EMBRYO DEVELOPMENT, SEED BANK POTENTIAL AND GERMINATION OF THE FEDERALLY ENDANGERED HERB OF PINE SAVANNAS, *THALICTRUM COOLEYI*

by

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ABSTRACT

Plant conservation efforts strongly rely on knowledge of species seed biology and viability for further propagation. Virtually nothing is known about the seed biology of the federally endangered *Thalictrum cooleyi* Ahles (Cooley's meadowrue, *Ranunculaceae*). *Thalictrum cooleyi* is a dioecious wind-pollinated herb with short-stalked, clustered achenes, native to the fire-adapted wet pine savannas of NC, FL, and GA. Congeners have underdeveloped embryos, some of which require cold stratification to germinate. I studied embryo presence and development, the effect of temperature (specifically, high heat and cold) on mean total germination rates, and the potential of *T. cooleyi* to form a seed bank. Stereo- (SM) and scanning electron microscopy (SEM) are both viable options for embryo evaluation, but SEM more readily identifies underdeveloped embryos and cavities. Half (50%) of 100 seeds had embryos, suggesting low viability. Both methods showed *T. cooleyi* to have small, linear underdeveloped embryos with peripheral attachment. The point of attachment is at the base of the carpel, suggesting basal placentation of ovules. My results showed that *T. cooleyi* has simple morphophysiological dormancy and seed biology characteristics similar to that of the rest of the family *Ranunculaceae*. Seeds were dormant at maturity; longer periods of cold (8 vs. 2 wk) at 1 ºC resulted in higher mean total germination. Moist cold breaks seed dormancy and embryos elongated upon subsequent exposure to germination conditions (25:15 ºC, light:dark, 14:10). Germination studies in the field, including burial of seed, showed that *Thalictrum cooleyi* can also form a seed bank of at least 1 year and overwintering is required for germination. High heat did not increase germination, but fire is necessary for maintaining an open canopy. Although low germinability remains a challenge for this species, seed propagation in cultivation and in the field hold promise for restoration efforts. Further research is necessary to ascertain long-term natural

seed bank potential, but my work confirms the presence and importance of seed banks in pine savannas, offering another tool for conservation efforts.

To my parents, Brian and Brandi Dietrick,
for lovingly tending my growth from seed to plant.

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Introduction

Global change is due to anthropogenic factors and has significant negative impacts on biological systems, including genetic diversity (Pauls et al. 2012), emergence and transmission of infectious diseases (Keesing 2010), water quality and marine life (Andersson et al. 2015), and loss of biodiversity (Chapin 2000; Ehrlich and Wilson 1991), notably plant species. The aim of conservation biology is to prevent loss of biodiversity and restore it. Plants are under-represented in conservation despite their vital roles in ecosystems (Velasco et al. 2015). Effective plant conservation requires an understanding of species habitats, their biology and their modes of reproduction. Seeds, the dispersal unit of flowering plant sexual reproduction, are critical to plant population persistence and are therefore critical subjects of plant conservation research (Silva et al. 2015). In particular, seed viability, dormancy, and germination are important characteristics of seed biology that contribute to knowledge of seed banks, the storage of viable but often dormant seeds in the soil (Fenner 1985).

I studied these characteristics of seed biology in *Thalictrum cooleyi* Ahles (Cooley's meadowrue, *Ranunculaceae*). Although federally endangered since 1989, its seed biology is unknown. The species is a dioecious wind-pollinated herbaceous perennial native to the fire-adapted wet pine savannas of North Carolina, Florida, and Georgia (USFWS 2008), a habitat which has been reduced to 3% of its original area (Frost 1993). When vegetative, the lax, leaning growth form of *T. cooleyi* hides it from view among dominant shrubs and grasses. Its leaves are ternately decompound or upper ternately compound with leaflets ranging from linear to elliptic, typically without lobes and with revolute margins. Cooley's meadowrue is best identified when flowering, between June and September. Male flowers have numerous stamens (1-2 mm long, acute or obtuse, filaments clavate, longer than anthers). Female flowers consist of 3-16 carpels

(stigmas 1-1.5 mm long). Fruits are small (4.5-6 mm long, 1.5-2 mm wide), sessile clustered achenes (Radford et al. 1968) that house the seeds.

Seeds characteristics, including dormancy type, vary widely. The current dormancy classification scheme is a modified version of Nikolaeva's (2001) with the metrics of seed/fruit coat permeability to water, seed physiological responses to temperature, and embryo morphology (Baskin and Baskin 2004). Seeds with morphophysiological dormancy (MPD) have underdeveloped but differentiated embryos and require pre-treatment to address the physiological component (Baskin and Baskin 2004). There are eight types of MPD, differentiated by the stratification that breaks seed dormancy, the time of embryo growth, and the ability of gibberellic acid to overcome dormancy. Walck et al. (1999) used a dissecting microscope to determine dormancy type and the viability of ungerminated seeds of the congener *T. mirable*. *Thalictrum mirable* has underdeveloped embryos and exhibits simple nondeep morphophysiological dormancy. Viable embryos, as confirmed by tetrazolium testing, were white and firm, while unviable embryos were soft and brown. Walck et al. (1999) achieved 100% germination in *T. mirable* after 12 wk of cold at 1 °C, and 2.0 mm was the critical embryo length for germination. Embryos remained underdeveloped until exposure to temperatures ≥ 15:6 ºC and after 8-12 wk of cold stratification. This pattern supports classifying seeds of *T. mirable* as physiologically dormant and highlights what could potentially be the dormancy type of *T. cooleyi*. Seeds of *Thalictrum cooleyi* are dormant at maturity but dormancy type has not been determined (Fortner 2015).

Seed banks store dormant but viable seeds in the soil under environmental conditions are favorable for germination. They hold promise as a method of restoration. We lack information on seed viability of most plant species of pine savannas (Glitzenstein et al. 2001), but seed banks

have been documented to occur/do exist in related pine communities (Cohen et al. 2004). Collecting soil cores is common to test seed bank potential, but burying bags of seed is more effective in spatially diverse ecosystems like the pine savanna (Coffey and Kirkman 2006). In their study of longleaf pine savanna species, Coffey and Kirkman (2006) also concluded that although some species have the potential to form short-term natural seed banks, conservation efforts in these ecosystems would require seed reintroduction.

Purpose

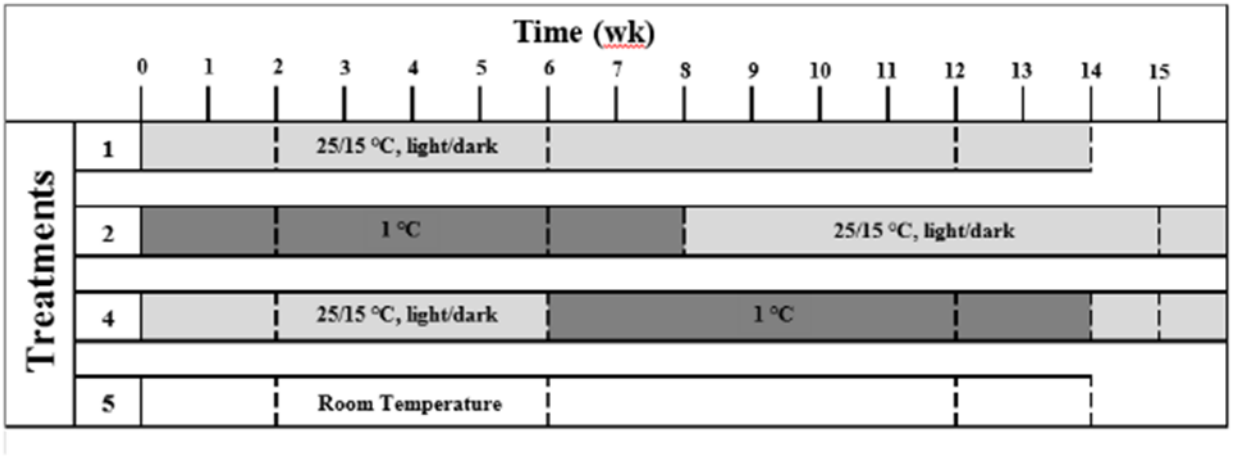
The purpose of this study was to aid restoration efforts of *T. cooleyi* and contribute to a growing body of knowledge on seed biology. I evaluated embryo presence and development, tested the effect of high heat (as might be experienced in a fire-adapted habitat) and length of cold stratification on mean total germination, and studied the seed bank potential of *T. cooleyi*.

Methods

Embryo Development Pilot Study

I first asked whether *T. cooleyi* embryos were underdeveloped at time of dispersal of seed and how environmental conditions might affect embryo development. I also compared two microscopy techniques for their ability to determine embryo condition and size. I used a Leica EZ4 HD stereo light microscope (SM) and a FEI Quanta 200 scanning electron microscope (SEM) to view the embryos of seeds from five pre-treatment groups at multiple time markers. As shown in Table 1, seeds ≥ 2.3 mg were divided as follows: 1) no stratification (absence of pre-treatment), 2) room temperature for 14 weeks (control), 3) six weeks of warm followed by 8 weeks of cold stratification, 4) room temperature for 8 weeks (control), and 5) eight weeks of cold stratification. Seeds were then evaluated via microscopy at 2, 6, 12, and/or 14 wk depending on the particular pre-treatment. At the end of each pre-treatment, all seeds were placed in a growth chamber and subjected to germination conditions: 25°C/15°C and 14:10 hr periods of light:dark.

Table 1: *Treatment and microscopy schedule for laboratory germination tests of Thalictrum cooleyi. Dashed lines indicate time markers for microscopy measurement. Treatment 1 = no stratification, alternating thermo- and photoperiod; Treatment 2 = cold stratification in dark followed by germination conditions, alternating thermo- and photoperiod; Treatment 4 = no stratification, alternating thermo- and photoperiod, then cold stratification in dark, followed by germination conditions, alternating thermo- and photoperiod; Treatment 5 = no stratification, room temperature, alternating photoperiod .Alternating thermoperiod: (25:15 ºC, 14:10). Alternating photoperiod: 14:10, light:dark.*



Both SM and SEM were used to compare and contrast their effectiveness in examining seed embryos. At any given time marker, four seeds from each pre-treatment group were taken to East Carolina University's Core Imaging Facility for microscopic viewing. Seeds were first prepared for viewing under the stereoscope by holding them in place with forceps and making a cut down the middle, from pointed end to pointed end, using a scalpel. The cut had to be as centered as possible in order to see the embryo. The forceps were then used to position each half of the seed—exposed side facing up—beside one another under the microscope. The following pictures were taken at 25x: a whole seed, a whole seed with measurements, the inside of the seed, and the inside of the seed with measurements. Measurements of the length (from point to point) and width (from widest part of one side to widest part of the other) were taken using Leica EZ4 software (Figure 1).

After photographing the seeds with the stereoscope, one-half of each seed was placed on a stub mount that had been taped with double-sided tape. Each half-seed was carefully placed side-by-side on the stub mount in similar orientation with the forceps. If an embryo was only present in one half of a seed, that half was the section placed on the stub mount. After organizing each seed on the stub mount, I placed a tiny piece of tissue paper on one corner of the stub mount so that I would not lose the orientation of my seeds while using the SEM. I also sketched the orientation and ID of each seed in my notebook to keep track of each seed.

The SEM was set on low vacuum, back-scattered electron mode at 20 KV and 0.45 Torr vacuum pressure. Two pictures were taken of each half-seed: one of the entire half-seed at or near 70x and a close-up of the embryo-containing (or lacking) side of the seed at varying magnifications.

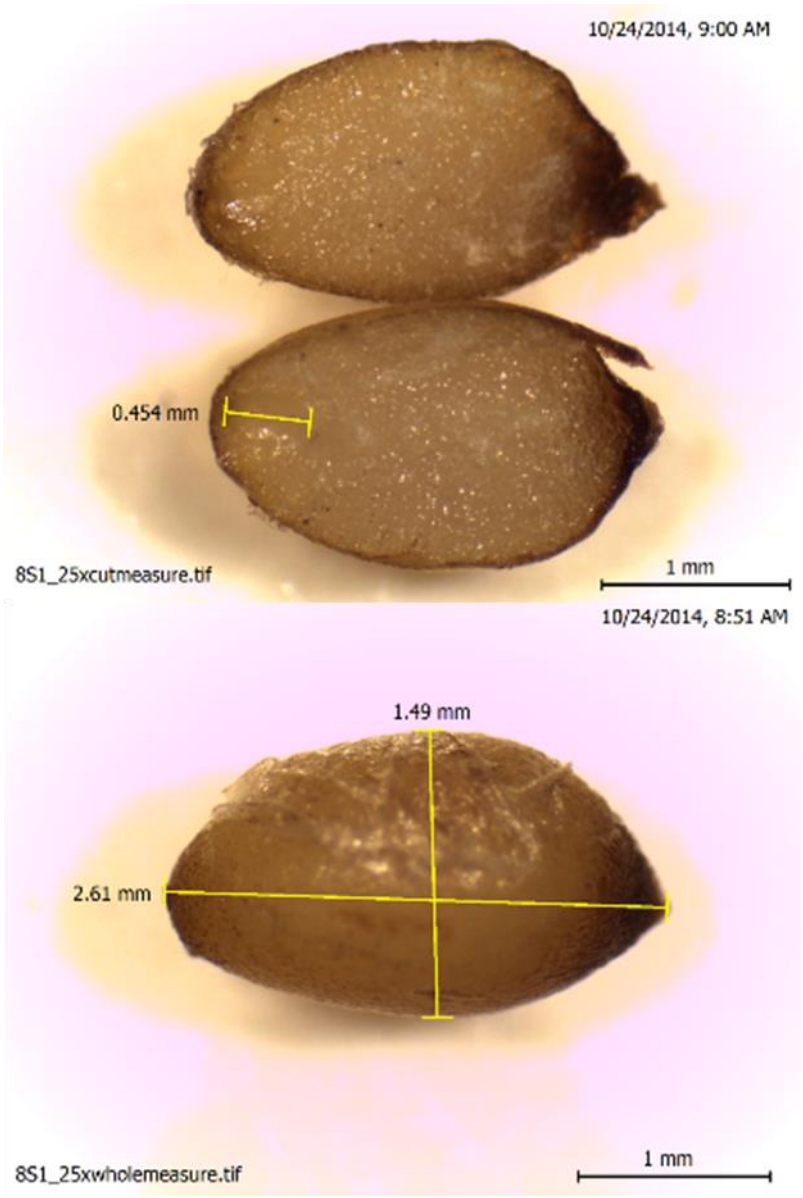


Figure 1: *Embryo and seed length measurements using Leica EZ Software.*

Heat Shock Germination Trials

I also asked whether heat increased germination of *T. cooleyi*, given its occurrence in fire-dependent pine-savannas. I used methodology of Dayamba et al. (2009) and Schwilk and Zavala (2012). I harvested and cleaned seeds from nine parent plants and randomly distributed them among four treatment groups: (1) heat shock followed by 8 wk of cold stratification, then germination conditions, (2) no heat shock followed by 8 wk of cold stratification, then germination conditions, (3) heat shock followed by germination conditions, and (4) no heat shock followed by germination conditions. Heat shock was 2.5 min in a convection oven. Germination conditions were 25 /15° C and 14:10 hr light:dark. Seeds were monitored daily for germination.

Buried Seed Bag Experiment: Seed Bank Potential

To determine the potential of *T. cooleyi* to form a natural seed bank, I conducted three experiments: buried bags in Pender County, NC, potted plants in Greenville, NC, and seeds at room temperature in the laboratory. I derived and modified methodology by Coffey and Kirkman (2006) to determine the potential of *T. cooleyi* to form a natural seed bank.

Buried Seed Bag Experiment: Pender County, NC

In October 2014, I harvested 300 *Thalictrum cooleyi* seeds which appeared large enough to contain viable embryos from 17 female plants at Shaken Creek and 14 at Sandy Run. I then filled three AquaClear® 70 Nylon Bags (100 µm) with 0.71 kg of play sand and 50 seeds—25 seeds from each of the two sites. Each bag of seeds represented 10 different maternal parent plants. I buried the bags 6 cm deep in three holes at the edge of a wooded area in Sandy Run. I marked bags above ground with a stake and left the string of each bag out of the ground.

After 7 months had passed, I retrieved the three bags (150 seeds) from Sandy Run and brought them to the laboratory for germination. I filled three plastic trays with 600 mL of autoclaved play sand and emptied each bag into a separate tray, evenly distributing the contents. I also prepared a control of 150 *Thalictrum cooleyi* seeds from the greenhouse of mass ≥ 2.2 mg (5 different females), divided into trays with sand in similar fashion. I allowed seeds to imbibe water by moderately moistening sand with deionized water and by covering trays with clear, plastic bags, fastened with binder clips. Trays were placed in a growth chamber 15:25°C 14:10 hr light:dark. It should be noted that I used greenhouse seeds because I was legally restricted to harvesting a certain number of seeds in the field, and excessive collection of rare seeds for research purposes could be detrimental to population persistence.

Buried Seed Bag Experiment: Potted Seeds in Greenville, NC

A week after harvest, I planted the remaining 150 seeds in pots in Greenville, NC. I filled 15 pots with moistened Jolly Gardener C/B soilless mix and placed them a 12 cm trench to prevent disturbances. Each pot was planted with five seeds from each site to represent ten genotypes. I cut and tightened shade cloth around each pot using plastic cable ties to prevent other species from establishing in the pots. Pots were watered weekly I recorded germination biweekly until switching to a daily schedule on May 30th. Only plants 1 cm in height were confirmed and counted as *Thalictrum cooleyi*.

Results

Embryo Development Pilot Study

Results are observations and data obtained from evaluation via stereo- and scanning electron microscopy, methods that may could useful in studying seed embryo morphology. *Thalictrum cooleyi* embryos were located on the stigmatic end of the seed; embryo attachment is therefore peripheral (Figure 2). Seeds of *T. cooleyi* contain small, underdeveloped linear embryos surrounded by water, fleshy, nuclear endosperm. The embryo comprised 14% of *T. cooleyi* seed length, independent of time and treatment, with most embryos being longer than wide. The darkened end of the seed (which used to be the ovule) is the point of attachment to the base (non-stigmatic end) of the carpel (which used to be the ovary) in *T. cooleyi.* Embryo presence did not differ across pre-treatments, and development was observed only in treatments 2 (8 wk cold at 1 ºC dark) and 4 (6 wk warm at 25:15 ºC, 14:10, light/dark then 8 wk cold at 1 ºC dark) at 15 wk during germination conditions (25:15 ºC, 14:10, light/dark). The mean length of embryos was 0.54 ± 0.032 mm prior to exposure to germination conditions, without exposure to cold stratification; this value is below the 2 mm threshold used by Walck et al. (1999) to indicate embryo elongation. Presence of a cavity near the embryo was only detected with SEM, though cavity presence was rare (Figure 3a; Figure 3b). Embryo presence and boundaries were more readily identified via SEM. Scanning electron microscopy after evaluation with stereomicroscopy confirmed that of 100 seeds across pre-treatments, only 50 appeared to have embryos.

Figure 2: *a) Thalictrum cooleyi achene with stigmatic end on right, b) the seed separated from the achene, stigmatic end on right and darkened end on left, and c) cross-section of the same seed with embryo and stigmatic end on right and darkened end on left.*

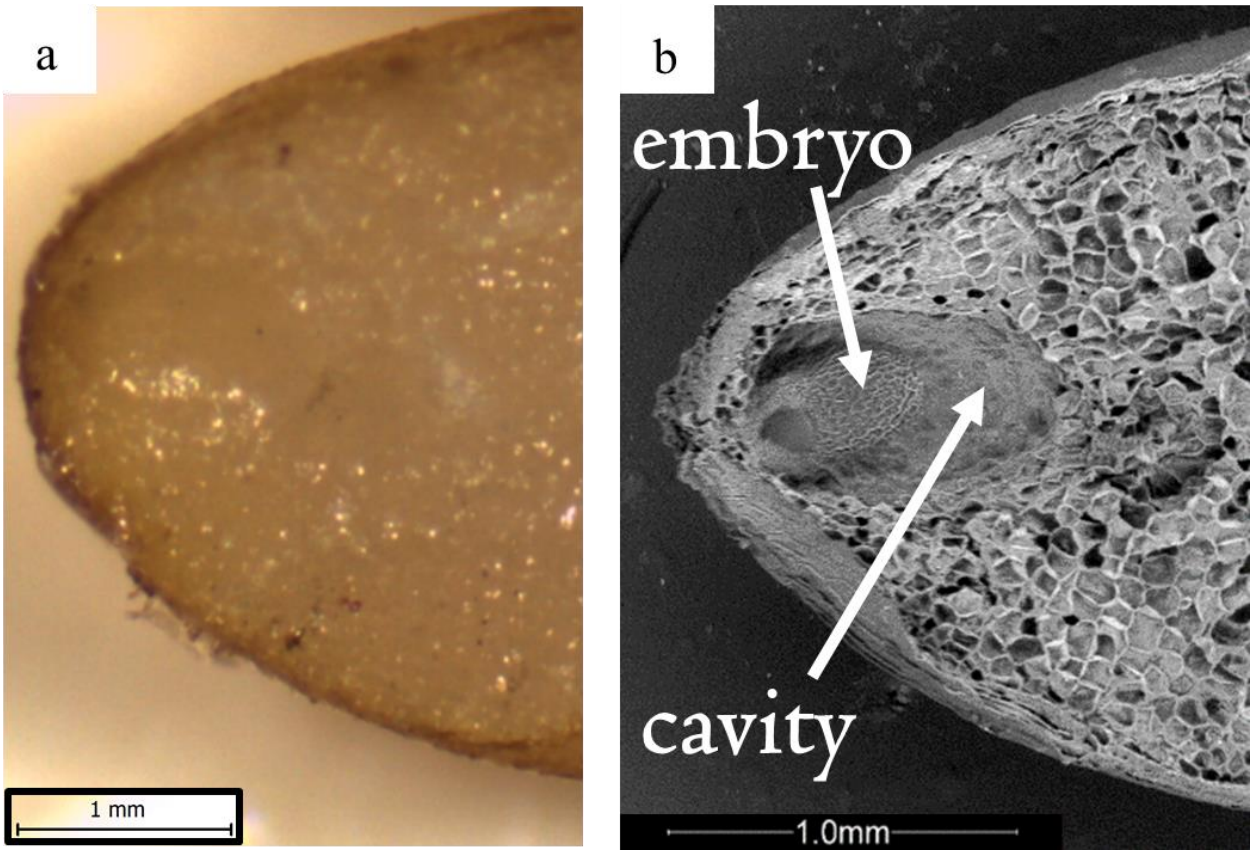


Figure 3: *a) seed examined via stereomicroscopy b) the same seed examined via SEM. While both microscopes detected the presence of an embryo, only the SEM showed the presence of a cavity. Photographs taken with the SEM clearly define seed tissue boundaries and allow for more confident study of embryo development.*

Heat Shock Germination Trials

This experiment was conducted to test the effects of high heat, as might be experienced in a fire-adapted habitat, and length of cold stratification on mean total germination of *T. cooleyi*. A single factor ANOVA showed statistical differences among the means of the four pre-treatment groups ($F_{calculated} = 20.145$, $F_{critical} = 4.006$, $P = 0.0004$). Heat did not appear to increase germination but length of cold stratification did. Visually, there was no difference in mean germination between the high heat and no high heat for seeds with 2 wk of cold pre-treatment groups. A t-test confirmed that high heat in the presence of 8 wk of cold also did not affect the mean number of germinant ($T_{stat} = -2.514$, $T_{critical} = 2.776$, $P = 0.066$). However, a t-test between the high heat with 2 wk of cold and no high heat with 8 wk of cold pre-treatment groups showed statistically significant differences among the means (T stat = -3.00416, T crit = 2.776445, P value = 0.039778); highest germination occurred on average following 8 wk of cold stratification in the absence of heat (Figure 4).

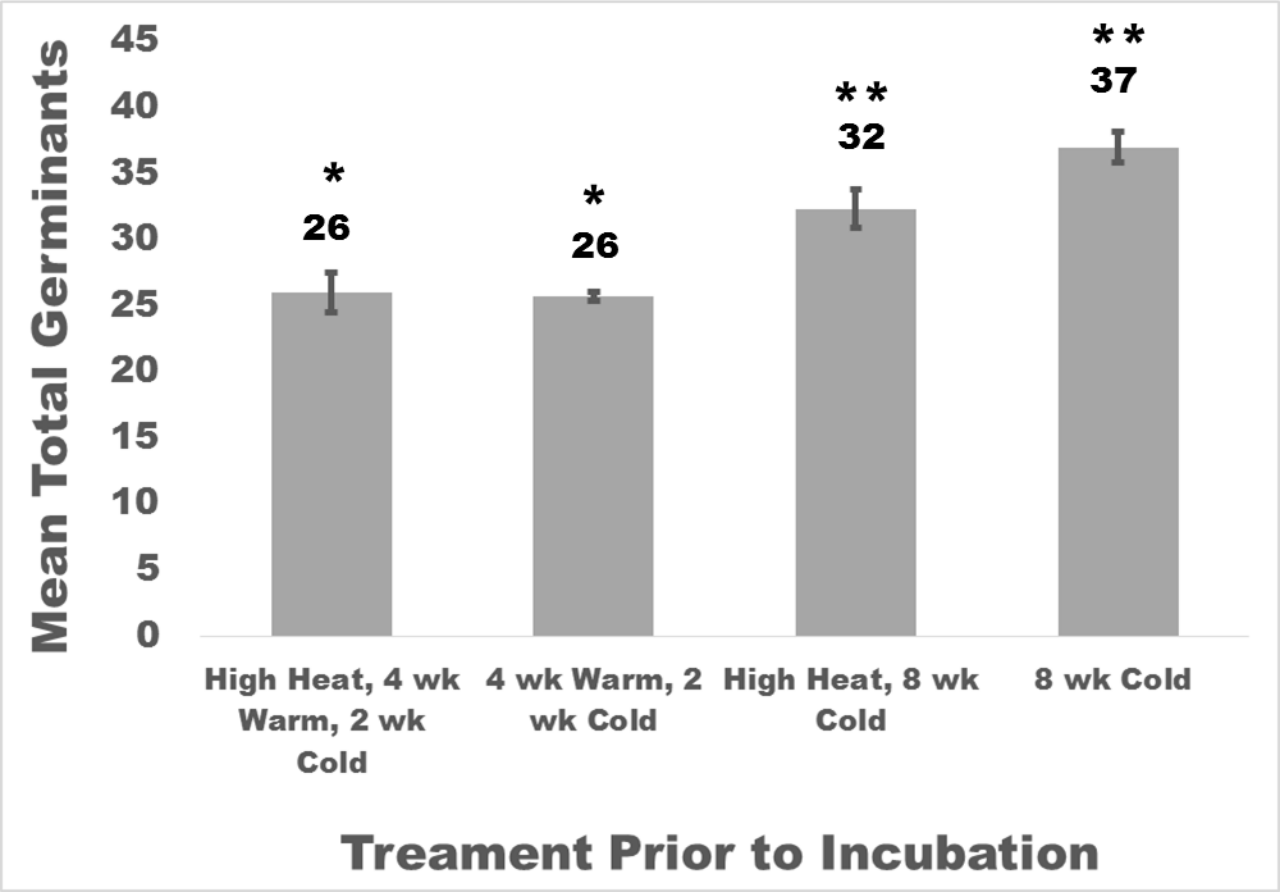


Figure 4: *mean total germination* (± SE) *of four pre-treatment groups characterized by the presence or absence of high heat and either a short (2 wk) or long (8 wk) period of cold stratification at 1 ºC. Asterisks indicate significant differences among means.*

Buried Seed Bag Experiment: Seed Bank Potential

I tested for seed bank potential because seed storage in the soil can be critical to plant population persistence and may be useful for restoration efforts of rare plants. Figure 5 compares total proportion of germinants in pots in Greenville, NC compared to those buried in bags in Pender County, NC and to the control. I used F-tests to compare variances between the transformed proportion of germinants in pots with those in buried bags as well as between the buried bags and the control. Variances were homogenous ($F_{(2,2)} = 12.6829$), meeting the assumptions of the analysis of variance (ANOVA). I then used a single-factor ANOVA to test the null of equal means among the three groups (pots vs. buried vs. control). The mean number of germinants differed among the treatments ($F_{(2, 6)} = 20.85$, $P = 0.00199$). The difference in standard error bars suggested that there may be differences between mean proportion of germinants (n = 50) between the potted plants and the control. I calculated a student T-test to verify higher germination in the buried bags than in the control ($F_{(1,4)} = 12.207$, $P = 0.0250$). Overall, there were significant differences between the means of both the pots and the bags when compared to the control, but not when compared to one another. The total proportion of germinants was essentially equally between the potted and the buried seeds, but average germination for these treatments greatly exceeded that of the control. It should also be noted that no seeds germinated while buried at Sandy Run.

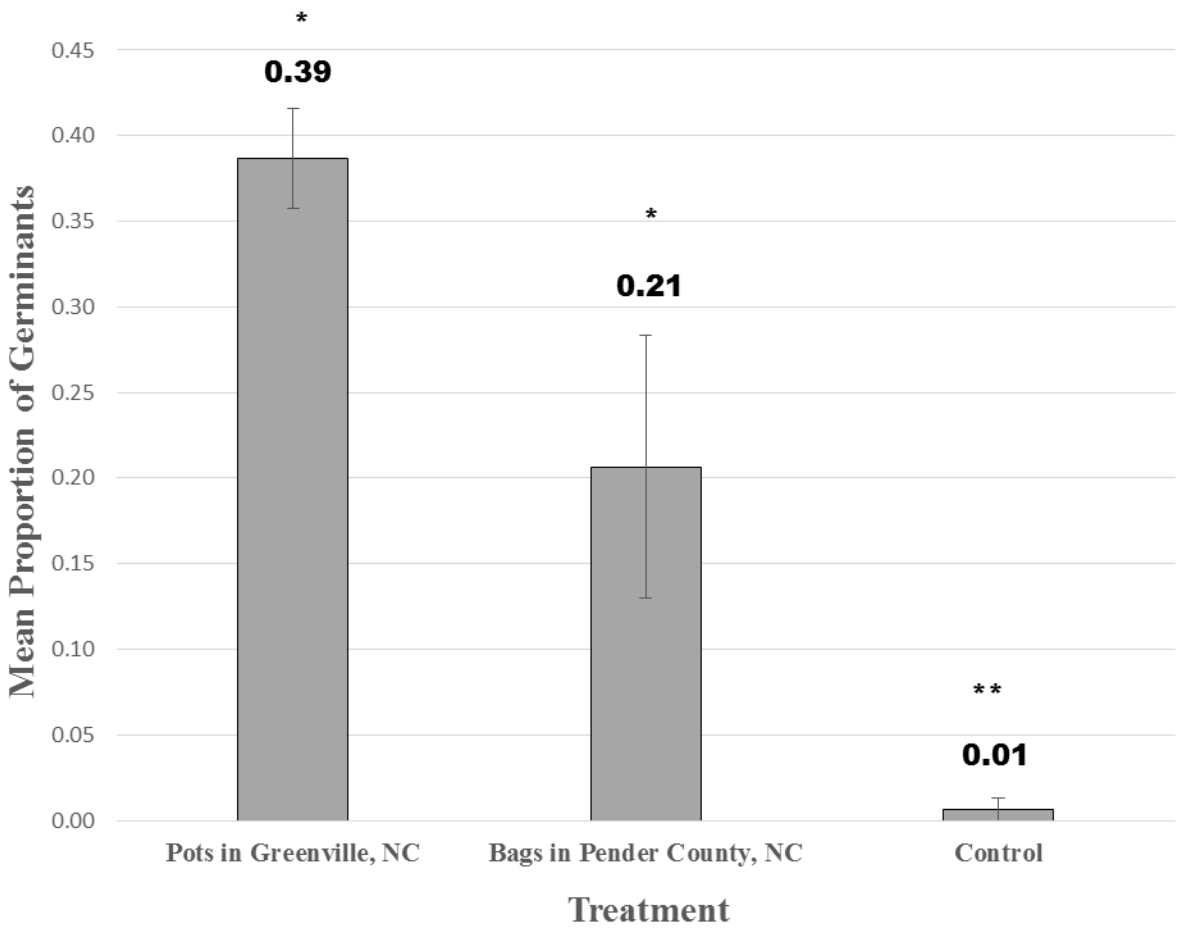


Figure 5: *A comparison of mean (± SE) proportion of germinants after 9 mo among potted seeds in Greenville, NC, buried seeds in Pender County, NC, and control seeds. Asterisks indicate significant differences among means.*

Discussion

Microscopic evaluation showed that *Thalictrum cooleyi* has seed biology characteristics similar to that of the rest of the *Ranunculaceae* family. Microscopic images taken of seed embryos across pre-treatments through time show that *T. cooleyi* embryos are linear and underdeveloped. This categorization is according to revisions of Martin's 1946 seed size classification system (Baskin and Baskin 2007). Observations that the seed is attached to the non-stigmatic end of the carpel suggests that *T. cooleyi* has basal placentation (Bhojwani and Bhatnagar 1974). *Thalictrum cooleyi* embryo morphology shares the same characteristics as the rest of the *Ranunculaceae* family, which has underdeveloped linear or rudimentary embryos with a large, watery, fleshy nuclear endosperm (Daskalova 2004; Martin 1946). The *T. cooleyi* gynoecium is multicarpellary and apocarpous.

Both stereo- and scanning electron microscopy show promise in evaluating embryo presence and development, although SEM offers additional knowledge of morphology. SM evaluation was quick and simple with built-in Leica EZ software. This software provides image descriptors, a scale bar, and measurements immediately after photographing. SEM clearly identified embryo borders and differentiated between tissue types, but set-up was more time consuming and complex. Embryo measurements between the Leica EZ software and Photoshop were very similar, but because embryos were small and underdeveloped, embryo presence was occasionally overestimated by the SM.

Thalictrum cooleyi seeds are dormant at maturity and require cold stratification at 1 °C. Longer periods of cold (8 wk) yielded higher germination rates (64-74%) than shorter periods (2 wk; 52% germination). This research corroborates the importance of cold asserted in previous studies, though germination at 25:15 ºC, light:dark, 14:10 after 8 wk of cold was lower (26.7 ±

1.76% ºC %) (Fortner 2015). In Fortner's study (2015), however, germination was slightly higher when cold was preceded by a period of warm stratification (33 ± 0.67% ºC %). She also found that giberellic acid also promoted germination, while light had no effect.

Seeds germinated readily after a winter outdoors, meaning that *T. cooleyi* can form a transient seed bank of at least 1 year (Walck et al. 2005). Congeners of *T. cooleyi* have the potential to form either a transient (T. *aquilegiifolium*) or persistent (*T. flavum, T. mirable*) seed bank (Walck et al. 1999). Longer-term seed banks may be possible and would require larger scale experiments with sequential harvest of buried seeds across many years. However, embryo growth did not occur until seeds were placed in germination conditions (15 °C) after cold stratification. Similar to *T. mirable* (Walk et al. 2011), *T. cooleyi* has simple morphophysiological dormancy. This type of dormancy is characterized by high germination temperatures (≥ 15 °C) and lack of embryo growth until in those conditions after cold stratification. Germination trials with longer cold pre-treatments could further distinguish *T. cooleyi* as having nondeep, intermediate, or deep MPD,

The mechanism whereby cold stratification breaks dormancy and stimulates germination is related to temperature effects on plant growth regulators (Finch-Savage and Leubner-Metzger 2006). Studies on other dormant species show association between seed dormancy and the plant hormones, abscisic acid (ABA), gibberellin, and cytokinin. ABA is part of the β inhibitor growth-inhibiting complex (Bennet-Clark and Kefford 1953) and is only one of many other identified inhibitors that may be responsible for dormancy (Wareing and Saunders 1971). Cytokinin is believed to be involved in bud elongation (Kuraishi et al. 1988). Increased gibberellin (GA) content in seeds exposed to warm temperatures after cold stratification could indicate that low temperatures function to promote gibberellin activity, though GA alone does

not cause dormancy break (Wareing and Saunders 1971). Thus, current models suggests that ABA controls dormancy and GA regulates germination, with levels of GA required for germination being influenced by levels of ABA during seed development (Baskin and Baskin 2004). However, more recent research proposes that both ABA and GA may play a role in dormancy and germination. This may also be true for *T. cooleyi*, though this cannot be confirmed without measuring plant hormone levels in this species.

High heat is not a germination requirement for *T. cooleyi*. Not all plant species of fire-dependent communities require high heat for germination, such as the grass *Euclasta condylotricha* Stapf (Dayamba et al. 2014). Fire may influence seed germination chemically as well as by associated high temperatures. Smoke from fire can be a germination requirement for some species of fire-adapted habitats (Dayamba et al. 2014) but was not tested in this study. Future research using smoke for germination should strongly consider the type of smoke used (aqueous or aerosol) and the combination of heat and smoke since both can yield differences in germination (Dayamba et al. 2014). Nonetheless, in the very least, fire still plays an important role in pine savannas and the pocosin-savanna ecotones where *T. cooleyi* occurs, by maintaining open canopies associated with this species (Fortner 2015) and understory richness (Veldman et al. 2014).

We lack knowledge of seedling and transplant success in the field, but this research suggests potential for species restoration of *T. cooleyi* from seedlings established outdoors after overwintering. Seeds germinated readily both indoors and outdoors after a period of cold. Seeds also survived multiple months in the refrigerator. Cryopreservation at very low temperatures in liquid nitrogen is suggested for long-term storage of seeds from endangered species and may prevent loss of viability over time suggesting that cryopreservation may be a feasible method of

storing seeds and preventing loss of viability over time (Pritchard 1995). Seedling establishment of *T. cooleyi* was successful when germinated seeds were planted in pots of Jolly Gardener C/B soilless mix. We moved plants outdoors for overwintering to promote flowering, though flowering did occur in the greenhouse. Previous research on seed banks in pine savannas also suggest that seed banks may be an important tool for restoration efforts and that numerous species of rare, dormant species can be germinated (Cohen et al. 2004).

Cooley's meadowrue is difficult to study as well as rare. There are a number of challenges to science-based conservation of *T. cooleyi*, including habit and habitat. Its fragile, leaning growth form (habit) makes it difficult to identify among shrubs and woody species of the wet pine savanna and pocosins. Extra care must be taken not to accidentally trample hidden *T. cooleyi* plants during field research. The species is most readily identifiable when it flowers during the summer months and seedlings are difficult to observe in the dense understory of the pocosin-savanna ecotone habitat. In North Carolina, *T. cooleyi* can be found in only three locations in Pender County: McLean's Savanna, Shaken Creek, and Sandy Run. These sites are somewhat remote locations prone to flooding during rains, sometimes inhibiting travel to and from the site, often highly overgrown with shrubs. These remote areas may protect *T. cooleyi* and habitat disturbance could potentially create microsites for this species (Gale 2010).

Low production of viable seed and germinability remain a challenge for *T. cooleyi* and its conservation. This species is functionally dioecious and wind-pollinated; thus, seed set is determined by availability of pollen as well as abundance of males, densities of plants, and population sizes. *Thalictrum cooleyi* may be pollen-limited (Fortner 2015). Even for seeds that are produced, less than 50% of *T. cooleyi* seeds have embryos. Inbreeding depression could also contribute to this high frequency of inviable embryos (Fortner 2015). Even for seeds with viable

embryos, germination is low. *Thalictrum cooleyi* may be a long-lived perennial with vegetative modes of reproduction (Fortner 2015).

Conclusions

Scanning electron microscopy (SEM) is more complex than (SM) but more readily identifies underdeveloped embryos. SM may be a more feasible alternative given its simplicity and convenience. However, microscopy can only identify embryo presence, not embryo viability.

Conservation agencies should utilize both vegetative and seed reproduction in restoration efforts of *Thalictrum cooleyi*. Seeds will germinate readily in ≥ 15 °C after a winter outdoors or ≥ 8 wk of cold stratification at 1 °C. Upon germination, seeds can be transferred to pots in Jolly Gardener C/B soilless mix and stored outdoors or in a heated greenhouse. Seed propagation should occur promptly after harvest as seeds may lose viability through time (Fenner 1985). Cryopreservation may have potential for seed preservation. Dormant but viable seeds persist in the soil for at least one year and may be germinated as well.

Restoration strategies focused on vegetative propagation as well as sexual reproduction via seed from explants or as recruits from the seed bank are critical for *Thalictrum cooleyi*. This species continues to be threatened by habitat destruction; continued efforts to reduce anthropogenic-driven loss of wet pine savannas will help conserve *T. cooleyi* and a number of other endangered species endemic to these few remaining species-rich ecosystems.

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