The Effect of Harvest Time on the Germination Rate of Cultivated Northern Wild Rice

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The Effect of Harvest Time on the Germination Rate of Cultivated Northern Wild Rice (*Zizania palustris*)

By

Jacques P. Duquette

A creative component submitted to the graduate faculty
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Plant Breeding

Program of Study Committee:
Dr. Shuizhang Fei, Major Professor
Dr. Thomas Lübberstedt

Iowa State University

Ames, Iowa

2019

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Abstract

Northern Wild Rice (*Zizania palustris*), a temperate aquatic grass, is a niche crop grown largely in northern Minnesota, northern California, and Canada. Seed at harvest is dormant and requires stratification in cold water before germination can begin. Germination rates from stratified seed harvested at the beginning of the season are low and rates increase as the harvest season progresses. Variation from year-to-year is present in commercial cultivars, but germination testing after minimal stratification can identify potential germination issues allowing for adjustment at planting. Germination rates are similar among multiple commercial and experimental lines.

Introduction

Northern Wild Rice (NWR) (Poaceae, *Zizania palustris var. interior*) is an aquatic grass native to shallow lakes, streams, and rivers of the mid-northern United States and southern Canada. Northern Wild Rice is a monocious, cross-pollinated plant that exhibits a main stem and tillering (Kovach and Bradford 1992; Oelke 2007). Besides *Zizania palustris*, the genus *Zizania* includes three other species, *Z. latifolia*, *Z. texana*, and *Z. aquatica*. *Z. palustris*, *Z. texana*, and *Z. aquatica* are all native to North America and have 2x = 2n = 30, while *Z. latifolia* originated from East Asia and has 2x = 2n = 34 (Walker, 2011).

The life cycle of the wild rice plant starts submerged and consists of germination after stratification in cold water (completely submerged) to break dormancy and then the growth of three submerged, non-waxy leaves. The emergence of a 4th wax-coated leaf (allowing the leaf to float on the water surface) indicates the plant has entered the floating leaf stage which will progress to the aerial stage with the stem growing erect out of the water, elongating, and developing tillers. The plant then enters the reproductive cycle starting with the boot stage, head emergence, flowering, pollination, seed maturation, and finally senescence. As the seed matures it will shatter from the panicle, falling to the bottom of the body of water to overwinter (Oelke, 2007).

The species is relatively resistant to self-pollination as its inflorescence consists of a single panicle per stem (main or tiller) with the one-flowered pistillate spikelets located above the one-flowered staminate spikelets. The female florets emerge first, and the receptivity of those florets is reduced by the time the male florets have emerged from the sheath and begun to shed pollen (Kovach and Bradford, 1992).
The seed of NWR is unorthodox (seeds that do not survive drying and/or freezing during ex-situ conservation) and is dormant at maturity, requiring stratification (cold, wet storage) for about three months to break dormancy and allow for germination. The seed of NWR is considered to be unorthodox because it has a sensitivity to desiccation. However, due to its native environment, NWR is quite tolerant of cold, and to some extent freezing temperatures (Kovach and Bradford, 1992). This is a trait necessary to survive the winters in its native environment but creates problems for storage of the seed. The unorthodox nature of the seed means that long term storage is difficult, with seed lasting only 1-2 years in storage before seed viability is lost (unpublished data). Because of this, seed sources for a breeding program have to be grown out and harvested each year, a considerable investment with the potential for loss of experimental lines if there is poor germination in a growing season.

Over several centuries, NWR has traditionally been harvested in wild stands by Native American populations from lakes, rivers, and streams for food. The species was also used as a food source for waterfowl by hunters and conservationists. Natural stand wild rice can also be harvested and transported to locations that do not have wild rice currently growing for the purpose of reseeding lakes and rivers to promote waterfowl health or to reintroduce wild rice for conservation efforts (Aiken et al., 1988; David, 2018; McAtee, 1917; USDA 2004). Commercial food companies showed interest in using NWR in the early 1900’s for packaged food products, but production fluctuations from traditional harvesters made it difficult for commercial interests to source a consistent supply of NWR every year. In the 1950’s the first commercial paddies (fields with dikes to hold water) were established to test the viability of growing NWR as a crop. With success, commercial production flourished and was used to change the use of previously unusable land into valuable agriculture land, adding revenue to areas that were previously economically vacant. There are currently three main areas of production in Minnesota: Aitkin, Clearbrook, and Waskish (Fig 1.1).
Fig 1.1 – Map of Minnesota showing the three main commercial growing areas (red dots): Aitkin area, Clearwater-Gonvick area, and Waskish area. Map from alabamamaps.ua.edu.

With the commercialization of NWR into a crop, the University of Minnesota was approached by Minnesota commercial growers and processors to work with them to help breed better cultivars. Research was then conducted on shattering resistance, yield improvement, disease resistance, lodging resistance, processing requirements, seed size, seed storage, and germination (Hayes et al., 1989; Oelke, 1982; Oelke, 2007). Many traits within NWR are still largely asynchronous (over a 10-14 days period) among all commercial cultivars. They include tillering, flower emergence, and pollen release as well as retaining the non-uniform ripening of seed on each head and between heads on the same plant, highlighting the need to develop more uniform cultivars.

As research has progressed on Northern Wild Rice, the shattering trait (where the seed falls off the panicle after it matures) was considered one of the greatest reasons for yield loss at harvest and great effort was undertaken to find and breed in the non-shattering trait. With breeding efforts to promote the non-shattering trait in commercial varieties showing success (Hayes et al., 1989; Oelke, 2007), studies were done to see if the new commercial cultivars had changes in seed maturity, dormancy, or germination. However, these studies were only conducted after full seed stratification and utilizing only one harvest date (Aitkins et al., 1987; Cardwell et al., 1978; Counts and Lee, 1991; Kurle et al., 1985; Simpson, 1966). No germination studies were available where germination was studied without stratification or with staggered sampling times during the harvest season.
New research could also have a direct impact on the cultural practices of NWR cultivation. Current cultural practice is for farmers to do test cuttings at the beginning of harvest (opening the field) to see if the drained NWR fields are ready to be harvested and to calibrate the machinery. Weather events are always a concern (rain that will soften the soil too much or wind damage that will lodge the plants causing loss of yield), so NWR farmers always attempt to harvest as early and quickly as possible, even if it means harvesting a lower percentage of mature seed. Seed harvested early in the season has often been used for seeding of fields (based on anecdotal evidence that early seed germinates at higher rates).

With no previous research done to observe the germination of seed without stratification, or how germination may change over the course of the harvest season, this study was done to determine how NWR seed germination is affected by harvest time. The objectives of this study were to determine: 1. seed germination rate over staggered harvest times; 2. seed moisture content at each harvest time; 3. seed germination rate after minimal and nominal stratification; and 4. the effect of all these three factors over several years and among several varieties.

**Materials and Methods**

*Plant Materials and Seed Harvest*

Two varieties, “Barron” and “Itasca-C12”, and two elite experimental lines, “FY-C20” and “PBML-C20”, were evaluated over the three years of this study. All were planted at the University of Minnesota North Central Research and Outreach Center (NCROC) in Grand Rapids, Minnesota in the month of May with harvest taking place each year after August 21st.

Each year, once physical maturity of the seed was noticeable (20% of the seed on the panicle), a one-three cup sample of physically mature seed (caryopsis filled and seed had hardened) would be randomly hand harvested from the main stem of the plant (stripping of the head by firmly gripping the base of the head and stripping it with the hand in a smooth motion without pulling off any part of the head other than the seed). Once started, sampling was continued every seven days until there were no plants left to sample. Because seed maturity is asynchronous within the plant head, there can be large variation in seed samples, ranging from completely empty to fully mature seed.

*Field Season Details*

2015: Variety “Itasca-C12” was planted in a 6m x 12m plot with eight passes of four-row, 38cm spacing with a planting density at harvest of six plants per row meter. Harvest was conducted over seven weeks.

2016: Variety Itasca-C12 intentionally planted for the 2016 year failed, but Itasca-C12 that grew as second-year volunteer plants in a poorly drained paddy from 2015 were available to use for
this study. Planting density at harvest was three plants/square meter. Harvest was conducted over seven weeks.

2017: Variety Itasca-C12 was grown in the same paddy as in 2016, but was also reseeded by broadcasting with variety Itasca-C12 at a rate of 11kg/hectare to increase planting density. Planting density at harvest was nine plants/square meter. Experimental lines “PBML-C20” and “FY-C20” were grown in a second paddy with each planted in a separate 6m x 12m plot with eight passes of four-row, 38cm spacing. Planting density at harvest was six plants per row meter. The variety “Barron” was grown in a third, separate paddy at a rate of 11kg/hectare (broadcast planted) with a planting density at harvest of nine plants/square meter. Harvest was conducted over six weeks.

Seed Moisture Content (SMC) Measurement: At each harvest in 2015 and 2016, a 5.0 g sample was weighed out, placed in paper coin envelopes, and put in a 60°C drying oven for 72hrs, then reweighed to obtain the dry weight of each sample. This was replicated four times. SMC was calculated as follows: ((fresh weight – dry weight)/fresh weight) x 100.

Seed Germination Test

After harvest, seed was stratified by being placed in water in Ziploc® bags (to completely submerge the seed) and stored at 3°C in a walk-in cooler. The length of stratification and the amount of seed used for testing varied among years (duration of stratification was determined by previous lab germination tests, unpublished data). In 2015, either 12 or 30 weeks of stratification was used and four replications, each of 50 seed per harvest date, were tested for germination. In 2016 and 2017, either 14 or 30 weeks of stratification was used and four replications, each of 20 seed per harvest date, were tested for germination.

Germination attempts consisted of placing the seed samples in labeled Ziploc® bags with water completely covering all the seed, then placing them in a growth chamber set at a day/night temperature of 18°C/9°C and day length of 15 hrs. Germination was attempted for 21 days. Seeds were scored as having germinated if the coleoptile had emerged and reached the length of at least 1cm. Swelling of the mesocotyl or simple emergence of the coleoptile without elongation was not scored as germinated.

Data Analysis

Data were analyzed with JMP Pro statistical software version 14.0.0 (SAS Inst. Inc, 2018). An analysis of variance (ANOVA) and least square means (LS-M) were generated for three years of Itasca-C12 germination testing, two years of Itasca-C12 SMC testing, and for 2017 all four varieties germination testing. Tukeys-Kramer HSD was used to calculate mean separation of genotypes between all varieties in 2017. A bivariate fit of germination rate by harvest week was used to calculate the fit across all genotypes germination rate for 2017. Plotting of unequal data was done with a lambda of 0.03 for smoothing.
Results

Seed germination percentage at harvest

When germination was attempted within 24 hrs of harvest, no germination was noted for any harvest week, any year, or any variety, without exception.

Germination percentages of Itasca-C12 from 2015-17 following stratification

Analysis of the Itasca-C12 variety (Appendix Table A.1) across all three years of harvest indicated that there were statistically significant differences in germination rates between harvest years (Fig. 1) and among weekly harvest times (Fig. 2). As harvest weeks progressed, variation in germination percentage decreased to the lowest point in the middle of the season and then began to increase (Fig. 3). There was no significant interaction between weekly harvest times and harvest years.

![LS-Means of Germination by Year](image)

Fig. 1 – Plot of LS-Means of variety “Itasca-C12” germination percentages by year. 2015 had a statistically significant lower germination rate overall than 2016 or 2017.
Fig. 2 – Plot of LS-Means for variety “Itasca-C12” germination percentages by harvest week for years 2015-2017. This illustrates the variation observed in seed germination by harvest week and by year of harvest.

Each error bar is constructed using 1 standard error from the mean.
Fig. 3 – Plot of LS- Means of “Itasca-C12” germination percentages for all three years by harvest week. As harvest weeks progressed, germination increased significantly. The shaded area shows that there is more variability at the beginning of harvest, a decrease in the middle of harvest, before variability begins to increase again near the end of harvest.

**Seed Moisture Content (SMC)**

Analysis of Seed Moisture Content (SMC) in the variety Itasca-C12 (Appendix Table A.2) over two years (2015 and 2016) indicated that there were significant differences between years and between weekly harvest times (Fig. 4). In contrast, there were no significant differences for the interaction of harvest week by year.
Fig. 4 – Plot of LS-Means of variety “Itasca-C12” years 2015-2016 seed moisture content by harvest week. As the harvest weeks progressed, seed moisture content decreased significantly.

The relationship between Seed Moisture Content (SMC) and germination percentage in Itasca-C12

Observation of Itasca-C12 germination rates over weekly harvest time compared to the Seed Moisture Content over weekly harvest time showed a strong negative correlation (Fig. 5).
Fig. 5 – Plot of Itasca-C12 three year LS-mean for germination rate by harvest week vs LS-Means for percent Seed Moisture Content (SMC) by harvest week. As SMC declined over the harvest weeks, germination rates increased, illustrating a strong inverse relationship.

2017 – variety comparison

In 2017 FY-C20 had a significantly lower difference in germination percentage than Barron, Itasca-C12, and PBML-C20 (Appendix Table A.5) (Table 1).

There were significant differences within varieties for germination percentages by weekly harvest times (Appendix Table A.3 and A.4), but there were no significant differences among varieties when germination rate by weekly harvest time was factored (Fig. 6).

Table 1 - LS-Means of germination percentages for all 4 varieties in 2017 by harvest week.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Wk1</th>
<th>Wk2</th>
<th>Wk3</th>
<th>Wk4</th>
<th>Wk5</th>
<th>Wk6</th>
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<tr>
<td>Barron</td>
<td>0.31±sd A</td>
<td>0.63</td>
<td>0.83</td>
<td>0.92</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>FY-C20</td>
<td>0.07±sd B</td>
<td>0.70</td>
<td>0.81</td>
<td>0.84</td>
<td>0.83</td>
<td>0.87</td>
</tr>
<tr>
<td>Itasca-C12</td>
<td>0.32±sd A</td>
<td>0.69</td>
<td>0.88</td>
<td>0.92</td>
<td>0.89</td>
<td>0.93</td>
</tr>
<tr>
<td>PBML-C20</td>
<td>0.41±sd AB</td>
<td>0.55</td>
<td>0.74</td>
<td>0.87</td>
<td>0.92</td>
<td>0.88</td>
</tr>
</tbody>
</table>
Fig. 6 – Plot of germination percentage LS-means by harvest week of all 4 varieties in 2017.

Discussion

Itasca-C12 years 2015-2017

The effect of dormancy on germination at harvest

Based on previous NWR germination studies (Cardwell et al., 1978; Counts and Lee, 1991; Simpson, 1966), the lack of germination at harvest for Itasca-C12 across all three years and across all varieties in 2017 was consistent with earlier findings. This can be explained by lack of stratification to break dormancy.

The effect of harvest year on seed germination rate

Analysis of the three years of data collected for Itasca-C12 (Appendix Fig. 1.1), across all harvest weeks by year, showed that there were significant differences between years. By plotting out the Least Square – Means (LS-M) for germination by year (Fig. 1), the overall germination rate in 2015 was significantly lower compared to 2016 or 2017. Because this species is relatively
new to commercialization and has considerable variation within the cultivar due to its out-crossing, this may be attributed to environmental effects. This effect was also observed in previously conducted unrelated experiments, where germination rates would vary widely from year to year, and was part of the impetus for this trial (unpublished data).

The effect of harvest week on seed germination rate

Plotting of the LS-M for germination by harvest week across all three years (Fig. 3) is consistent with a linear model for germination of an annual species over a typical harvest period, with a continued rise in germination percentage over time as seed in the population reaches maturity during the season (Atkins et al., 1987; Oelke et al., 1982). There is greater variability in germination percentage early in the harvest, but it declines in the middle of the season as seed matures. The variability begins to increase late in the harvest season, potentially caused by desiccation of the earliest maturing seed (Kovach and Bradford, 1992).

When the average germination rate was plotted vs. harvest week by year, it was noted that there was variability from year-to-year (Fig. 2). Each year’s germination curve illustrates the expected germination rates over harvest weeks: low germination at the earliest harvest date, a rise in germination rate as the seed matures, and a final plateau as the harvest season comes to an end (Atkins et al., 1987; Oelke et al., 1982). This established germination curve provides more precise metrics for the optimal time for harvest if maximizing germination is the primary goal. It also shows that the environment still has a large effect on the variation in germination percentages by year highlighting the need to develop more uniform cultivars.

The effect of stratification duration on seed germination rate

Previous studies involving stratification duration showed that differences between treatments could be expected (Counts and Lee, 1991; Simpson, 1966). The LS-M germination rate at 12/14 weeks and at 30 weeks of stratification were plotted by year. The 14 week test closely matches the 30 week test across all harvest weeks in two of the three years. This shows that once the seed has been exposed to stratification sufficient to break dormancy, it maintains that germination rate through the stratification treatment. However, variability is still an issue as illustrated in 2016, where the 30 weeks data points and their respective 14 weeks data points do not match well in three places because the germination rate has changed over storage time (Fig. 7). In contrast, the germination rate in 2015 was significantly lower than in 2016 or 2017, but the harvest week germination rates still match well from 12 weeks to 30 week stratification.
The effect of Seed Moisture Content (SMC) on germination rate

The data analysis (Appendix Table A.2) run on the 2015-2016 data for Itasca-C12 across all harvest weeks showed significant differences among harvest weeks and among the test years. The Least Square Means (LS-M) plot (Fig. 4) shows that SMC drops significantly as harvest weeks progressed, which is expected as the seed matures and prepares to overwinter. The concern is that as the SMC reduces, it will reach a critical point where seed germination will drop rapidly due to fatal desiccation. (Kovach and Bradford, 1992; Kurle et al., 1985).

The correlation between Seed Moisture Content (SMC) and germination rate

Does the non-shattering trait in commercial cultivars lead to lower germination rates due to the trait keeping the seed on the panicle longer? This greater exposure to a drying fall environment might allow the seeds to reach fatal levels of desiccation before being harvested. There was a strong negative correlation between germination rate and SMC in Itasca-C12 (Fig. 5). This relationship shows that although NWR seed is sensitive to desiccation, a SMC of ~ 20% still
provides for a high germination rate (Kovach and Bradford, 1992). Germination was not affected by loss in SMC over the course of the harvest season, showing that non-shattering cultivars do not expose the seed to fatal levels of desiccation.

The effect of cultivar on seed germination

The four variety data from 2017 were analyzed and indicated that there was a significant difference between the overall mean germination rate of FY-C20 when compared to the other three varieties. Further analysis showed that when the data was separated by variety for the germination percentage of each harvest week, no significant difference among the varieties was found. Significant differences were only found within the varieties among harvest weeks. When the Least Square Means (LS-M) for each variety was plotted (Fig. 6), FY-C20 showed a significant difference due to a particularly low germination rate for harvest week one. This can be attributed to FY-C20’s breeding objective as a later maturing variety. After harvest week one, there was no significant difference in overall germination among varieties. It can be speculated that if initiation of harvest had been delayed by a few days, then a significant difference may not have been observed among the varieties.

Conclusion

Seed harvested early in the season is expected to have a lower germination rate, with higher germination rates expected as seed is harvested later in the season. The trends established in this study can be applied across multiple NWR varieties to predict germination percentages after minimal stratification and to show desiccation tolerance over the harvest season. However, the diversity in germination rate shown in this study is not a preferred characteristic and will require further research to improve in subsequent commercial varieties.

References


Acknowledgements

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• Henry Schumer – University of Minnesota (retired)
• Amanda Monson – University of Minnesota
• Dr. Raymond Porter – Huntington University
• Minnesota Cultivated Wild Rice Council
Appendix

Analysis of Variance Table

Response: germpercnt

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<th></th>
<th>DF</th>
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<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
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<td>3.5741</td>
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<td>Year</td>
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<td>81.5442</td>
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Table A.1 – ANOVA of Itasca-C12 over 3 years germination data comparing the weekly harvest date, the harvest year, and the interaction between the weekly harvest and the year.

Analysis of variance table

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
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<th>Mean Sq</th>
<th>F value</th>
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A.2 – ANOVA of Itasca-C12 over 2 years Seed Moisture Content comparing the weekly harvest date, the harvest year, and the interaction between the weekly harvest and the year.

Analysis of Variance Table

Response: germpercnt

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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A.3 – ANOVA of all 4 varieties for the 2017 germination data comparing the varieties to each other, the weekly harvest date, and the interaction between the variety and the weekly harvest date.
Table A.4 – ANOVA and Bivariate fit of germination by variety and germination by weekly harvest date with Tukey-Kramer HSD comparison.
Table A.5 – ANOVA of germination by variety for the entire 2017 season with Tukey-Kramer HSD comparison.
Pic A.1 - Northern Wild Rice seed immediately after harvest. Note the dark coloration of the hull, with a slight purple iridescence. Seed size and coloration are variable within any variety.
Pic. A.3 – Harvest week sample of NWR hand collected at the NCROC in Grand Rapids, MN. Notice the variation in color of the seed hull. Tan or brown seed hulls generally indicate physiological maturity but may not indicate reproductive maturity.
Pic. A.4 – Field of NWR at head emergence in Aitkin, MN.
Pic. A.5 – Field of variety “Barron” at week one in the harvest season near Aitkin, MN.
Pic. A.6 – NWR variety Itasca-C12 panicles at harvest. Notice variation in coloration of flowers, seed, and maturity of panicles.
Pic. A.7 – NWR going aerial at research paddies at the NCROC in Grand Rapids, MN.
Pic. A.8 – NWR near harvest at NCROC research paddies in Grand Rapids, MN. Notice the variation in flower color and panicle shape still inherent in varietal lines.