8	6.5	0.9	1.8	7.3	1.2	1.8	6.9	1.0	1142	1146
Voyager (G/I)	1.6	6.0	1.3	2.1	7.9	1.5	1.9	7.0	1.4	1560	1559
K-1 C5	1.8	5.6	1.5	2.1	7.3	1.6	1.9	6.5	1.6	1601	1643
K2 (Godward)	1.6	5.9	1.5	2.1	8.0	1.2	1.8	7.0	1.3	1451	1450
Franklin (MCIA)	1.6	5.0	1.7	1.8	6.6	1.7	1.7	5.8	1.7	1595	1699
Petrowske BB	1.8	5.7	1.6	2.2	8.1	1.5	2.0	6.9	1.5	1654	1659
FSSR-C6	1.8	5.3	1.6	2.3	8.1	1.4	2.0	6.7	1.5	1531	1554
PLAR	1.7	6.4	1.6	2.4	8.8	1.5	2.1	7.6	1.5	1810	1749
NACH-B	1.8	5.8	1.5	2.0	8.0	1.4	1.9	6.9	1.4	1598	1601
K-2(Pi)F3	2.3	5.8	1.6	2.5	7.1	1.5	2.4	6.4	1.5	1564	1613
PBB-C1	2.3	5.6	1.6	2.3	8.0	1.6	2.3	6.8	1.6	1711	1722
PB(M)C2	2.7	5.9	1.4	2.3	7.9	1.5	2.5	6.9	1.5	1605	1610
PBP-C1	2.4	5.7	1.3	2.5	7.7	1.5	2.4	6.7	1.4	1469	1493
K-1(Pi)F3	2.1	4.9	1.4	2.5	7.7	1.9	2.3	6.3	1.6	1700	1757
M1 (Manomin)	2.2	5.6	1.1	2.4	7.2	1.2	2.3	6.4	1.1	1157	1208
MK-1(Pi)F3	2.5	6.2	1.2	2.5	7.7	1.6	2.5	7.0	1.4	1541	1538
PM3(E)C3	2.3	6.6	1.3	2.4	9.8	1.7	2.4	8.2	1.5	1996	1883
MK-2(Pi)F3	2.3	5.3	1.4	2.4	8.0	1.5	2.4	6.6	1.5	1573	1600
SS-C5 short	2.5	5.7	1.3	3.0	8.1	1.2	2.7	6.9	1.3	1380	1385
mean	2.1	5.8	1.4	2.3	7.9	1.4	2.2	6.8	1.4	1520	1529
LSD(5%)	0.4	1.1	0.3	0.4	1.3	0.3	0.3	0.8	0.2	229	222

[a] Dry weight of seed ÷ number of panicles harvested. [b] Grain yield at 40% moisture.
[c] Grain yield at 40% moisture, adjusted for stem density.

Response of Pistillate Plants to Topdressed Nitrogen (with P.R. Bloom)

This experiment is a follow-up to the previous year's experiment, in which we compared the response to nitrogen of 6 breeding populations having differing proportions of pistillate plants. We were seeking a second year of data to fulfill our objectives, which were: 1) to test and compare the nitrogen response of populations with varying proportions of pistillate and normal plants, and 2) to compare the response of pistillate and normal plants within mixed populations.

Methods. This year, we compared 12 populations which we expected to have near zero, 1/6, 1/3, or 1/2 pistillate plants (Table 5). The actual proportions were somewhat different, generally less than expected. However, P-1/6 and P-1/3 had more pistillate plants than expected. They were blends of PM3-N C3 and PM3(E)C3, in a 2:1 and a 1:2 ratio, respectively. PM3-N C3 had much lower seed viability relative to PM3(E)C3, resulting in a greater proportion of pistillate plants than expected in these blends. For the purposes of presenting these results, we considered all the entries with 20% or more actual pistillate plants to be pistillate entries, while the rest we considered non-pistillate. The latter group, consisting of Franklin, K-1 C5, and PM3-N C3, allowed us to see the N-response of non-pistillate populations (or cultivar, in the case of Franklin).

Table 5 Expected and actual pistillate frequencies of entries in the nitrogen-response experiment, 1993.

Entries	%P expected	%P actual
Franklin	1	0.5
K-1 C5	1	0.7
PM3-N C3	6	12
K-1(Pi)F3	33	22
K-2(Pi)F3	33	28
P-1/6	17	33
MK-1(Pi)F3	33	34
MK-2(Pi)F3	33	35
PLAR	50	41
PM3-P	50	43
PM3(E)C3	50	44
P-1/3	33	45

The design, like last year's experiment, was split plot, with entries being the main plots and topdress treatments the subplots. The plots were spring-planted at 2 locations, Grand Rapids (NCES) and Aitkin (Vomela farm). At each location there were 3 replicates, with 4 sub-plots of each variety planted adjacent to each other in each rep. Each subplot consisted of four 10-foot rows, with 12 inches between rows within plots and 30 inches between adjacent plots. After plants had reached the aerial leaf stage, plots were thinned and transplanted as needed to obtain relatively uniform stands. All sub-plots were treated in an identical fashion until separated into 4 topdress treatments: 0, 30, 60, and 90 lb/A of N as urea. Topdress treatments began at the late boot stage and were split into applications of 30 lb/A at each of 3 times (see Table 6).

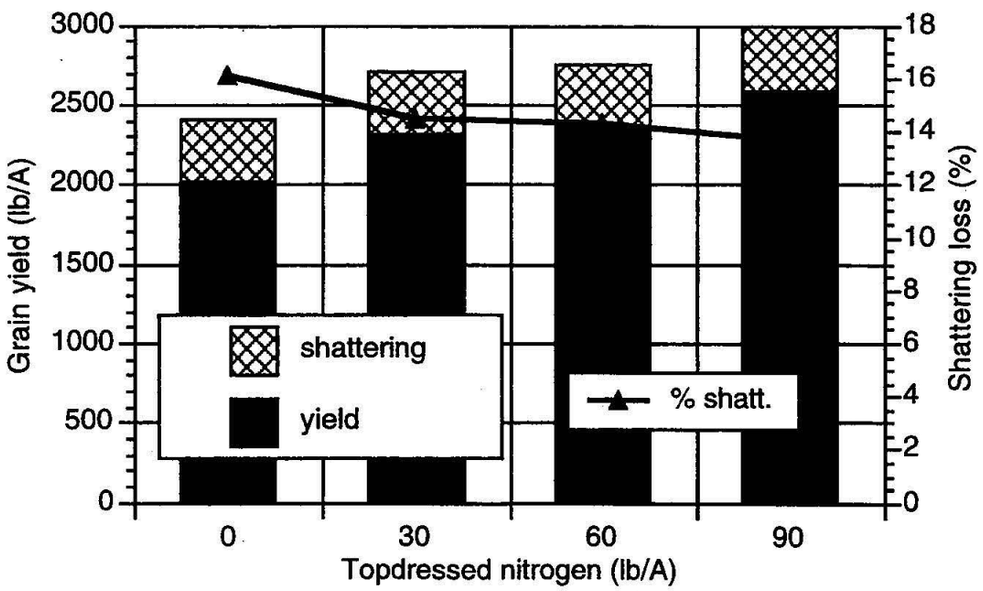
Plots were harvested by hand, keeping pistillate heads and normal heads in separate fractions for each plot, to be threshed and weighed separately. Shattered seed was collected from plots placed between the rows. Harvested seed and shattered seed estimates were combined to calculate potential yield; these three variables were adjusted to a 40% moisture basis, and used to calculate percent shattering loss. In each plot, the numbers of plants and panicles harvested were also counted separately (pistillate vs. normal) to calculate stand density and yield per head of each fraction.

Table 6 Topdress application schedule for fertility trial

Application time	0 lb/A plots	30 lb/A plots	60 lb/A plots	90 lb/A plots
	lb/A applied			
late boot	0	0	30	30
early flowering	0	30	0	30
mid flowering	0	0	30	30

Results and Discussion. We saw a positive response to topdressed nitrogen averaged over all 12 entries (Fig. 1). Shattering remained relatively constant in absolute terms, resulting in a decrease in percent shattering, since the total yield (by which actual shattering loss was divided to calculate percent shattering) increased.

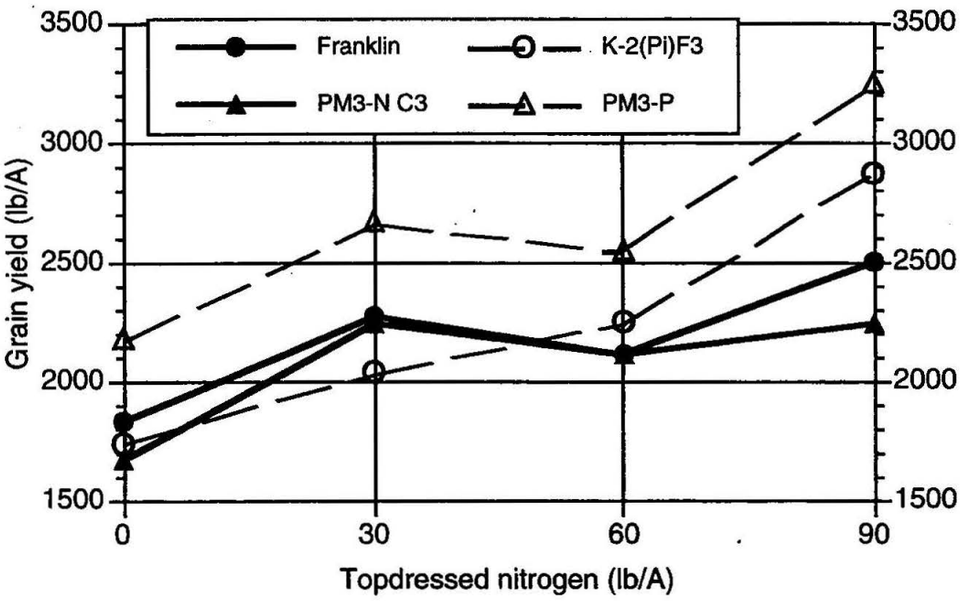
Fig. 1 Response of grain yield and shattering to nitrogen
Average of 12 entries--1993



Apparently the additional grain produced in response to nitrogen did not contribute significantly to the amount of grain lost. As we will point out later, this was probably due to the addition of grain from tillers which may not have matured enough to shatter by the time of harvest.

Four examples of individual varieties' responses are shown in Figure 2. The yield of Franklin increased as topdress was added, by 630 lb/A over the range of 90 lb/A of N. However, K-2(Pi)F3, showed a greater rate of increase. This pistillate population (selected from Franklin) gained 1130 lb/A of grain in response to 90 lb/A of added N, in spite of having only 28% pistillate plants (see Table 5). PM3-N C3, having only 12% pistillate plants, still increased by 570 lb/A, while PM3-P (43% pistillate) increased by 1070 lb/A when 90 lb/A of N was added. PM3-P achieved the highest yield of all the entries, 3240 lb/A at the 90 lb/A rate of topdressed nitrogen.

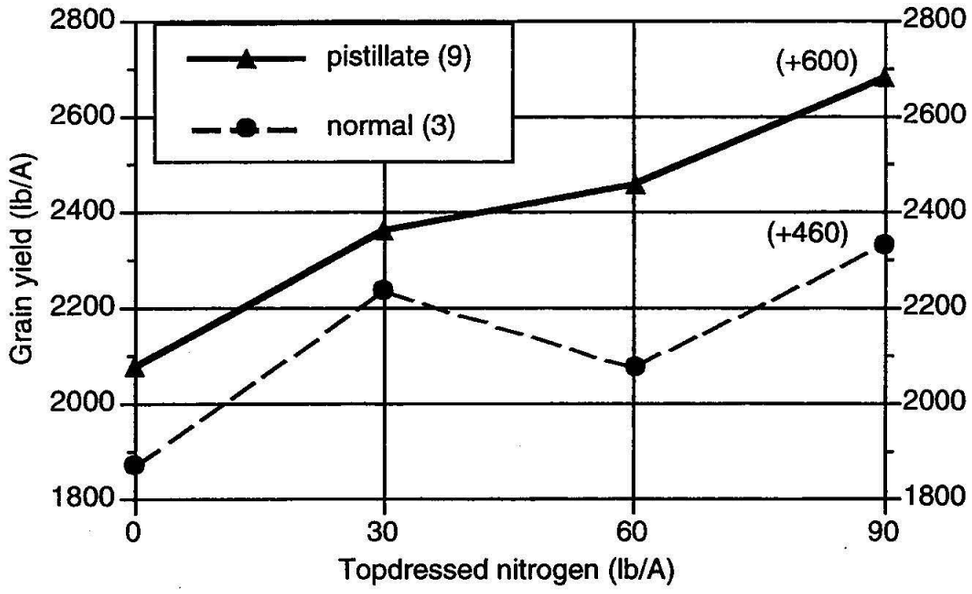
Fig. 2 Response of grain yield to nitrogen
Four entries--1993



When we compare the average of all 9 pistillate entries to the average of the 3 normal entries (Figure 3), we see differences in their responses (although not statistically significant). Consistent with the individual entries shown in Figure 2, the normal entries produced additional grain (by 460 lb/A), but the pistillate entries even more so (by 600 lb/A). The normal entries tend to level off after the initial 30 lb/A of nitrogen, whereas the pistillate entries continued to increase, achieving an average yield level 350 lb/A greater than the normal group. The pistillate entries had a 200 lb/A advantage when no topdressed nitrogen was applied. The slight dip in the lines, especially pronounced for

the normal group, may have been due to the way the topdresses were applied. There was a gap of about 4 weeks between the first 30 lb/A application and the last one. Perhaps growth was stimulated by the first application during the boot stage, but grain fill was diminished until the later application.

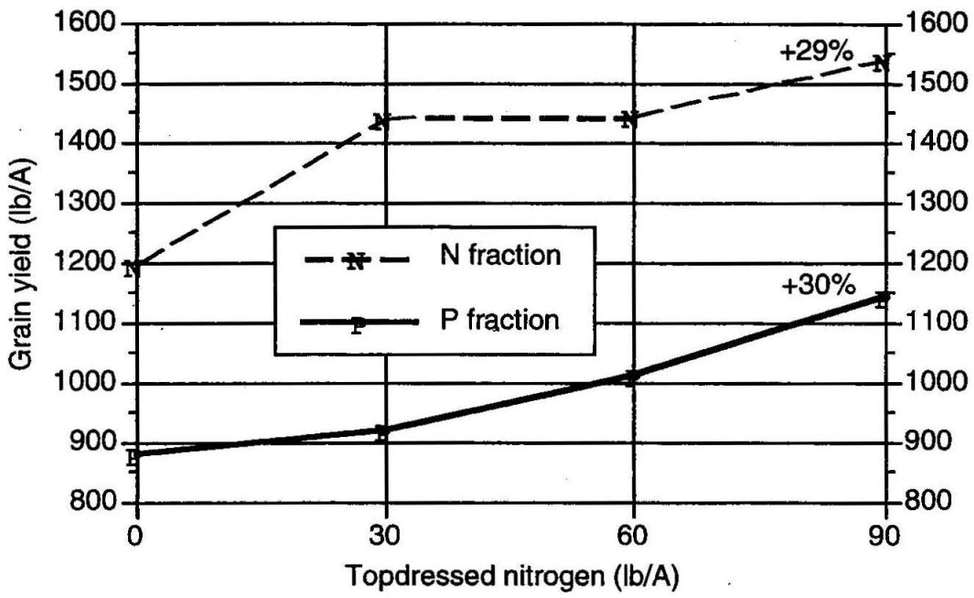
Fig. 3 Response of grain yield to nitrogen
Normal vs. pistillate entries--1993



The pistillate and normal fractions within the mixed (pistillate) populations parallel the responses of the pistillate and normal entries (Figure 4). The normal fraction increased in grain yield with the first 30 lb/A of N, then leveled off somewhat. The pistillate fraction increased steadily throughout the range of topdress treatments. Although the relative increase from the first to the last treatment was similar (30%), the pistillate fraction was still increasing at the same rate at the higher topdress levels, indicating that yield potential had still not been reached by pistillate plants. Soil and plant sampling prior to the topdress treatments had revealed depletion of nitrogen to near zero, especially in the third rep (data not shown). This depletion of basal nitrogen prior to the reproductive period, when topdresses were applied, may have tended to equalized the yield potential of the two plant types.

The average number of pistillate plants in the 9 pistillate entries which yielded data for Figure 4 was substantially less than half (36%), ranging from 22 to 45% (see Table 5). This accounts for the lower contribution of the pistillate fraction compared to the normal fraction--most of the plants in each plot were of the normal type.

Fig. 4 Response of fractional grain yield to nitrogen
Normal & pistillate plant fractions of 9 entries--1993



When expressed in terms of yield per panicle, the productivity of the pistillate plant type is far greater than the normal (Figure 5). This advantage was about 30-40% in this experiment. Both pistillate and normal panicle yields increased with added nitrogen, indicating greater grain fill, since shattering was unaffected. Although the pistillate panicles seemed to be adding grain at a greater rate than the normals over the first two topdresses, they decreased slightly upon adding the third topdress.

Figures 6 and 7 may explain this decrease. Overall the number of plants increased only slightly, perhaps due to increased health resulting in decreased mortality (Figure 6). But there was a greater increase in panicle density as nitrogen was added, indicating more tillering. Tillers (or panicles) per plant responded differently for pistillate and normal plants (Figure 7). Normal plants stopped producing tillers after the first topdress, while pistillate plants didn't really get going until the second or third topdresses were applied. Because wild rice tillers appear very asynchronously (over a period of time) the pistillate tillering response might be expected to add to the total number of panicles harvested, while bringing down the average per panicle because of the immaturity of the later panicles. This may have masked any increase in grain fill of earlier pistillate panicles.

Pistillate plants have been observed to flower later than normal plants. This would result in less mature grain overall in the pistillate fraction, as indicated by the higher moisture content of the pistillate grain at harvest (Figure 8). This lends weight to the idea that the pistillate plants in this experiment were still producing and filling tillers when harvest occurred. Again, pistillate panicles may be continuing to respond to added nitrogen, but this response would be masked, in the data presented, by the lesser contribution of later tillers.

Fig. 5 Response of grains per head to nitrogen
Normal and pistillate fractions, 9 entries--1993

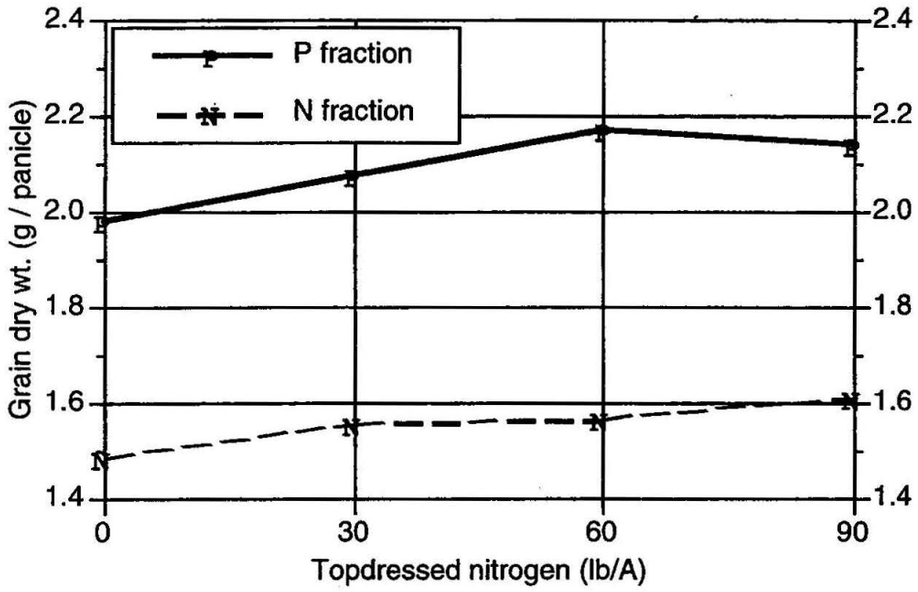


Fig. 6 Response of plant and panicle density to nitrogen
12 entries--1993

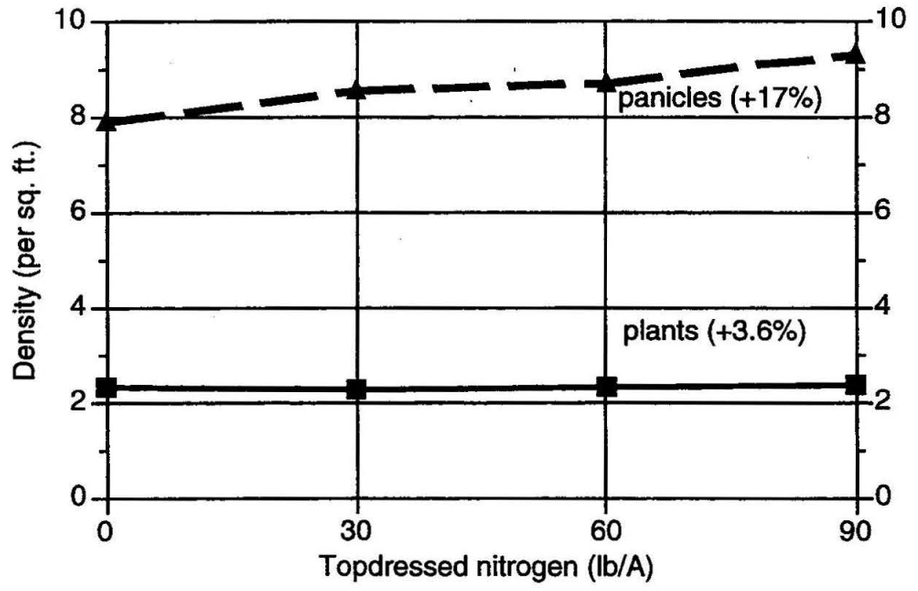


Fig. 7 Response of heads per plant to nitrogen
Normal and pistillate plants, 9 entries--1993

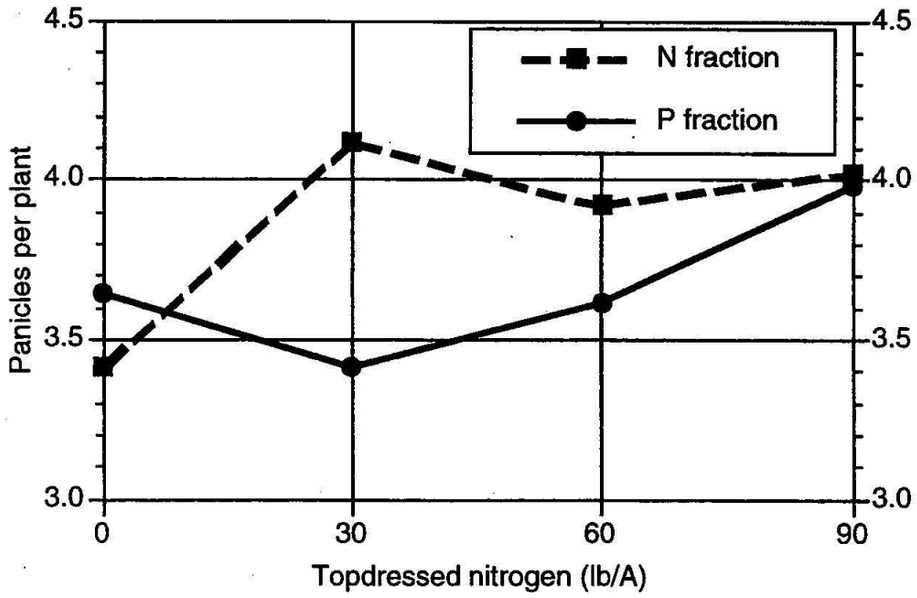
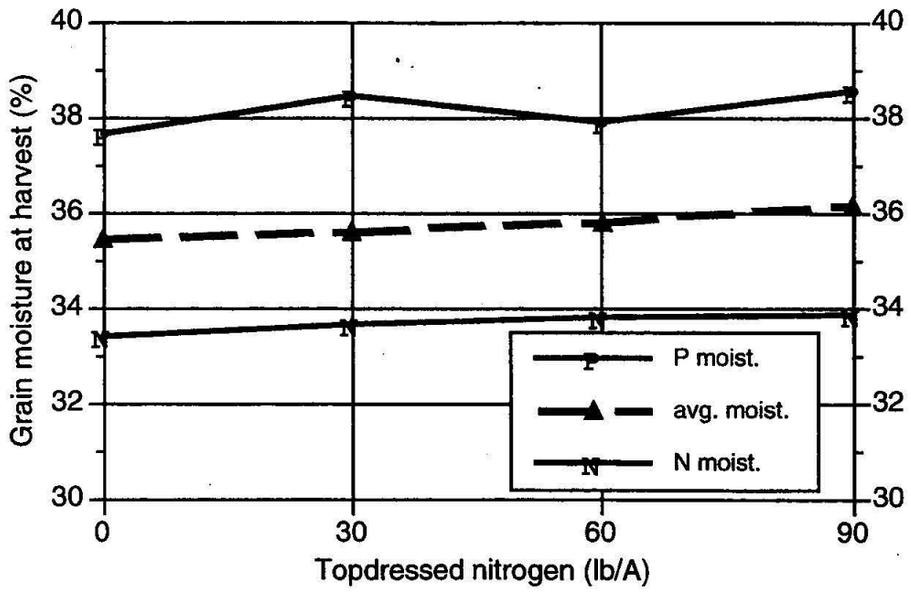


Fig. 8 Response of grain moisture to nitrogen
Normal & pistillate fractions--1993



We conclude, then, that 1) both pistillate and normal populations show a response to nitrogen topdressing, and 2) pistillate panicles may respond better to topdressed nitrogen than normal panicles. More specific data on yield of earlier pistillate (and normal) panicles are needed to confirm the second conclusion. But the bottom line is: pistillate populations yield well, and even more so when nitrogen is added.

Breeding for Resistance to Fungal Brown Spot (with R.F. Nyvall)

We are continuing to refine field inoculation techniques to give a reliable, relatively uniform disease pressure. We inoculated individual leaves of approximately 700 half-sib families representing 3 populations. Although fungal brown spot symptoms were minimized by the unfavorably cool temperatures this summer, Dr. Nyvall was able to consistently isolate both of the pathogens applied (Bipolaris oryzae and B. sorokiniana) from lesions on the inoculated leaves, showing that our inoculation methods were successful. Less successful, however, were the evaluations of the disease symptoms. Individual leaves were collected and frozen for later ratings, but the process is time-consuming. Plots were also rated as a whole using a 1-5 scale, but Fungal Brown Spot symptoms were not severe enough for a reliable evaluation. Next year we plan to inoculate whole rows with a more general application of the pathogen.

In addition to evaluating half-sib families, we are increasing a population selected for resistance two years ago, PBR-C1. Other possible sources of resistance may include Zizania aquatica from two locations: the long-seasoned 'Suwannee River' population, which is being backcrossed to Z. palustris cultivars, and 'Embarrass River' from Wisconsin, on which we observed fewer lesions than adjacent populations, from which We have obtained F_1 hybrids with PBP-C1, Franklin, and "Mudhen Lake', a Z. palustris var. palustris collection from Wisconsin. When F_2 populations of these crosses are available, we will evaluate them for field resistance and analyze DNA samples for molecular markers associated with differences in disease reaction.

Hybridization Activities

Much of our effort in the field this year was devoted to improving crossing and selfing techniques, and in obtaining crossed and selfed seed from a number of populations. This took place in lieu of extensive selection within our populations, since the unusual growing season did not allow full expression of the major traits of interest (i.e. shattering and disease resistance). We have been continuing to refine pollination methodology, including the use of lighter weight bags to minimize bending or breaking of stems and to decrease temperature and humidity inside the bags. Since wild rice pollen is short-lived (1-2 hours), we need to enhance our probability of success using these and other improvements, without sacrificing the reliability of crosses.

Our pollination efforts have yielded 58 full-sib families and 12 S_1 families of K-1 C5 (a shattering resistant population), 21 S_1 families of SS-C5 (selected for sturdy stems), 46 full-sib families from bottlebrush x normal crosses as well as 44 S_1 families from NBB-

C1 (selected for bottlebrush and shattering resistance), 34 full-sib families and 25 S_1 families from MNDJ, 19 MSS(Pi) S_1 families (pistillate and sturdy traits), 45 FSSR-C7 S_1 families (shattering resistance), 24 full-sib families from pistillate x normal crosses in MK-1(Pi)F3 as well as 71 S_1 families, and a number of other families from cross- or self-pollinations. We have also collected over 1000 half-sib families from various sources.

Z. aquatica x Z. palustris backcrosses. Currently we are using selfed BC_2 and BC_3 lines which have carried the nondormancy trait to make further backcrosses. In addition, we have a BC_1F_3 non dormant population which we are increasing for further study and development.

Molecular Mapping Populations. In addition to the crosses mentioned above, we have made a number of other crosses to develop mapping populations. To form mapping populations which will segregate for shattering, we have crossed 'Shovel Lake' and 'Dora Lake', local Z. palustris var. interior populations, with Franklin, Petrowske Bottlebrush, NR, and NACH-B, in addition to the populations mentioned above. Also, we crossed 'Jericho Lake Creek' an Illinois Z. aquatica population, with both Franklin and MSS(Pi); 'Miller Woods', an Indiana Z. palustris var. interior population, with Mudhen Lake and Franklin. To conserve the parent population genes for mapping, we have also selfed Dora Lake, Shovel Lake, Mudhen Lake, Embarrass River, Jericho Lake Creek, and the pistillate populations. Crosses from Shovel Lake, Dora Lake, and White Elk Lake to Franklin from last year's greenhouse did not produce enough F_1 plants to give useful mapping populations. New crosses being made in the greenhouse this year include NorCal-3 x Dora Lake, Suwannee River x NorCal-3, and others. When these populations reach the F_2 (S_1) generation, we will sample DNA from plants and note their phenotypes for RFLP analysis. The S_1 plants will be diallel-crossed to carry the populations forward for future work. Also, tester lines are being developed which carry one or the other major shattering gene. We are currently making crosses and selfs of the S_2 plants to determine which lines are complementary. These may also be used in mapping studies for the shattering trait.

Acknowledgments

We thank Tom Godward, Manomin Development Co. (Art Hedstrom), John Gunvalson and Paul Imle, Clearwater Wild Rice (Rod Skoe and Don Barron), for providing seed for these experiments and for selection. We also thank the Minnesota Cultivated Wild Rice Council and the growers of Minnesota for continued support of this project, and the Plant Breeding Advisory Committee appointed by the Council, for input and advice. Council funding also supported the fertility research with Dr. Bloom and Dr. Grombacher's efforts on the mapping and breeding projects. We also thank Dr. David Rabas, Head of the North Central Experiment Station, for being a strong supporter. We are grateful for the labors of Pete Kampen, Mark Omelia, Ted Goggleye, and Ben Smith. This project is funded primarily by a grant from USDA-CSRS, and by funds at the North Central Experiment Station.

1993 REPORT OF THE MINNESOTA WILD RICE MOLECULAR GENETICS PROJECT

Dr. A.W. Grombacher and Dr. R.L. Phillips[1]

This project began on January 4, 1993 with the arrival of Dr. Grombacher at Dr. R.L. Phillips' molecular genetics laboratory at the University of Minnesota. The 1993 goals of the project were to 1. Increase our understanding of the genetics of wild rice; 2. Adapt the RFLP procedures to wild rice DNA samples; and 3) Initiate the development of mapping populations that would be useful in genetic studies and in aiding the plant breeding program at the North Central Experiment Station in Grand Rapids.

Little is known about the cytogenetics and the molecular genetics of wild rice and its relatives. Immature panicles were preserved for microscopic evaluation of the wild rice chromosomes. The chromosomal preps showed normal pairing during meiosis. Observance of normal pairing is a good sign, because it indicates that distorted ratios due to meiotic disruptions should not occur in the mapping studies. Meiotic observations suggest one or two chromosomes might be associated with the nucleolus. In situ hybridizations will tell how many chromosomes actually possess a nucleolus organizer region, the site of genes required for protein synthesis.

Flow cytometry work done in cooperation with Dr. Kowles at St. Mary's College in Winona, Minnesota has shown wild rice nuclei to be at least 4 to 5 times smaller than maize nuclei. These observations indicate the DNA content of wild rice to be much lower than the DNA maize content. Further analysis will be performed.

Wild rice DNA clones for constructing a RFLP map are now available. A genomic library (designated PAWG = probes of aquatica wild rice genomic) was constructed with DNA from "Franklin", the latest experiment station varietal release. The DNA was digested with the restriction enzyme, *PstI*, and fractionated on a sucrose gradient. The range of sizes of the cloned DNA fragments is 0.5 to 6.0kb with an average size of 3.25kb. 4,365 clones were isolated. Dot blots indicate approximately 70% of the clones are single or low copy sequences, therefore about 3,000 clones will be available for the mapping work. The wild rice PAWG clones have been used on screening blots with polymorphisms being identified in the K2(2)C5, K2(2)C3Pi, and K2(1)C4Pi populations. Screening of the PAWG clone library has begun with the extraction of the first 280 clones.

Maize clones (UMC and BNL) have been used as RFLP probes on wild rice. Polymorphisms have been detected among wild rice samples. We are testing the relationship between the pistillate character of wild rice and the UMC clones near the tassel seed trait of maize lines.

The first ten white rice clones from Tanksley's Laboratory at Cornell have been successfully transformed into E. coli strain (DH10B) for inclusion in the synteny test between wild rice and white rice. Synteny refers to the occurrence of genes in one chromosome, and current information indicates genes in large blocks occur together in different grass species.

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[1]University of Minnesota/North Central Experiment Station

A crossing nursery was set up to develop mapping populations necessary for the RFLP project. The populations include PM3xLaRonge, K2(1)C3Pi, K2(2)C4Pi, K2(2)C5, Franklin, Miller Woods, Embarrass River, and Jericho Lake Creek. B73, Tx303, and Co159 are maize inbred lines being extracted for confirming the identity of the maize clones. A detailed review of the 1993 molecular crosses is listed in the plant breeding report.

Extraction of the DNA samples collected during the summer is ongoing. Protocols for producing better gels and membranes containing the populations' DNA samples are being improved, so that screening blots can be more accurately scored.

The conclusions drawn from this first year of the project are: 1) Polymorphisms have been detected in wild rice populations using maize clones; 2) PAWG clones show polymorphism when hybridized to genomic wild rice DNA; 3) wild rice chromosomes have normal pairing in meiosis with no abnormalities; and 4) wild rice nuclei have been observed to be at least 4 to 5 times smaller than corn nuclei, suggesting that the wild rice DNA content is similar to white rice (O. sativa).

Acknowledgements

We would like to thank Dr. R.A. Porter for sharing his expertise and making field space and project materials available to us. Our appreciation is expressed to Henry Schumer (field plot coordinator), and Bruce MacGregor (junior scientist) for their assistance and advice throughout the season, and to Suzanne Livingston (associate scientist), Jayanti Suresh (associate scientist), Lisa Gulbranson (junior scientist), Dr. Bai-Chi Wu and Dr. Shahryar Kianian (oat RFLP posdocs), Ted Goggleye, Mark O'Melia, John Kenny, and Scott Ommen (undergraduate assistants).

Scab of Cultivated Wild Rice in Minnesota Caused by Fusarium spp.

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ABSTRACT

Nyvall, R. F., Mirocha, C. J., Porter, R. A., and Percich, J. A. 1994. Scab of cultivated wild rice in Minnesota caused by Fusarium spp.

Fusarium spp. were frequently isolated from wild rice (Zizania palustris) seed grown both in wild rice paddies and in lakes. No Fusaria were isolated from wild rice seed that was stored in water immediatedly after harvest but were frequently isolated from wild rice seed dried to 20-21 % moisture then stored either at -20 or 4 C. The most common fungus isolated was F. graminearum. Other Fusaria isolated were F. anthrophilum, F. culmorum, F. moniliforme, F. sporotichioides, and F. subglutinans. Nivalenol and dioxynivalenol were produced by F. graminearum cultures but was not isolated from the seed sample the cultures were isolated from.

Cultivated wild rice (Zizania palustris L.) is grown on approximately 6,882 ha in Minnesota. Diseases have been an important factor in the cultivation of wild rice since 1961 (2). However, most research efforts have concentrated on fungal brown spot which is considered to be the most severe disease of cultivated wild rice in Minnesota.

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 (6). During 1993, 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.

Fusarium spp. have not previously been reported to be isolated from cultivated wild rice seed. A Fusarium sp. had been reported isolated from the roots of cultivated wild rice grown in Minnesota in 1970 (4). However, this information lacked data and was never published. Further investigations were conducted to determine the incidence and identity of seedborne Fusaria in cultivated and lake wild rice, effect of storage conditions, and production of toxins.

MATERIALS AND METHODS

To determine the incidence of scab, samples of seed grown in 1993 were obtained from the following conditions: a) seed dried to 20-21% seed moisture for 2-3 days at 21-24 C then stored at 4 C, b) seed dried to 20-21 % moisture for 2-3 days at 21-24 C then stored at -20 C, c) seed immersed in water after harvest and stored at 4 C, and d) seed processed immediately after harvest. Processed seed was obtained from a grocery store. Processing involves placing the caryopsis at about 94 C for approximately 1.5 hr, the temperature

then rises to 117-120 C for .5 hr when the heating stops. Seed samples were obtained from both cultivated and lake-grown wild rice; however, it was not possible to obtain an equal number of samples from each source.

Isolation of fungi was done from 300 whole seeds, 100 palea and lemma, and 100 caryopsis per sample. Whole seeds were surface treated by washing in running tap water (7 C, nontreated with chlorine) for 2 hr. Seeds were then placed in a 1:1 solution (v/v) of 1% sodium hypochlorite (NaOCl) and 75% ethanol (ETOH) for 3 min, rinsed in sterile disilled water and placed on potato dextrose agar adjusted to pH 4.5 with 50% lactic acid.

Isolation from palea and lemma, and caryopsis was done by washing whole seeds in running tap water for 2 hr then separating the palea and lemma from the caryopsis with forceps and surface treating them similar to whole seeds. The washing of whole seeds for two hours facilitated the easy separation of the palea and lemma from the caryopsis. Because the palea and lemma have less mass than the whole seed or the caryopsis, different treatment times of .5, 1, 3, and 5 min in the NaOCl and ETOH solution were attempted with 3 min being the most satisfactory.

Seeds, palea and lemma, and caryopsis were incubated at 22-26 C under cool, white flourescent lights and beginning at 7 days, exposed for the next 7 days to UV light (360 nm) for 30 min daily to enhance sporulation. Cultures were identified to species after 14 days. Cultures not identified immediately were placed on APDA slants and identified when convenient.

One sample of wild rice seed obtained from a lake that had been stored at -20 C and three F. graminearum Schwabe cultures isolated from this seed source were examined for presence of nivalenol and dioxynivalenol as follows. Samples ground in a mill for 1 minute then placed in flask to which extraction solvent (acetonitril:water, 84:16 v/v) added and shaken for 1 hr. Extract filtered through Whatman # 4 filter paper and added to a charcoal column previously activated with extraction solvent. The eluate was evaporated to dryness and analysized by HPLC (Shimadzu SLC-6A at 229 nm)

Identification of Fusarium to species was done using the key by Nelson et al (5). Identification of fungi other than Fusarium spp. was done using the key by Barnett (1).

RESULTS

Seed sources stored either at -20 or 4 C were considered representative of wild rice seed sources grown in Minnesota in 1993. Fusarium spp. were consistently isolated from all seed sources regardless of their origin (Table 1). Additionally, there was no difference in incidence of Fusaria isolated from wild rice seed dried to 20-21 % moisture content and stored at either -20 or 4 C; however, Fusarium spp. were not isolated from seed stored in water after harvest. Instead Mucor sp., Geotrichum sp., and yeast (Saccharomyces spp.) were consistently isolated from all seed samples stored in water. Although Fusarium spp. were infrequently isolated from processed seed, F. graminearum was still isolated from .6 % of the seed that was sampled even after being subjected to the high temperatures necessary for the parching process.

Fusarium spp. were consistently isolated from all seed structures and were present in the caryopsis regardless of seed source or temperature of storage (Table 2). Generally, if there is a high incidence of Fusaria isolated from whole seed, Fusaria will be isolated from the palea and lemma, and caryopsis also.

Fusarium graminearum was the predominant species isolated from whole seed, palea and lemma, and caryopsis of wild rice seed (Table 3). Other Fusarium spp. that were present were F. anthophilum (A. Braun) Wollenw., F. culmorum (Wm. G. Sm.) Sacc., F. moniliforme J. Sheld., F. sporotrichioides (Sherb.), and F. subglutinans (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun, & Marasas. However, these species were infrequently isolated. Fusarium anthrophilum, F. culmorum, F. sporotrichioides and F.

subglutinans were isolated only from seed gathered from paddies and were not isolated from lake wild rice sources.

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

Because 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 (Fig. 1). Some seeds have a light pink discoloration that is apparently due to mycelial growth of Fusaria. Fusarium spp. were isolated from 100 % of seeds that displayed scab symptoms and from 1-26 % of seeds that did not display symptoms.

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.); however, F. semitectum Berk. & Ravenel has been implicated in a necrosis that partially or totally covers the surface of kernels. A similar necrosis develops from flowering to ripening (7). Fusarium moniliforme has been commonly isolated from white rice seed where it has been implicated in reducing germination (3).

The unusally 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. Disease observation notes (Unpublished) and 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 regardless of where the plants were grown.

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 excercised with wild rice. However, 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 condusive to development of scab.

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Table 1. Percentage of Fusarium spp. isolated from whole wild rice seed obtained either from paddies or lakes and subjected to different storage conditions after harvest or processed

Source	Storage condition	Percentage Fusarium spp.[a]
Lake	-20 C, 20-21 % seed moisture	51
Paddy	-20 C, 20-21 % seed moisture	18
Lake	4 C, 20-21 % seed moisture	81
Lake	4 C, 20-21 % seed moisture	46
Paddy	4 C, 20-21 % seed moisture	62
Paddy	4 C, 20-21 % seed moisture	99
Lake	4 C, immersed in water	0
Paddy	4 C, immersed in water	0
Paddy	4 C, immersed in water	0
Lake	Processed	.6
Paddy	Processed	.6

[a] Isolations were made from 300 seeds per sample.

Table 2. Percentage of Fusarium spp. isolated from whole seed, palea and lemma, and caryopsis of wild rice seed harvested from a paddy and a lake source. Seed was dried to 20-21 % moisture for 2-3 days at 21-24 C then stored either at -20 C or 4 C.[a]

Source (Storage Temperature)	Percentage Fusarium spp. Whole seed	Palea and lemma	Caryopsis
-20 C			
Paddy	16	24	3
Lake	51	28	4
4 C			
Paddy	62	23	34
Paddy	99	96	97
Lake	81	35	54
Lake	46	36	2

[a]
Isolations were made from 300 whole seeds and 100 palea and lemma, and caryopsis.

Table 3. Percentage of Fusarium spp. isolated from whole seed, palea and lemma, and caryopsis of wild rice seed gathered either from paddies or lakes

Species (source)	Whole seed [a] %	palea and lemma %	caryopsis %
Paddy			
anthrophilum	>1	0	0
culmorum	>1	0	0
graminearum	58	48	45
moniliforme	2	>1	>1
sporotrichioides	>1	0	0
subglutinans	1	0	0
Lake			
anthrophilum	0	0	0
culmorum	0	0	0
graminearum	59	33	19
moniliforme	>1	>1	1
sporotrichioides	0	0	0
subglutinans	0	0	0

[a]
Isolations were made from bulked 900 whole seeds, 300 palea and lemma, and 300 caryopsis stored at -20 C and 4 C.

Control of Fungal Brown Spot of Paddy Wild Rice by Propiconazole (Tilt) in Minnesota in 1993

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North Central Experiment Station
Grand Rapids, MN

Purpose: The purpose of the 1993 propiconazole study was to determine the proper timing for application to cultivated wild rice to control fungal brown spot caused by *Bipolaris sorokiniana* and *B. oryzae*.

Materials and Methods: Two field sites were selected at Clearwater Farms by Clearwater, MN and Godward Farm by Aitkin, MN. Four treatments were replicated three times. The treatments were 1) no Tilt; 2) Tilt applied late only (6 oz. applied about 2 weeks after first application); 3) Tilt applied early only (6 oz. applied at boot stage); and 4) Tilt applied twice (6 oz. application at boot stage and about 2 weeks later). All applications were done by air.

Each treatment was 20 x 4 ft. A frame made of 1/2 in. pipe was constructed for each treatment (20 x 4 x 8 ft). Heavy plastic was then placed over each frame just prior to application and removed after application of Tilt.

Yields were done by harvesting an area of 22 in. (corresponding to harvester width) by 20 ft. from each plot with a plot harvester. The entire plant was then threshed in a stationary combine to obtain seed. Seed was then put through a fanning mill twice to remove foreign matter. Seed was dried in an oven for seven days at 45° C. The remainder of the plant was dried for 30 days to obtain dry weight of biomass which was used to calculate harvest index. Recovery of *Bipolaris* spp. was done by surface treating 100 dry seeds for 3 minutes in a 1:1 solution of 75% ETOH and 1% NaOHC1 then placing on sterile filter paper moistened with sterile distilled water.

Disease ratings were taken at harvest. Incidence and severity of fungal brown spot was measured by number and size of lesions on the uppermost leaf of 50 plants/treatment using a modified Clive James Key (Septoria Leaf Blotch). The disease rating is as follows:

Rating	Percent of Diseased Leaf Area
1F	0.5
1	1.0
1+	3.0
5	5.0
5+	10.0
25	25.0
25+	35.0
50	50.0

Results: Results are from the Clearwater Farm site only since the frames at the Godward Farm were destroyed by wind.

Treatment	Yield Finished Lbs/A	Disease Rating	Percent Recovery of Bipolaris from Seed
6 oz. applied early only	624	8.2	5
6 oz. applied late only	751	4.4	5
6 + 6 oz.	735	4.2	2
Control	620	6.5	5

Conclusions:

1. Tilt applied late only was similar in yield and disease ratings to two applications.

2. An early application of Tilt is an unsatisfactory means of disease control yielding the same results as no Tilt.

3. Two applications prevented seedborne infection by *Bipolaris spp.* However, the low incidence and any affect on seed quality is probably negligible.

Discussion: The 1993 growing season was extremely moist and cool. However, fungal brown spot was at a relatively low incidence but bacterial leaf spots were at a high incidence. On the basis of this data, it could be concluded that under weather conditions in 1993 one late application of Tilt is as effective as two Tilt applications. An early application of Tilt only is an ineffective means of disease control.

Status of Fungal Brown Spot of Wild Rice in 1993

Robert Nyvall, Professor; James Percich, Professor; and Jason Brantner, Research Associate.

Fungal brown spot was not as prevalent or severe during 1993 as in past years. Because of the unusually wet and cool weather, bacterial leaf blights were extremely common. Disease incidence was determined by isolating from 100-300 samples at each sampling location.

The following data indicates there was a low incidence of fungal brown spot in the summer of 1993. The most prevalent organism was *Bipolaris sorokiniana*. Disease can occur in isolated fields where wild rice had not been previously grown (Lindquist). In the latter example, the disease may have originated from infected oat seed mixed with rice seed at planting, infected nearby grass, or both.

Location		Spots or seed Bipolaris spp. isolated from (%)	
		B. sorokiniana	*B. oryzae*
Clearwater	(wild rice 7/2)	2	0
	(grass 7/2)	1	0
	(wild rice 9/9)	0	0
Lindquist	(wild rice seed 5/26)	0	0
	(oat seed 5/26)	1	0
	(wild rice 8/16)	2	0
	(wild rice 9/9)	8 (stem), 10 (leaves)	1
Berger	(8/16)	0	0
Brink	(8/16)	0	0
	(9/9)	8	10
	(9/27)	2 (leaves), 1 (stem)	0
Godward	(9/9)	2 (leaves)	2 (stem)
Leonhardt	(9/9)	0	0
Manomin	(9/9)	12 (leaves), 2 (stem)	0

Summary of Research on Diseases of Wild Rice in 1993

Robert F. Nyvall, James A. Percich, Jason R. Brantner, and Raymond A. Porter

1. Trapping of spores to determine origin and survival of inoculum.
2. Screen cultivars for resistance to fungal brown spot utilizing the toxin ophiobolin.
3. Survival and sources of inoculum.
4. Use of Tilt to control fungal brown spot.
5. Determination of a best method of growing inoculum and inoculation procedure inducing disease of wild rice cultivars in test plots.
6. Plant disease nursery.
7. Disease incidence and severity in growers fields.
8. Survival of *Bipolaris oryzae* and *B. sorokiniana* in infested residue.
9. Determination of what happens to fungal spores of causal organisms.
10. Scab caused by *Fusarium* species. A new disease of cultivated wild rice.

Effect of Continuous and Intermittent Wet Periods at Various Temperatures on Infection of Wild Rice by *Bipolaris oryzae.*

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ABSTRACT

Percich, J. A., Nyvall, R. E., Kohls, C. L., and Malvick, M. K. 1994. Effect of continuous and intermittent wet periods a various temperatures on infection of wild rice by *Bipolaris oryzae.*

Infection of wild rice (*Zizania palustris*) flag leaves by *Bipolaris oryzae* was studied at temperatures of 5-35 C and wet periods of 2-36 hr after inoculation. Lesion densities (lesions/ cm^2) increased with increasing wet periods depending on optimum temperature. High rates of infection occurred at 25 and 35 C with continuous wet periods of 16, 18, 20 and 24 hr, respectively. There were no lesions at 5 and few at 10 C. Lesion densities declined when wet periods of 2, 4, or 8 hr were interrupted by dry periods of 4, 6, 8 or 12 hr followed by a final 14 hr of wetness. Lesion densities decreased at all temperatures with increased dry periods regardless of the initial wet period. The interaction of dry period length x wet period length x temperature was significant at the O.5% level.

LITERATURE REVIEW

Fungal brown spot of wild rice caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker is a severe disease that occurs on leaves, stems and flowers of cultivated wild rice (*Zizania palustris* L.) in Minnesota (10). Under favorable environmental conditions for disease, fungal brown spot can cause up to 67% yield loss (11, 12). The effect of environment (sunlight, moisture, and temperature) and plant nutrition on infection by *B. oryzae* has been investigated for brown spot disease of rice (*Oryzae sativa* L.), but results are often contradictory (1, 2, 4, 5, 6, 7, 8, 9, 10. 20). *Bipolaris oryzae* isolates from the Southern U. S. were morphologically and physiologically distinct than those from the Philippines (17).

Conidial germination behavior of *B. oryzae* differs if it isolated from rice or wild rice. In general, conidia of *B. oryzae* from rice, regardless of isolate and/or

source, germinate on a selective medium at 16-40 C with optimal germination and growth occurring at 28 C (17). Successful infection of rice, however, occurs at temperatures of (20-30 C) with optimal infection at 20-25 C with 4 hr or more of continuous moisture (100% RH) (9, 21). Whereas conidial germination of *B. oryzae* isolates from wild rice can germinate at 5-45 C on water agar with optimal infection at 28-30 C (96-100% RH) after 8 hr (16).

Currently, fungicide spray scheduling to control fungal brown spot of cultivated wild rice is based on either a calendar schedule beginning in early July or when forecast weather is thought to be favorable for severe disease to occur (11, 12, 18). A better understanding of the environmental conditions necessary for infection and disease progress in commercial fields in Minnesota is needed or consistent and effective integrated management of fungal brown spot will remain illusive (9, 15).

There have been no studies of the effect of environment on infection of wild rice by *B. oryzae*. The objectives of this study were to determine the effects of a) temperature under conditions of continuous wetness and b), intermittent wet and dry periods at various temperatures on infection of wild rice by *B. oryzae*.

MATERIALS AND METHODS

Dew chambers were constructed of polyvinyl chloride (PVC) pipe covered with clear polyethylene plastic (4 mil) by the method of Krupinsky and Scharen (14). These chambers were assembled inside environmental growth chambers (1.2 x 2.3 x 1.7 m) (Integrated Development & MFG., Environmental Growth Chambers, P.0. Box 407, Chagrin Falls, OH). Pipe (PVC, 5 cm diam) had holes (1.3 mm diam) placed at 10 cm intervals above the plants to evenly distribute mist. A single mister (Herrimidifer Co, Inc., Model 707 SM, Lancaster, Penn) was connected to one end of the pipe. Plastic lined wooden frames, 13 cm in depth were placed in the dew chambers and filled with water.

Wild rice seed (cultivar K-2), was stored for 4 mo at 2 C then germinated in tap water at 24 C. Seedlings were placed individually in plastic pots (15 cm diam) previously filled within 2 cm of the top with a soil mix (7 parts field soil: 3 sand: 2 peat: 1 manure), pH 6.9 amended with 3.5 g of a 10-10-10 fertilizer. The remaining 2 cm of each pot was filled with washed silica sand to control growth of algae. Pots were then placed in wooden frames (91 x 71 cm) lined with four layers of 4 mil black polypropylene plastic. The frames were filled with tap water to a depth of 13 cm that was maintained throughout the experiment. Supplemental lighting was a mixture of 60 W incandescent and 160 W cool white and Gro-lux fluorescent bulbs (Sylvania , Danvers, AMA) (ratio 5:5:3) for 16.5 hr at 300 μM m^{-2} s^{-1}, measured at midfoliage with a LiCor Quantum Radiometer Photometer (Model L1-185, Lincoln, NE 68504). An additional granular 2.5 g of urea (46-0-0) fertilizer was placed into the water of each flat during the early boot stage of plant development.

The middle portion of uppermost fully expanded leaf (8-10 cm in length) from plants in the boot stage of development was delineated as the inoculation areas with a permanent felt tip marker. Each inoculation area was uniformly inoculated by hand with a conidial suspension until lightly wet as described by Browder (3). The isolate of *B. oryzae* (BO 8305) used in this study was originally isolated from a plant in a commercial Minnesota field in 1983. Conidia were produced on PDA (Difco Corp., Detroit, MI) in 100 x 15 mm plastic petri dishes for 4 wk at 24 C in the dark. Conidia were removed from cultures by suspending them in Soltrol 120 oil (Phillips Chem. Co., Borger, TX 79007), and their concentration adjusted to approximately 1.0×10^5 conidia/ml.

Continuous and intermittent wet period treatments were incubated at 5, 10, 15, 20, 25, 30 or 35 C. Prior to placement in the dew chambers, plants were misted with a mixture of 1 ml Tween 40 (Polyoxyethylene titan monopalmitate, Sigma Chem. Co.) per 3.8 L of deionized water. In the Continuous wet period, plants were wet for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, and 36 hr after inoculation. In the intermittent wet period, plants were initially kept wet for 2, 4, or 6 hr followed by dry periods (85% RH) when plants were removed from the dew chamber for 4, 6, 8, 10, 12, or 14 hr. After the dry periods, plants were placed in the dew chamber for a final 14 hr wet period. After each wet period, plants were returned to the greenhouse for lesion development. Each treatment consisted of 11 plants and was replicated three times.

Seven days after initiation of the wet periods, the inoculated areas were excised from the plants, placed in breeding head bags, pressed and dried at 24 C to determine lesions (lesions / cm^2) later. Pressed samples were rehydrated for 30 min in 250 ml water mixed with 0.3 ml Tween 40 and examined with a Quebec colony counter (model 3327, American Optical, Scientific Instrument Division, Buffalo, NY 14215). Lesions averaged 0.5 to 1.5 mm dia after 20 and 36 hr incubation, respectively at temperatures above 15 C. Leaf area measurements were determined with a portable area meter and transparent belt conveyer accessory (Model L1-3000 and L1-3050A Li-cor Inc., Lincoln, NE).

Data was analyzed using the IVAN statistical package, an interactive computer program for factorial design analysis (21), and the University of Minnesota's Cyber 74 computer.

RESULTS

Disease occurred at temperatures of 10-35 C and continuous wetness periods of 10-36 hr with maximum numbers of lesions/cm^2 occurring at 25 C and 24 hr of wetness (Table 1). No disease occurred at 5 C and little at 35 C. Continuous moistures of 2-8 hr, regardless of temperature did not result in disease. However, lesion density increased at continuous wet periods up to 24 hr but decreased at wet

periods of 28-36 hr. The analysis of variance (ANOVA) for the continuous wet period study (Table 2) demonstrated factors, such as length of the wet period, temperature at incubation and the interaction of wet period and temperature of infection had highly significant (0.005 level) effects on lesion density in a linear fashion.

Lesion density in the Intermittent wet period study generally increased with decreasing dry periods and increasing temperature of 15-25 C (Table 3). Lesion density declined at 30 and 35 C. No lesions developed at 5 or 10 C, regardless of the length of the wet and dry periods. The ANOVA for the intermittent wet period study indicated temperature was highly significant (0.005 level) related to lesion density (Table 4). Lengths of the initial wet and dry periods, at a given temperature, were negatively related to the lesion density in a linear manner. Thus, increasing the length of the wet and dry period from 4 and 12 hr, respectively, caused a decrease in the number of lesions per cm^2 at incubation temperatures of 15-25 C.

The lack of significance of the wet period x dry period x temperature interaction in the ANOVA indicates the temperature of incubation during the short initial wet period did not significantly influence the effect of wet period on lesion density. The interaction of dry period length and temperature of incubation was significant (P=0.005). If all three factors, wet period (W), dry period (D), and temperature of incubation (T), are taken into consideration together in a W x D x T their interaction is significant (P=0.005).

DISCUSSION

Significant disease development by *B. oryzae*, indicated by lesion numbers in the continuous wet period treatments occurs in a narrower temperature range than germination. *Bipolaris oryzae* germinated 80-90% in 2 hr at temperatures of 15-35 C, however, significant levels of infection require at least 12 hr of continuous wetness. Germination of *B. oryzae* on wild rice at 5 or 10 C is very slow with little infection at these temperatures even after continuous wet periods of 24 hr or more. At 10 C at least 18 hr of continuous wetness was required for even a very low level of successful infection. *Bipolaris oryzae* conidia are multicellular and initially germinate bipolarly (5) to create more than one point of infection (16). However, the importance of the middle conidial cells to infection during intermittent wet periods has not been investigated. Additionally, *B. oryzae* isolates differ in the speed at which conidial germination and subsequent infection takes place (17). Consequently, these two factors may be important in a paddy environment where long nightly wet periods are common (9, 11).

The intermittent wet period results for *B. oryzae* on wild rice are similar to those of *Coccomyces hiemalis* Higgins on sour cherry (6). Lesion density from the intermittent wet period study was lower that equivalent continuous wet period length in both cases. Both pathogens had fewer successful infections with increasing

lengths of dryness after initial wet periods. Lesion formation by *B. oryzae* on wild rice cultivar K-2 occurs by 24 hr after inoculation. Hyphae emerged through the cuticle and stomata by 48 hr (96 - 100% RH, 28 - 30 C) and conidiophore initials and mats of hyphae on the cuticle surface occurred at 48-72 hr (96 - 100% RH) (16). Isolates of *B. oryzae* from rice, *Oryzae sativa* L, produced conidiophores 5 - 14 hr after inoculation at 100% RH (20). Because periods of high relative humidity occur frequently in wild rice stands it is likely the latent period of *B. oryzae* during July and August in Minnesota may frequently be only 6 - 8 days (11). Also, because wild rice is an aquatic plant the microenvironment in the paddy is different from that of dryland cereal. Dew periods in Minnesota may occur nightly and are often 10 hr or more in the middle of the plant canopy (12). Cultivated wild rice tillers profusely and at maturity may be greater than 2 m in height with leaves 1 m long to produce an understory of densely packed leaves and stems, that is characterized by long dew periods and poor penetration by wind or aerially applied fungicide. The upper canopy is less dense and has shorter dew periods, good air circulation and excellent fungicide penetration (11). Thus, because of differences in wet periods and fungicide deposition, the flag leaf may have only a few small lesions, while leaves in the understory may have a very high disease severity rating (12, 18).

Experiments are underway to study the survival, primary inoculum dispersal, early infection, and subsequent spread of *B. oryzae* in commercial wild rice fields in Minnesota.

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Table 1. Mean number of lesions/cm^2 on the flag leaf of *Zizania palustris* infected with *Bipolaris oryzae* at various temperatures and periods of continuous wetness.

Continuous wet periods (hr)	Incubation temperature (C)					
	10	15	20	25	30	35
10	0.0	0.0	0.5[z]	1.0	2.0	0.1
12	0.0	0.0	2.1	8.0	4.5	0.1
14	0.2	2.0	3.6	9.6	6.8	0.6
16	0.2	2.4	4.3	12.6	9.4	0.4
18	0.3	2.5	4.8	16.2	11.4	0.2
20	0.5	2.6	3.8	22.6	15.8	0.3
24	1.2	2.5	3.0	22.7	18.6	0.4
28	0.4	3.2	3.1	20.0	14.0	0.1
36	0.3	2.5	3.5	14.6	11.0	0.2

[z]Mean value of 11 plants in each of three replications.

Table 2. The analysis of variance for lesion density data from the continuous wet period infection study of *Bipolaris oryzae* on wild rice.

Source	df	Mean Square	F^z
Wet period	13	136.25	22.67***
linear	1	1,374.79	212.64
remainder	12	422.61	
Temperature	6	435.73	68.66***
Wet x temperature	78	42.18	5.23***

[z]Two asterisks indicate that the F value was significant at $P<0.005$.

Table 3. Mean number of lesions/cm² on the flag leaves of *Zizania palustris* infected with *Bipolaris oryzae* at various temperature and intermittent wet and dry periods. The dry periods were followed by 14 hr of continuous wetness.

Wet hr	Dry hr	Incubation temperature (C)				
		15	20	25	30	35
2	4	0.8[z]	0.5	11.5	7.3	0.1
	6	5.1	0.7	4.2	5.7	2.6
	8	1.4	0.2	11.2	1.9	0.8
	10	1.6	1.1	3.2	0.6	0.5
	12	0.2	0.4	1.3	0.1	0.5
4	4	0.3	0.1	10.0	5.5	2.2
	6	1.0	1.7	8.8	0.2	1.9
	8	0.2	1.1	4.5	1.1	0.7
	10	0.3	0.6	1.0	0.0	0.0
	12	0.4	0.1	0.3	0.0	0.0
6	4	1.0	0.0	7.8	1.3	0.2
	6	1.0	0.4	9.4	2.7	0.4
	8	0.5	0.3	7.2	2.0	0.1
	10	1.5	1.3	3.4	0.7	0.0
	12	0.0	0.6	0.2	1.2	0.1

[z]Mean of eleven flag leaves in each of three replications.

Table 4. Analysis of variance for lesion density data from the intermittent wet period infection study of *Bipolaris oryzae* on wild rice.

Source	df	Mean Square	F^z value
Wet period	2	8.06	4.38
linear	2	12.66	6.88**
remainder	1	3.45	
Dry period	4	33.19	18.03
linear	1	128.56	69.85***
remainder	3	4.20	
Temperature	6	115.08	62.53***
Wet x dry	8	2.14	1.16
linear x linear	1	8.26	4.49**
remainder	7	8.86	
Wet x temperature	12	2.51	1.37
Dry x temperature	24	14.37	7.80***
Wet x dry x temperature	48	3.37	1.83
Error	105	1.84	

[z]Two asterisks indicate that the *F* value was significant at $P<0.05$; Three asterisks at $P<0.005$.

DATE: January, 1994

TITLE: Waterfowl, nongame birds, and invertebrates associated with cultivated wild rice paddies in northwest Minnesota.

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Red Lake Watershed District
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U.S. Fish and Wildlife Service (Nongame Bird Conservation and North American Waterfowl Plan Programs)
Minnesota Department of Natural Resources
Wallace Dayton
Minnesota Waterfowl Association
Clearwater County Soil and Water Conservation District

THIS IS A SUMMARY REPORT OF THE 1993 FIELD SEASON. RESULTS CONTAINED HEREIN ARE PRELIMINARY AND SUBJECT TO REFINEMENT AS MORE DATA ARE COLLECTED. RESULTS SHOULD NOT BE CITED WITHOUT PERMISSION OF THE AUTHORS.

OVERVIEW

In 1992, about 17,000 acres of wild rice were cultivated in Minnesota paddies. A number of waterfowl use these paddies, beginning with spring migrants in April, followed by breeding birds in late spring and summer, then fall use by migrants after paddies are again flooded in September and October. Wildlife-related studies of cultivated rice paddies in Minnesota have been limited to 2 which examined spring breeding waterfowl use. However, a number of studies have been carried out on water quality and agronomic production practices, mostly conducted by Bemidji State University, Minnesota Pollution Control Agency, and the University of Minnesota through the North Central Experiment Station at Grand Rapids. This study was designed to address primarily wildlife-related issues of cultivated wild rice production but that necessitates evaluating other aspects of the paddy environment such as water quality, aquatic invertebrates, vegetation besides wild rice, and land use adjacent to wild rice paddies. Discussions were initiated in the summer of 1992 with a variety of individuals and groups having an interest in wild rice/wildlife issues. Major issues were identified and refined into those which needed more information for resource management decision making and those which were researchable, given available resources of personnel, equipment, study area, and funding. A project was approved through the Minnesota Agricultural Experiment Station, and organized through the Northwest Experiment Station at Crookston where the principal investigator has a joint appointment. The overall project has the following objectives:

1. To evaluate waterfowl production and use of cultivated wild rice paddies in northwest Minnesota.

2. To inventory invertebrate populations in cultivated wild rice paddies and evaluate effects of associated agricultural practices.

3. To inventory nongame bird use of cultivated wild rice paddies and assess the value of paddies as habitat for black terns, American bitterns and Wilson's phalaropes.

The intensive study area is located about 12 miles northeast of Oklee in Polk, Pennington, and Clearwater Counties. The terrain is quite flat, occurring in the lake plain of Glacial Lake Agassiz, with organic soils derived from reed-sedge peat. The study centers on a block of land containing about 1600 acres of wild rice distributed in about 40 paddies, owned by the Gunvalson brothers, Paul Imle, and Gully Farms. The study area is along the Clearwater River which serves as the water source for rice production. A variety of idle brushland, pastureland, small grain fields, woodlands, and Conservation Reserve Program (CRP) land is distributed throughout the study area. The original vegetation of the area was poorly-drained lowlands dominated by shallow marshes and grass-sedge communities. Better-drained, low ridges in the area were dominated by bur

oak savannas, and scattered occurences of oaks with intermixed aspen are currently present. The study area is representative of the prairie and prairie-forest transition area where about half of the cultivated wild rice is produced in Minnesota. The study area is also positioned in a major waterfowl migration corridor of the prairie pothole and lake country of western Minnesota, about 40 miles south of the Agassiz National Wildlife Refuge and Thief Lake Wildlife Management Area. It is adjacent to an extensive area of natural wetlands on the Red Lake Indian Reservation.

WATER QUALITY

Water sampling was commenced on 17 May and continued twice-monthly until 25 August, when water levels had been drawn down from paddies. Samples were taken at 5 sites along the Clearwater River; upstream from, within, and downstream from, the cultivated wild rice growing area. Water samples were also taken from the 13 paddies selected for the invertebrate study. The following water quality parameters were measured: temperature, conductivity, pH, dissolved oxygen, alkalinity, turbidity, chemical oxygen demand (COD), nitrate, ammonia, total Kjeldahl nitrogen (TKN), phosphorus (reactive, organic, total), total suspended solids, volatile solids, color, and fecal coliform counts. Samples were analyzed at the Soil and Water Laboratory at the University of Minnesota, Crookston (certified by the Minnesota Department of Health). Four of the 5 river sampling sites had been similarly sampled for a full year by the Red Lake Watershed District previous to this study and those data will be combined with our data for a 15-month period.

INVERTEBRATE STUDY OF CULTIVATED WILD RICE PADDIES IN NORTHWEST MINNESOTA

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This is a brief summary, including preliminary results, of the invertebrate sampling program conducted during the summer of 1993, by the Minnesota Cooperative Fish and Wildlife Research Unit, University of Minnesota.

Background Information

Commercial wild rice production involves several natural resource issues. Rice paddies are known to attract large numbers of migrant and breeding waterfowl, as well as other birds. Paddies provide a valuable source of food for waterfowl in the form of wild rice seeds, and aquatic invertebrates which also occupy the paddies. These aquatic invertebrates are critically important in the diets of waterfowl and other migratory birds. They also can indicate overall water quality. During the rice growing season, water which occupies rice paddies may be contaminated by agricultural pesticides through drift during spray operations and direct over spray. Pesticides can be lethal to the aquatic invertebrates that ducklings, breeding ducks, and other birds rely on as a food source (Swanson et al., 1979, Swanson and Meyer, 1979). The subsequent return of water from paddies to rivers, after possible exposure to pesticides, raises questions concerning water quality. Invertebrate communities present in wetlands/paddies reflect their ability to support waterfowl and other wildlife and indicate overall wetland/paddy health.

The invertebrate sampling during the 1993 field season was designed to characterize the invertebrate communities of commercially harvested wild rice paddies. The data should indicate differences between invertebrate communities occupying the ditches surrounding the paddies and those occupying the central portion of the paddies.

Materials and Methods

A large part of wetland research focuses on invertebrates because of their sensitivity to chemicals and their importance as a waterfowl food source. Accurate characterization of aquatic invertebrate communities is very dependent on the sampling techniques and devices used. Several sampling devices are currently used for aquatic invertebrate sampling. Each of these devices is designed for sampling specific types of invertebrates and are used in different types of aquatic habitats (Merritt and Cummins 1978, Kaminski and Murkin 1981, Swanson 1978, 1983). Since no single type of device is capable of accurately sampling each of the diverse species of invertebrates present, we feel that the most successful studies incorporate more than one sampling device and method.

For the purpose of this project, two types of sampling devices were used; activity traps (ATs), and emergent traps (ETs). Activity traps are suspended in the water column where they capture nektonic, planktonic, and epibenthic invertebrates. These traps also sample nymphs of emergent invertebrates in the water column. Emergent traps capture emerging adult invertebrates at the water surface.

Using maps provided by Gunvalson/Imle farms, 13 paddies were randomly selected for invertebrate sampling. Using random numbers, random entry points were determined for each paddy that was sampled. Transects were placed on each of these random entry points with two ATs and two ETs placed on each transect. Half of the traps were placed in the ditches, while half were placed in the main body of the paddy. For the purpose of our sampling it was assumed that the main body of each paddy was homogeneous in structure. This assumption also applies to the ditches surrounding each paddy. Sampling began on 6 May and continued until 28 July. Activity traps were set for a 24-hour period and collected bi-weekly, while ETs were set for a 72-hour period and collected once per week.

The invertebrates were preserved for later identification. Most invertebrates will be identified to the taxonomic level of Family. Final tabulation of the invertebrate data will be completed by March of 1994. Table 1 shows a list of taxa which have been identified to this point.

Accomplishments

Preliminary examination of samples indicates the most abundant invertebrates to be Notostraca (tadpole shrimp), Ostracoda (seed shrimp), Conchostraca (clam shrimp), and Dytiscids (water beetles).

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Table 1. List of invertebrate taxa identified in wild rice paddies through 23 December 1993 for the 1993 field season.

Water beetles	Water bugs	Dragonflies	Damselflies
Dytiscidae	Corixidae	Libellulidae	Lestidae
Gyrinidae	Belostomatidae		
Hydrophilidae	Notonectidae		
Haliplidae			
Mayflies	**Mosquitoes**	**Midge/flies**	**Crustaceans**
Baetidae	Culicidae	Chironomidae	Talitridae
Siphlonuridae		Ceratopogonidae	Notostraca
			Conchostraca
			Cladocera
			Ostracoda
			Copepoda
Snails	**Minnows**	**Leeches**	**Spiders**
Lymnaeidae	Gasterosteidae	Hirudinea	Hydracarina
Physidae	Umbridae		
Planorbidae	Cyprinidae		

CULTIVATED WILD RICE PADDIES AND THEIR RELATIONSHIP TO WATERFOWL IN NORTHWESTERN MINNESOTA - 1993 SUMMARY

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Introduction

The value of wild rice seeds as a preferred duck food has been frequently cited. Rogosin (1951) classified wild rice as one of the important duck foods in the United States and Canada, and Martin and Uhler (1939) called it one of the best known of North American duck foods. Cultivated wild rice is produced in diked paddies that are flooded in the spring and drained in late summer. Water levels are maintained at about 12 inches throughout most of the growing season (late May - late July). Paddies are attractive to waterfowl due to abundant invertebrates, sago pondweed (another well known duck food), and other aquatic plants. Sorenson (1973) and Johnson (1976) found that large numbers of migrant waterfowl species, as well as many breeding duck pairs, used Minnesota's cultivated rice paddies during spring. Further analysis however, is necessary to evaluate the full extent of the benefits that migratory and breeding waterfowl are deriving from this agricultural practice. Data on waterfowl production, brood survival, and habitat use of paddies and associated uplands are critical to gaining a better understanding of wild rice paddy - waterfowl relationships.

Our intensive study area is located in northeast Polk, northwest Clearwater and southeast Pennington counties, about 12 miles northeast of Oklee, Minnesota. It is positioned along a major waterfowl migration corridor of the Prairie Pothole and Lake Country of western Minnesota. Agassiz National Wildlife Refuge and Thief Lake Wildlife Management Area are located about 30 and 40 miles to the northwest, respectively. The study centers on a 10 section block of land containing about 1600 acres of wild rice, distributed in some 40 paddies. The land is owned and operated by the Gunvalson brothers (John, Jim and Ken), and Paul Imle. A variety of idle brushland, pastureland, small grain fields, woodlands, and CRP land is distributed throughout the study area. This area is representative of the prairie - forest transition area, where about half of the commercial wild rice is produced in Minnesota.

Methods

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

Species composition and density (number of birds per flooded paddy acre) were recorded along a pre-selected, 5 mile census route. This route included 17 paddies of various size and shape, representing about 780 acres of paddies. The route was run weekly, with counts alternating between mornings (0700-1000) and evenings (1700-

2000) when waterfowl were most active. Data were recorded using a combination of the single point observation method and circuit count (Hammond 1970). Data collection began on 3 April, when open water began to appear on some of the paddies, and continued through 17 August, the onset of wild rice harvest. Census routes were resumed on 23 September, once fall flooding of some of the paddies along the original census route had begun, and continued through October.

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

Breeding population census

To census the breeding population of ducks associated with wild rice paddies, a method similar to that used by the Wetlands Wildlife Research Unit, Minnesota Department of Natural Resources, Bemidji (Todd Eberhardt, *pers. commun.*) and the Agassiz National Wildlife Refuge (Gary Huschle, *pers. commun.*) was used. Five census routes were chosen throughout the study area where vehicle access on dikes or roads was good. All ducks were counted within a 200-meter strip out from the vehicle in flooded paddies from 0610 to 0851 on 23 May. Only the length of flooded paddy edge was used in calculating the total area of the census.

Nest searching

Intensive nest searching began on 14 May, after some of the upland-nesting hens began incubation. Nest searches were conducted in one of two ways. Open, upland cover (i.e., C.R.P. land) was searched by dragging a 50-foot length of cable between 2 all-terrain vehicles (Klett et al. 1986). Potential nesting cover that was inaccessible to cable-dragging was systematically searched by researchers on foot, using willow sticks to flush nesting hens. Islands, paddy dikes, and brushy areas were searched in this manner.

Nests were marked 10 feet to the north with a 6-foot willow stake, and flagged with surveyor's tape. The following data were recorded for nests: date found, location on base map, visual obstruction reading (VOR) using a Robel pole (Robel et al. 1970a), 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, a return visit was made at an estimated incubation stage of 20 days. At this time, the hen was flushed, nest data updated, and a walk-in nest trap (Dietz, et al.) placed over the nest. The trap was visited about 3 hours later,

allowing the hen time to find her way through the funnel-like opening of the trap and resume incubation. The trap was approached rapidly from the direction the opening faced, and the hen was usually captured.

Once captured, the hen and the nest trap were immediately removed from the nest site to reduce the amount of disturbance to surrounding cover. Processing was done 50 to 75 yards from the nest site. Hens were first leg-banded, then radio transmitters (source: Advanced Telemetry Systems, Esko, MN) were attached at shoulder level along the midline of the back following procedures similar to those used by Mauser and Jarvis (1991) and refined by Gary Krapu, Northern Prairie Wildlife Research Center, USFWS, Jamestown, ND. A stainless steel anchor was inserted just below the skin and 3 sutures used to secure the transmitter. Average processing time was around 20 minutes per hen. After processing, hens were mildly anesthesized using methoxyflurane to reduce the risk of abandonment after being returned to the nest. Researchers quickly left the nest site without flushing the hen from the immediate area.

Processing newly-hatched ducklings

Mallard nests were visited near their predicted hatch date (24-25 days incubation). If the nesting hen had been radioed, this visit occurred while she was absent from the nest. During this visit, a more accurate hatch date could be estimated. On the predicted hatch day , the hen was flushed from the nest and the newly-hatched ducklings were processed. The entire brood, or those completely out of the eggs, were placed in a container, covered with a towel, and removed from the nest. All recovered ducklings were web-tagged, and 2 to 5 randomly chosen ducklings were equipped with transmitters, using procedures similar to those described for hens. After processing, ducklings were returned to the nest bowl and covered with a towel until their activity level had dropped to a point where there was minimal risk of ducklings leaving the nest bowl before return of the hen.

Monitoring movement and survival of radio-marked hens and broods

Once radioed, adult hens were located daily to monitor both movement and nest status. Locations were determined by triangulation from 2 or 3 points identifiable on base maps. Broods were tracked from nests to their first wetland and then located daily. 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 hens and broods were monitored throughout the summer until fledging, mortality, or loss of radio signals. Visual sightings were obtained whenever possible to verify the survival of non-radioed ducklings. Data collected on radioed hens and broods were used to determine movements, habitat use, and survival of broods.

Preliminary Results

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

Census route data collection began on 3 April, when open pockets of water first began to appear on paddies flooded the previous fall. Although little open water was available at that time, what was available held large numbers of waterfowl; particularly mallards, pintails, and Canada geese. Species richness, as well as total numbers of birds counted, began to increase on 10 April, when most of the fall-flooded paddies contained some open water and spring pumping had begun to fill other paddies (Fig. 1). Tundra swans and northern shovelers made up the bulk of new arrivals during the second week in April.

Peak total numbers and number of species occurred around late-April with the arrival of blue-winged teal, American wigeons, ring-necked ducks, lesser scaup, canvasbacks, and American coots. The spring waterfowl density peaked at 9.1 birds per flooded paddy acre on 18 April and the highest number of species occurred on 2 May, with 16 species. By mid-May, most of the more northern breeders (pintails, tundra swans, and most of the divers) had departed, and waterfowl density began to decrease, stabilizing at about 10% of peak spring density. Species recorded throughout the nesting and brood-rearing periods were primarily mallards, blue-winged teal, northern shovelers, wood ducks, American coots, and Canada geese. Also persisting into early summer, were smaller numbers of gadwalls, American wigeons, green-winged teal, redheads, and ring-necked ducks.

By mid-June, emergent vegetation greatly restricted visibility on the paddies. Entire paddies could no longer be viewed, and birds counted were those present in paddy ditches (perimeter and interior), or in areas of sparse vegetation near the census route. As the growing season progressed, it became increasingly difficult to view birds in paddies, and counts from mid-June through harvest were very conservative. A noticeable increase in density occurred toward the end of July, due to large numbers of broods observed in paddy ditches as paddies were being drawn down in preparation for harvest.

To estimate fall migrant use, the census was resumed on 23 September, the onset of fall-flooding. Large concentrations of waterfowl began using paddies as soon as flooding began, and peak fall densities exceeded the highest spring densities recorded (Fig. 1). On the last 2 censuses (21 and 28 October), I recorded densities in excess of 20 birds per flooded paddy acre.

In an attempt to quantify the amount of post-harvest residual rice seeds and other foods remaining in paddies following harvest, and that is available to waterfowl, core samples were collected from several representative paddies, and are currently being analyzed.

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

Breeding population

The breeding population of ducks was estimated from a total of 12.2 miles (19.6 km) of 200-meter strips along paddy edges. A breeding population density of 674.9 ducks per square mile of flooded paddy was estimated with mallards the predominant species (53.3%), followed by blue-winged teal (24.8%), northern shovelers (10.0%) and lower numbers of several other species (Table 1). Some species recorded on the breeding population census were not documented to breed by the observation of nests or broods during the course of later field work.

Nesting

During the 1993 field season, 136 upland waterfowl nests were located, with 77 found while cable-dragging 634 acres of upland habitat, yielding an efficiency of 1 nest located for every 8.26 acres searched. The remainder of the nests were located incidental to other field work or by searching potential nesting cover on foot. The peak of nest initiation for upland nesting species (mallards, blue-winged teal, northern shovelers, and gadwalls) occurred during the second week of May (Fig. 2). About 26% of all upland nesting duck nests were initiated in this period, so the peak of hatching occurred during mid-June with ducklings fledging during late July or early August. Mallard peak nest initiation was during the second week of May (9 of 46 initiated). It remained relatively constant through the second week of June then tapered off with the last observed nest initiated the first week in July. Blue-winged teal nest initiation also peaked during the second week in May, declined until the second week in June, then showed a slight increase, probably due to renesting. Most of the northern shoveler nests were initiated during May (weeks 2, 3, and 4), with only 2 of 14 nests initiated in June. Only 4 gadwall nests were located and were initiated evenly between the last 2 weeks in May and the first 2 weeks in June.

On 29, 30 and 31 May, the emergent vegetation (mainly cattail) along perimeter and interior ditches of about 700 acres of paddies was nest searched via canoe. Overwater nests of 58 American coots, 1 pied-billed grebe and 1 canvasback were located. Due to time constraints of other field work, nesting success was not determined except for the canvasback nest.

Nest success was determined for all waterfowl found nesting on the study area in 1993 (Table 2). Apparent nest success was 34.4%, with 44 of the 136 nests surviving to hatch. Considering upland nesting species only (blue-winged teal, mallard, northern shoveler, and gadwall), apparent nest success was 30.5%, and Mayfield(.40) nest success was 22.7%. Shovelers were most successful in nesting, showing apparent and Mayfield success of 71.4 and 37.1%, respectively. Mallards showed the lowest success,

with apparent nest success of 15.2% and Mayfield success of 9.5%.

Nest site selection by individual hens seemed to influence success or failure of a given nest. Those hens which nested on islands or in large blocks of cover showed an apparent nest success of 41.5%, and those which nested on roadsides, paddy dikes, or other strip covers, showed only 7.1% apparent nest success. Using a 2X2 test of independence, I found that nest success was significantly dependent on nest site selection by upland-nesting hens (Chi-square = 11.406, $p < .001$). Using backward-stepwise linear regression techniques, with 10 predictor variables for the criterion variable (hatch/no hatch), I found that several investigator-influenced variables, in addition to nest site selection by hens, made up a significant portion of predicting whether or not a nest would hatch. 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 whether a nest would be successful ($t > 2.0$, 120df, $p < .05$).

Predation was the major cause of nest failure, accounting for around 81% of unsuccessful nests. Abandonment, agricultural practices, and investigator disturbance accounted for the remaining losses (Table 3).

Capture, processing, and radio tracking mallard hens and broods

During the nesting season, 11 mallard hens were captured on nests and tracked for a total of 300 radio-days. Five of the radioed hens were successful in their nesting attempts and 3 of these also had their broods captured and radioed at the nest (Table 4). A total of 22 mallard ducklings were equipped with radio transmitters, with 15 captured on the nest at hatching, representing 4 separate broods. Three of the radioed broods belonged to hens which had been previously radioed and the remaining brood was radioed at the nest of an unradioed hen. Ducklings captured and processed at the nest site were tracked for a total of 307 radio-days.

All 4 broods were located on a wild rice paddy within 24 hours of being radioed. Distance traveled from the nest to their first wetland varied from several feet (island nesters) to over a mile. No radioed ducklings were lost during over-land travel. Three of the 15 ducklings radioed at the nest were known to fledge and 2 additional ones were presumed to have fledged. These 2 ducklings were still alive at the end of monitoring (25 August) and were within 2 weeks of fledging. Between 20 and 30% of the ducklings radioed at the nest site and raised on cultivated wild rice paddies survived to fledging (Table 5). Seven 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 radio days, but none survived to fledge.

On 24 August, an aerial search for "lost" birds was conducted of the intensive study area plus a 4-mile fringe area. Few wetlands other than rivers were present outside of this

area except the Red Lake Indian Reservation to the northeast, where the southeast corner to the edge of the Lower Red Lake was searched. One radio-tagged hen was trapped on 21 September by staff at the Agassiz NWR (Parker site). This represented a straight-line movement distance of about 35 miles.

A total of 180 incidental brood sightings were recorded on paddies, representing mallard, blue-winged teal, northern shoveler, wood duck, American coot, and Canada goose. On 4 August, over 50 duck broods were observed in paddy ditches after water levels had been drawn down from paddy interiors. Duckling ages ranged from Class IA to Class III.

Summary and Plans for the 1994 Field Season

Substantial progress was made in the 1993 field season in becoming familiar with the study area, refining logistic aspects of field procedures, and collecting useful data on waterfowl use and production in association with cultivated wild rice paddies. Wild rice paddies were found to serve as attractive stop-over habitat for migratory waterfowl and, along with their associated upland habitats, contributed to Minnesota's waterfowl production by providing adequate nesting cover and brood-rearing habitat.

In analyzing the first season's data, I've been able to identify several aspects of the study which deserve further attention in order to more accurately assess the value of cultivated wild rice paddies as waterfowl habitat.

The recent land use history of the paddies included in our census route will be better known. Some paddies seemed to be more attractive to waterfowl than others and this may be due to different farming practices (i.e., fall vs. spring-flooding and moldboard vs. chisel-plowing). Detailed information on paddy characteristics may help to explain differential use of paddies and allow comparisons between paddies and between groups of paddies in terms of waterfowl usage.

A substantial number of waterfowl nests were located and monitored during the first field season. Now that I'm familiar with available nesting cover and the apparent preferences of nesting species, I expect to increase the efficiency of locating nests in the upcoming field season.

With a better understanding of nesting chronology, the development of an efficient nest trap, and a field season's worth of experience in egg candling and radio attachment, I will strive to minimize the potential human influences being introduced into our work. A new trapping technique being developed may allow the capture of hens and their entire brood after they have reached the rice paddies. This could provide a larger sample of radioed ducklings.

Detailed maps will be developed to allow better assessment of daily movements, home ranges, and "preferred areas" of brood hens with broods on paddies. Further consideration will also be given to the relationship between the complex of paddies that we are working on and Agassiz National Wildlife Refuge. The radioed hen captured at Agassiz during their fall banding work, suggests that birds breeding in our study area may be using Agassiz as a molting area or as an alternate refuge during wild rice harvest.

When considering the potential benefits that waterfowl derive from cultivated wild rice, one must consider the extent to which it fulfills essential habitat requirements. Although the cultivated wild rice paddies of northwestern Minnesota are not designed specifically for waterfowl, my initial findings suggest that they may play an important role in fulfilling some of the needs of the area's breeding and migratory waterfowl.

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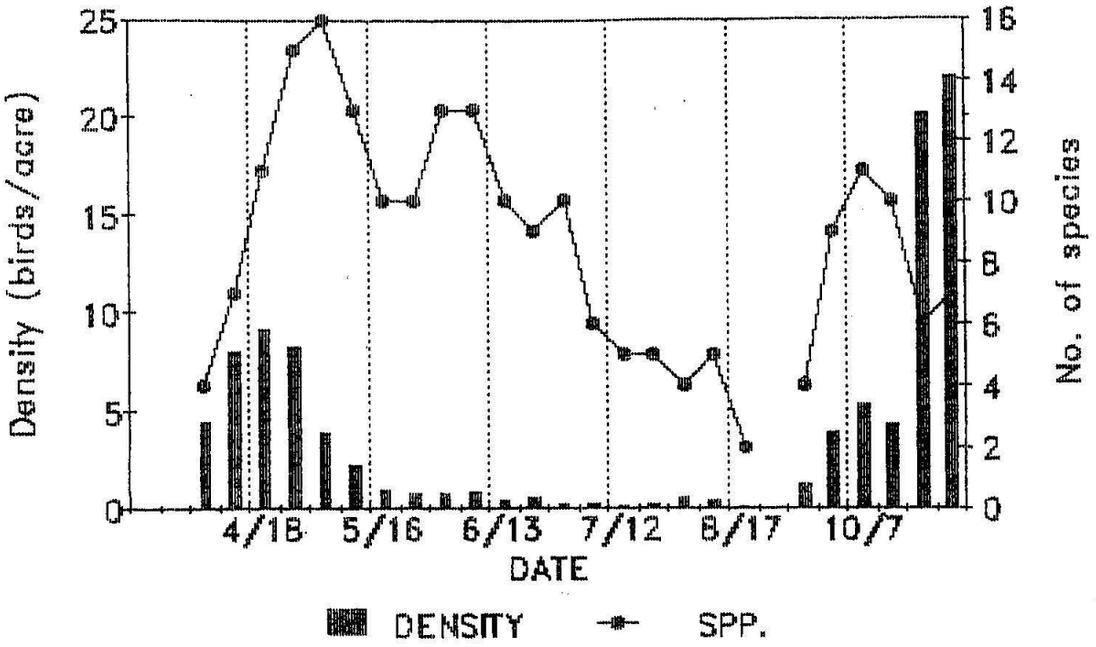

Figure 1. Waterfowl density and species richness along a census route in wild rice paddies, 1993.

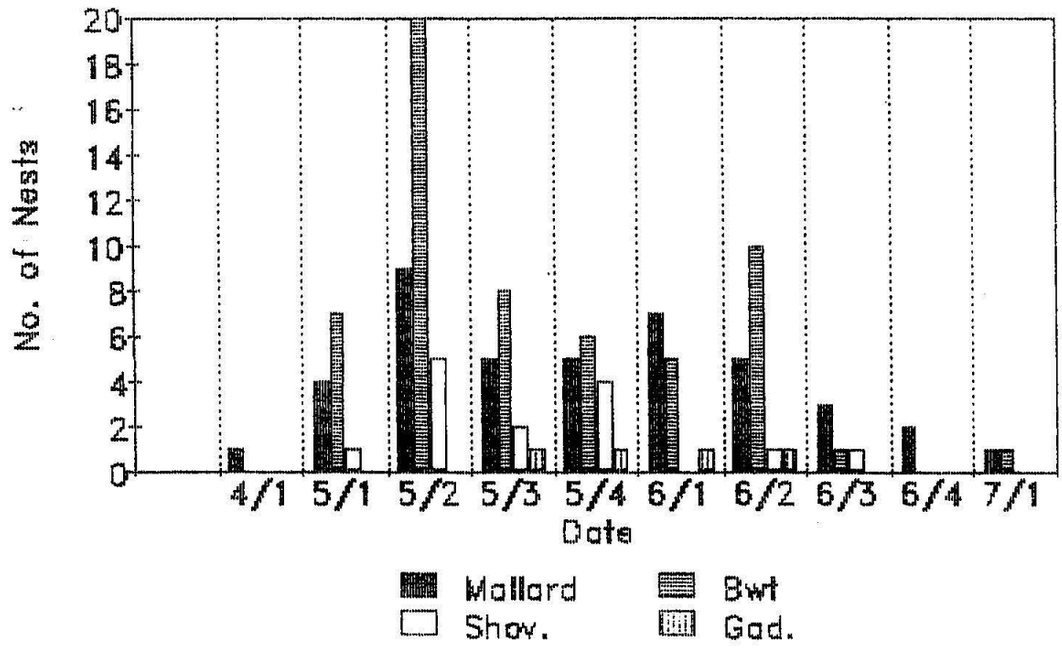

Figure 2. Nest initiation dates for upland-nesting ducks associated with wild rice paddies, 1993.

Table 1. Duck breeding populations associated with cultivated wild rice paddies in northwest Minnesota, 23 May, 1993.

Species	Density (birds/square mile)[1]	%
Mallard	359.9	53.3
Blue-winged teal	167.4	24.8
Northern shoveler	67.2	10.0
Wood duck	19.8	2.9
*Scaup	14.5	2.1
Gadwall	13.2	2.0
*Redhead	11.9	1.8
*Northern pintail	11.9	1.8
*American wigeon	2.6	0.4
*Ring-necked duck	2.6	0.4
Canvasback	2.6	0.4
*Ruddy duck	1.3	0.2
Total	674.9	

*Nests and/or broods of these species were not observed in subsequent field work.
[1]Density data are birds per unit area of paddies.

Table 2. Nest success for waterfowl associated with wild rice paddies, 1993.

Species	No. nests located	No. successful	Apparent success	Mayfield success
Blue-winged teal	67	22	32.8%	18.2%
Mallard	46	7	15.2%	9.5%
N. shoveler	14	10	71.4%	37.0%
Gadwall	4	1	25.0%	N.A.
Canvasback	1	1	100.0%	N.A.
Canada goose	4	3	75.0%	N.A.
Total	136	44	$\bar{x}$ = 32.4%	

Table 3. Summary of probable causes of waterfowl nest failures associated with wild rice paddies, 1993.

Probable cause	Number of lost nests
Abandonment	10
Depredation	74
raccoon	(17)
mink	(10)
skunk	(29)
Franklin's ground squirrel	(15)
unknown predator	(3)
Human activity	7
Total	91

Table 4. Data summary of radioed mallard hen nesting near wild rice paddies, 1993.

Frequency	Nest Fate	Days monitored	Comments
151.253	successful	22	brood unradioed
151.237	depredated	14	
151.295	abandoned	27	
151.267	successful	23	brood unradioed
151.245	abandoned	5	
151.275	successful	64	brood radioed
151.283	depredated	27	
151.305	abandoned	15	
151.225	successful	44	brood radioed
151.215	successful	24	brood radioed
150.995	depredated		

Table 5. Summary of monitoring data for radio-tagged mallard ducklings associated with wild rice paddies, 1993.

Frequency	Days monitored	Period monitored	Fate
Radioed at nest			
150.965 (brood 1)	60	(6/8 - 8/5)	fledged
151.015 (brood 1)	60	(6/8 - 8/5)	fledged
159.933 (brood 1)	54	(6/8 - 8/1)	fledged
151.083 (brood 2)	5	(7/16 - 7/21)	depredated (mink)
151.045 (brood 2)	4	(7/16 - 7/20)	depredated (mink)
150.805 (brood 2)	5	(7/16 - 7/21)	depredated (mink)
150.873 (brood 2)	4	(7/16 - 7/20)	depredated (mink)
150.975 (brood 3)	21	(7/16 - 8/4)	depredated (?)
150.885 (brood 3)	8	(7/16 - 7/24)	radio failure
151.055 (brood 3)	39	(7/16 - 8/22)	probable fledge
150.825 (brood 3)	39	(7/16 - 8/22)	probable fledge
151.025 (brood 4)	2	(7/27 - 7/29)	depredated (?)
150.995 (brood 4)	3	(7/27 - 7/30)	depredated (mink)
151.005 (brood 4)	2	(7/27 - 7/29)	depredated (mink)
150.815 (brood 4)	1	(7/27 - 7/28)	radio failure
Incidental broods			
150.955 (brood A)	2	(5/23 - 5/25)	died of exposure
151.195 (brood A)	2	(5/23 - 5/25)	died of exposure
150.955a (brood B)	2	(5/26 - 5/28)	died of exposure
151.075 (brood C)	3	(5/28 - 6/1)	depredated (mink)
150.925 (brood C)	3	(5/28 - 6/1)	depredated (turtle ?)
150.955b (brood D)	3	(5/30 - 6/3)	died of exposure
151.195a (brood D)	3	(5/30 - 6/3)	depredated (mink)

NONGAME BIRDS

Dan Svedarsky

A singing male census of nongame birds was conducted along a 5-mile route of roads and dikes within a portion of the study area considered representative of cultivated wild rice paddies and associated habitats. Censuses were taken in early morning on 30 May, 5 June and 22 June. Other than black terns, most birds were associated with emergent vegetation (primarily cattail) along paddy edges and upland habitats of; paddy dikes and roads, adjacent grass fields, grass-brush areas, and small groves of trees, principally trembling aspen and bur oak. The following species were recorded in general order of abundance: red-winged blackbird, savannah sparrow, song sparrow, brown-headed cowbird, common yellowthroat, killdeer, American goldfinch, clay-colored sparrow, common grackle, American robin, yellow warbler, warbling vireo, least flycatcher, alder flycatcher, sharp-tailed sparrow, common flicker, yellow-headed blackbird, and eastern kingbird.

Featured nongame bird species in this study were Wilson's phalaropes, black terns and American bitterns. Black terns are described separately and the following accounts summarize observations for Wilson's phalaropes, American bitterns, and other noteworthy species:

Wilson's phalarope: Small numbers observed throughout the study area along saturated mud flat habitats of paddies but no nests were recorded. One instance of "broody" behavior was observed on 21 July.

American bittern: Seven nests were found when cable-dragging upland fields associated with wild rice paddies; nests were commonly over 0.5 mile from paddies. Three of the 7 nests hatched but fledging success is unknown. Adults were commonly observed feeding along paddy ditches and occasionally in the interior of paddies.

Sandhill crane: Three crane nests were located near paddies; 2 hatched and the other was abandoned after a second nesting attempt. Adults and young were observed along the edge of the intensive study area indicating that a minimum of 2 additional, successful nests were present for a density of about 1 breeding pair per square mile in the general area where cranes were nesting.

Northern harrier: Five nests were found and 2 were successful. Four nests were in large expanses of dense grass cover and 1 was along a paddy dike.

Marbled godwit: Low numbers of adults were regularly observed in late April and May feeding along paddy edges and adjacent hayfields or pastures. One unsuccessful nest was located in a field which had been hayed the year before.

American avocet: As many as 3 pairs of avocets were observed throughout May with 1 nest established along the edge of a large paddy where an expanse of unvegetated peat was exposed. The nest was incubated for about 2 weeks then abandoned around 1 July. Avocets foraged in paddies containing 6 to 10 inches of water.

A variety of shorebirds, occasionally in considerable numbers, foraged along paddy edges on saturated peat flats and shallow water areas. On 2 October, about 400 greater and lesser yellowlegs, pectoral sandpipers, "peeps", and lesser golden or black-bellied plover were feeding in newly-flooded paddies. On 5 October, a scattered group of "plovers" were feeding in a freshly-cultivated paddy and later analysis of core samples from this paddy indicated an abundance of crane fly larvae (Tipulidae) about 2.5 cm in length, which are believed to be what the plovers were feeding on. On 8 October, about 125 plovers and 30 greater yellowlegs were resting and feeding in a paddy being flooded. The air temperature was about 25°F.

NEST CHARACTERISTICS AND NESTING SUCCESS OF BLACK TERNS IN CULTIVATED WILD RICE PADDIES IN NORTHWEST MINNESOTA

Dan Svedarsky

This progress report describes findings of the first year of a two-year study. The goal is to better understand the nesting ecology of black terns in cultivated wild rice paddies. This study is part of a larger study evaluating water quality effects, invertebrate populations, waterfowl use, and nongame bird use associated with cultivated wild rice paddies.

Objectives

1. To evaluate the nesting ecology of black terns in cultivated wild rice paddies in northwest Minnesota as to: nesting chronology, nest characteristics, nesting success, and likely causes of nest failure.

2. To evaluate the effects of agricultural practices on summer black tern use of wild rice paddies and identify possible management practices that would enhance nesting success.

Methods

Field study methods were essentially the same as those of Stephen Maxson (1992) who is conducting a three-year study of black tern habitat selection and nesting success at the Agassiz National Wildlife Refuge located about 48 km northwest of this study area. Field observations for the overall study commenced in early April. A roadside survey was conducted on 29 May and all black terns flying or sitting near paddies were counted.

Nests were found by noting black tern activity from roads and paddy dikes then searching with a small duck boat in early June when water depths were 25-38 cm and later with floating, pontoon skiis and "ski" poles fashioned from hollow, plastic electrical conduit. Black tern nests were marked with a 4-m orange-flagged ash pole placed 5 m north of the nest. The following nest data were collected: nest substrate (composition of nest), dominant emergent vegetation within 2 m of the nest, distance to "open" water (most nests were on floating peat hummocks so this distance was that to water depths of at least 2.5 cm), distance to the nearest black tern nest if less than 30 m, percent of open water within 1 m of nest, water depth at a distance of 1 m on four sides of the nest, and nest visibility (measured with a density cube). The density cube, modified from Jones (1968), was placed on the nest and readings taken from the 4 cardinal directions.

The number of squares (25 possible) at least 50% visible from a distance of 5 m and a sighting height of 0.5 m were counted on each of the four sides.

Eggs were floated to determine incubation stage (Westerkov 1950) and revisited shortly after the projected hatch date to determine nest fate. A nest was considered to have hatched if at least one egg hatched. Nesting success was determined using the Mayfield method (Mayfield 1975). An attempt was made to determine the cause of failure at unsuccessful nests.

Results and Discussion

Black terns were first observed on the study area on 22 May when a group of 20 were noted. A total of 93 black terns were counted on a 29 May census (Maxson 1992) when 67 paddies were surveyed from roads. Censused paddies ranged from 4 to 100 ha and totaled about 1200 ha. Over 120 black terns were observed on 12 June feeding over a 20-ha paddy which were either migrants passing through or late arrivals which nested in the study area.

Nest building was first observed on 5 June with the majority of nests initiated during the second week of June (Fig. 1) when wild rice plants were in the floating leaf stage and had not yet emerged above water. A total of 46 nests were found (Table 1) with the estimated initiation date commencing around 6 June; somewhat later than the earliest nests found by Maxson (1992) who found 12 initiated in late May. It is unknown if late nests (July) were renests or first nests of younger and/or late arriving birds.

Attempts were made to check nests near the projected hatch date but this was not always possible due to logistical and access problems. After water levels became too shallow to use a canoe or duckboat, access was very difficult when trying to wade to nests since the peat substrate was quite soft to a depth of about 1 m. Initially, conventional water skiis were modified by taping blocks of styrofoam onto them but these were not buoyant enough to hold up the investigator in most paddies. Eventually a set of homemade, pontoon skiis were borrowed from the MN D.N.R. Wetlands Wildlife Research Unit consisting of fiberglass-coated .3 x .3 x 1.5 m blocks of styrofoam fashioned to be attached similar to snowshoes. "Ski poles" were made from 1.5 m lengths of PVC electrical conduit and, together with the pontoon skiis, made it possible to access nest sites. A concern was not to damage developing wild rice plants but the hollow stems of rice plants allowed plants to straighten up within 1-2 days after checking nests if 3-10 cm of water was present. Nest checking was terminated however, on 21 July due to paddy water levels being mostly drawn down and the rapidly maturing wild rice plants. Of 46 nests found, a minimum of 19 contained at least one hatched egg for an apparent nest success of 41.3% (Table 1). Mayfield nesting success of 34.0% was determined from a sample of 35 nests which compares to 32.8% for a sample of 84 nests at Agassiz NWR (Maxson 1992). Overall, wild rice paddies were similar in water depth, levelness, and vegetation but there were some differences. Some paddies had

deeper water (>0.3 m) in areas, contained dense patches of water plantain (*Alisma plantago-aquatica*) in shallow water areas, and there was occasional variation between paddies as far as to the timing of wild rice plant development and plant density. No obvious relationship was noted however, between nest site selection and habitat characteristics. It appeared that the availability of small peat hummocks or a clump of floating plant debris for nest placement was more important than the general setting around nest sites. Habitat variables of unsuccessful and successful black tern nests were similar (Table 2). Determining the cause of nest failures was difficult, but predation was attributed to mink when shell fragments with small teeth marks, and tracks were present nearby. Eggs in some nests simply disappeared and could have been taken by raccoon, mink, or gulls. Swamping due to heavy rain and high wind could also have been a factor, although no eggs were found which had floated out of nests.

Young usually stayed in the nest the first 1 or 2 days after hatching, but would then swim 1-2 m from the nest and hide in vegetation as adults would give alarm calls at my approach. Young were well camouflaged and difficult to find, particularly after they became more mobile, 4-5 days after hatching. In a few cases when the nest could not be checked close to the hatch date, the appearance of the nest (used to brood young) and the behavior of mobbing adults were used to judge whether a nest had successfully hatched a young.

Fledging success was difficult to determine due to the hiding ability of older chicks, the growth of the wild rice, and the concern for damaging the crop by accessing nests later in July. Mink, raccoon, and American bitterns could prey upon young terns and bitterns were observed on occasion in paddy interiors being mobbed by black terns. The first flying juvenile tern was observed on 21 July. A group of 70-80 adults (some molting) were observed feeding and resting around a large exposed peat flat from 7-10 July. Their breeding status was unknown but some may have been unsuccessful at nesting.

Adults were observed to feed on tadpole shrimp (Notostraca) and minnows in the open expanses of paddies and along paddy ditches (interior and perimeter). Adults foraged almost exclusively along ditches later in the summer after the interior of paddies had developed thick stands of wild rice. Occasional open water areas in rice stands were commonly utilized by several foraging adults in mid summer. Some ditches developed a dense surface growth of duckweed (*Lemna minor*) and presumably made minnows unavailable as adults ceased foraging there when this occurred.

Plans for 1994 field season

1. Monitor from 50-75 nests to observe the range of environmental conditions used for nesting and identify possible correlates of nest site selection and nesting success. Consider using an air boat for more efficient nest searching.

2. Use taller, nest-marking stakes to better facilitate nest relocation when wild rice plants near maturity. Also, stakes should be double flagged and numbered near the base of flagging rather than the ends due to the possibility of wind damage to flags.

3. Better define the relationship between wild rice production practices and black tern nesting and production.

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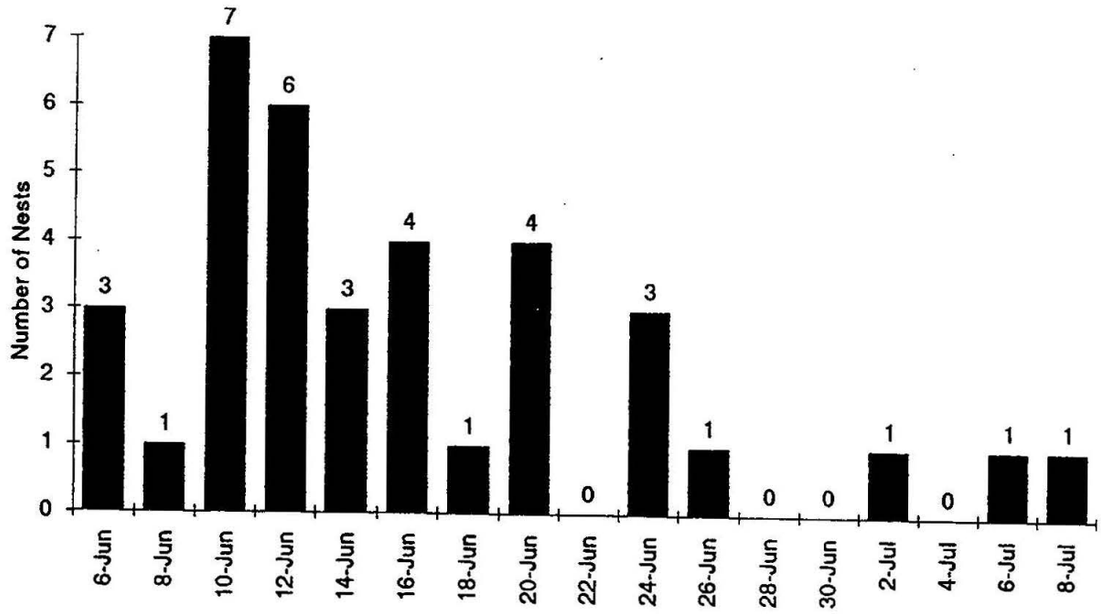

Figure 1. Estimated nest initiation dates for black terns in cultivated wild rice paddies in northwest Minnesota, 1993.

Table 1. Nest fate and success of black tern nests in cultivated wild rice paddies in northwest Minnesota, 1993.

No. nests monitored	46
No. hatched	19
No. depredated	19
No. abandoned	3
No. of unknown fate	5
Apparent success (minimum)	41.3%
Mayfield success	34.0%

Table 2. Summary of habitat variables at black tern nests in cultivated wild rice paddies in northwest Minnesota, 1993.

	Successful	Unsuccessful	Overall	Range
No. nests	19	27	46	
Distance to nearest nest in colony (m)[a]	11.1 (6.4)[b]	10.7 (5.4)	10.9 (5.6)	3-29
Water depth (cm)	8.6 (7.6)	8.5 (11.3)	8.5 (9.8)	0-60
Distance to open water (m)[c]	0.5 (0.9)	0.4 (0.5)	0.4 (0.6)	0.1-4.0
% open water within 2 m radius[d]	3.6 (1.6)	4.0 (1.2)	4.0 (1.3)	1-5
Nest visibility[e]	59.6 (30.9)	67.5 (25.1)	60.4 (30.5)	8-100
Tallest vegetation within 1 m (cm)	41.2 (25.0)	33.6 (14.1)	35.8 (20.0)	0-102

[a] Does not include nests more than 30 m from nearest neighbor.

[b] Values are mean (std. dev.).

[c] Distance from nest (usually on peat hummock or floating vegetation) to water.

[d] 1=0-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%.

[e] Total squares visible on density cube. Maximum visibility = 100.

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PROJECT COMMUNICATIONS

This project summary report will be circulated to all sponsors, cooperators and interested parties with a request for feedback information. Preliminary reports were given to representatives of the Minnesota Ornithologists' Union on 25 June; a group of growers, officials of the Cultivated Wild Rice Council, and media representatives on 24 August; and to the annual meeting of the Minnesota Ornithologist's Union in Minneapolis on 4 December. Additional reports will be given to the Institute for Ecological Studies (U of N. Dakota) on 7 February, to the annual meeting of the Cultivated Wild Rice Council on 10 March, and to the Board of the Red Lake Watershed District at a spring meeting.

PLANS FOR 1994

1. Develop detailed land use map of study area using Geographic Information Systems techniques.

2. Continue migrant and breeding waterfowl study as in 1993 but strive to radio-tag more mallard broods to better document habitat use and fledging success.

3. Field test a new trap design for trapping unradioed mallard hens and broods along paddy edges.

4. Complete analysis of water quality data and combine with the 12-month data set collected by the Red Lake Watershed District.

5. Develop reference collection of aquatic plants (herbarium specimens, seeds, and tubers) associated with wild rice paddies.

6. Video coverage of field work.

7. Record detailed phenology log of biological events, agricultural operations, and research activities for the field season.

8. Intensify efforts to document movements of radio-tagged mallard hens in July and August which have lost or fledged broods.

9. Conduct aerial waterfowl survey at spring migration peak.

10. Intensify emphasis on American bittern nesting ecology.

11. Complete data analysis of paddy core samples collected to evaluate the extent of food resources available after cultivation.

12. If possible, develop a field method of evaluating the effects of malathion application on aquatic invertebrates and ducklings.

13. Monitor from 50-75 black tern nests to better observe the range of variables affecting production and investigate a more efficient means of access using a small air boat or "Go-Devil" outboard motor.

PRODUCTION AND EVALUATION OF VARIOUS PORK BREAKFAST SAUSAGE/WILD RICE MIXTURES[1]

J.A. Rivera[2], P.B. Addis, R.J. Epley, A.M. Salih and B.B. Breidenstein

ABSTRACT

Wild rice was cooked with water to two targeted levels of hydration (1:2, 1:4); each added at a level of 0 (control), 15 and 30 percent to low-, intermediate-, and high-fat, coarse ground pork trimmings (20, 35 and 50 fat percentage). Proximate analysis of the fifteen sausage mixtures showed decreases in percentages of fat and protein, and increases in percentages of carbohydrate and moisture, as the level of wild rice increased. Thiobarbituric acid reactive substances (TBARS), a chemical measure of rancidity, were significantly reduced during frozen storage ($p<0.01$) by addition of hydrated wild rice. Sensory scores were higher in texture, juiciness, flavor, visual appeal and overall liking; and lower in rancidity, toughness and cohesiveness when wild rice was added to the formulation.

INTRODUCTION

Pork breakfast sausage typically contains a minimum of 38 percent fat. The fat content can be significantly reduced by simply selecting trimmings with a low fat content. However, when the fat content is reduced, the moisture loss during cooking will be significant. Wild rice has the potential to bind the moisture present in lean tissue during cooking and thus results in a cooked product of improved juiciness.

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Wild rice starch offered less resistance towards swelling at 60 and 70°C compared to wheat starch (Lorenz, 1981). Minerich *et al.* (1991) found that when cooked wild rice was combined with ground beef, a more desirable product with more favorable cooking yields than regular ground beef resulted. Minerich *et al.* (1991) also observed decreases in thiobarbituric acid reactive substances (TBARS) in ground beef patties with wild rice over 100 percent ground beef patties during refrigerated and frozen storage, indicating possible antioxidant effects. Further work by Wu *et al.* (1994) revealed that phytic acid in wild rice was one of the antioxidants retarding lipid oxidation in meat/wild rice mixtures. Wild rice contains 2.2% (w/w) dry weight of phytic acid (Graf and Eaton, 1990).

Wild rice is a good source of the B-vitamins thiamin, riboflavin and niacin and contains common minerals in amounts comparable to those in oats, wheat and corn (Lorenz, 1981). Wild rice has a gross composition of 14% protein, 75% carbohydrates, 1% ash, 1% crude fiber and 1% fat (Anderson, 1976).

Addis (1986) stated that deleterious changes in foods caused by lipid oxidation may be detrimental to the health of consumers. Since pork fat and lean tissues are highly susceptible to rancidity, wild rice offers the potential to significantly increase the shelf life of pork breakfast sausage. Therefore, the objectives of this research were to evaluate the effects of wild rice level, degree of hydration and fat level on the physical, chemical and sensory characteristics of pork breakfast sausage after frozen storage.

MATERIALS AND METHODS

Materials

Grade A wild rice was obtained from New Frontier Foods, Inc., Aitkin, Minnesota. Pork trim from normal slaughter weight barrows and gilts was obtained from Seaboard Farms/Farmstead Foods, Albert Lea, Minnesota. The pork sausage seasoning mix was obtained from Heller seasonings & Ingredients, Inc., Bedford Park, Illinois.

Preparation of pork sausage/wild rice mixtures

Grade-A Minnesota paddy wild rice was hydrated at targeted ratios of 2:1 and 4:1 (w/w) water to wild rice by cooking in an excess of boiling water for 55 and 105 minutes, respectively. The actual ratios were 2.3:1 and 3.54:1 for the first batch and 1.88:1 and 3.18 for the second batch (replication). In both cases, the cooked wild rice was drained, rinsed twice with cold water, drained and refrigerated prior to mixing with the pork trimmings.

Low, intermediate and high fat, fresh-pork trimmings, containing approximately 20, 35 and 50% fat respectively, were obtained from the local pork processor and transported to the University of Minnesota Meat Science Laboratory. After overnight storage in a 1°C cooler, refrigerated pork trimmings were immediately coarse ground through a 1.27 cm plate with a Hobart grinder (The Hobart MFG, Co., Troy, Ohio). Coarse ground pork trimmings were mixed separately for 1 minute in a Leland Food Mixer (Leland Detroit Manufacturing Co., Detroit, Michigan). After mixing, a commercial pork sausage seasoning mix was added at a level of 2.2% of meat block to each fat level and hand mixed. Fifteen pork sausage/wild rice mixtures were formulated by adding 0(control), 15 and 30% wild rice (each at 2:1 and 4:1 hydration level) to the coarse ground pork trimmings (20, 35 and 50% fat). Pork sausage/wild rice mixtures were ground through a 0.32 cm plate, stuffed with a water-powered sausage stuffer (E.F. Zuber Engineering & Sales Co., Minneapolis, Minnesota) in 454 g chubs, frozen and stored at -23°C. The entire procedure was replicated.

Sensory Evaluation

A panel of twelve graduate students (ages 21-45, 6 females, 6 males) participated in this study. Panelists were students from the Department of Food Science and Nutrition of the University of Minnesota. Panelists were trained in a one hour session using fresh pork sausage and a 5 month old pork/wild rice sausage to demonstrate large differences in attributes to be evaluated. Attributes were thoroughly explained to the panelists. Panelists familiarized themselves with the samples and the evaluation techniques.

Sensory evaluation of the fifteen pork sausage/wild rice mixtures was conducted after 0, 3 and 6 months of frozen storage. Samples were evaluated for rancidity, juiciness, toughness, cohesiveness, wild rice flavor, and amount of wild rice using 10 cm line scales labeled at the ends with "low" and "high". Labels of "dislike very much" and "like very much" where placed at the ends of the same 10 cm scales for visual appeal, flavor, texture and overall liking.

Sensory sample preparation

Samples were thawed at 5°C for 24 hr and formed into 100 g patties. Patties were pan fried to an internal temperature of 70 to 75°C. Patties were turned after 2,2,2,1 and 1 min on a side. Each patty was partitioned into four equal pieces of 20-25 g serving size. Samples were served immediately after cooking.

Samples were served in 28 g plastic cups labeled with three random digit numbers. Samples were divided into three sets (5 samples of the same fat level in each set). Samples to be evaluated were randomly ordered in each set. Sets were randomly assigned to the panelists. Panelists were presented with one

sample at a time and were given 2 to 3 minutes per sample to complete the sensory evaluation. After each set of samples were evaluated, panelists waited for 5 minutes before the next set of five samples was served. Panelists were asked to rinse their mouths with water between samples. Analysis of a replicated repeated measures design using pork sausage/wild rice mixtures as whole plots and extraction from frozen storage as repeated measures in time was used to analyze data and orthogonal contrasts were used to compare treatments, both using the statistical program Statistix (1985).

Chemical analysis of pork sausage/wild rice mixtures

The fifteen pork sausage/wild rice mixtures were analyzed in duplicate according to A.O.A.C procedures for moisture (1984,24.003a), fat (1984,24.005b) and protein (1984,24.027). Carbohydrate was calculated by difference from proximate analysis.

The fifteen treatments were analyzed in duplicate for TBARS, according to method of Salih *et al.* (1987) at 0, 3 and 6 months of frozen storage. Data were subjected to analysis of variance. Orthogonal contrasts were used to compare treatments.

RESULTS AND DISCUSSION

Effect of wild rice level, degree of hydration and fat level on physical, chemical and sensory characteristics of different pork breakfast sausage/wild rice mixtures during frozen storage.

Proximate Analysis

Proximate analysis of the uncooked pork/wild rice breakfast sausage mixtures showed decreases in percentage fat and protein, and increases in percentage carbohydrates and moisture as the level of wild rice increased (Table 1). This agrees with the results of Minerich *et al.* (1991).

TBARS

Table 2 summarizes the effects of the different variables on TBARS. TBARS values were significantly affected by replication, level of fat, wild rice level and storage time. The same table also shows the significant effects on TBARS of a two way interaction between fat level and wild rice level. Addition of wild rice at levels of 15 and 30 percent was more effective in lowering the TBARS values at the medium- and high-fat treatments when compared to the low-fat treatments (Table 3). Similar results were obtained by Minerich *et al.* (1991) when adding wild rice to ground beef. Another significant interaction was also found between fat level and storage time. Higher TBARS values were obtained for the high-fat treatments after 3 and 6 months of

frozen storage when compared to the low- and medium-fat TBARS measured after the same frozen storage time.

In general, addition of hydrated wild rice at levels of 15 and 30 percent to low-, medium- and high-fat pork meat significantly retarded lipid oxidation after frozen storage ($p<0.01$). Orthogonal contrasts showed that TBARS were significantly lowered ($p<0.01$) when wild rice was increased from 15 to 30 percent (w/w), wild rice to meat. No significant differences were found in TBARS values by hydrating the wild rice at levels of 2:1 and 4:1 (w/w), water to wild rice ($p>0.05$). This finding certainly represents an economic advantage, since a higher hydration level can significantly contribute to lower production costs without detrimental effects on the quality and storage stability of the product.

In all treatments at month 0 of frozen storage; low- and high-fat level after month 3 of frozen storage; and low-fat level after month 6 of frozen storage, a slight increase (non-significant) in TBARS values was noted for the 4:1 (w/w) hydration level when compared to the 2:1 (w/w) hydration level (Tables 4,5 and 6). A higher hydration level could have increased the mobility of the lipid radicals with other components in the food system, causing the observed increase in TBARS (Finley and Given, 1986).

Sensory attributes

Rancidity

The level of fat, level of wild rice and replication significantly affected the rancidity values of the different pork sausage/wild rice mixtures as scored by the panelists. The storage time did not have a significant effect on rancidity (Table 2).

Panelists scored rancidity lower for treatments with the wild rice added than with no wild rice added for the three fat levels ($p<0.01$), as indicated by the orthogonal contrasts. No significant difference in rancidity scores was found when wild rice was increased from 15 to 30 percent in the sausage formulation ($p>0.05$). Failure of the panelists to detect differences in rancidity when the product was formulated with wild rice at two different levels reveals a possible masking effect of wild rice on rancidity. Panelists could also have developed a preference for a rancid taste during the study, hence affecting the rancidity scores. This lack of specificity by the panelists on rancidity scores was certainly reflected by a 0.51 correlation coefficient between sensory scores and TBARS. However, the low correlation coefficient could also be due to the TBARS method used. Salih *et al.* (1987) reported a correlation coefficient of 0.85 when this method was used to monitor lipid oxidation in poultry products.

Juiciness

Juiciness was significantly affected by fat level, wild rice level and storage time (Table 2). An important interaction was found between the fat level and storage time. Juiciness scores reached their maximum value for the medium- and high-fat treatments at month 0 of frozen storage, then decreased almost at equal values after 3 and 6 months of frozen storage. A possible explanation to this loss of juiciness could be due to water migration occurring during the storage time. Some of the bound water may have become available as free water as a result of the cell membrane disruption caused by the grinding step in the sausage manufacture. Therefore, water released from the muscle tissue that was not bound by the wild rice could have been evaporated during the cooking step.

In general, panelists scored juiciness higher for treatments with wild rice added when compared to controls ($p<0.05$). Despite the decrease of juiciness over frozen storage time, the addition of wild rice to the sausage formulation helped to maintain a more desirable level of juiciness when compared to controls. Juiciness scores for treatments with 15 percent wild rice were significantly higher from those treatments with 30 percent wild rice ($p<0.01$). Again, this could be due to some water migration that occurred during storage time. Panelists scored juiciness higher when wild rice was hydrated at 4:1 (w/w), water to wild rice, than 2:1 ($p<0.01$).

Toughness

Toughness of the pork sausage/wild rice mixtures was significantly affected by replication, level of fat and level of wild rice (Table 2). A significant interaction was found between level of fat and storage time. Lower toughness scores were assigned by the panelists to medium- and high-fat level treatments at 0,3 and 6 months of frozen storage when compared to the low-fat level treatments at the same storage time. The lowest toughness value was reached at the high-fat level after 6 months of frozen storage. Some tenderization effect may have occurred during frozen storage in all sausage mixtures; however, a lower lean/fat ratio in treatments formulated with 35 and 50 percent fat may have also resulted in lower toughness scores by panelists.

Lower toughness scores were assigned to the treatments with added wild rice than treatments with no wild rice added ($p<0.01$). Toughness scores for treatments with 15 percent added wild rice were significantly higher than those scores assigned to treatments with 30 percent added wild rice ($p<0.01$). Toughness scores for treatments with the 4:1 (w/w) hydration level were also significantly lower than scores for the 2:1 (w/w) hydration level ($p<0.01$).

Cohesiveness

Cohesiveness of the pork sausage/wild rice mixtures was significantly affected by the level of wild rice and storage time (Table 2).

The cohesiveness of the sausage mixtures at all fat levels decreased when the wild rice was added to the formulation ($p<0.01$). The larger the percentage of wild rice, the less cohesive the product became ($p<0.01$). Cohesiveness scores for treatments with a hydration level of 2:1 (w/w) were significantly higher than scores from treatments with a 4:1 (w/w) hydration level ($p<0.05$). The practical importance of these findings consists in the use of an appropriate rice level and hydration degree for the sausage formulation without detrimental effects in the product functionality and overall quality.

Texture

Texture was significantly affected by fat level, wild rice level and storage time (Table 2).

Texture was improved by addition of wild rice to the formulation. Panelists consistently scored higher those treatments with wild rice compared to controls for the three fat levels at storage times of 0,3 and 6 months ($p<0.01$).
Texture scores for treatments with 15 percent added wild rice were significantly higher from those with 30 percent added wild rice ($p<0.05$). The panelists actually preferred treatments with 15 percent over the 30 percent wild rice added treatments. No significant differences in texture were found between hydration levels of 2:1 (w/w) and 4:1 ($p>0.05$).

Flavor

Flavor was significantly affected by fat level, wild rice level and storage time. An important interaction was found between wild rice level and storage time (Table 2). Flavor scores decreased consistently over frozen storage time at 0 percent level of wild rice. However, addition of wild rice at levels of 15 and 30 percent to the sausage formulations resulted in higher flavor scores. Maximum flavor scores were obtained for 15 and 30 percent wild rice added at month 0 of frozen storage (Tables 4,5 and 6).

Flavor in samples with wild rice was significantly improved when compared to controls ($p<0.01$). However, the addition of 30 percent wild rice to the formulations did not affect the flavor significantly over the samples with 15 percent wild rice ($p>0.05$). Also, no significant differences in flavor were found between samples with the 4:1 (w/w) hydration level and 2:1 hydration level ($p>0.05$). Therefore, a higher hydration level such as 4:1 (w/w) combined with a low wild rice percentage such as 15 percent, can result in a product manufactured at low cost with improved flavor characteristics. Wild rice was shown to be very useful at levels

of 15 percent with a 4:1 (w/w) degree of hydration, against rancidity in medium- and high-fat treatments. The addition of wild rice evidently helped to improve and retain the overall flavor of the sausage mixtures over frozen storage time.

Wild rice flavor

The level of wild rice and degree of hydration significantly affected wild rice flavor. A two way interaction between fat level and storage time was also found significant (Table 2). Wild rice flavor scores increased over frozen storage time as the level of fat increased from 20 through 50 percent. Wild rice scores were higher at all three fat levels after 3 and 6 months of storage time when compared to scores at month 0 of frozen storage. At a level of 35 percent fat, wild rice flavor scores reached their maximum value after 3 and 6 months of frozen storage time. However, at a level of 50% fat, wild rice flavor scores reached their maximum value after month 3 of storage time.

Wild rice flavor was clearly detected in all treatments by the panelists. The lowest scores were assigned in all cases for the control samples ($p<0.01$). Panelists assigned higher scores at all fat levels for the 30 percent added wild rice samples when compared to the 15 percent added wild rice samples ($p<0.01$). Panelists also indicated a stronger wild rice flavor for the treatments with the 2:1 (w/w) hydration level than the 4:1 (w/w) hydration level ($p<0.01$). A loss of flavor in wild rice could have occurred as a result of a longer cooking time to attain a higher hydration level, 4:1 (w/w). Also, a hydration level of 4:1 (w/w) certainly increased the ratio of water to wild rice causing a dilution effect in the flavor compounds.

Perception of wild rice amount

Amount of rice in the pork sausage/wild rice mixtures was significantly affected by level of wild rice and degree of hydration (Table 2).

In all cases, panelists were able to distinguish treatments with wild rice added compared to treatments with no wild rice added ($p<0.01$). Panelists assigned higher scores for treatments with 30% wild rice than treatments with 15% wild rice ($p<0.01$). Panelists also scored amount of rice higher for the 2:1, w/w hydration level than the 4:1, w/w ($p<0.01$). A high hydration level of 4:1 (w/w) certainly contributed to a major softening of the wild rice grain than a 2:1 hydration level, by binding more water and disrupting its own structure.

Visual appeal

Visual appeal as evaluated by panelists was significantly affected by replication, fat level, wild rice level and storage time (Table 2). An important interaction was found between fat level and storage time. Lower visual appeal scores were obtained

at all three fat levels (20, 35 and 50 percent) after 6 months of storage time when compared to scores from 0 and 3 months of frozen storage. The highest scores for visual appeal corresponded to month 3 of storage time for the 20 and 50 percent fat levels (tables 4,5 and 6).

Addition of wild rice at the level of 15 and 30 percent to the three different fat levels notably improved the visual appeal of the sausage mixtures when compared to the control samples ($P<0.01$). Treatments formulated with 15 percent wild rice were not significantly different from those treatments formulated with 30 percent wild rice ($p>0.05$). However, treatments with the lower hydration level of 2:1 (w/w) were scored higher for visual appeal when compared to the 4:1 (w/w) hydration level ($p<0.01$). In most cases, the presence of wild rice helped to retain some of the visual appeal when compared to the controls after frozen storage (Tables 4,5 and 6).

Overall liking

Overall liking was significantly affected by level of fat, level of wild rice and storage time (Table 2). One important interaction was found between fat level and wild rice level. Addition of wild rice at levels of 15 and 30 percent had a better effect on overall liking at the high-fat level treatments when compared to the low- and medium-fat level treatments. However, the highest scores for overall liking were assigned by the panelists to the low-fat treatments formulated with 0,15 and 30 percent wild rice. Another significant two way interaction was found between fat level and storage time. Panelists scored the highest overall liking values for all three fat levels (20,35 and 50 percent) at month 0 of storage time when compared to scores from months 3 and 6 of frozen storage. The lowest scores for overall liking were obtained for all fat levels after month 3 of storage time (Tables 4,5 and 6).

Overall liking improved in those treatments formulated with wild rice. Regardless of the wild rice percentage and the hydration level, all scores for the three different fat levels at months 0,3 and 6 were higher for products containing wild rice than scores for the controls ($p<0.01$). Scores for overall liking from treatments with 15 percent wild rice added were not significantly different from those treatments with 30 percent wild rice added ($p>0.05$). Addition of wild rice certainly helps to preserve the overall liking of the sausage mixtures at the three fat levels (20,35 and 50 percent). Despite the detrimental effect of storage time on overall liking scores, adding wild rice at a level of 15 percent and at a hydration level of 4:1 (w/w) to a medium or high-fat level (35 and 50 percent), makes it possible to manufacture a high quality product of improved sensory attributes, good functionality, low cost and satisfactory shelf-life.

CONCLUSIONS

Addition of hydrated wild rice (2:1 and 4:1, w/w) to coarse ground pork trimmings (20,35 and 50 percentage fat) at levels of 15 and 30 percent, notably improved sensory scores of texture, juiciness, flavor, visual appeal and overall liking when compared to pork sausage with no added wild rice. Lower scores for toughness and rancidity were obtained from treatments with wild rice compared to control treatments ($P<0.01$). Compositional analysis of the sausage mixtures revealed that as the level of wild rice increased, the percentage fat and protein decreased with an increase in percentage carbohydrate and moisture. Significant reductions during frozen storage in TBARS values ($p<0.01$) were observed when wild rice was incorporated into the formulations. A more nutritious, low fat, low calorie product with superior sensory attributes and satisfactory shelf life can be produced by adding hydrated wild rice to pork sausage.

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Table 1. Means of proximate analysis of the uncooked pork/wild rice sausage mixtures.

Treatment[a]	compositional analysis, %[b]			
	moisture	fat	protein	carbohydrate[c]
20:0:0	63.9	17.5	17.8	0.7
20:15:2	63.1	17.4	15.1	3.9
20:15:4	65.9	15.0	15.5	3.6
20:30:2	64.4	15.2	14.2	6.2
20:30:4	67.6	14.6	13.5	4.3
35:0:0	51.3	34.2	12.6	1.9
35:15:2	53.6	31.4	11.8	3.2
35:15:4	53.4	32.7	11.9	1.9
35:30:2	54.4	31.1	11.1	3.5
35:30:4	56.7	27.7	10.3	5.2
50:0:0	43.2	45.3	11.2	0.3
50:15:2	43.2	43.7	9.8	3.3
50:15:4	45.5	42.9	11.1	0.4
50:30:2	48.6	36.3	8.9	6.3
50:30:4	49.3	39.4	7.8	3.5

[a] Fat level(%):wild rice level(%):hydration level

[b] Duplicate determinations

[c] Carbohydrate calculated by difference

Table 2. Analysis of variance of TBARS and sensory attributes.

Source of variation [a]	df [b]	Attributes										
		TBARS	Rancidity	Juici-ness	Tough-ness	Cohesive-ness	Texture	Flavor	Wild rice flavor	Amount of rice	Visual appeal	Overall liking
Rep (A)	1	***	***	NS	***	NS	NS	NS	NS	NS	***	NS
Fat (B)	2	***	*	***	***	***	***	***	NS	NS	**	***
Hrice (C)	4	***	***	***	***	***	***	***	***	***	***	***
B*C	8	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Time (D)	2	***	NS	***	NS	***	*	**	NS	NS	*	***
B*D	4	***	NS	*	**	NS	NS	NS	*	NS	**	*
C*D	8	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
B*C*D	16	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A*B*C*D (E)	30											

[a] Abbreviations used: Rep(A)= replication, Fat(B)= fat level, Hrice(C)= hydration level and wild rice level, Time(D)= storage time, (E)= error and TBARS= thiobarbituric acid reactive substances.

[b] Degrees of freedom

* significant at $p<0.05$

** significant at $p<0.01$

***significant at $p<0.001$

Table 5. Month 3-means of sensory scores of pork/wild rice sausage as influenced by fat, wild rice and hydration levels.

	Sensory scores [a]								
Treatment[b]	Juici-ness	Tough-ness	Cohesive-ness	Texture	Flavor	Wild rice flavor	Amount of rice	Visual appeal	Overall liking
20:0:0	4.2	3.7	5.6	5.6	3.6	4.3	2.7	2.6	4.6
20:15:2	4.5	3.9	4.8	4.7	4.3	5.0	2.4	4.2	3.6
20:15:4	4.4	3.9	4.5	4.8	3.6	4.2	2.0	5.7	3.8
20:30:2	3.1	2.7	4.0	4.2	3.9	5.5	2.2	3.9	3.1
20:30:4	4.1	3.7	4.4	4.1	3.6	4.5	2.3	5.9	4.0
35:0:0	4.0	3.2	5.7	5.3	2.7	4.0	3.1	2.2	4.4
35:15:2	4.2	4.0	4.5	4.4	4.6	4.9	2.7	3.9	3.7
35:15:4	4.6	3.6	5.2	4.8	4.0	5.0	2.6	4.4	3.0
35:30:2	3.4	3.1	4.1	4.1	3.9	4.1	2.3	6.8	3.2
35:30:4	3.8	3.3	4.8	5.0	3.9	4.9	2.4	4.0	3.7
50:0:0	4.6	3.2	5.5	5.3	3.3	4.4	2.6	4.0	4.0
50:15:2	4.3	3.8	4.7	5.0	4.2	5.1	2.4	3.7	4.0
50:15:4	4.6	3.4	5.0	4.7	3.5	5.1	2.4	5.4	3.8
50:30:2	3.4	2.8	4.1	4.0	3.8	4.9	2.3	6.4	3.3
50:30:4	4.1	3.6	4.6	4.6	4.0	4.9	2.4	5.4	4.1

[a] Means based on a 10 cm line scale where 0=low and 10=high

[b] Fat level(%):wild rice level(%):hydration level

Table 6. Month 6-means of sensory scores of pork/wild rice sausage as influenced by fat, wild rice and hydration levels.

	Sensory scores [a]								
Treatment[b]	Juici-ness	Tough-ness	Cohesive-ness	Texture	Flavor	Wild rice flavor	Amount of rice	Visual appeal	Overall liking
20:0:0	4.5	3.6	5.1	5.4	3.4	4.4	2.9	2.6	4.4
20:15:2	3.7	3.4	4.2	4.6	3.8	4.7	2.5	3.2	3.7
20:15:4	4.1	3.7	4.7	5.1	3.9	4.6	2.4	2.3	4.2
20:30:2	3.4	3.2	3.6	4.0	3.9	5.5	2.6	4.6	3.5
20:30:4	3.7	3.3	4.3	4.4	3.8	5.0	2.6	2.8	3.5
35:0:0	4.2	3.2	5.3	5.8	3.4	4.0	3.3	2.5	4.5
35:15:2	4.6	3.4	4.8	4.9	4.8	6.3	2.9	4.7	3.8
35:15:4	4.0	3.1	5.0	5.2	4.1	5.0	2.7	3.9	4.4
35:30:2	3.4	2.8	3.6	3.7	4.0	5.3	3.0	5.9	2.9
35:30:4	3.6	3.1	4.3	4.7	4.1	5.5	2.9	4.0	3.9
50:0:0	3.9	2.6	5.8	4.9	2.6	3.0	3.7	1.8	5.1
50:15:2	3.9	3.1	4.6	4.8	4.4	5.2	2.7	4.1	3.9
50:15:4	3.7	2.6	5.1	5.0	3.5	4.5	3.0	2.5	4.5
50:30:2	3.0	2.5	3.6	3.6	3.5	4.4	3.5	3.7	3.6
50:30:4	4.0	3.3	4.5	4.8	4.4	5.3	3.3	3.6	4.0

[a] Means based on a 10 cm line scale where 0=low and 10=high

[b] Fat level(%):wild rice level(%):hydration level