

INSIGHTS INTO THE HABITAT AND BREEDING BIOLOGY OF THE FEDERALLY  
ENDANGERED HERBACEOUS PLANT, *THALICTRUM COOLEYI* AHLES

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*Thalictrum cooleyi* Ahles (Cooley's meadowrue, Ranunculaceae) is a dioecious species, endemic to fire-dependent, wet pine savannas of the southeastern US. *T. cooleyi* was listed as federally endangered in 1989; knowledge of habitat and breeding biology are crucial to conservation. I studied NC populations of *T. cooleyi* and examined (1) woody species associates, (2) breeding system, (3) sex ratios, and (4) potential pollen limitation. I also assessed seed germination requirements and vegetative propagation potential for *T. cooleyi*.

To document woody species associates of *Thalictrum cooleyi*, I sampled 104-3 m<sup>2</sup> quadrats and measured percent canopy closure, identified woody understory species, and estimated their percent cover. Logistic regression showed that *T. cooleyi* presence decreased as canopy closure increased. In contrast, *T. cooleyi* was more likely to occur as woody shrub cover increased. Canopy closure and woody understory cover were not associated with density, total number, and flowering to vegetative ratio of *T. cooleyi* plants. Only *Morella cerifera* L. of the woody understory was a significant predictor of *T. cooleyi* presence (Wald = 13.823, df = 1,  $P < 0.001$ ).

Deviations from strict dioecy are uncommon in *Thalictrum cooleyi*; only 5% of female plants surveyed had hermaphroditic flowers ( $n = 375$ ). Sex ratios differed significantly from 1:1

and were male-biased. Despite male-bias, my supplementation experiments found evidence for pollen limitation to seed set.

*Thalictrum cooleyi* seeds are dormant at maturity, requiring cold stratification at 1 °C to break dormancy. Germination rates were higher for seeds that received warm then cold stratification, compared to cold only ( $33 \pm 0.67\%$  vs.  $26.7 \pm 1.76\%$ , respectively). Gibberellic acid can break dormancy without cold stratification. Light exposure during incubation is not a requirement for germination. Overall, germination rates were low ( $\leq 33.3 \pm 0.67\%$ ), likely due to low seed viability. *Thalictrum cooleyi* can be propagated vegetatively from caudex and rhizome divisions.

My work supports concerns that habitat loss (i.e., transition of open pine savanna to dense forest), pollen limitation to seed set, and poor seed viability threaten the recovery of *Thalictrum cooleyi*. Conservation efforts will benefit from this new knowledge on seed germination and vegetative propagation protocols for *T. cooleyi*.



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By

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*Dedicated to my two favorite guys in the world;  
without your support, this would not have been possible.*

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## INTRODUCTION

Prior to European settlement in North America, longleaf pine ecosystems were widespread, covering an estimated 30 million hectares (Frost 1993). Longleaf pine savannas, in particular, are characterized by an open canopy of pine trees with an understory dominated by graminoids and a high diversity of herbaceous plants (Veldman et al. 2014). The sparse understory is maintained primarily by frequent fires, which were once a common occurrence in the coastal plains of the southeastern United States, but now occur mostly through prescribed burns (Glitzenstein et al. 1995). These diverse ecosystems are now estimated to cover a mere 3% of their former range, in part due to fire suppression and other anthropogenic pressures (Frost 1993).

The loss of pine savanna ecosystems is a significant threat to biodiversity. Pine savannas contain high species diversity of herbaceous plants, particularly in the wetter habitats. The plant species richness of a mesic pine savanna within the Green Swamp Preserve, NC was found to be 35 species per m<sup>2</sup>, greater than that recorded for any other North American ecosystem (Walker and Peet 1984). These ecosystems also harbor large numbers of rare and endemic plant species. There are 1630 vascular plant taxa endemic to the Coastal Plain regions of the southeastern US, of which 1000 taxa are found only in longleaf pine habitats (Sorrie and Weakley 2006). In general, it is endemic species which are most at risk of extinction due to their limited geographic ranges (Sorrie and Weakley 2006). Habitat loss within the limited ranges of endemic species can be a driver for rarity and risk of extinction (Dirnböck et. al 2011).

*Thalictrum cooleyi* Ahles, Cooley's meadowrue (Ranunculaceae), is an herbaceous perennial angiosperm, endemic to the southeastern US coastal plain. *T. cooleyi* was considered rare when first described in 1957 and later listed as federally endangered in 1989 (USFWS

1994). The species occurs in North Carolina (9 populations), Georgia (2 populations), and Florida (1 population) (USFWS 2008). *T. cooleyi* is associated with circumneutral wet pine savannas dominated by *Pinus palustris* Mill. and *P. serotina* Michx. and adjacent pocosin-like habitats (USFWS 2008; Schafale 2012). *T. cooleyi* also occurs in disturbed areas such as powerline right-of-ways and roadside ditches (USFWS 2008). When in a vegetative phase, *T. cooleyi* can be cryptic with a rosette of pinnately compound glabrous leaves often obscured by surrounding vegetation. *T. cooleyi* leaflets tend to be more linear than other *Thalictrum* species and range from lobed to unlobed. When flowering (June-August), *T. cooleyi* rises above surrounding vegetation as a 1m tall, open panicle often supported by shrubs such as inkberry (*Ilex glabra* (L.) A.Gray) and wax myrtle (*Morella cerifera* (L.) Small).

The breeding system of *Thalictrum cooleyi* is considered dioecious with functionally male and female flowers occurring on different plants. Staminate (male) flowers have numerous unfused filaments (2-3 mm long), ranging from white to pale lavender and topped with bright yellow anthers (1.5 mm long, Figure 1). Each pistillate (female) flower has on average 13 separate carpels ( $\pm$  SE = 0.055,  $n = 60$ ) of a greenish hue with white pubescent stigmas, 1.5-2 mm long. The lack of petals (apetalous) in both sexes, highly papillate stigmas, and smooth pollen grains suggest wind pollination (Figure 1), although insects have been seen visiting male flowers (pers. obser.). Flowering occurs from June-September, with males beginning to flower first and female plants a couple weeks later. *T. cooleyi* fruits are ellipsoid achenes (4.5-6 mm long and 1.5-2.5 mm wide) which mature from August-October. *T. cooleyi* is known to have a ploidy level of  $2n = 210$ , which is 30 times higher than any other species in the genus (Park 1992).



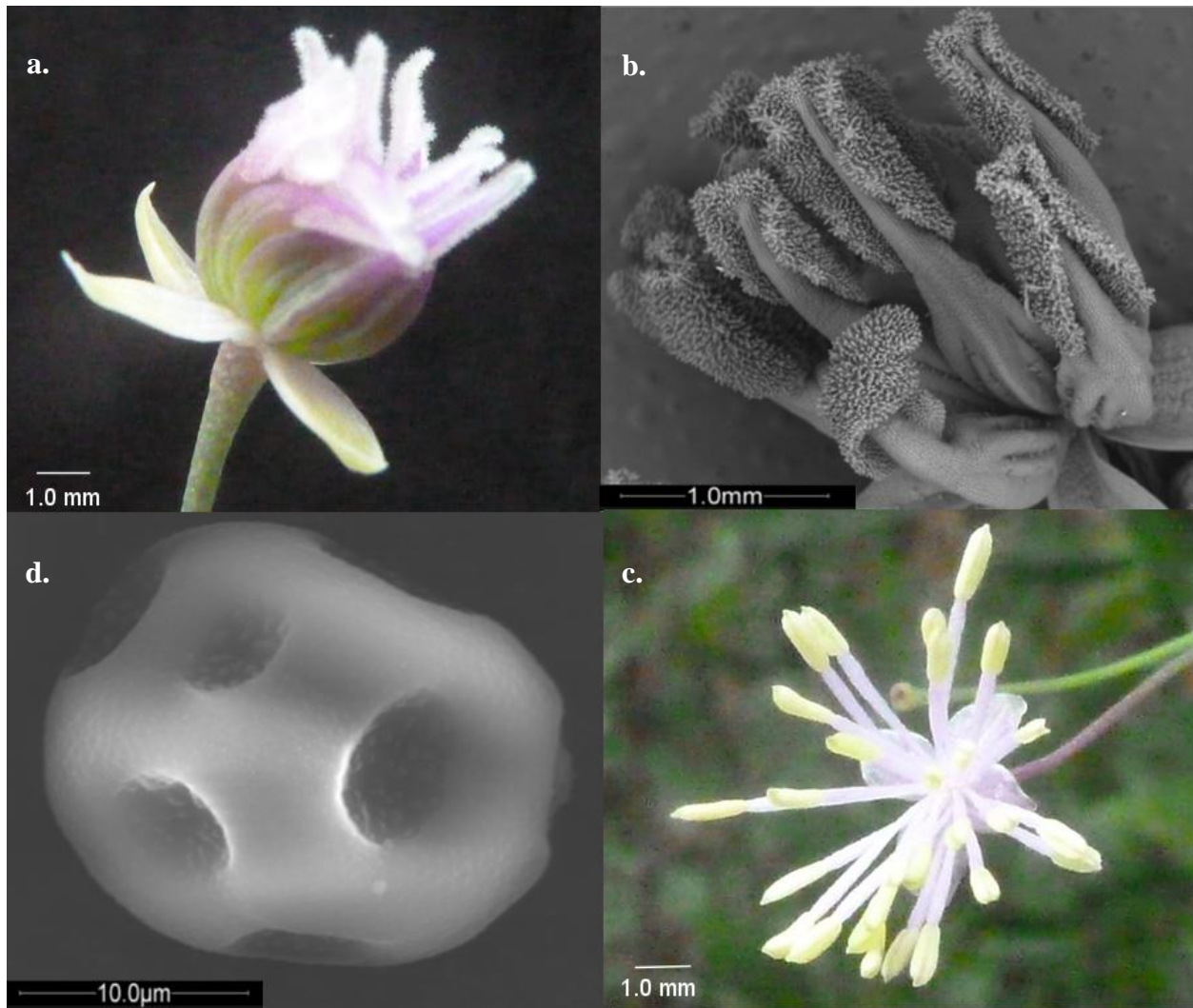


Figure 1. *Thalicttrum cooleyi* flower and pollen morphology. Clockwise from top left: (a) Female flower, (b) Female flower (Scanning Electron Micrograph (SEM) 56X), (c) Male flower, and (d) Pollen grain (SEM, 7000X)

## Woody Plant Associates

One of the primary threats to *Thalictrum cooleyi* is the successional transition of open pine savannas to a dense forest habitat in the absence of fire (USFWS 2008). Pine savannas have a sparse canopy with treeless gaps created by natural events such as fire, windthrows, and hurricanes (Gilliam et al. 2006). Trees in the pine savanna overstory exert a strong influence on the abundance and richness of herbaceous plants below in the understory layer (Platt et al. 2006; Veldman et al. 2014). Canopy trees can limit understory vegetation indirectly by their effects on light availability and directly through competition for soil water and nutrients (Harrington et al. 2003). In a study on the effects of overstory thinning, an increase in light availability was the primary cause of an increase in forb and grass cover (Harrington and Edwards 1999). Plant reproductive output has also been associated with light availability, with higher rates of flowering and seed set as light levels increase (Horton et al. 2010). Canopy closure, the proportion of sky hemisphere obscured by vegetation when viewed from a single point, is used as an indirect assessment of the understory light climate (Jennings 1999). Various techniques have been used to measure canopy closure with varying degrees of cost and accuracy, including: sighting tubes, hemispherical photographs, and most recently aerial LiDAR imagery (Beaudet and Messier 2002; Fiala et al. 2006; Moeser et al. 2014). A data set compiled from numerous studies of pine savannas of the southeastern USA (256 sites at three locations) found that mean tree canopy cover ranged from 30-78 % with a mean cover of 56% (Veldman et al. 2014).

Fire in pine savannas helps maintain a diverse herbaceous layer by suppressing the growth of mid-story hardwood trees and woody shrubs (Robertson and Hmielowski 2014). Yet, in the fire-maintained savannas where *Thalictrum cooleyi* occurs, it is often found beneath shrubs (pers. obser.). Depending on the frequency and intensity of fire, a minor woody component can

still exist due to post-fire regeneration of surviving plants and new seedling establishment (Greenberg et al. 1995). Woody understory plants can have negative or positive effects on the surrounding herbaceous layer (Filazzola and Lortie 2014). Woody shrubs can reduce species richness of herbaceous understory plants by alteration of fire regimes through suppression of highly flammable species such as wiregrass (Freeman and Jose 2009). Herbaceous plants can also be negatively affected by woody shrubs through direct competition for light and nutrients (Glitzenstein et al. 2003). In some environments, however, shrubs can serve as “nurse plants” that facilitate the growth and survival of other species by ameliorating environmental stress (Filazzola and Lortie 2014; Tian and Wang 2014). Shrubs can create microsites favorable to the growth of other species through their influence on light levels, soil nutrients, and water availability (van Zonnefeld et al. 2012; Navarro-Cano et al. 2015). Researchers found that nurse shrubs also facilitated the vegetative recovery of herb and woody species after a disturbance that simulated herbivory or fire (Raffaele and Veblen 1998). A better understanding of the relationship between *T. cooleyi* and surrounding vegetation could help land managers develop restoration protocols.

### **Breeding System**

Breeding systems affect seed success and genetic structure, thus, effective conservation requires we understand these traits, particularly for rare plants. Plant breeding systems include components such as structure and attributes of individual flowers, the production and dispersal pattern of pollen, receptivity of female organs to various pollen donors, as well as seed characteristics and dispersal (Richards 1986). The sexual expression of flowers, an important component of the breeding system, can be determined by genetic (e.g., heteromorphic sex chromosomes) or physiological mechanisms, environmental conditions, plant size, or a

combination of factors (Stehlik et al. 2008; Aryal and Ming 2014). Dioecy, which evolved from hermaphroditism in flowering plants, is an uncommon breeding system, occurring in an estimated 6% of species (Barrett 2013). Dioecy can be considered inefficient, compared to hermaphroditism, because only half of the genets produce seeds (Richards 1986). Seed set can also be limited in dioecious species by factors that prevent pollen transfer between the sexes, including a lack of pollinators, unbalanced sex ratios, and distances between the sexes (Larson and Barrett 2000; Shelton 2008; Wang et al. 2013). Some species, however, are not strictly dioecious and within a population can have pistillate and hermaphroditic individuals (gynodioecious), staminate and hermaphroditic individuals (androdioecious) or exhibit a range of sexual morphs known as polygamodioecy (Sakai and Weller 1991).

There is a diversity of breeding systems within the estimated 190-196 unique species of *Thalictrum* (Soza et al. 2012). Phylogenetic evidence suggests that dioecy evolved from hermaphroditism multiple times within the genus *Thalictrum* (Soza et al. 2012). Some species of *Thalictrum* have flowers that are hermaphroditic in appearance, but contain non-functioning (or vestigial) male or female reproductive organs (i.e., cryptic dioecy; Davis 1997). Penny and Steven (2009) found that *T. macrostylum* Small & A. Heller has perfect flowers with inaperturate pollen, rendering them functionally female, and thus this species is androdioecious. Although considered dioecious, *T. cooleyi* can produce some perfect flowers on either male or female plants (pers. obser.). If the perfect flowers in *T. cooleyi* are functional, they could help contribute to overall seed set and thus population persistence. Knowledge of the breeding system of *T. cooleyi* can also contribute to our overall understanding of the evolution of dioecy from hermaphroditism within the genus.

## Sex Ratios

In dioecious plant populations, there are often unequal numbers of female and male flowering plants (Barrett et al. 2010). This is in contrast to theoretical predictions that natural selection will act to maintain a 1:1 sex ratio (Fisher 1930; Edwards 2000). The factors that lead to unequal sex ratios are not well understood although certain correlates of biased sex ratios have been found including: pollen and seed dispersing mechanisms, clonality, and sex chromosomes (Field et al. 2013). A survey of 243 dioecious species found that male-bias is associated with long-lived plants, biotic seed dispersal, and fleshy fruits, whereas female-biased ratios are correlated with clonal growth, abiotic pollen dispersal, and sex chromosomes (Field et al 2013). In general, male-biased flowering sex ratios occur twice as often as female-biased ratios (Field et al. 2013). For many dioecious species, females have a higher reproductive cost compared to males (Ward 2007) due to the greater cost of ovule production and seed set. This differential cost of reproduction can lead to male-biased sex ratios through higher mortality rates for females compared to males (Barrett et al. 2010). Sex ratios can also vary along environmental gradients, such as altitude or moisture, often with female plants present in more favorable growing conditions (Bierzychudek and Eckhart 1988; Eppley 2001; Van Drunen and Dorken 2012).

Regardless of the underlying mechanisms responsible for biased sex ratios, deviations from 1:1 can negatively affect pollination and thus seed set (Buckel et al. 2012). In the endangered species *Otellia acuminata* Dandy, an aquatic dioecious plant, there was a significant reduction in seed set in female-biased populations compared to those with higher proportions of males (Xia et al. 2013). In extreme cases, the success of a population can be compromised if the female: male ratio is highly unbalanced (Abe et al. 2008). The negative effects of biased sex ratios can also be more pronounced in small, fragmented populations (Wall et al. 2013), typical

of many endangered species such as *Thalictrum cooleyi*. Currently, we lack detailed surveys of sex ratios in *T. cooleyi* populations.

### **Pollen Limitation**

For many plant species, successful seed set and seedling recruitment are crucial to population growth and persistence (de Kroon et al. 1986; Lavergne et al. 2004; Münzbergová 2013). Seed set can be limited by several factors: initially, lack of pollination, and subsequently, inadequate resources for the developing ovule (i.e., resource limitation), ovule abortion, and seed predation (Fenner and Thompson 2005). Low pollen availability has been found to be the limiting factor to seed set in numerous taxa (Friedman and Barrett 2009). In a literature review, pollen limitation was observed in 62% of the 258 taxa studied (Burd 1994). This phenomenon is present in species with abiotic (e.g., wind) and biotic (e.g., animal) modes of pollen transfer (Ågren 1996; Hesse and Panell 2011). Burd (1994, 2009) attempted to explain why pollen limitation is so common by proposing: “the ovule-packing hypothesis”. This hypothesis posits that a stochastic pollen environment has selected for flowers to over-produce ovules relative to an average pollen load. Certain breeding systems are less prone to pollen limitation, however, specifically those in which plants are both hermaphroditic and self-compatible (Knight et al. 2005).

Dioecious species could be particularly susceptible to pollen limitation because of the separation of male and female sexual functions (Corff et al. 1998; Larson and Barrett 2000). Morphological differences between male and female flowers, such as a lack of pollinator reward in females and showier male flowers, can limit pollinator visits to females flowers (Ashman 2000; Van Etten and Chang 2014). For animal-pollinated dioecious species, this sexual dimorphism can lead to pollen limitation and a higher extinction risk if pollinator abundance is

low (Vamosi and Otto 2002). Because pollen travels limited distances, dioecious species that are wind-pollinated have also been found to be pollen limited, particularly in small, low-density populations (Fox 1992; Davis 2004). Steven and Waller (2007) examined low- and high-density populations of wind-pollinated *Thalictrum fendleri* Engelm ex A. Gray and *Thalictrum dioicum* L. The authors found that pollen limitation reduced seed set only in the low-density populations. In their study, rates of seed set decreased as the distance to the nearest male plant increased. Pollen limitation could also be one explanation for limited seed set in *T. cooleyi* populations.

### **Seed Germination**

Successful seed germination and establishment (ecesis) are critical for species conservation. Little is known of *Thalictrum cooleyi* seed ecology, but previous work suggests seeds may be dormant at maturity. An unpublished study on *T. cooleyi* tested the effects of a chilling pre-treatment on seeds and obtained no germination (Goodnight 1990). The seeds of many temperate plant species exhibit dormancy to prevent germination in unfavorable conditions and require seasonal changes in temperature and moisture to break dormancy; factors required to break dormancy vary among species (Baskin and Baskin 1998). Seed dormancy can be broadly categorized in three ways: (1) the seed is immature when dispersal occurs and additional growth/differentiation is required prior to germination (morphological), (2) the seed has an impermeable protective coating and the embryo is dry until seed coat is broken and water can enter to facilitate germination (physical), or/and (3) germination is prevented until a chemical change occurs within the seed (physiological) (Baskin and Baskin 1998). Dormancy types are not exclusive; seeds can exhibit multiple types of dormancy (Fenner and Thompson 2005). At maturity, *T. mirabile* seeds have underdeveloped (small) embryos and physiological dormancy, a condition known as morphophysiological dormancy (Walck et al. 1999).

Under controlled conditions, dormancy can be overcome in many species by a process called stratification, which involves subjecting seeds to a period of moist-prechilling, moist-warm conditions or a combination of both treatments (Baskin and Baskin 1998). In *Thalictrum mirabile*, seeds germinated best with an initial period of warm stratification followed by cold stratification at 1 °C and incubation in warm conditions  $\geq 15$  °C (Walck et al. 1999). Light exposure also affects seed germination for some species. Depending on numerous other factors (e.g., temperature and timing of exposure), light can either inhibit or encourage dormancy break (Baskin and Baskin 1998). Pre-treatments used to break dormancy, such as light and temperature, have been found to affect the ratio of abscisic acid (a germination inhibitor) and gibberellin concentrations within the seed (Amat et al. 2014). Gibberellins can occur naturally as plant hormones and can play an important role in seed germination in some species (Derx et al. 1994; Finkelstein et al. 2008). The application of exogenous gibberellic acid (GA<sub>3</sub>) can break dormancy in seeds which are unresponsive to other pre-treatments (Sivakumar et al. 2006; Payal et al. 2014). In a study of *T. dioicum*, *T. rochebrunianum*, and *T. tuberiferum*, seed germination was low and protracted unless seeds were exposed to GA<sub>3</sub> (Deno 1993).

### **Vegetative Propagation**

In fire-adapted habitats, frequent fires kill the top growth of herbaceous and woody plants and post-fire regeneration may occur from buds found on below-ground stems called rhizomes (Lemon 1949; Greenberg et al. 1995). Although *Thalictrum cooleyi* occurs in historically fire-dependent habitats, the effects of fire on *T. cooleyi* are unknown. Like many fire-adapted species, however, *T. cooleyi* can regenerate via rhizomes. *T. cooleyi* can also grow vegetatively from buds along a basal stem structure called a caudex. These buds can form leaves, roots, or new caudices with the potential of becoming a clone of the mother plant (pers. obser.).



The ability of plants to regenerate and reproduce vegetatively has been utilized by conservationists as a reliable alternative to seed propagation for endangered species (Cenkci et al. 2009; Kunz et al. 2014). The physiology of rhizomes, i.e., the presence of belowground buds, makes them an effective means of vegetative propagation in many plant species (Nivot et al. 2008). Rhizomes can be cut into multiple segments, each section with one or more intact buds from which new shoots and roots can occur (Joshi et al. 2004). The survival of rhizome segments can be increased by stimulating root growth with the plant hormone, auxin (Kandari et al. 2011). In the woodland herbs *Asarum canadense* L. and *Sanguinaria canadensis* L., root growth increased three and four-fold with the use of the auxin, indole-3-butyric acid (Nivot et al. 2008). Cultivating threatened and endangered plants through vegetative propagation, which is often more efficient than by seed, could be one way to restore natural populations (Butola et al. 2010).

Continued threats to *Thalictrum cooleyi* include habitat loss, fire suppression, and possibly limited seed set and low seed viability. Based on the USFWS Five Year Review, *T. cooleyi* has little published information and has “not been monitored in enough detail or with sufficient frequency nor has enough detailed data been collected to predict long term population trends” (USFWS 2008). My research addressed issues related to the reproductive biology of *T. cooleyi*, its microhabitat, and propagation techniques. Specific questions addressed: (1) Is there a characteristic canopy closure and woody shrub presence associated with *T. cooleyi*?, (2) What is the breeding system of *T. cooleyi*?, (3) Do sex ratios differ from 1:1 in populations of *T. cooleyi*?, (4) Does pollen availability limit seed set in *T. cooleyi*?, (5) What factors control seed germination?, and (6) Does propagation of vegetative organs hold promise for restoration?

## METHODS

### Study Sites

The North Carolina Natural Heritage Program recognizes 25 subpopulations, also known as elemental occurrences (EO), of *Thalictrum cooleyi* (USFWS 2008). My field work was conducted in 2013 and 2014 at three *T. cooleyi* subpopulations in the coastal plain region of southeastern NC (37° 24' 26.0244"N, 122° 6' 25.7832"E): (1) Shaken Creek Preserve-Fill Rd. (EO 32.30, Pender Co.), (2) McLean Savanna (EO 18, Onslow Co.), and (3) Sandy Run Swamp Powerline (EO 24.4, Pender Co.). Shaken Creek Preserve and McLean Savanna, both owned by The Nature Conservancy, represent high quality pine savannas of the very wet loamy variety (Schafale 2012). These sites are routinely burned and have an open canopy of *Pinus palustris* Mill. and *Pinus serotina* Michx., along with a diverse understory dominated by grasses such as *Ctenium aromaticum* (Walter) Alph. and *Sporobolus teretifolius* Harper. The soils at Shaken Creek-Fill Rd. are Foreston loamy fine sand, while McLean Savanna has Woodington fine sandy loam (NRCS 2014). These seasonally saturated soils support large patches of *Sarracenia flava* L. in the wettest areas. The shrubs *Morella cerifera* L. and *Ilex glabra* (L.) A. Gray occur intermittently throughout.

The third population, managed by the North Carolina State Parks and Recreation, is part of the Sandy Run Savannas State Natural Area. At this distinctive site, *Thalictrum cooleyi* plants occur under a powerline on the edge of Sandy Run Swamp. The area has been maintained through mowing and burning, leading to savanna-like habitat with an absence of overstory trees. The ground layer contains graminoids such as *Cladium jamaicense* Crantz, *Rhynchospora latifolia* (Baldwin ex Elliott) W.W. Thomas, and the federally endangered *Carex lutea* LeBlond

(NCNHP 2012). Forb species include *Polygala lutea* (L.), *Eupatorium rotundifolium* (L.), and *Scutellaria integrifolia* (L.). The soil is Foreston loamy fine sand (NRCS 2014).

### **Plants Harvested from Natural Populations for Research in Greenhouse**

In 2013, I harvested plants (with the appropriate permits) from three robust *Thalictrum cooleyi* subpopulations. On 12 June 2013, I excavated plants from Mark Pine Bay (EO 25.9,  $n = 18$ ) a site in Columbus county and later on 9 and 11 July 2013 at McLean Savanna ( $n = 16$ ) and Shaken Creek Preserve ( $n = 11$ ). Within 24 h of being dug, plants were transferred into pots with a 3:1:1 ratio of sterile potting soil (Fafard<sup>TM</sup> 3-B): sand: perlite. Plants were placed in an environmental growth chamber (Conviron A-1000AR) with a daily 14/10 h alternating thermoperiod of 25/15 °C and light/dark photoperiod. In August 2013, plants were moved to a climate-controlled greenhouse, where temperatures do not go below 10 °C in the winter, but can reach 38 °C in the summer. Plants were kept moist and initially fertilized with 200 ml of a dilute solution of Miracle-Gro<sup>TM</sup> 24-8-6 (1.5 tsp per 6.6 L of tap water), monthly when not in flower and weekly when flowering. In August 2014, I switched to Osmocote<sup>TM</sup> 19-6-12 slow-release granules (1.5 tsp per 6.6 L pot, every 6 months) for ease of application.

In 2013, these field excavated plants flowered from June-November in the greenhouse and then set seed without assisted pollination. In order to stimulate flowering again in 2014, we randomly selected 19 plants to be vernalized, exposed them to prolonged exposure to cold temperatures inside an unheated greenhouse from January-April 2014, then returned the vernalized plants to the climate-controlled greenhouse. These plants began flowering on 9 June 2014 and set seed. Plants that remained in the climate-controlled greenhouse over winter (i.e., not vernalized) did not flower in 2014.

## Woody Plant Associates

In order to document percent canopy closure and percent cover of woody understory species, I established belt transects and sampling quadrats at Shaken Creek-Fill Rd and McLean Savanna. In mid-June 2014, I used meter tapes to create a total of 18 belt transects spanning the entire area in which *Thalictrum cooleyi* occurs at Shaken Creek-Fill Rd. (10 transects) and most of the area where it occurs at McLean Savanna (8 transects). Transects were spaced 10 m apart with dimensions of 6 m × 25 m long, representing overall coverage areas of 1500 m<sup>2</sup> (Shaken) and 1200 m<sup>2</sup> (McLean). For each transect, a starting point for sampling was established by recording a GPS point and installing a PVC tube. I used a stratified random sampling design to establish a total of 104 quadrats (1 m × 3 m) within the belt transects at both sites (Shaken = 52, McLean = 52). Quadrat locations were chosen by selecting a random number to represent a distance along the 25 m transect, the distance being the spot on which we placed a quadrat.

During the summer of 2014 (June-August), I estimated percent canopy closure and percent cover of woody understory species for quadrats at Shaken Creek-Fill Rd. and McLean Savanna. To measure canopy closure, I used a hand-held convex spherical densiometer (Forest Densiometers, Rapid City, South Dakota- Model- A) in which a hemispherical image of the canopy above is projected onto a graticuled mirror (Lemmon 1956; Baudry et al. 2014). Following the methods of Lemmon (1956), I stood in each quadrat center and took a total of four measurements, one while facing each cardinal direction. For each measurement, I mentally divided each of the 24 squares engraved onto the mirror into four smaller squares and counted the number of squares that were obstructed by vegetation (96 possible squares). The four values were each multiplied by 1.04 to obtain percent canopy closure and then averaged together to obtain a percent cover value for each quadrat. I also documented the woody species present in

the understory (height < 2.5 m and diameter at breast height, dbh < 7.5 cm) and visually estimated their respective percent cover within each quadrat.

The three cover measures (overall percent canopy closure, percent cover of individual woody species, and percent cover of all woody understory species in a quadrat combined) were pooled across sites and averaged for quadrats with and without *Thalictrum cooleyi*. I used a logistic regression to test whether mean percent canopy closure and percent cover of woody understory species (combined) were predictors of the occurrence of *T. cooleyi*. The four woody species with highest percent cover were analyzed individually with logistic regression. I also used Pearson's correlation coefficient to test for a correlation between percent canopy closure and percent cover of woody understory species combined.

Variables were tested for normality and equality of variances to meet model assumptions; all analyses were performed in SPSS 22.0 (2013). Means are reported  $\pm 1$  SE throughout.

### **Plant Density**

To describe plant density, I surveyed the aforementioned 52 (1m  $\times$  3m) quadrats at McLean Savanna and Shaken Creek-Fill Rd for the presence or absence of *Thalictrum cooleyi*. If present, a plant was counted as a unique individual if it was found more than 5 cm from its nearest neighbor; I also noted if the plant was flowering or vegetative. An ANOVA was used to compare mean density/m<sup>2</sup> of *T. cooleyi* plants at Shaken Creek Preserve-Fill Rd and McLean Savanna. Prior to analyses, an outlier (a quadrat with 22 plants) from Shaken Creek Preserve that was significantly higher than the mean  $11 \pm 2$  plants per quadrat was removed to meet assumptions of normality. I used linear regression to test if percent canopy closure and percent cover of woody understory species combined are predictors of the mean density (plants/m<sup>2</sup>) of *T. cooleyi* and the ratio of flowering: vegetative plants. Quadrats lacking either a flowering ( $n$

= 1) or vegetative plant ( $n = 4$ ) were excluded from the analysis. A Poisson regression was used to test these variables as predictors of the total number of *T. cooleyi* plants per quadrat.

### **Sex Ratios and Hermaphroditic Flowers**

Due to the difficulty in distinguishing individual plants (genets) in the field, most sex ratio surveys are based on numbers of flowering stems (ramets) rather than unique individuals (Barrett et al. 2010; Munné-Bosch 2015). On 7-8 July 2013, I documented sex ratios at Shaken Creek-Fill Rd. and McLean Savanna by counting the number of female and male flowering stems. An effort was made to document all flowering stems by walking the entire area in which *Thalictrum cooleyi* occurs at these sites. I repeated the sex ratio surveys on 7-8 July 2014 at both Shaken Creek-Fill Rd. and McLean Savanna, and also added the Sandy Run Swamp Powerline site on 25 July 2014. I either walked the established belt transects (Shaken and McLean) or the entire area (at the Powerline site, where *T. cooleyi* occurs in a small linear strip), noting the number of female and male flowering stems. In 2014, I also noted the number of female flowering stems with perfect flowers and the proportion of perfect flowers relative to the total number of open flowers on each stem. I used  $\chi^2$  Goodness of Fit tests to compare sex ratios among sites (Shaken Creek Preserve-Fill Rd., McLean Savanna, and Sandy Run Swamp Powerline) and also from two sampling dates at Shaken Creek Preserve to ask if sex ratios vary throughout a flowering period (SPSS 22, 2013).

During the summer of 2014, I also recorded the occurrence of perfect flowers on female greenhouse plants. In order to test if perfect flowers produce viable pollen and are self-compatible, I used organza to bag 25 perfect flowers on a single female prior to stigma receptivity. The experiment was conducted in a greenhouse room without male plants to ensure the only pollen source was from perfect flowers on that particular female.

## **Pollen Limitation**

I conducted pollen supplementation experiments in 2013 and 2014 to determine if pollen is a limiting factor for seed set in *Thalictrum cooleyi* populations. The 2013 experiment was conducted at McLean Savanna, Shaken Creek-Fill Rd., and Sandy Run Swamp Powerline. Selected plants had one flower hand-pollinated with fresh mixed pollen from at least three males and another flower left unmanipulated as an open-pollinated control. This was performed on 7-8 July at the Sandy Run Swamp Powerline ( $n = 19$  paired flowers) and Shaken Creek-Fill Rd. ( $n = 16$  paired flowers) and 17 July at McLean Savanna ( $n = 11$  paired flowers). I tagged flowers with threads of differing colors based on treatment. I returned 2-3 wk later to count the number of maturing carpels at Shaken Creek and Sandy Run Swamp Powerline on 24 July 2013 and at McLean Preserve on 30 July 2014. The proportion of maturing carpels relative to the total number of carpels was used to quantify seed set for hand-pollinated vs. open-pollinated flowers. For each female plant in the pollination study, I also documented covariates known to affect seed set: (1) height of flowering female plants, (2) distance to the nearest male, (3) number of male plants, and (4) flowers within 2 m of female plants. I pooled seed set data across sites for each treatment group (hand- vs. open-pollinated) given that sample sizes were somewhat low at each site. I used a mixed model analysis with a variance components error structure to compare seed set between hand- and open-pollinated flowers (SPSS 22.0, 2013). The model included pollen supplementation treatment and covariates listed above as fixed factors along with plant as a random factor.

The pollen supplementation experiment was repeated with modifications in 2014 at Shaken Creek-Fill Rd and McLean Savanna. On 7 July 2014 (Shaken Creek) and 8 July 2014 (McLean Savanna), each sample plant received a single treatment (hand- vs. open-pollinated)

on all flowers that were receptive. Unreceptive or pollinated stigmas become brown; I scored flowers as receptive if all stigmas were white. In total, I hand-pollinated flowers on 32 and 20 plants at Shaken Creek and McLean Savanna, respectively. Mixed pollen loads were collected from at least three different nearby male plants. Each treated plant had a paired control plant on which I tagged unmanipulated flowers (i.e., open-pollinated) that were in the same stage of receptiveness as hand-pollinated flowers. I returned approximately 6 wk later to count maturing carpels at each site. In addition to the covariates measured in 2013, I also included the number of open flowers on each sample female and the number of additional female plants within 2 m. This design is similar to that used by Steven and Waller (2007). For the 2014 experiment, hand- and open-pollinated flowers were on separate plants (i.e., independent), so I compared seed set between treatments with a general linear model (GLM, SPSS 22.0, 2013). For the GLM, I included the covariates (height of flowering female plants, distance to the nearest male, number of male plants, flowers within 2 m of female plants, number of open flowers on each sample female, and the number of additional females within 2 m) along with the fixed factors, treatment, and site. Because pollen travels limited distances, the number of plants that occur in a given area (i.e., plant density) can influence the amount of pollen females receive and thus seed set (Steven and Waller 2007).

### **Seed Collection and Seed Germination Studies**

I performed two seed germination experiments in 2013-14 and 2014-15 to test for seed dormancy at maturity and to determine which factors are most important to germination of *Thalictrum cooleyi* seeds. Seeds were collected, as they matured (i.e., when brown but before falling from plant), from greenhouse plants. Seed collection was protracted (August – November 2013 and September – October 2014) because seed maturation occurred at different



times both within and among plants. Seeds were sourced from 10 genotypes for the first experiment and four genotypes in 2014-15. Upon harvest, seed coats were removed from each achene and seeds were stored dry at room temperature until the experiments began on 22 January and 16 September 2014. In 2013, all seeds were massed to the nearest 0.0001mg (Cahn model E-15, Cerritos, CA). In 2014, I massed a subset of seeds from each plant or enough to be able to visually select seeds  $\geq 2.5$  mg. I randomly selected seeds with a mass  $\geq 2.5$  mg for use in both germination trials; our pilot work suggested that seeds smaller than 2.5 mg were unfilled and would not likely germinate.

For both sets of germination experiments in 2013 and 2014, stratification and incubation conditions were similar to those used on the congener, *Thalictrum mirabile* Small (Walck et al. 1999). Cold stratification took place inside a lab refrigerator at 1 °C; uncovered seed trays were exposed to light only for brief periods when the door was opened. Incubation and any resultant germination took place inside an environmental growth chamber under a daily 14/10 h cycle of alternating photoperiod (light/dark) and thermoperiod (25/15 °C). Plate wells and petri dishes were filled with QUIKRETE™ Play Sand that had been autoclaved at 250 °C for 25 min and then moistened with distilled water.

In the first experiment (begun on 22 January 2014), my treatments included exposure to gibberellic acid (GA<sub>3</sub>), presence or absence of cold stratification, and length of cold stratification (8 wk vs. 12 wk, Figure 2). Seeds were germinated in 24-well plastic tissue culture plates (2 cm diameter  $\times$  1.5 cm depth). For seeds not cold stratified prior to incubation, plates were stored dry at room temperature for 8 or 12 wk. In order to test for the effects of light on germination, each treatment (not GA<sub>3</sub>) included three plates ( $n = 72$ ) covered with aluminum foil to exclude light

		Time (wk)													
Year	Treatments	0	2	4	6	8	10	12	14	16	18	20	22	24	26
2013-14	No Cold	25/15 °C, light/dark or dark, moist													
	Gibberellic	25/15 °C, light/dark, moist													
	8 wk Cold	1 °C, dark, moist				25/15 °C, light/dark or dark, moist									
	8 wk Control	Room Temp, dark, dry				25/15 °C, light/dark or dark, moist									
	12 wk Cold	1 °C, dark, moist						25/15 °C, light/dark or dark,							
	12 wk Control	Room Temp, dark, dry						25/15 °C, light/dark or dark,							
2014-15	No Cold	25/15 °C, light/dark, moist													
	8 wk Cold	1 °C, dark, moist				25/15 °C, light/dark, moist									
	8 wk Control	Room Temp, dark, dry				25/15 °C, light/dark, moist									
	6 wk/8 wk Warm/Cold	25/15 °C light/dark, moist				1 °C, dark, moist				25/15 °C, light/dark or dark, moist					
	14 wk Control	Room Temp, dark, dry						25/15 °C, light/dark, moist							

Figure 2. Treatments and the corresponding environmental conditions for the *Thalictrum cooleyi* seed germination experiments. In 2013-14, each treatment (excluding Gibberellic Acid) included trays incubated in alternating light/dark or in 24 h darkness. Time zero was treatments initiation on 22 January 2014. In 2014-15 time zero was when each treatment began, 31 October 2014 (16 September 2014 for the No Cold treatment).

and three uncovered plates ( $n = 72$ ). Seeds had been stored at room temperature from 79-142 d (dependent on harvest date) prior to the start of treatments.

Treatments were modified for the second experiment (16 September 2014) due to a smaller seed harvest compared to the previous year; these treatments were chosen based on results from the initial trial. Treatments included presence or absence of cold stratification (0 vs 8 wk) and the addition of 6 wk warm conditions prior to 8 wk cold (incubated in alternating light/dark or dark, Figure 2). The 6 wk warm pre-treatment occurred in the same chamber as incubation (14 /10 h daily cycle of 25/15 °C light/dark). For seeds not cold stratified prior to incubation, dishes were stored dry at room temperature for 8 or 14 wk. Seeds for each treatment were germinated in three petri dishes of either 25 (no cold), 35 (6 wk warm/8 wk cold; dark), or 50 seeds (0 wk cold; 8 wk cold stratification; 6 wk warm/8 wk cold; light). Seeds had been stored at room temperature between 1-37 days (dependent on harvest date) prior to the start of treatments.

For the 2013-14 seed germination experiment, seeds were sown in individual wells allowing me to treat each seed as an independent replicate. Seed germination rates, therefore, were calculated as the number of germinated seeds out of the total number of individual seeds for a particular treatment. In 2014-15, each petri dish was treated as a replicate ( $n = 3$ ) and their germination rates were averaged for the mean germination rate of each treatment. Seed germination data from both years were analyzed with logistic regression to determine which treatments were the best predictors of whether or not a seed germinated (SPSS 22.0, 2013). Treatment was included as a categorical factor. For the 2013-14 regression, I also included as covariates seed mass and time since harvest (wk) to account for the age of seeds when they were exposed to favorable germination conditions (i.e., incubation). As a reference variable by which to compare all other treatments, I used the treatment in which seeds did not receive a cold pre-

treatment or light exposure during incubation. In order to test for the significance of the length of cold exposure on germination, I did a separate logistic regression using the 8 wk cold stratified (dark) treatment as the reference variable. In the regression analysis of 2014-15 data, treatment was the only predictor variable. For this experiment, all seeds were incubated in the light except for a 6 wk warm/8 wk cold treatment, which was incubated in the dark. As a reference variable, therefore, I used the treatment in which seeds did not receive a cold pre-treatment but were exposed to light during incubation. To test for differences between light and dark treatments on germination rates, an additional logistic regression was performed in which treatment variables were compared to the treatment with seeds incubated in the dark (6 wk warm/8 wk cold).

In order to assess the effects of variable light exposure and temperatures in the field on seed germination, we also placed seeds inside an unheated greenhouse. On 12 January 2014, three replicates of 10 seeds each, were planted in potting soil (Fafard<sup>TM</sup> 3-B) inside separate pots and watered regularly.

### **Vegetative Propagation**

In January 2014, I conducted an experiment to determine if *Thalictrum cooleyi* could be propagated from vegetative parts. I randomly selected 20 different genotypes from the greenhouse plants for this experiment. Depending on the condition of a particular plant, one or more vegetative parts (propagules or organs) were used: (1) roots, (2) caudex divisions, (3) offsets, and (4) rhizome divisions. For root divisions, yellowish, fleshy roots (3-5 mm in diameter) were removed from 16 plants and cut into two 5 cm long segments (Figure 3a). Half of the 32 root segments were selected to receive the plant growth hormone indole-3 butyric acid (IBA), while the others did not. Caudex divisions were obtained by cutting a caudex (at least 2



Figure 3. The various plant parts (propagules) utilized in a *Thalictrium cooleyi* vegetative propagation experiment included roots, caudex divisions, offsets, and rhizome divisions. (a) Root system of *T. cooleyi*, (b) Caudex division showing upper (crown at left) and lower segments (at right), (c) New crowns (offsets) removed from caudex, and (d) Rhizome (see arrow) with two crowns (circles).

cm in length) in half for a total of 28 caudex propagules (Figure 3b). A subset of the upper caudex (crown) segments was selected without bias to be dusted with IBA ( $n = 8$ ) while the others did not receive IBA ( $n = 6$ ). Since the primary function of IBA is to stimulate root growth (Kandari et al. 2011), none of the lower caudex segments received IBA due to the large number of intact roots at the time of planting. For some plants, I removed what appeared to be new crowns forming on the original caudex (offsets) and IBA was applied to five of the 11 offsets (Figure 3c). I also divided plants attached to one another via below-ground stems (with crowns intact), none of which received IBA (rhizome divisions, Figure 3d).

If present, leaves were removed from all propagules prior to potting. I obtained a total of 46 propagules, which were potted in a 3:1:1 mixture of Fafard 3-B<sup>®</sup> potting soil: sand: perlite, placed in the greenhouse, and watered regularly. Growth responses were recorded using the variables: (1) percent survival, (2) number of leaves, and (3) leaf length. The propagation experiment ended after 4 mo (May 2014) with collection of the final growth response data. Descriptive statistics were calculated for the growth responses (% survival, number of leaves, and leaf length) of each propagule with and without IBA.

## RESULTS

### Woody Plant Associates

*Thalictrum cooleyi* occurred in quadrats with a lower mean percent canopy closure compared to those without (Table 1); however, the mean percent cover of woody understory species was similar ( $44 \pm 4.6$  % vs.  $41 \pm 4.0$  %, with vs. without, respectively). Site was unrelated to *T. cooleyi* presence in a logistic regression (Wald = 0.438, df = 1,  $P = 0.508$ ). Percent canopy closure and percent cover of woody understory species, however, were both significant predictors of the occurrence of *T. cooleyi* (Table 2). Specifically, *T. cooleyi* was more likely to occur as canopy closure decreased ( $B = -0.908$ ). Increases in woody understory cover were positively associated with *T. cooleyi* occurrence (Table 2). From the logistic regression, we also can interpret the odds-ratios ( $e^B$ ). For every 1% increase in canopy closure, the odds of *T. cooleyi* occurrence decreased by 9.3%. Similarly for every 1% increase in cover of woody understory, there was a 2% increase in the odds of *T. cooleyi* presence. There also was a positive correlation between percent canopy closure and percent cover of woody understory species ( $r = 0.439$ ,  $P < 0.001$ ).

The four most common woody understory species found in all quadrats were *Acer rubrum* L., *Ilex glabra* L., *Liquidambar styraciflua* L., and *Morella cerifera* (L.) Small (Table 3). For quadrats with *T. cooleyi*, *M. cerifera* occurred most often (93.8 % of quadrats) and also had the largest percent cover ( $22 \pm 3.94$ %). Of the four most common woody species, *M. cerifera* was the only significant predictor of the occurrence of *T. cooleyi* (Wald = 13.823, df = 1,  $P < 0.001$ ; Table 2).

Table 1. The range and mean values ( $\pm 1$  SE) for percent canopy closure and percent cover of woody understory species in quadrats with and without *Thalictrum cooleyi* (THACOO) at Shaken Creek Preserve-Fill Rd. and McLean Savanna combined.

Variable	WITH THACOO ( <i>n</i> = 32)		NO THACOO ( <i>n</i> = 72)	
	Range	Mean	Range	Mean
% Canopy Closure	1 - 42	22 $\pm$ 2.0	10 - 62	34.0 $\pm$ 1.7
% Cover Woody Understory Species	1 - 85	44.0 $\pm$ 4.6	0 - 100	41.2 $\pm$ 4.0



Table 2. Logistic regression results comparing mean percent canopy closure, mean percent cover of woody understory species, and presence of the woody shrub *Morella cerifera* on the probability of *Thalictrum cooleyi* occurrence in quadrats at Shaken Creek-Fill Rd. and McLean Savanna. B = coefficient, Wald =  $(B/SE)^2$  used to compute the test statistic distributed as a chi-squared,  $e$  = base of natural log. All other Wald statistics exceeded  $P > 0.05$ .

Variable	B	SE	Wald	df	P	$e^B$
% canopy closure	-0.098	0.024	16.397	1	0.001	0.907
% cover of woody understory species	0.020	0.009	4.958	1	0.026	1.021
% <i>Morella cerifera</i>	0.066	0.018	13.823	1	0.001	1.068

Table 3. The four most common woody understory species in 52 quadrats each at Shaken Creek Preserve-Fill Rd. and McLean Savanna. The percentage of quadrats in which species occur and their mean percent cover ( $\pm 1$  SE) are listed for quadrats with and without *Thalictrum cooleyi* (THACOO).

Woody Species	WITH THACOO ( <i>n</i> = 32)		NO THACOO ( <i>n</i> = 72)	
	Occurrence (% of quadrats)	% Cover	Occurrence (% of quadrats)	% Cover
<i>Acer rubrum</i>	37.5	3.5 $\pm$ 1.59	45.8	7.8 $\pm$ 1.78
<i>Ilex glabra</i>	59.4	8.6 $\pm$ 2.21	72.2	22.7 $\pm$ 2.67
<i>Liquidambar styraciflua</i>	34.4	5.4 $\pm$ 2.29	29.0	12.5 $\pm$ 1.48
<i>Morella cerifera</i>	93.8	22.0 $\pm$ 3.94	49.0	11.3 $\pm$ 1.33

## Plant Density

*Thalictrum cooleyi* was absent from most of the sampling quadrats (72 of 104, 69.2%). The mean density of plants at Shaken Creek-Fill Rd. and McLean Savanna was  $3.04 \pm 0.34$  plants/m<sup>2</sup> ( $n = 31$ ). The average ratio of flowering: vegetative plants per quadrats was nearly equal (1.26,  $n = 27$ ). I performed an ANOVA that showed mean density did not differ significantly between Shaken Creek-Fill Rd. and McLean Savanna ( $F(1,30) = 0.540$ ,  $P = 0.468$ ). Linear regression showed that mean percent canopy closure and percent cover of woody understory species were not significant predictors of mean density (model  $F(1,30) = 0.025$ ,  $P = 0.975$ ) or of the ratio of flowering: vegetative plants (model  $F(1,26) = 0.329$ ,  $P = 0.723$ ). Based on the Poisson regression, mean percent canopy closure and percent cover of woody understory species also were not significant predictors of the total numbers of *T. cooleyi* plants in a quadrat ( $P = 0.646$  and  $0.082$  for canopy closure and woody cover, respectively).

## Sex Ratios and Hermaphroditic Flowers

In 2013, there was nearly a 1:1 sex ratio at McLean Savanna; in contrast, male plants outnumbered females nearly 2:1 at Shaken Creek-Fill Rd (Table 4). In 2014, sex ratios were male-biased at all three sites, with nearly twice as many males as females (Table 4). Shaken Creek-Fill Rd. had the largest difference between females and males (females: males = 0.55). Differences in sex ratios, however, were not statistically significant among Shaken Creek-Fill Rd., McLean Savanna, and Sandy Run Swamp Powerline ( $\chi^2 = 4.372$ ,  $df = 2$ ,  $P > 0.05$ ). Perfect flowers were uncommon in these three *Thalictrum cooleyi* populations. In 2014, 21 of 375 female flowering stems surveyed (5 %) at Shaken Creek Preserve-Fill Rd. and McLean Savanna had perfect flowers. Although a small proportion of stems produce hermaphroditic flowers, for

Table 4. Female: male flowering sex ratios at three North Carolina *Thalictrum cooleyi* populations. The total number of flowering stems surveyed at each site appears in parentheses.

Site	Sex Ratio	
	2013	2014
McLean Savanna	0.92 ( <i>n</i> = 333)	0.65 ( <i>n</i> = 456)
Shaken Creek Preserve-Fill Rd.	0.63 ( <i>n</i> = 140)	0.55 ( <i>n</i> = 370)
Sandy Run Swamp Powerline	N/A	0.67 ( <i>n</i> = 184)

those that do, perfect flowers represent on average  $50.2 \pm 0.07\%$  ( $n = 21$ ) of the total number of open flowers on a given day.

Under greenhouse conditions, perfect flowers also were produced by otherwise predominantly female and male flowering plants. For three female plants surveyed in the greenhouse on 23 July 2014, on average,  $40 \pm 0.10\%$  of open flowers on a plant were hermaphroditic. Perfect flowers produced an average of  $8 \pm 0.23$  carpels and  $4 \pm 0.25$  stamens ( $n = 95$  flowers). In contrast, the mean number of carpels for an imperfect female flower was  $13 \pm 0.46$  ( $n = 60$ ). Perfect flowers produced viable pollen and did self-pollinate. In the greenhouse, seven of 25 bagged perfect flowers produced seed with an overall average percent seed set of  $11.1 \pm 0.05\%$  ( $n = 25$  flowers). In comparison, female flowers in the greenhouse produced an average percent seed set of  $45 \pm 0.01\%$  ( $n = 486$  flowers). The viability of seeds from perfect flowers, however, is unknown.

### **Pollen Limitation**

For both studies (2013 and 2014), mean seed set in hand-pollinated flowers was considerably higher than in open-pollinated controls, indicating pollen limitation (Table 5). In 2013, only hand pollination rather than covariates explained differences in seed set between hand- and open-pollinated flowers ( $F(1, 31) = 21.139, P < 0.001$ ). For the 2014 study, results of the general linear model also showed that hand pollination was the only factor that explained significant variation in seed set ( $F(9, 82) = 86.344, P < 0.001$ , Table 6).

### **Seed Germination**

Based on low germination rates (2013-14 and 2014-15) for seeds not cold stratified prior to incubation, most *Thalictrum cooleyi* seeds are dormant at maturity (Figure 4; Figure 5). In 2013-14, mass and weeks since harvest were not significant predictors of seed germination in a

Table 5. Results of 2013 and 2014 pollen supplementation experiment showing seed set for hand- vs. open-pollinated flowers at three *Thalictrum cooleyi* sites. Reported seed set values are means  $\pm$  1 SE.

Seed Set				
	2013		2014	
Site	Hand-Pollinated	Open-Pollinated	Hand-Pollinated	Open-Pollinated
McLean Savanna	0.62 $\pm$ 0.10 (n = 11)	0.27 $\pm$ 0.07 (n = 11)	0.78 $\pm$ 0.05 (n = 16)	0.36 $\pm$ 0.06 (n = 15)
Shaken Creek-Fill Rd.	0.73 $\pm$ 0.08 (n = 12)	0.40 $\pm$ 0.07 (n = 11)	0.77 $\pm$ 0.02 (n = 31)	0.33 $\pm$ 0.04 (n = 30)
Sandy Run Swamp Powerline	0.59 $\pm$ 0.08 (n = 7)	0.49 $\pm$ 0.09 (n = 4)	N/A	N/A

Table 6. ANOVA comparison of mean seed set between hand- vs. open-pollinated flowers, based on treatment and other factors known to affect seed set.

Source	Type III				
	Sum of Squares	df	Mean Squares	<i>F</i>	Sig.
Corrected Model	7.797	9	0.866	15.466	0.000
Height	0.067	1	0.067	1.189	0.279
Nearest male	0.025	1	0.025	0.446	0.506
Males within 2 m	0.031	1	0.031	0.556	0.458
Male flowers within 2 m	0.123	1	0.123	2.191	0.143
Female flowers	0.186	1	0.186	3.328	0.072
Females within 2m	0.035	1	0.035	0.618	0.434
Site	0.004	1	0.004	0.074	0.786
Treatment	4.836	1	4.836	86.344	0.000
Site * Treatment	0.012	1	0.012	0.215	0.644
Error	4.593	82	0.056		
Total	50.770	92			

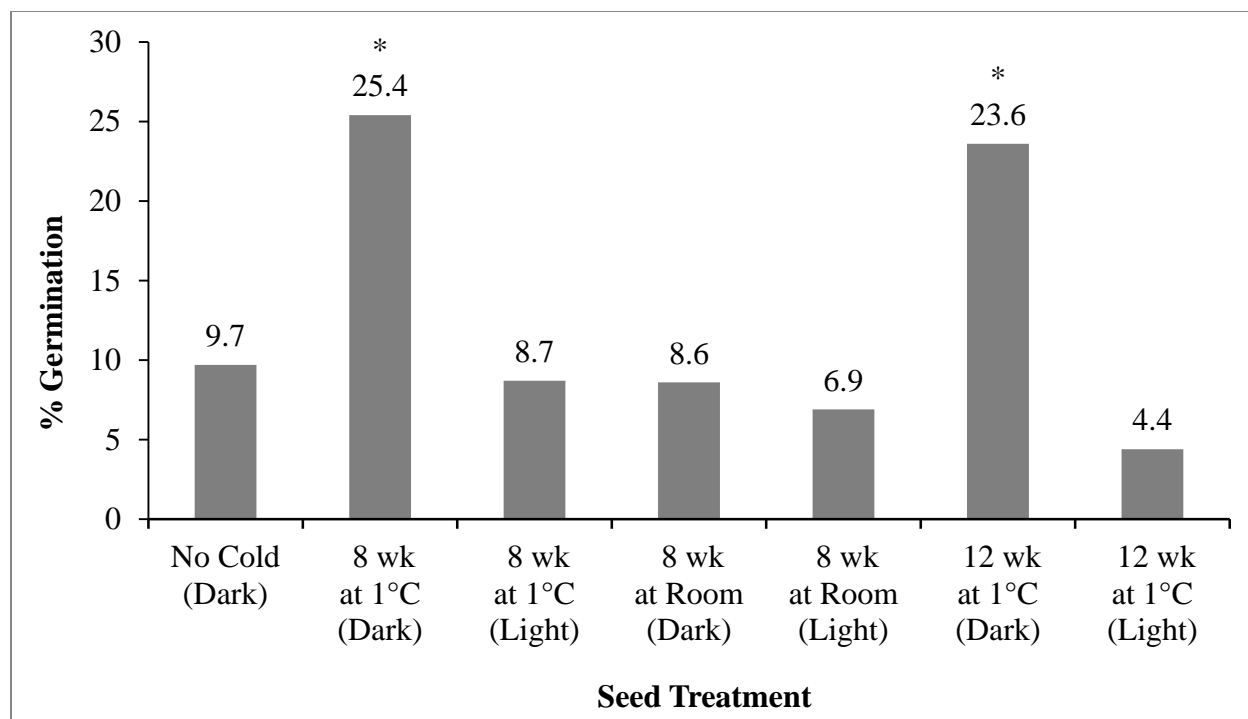


Figure 4. 2013-14 percent seed germination in *Thalicttrum coleyi* based on cold stratification (none or length in wk) and light exposure during incubation (14 h/10 h light/dark or 24 h dark). The 12 wk at room temperature (Light and Dark) treatment data and No Cold/Light were omitted due to low germination (1.4 %, 2.8 %, and 1.4%, respectively). Asterisks denote  $P < 0.05$  when compared to the No Cold/Dark reference variable in a logistic regression.



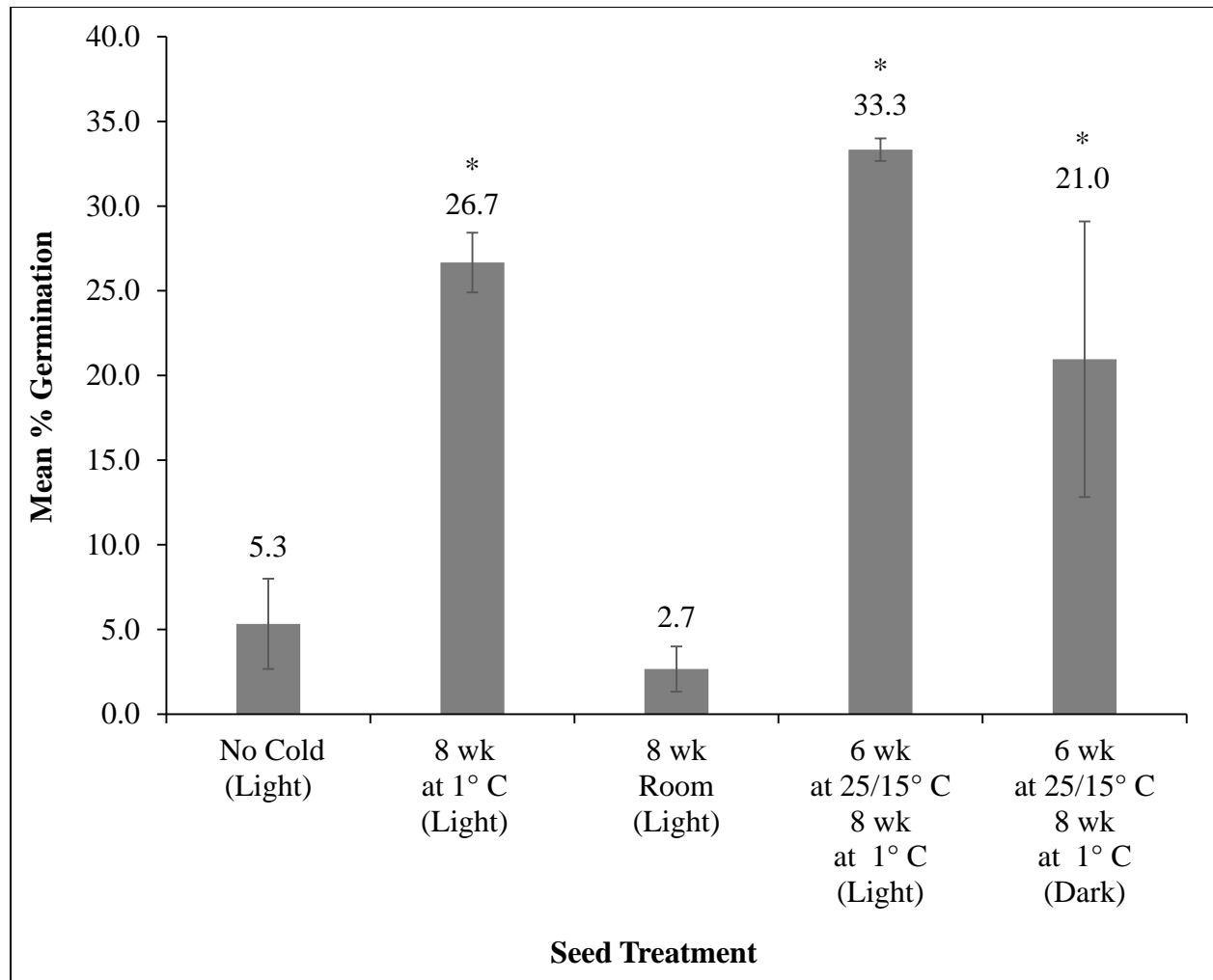


Figure 5. 2014-15 mean percent seed germination in *Thalictrum cooley* based on cold stratification (none or length in wk) and light exposure during incubation (14 h/10 h light/dark or 24 h dark). Standard error bars are given with mean values above. Asterisks denote  $P < 0.05$  when compared to the No Cold/Light reference variable in a logistic regression.

In 2014, seeds were again more likely to germinate if exposed to low temperatures prior to incubation when compared to seeds which did not receive cold stratification (Table 8). Seeds germinated best when exposed to 6 wk warm prior to 8 wk of cold stratification and incubated in light ( $33.3 \pm 0.67\%$ , Figure 5). The 8 wk cold stratified seeds incubated in light also had high germination rates ( $26.7 \pm 1.76\%$ , Figure 5), but were less likely to germinate when compared to 6 wk warm/8 wk cold (Light) (Table 8). When the 6 wk warm/8 wk cold treatments (light, dark) were compared to one another (using the light treatment as the reference variable), seeds incubated in the dark were less likely to germinate than those incubated in light (Table 8).

Table 7. Results of two logistic regressions comparing the effects of seed mass, germination treatment, and weeks since harvest on the probability of *Thalictrum cooleyi* germination in 2013-14. (a) The No Cold/Dark and (b) 8 wk Cold/Dark treatments were used as reference variables. B = coefficient, Wald =  $(B/SE)^2$  used to compute the test statistic distributed as a chi-squared,  $e$  = base of natural log. All other Wald statistics exceeded  $P > 0.05$ .

(Reference Variable)						
Treatment						
(a) (No Cold/Dark)	B	SE	Wald	df	P	$e^B$
Gibberellic Acid	1.668	0.542	9.479	1	0.002	5.302
8 wk Cold (Dark)	2.256	0.749	9.076	1	0.003	9.545
12 wk Cold (Dark)	2.356	0.957	6.054	1	0.014	10.544
(b) (8 wk Cold/Dark)						
8 wk Cold (Light)	-1.133	0.524	4.671	1	0.031	0.322
12 wk Cold (Light)	-1.572	0.708	4.928	1	0.026	0.208
8 wk Room (Dark)	-2.180	0.782	7.780	1	0.005	0.113
12 wk Room (Dark)	-1.968	0.829	5.634	1	0.018	0.140
8 wk Room (Light)	-1.658	0.596	7.735	1	0.005	0.190
12 wk Room (Light)	-2.759	1.085	6.463	1	0.011	0.063
No Cold (Dark)	-2.256	0.749	9.076	1	0.003	0.105
No Cold (Light)	-3.943	1.159	11.573	1	0.001	0.019

Table 8. Results of two logistic regressions comparing the effects of seed treatments on the probability of *Thalictrum cooleyi* germination in 2014-15. (a) The No Cold/Light and (b) 6 wk Warm/8 wk Cold/Light treatments were used as reference variables. B = coefficient, Wald =  $(B/SE)^2$  used to compute the test statistic distributed as a chi-squared,  $e$  = base of natural log. All other Wald statistics exceeded  $P > 0.05$ .

(Reference Variable)						
Treatment						
(a) (No Cold/Light)	B	SE	Wald	df	P	$e^B$
8 wk Cold (Light)	1.865	0.546	11.662	1	0.001	6.455
6 wk Warm/8 wk Cold (Light)	2.183	0.542	16.208	1	0.001	8.875
6 wk Warm/8 wk Cold (Dark)	1.549	0.567	7.457	1	0.006	4.705
(b) (6 wk Warm/8 wk Cold/Light)						
6 wk Warm/8 wk Cold (Dark)	-0.635	.296	4.603	1	0.032	0.530
8 wk Room (Light)	-2.904	.536	29.403	1	0.001	0.055
No Cold (Light)	-2.183	.542	16.208	1	0.001	0.113

## **Vegetative Propagation**

Overall, 33 out of 46 propagules survived (72%), although survival rates varied based on propagule and IBA treatment (Table 9). The root division propagules failed to initiate any new growth, regardless of whether or not they received IBA. Four out of six crown segments survived without IBA (67%). Twelve out of 14 of the lower segments with the majority of roots intact survived (85.7%). Four out of six offsets not treated with IBA survived, while only one out of five offsets survived of those treated with IBA. The majority of rhizome divisions survived (67%)

Growth responses also varied depending on the propagule and IBA treatment (Table 9). The lower caudex, with original root system intact and no IBA, had the highest mean number of leaves at  $12.1 \pm 1.93$ , while the offsets not treated with IBA had the lowest with  $2.8 \pm 1.31$ . The average leaf length was similar for most propagules (ranging from 23.58-27.25 cm), except for the offsets with no IBA, which had the lowest average leaf length at  $9.0 \pm 4.64$ .

Table 9. A comparison of survival and growth responses of *Thalictrum cooleyi* vegetative propagules after 4 wk in a temperature-controlled greenhouse. All values are means  $\pm$  SE and sample sizes are in parentheses.

Propagule	% Survival	Growth Responses	
		Leaves Produced $\bar{X} \pm SE$	Leaf Length (cm) $\bar{X} \pm SE$
Crown Caudex (no IBA)	67 ( $n = 6$ )	6.5 $\pm$ 1.66	25.2 $\pm$ 0.44
Crown Caudex (IBA)	75 ( $n = 8$ )	7.8 $\pm$ 1.87	23.6 $\pm$ 4.64
Lower Caudex (no IBA)	86 ( $n = 8$ )	12.1 $\pm$ 1.93	25.3 $\pm$ 2.29
Offset (no IBA)	67 ( $n = 6$ )	2.8 $\pm$ 1.31	9.0 $\pm$ 4.64
Offset (IBA)	20 ( $n = 5$ )	-	-
Rhizome Division (no IBA)	67 ( $n = 6$ )	4.7 $\pm$ 1.58	18.4 $\pm$ 5.77

## DISCUSSION

### Woody Plant Associates

I observed a negative association between *Thalictrum cooleyi* and increasing canopy closure, based on the logistic regression results. *T. cooleyi* did not occur in quadrats with a canopy cover greater than 42% and the mean canopy cover for quadrats with *T. cooleyi* was smaller yet,  $22 \pm 2.0$  %. Overstory trees can influence the occurrence and abundance of herbaceous understory species through various means, including their role in determining the light environment below their canopy (Pecot et al. 2007). An increase in light transmission to the understory due to tree gap openings has been associated with an increase in the numbers of less abundant, small-stature forbs like *T. cooleyi* in pine savannas (Platt et al. 2006). Although there was a negative association between canopy cover and *T. cooleyi* presence at my study sites, canopy closure (an indirect measure of the understory light environment) was not a significant determinant of the total number or density (plants/m<sup>2</sup>) of *T. cooleyi* plants. Additionally, canopy closure was not associated with whether or not *T. cooleyi* plants were flowering. This could be due to the fact that at these sites, *T. cooleyi* is entirely absent from areas with canopy closure > 50%, and where plants do occur, they are not light-limited.

My research suggests that *Thalictrum cooleyi* is more likely to occur as shrub cover increases. While *T. cooleyi* plants are more often found under a relatively open canopy, plants are also not common in highly exposed microsites. Of the 32 quadrats in which *T. cooleyi* was found, only three had a canopy closure < 10%. Woody shrubs may facilitate the growth and persistence of *T. cooleyi* by providing a favorable microclimate in which seeds can germinate and later by protecting plants from full sun exposure. Nurse plants have primarily been studied in arid climates where they have been found to be important in ameliorating the scarcity of water

and thermal stress (Armas et al. 2011; Egawa and Tsuyuzaki 2015). Nurse shrubs, in particular, have been found to improve post-fire restoration of woody species in Mediterranean pine-oak forests (Siles et al. 2010). In a wiregrass-dominated longleaf pine savanna, Iocona et al. (2012) tested the facilitative effects of *Aristida stricta* Michx. along a moisture gradient from xeric to mesic. The results did not support their hypothesis that shading from *Aristida stricta* plants would have facilitative effects on seedling recruitment. The researchers did confirm that seedling recruitment in longleaf pine savannas is generally limited by water availability, as evidenced by higher seedling recruitment in mesic compared to xeric sites, making facilitation a possibility.

It is likely that the association with *Thalictrum cooleyi* and increasing woody understory cover is driven by its co-occurrence with the evergreen shrub *Morella cerifera*. Of the most commonly occurring woody plants at my study sites, only *M. cerifera* was a significant determinant of the occurrence of *T. cooleyi*. *M. cerifera* is a nitrogen-fixing shrub that can colonize poor soils and be a dominant species in coastal plain forests (Permar and Fisher 1983; Shiflett et al. 2014). Nitrogen-fixing shrubs have been found to facilitate the growth of other plants by creating areas of fertility in otherwise nutrient poor habitats (Gosling 2005; Bonanomi et al. 2008). The soils at Shaken Creek Preserve and McLeans Savanna are mapped as Foreston loamy fine sand and Woodington fine sandy loam; both types are known for low natural fertility and poor nutrient holding capacity (USDA 1990). It is possible that *M. cerifera* facilitates the occurrence of *T. cooleyi* through its nitrogen-fixing capabilities.



## Sex Ratios and Hermaphroditic Flowers

Sex ratios within select *Thalictrum cooleyi* populations are male-biased with twice as many males as females. Biased sex ratios are common in dioecious plant species, although the causes for deviations from 1:1 are not well understood. In one review, unbiased sex ratios were found in only 33% of the 126 species surveyed (Barrett et al. 2010). Numerous studies have documented negative consequences of female-biased populations, such as pollen limitation to seed set (Buckel et al. 2012; Myers-Smith and Hik 2012), while few have shown any negative effects due to male-biased sex ratios. In one study, as male-bias increased (ranging from 10-90% males), seed set improved until severe male-bias resulted in higher variability in the reproductive output of female plants (Xia et al. 2013). Fewer females overall also means there are fewer plants directly contributing seeds for seedling recruitment in *T. cooleyi* populations.

At my study sites, sex ratios varied among populations and from one year to the next within the same population. In my 2013 survey, I found 1:1 sex ratios at McLean Savanna, but at Shaken Creek Preserve, ratios were male-biased. In 2014, however, sex ratios were male-biased at all sites. I did use slightly different sampling methods for sex ratio surveys in 2013 compared to 2014, which could have resulted in different sex ratios between years at McLean Savanna. This is unlikely, however, both methods were thorough and sex ratios did not differ in 2013 and 2014 at Shaken Creek Preserve.

Temporal variation in sex ratios within a plant population can result from sex switching of individuals or differential mortality and flowering rates between female and male plants (Richardson and Clay 2001). Some plant species can change sex over time to ensure reproductive success, a condition known as sequential hermaphroditism (Sinclair and Freeman 2012). Sex-switching may occur during different stages of growth, as is the case in *Arisaema*

*triphylum*, or in response to environmental stressors (Srivastava and Banerji 2012). Sex reversal was not observed in the congeners *Thalictrum dioicum* or *T. macrostylum*, suggesting that sex is in part determined genetically in the genus and an unlikely explanation for temporal variation in sex ratios (Di Stilio et al. 2005; Penny 2014).

Temporal variation in sex ratios may be the result of different probabilities of flowering between the sexes among years or variation in flowering stem production between males and females. Because of differences in the cost of reproduction, the magnitude of ramet and flower production from one year to the next can differ between the sexes (Matsushita et al. 2011). In some species, male plants produce more flowers and flower at a greater frequency than females, leading to male-biased flowering sex ratios (Queenborough et al. 2007). We currently do not know if mortality, flower production, or flowering rates differ between the sexes in *T. cooleyi*. Long-term demographic studies on Cooley's meadowrue would help discern this.

*Thalictrum cooleyi* does indeed have a dioecious breeding system, however, like many other dioecious taxa, dioecy is incomplete ("leaky" dioecy or subdioecy, Spigler and Ashman 2011). In a literature review, Ehlers and Bataillon (2007) found 32 subdioecious species in 21 different families in which hermaphroditic individuals represent a minimum of 5% of a population. The labile expression of flower "gender" within dioecious species has been attributed to environmental factors, such as resource limitation and water stress (Ainsworth 2000; Adam et al. 2011). My work suggests that female plants with hermaphroditic flowers are uncommon individuals in *T. cooleyi* populations (5 % of flowering stems surveyed). However, on those female plants with hermaphroditic flowers, on any given day, those perfect flowers can represent up to half of the open flowers on an individual flowering stem. We did not survey male plants in the field for perfect flowers; stamens are readily deciduous, making it difficult to

discern hermaphroditic flowers once pollen was released. There were a few perfect flowers on male greenhouse plants that did produce achenes, however. Female plants of *Thalictrum pubescens* Pursh and *T. macrostylum* both produce perfect flowers with non-functional pollen (Davis 2004). Numerous other studies, however, have shown hermaphroditic individuals of subdioecious plant species to be self-compatible, thus providing a reproductive advantage in pollen-limited environments over strictly female morphs (Ehlers and Bataillon 2007). My bagged pollination experiment showed that perfect flowers on females of *T. cooleyi* have functional pollen and are self-compatible. Future studies will be needed to determine if seeds from self-pollinated perfect flowers are viable. Despite being uncommon, functional hermaphroditic flowers in *T. cooleyi* populations could contribute to overall seed set and thus population persistence.

### **Pollen Limitation**

In these study populations, seed set in *Thalictrum cooleyi* is pollen limited, despite overall male-biased sex ratios. Pollen limitation to seed set can be variable among populations and within the same population from one year to the next (Burd 2008). I found that *T. cooleyi* plants were pollen limited at all sites across both years of my study. Others have found that seed set decreases (i.e., pollen limitation increases) with increasing distances between female and male plants (Merrett and Robertson 2012; Wang 2013). Steven and Waller (2007) found that seed set increased with decreasing distance to the nearest male in low-density ( $0.34 \text{ plants/m}^2$ ), but not high-density populations ( $0.62 \text{ plants/m}^2$ ) of *Thalictrum fenderli*. Seed set for open-pollinated flowers of *T. fenderli* was significantly lower in the low-density ( $0.275 \pm 0.226$ ) compared to high density populations ( $0.523 \pm 0.269$ ). Surprisingly, however, the large differences in seed set between my hand- and open-pollinated *T. cooleyi* plants could not be

attributed to distance from the nearest male or number of male flowers within 2 m of female plants. I found overall densities of *T. cooleyi* flowering plants (as opposed to mean density within quadrats) to be low compared to those reported for *T. fendleri*, with 0.17 and 0.33 plants/m<sup>2</sup> at Shaken Creek-Fill Rd. and McLean Savanna, respectively. At these sites, however, mean seed set for open-pollinated plants was slightly higher than values reported for low-density populations of *T. fendleri* (Shaken:  $0.33 \pm 0.04$ , McLean:  $0.36 \pm 0.06$ ).

Pollen limitation to seed set is not only caused by low pollen availability, but also poor quality pollen being deposited onto stigmas (Alonso et al. 2012). A pollen grain is said to be of low quality if individual plant fecundity is inhibited by genetic factors related to the pollen source (Chacoff et al. 2008). Mutations during pollen grain development can lead to abnormally small grains and in extreme cases, male sterility (Liu and Fan 2013). In *Cucurbita texana*, pollen grains from inbred plants had slower pollen tube growth and sired fewer seeds compared to outbred plants (Jóhannsson et al. 1998). I attempted to control for the genetic aspect of poor pollen quality by using a mixture of pollen collected from at least three unique males to ensure genetic diversity. It could be that the increase in seed set for hand-pollinated flowers was not due to larger quantities of pollen, but rather an increase in the genetic diversity of pollen donors (i.e., higher quality pollen) compared to the open-pollinated controls (Ashman et al. 2004).

The implications of pollen limitation and resultant low seed set for seedling establishment and population persistence of *Thalictrum cooleyi* are unknown at this point. The consequences of limited seed set on plant populations can vary among species (Ashman et al. 2004). For many long-lived perennials, factors such as survival and clonal growth are more important than seed set for population persistence (Castro et al. 2014). Additional information on the contribution of

seed set and clonal growth to population growth is needed to fully understand the implications of pollen limitation for *T. cooleyi*.

### **Seed Germination**

Similar to many temperate plant species, *Thalictrum cooleyi* seeds are dormant at maturity and require a period of moist cold conditions to break dormancy (Baskin and Baskin 1998; Jones and Kaye 2015). In the field, *T. cooleyi* seeds mature in fall and most likely delay germination until the following spring after exposure to the moist cold conditions of winter. Indoors, dormancy can be broken with 8 wk of moist cold stratification inside a darkened refrigerator at 1 °C with resultant germination after seeds are incubated at 25/15 °C. For species with morphophysiological dormancy (present in other members of the genus *Thalictrum*), a period of warm stratification (25/15 °C) prior to cold stratification improves germination rates (Walck et al. 1999; Hawkins et al. 2010). I found that germination rates were only slightly higher for the 6 wk warm/8 wk cold stratified seeds (33.3%) compared to those cold stratified for 8 wk only (26.7%). If time limited, land managers and nursery growers can forego the warm stratification pre-treatment, thus reducing the stratification period by 6 wk. Alternatively, GA<sub>3</sub> can be used to break seed dormancy in the absence of cold stratification. The germination rate for seeds which imbibed GA<sub>3</sub> prior to incubation in light (31%) was similar to the highest rate I obtained for cold stratified seeds (33.3%).

It is likely that *T. cooleyi* seeds do not have strict light requirements for seed germination. Differences in design and protocol prohibit statistical comparison of germination rates across years, however, in my first experiment (2013-14), seeds germinated better when incubated in dark, whereas germination rates were higher for seeds incubated in light in the second experiment (2014-15). Low overall germination rates and fewer treatments in the second experiment make it

difficult to draw conclusions about the optimal light requirements for seed germination in *T. cooleyi*. In the 2013-14 experiment, the germination rates for seeds incubated in the dark are based on a few seeds which germinated (ranging from 2-18) out of the 72 seeds in each treatment. In the 2014-15 experiment, a small seed harvest resulted in only a single treatment group in which seeds were incubated in the dark. Other members of the genus also do not appear to need light for germination. Seeds of the congener *Thalictrum mirabile* germinated well in light or dark (92 vs. 88%, respectively) after cold stratification for 12 wk at 1 °C (Walck et al. 1999).

Seed germination rates in *Thalictrum cooleyi* were low regardless of pre-treatment and incubation conditions (33% being the highest). In comparison, Walck et al. (1999) achieved 100% germination rates in *T. mirabile* seeds that were stratified for 6 wk warm followed by 12 wk cold. Many of my *T. cooleyi* seeds molded soon after being placed in the growth chamber, suggesting non-viable embryos (Baskin and Baskin 1998; Perez and Norcini 2010). In the 2014-15 experiment, 65% of 780 seeds molded before germination. The reason for high numbers of non-viable seeds in *T. cooleyi* is not known; low levels of genetic variation and associated homozygosity of recessive lethal alleles may be at play. In the dioecious species *Silene latifolia*, inbreeding depression was expressed as low seed germination rates when seeds were sired by half-sib, full-sib, and second-generation-sib (related) males (Richards 2000).

The potential sires of the seed used for my experiments were represented by 16 males from three sites in 2013-14 and four males from three sites in 2014-15. Because pollination in the greenhouse occurred passively without manipulation, I do not know which male plants contributed pollen for seed set by specific females. Furthermore, the genetic relatedness of maternal and paternal plants in the greenhouse is unknown. While it is possible that genetic limitations could have resulted in low seed viability, we cannot make definitive conclusions based

on this study. Future work could include genotyping of greenhouse plants and controlled pollination studies to help determine causes for low seed viability in *Thalictrum cooleyi*.

In some species, seed viability declines during dry storage at room temperature, while in others viability is not affected (Wang et al. 2010; Brits et al. 2015). It has been suggested that *Thalictrum* seeds do not tolerate dry storage well (Deno 1993). For my experiment, seeds were at room temperature (dependent on harvest date) anywhere from 79-142 d (2013-14) or 1-37 d (2014-15) prior to the start of treatments. It is possible that seeds lost viability in storage over time, contributing to the high number of non-viable seeds which molded. However, the time since harvest, as a covariate in the mixed model analysis, was not a significant determinant of whether or not *T. cooleyi* seeds germinated. Furthermore, seeds for my second experiment (2014-15) spent substantially less time at room temperature than those in the first experiment, yet germination rates were not significantly higher. Based on my experience, future studies can shorten the amount of time seeds spend in dry storage by massing a subset rather than each individual seed and finding techniques that speed up the harvest/cleaning process. Given that seed germination rates are low for *T. cooleyi*, any field harvesting of seeds should be done conservatively, so as not to negatively impact seedling recruitment. A more appropriate source for *T. cooleyi* seeds may be from plants in cultivation, given how well field transplants flowered and set seed.

## **Vegetative Propagation**

Vegetative propagation could be part of an effective restoration program for *Thalictrum cooleyi*. Plants can be readily propagated from rhizome and caudex divisions without the use of IBA. For some species, rhizome divisions can be planted directly in the ground rather than grown in pots, thus making restoration efforts more efficient (Schoonover et al. 2011). Further studies will be needed to determine the most appropriate method for introducing propagated *T. cooleyi* plants into natural populations. While vegetative propagation does not provide the genetic variability of seeds, it can still be a useful restoration tool, particularly if seed germination rates are low as reported for *T. cooleyi* (Fenner and Thompson 2005).

## **Conclusions**

Loss of global biodiversity continues at astonishing rates and efforts to reverse this trend need to be strengthened (Butchart et al. 2010). Wet pine savannas, the unique yet diminished habitats in which *Thalictrum cooleyi* occurs, are considered hotspots for endemism and plant species diversity (Walker and Peet 1984; Sorrie and Weakley 2006). *T. cooleyi* is one of the rare species that contributes to this diversity. *T. cooleyi* populations are threatened by habitat loss, fire suppression, and based on my findings, limited seed set and low seed viability. Despite these threats, *T. cooleyi* is believed to have a high potential for recovery given that appropriate conservation measures are taken (USFWS 2008).

We must ensure the stability of existing *Thalictrum cooleyi* populations. The first step is to understand more about the specific habitat requirements and basic breeding biology of *T. cooleyi*. My research sheds light on the association between *T. cooleyi* and the pine savanna overstory, suggesting the maintenance of an open canopy, possibly through fire management, should be priority. This work also raises intriguing questions about the relationship between *T.*



*cooleyi* and woody understory plants, such as *Morella cerifera*, and the effects on seedling establishment and nutrient availability. Future conservation efforts will also need to address low seed set and poor seed viability in *T. cooleyi*. The addition of plants to existing *T. cooleyi* populations to increase densities and genetic diversity may be beneficial to seed set. I have also shown that low seed germination rates in *T. cooleyi* can be augmented with vegetative propagation via rhizomes. Informed, science-based management holds promise for conservation of rare taxa like *Thalictrum cooleyi* and their imperiled habitats

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