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Note

Desiccation tolerance of introduced flowering rush (*Butomus umbellatus*)

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INTRODUCTION

The ability of aquatic or wetland plants to resist or withstand (i.e., tolerate) desiccation could be critical for surviving stressful periods of drying, such as during seasonal drought, operational dewatering of reservoirs, or overland transport by animals or humans. This has received some attention because of its importance in determining viability of fragments of some aquatic plant species (Jerde et al. 2012, Barnes et al. 2013, Bickel 2015), of which many of the most harmful invaders can be spread by transport on watercraft trailers (Johnson et al. 2001, Rothlisberger et al. 2010). Within invading species' populations, genetic differences due to separate introductions or other genetic selection events (e.g., founder or bottleneck processes), can lead to substantial differences in key traits (Hodgins et al. 2018), which can result in differences in response to management. For example, genetic differences between introduced populations of hydrilla (*Hydrilla verticillata* L.f. Royle) might be responsible for lack of biological control success in certain areas (Grodowitz et al. 2010, Harms and Grodowitz 2011), and hybridization in some watermilfoil (*Myriophyllum* spp.) populations has been linked to variation in response to herbicides (Larue et al. 2013, Parks et al. 2016, Thum et al. 2017). Differences between genetic lineages of an invasive plant have not been examined with regard to desiccation, but differences in the ability of genotypes to survive periods of drying has implications for risk assessments and prioritization of management, particularly if the geographic distribution of introduced genotypes is known. Drawdowns, the management strategy of reducing water level within a waterbody for a period of time to impact viability of target plants (Poovey and Kay 1998, Barrat-Segretain and Cellot 2007, Dugdale et al. 2013, Hussner et al. 2017), or operations that dewater reservoirs for flood control and fish and wildlife habitat, can have varying impacts on the target species, depending on the degree and duration of dewatering and if there are genetic-based differences in tolerance to drying. Although desiccation tolerance has been compared among multiple species of interest (e.g., Barnes et al. 2013, Coughlan et al. 2018), to date there has not been a study to determine whether it varies within an invasive plant species, although some research has examined other species, such as the agriculturally important sorghum [*Sorghum bicolor* (L.) Moench] (Basnayake et al. 1993).

The Eurasian aquatic plant, *Butomus umbellatus* L. (flowering rush) has been introduced into the United States multiple times, which is evident in the genetic diversity in invaded populations. There are currently eight Amplified Fragment Length Polymorphism (AFLP) genotypes (G1, G2, G3, G4, G5, G6, G7, G8) within two cytotypes (diploid and triploid) present in North America (X. X. Gaskin, pers. comm.). Although there is some overlap in geographic distribution between genotypes in the upper midwestern and northeastern United States, they are mostly separated with the common triploid genotype G1 present in the Pacific northwest and the common diploid genotype G4 in the northeast and midwestern United States (Eckert et al. 2003). All other genotypes are relative rare, only represented by one or two populations in the US (X. X. Gaskin, pers. comm.). In the United States, flowering rush spreads primarily through vegetative growth and fragmentation although viable seeds can be produced by diploid plants in some cases (Lui et al. 2005). Flowering rush grows in a variety of substrates and water depths but is capable of surviving periods of drying due to natural or scheduled drawdowns (Parkinson et al. 2010, Madsen et al. 2017). Additionally, grubbing/uprooting of flowering rush plants by vertebrates can increase downstream spread of fragments (Harms and Shearer 2015). To date, there has been no examination of desiccation tolerance within flowering rush, nor a comparison between introduced genotypes. In the current study, we examined differences between two North American flowering rush genotypes (G1 and G4) with regard to their ability to resprout following periods of drying. We used replicate G1 and G4 populations in a common garden to determine whether viability of dried plants differs between introduced genotypes.

MATERIALS AND METHODS

This experiment was conducted in a greenhouse at the US Army Engineer Research and Development Center, Vicksburg, MS (32.31°N, 90.87°W). Flowering rush plants were originally collected from field sites in 2015 to 2016,

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then propagated in culture over 2 yr before experiments began. We used the following populations for this work: Lake Pend Oreille, ID (N48.18, W116.24; G1); Columbia River, WA (N46.25, W119.22; G1); Mississquoi River, VT (N44.95, W73.16; G1), and Oswegatchie River, NY (N44.69, W75.49; G4); Killdeer Pond, OH (N40.80, W83.37; G4); and Unity Island, NY (N42.93, W78.91; G4). All plants were maintained in a common garden, and genotype was determined prior to experimentation (X. X. Gaskin, pers. comm.). Propagating plants over multiple years in a common garden was done to reduce maternal effects and ensure we were measuring the effect of interest (desiccation tolerance), not lingering effects of the environment where they were collected (Roach and Wulff 1987). Plants were cultured in commercial topsoil amended with slow-release fertilizer[1] in outdoor tanks. Prior to the experiment, plants were harvested and propagated in the same topsoil in seed starter trays. Diploid (G4) plants typically produce very little rhizome material but abundant bulbils (Eckert et al. 2000, Lui et al. 2005), so we planted bulbils in trays, allowed them to sprout and grow for 3 to 4 wk, and then used those plants. G1 plants were harvested from culture, rhizomes were separated into approximately 4- to 5-cm segments which contained buds, then planted in seed-starter trays to sprout. G1 rhizomes were planted approximately 3 wk after diploid bulbils so that resulting plants would be similar sizes at the beginning of the experiment.

Whole plants were sorted into similar sizes (~30 cm tall), weighed, and laid on an aluminum wire table to dry in a greenhouse covered with 50% shade fabric. Initially (0 hr), 25 plants per population were weighed and placed on the table. Thereafter, weights were recorded at 4 h, 24 h (1 d), 48 h (2 d), 120 h (5 d), and 240 h (10 d) drying. After weighing at each time period, five plants per population (desiccated leaves intact) were randomly selected and placed into 1-L plastic observation containers with 500-ml, carbon-filtered, municipal-delivered tap water and observed for sprouting. Observations were continued for 30 d after the last time period of 10 d of exposure to desiccation. Water was replaced in observation containers every 2 to 3 d. Sprouting was identified as new green growth which persisted for several days. Temperature on the greenhouse table was monitored every 5 min with a temperature data logger[2] enclosed in a vented box, which allowed air movement over the data logger but minimized solar heating. Average temperatures during the study period were 30.1 ± 0.44 C (day) and 25.95 ± 0.25 C (night). For each plant, the following data were recorded: initial (fresh) mass, mass at each experimental time point, mass at time point when placed in water, and sprouting success (yes/no). For plants that successfully sprouted, days until sprouting was determined.

Statistical approach

First, we tested whether there were differences in the desiccation rate between the common genotypes G1 and G4. For each population, exponential decay functions were fitted for each plant weighed on all dates (i.e., those that were harvested on day 10) and the unitless decay rate parameter (i.e., population-specific desiccation rate; *b*) was determined (Jerde et al. 2012). Then, we used a general linear mixed model (GLMM) to test for genotype differences in *b*. In the GLMM, desiccation rate (*b*) was the dependent variable, genotype was a fixed effect, and population was nested within genotype as a random effect. Because initial plant size might account for some variation in desiccation tolerance, initial plant weight was included as a covariate in the GLMM. If differences in the rate of drying existed, it could explain viability differences at various time points of the experiment. Next, to determine whether there are differences in desiccation tolerance (i.e., ability to resprout after drying) between common flowering rush genotypes, we used a generalized linear mixed model (GLZ) with binary error distribution and log-link function. Successful sprouting (y/n) was the dependent binary variable in the model; genotype, days dried, and the genotype by days-dried interaction were included as fixed variable predictors. Additionally, population was nested within genotype as a random variable. Finally, we tested whether the time it took plants to sprout after being placed in fresh water was related to the duration of drying and whether the relationship between drying time and time to sprouting varied by genotype. For this, we used a GLMM with genotype, days dried, and the genotype by days dried interaction as fixed variables, population nested in genotype as a random variable, and initial plant weight as a covariate. Statistical analyses were conducted with SAS ver. 9.4 or Statistica ver. 12 and significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The desiccation rate parameters of populations ranged between −0.8426 (Oswegatchie River) and −1.2249 (Mississquoi River) but were not significantly different between genotypes (Figure 1A; $F = 1.63$, $df = 1, 3.7$, $P = 0.27$). Measured rates are at the low end of those determined for a number of invasive aquatic plants, although a direct comparison of desiccation rate between this and other published studies (e.g., Jerde et al. 2012, Barnes et al. 2013, Coughlan et al. 2018) is not possible because of variation in drying protocols, in particular the environment in which drying takes place. Others have used forced-air to simulate overland transport on watercraft trailers or conducted studies in controlled-climate rooms (Jerde et al. 2012, Barnes et al. 2013, Bickel 2015, Coughlan et al. 2018). Although we did not apply forced-air to drying plants, we believe that allowing plants to dry at ambient temperatures is likely to realistically reproduce temperature fluctuations that plants might experience during summer conditions, and elevated temperatures in combination with desiccation might be a better predictor of survival and viability than desiccation alone (Coughlan et al. 2018). Given that some flowering rush-infested sites experience operational drawdowns during winter (Madsen et al. 2017), it could be important to consider response of plants to drying under winter conditions (e.g., freezing temperatures) as well.

Resprouting of plants after drying was dependent on the degree of desiccation/drying duration and decreased as the

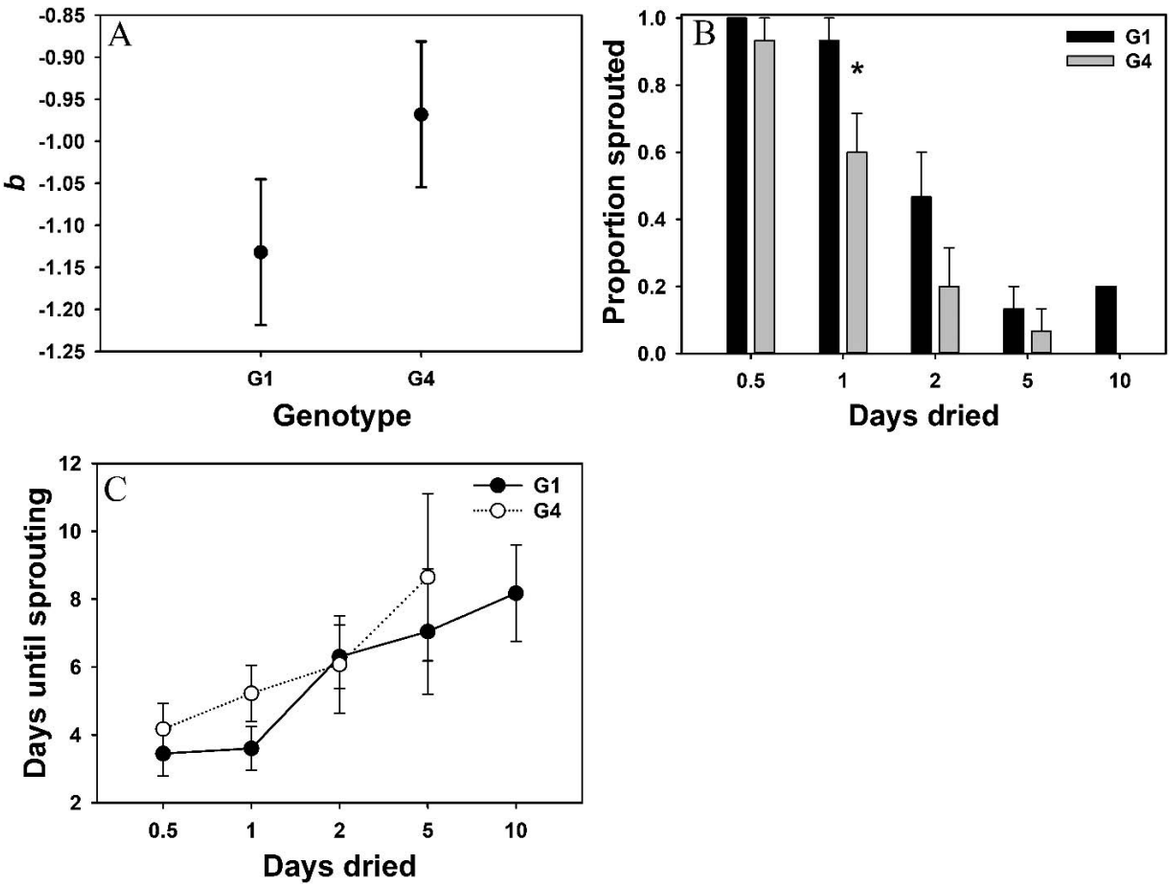

Figure 1. (A) Mean ± SE desiccation rate parameter (*b*) for flowering rush populations, (B) the mean ± SE proportion of flowering rush plants that sprouted, and (C) days until sprouting, after experiencing various drying times in a greenhouse study. In (B), the asterisk denotes a marginally insignificant difference between mean sprouting at 24 h (df = 1,134, F = 3.65, $P = 0.058$).

drying time increased (Figure 1B). There were no statistical differences between G1 (triploid) and G4 (diploid) plants with regard to desiccation tolerance overall ($F < 0.01$, df = 1,4, $P = 0.98$) but the number of days dried was a significant predictor of sprouting ($F = 87.82$, df = 4,134, $P < 0.001$). Additionally, the interaction between drying duration and genotype was nonsignificant ($F = 0.24$, df = 3,134, $P = 0.87$). Differences between G1 and G4 plant survival were minor and insignificant at early (after 4 h) and late (5 and 10 d) time points. However, at 24 h, mean sprouting of G1 plants was 35% higher (0.93 vs. 0.60) than G4 plants. Likewise, at 48 h, sprouting of G1 plants was more than double that in G4 plants (0.47 vs. 0.20). Survival differences might be related to the biomass differences between G1 and G4 plants used in our study; G1 plants were slightly larger at initiation of the experiment (G1 initial weight: 7.3 ± 0.54 g; G4 initial weight: 4.87 ± 1.92 g), but mass differences were reduced by 1 (G1: 2.4 ± 0.17 g; G4: 1.78 ± 0.93 g), and 2 d (G1: 1.65 ± 0.11 g; G4: 1.16 ± 0.55 g) of drying.

Importantly, despite the harshness of our experimental setup, survival of G1 plants was still positive after 10 d (Figure 1B). In fact, plants of both genotypes sprouted after drying for as long as 5 d. These results support the conclusion that drawdown and desiccation might not be adequate to kill flowering rush plants, depending on the degree of drying experienced during the drawdown. Because our experiment was conducted with plants drying in the air, and not drying in soil, the rates of desiccation were much higher and the amount of sprouting likely lower than would be observed in the field. Thus, the time frame in which we observed differences between genotypes in a greenhouse (1 to 5 d) might translate into a much longer duration in relevant field settings, depending on field desiccation rates. Other scenarios in which desiccation tolerance might be important would be when herbivory by vertebrates (i.e., muskrats, waterfowl) leads to uprooted plants that strand on the shore or drying related to transport on watercraft trailers. Our study demonstrates a great capacity for survival even with extreme drying. The microclimates experienced by uprooted plants on shore are likely humid/ damp, leading to higher survival over a longer period than we report here. No difference was found between genotypes for the length of time needed to observe sprouting during the experiment ($F = 0.02$; df = 1, 3.52; $P = 0.89$) or the interaction between genotype and duration of drying ($F = 1.35$, df = 1, 61.5, $P = 0.25$). However, the duration of drying was a significant predictor of the length

of time needed to observe sprouting (Figure 1C; $F = 9.40$, df $= 1,62$, $P = 0.003$).

Our approach to use a common garden, with plant clones from geographically diverse populations, allows for a robust test of desiccation tolerance between flowering rush genotypes. Although our sample size was limited (three G1 and three G4 populations), this work suggests that despite a lack of differences in the rate of desiccation between genotypes, there might be differences in survival at intermediate time points. Additionally, we used whole plants to test effects of desiccation on resprouting, but it might be appropriate in the future to focus on desiccation and viability of bulbils (diploid populations) and rhizome buds (diploid and triploid populations), because disturbance that causes fragmentation of these parts has been suggested as the likely mode of spread (Parkinson et al. 2010).

Preventing establishment of invasive plants by identifying and managing spread pathways saves time and money that might otherwise go towards managing infestations where established (Leung et al. 2002, Hulme 2009, Epanchin-Niell 2017). Overland transport of vegetative propagules by wildlife or transport on watercraft trailers that move between waterbodies are pathways for spread of clonal aquatic and wetland plants (Rothlisberger et al. 2010). Therefore, an understanding of how aquatic species respond to drying conditions could inform risk assessments of new and emerging species or offer the ability to better tailor management strategies for species with genetically distinct lineages, as in the case of flowering rush in the United States. Intraspecific differences in desiccation tolerance could lead to variable management success if drying or drawdowns are used to control invasive aquatic plants. Although our study demonstrates variability in survival between introduced cytotypes of flowering rush, next research steps should focus more on field conditions to generate conclusions that can be more directly applied by aquatic vegetation managers.

SOURCES OF MATERIALS

[1]Osmocote®, 15–9–2, Scotts Miracle-Gro, Marysville, OH XXXXX.

[2]HOBO® Pendant Temperature data logger, Onset®, Cape Cod, MA XXXXX.

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