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Effects of Hot Water on Breaking Seed Dormancy of the Endangered Kankakee Mallow, *Iliamna remota* Greene (Malvaceae)

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Abstract: *Iliamna remota* Greene (Kankakee mallow, Malvaceae) is listed as endangered in Illinois and is endemic to Langham Island in the Kankakee River, Kankakee County, Illinois. Information on ways to break seed dormancy of *I. remota* would be useful for restoration and management. The purpose of this study was to determine if hot water at different temperatures and for different lengths of time would break seed dormancy. Seeds were dipped for 60 seconds into water at 70, 80, 90 or 100°C with 22°C dips as a control. In another trial, seeds were dipped into 80°C for 0, 10, 20, 30 or 60 seconds. Each treatment included six replications with 25 seeds each. Seeds were germinated in Petri dishes in a seed germinator at 25°C with continuous light. Germinated and fungal contaminated seeds were counted for three weeks. When comparing temperatures with dips for 60 seconds, germination percentages were significantly greatest with dips at 80°C (63%), lower at 70°C (46%), still lower at 90°C (29%), and lowest at 100°C (15%) or control (9%). When comparing times with dips at 80°C, 20 seconds was the best time. Hot water dips for 60 seconds at all temperatures significantly decreased contamination (< 5%) relative to the control (15%). Thus, hot water dips at 80°C provided the best hot water dip for breaking seed dormancy of *I. remota* when using seeds to grow plants for restoration. In addition, our findings suggest that high soil temperatures created during burns for management could enhance seed germination in soils.

Introduction

Iliamna remota Greene (Kankakee mallow, Malvaceae) is a state endangered species in Illinois, endemic to Langham Island in the Kankakee River, Kankakee County, Illinois (Herkert and Ebinger 2002, Illinois Endangered Species Protection Board 2005, Schwegman 1991, USDA-NRCS 2007). The species was first discovered on the island by Reverend E. J. Hill in 1872 (Sherff 1946, Strausbaugh and Core 1932). In 1966, Langham Island was dedicated as an Illinois Nature Preserve with the sole purpose of preserving the only known native population of *I. remota* (McFall and Karnes 1995). Populations also occur in Indiana and Virginia where it is presumed to be introduced by efforts of the Wild Flower Preservation Society of Chicago in the 1920s when seeds were spread along railroads (Glass *et al*. 2003, Jacobs 1992, Keener 1964, Sherff 1949, Swinehart and Jacobs 1998). *Iliamna corei* (Sherff) Sherff recently was reported as a synonym of *I. remota* based upon DNA analysis (Bodo Slotta and Porter 2006), although an earlier study with DNA analysis concluded that *I. corei* was a subspecies of *I. remota* (Stewart *et al*. 1996). *Iliamna corei* (*I. remota*) occurs in only one location in Virginia (Baskin and Baskin 1997). Another related species, *I. longisepala* (Torr.) Wiggins, is a rare endemic forb known from three counties in Washington (USDA-NRCS 2007).

In Illinois, *I. remota* plants are found on slopes with rocky, gravelly or sandy soils that are well drained (Glass *et al*. 2003, Schwegman 1984). Plants of *I. remota* are shade intolerant requiring full sun, and hence perform best in open, sunny areas (Baskin and Baskin 1997, Glass *et al*. 2003, Keener 1964, Schwegman 1984). Management to maintain *I. remota* on Langham Island began in 1983 to keep these areas shade-free involving removal of invasive shrubs (especially *Lonicera maackii* (Rupr.) Maxim. and *Rosa multiflora* Thunb.) via herbicide and burning (Glass *et al*. 2003, Schwegman 1991).

Iliamna remota reproduces both from vegetative structures and from seeds. Plants can spread vegetatively

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from thick, vigorous rootstocks, although this reproduction varies with habitat (Wadmond 1932). Root crowns develop in September for the next year's growth, and these buds usually are not harmed by freezing or by fire (Schwegman 1984). New plants also are reported to propagate easily from seeds when being used to cultivate plants for use in state parks, roadsides or private lands (Flood *et al*. 2000). On Langham Island, plants flower in July to August with pink-purple flowers that are most likely pollinated by insects, and small but heavy seeds are shaken from capsules in the end of September (Glass *et al.* 2003, Schwegman 1984). Seeds of *Iliamna remota* remain in the seed bank for at least 10 years (Glass *et al*. 2003), and remained viable for at least 4 years (Swinehart and Jacobs 1998). Schwegman (1984) reported that older seeds (2+ years old) germinated more readily than fresh seed. Although seeds are present, seedlings often are only found following an apparent stimulation by fire on Langham Island (Glass *et al*. 2003), which may be due to breaking of seed dormancy.

Iliamna remota seeds are dormant and may require treatment to break dormancy. Without seed pretreatment, only a few ($\leq$10%) *I. remota* seeds germinated (McDonnell, pers. obs.). However, when seeds were exposed to cold moist stratification (Flood *et al*. 2000), scarification (Swinehart and Jacobs 1998, Wadmond 1932), and hot water (Wadmond 1932, Hilscher and Preece 1994, McDonnell *et al*. 2006), higher seed germination than controls resulted. Unfortunately replicated trials were not included in these reports with warm or hot water. Dry heat scarification resulted in $>$ 80% germination when seeds were treated at 80°C for 30 or 60 minutes. *Iliamna corei (I. remota)* seeds subjected to 1 to 13 soaks in sulfuric acid for 1-hour periods followed by rinses with tap water resulted in as high as 90% germination. *Iliamna corei (I. remota)* seeds dipped in boiling water for 1, 3, 5, 10, and 20 seconds germinated as high as 93%, whereas germination declined for seeds dipped in boiling water longer than 20 seconds. Seeds dipped in boiling water for 50 seconds or longer were mostly killed (Baskin and Baskin 1997).

Burning also may aid the ability of *I. remota* to reproduce from seeds as dense stands of seedlings were found during July in areas where brush piles that were burned in April had created hot spots at Langham Island (Schwegman 1984). For *Iliamna corei (I. remota)* in greenhouse studies, seeds on the surface of soil in flats germinated when subjected to fire, breaking dormancy induced by a durable (hard) seed coat (Baskin and Baskin 1997). For *Iliamna longisepala*, fire also broke seed dormancy and more germination occurred when burn severity was higher rather than lower, and more occurred with fall burns than spring burns, when none were found (Harrod and Halpern 2005). Given the effects that fire has on increasing seedling numbers at Langham Island and seed germination in related species, further work is needed to determine how high temperatures could affect seed dormancy of *I. remota*.

Seeds of *I. remota* germinate poorly without treatments to break dormancy, and fire is reported to stimulate germination of these seeds. For related species, high temperatures including hot water dips effectively broke dormancy. The objective of this study was to determine how water dips at temperatures of 22 (control), 70, 80, 90 or 100°C, and for times of 0 (control), 10, 20, 30 or 60 seconds at 80°C affect seed germination of *I. remota*. This information should be useful for propagating plants in controlled environments for conservation and restoration efforts.

MATERIALS AND METHODS

Iliamna remota seeds were obtained from Prairie Moon Nursery (Winona, MN) in April of 2005. Seeds were purchased commercially rather than collected from natural populations such as Langham Island to eliminate possible negative impacts of seed removal from natural areas. Seeds were stored in a seed desiccator at 4°C and $\leq$ 40% relative humidity until used for this study in January 2006.

Temperature treatments

Seeds were dipped in 22 (room temperature control), 70, 80, 90 or 100°C water for 60 seconds. Twenty-five seeds were placed in each of six plastic Petri dishes (100 $\times$ 15 mm) for each temperature treatment. Petri dishes contained 3 sheets of Whatman #1 (Fisherbrand®, Pittsburg, PA) filter paper (90 mm) with 6 ml of distilled water. Seeds were dusted with a fungicide, thiram powder (50% active ingredient, tetramethylthiuram disulfide). Petri dishes were sealed with Parafilm® "M" Laboratory Film (Pechiney Plastic Packaging, Chicago, IL) to prevent moisture loss. Dishes were placed randomly in a plastic Rubbermaid® tub (33 cm $\times$ 24 cm $\times$ 10 cm) in a Percival Scientific® (Perry, IA) seed germinator with a mean ($\pm$ SE) temperature of 25 $\pm$ 0°C and continuous light (17 $\pm$ 1 μmol/m^2/sec). Light was recorded in nine random areas inside the seed germinator with an Apogee® (Logan, UT) quantum sensor. Numbers of germinated (as measured by radicle emergence) and fungal contaminated seeds were recorded daily for 20 days when germination percentages had reached a plateau. Contamination counts help assess what percentage of seeds may have not germinated due to fungal contamination as opposed to other factors.

Time treatments

Seeds were dipped in 80°C water for 0, 10, 20, 30 or 60 seconds. The same germination protocol was

Table 1: Mean (± SE) percentage of seeds germinated or contaminated after 20 days following water dips for 60 seconds at different temperatures. ANOVA results reported. Different lower case letters denote significant differences within a column (Duncan's multiple range test).

Temperature (°C)	Germination (%) F = 27.49; P < 0.001	Contamination (%) F = 10.55; P < 0.001
22 (control)	9.3 ± 2.0 d	14.7 ± 3.4 a
70	46.0 ± 4.0 b	4.7 ± 2.4 b
80	63.3 ± 4.3 a	0.7 ± 0.7 b
90	29.3 ± 7.0 c	0.0 ± 0.0 b
100	14.7 ± 2.0 d	0.7 ± 0.7 b

utilized for testing time as for temperature. Numbers of germinated and contaminated seeds were recorded weekly for three weeks when germination percentages had reached a plateau.

Statistical analyses

Means and standard errors were calculated for the number of germinated and contaminated seeds. All data were analyzed using Analysis of Variance (ANOVA) followed by Duncan's multiple range test at 5% level to determine significance. Data were analyzed with SPSS 15.0 for Windows (2006) and Microsoft® Office Excel version 12 (2007).

RESULTS

Temperature treatments

A significantly greater percentage of seeds germinated after 20 days when dipped in water at 80°C (63.3%) followed by 70°C (46.0%), and then 90°C (29.3%) (Table 1). The lowest percentage of seeds germinated when dipped in a control treatment (22°C) or 100°C water, which did not significantly differ from each other. Seeds dipped in 70°C or 80°C water also germinated at a more rapid rate compared to other temperatures (Figure 1). Dipping the seeds in hot water treatments also decreased the number of fungal contaminants as the control (14.7%) had significantly more contamination compared to other treatments (Table 1).

Time treatments

Seeds dipped in 80°C water for 20 seconds resulted in significantly higher percent germination (54.7%) compared to all other soaking times (Table 2). Although percent germination was not significantly different among seeds exposed to 10, 30 or 60 seconds, seeds that were not subjected to hot water (control) resulted in significantly lower percent germination (Table 2). Germination rates were slightly higher with dips for 20 seconds relative to other times, and much lower for the control relative to all other times (Figure 2). Contamination was not significantly different between treatments for different times (Table 2).

DISCUSSION

Germination varied following dips of seeds into water of different temperatures. The 70°C water dip had a lower number of seeds germinated than the 80°C water dip, but still a greater number of seeds germinated compared to other temperatures. The optimal temperature (80°C) in this study closely parallels the temperature (82°C) used by Hilscher and Preece (1994), although germination percentages or tests of other temperatures and times were not reported in

Table 2: Mean (± SE) percentage of seeds germinated or contaminated after three weeks following water dips at 80°C for different times. ANOVA results reported. Different lower case letters denote significant differences within a column (Duncan's multiple range test).

Time (seconds)	Germination (%) F = 14.88; P < 0.001	Contamination (%) F = 0.72; P = 0.585
0 (control)	9.3 ± 1.7 c	8.7 ± 3.3 a
10	38.7 ± 6.2 b	10.0 ± 4.8 a
20	54.7 ± 3.5 a	7.3 ± 2.6 a
30	40.7 ± 4.4 b	5.3 ± 3.0 a
60	33.3 ± 4.3 b	2.7 ± 2.7 a

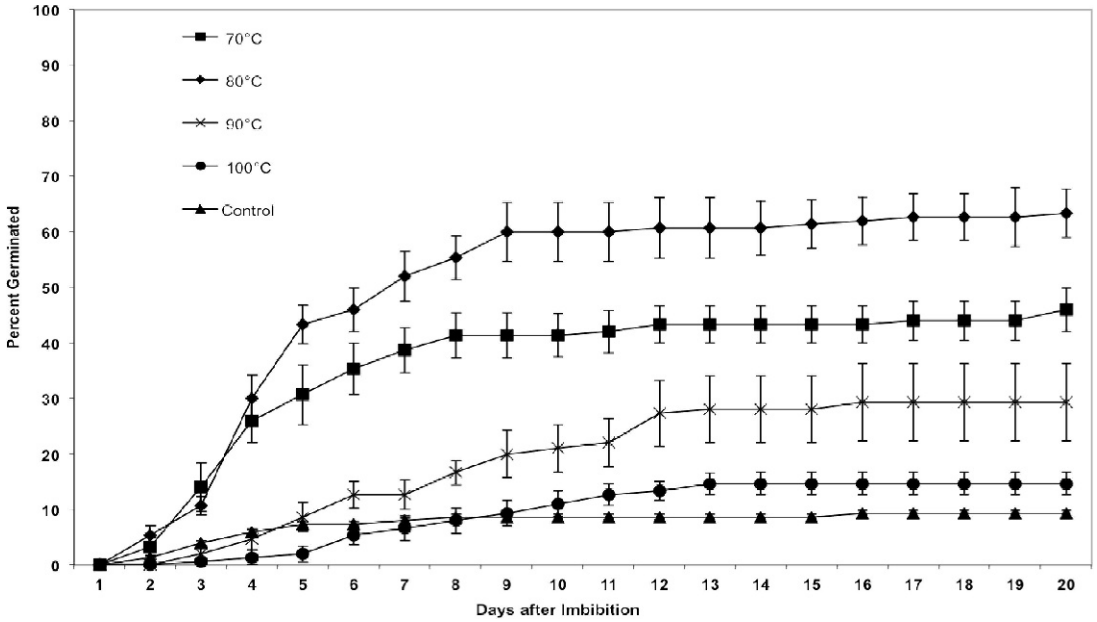

Figure 1. Mean percentages (± standard errors) of seeds germinated per day following water dips for 60 seconds at different temperatures.

their study. Seeds dipped in both 70 and 80°C water germinated earlier than seeds exposed to all other temperatures, indicating effective temperatures for breaking dormancy. Temperatures above 80°C may have been too hot, thus injuring or killing embryos in seeds, and resulting in lower germination percentages. Hence hot water dips at different temperatures affected germination.

When different times were tested at 80°C, seed germination was significantly higher after a 20 second exposure (54.7%) compared to other times. The length of time per water dip also affected the percentage of *I. corei (I. remota)* seeds germinated, when the number of *I. corei* (*I. remota)* seeds germinating declined after being dipped for longer than 20 seconds in boiling water (Baskin and Baskin 1997). In our study, the percentage of germinated *I. remota* seeds declined after being dipped for longer or less than 20 seconds in 80°C water. Also, control seeds (those not dipped in hot water) resulted in significantly lower percent germination (9.3%) compared to seeds soaked in hot water. Control seeds of *I. corei (I. remota),* displayed even lower (1%) germination compared to the percentage (9.3%) of *I. remota* seeds that were dipped in room temperature water in our study (Baskin and Baskin 1997). These studies show that not treating seeds with hot water significantly decreases germination for *Iliamna remota*.

An inconsistent finding occurred after subjecting seeds to dips in 80°C water. In the initial temperature trial experiment (60 seconds), percent germination was 63.3%, but in the time trial experiment (60 seconds), percent germination was considerably lower (33.3%) when dishes were checked only once a week resulting in less frequent water additions. For temperature trials, dishes were checked daily, and additional water was added as needed. However, for time trials, dishes were checked only once a week, so water additions were less frequent. These differences in frequency of water additions may have affected germination in temperature vs. time trials.

Temperature and soaking time played a role on the incidence of seed-borne contaminants. The greatest percentage of contamination (14.7%) was in the treatment where seeds were dipped in room temperature (22°C) water for 60 seconds, as hot water treatments had significantly lower contamination compared to the room temperature control. Thus, dipping seeds in hot water effectively reduced contaminants compared to dipping seeds in room temperature water. The percentage of contamination was not significantly different between time treatments.

A significantly higher percentage of seeds germinated when dipped in water at 70 to 90°C compared to those exposed at ambient temperature or 100°C. Thus, hot water treatments were effective in breaking dormancy of *I. remota* seeds. Some species within Malvaceae are known to possess durable (hard) seed coats prone to weakening by hot water exposure

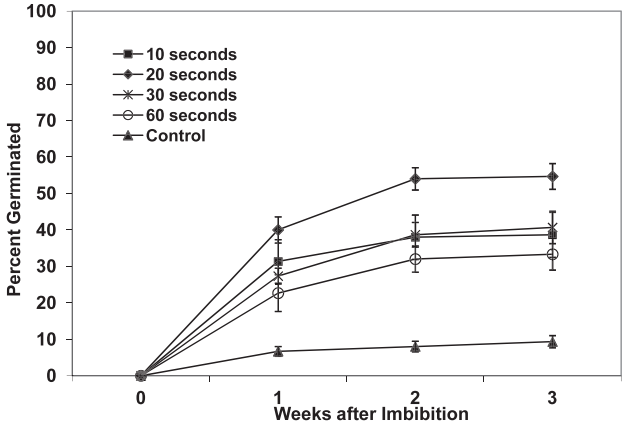

Figure 2. Mean percentages (± standard errors) of seeds germinated per week following water dips at 80°C for different times.

(Baskin and Baskin 1997). In this study, hot water effectively broke dormancy of *I. remota* seeds suggesting that physical seed dormancy in this species is attributed to a durable (hard) seed coat. Studies that test water imbibition following mechanical scarification would lend further support for this hypothesis. If true, overcoming seed coat-related dormancy *in situ* would have implications for the conservation of this species. For example, seed dormancy could conceivably be removed by higher temperatures triggered by fires, by freezing and thawing cycles, by wetting and drying, or a combination of these factors.

The information presented in this study should be useful for restoration and management of this rare species. For restoration purposes, *I. remota* may be grown in controlled environments where seed germination will be enhanced with hot water dips as reported in this study. For management of the *I. remota* population on Langham Island, previous studies showed that controlled burns dramatically increased seedling development (Glass *et al*. 2003). This increase following burns could relate to breaking seed dormancy from high temperatures (70–90°C) as temperatures found to increase germination are within the range of soil temperatures reported during fires in other habitats such as 50–225°C (Davis *et al.* 1989), 65–115°C (Auld 1986), and 70–125°C (Shea *et al.* 1979). Baskin and Baskin (1997) found that fire was a natural treatment for *I. corei (I. remota)* to break seed dormancy. Hence, controlled burns near populations of *I. remota* could help to break seed dormancy, in addition to control for woody invasive species. Thus, hot water may be useful for production of plants for use in restoration.

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