4B-Campbell-Martinez-2ndPlace.doc

Seed Germination of *Crocanthemum arenicola* (Coastalsand Frostweed)[©a]

Gabriel Campbell-Martínez[b], Anna Dicks, Mack Thetford and Debbie Miller

University of Florida, 5988 Highway 90, Building 4900, Milton, Florida 32583, USA

[b]Email: camp5595@ufl.edu

Keywords: Physical dormancy, *Helianthemum*, beach dune, scarification, endemic

SUMMARY

Crocanthemum arenicola (coastalsand frostweed) is a southeastern USA native plant with ornamental and restoration potential. Propagation information for this plant is lacking. However, other species of Cistaceae have seeds with physical dormancy that can be alleviated by scarification. The objective of the present study was to test the effects of scarification via sandpaper abrasion with an electric seed scarifier and photoperiod on germination of *C. arenicola*. Scarification for 50-200 seconds with an electric seed scarifier lined with sandpaper was sufficient to break physical dormancy with ≥90% germination for graded seed. Additionally, no difference in germination (63-64%) was detected between non-graded seeds exposed to a 12-hour photoperiod or left in the dark.

INTRODUCTION

Crocanthemum is a new world genus composed of 15 species within Cistaceae, the majority of which are native to the southeastern USA (Sorrie, 2011; USDA and NRCS, 2018). Plants are fire-tolerant perennial herbs to subshrubs that resprout readily from a woody caudex (eFloras, 2018). The species of interest in this study, *C. arenicola* (coastalsand frostweed), is an

---

[a]Second Place- Graduate Student Research Paper Competition

herbaceous, perennial groundcover species found on beach dunes, scrub, and sandhill plant communities (Wunderlin and Hansen, 2011; Fig. 1). It is endemic to 12 coastal counties of the Florida panhandle, Mississippi, and Alabama (USDA and NRCS, 2018). This plant has 2 types of flowers, showy (chasmagamous) yellow flowers and non-showy (cleistogamous) flowers (eFloras, 2018; Fig. 1).

Propagation information for *C. arenicola* is lacking. However other *Crocanthemum* and the closely related *Helianthemum* species have been reported to have physical dormancy that may be alleviated via scarification. It has been proposed to move *Crocanthemum* species from the *Helianthemum* genus (Sorrie, 2011). Mechanical scarification via sandpaper abrasion has been reported as an effective form of dormancy alleviation for several species of *Helianthemum* (Pérez-García et al., 1995; Pérez-García and González-Benito, 2006; Yeşilyurt et al., 2017). In these studies, seeds germinated in both light and dark conditions with mixed results. The objective of the present study was to describe basic information about seeds and fruits, physical dormancy, and germination for *C. arenicola*. We hypothesized that seeds of this plant are physically dormant and that photoperiod may affect germination. Additionally, we informally discuss a success story of containerized production of this plant from stem cuttings.

MATERIALS AND METHODS

Stock Plants. Stock plants were derived from spring-collected softwood-stem cuttings from wild plants growing in secondary dunes on Santa Rosa Island and Perdido Key, Florida. Rooted cuttings were grown in 1-gallon plastic containers with a 2:1 mix of pine bark:Sungro MetroMix® 830 and top-dressed with 0.5 tablespoon of Osmocote® 18-6-12. Plants were hand watered as needed and subjected to a natural photoperiod in a research greenhouse (University of Florida, Milton Campus).

Fruit and Seed Collection, Characteristics, and Storage. Fruits were collected from stock plants as fruits turned brown and fell off or as fruits fell off when the plant was shaken slightly throughout the fall and winter of 2017. Seeds were separated from fruit by hand and air dried under laboratory conditions (~25ºC and 70% RH) for at least 2 weeks. A total of 30 fruit were used to determine average number of seeds per fruit. A total of 18 samples, each containing 20 seeds, were weighed (mg) to determine average seed weight. Seeds were placed in an airtight jar in the dark for storage.

Imbibition Experiment. An imbibition experiment was conducted on scarified and non-scarified (control) seeds to determine if physical dormancy was present. Seeds were scarified using an electric seed scarifier (Seedburo Equipment, Des Plaines, Illinois) lined with sandpaper (80 grit aluminum oxide, GatorGrit^{TM}, USA) for 50 seconds. Control seeds received no sandpaper abrasion. Seeds were placed in Petri dishes on two sheets of blotter paper that were saturated with 5 mL of distilled water and maintained at room temperature (25ºC) in a laboratory. Four replicate dishes, each with 90 seeds, were used per treatment. Seed mass was measured to the nearest 0.01 mg after 0, 0.25, 0.5, 1, 2, 3, 6, 12, and 24 hours. Water was added when the film of water around the seeds decreased to <1 mm.

Scarification Experiment. An experiment was conducted to determine the time of abrasion with sandpaper in an electric seed scarifier - needed to break physical dormancy and allow for germination. Seeds for this experiment were graded and seeds which appeared empty, deformed, or discolored were discarded. All seeds were surface sterilized via a 1-minute 70% isopropyl bath (10 mL in a 50 mL glass beaker), followed by a 10-min bath in sodium chloride (5 mL of 8.25% sodium hypochlorite solution diluted with 45 mL distilled water), and finally triple rinsed with distilled water. Additional seeds were not surface sterilized to determine if the

sterilization process alone would break physical dormancy. Four Petri dishes per treatment, each with 25 seeds, were kept in the dark (double wrapped in aluminum foil) and maintained at room temperature in a laboratory. Germination percentage was recorded after 14 days.

The experimental design was a single factor experiment with 5 (0, 50, 100, 150 and 200 seconds of scarification) treatment levels plus a non-scarified, non-sterilized control. Experimental units were Petri dishes containing seed, and germination percentages per dish was the response variable. Data were transformed prior to analysis via an arc-sine square-root transformation. Statistical analysis were conducted using RStudio 1.1.419. Data were subjected to a one-way analysis of variance to determine differences among treatments. Differences among means were determined using the least squares means package with a Bonferroni correction at an alpha level $\leq 5\%$.

Photoperiod Experiment. An experiment was conducted to test the effects of photoperiod on germination. Ungraded and non-sterilized seed were scarified using an electric seed scarifier lined with sandpaper for 50 seconds. Seeds were exposed to a 12-hour photoperiod of cool-white fluorescent light or kept in the dark. There were five replicate Petri dishes containing 25 seeds for each treatment. An additional 2 Petri dishes with non-scarified seeds were exposed to dark to confirm that seeds remained physically dormant throughout the experiment. Petri dishes were placed in growth chambers at 25ºC and evaluated for germination (1 mm radical emergence) and disease (presence of contamination) tri-weekly for 28 days. Petri dishes wrapped in aluminum foil were unwrapped for no more than 5 minutes and exposed to ambient laboratory lighting.

Data for this experiment were analyzed using semi-parametric (Cox regression models) and non-parametric (Kaplan-Meir estimators) time-to-event analyses. These methods were used

in lieu of an analysis of variance because we had several data collection dates and removed diseased seeds throughout the experiment. More information about time-to-event analysis and its utility in germination studies is provided by Allison (2010) and McNair et al. (2013), respectively. The experimental unit was a seed and germination was the response variable. Seeds that germinated during the course of the experiment were coded as 1. Diseased seeds were coded as 0 at infection day. Seeds which did not germinate by day 28 were coded as 0. Cox regression model construction (analyzed using PROC PHREG in SAS 9.4) and Kaplan-Meier estimates of the survivor functions (analyzed using survival package in RStudio 1.1.419) were implemented using the same methods as described in Campbell-Martínez et al. (2017).

RESULTS

Fruit and Seed Characteristics. Fruits are 3-valved capsules with stellate trichomes on the distal end. There were 10.9 ± 3.2 seeds per fruit. Average seed weight was 0.68 ± 0.05 mg. The number of seeds per fruit ranged from 3 to 19. Within fruits, seeds form a discrete unit which remains intact in a spherical structure that may be disassembled upon the application of pressure (Fig. 2). The vast majority of seeds were <2 mm wide (Fig. 2).

Imbibition. Scarified seed imbibed 3 times the amount of water in 24 hours than did non-scarified seed (Fig. 3). However, a small portion of non-scarified seeds did imbibe by hour 24. Imbibed seeds were easily identifiable by the naked eye as they were more swollen and lighter in color and seed testas were more translucent than non-imbibed seeds.

Scarification Experiment. Regardless of time of abrasion in the seed scarifier (50 – 200 seconds), germination percentages for scarified seeds (≥90%) were significantly higher than non-scarified seeds (9-11%) (Fig. 4; Table 1). There was no difference in germination between non-

scarified seed which were surface sterilized or non-surface sterilized - indicating our sterilization process did not break dormancy (Fig. 4).

Photoperiod Experiment. Photoperiod had no effect on germination probability across time (Fig. 5; Table 2). Germination began at week 1 and continued through the end of the experiment (Fig. 5). At the end of the experiment, 63% and 64% of scarified seed germinated, respectively, under light and dark conditions (Fig. 6). Only a small portion (14%) of non-scarified seeds germinated by the end of the experiment (Fig. 6). Disease ranged from 2-8% (Fig. 6).

DISCUSSION

Seeds of *C. arenicola* are large enough to be seen with the naked eye but may be too small to be sown by hand. Seeds are orthodox and may be dried and stored without complicated procedures. Like other seeds of Cistaceae, seeds of *C. arenicola* have physical dormancy due to a water-impermeable seed coat (Thanos et al., 1992). However, a small amount of *C. arenicola* seed germinate readily without pretreatment (i.e., are not dormant) as has been reported for *C. scoparium* (Keely, 1985). Similar to the closely related *Helianthemum,* seed dormancy of *C. arenicola* can be alleviated via abrasion by sandpaper (Pérez-García et al., 1995; Pérez-García and González-Benito 2006). Likewise, other *Helinathemum* have germinated readily with exposure to light (Escudero et al., 1997) or in the dark (Yeşilyurt et al., 2017) - as was the case for *C. arenicola*. We recommend at least 50 seconds of abrasion within an electric seed scarifier prior to sowing with or without light exposure for seeds of *C. arenicola.*

The authors have successfully grown containerized plants of *C. arenicola* (some of the stock plants used in this study). Rooted stem cuttings (no auxins were used on stem cuttings) have been successfully transplanted to multiple container types (3.5 in. square and tall, 4 in. -

round, 4 in. wide, and 1-gal pots); extensive root growth occurred in pots within weeks to a few months. Plants were grown using a mix of peat-based horticulture media (Sungro MetroMix® 830) and milled pine bark with fines at a 2:1 ratio. Plants were kept within a climate-controlled greenhouse during the winter months of 2018 and flowered prolifically for extended periods of time (Fig. 1). Plants responded well (vigorous growth and flower and seed production) to moderate amounts of slow-release fertilizer (1 teaspoon 18-6-12 Osmocote® per gallon) and fertigation (100 ppm N Peters Professional® 20-20-20). Plants tolerated frequent substrate saturations as well as frequent periods of substrate drying. Aphids and white flies were seen feeding on plant tissue but did not appear to diminish the appearance, flowering, or fruiting of stock plants. Insect populations were kept low by the application of horticultural oils and soaps as needed. The use of the southeastern USA native *Crocanthemum* in restoration and as an ornamental plant is currently limited. This limited use is possibly due to a lack of propagation, production and out-planting information. Here, we present information that informs the grower on how to collect, store, and germinate, *C. arenicola* seeds as well as how to produce containerized plants of *C. arenicola*.

Literature Cited

Allison, P. (2010). Survival Analysis Using SAS®: A Practical Guide, 2nd ed. SAS Institute Inc., Cary.

Campbell-Martinez, G., Thetford, M., Miller, D., and Pérez, H.E. (2017). Follicle maturity, seed size, temperature and photoperiodic effects on seed germination of *Asclepias humistrata* (sandhill milkweed). Seed Sci. Tech. 45:1-17.

eFloras. (2018). http://www.efloras.org [accessed 15 September 22018] Missouri Botanical Garden, St. Louis, MO and Harvard University Herbaria, Cambridge, MA.

Escudero, A., Carnes, L.F., and Pérez-García, F. (1997). Seed germination of gypsophytes and gypsovags in semi-arid central Spain. Journal of Arid Environments 36:487-497.

Keeley, J.E., Morton, B.A. Pedrosa,A. Trotter, P. (1985). Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. J.Ecol. 73:445-458.

McNair, J.N., Sunkara, A., and Frobish, D. (2013). How to analyze seed germination data using statistical time-to-event analysis: non-parametric and semi-parametric methods. Seed Sci. Res. 22:77-95

Pérez-García, F. and González-Benito, M.E. (2006). Seed germination of five *Helianthemum* species: Effects of temperature and presowing treatments. J. Arid Environ. 65:688-693.

Perez-Garcia, F., Iriondo, J.M., Gonzalez-Benito, M.E., Carnes, L.F., Tapia, J., Prieto, C., Plaza, R., and Perez, C. (1995). Germination studies in endemic plant species of the Iberian Peninsula. Israel J. Plant Sci. 43:239-247.

Sorrie, B.A. (2011). Transfer of North American *Helianthemum* to *Crocanthemum* (Cistaceae): new combinations. Phytologia 93:270-271.

Thanos, C.A., Georghiou, K., and Kadis, C. (1992). Cistaceae: A plant family with hard seeds. Israel J. Plant Sci. 41:251-263.

USDA and NRCS. (2018). The PLANTS Database, http://plants.usda.gov [accessed 15 April 2018]. National Plant Data Team, Greensboro, NC 27401-4901 USA.

Wunderlin, R.P. and Hansen, B.F. (2011). Guide to the vascular plants of Florida. 3rd ed. Gainesville (FL): University Press of Florida.

Yeşilyurt, E.B., Erik, S., and Tavşanoğlu, Ç. (2017). Inter-population variability in seed dormancy, seed mass and germination in *Helianthemum salicifolium* (Cistaceae), a hard-seeded annual herb. Folia Geobotanica 52:253-263.

Table 1. Analysis of variance table (analyzed using RStudio 1.1.419) indicating significance of scarification treatments on germination of *Crocanthemum arenicola*. Seeds were germinated at 25°C for 28 days.

	df	Sum of squares	Mean Square	F-value	P-value
Scarification	5	5.677	1.1355	107.7	<0.0001
Error	18	0.19	0.0105		

Table 2. Effects of photoperiod on germination probability of *Crocanthemum arenicola.* Seeds were germinated at 25°C for 28 days. Data were analyzed via cox regression models using PROC PHREG in SAS 9.4

Treatment	Coefficient (βi)	SE of (βi)	Wald χ^2	*P*-value	Hazard Ratio[1]
Photoperiod (Dark vs. Light)[2]	<0.01	0.11	<0.01	0.99	1.00
Experiment Run (Run 1 vs Run 2)[3]	0.06	0.11	0.27	0.60	1.06

[1]Hazard ratios > or < one with p value ≤0.05 indicate increased or decreased relative germination probability.
[2]The second row represents comparisons of seeds germinated in the dark or light (12-hour photoperiod).
[3]The third row represent comparisons between experiment runs.

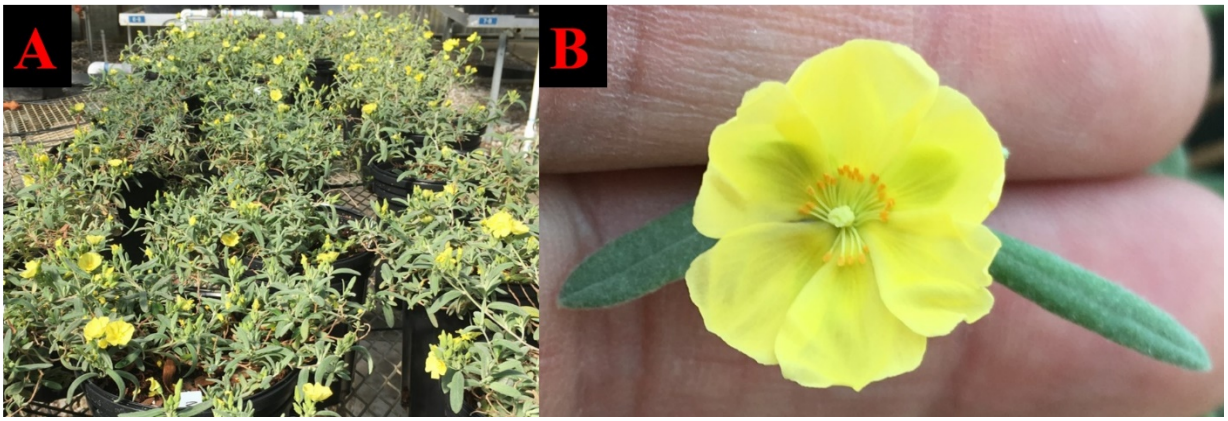

Figure 1. (A) 1-gal containerized plants and (B) chasmagamous flower of *Crocanthemum arenicola*.

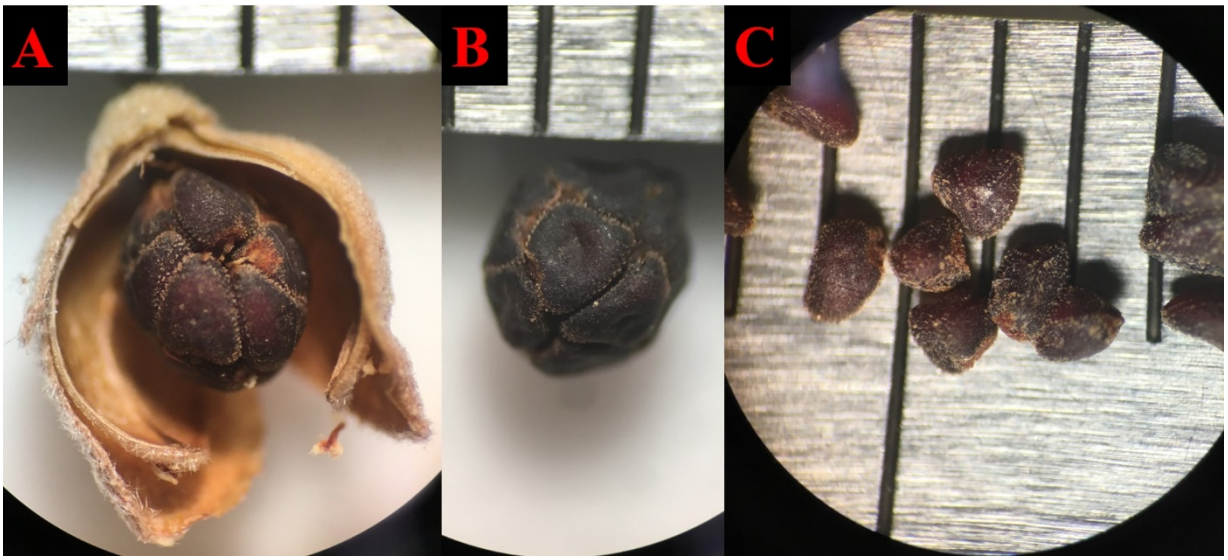

Figure 2. Fruit (A), intact cluster of seeds (B), and individual seeds (C) of *Crocanthemum arenicola* (coastalsand frostweed). Note 1-mm lines for reference.

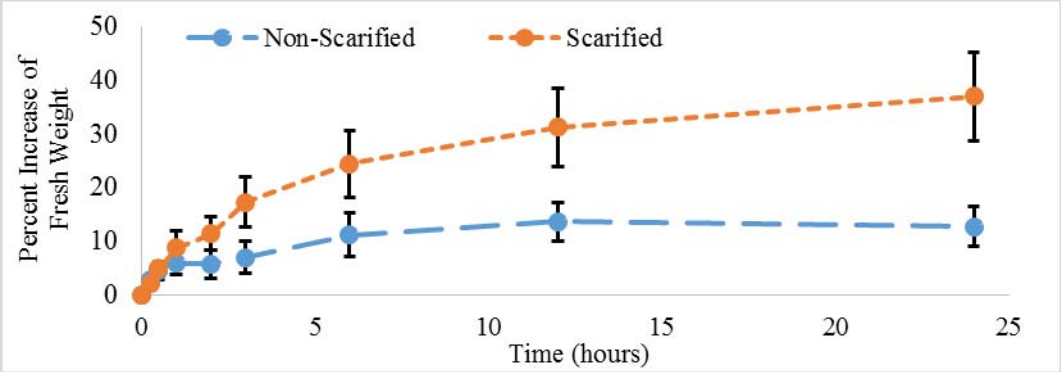

Figure 3. Seed imbibition curves for non-scarified (blue line) and scarified (orange line) *Crocanthemum arenicola* seeds. Scarified seeds were placed in an electric seed scarifier lined with sandpaper for 50 seconds while control seeds received no treatment.

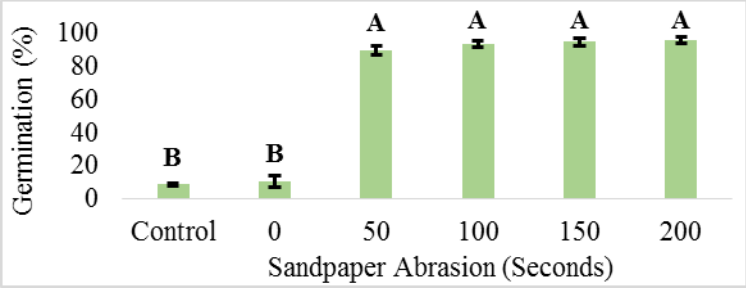

Figure 4. Effects of surface sterilization and exposure to abrasion by sandpaper in an electric seed scarifier on germination percentages of *Crocanthemum arenicola*. Control seeds were not surface sterilized or scarified. Seeds were classed as germinated following a 1 mm protrusion of the radicle. Seeds were placed in the dark at room temperature (~25ºC) and observed for 14 days. Different letters above bar graph denote significant ($P \leq 0.05$) treatment differences using a pairwise comparison test with a Bonferroni correction (analyzed using lsmeans package in RStudio 1.1.419).

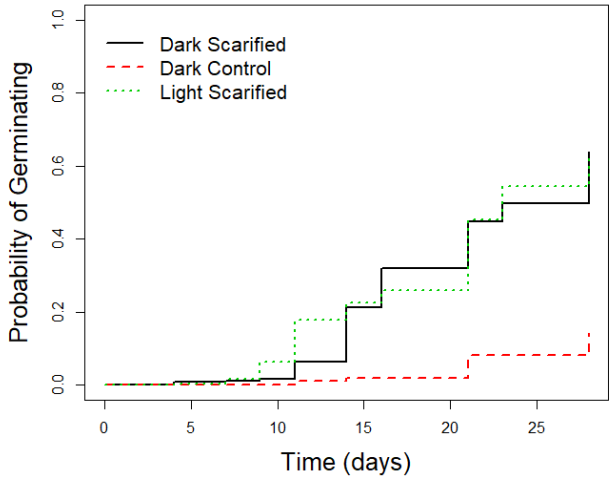

Figure 5. Inverted Kaplan-Meier curves (analyzed using survival package in RStudio 1.1.419) showing effects of photoperiod (seeds were either exposed to a 12-hour photoperiod or left in the dark) on germination probability of *Crocanthemum arenicola*. Ninety-five percent confidence intervals are excluded for clarity. Seeds were germinated within growth chambers at 25ºC for 28 days. Control seeds were non-scarified.

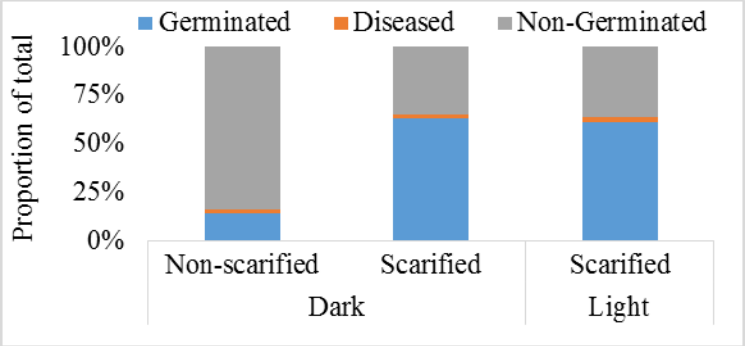

Figure 6. Effects of photoperiod (seeds were either exposed to a 12-hour photoperiod or left in the dark) and scarification on relative percentages (proportion of total) of germinated, diseased, non-germinated seeds of *Crocanthemum arenicola*. Seeds were classed as germinated following a 1 mm protrusion of the radicle and diseased when seeds showed visual pathogen infection. Seeds were placed in a growth chamber in the dark at 25ºC for 28 days.