Aquatic Botany 94 (2011) 113–118

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Effects of water depth and seed provenance on the growth of wild rice (*Zizania aquatica* L.)

Rebecca C. Tucker[a,*], Michael J. Zanis[a], Nancy C. Emery[a,b], Kevin D. Gibson[a]

[a] Department of Botany and Plant Pathology, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054, USA
[b] Department of Biology, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054, USA

ARTICLE INFO

Article history:
Received 22 May 2010
Received in revised form 7 December 2010
Accepted 10 December 2010
Available online 21 December 2010

Keywords:
Zizania aquatica L.
Wild rice
Water depth
Seed provenance
Restoration
Conservation

ABSTRACT

Annual wild rice (*Zizania aquatica* L.), a species of conservation concern, is an ecologically and culturally important aquatic grass found in stands in the near shore habitats of lakes and rivers in the Midwest and along the eastern coast of North America. This study examined the effects of water depth and seed provenance on the early growth of three Indiana wild rice stands (collected from two lakes) under greenhouse conditions in 2009. Plants were grown at water depths of 46 cm, 23 cm, 0 cm, or −15 cm and harvested either at the first floating leaf stage or at 48 days after transplanting. Wild rice growth was affected by both water depth and seed provenance. The dry weight of roots, stems, leaves, and inflorescences, total biomass, number of tillers, number of leaves, and total leaf area were the lowest in the −15 cm treatment. These vegetative growth parameters also decreased with increasing water depth from the 0 cm treatment. Differences in growth between seed sources were found, supporting the hypothesis that genetic differences among relatively isolated wild rice stands may influence the success of efforts to conserve this species.

1. Introduction

Zizania aquatica L. and *Zizania palustris* L. (commonly known as annual and northern wild rice, respectively) are emergent annual grasses found in near-shore lake and river habitats of North America (Terrell et al., 1997; USDA, 2010). Although distributed across more than twenty degrees of latitude (Minnesota to Florida, including Indiana), *Z. aquatica* is rare throughout most of its range. Wild rice stands provide habitat and food for many species of waterfowl and small mammals and important ecosystem functions such as sediment stabilization (Steeves, 1952; Aiken et al., 1988; Meeker, 1996; Thompson and Luthin, 2004). *Z. aquatica* and *Z. palustris* are harvested by humans; the latter species has been domesticated and can be grown under paddy conditions (Hayes et al., 1989; Oelke, 1993). Wild rice is also a vital link to the past generations, cultural identities, and current religious beliefs for several Native American tribes in the upper Midwest of the United States (Steeves, 1952; Aiken et al., 1988; Vennum, 1988). Thus, government agencies, Native American groups, and non-profit organizations (i.e. the Minnesota and Wisconsin Departments of Natural Resources, the USDA Natural Resources Conservation Service, the Great Lakes Indian Fish and Wildlife Commission, and the Nature Conservancy) are working to conserve and restore wild rice in the Midwest.

Environmental gradients, such as water depth, are critical factors influencing the distribution and abundance of aquatic species as well as the success of restoration efforts (Peden, 1982; Weichel and Archibold, 1989; Counts and Lee, 1990; Kennedy et al., 2006; Emery et al., 2009; Pillsbury and McGuire, 2009). Water level has been implicated as a key factor affecting wild rice growth and reproduction. For example, Pillsbury and McGuire (2009) surveyed sixty wild stands of *Z. palustris* populations in Wisconsin and Minnesota and reported correlations between water depth and stand size with high population densities occurring at a depth of 69.9 cm (±25.5 cm). Thomas and Stewart (1969) found that *Z. palustris* plants grown at a water depth of 32 cm took longer to reach anthesis and senescence, were taller, and allocated more biomass to leaves than plants that were not submerged. Although Stevenson and Lee (1987) did not examine growth of plants at the water surface, they concluded that the vegetative and reproductive production of *Z. aquatica* was significantly reduced when plants were transferred at any growing stage from a level of 45 cm below the water to a depth of 75 cm or greater.

Seed provenance, the specific lineage or place of origin from which seeds are to be collected, may also contribute to plant performance in restoration efforts, particularly in species with limited population size and gene flow (Ellstrand and Elam, 1993;

* Corresponding author. Tel.: +1 765 494 4614; fax: +1 765 494 0363.
E-mail address: rtucker@purdue.edu (R.C. Tucker).

0304-3770/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquabot.2010.12.001

Hufford and Mazer, 2003; McKay et al., 2005). For example, Bischoff et al. (2006) found that seed from four species of wildflowers had different rates and times of germination depending on their originating sources. Seed provenance has also been shown to affect the responses of both terrestrial and aquatic plants to external influences such as water availability (O'Brien et al., 2007), competition (Gustafson et al., 2004), tidal regimes (Van Katwijk and Wijgergangs, 2004), and other site-specific conditions (Renison et al., 2005). Gene by environment interactions have not been examined extensively in *Zizania* sp., but similar studies suggest that these details may be important considerations in wild rice restoration.

Although *Z. aquatica* may occupy a greater geographic range than *Z. palustris,* relatively little research has been conducted on this species. This study used controlled greenhouse experiments to assess the effect of water depth and seed provenance on the growth of *Z. aquatica.* We hypothesized that (1) wild rice biomass would decrease with increasing submergence depth and would be greater for submerged plants than for plants exposed to intermittent soil drying, and (2) seed from different provenances would differ in their response to water depth.

2. Methods

2.1. Seed collection and germination

Z. aquatica seeds were collected in Indiana during September 2008 from a single stand in the Jasper-Pulaski Fish and Wildlife Area (41°9′28.34″N, 86°56′42.84″W), and from two stands in the Tri-County Fish and Wildlife Area (41°21′43.68″N, 85°40′33.36″W). The Jasper-Pulaski stand occurred both near shore and in the shallow middle of the marsh. This stand was the largest of the three during the sampled year; however, personal communications with the rangers at the wildlife area suggest that the population had recovered from a reduction in stand size within the last twenty years. The two Tri-County stands were found in separate areas approximately 200 m apart along the shore line of the Hammond and Allen–Rothenberger Lake system.

Seeds were stored in reverse osmosis water at 4–7 °C for three months to break dormancy (Horne and Kahn, 2000). Germinated seeds were planted in seedling trays and allowed to grow for three weeks to between 8 and 12 cm in height to avoid maternal effects. Thirty-two seedlings were chosen from each of the three seed sources (abbreviated JP, AR-1, and AR-2, respectively) and transplanted individually into 12 cm diameter by 14 cm deep pots filled with water-saturated Metro-Mix 510 commercial soil media (30–40% Canadian sphagnum peat moss from Sun Gro Horticulture Canada Ltd., Vancouver, British Columbia, Canada).

2.2. Greenhouse experiment design

A mesocosm experiment was used to test the hypotheses under greenhouse conditions at Purdue University in West Lafayette, IN. The design was a randomized complete block with four blocks and two main factors (water depth and seed provenance), with two replicates of each factor combination to allow for two harvests. Each block consisted of one 570 L water-filled tank containing a PVC "cage". The cage allowed the pots to be placed so that the top of the soil was 0 cm, 23 cm, or 46 cm (±2 cm) below the water (surface, mid, and bottom treatments, respectively). Pots were also placed above the water (−15 cm water depth, referred to as the drought treatment). Plants in the drought treatment were watered to soil saturation every ten days.

Two complete replications of the experiment were completed in the same greenhouse. These two replications, called "runs", were initiated on February 13, 2009 and on March 20, 2009. Plants were grown under natural and artificial light (one 400 W high-pressure sodium lamp per tank) with a 14 h day/10 h night schedule (to simulate the day length during Indiana summers) for 48 days after transplanting (DAT). Air and water temperatures, measured weekly (YSI Pro20 multiple sensor, YSI Inc., Yellow Springs, OH), averaged 26 ± 2 °C and 21 ± 2 °C, respectively; differences in temperature among runs and depths were not detected. Light was measured at 15 cm above and at 23 cm and 46 cm below the water surface at the start of each run. Light initially reaching pots in the mid and bottom treatments was reduced from 670 ± 76 μmol m^{-2} s^{-1} above the surface to 390 μmol m^{-2} s^{-1} (58%) and 185 μmol m^{-2} s^{-1} (28%) in the first run, respectively. In the second run, light decreased from 1300 ± 87 μmol m^{-2} s^{-1} above the surface to 475 μmol m^{-2} s^{-1} (37%) in the bottom treatment, but light was no lower at the mid depth than at the surface. Water levels were maintained at a constant depth throughout the experiment.

2.3. Data collection

Height (from the soil surface to the tip of the longest leaf) was measured each week. Plants were randomly chosen to be harvested when leaves from a majority of plants in the bottom treatment reached the water surface (27 DAT) and at the first signs of flowering (48 DAT). Harvested plants were separated into component parts (roots, stems, leaves, and immature inflorescences, if present) and dried at 60 °C for one week before being weighed. Leaf area (LA) was determined before drying with a leaf area meter (LI-3100, LI-COR Biosciences., Lincoln, NE). Root weight ratio (root dry weight/total dry weight, RWR), leaf weight ratio (leaf dry weight/total dry weight, LWR), and specific leaf area (LA/leaf dry weight, SLA) were calculated for each plant.

2.4. Data analysis

PROC CORR (in SAS) was used to examine correlation between response variables. Once these relationships were established, MANOVAs (PROC GLM with MANOVA option) were used to assess the effect of the independent variables and their interaction on linear combinations of the dependent variables. Wilks' lambda statistic with an overall alpha of 0.05 was used to test significance. Mixed model ANOVAs (PROC MIXED) were then used to assess the specific effects of the random variables run and block and the fixed factors harvest time, depth, and seed provenance on the following variables: root, stem, leaf, inflorescence, and total dry weight, LA, SLA, RWR, LWR, height, and number of leaves and tillers. Data were pooled across runs and reanalyzed if no interaction was detected between run and other variables. Pair-wise comparisons were conducted using the Tukey–Kramer test with a Tukey adjustment to maintain an overall alpha of 0.05. Stepwise regression was used to assess the relationship between plant height and the following variables: run, site, and sampling date. The significance level for both entry and exit into the model was set at 0.05. Data used for regression analyses were square root transformed before regression to meet homogenous variance and normal distribution requirements. Statistical analyses were performed with the SAS 9.1.3 software package (SAS Institute Inc., Cary, NC, USA) and SigmaPlot 11 (Systat Software Inc., Chicago, IL, USA).

3. Results

Dry weight variables (leaf, stem, total) were highly correlated with each other and with leaf area at both harvests (Table 1). Dry weight variables (leaf, stem, total) and leaf area were also highly or moderately correlated with the number of tiller and leaves at

Table 1
Pearson percent correlation coefficients for dependant variables with results from Harvest 1 in the upper right half and results from Harvest 2 in the lower left half. Coefficients with *p*-values >0.05 are represented as not significant (NS) and coefficients with *p*-values <0.0001 are presented in bold.

	Root DW	Stem DW	Leaf DW	Infl. DW	Total DW	LA	SLA	RWR	LWR	Height	No. of tillers	No. of leaves
Root DW	–	**0.7**	**0.7**	–	**0.9**	**0.7**	NS	0.4	−0.3	−0.2	**0.7**	**0.7**
Stem DW	**0.8**	–	**0.9**	–	**0.9**	**0.8**	−0.4	−0.3	NS	0.2	**0.7**	**0.7**
Leaf DW	**0.8**	**0.8**	–	–	**0.9**	**1**	−0.3	−0.3	0.4	0.3	**0.7**	**0.8**
Infl. DW	**0.4**	**0.7**	0.3	–	–	–	–	–	–	–	–	–
Total DW	**0.9**	**1**	**0.9**	**0.6**	–	**0.9**	−0.3	NS	NS	NS	**0.8**	**0.8**
LA	**0.8**	**0.8**	**1**	0.2	**0.8**	–	−0.2	−0.3	0.4	0.3	**0.8**	**0.8**
SLA	NS	−0.2	−0.2	NS	−0.2	NS	–	0.2	−0.2	NS	NS	−0.3
RWR	0.4	NS	NS	NS	NS	NS	−0.2	–	−**0.9**	−**0.7**	NS	NS
LWR	−**0.5**	−**0.6**	−0.3	−**0.6**	−**0.6**	NS	0.3	−**0.6**	–	**0.7**	NS	0.2
Height	**0.4**	**0.6**	**0.7**	0.3	**0.6**	**0.7**	NS	−0.4	NS	–	NS	NS
No. of tillers	**0.8**	**0.6**	**0.7**	**0.4**	**0.7**	**0.8**	NS	NS	−0.3	0.4	–	**0.9**
No. of leaves	**0.8**	**0.7**	**0.7**	0.4	**0.8**	**0.7**	NS	0.3	−0.4	0.3	**0.9**	–

both harvests (Table 1). Inflorescence DW was moderately correlated with all variables except SLA and RWR. Plant height was only moderately correlated with dry weight variables at both harvests; height was negatively correlated with root DW at the first harvest and with RWR at both harvests (Table 1). LWR and RWR had low or moderate correlation with several variables at both harvests (Table 1). RWR was negatively correlated with stem and leaf DW at the first harvest but not at the second (Table 1). LWR was negatively correlated with dry weight variables at the second harvest reflecting the larger contribution of root and stem DW to total DW once plants emerged from the water (Table 1). LWR was also negatively correlated with the number of tillers and leaves at the second harvest (Table 1). In general, few tradeoffs among traits were detected at either harvest except that partitioning to leaf DW decreased as plants grew larger, i.e. greater DW, more tillers, at the second harvest and partitioning to root DW decreased as height increased.

Water depth, seed provenance, and their interaction significantly affected the composite variable in both harvests according to MANOVA (Table 2). In the univariate ANOVAs, significant interactions between water depth and seed provenance were only found for the number of tillers and number of leaves (Table 3). Differences among provenances in tiller or leaf production were not detected between AR-1 and AR-2 at any depth, but JP plants produced fewer tillers and leaves than AR-1 and AR-2 plants in the surface treatment at both harvests (Table 4). JP also produced fewer tillers and leaves than AR-1 and AR-2 plants in the mid treatment at the second harvest (Table 4).

Total and component (root, stem, and leaf) dry weights were greater for plants grown at the water surface than for plants in all other treatments (Fig. 1). Total dry weight decreased with increasing water depth at both harvests. Root dry weight did not differ among plants in the drought, mid, or bottom treatments at the first harvest. In the second harvest, however, plants in the mid treatment produced significantly more roots than plants in the drought and bottom treatments. Differences in stem and leaf biomass were not detected between the drought and bottom treatments in the first harvest, but the drought treatment produced significantly less biomass at the second harvest. Inflorescence dry weight was greater for plants grown at the surface than for plants in the drought and bottom treatments (Fig. 1).

LA decreased with depth from the surface and was lowest for plants in the drought treatment at both harvests (Table 5). SLA at both harvests was greater in the bottom treatment than in the drought and surface treatments at both harvests (Table 5). No differences in RWR were detected between the surface and submerged treatments at the first harvest. However, RWR decreased with depth from the drought treatment at the second harvest (Table 5). No differences in LWR were detected among treatments in the first harvest, but LWR increased with decreasing water depth at the second harvest (Table 5).

Plants in the mid and bottom treatments emerged from the water at 12 ± 1.2 DAT and 27 ± 1.7 DAT, respectively for the first run and 6 ± 0.5 DAT and 15 ± 0.5 DAT, respectively for the second

Table 2
Summary statistics from the MANOVA analyses of the effect of depth, seed provenance, the interaction of the two factors, and block on the response variables simultaneously. Degrees of freedom (DF), *F*-values, and *p*-values calculated from the Wilks' lambda test statistic (alpha = 0.05).

	Num, den DF	Wilks' λ	*F*-value	*p*-value
Harvest 1				
Depth	30, 197	0.035	14.08	<0.0001
Site	20, 134	0.582	2.08	0.0074
Depth × site	60, 356	0.302	1.53	0.0109
Block	30, 197	0.623	1.15	0.2814
Harvest 2				
Depth	33, 204	0.003	37.2	<0.0001
Site	22, 138	0.473	2.85	0.0001
Depth × site	66, 375	0.237	1.75	0.0007
Block	33, 204	0.625	1.07	0.3743

Table 3
Summary for two-way mixed model ANOVA analyses with *p*-values for each test. For both harvests, numerator (error) DF were 3, 2 and 6 for depth, site and the interaction term, respectively and denominator (error) DF were 76 and 79 for Harvests 1 and 2, respectively; *p*-values <0.05 are presented in bold.

	Depth	Site	Depth × site
Harvest 1			
Root DW	**<0.0001**	**0.0491**	0.8391
Stem DW	**<0.0001**	0.4437	0.871
Leaf DW	**<0.0001**	0.0685	0.5633
Total DW	**<0.0001**	**0.044**	0.7913
LA	**<0.0001**	**0.0084**	0.4244
SLA	**<0.0001**	0.2006	0.9877
RWR	**0.0083**	0.9039	0.9756
LWR	0.0891	0.6651	0.9616
Height	**<0.0001**	0.4734	0.924
Number of tillers	**<0.0001**	**<0.0001**	**<0.0001**
Number of leaves	**<0.0001**	**0.0002**	**<0.0001**
Harvest 2			
Root DW	**<0.0001**	**0.0296**	0.3474
Stem DW	**<0.0001**	0.5734	0.6142
Leaf DW	**<0.0001**	0.0969	0.499
Inflorescence DW	**0.0001**	0.1295	0.5719
Total DW	**<0.0001**	0.1924	0.7944
LA	**<0.0001**	**0.0026**	0.2963
SLA	**0.0191**	0.9599	0.7785
RWR	**<0.0001**	**0.0038**	0.8922
LWR	**<0.0001**	0.7684	0.8344
Height	**<0.0001**	0.089	0.2077
Number of tillers	**<0.0001**	**<0.0001**	**0.0002**
Number of leaves	**<0.0001**	**<0.0001**	**<0.0001**

Table 4
The effect of the interaction between seed provenance and water depth on the number of tillers and leaves at Harvests 1 and 2. Values are least square means; parentheses enclose standard errors of the means. Values in rows with the same letter indicate that significant differences were not detected (alpha = 0.05).

	JP	AR-1	AR-2
Harvest 1			
Number of tillers			
Drought	1.0 (0.08) a	1.1 (0.08) a	1.0 (0.08) a
Surface	1.4 (0.3) b	3.5 (0.3) a	3.5 (0.3) a
Mid	1.0 (0.1) a	1.3 (0.1) a	1.3 (0.1) a
Bottom	1.0 (0.0) a	1.0 (0.0) a	1.0 (0.0) a
Number of leaves			
Drought	5.9 (0.4) a	6.3 (0.4) a	5.9 (0.4) a
Surface	8.1 (1.3) b	15.0 (1.3) a	15.5 (1.3) a
Mid	5.8 (0.6) a	6.9 (0.6) a	6.9 (0.6) a
Bottom	4.9 (0.6) a	5.0 (0.6) a	5.4 (0.6) a
Harvest 2			
Number of tillers			
Drought	1.0 (0.0) a	1.0 (0.0) a	1.0 (0.0) a
Surface	2.6 (0.4) b	4.4 (0.4) a	5.1 (0.4) a
Mid	1.7 (0.3) b	2.9 (0.3) a	2.9 (0.3) a
Bottom	1.0 (0.2) a	1.5 (0.2) a	1.5 (0.2) a
Number of leaves			
Drought	7.0 (0.3) a	6.8 (0.3) a	7.1 (0.3) a
Surface	16.8 (1.7) b	27.0 (1.7) a	27.0 (1.7) a
Mid	8.6 (1.0) b	13.0 (1.0) a	13.3 (1.0) a
Bottom	6.4 (0.9) a	9.0 (0.8) a	6.9 (0.8) a

run. Submerged plants were significantly taller than plants in the surface treatments at the first harvest but not at the second harvest (Table 5). The drought treatment produced the shortest plants at both harvests. When height was analyzed through weekly measurements from 6 DAT to 48 DAT, site, run, and DAT combined explained 90% of the variation in plant height over time for the surface and bottom depth treatments (Table 6). Site and DAT explained 60% of the variation in height for the drought treatment while run and DAT explained 80% of the variation in the mid treatment (Table 6).

Differences among sites in total dry weight were only detected at the first harvest (Fig. 2). JP plants produced less root dry weight than AR-2 and AR-1 plants at the first and second harvests, respectively (Fig. 2). Differences in stem and leaf dry weights among sites were not detected at either harvest. Inflorescence dry weights did not differ among different sites at the second harvest. LA was smaller for JP plants than for plants from at least one AR site at both harvests (Table 7). No differences were found among sites for SLA, LWR, or height for either harvest. Root weight ratio did not differ among sites at the first harvest; however, AR-1 plants partitioned more biomass to roots than JP plants at the second harvest. There were no significant differences between any of the sites in their time to emergence ($p > 0.1$).

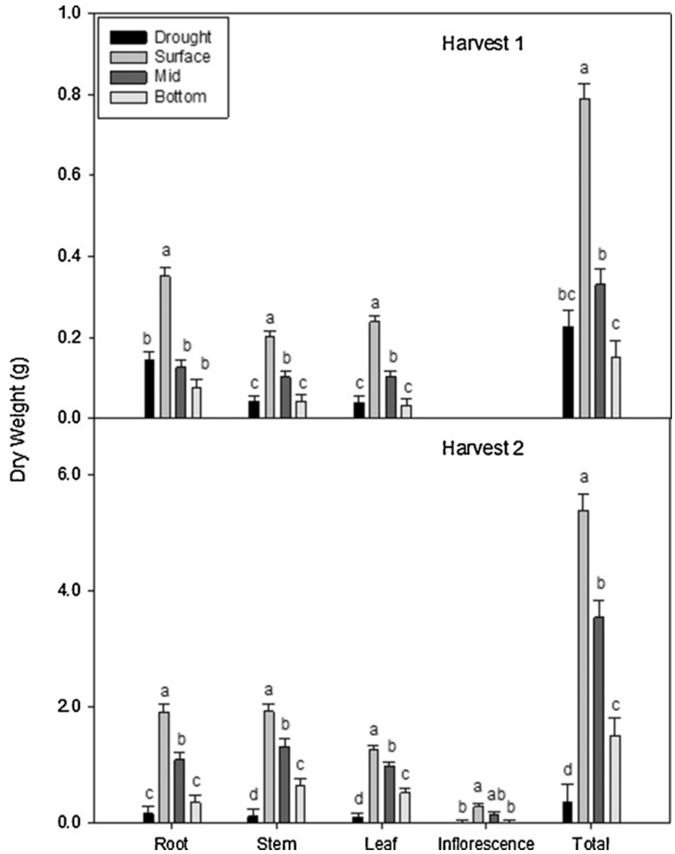

Fig. 1. The effect of water depth on plant dry weight at 27 DAT (Harvest 1) and 48 DAT (Harvest 2). Values are least square means and error bars are standard errors of the means. For each variable and harvest, columns with the same letter indicate that significant differences among means were not detected (alpha = 0.05).

Table 6
Regression equations, determined by a stepwise approach with selection criteria = 0.05, calculated for the weekly height measurements from 6 DAT to 48 DAT where the response variable y = height and the prediction variables x_1 = site, x_2 = run, and x_3 = DAT. Height data were square-root transformed before regression. N is the number of individuals used in the regression and the r^2 is the coefficient of determination for each full regression.

Treatment	N	Intercept	$b(x_1)$	$b(x_2)$	$b(x_3)$	r^2
Drought	164	3.1	0.2	–	0.05	0.6
Surface	166	3.0	−0.1	0.6	0.1	0.9
Mid	158	3.4	–	0.9	0.1	0.8
Bottom	155	−0.05	0.4	2.0	0.1	0.9

Table 5
The effect of water depth on leaf area (cm^2), specific leaf area ($cm^2\ g^{-1}$), root weight ratio (RWR), leaf weight ratio (LWR), and height (cm) at 27 DAT (Harvest 1) and 48 DAT (Harvest 2). Values are least square means pooled over the three sites of origin; parentheses enclose standard errors of the means. Within each harvest, treatments in a row with the same letter were not significantly different (alpha = 0.05).

	Drought	Surface	Mid	Bottom
Harvest 1				
LA (cm^2)	14 (5.0) c	79 (4.7) a	40 (4.7) b	17 (5.1) c
SLA ($cm^2\ g^{-1}$)	364 (42.8) b	347 (41.1) b	425 (41.1) b	640 (43.6) a
RWR	0.6 (0.05) a	0.5 (0.05) ab	0.4 (0.05) b	0.4 (0.05) b
LWR	0.20 (0.03) a	0.28 (0.03) a	0.31 (0.03) a	0.29 (0.03) a
Height (cm)	24 (3.4) c	45 (3.2) b	59 (3.2) a	64 (3.5) a
Harvest 2				
LA (cm^2)	23 (20.7) c	321 (20.3) a	274 (20.3) a	142 (20.7) b
SLA ($cm^2\ g^{-1}$)	246 (30.7) b	251 (30.0) b	291 (30.0) ab	370 (30.7) a
RWR	0.42 (0.02) a	0.34 (0.02) b	0.28 (0.02) c	0.18 (0.02) d
LWR	0.27 (0.02) c	0.26 (0.02) c	0.33 (0.02) b	0.40 (0.02) a
Height (cm)	34 (3.6) b	83 (3.5) a	89 (3.5) a	95 (3.6) a

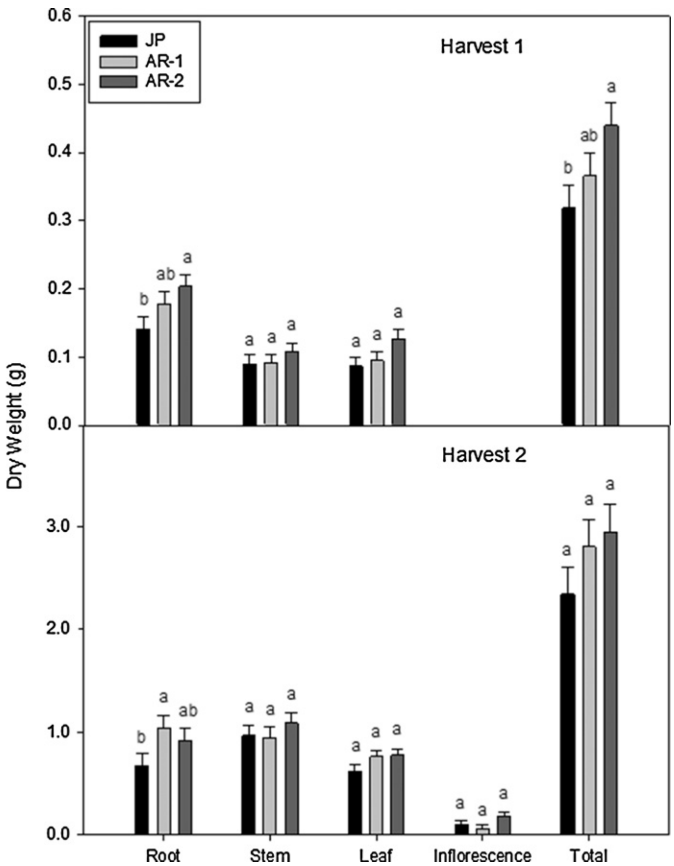

Fig. 2. The effect of site (seed provenance) on plant dry weight at 27 DAT (Harvest 1) and 48 DAT (Harvest 2). Values are least square means and error bars are standard errors of the means. For each variable and harvest, columns with the same letter indicate that significant differences among means were not detected (alpha = 0.05).

Table 7
The effect of seed provenance on leaf area (LA) (cm^2), specific leaf area (SLA) ($cm^2\ g^{-1}$), root weight ratio (RWR), leaf weight ratio (LWR), and height (cm). Values are least square means pooled over the four depth treatments; parentheses enclose standard errors of the means. Within each harvest, treatments in a row with the same letter were not significantly different (alpha = 0.05).

	JP	AR-1	AR-2
Harvest 1			
LA (cm^2)	28 (4.3) b	38 (4.3) ab	47 (4.2) a
SLA ($cm^2\ g^{-1}$)	394 (37.6) a	473 (37.5) a	465 (37.0) a
RWR	0.5 (0.05) a	0.5 (0.05) a	0.5 (0.05) a
LWR	0.3 (0.03) a	0.3 (0.03) a	0.3 (0.03) a
Height (cm)	47 (2.9) a	46 (2.9) a	51 (2.9) a
Harvest 2			
LA (cm^2)	142 (18.3) b	215 (18.0) a	213 (18.3) a
SLA ($cm^2\ g^{-1}$)	286 (26.5) a	287 (26.0) a	296 (26.5) a
RWR	0.27 (0.02) b	0.34 (0.02) a	0.32 (0.02) ab
LWR	0.3 (0.01) a	0.3 (0.01) a	0.3 (0.01) a
Height (cm)	70 (3.1) a	77 (3.0) a	79 (3.1) a

4. Discussion

The three stands of wild rice used in this study grew optimally in saturated, but not submerged, soils. Plants in the drought treatment exhibited signs associated with low water availability (reduced growth and partitioning to roots at the expense of leaves) while submerged plants exhibited signs related to low light availability (reduced growth, partitioning to leaves at the expense of roots, and increased height). These results are consistent with those recorded for *Z. aquatica* (Weber and Simpson, 1967; Thomas and Stewart, 1969), *Z. palustris* (Gemma et al., 1993), *Typha* spp. (Grace, 1989), *Leersia oryzoides* (Pierce et al., 2007), *Oryza sativa* L. (Zeng et al., 2003), and other aquatic plant species (Casanova and Brock, 2000; Warwick and Brock, 2003; Kercher and Zedler, 2004). *In situ* wild rice stands at the JP and AR sites were generally found rooted in less than 0.5 m of water but not above the water line (R. Tucker personal observations), suggesting that abiotic stress (low water availability for non-submerged plants and low light availability for submerged plants) may restrict these Indiana wild rice stands to a narrow range of water depths. Efforts to conserve or restore wild rice populations should focus on maintaining the water depth in rice stands within this range.

Differences in time to emergence between runs can be attributed to higher light levels in the second run than in the first run. In our experiment, seedlings were allowed to grow between 8 and 12 cm tall before being submerged. It seems likely that time to emergence would have been longer, and differences in biomass production between submerged and surface treatments greater, if plants were submerged at germination. However, Stevenson and Lee (1987) moved *Z. aquatica* plants from one depth to other depths at different stages in their phenology and found that the final biomass of *Z. aquatica* plants was not affected by the stage at which plants were placed in deeper water.

In addition to limits placed on its growth by abiotic factors, *Z. aquatica* establishment and development may be highly influenced by competition with other aquatic plant species. Rooted floating and aquatic emergent plants, such as spatterdock (*Nuphar advena* Ait.), water lily (*Nymphaea* spp.), pickerelweed (*Pontederia cordata* L.) and arrow arum (*Peltandra virginica* (L.) Kunth), inhabit the same near-shore environment as wild rice, but develop earlier in the season and may shade developing wild rice seedlings even in shallow water (Winterringer and Lopinot, 1977; R. Tucker personal observations). Once mature, wild rice may overtop these species, but during early establishment it may be sensitive to competition. Restoration and conservation efforts could benefit from the management of other vegetation until the wild rice is well established for the year or the appropriate choice of seedling location to minimize the impact of early season competition.

Seed provenance was also found to influence the vegetative growth of these *Z. aquatica* populations. Jasper-Pulaski produced less biomass and lower leaf area at the first harvest and fewer leaves and tillers at both harvests than the Allen–Rothenberger populations. Differences among plants in early growth may affect their ability to compete for resources and reproduce (Ellison and Rabinowitz, 1989; Weiner, 1990). Cumulatively, these results support the hypothesis that Indiana populations of wild rice differ in their response to water depth. Although the two AR stands were found in physically distinct areas that were separated by approximately 200 m, AR-1 and AR-2 had similar responses to water depth. AR-1 and AR-2 may share genetically similar traits that relate to depth tolerance while JP appears to be a distinctly separate population in regard to this environmental stress. In a study of isozyme markers in seventeen *Z. palustris* populations in northern Wisconsin, Lu et al. (2005) found high variation in the genetic diversity levels between lake populations, suggesting limited gene flow on a landscape level among lakes. Research is needed to determine the effect of seed provenance and genetic diversity on the success or failure of *in situ* wild rice restoration projects.

Acknowledgements

This study was made possible by the constant support provided by the faculty, staff, and students of the Botany and Plant Pathology Department at Purdue University, particularly those who assisted in both the logistic and physical setup of the greenhouse needed for each run. We are grateful for the generous access to seed, habitat, and knowledge extended to us by the Indiana Department of Natural Resources and its employees. We appreciate the efforts by Dr. Jan Vermaat and two anonymous reviewers to improve the paper.

References

Aiken, S.G., Lee, P.F., Punter, D., Stewart, J.M., 1988. Wild Rice in Canada. NC Press Limited, Toronto.

Bischoff, A., Vonlanthen, B., Steinger, T., Müller-Schärer, H., 2006. Seed provenance matters – effects on germination of four plant species used for ecological restoration. Basic Appl. Ecol. 7, 347–359.

Casanova, M.T., Brock, M.A., 2000. How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? Plant Ecol. 147, 237–250.

Counts, R.L., Lee, R.F., 1990. Patterns of variation in Ontario wild rice (*Zizania aquatica* L.) 4. Influence of regional and local environmental factors on variation within and among field populations. Aquat. Bot. 36, 193–205.

Ellison, A.M., Rabinowitz, D., 1989. Effects of plant morphology and emergence time on size hierarchy formation in experimental populations of two varieties of cultivated peas (*Pisum sativum*). Am. J. Bot. 76, 427–436.

Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst. 24, 217–242.

Emery, N.C., Stanton, M.L., Rice, K.J., 2009. Factors driving distribution limits in an annual plant community. New Phytol. 181, 734–747.

Gemma, T., Miura, H., Hayashi, K., 1993. Effects of water depth and temperature on the seedling growth of wild rice, *Zizania palustris* L. Jpn. J. Crop Sci. 62, 414–418.

Grace, J.B., 1989. Effects of water depth on *Typha latifolia* and *Typha domingensis*. Am. J. Bot. 76, 762–768.

Gustafson, D.J., Gibson, D.J., Nickrent, D.L., 2004. Competitive relationships of *Andropogon gerardii* (Big Bluestem) from remnant and restored native populations and select cultivated varieties. Funct. Ecol. 18, 451–457.

Hayes, P.M., Stucker, R.E., Wandrey, G.G., 1989. The domestication of American wildrice (*Zizania palustris*, Poaceae). Econ. Bot. 43, 203–214.

Hufford, K.M., Mazer, S.J., 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends Ecol. Evol. 18, 147–155.

Horne, F.R., Kahn, A., 2000. Water loss viability in *Zizania* (Poaceae) seeds during short-term desiccation. Am. J. Bot. 87, 1707–1711.

Kennedy, M.P., Murphy, K.J., Gilvear, D.J., 2006. Predicting interactions between wetland vegetation and the soil-water and surface-water environment using diversity, abundance and attribute values. Hydrobiologia 570, 189–196.

Kercher, S.M., Zedler, J.B., 2004. Flood tolerance in wetland angiosperms: a comparison of invasive and noninvasive species. Aquat. Bot. 80, 89–102.

Lu, Y., Waller, D.M., David, P., 2005. Genetic variability is correlated with population size and reproduction in American wild-rice (*Zizania palustris* var. *palustris*, Poaceae) populations. Am. J. Bot. 92, 990–997.

McKay, J.K., Christian, C.E., Harrison, S., Rice, K.J., 2005. "How local is local?" – a review of practical and conceptual issues in the genetics of restoration. Restor. Ecol. 13, 432–440.

Meeker, J.E., 1996. Wild-rice and sedimentation processes in a Lake Superior coastal wetland. Wetlands 16, 219–231.

O'Brien, E.K., Mazanec, R.A., Krauss, S.L., 2007. Provenance variation of ecologically important traits of forest trees: implications for restoration. J. Appl. Ecol. 44, 583–593.

Oelke, E.A., 1993. Wild Rice: Domestication of a Native North American Genus. New Crops. Wiley, New York, pp. 235–243.

Peden, D.G., 1982. Factors associated with growth of wild rice in northern Saskatchewan. Arctic 35, 307–311.

Pierce, S.C., Pezeshki, S.R., Moore, M.T., 2007. Ditch plant response to variable flooding: a case study of *Leersia oryzoides* (rice cutgrass). J. Soil Water Conserv. 62, 216–224.

Pillsbury, R.W., McGuire, M.A., 2009. Factors affecting the distribution of wild rice (*Zizania palustris*) and the associated macrophyte community. Wetlands 29, 724–734.

Renison, D., Cingolani, A.M., Suarez, R., Menoyo, E., Coutsiers, C., Sobral, A., Hensen, I., 2005. The restoration of degraded mountain woodlands: effects of seed provenance and microsite characteristics on *Polylepis australis* seedling survival and growth in Central Argentina. Restor. Ecol. 13, 129–137.

Steeves, T.A., 1952. Wild rice: Indian food and a modern delicacy. Econ. Bot. 6, 107–142.

Stevenson, S.C., Lee, P.F., 1987. Ecological relationships of wild rice, *Zizania aquatica*. 6. The effects of increases in water depth on vegetative and reproductive production. Can. J. Bot. 65, 2128–2132.

Terrell, E.E., Peterson, P.M., Reveal, J.L., Duvall, M.R., 1997. Taxonomy of North American species of *Zizania* (Poaceae). SIDA Contrib. Bot. 17, 533–549.

Thomas, A.G., Stewart, J.M., 1969. The effect of different water depths on the growth of wild rice. Can. J. Bot. 47, 1525–1531.

Thompson, A.L., Luthin, C.S., 2004. Wetland Restoration Handbook for Wisconsin Landowners. Bureau of Integrated Science Services Wisconsin Department of Natural Resources, Madison.

USDA, 2010. Plants profile: *Zizania* genus. In: Plants Database. United States Department of Agriculture, Natural Resources Conservation Service, http://plants.usda.gov/java/ClassificationServlet?source=profile&symbol=ZIZAN&display=63.

Van Katwijk, M.M., Wijgergangs, L.J.M., 2004. Effects of locally varying exposure, sediment type and low-tide water cover on *Zostera marina* recruitment from seed. Aquat. Bot. 80, 1–12.

Vennum, T., 1988. Wild Rice and the Ojibway People. Minnesota Historical Society Press, St. Paul.

Warwick, N.W.M., Brock, M.A., 2003. Plant reproduction in temporary wetlands: the effects of seasonal timing, depth, and duration of flooding. Aquat. Bot. 77, 153–167.

Weber, R.P., Simpson, G.M., 1967. Influence of water on wild rice (*Zizania aquatica* L.) grown in a prairie soil. Can. J. Bot. 47, 657–663.

Weichel, B.J., Archibold, O.W., 1989. An evaluation of habitat potential for wild rice (*Zizania palustris* L.) in northern Saskatchewan. Appl. Geogr. 9, 161–175.

Weiner, J., 1990. Asymmetric competition in plant populations. Trends Ecol. Evol. 5, 360–364.

Winterringer, G.S., Lopinot, A.C., 1977. Aquatic Plants of Illinois. Department of Registration & Education, Illinois State Museum Division and Department of Conservation, Division of Fisheries, Springfield.

Zeng, L., Lesch, S.M., Grieve, C.M., 2003. Rice growth and yield respond to changes in water depth and salinity stress. Agric. Water Manage. 59, 67–75.