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Germination Trends of American Chaffseed, Schwalbea Americana L., and Factors Affecting First-Year Seedling Development

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GERMINATION TRENDS OF AMERICAN CHAFFSEED, *Schwalbea americana* L.,
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by
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ABSTRACT

Following centuries of exploitation and fire suppression, longleaf pine systems are now the focus of many conservation efforts. Efforts to restore populations of *Schwalbea americana* L. in longleaf pine savannas have been met with frustratingly low recruitment. While past studies have briefly quantified germination rates for *Schwalbea*, there have not been any studies yet that truly investigate this plant's germination requirements. Additionally, there has been little research into characterizing the parasitic relationship between *Schwalbea* and its various host species. We conducted a germination study in a growth chamber that investigated *Schwalbea*'s germination rate and time to germinates as response variables using stratification and time between dispersal and sowing as independent variables. We found that a cold stratification significantly influenced germination rates, and after allowing one or two months to pass between dispersal and sowing the seeds, we observed a stronger germination response following stratification, and shorter time to germinate. Additionally, we explored the post-germination development of *Schwalbea* seedlings at varying levels of host and resource availability. Host presence resulted in greater resource allocation to storage and regenerative organs (i.e. thickened roots and dormant buds) with higher moisture levels amplifying this effect. Knowledge of these germination and seedling development trends may help facilitate future conservation and reintroduction efforts and may be used to inform future studies with the goal of facilitating *in situ* recruitment.

DEDICATION

This thesis is dedicated in part to my late father, Harold Lee Miller III, who helped shape me into the person I am today. He taught me to notice the tiny things that make our natural world so incredibly complex, beautiful, and worth caring for. I'd also like to dedicate this to my daughter. Calla, I hope that our collective efforts to conserve our natural world will allow you to see, experience, and cherish the same beautiful Nature that your Papa Lee shared with me.

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I would like to acknowledge and extend my thanks for my advisor and committee chair, Dr. Don Hagan. His guidance, knowledge and patience has been a highlight of my graduate school experience.

Thanks also to Dr. Patrick Hiesl and Dr. Jess Hartshorn. I gained so much knowledge from you, and truly enjoyed learning from you both, and I am so appreciative of you both being on my committee.

To my wife, your support and encouragement through this process has meant more than I can say. I could not ask for anything more than all you have done for me. Similarly, I want to thank my mom, siblings, grandparents, and extended family for your interest and support in my journey through graduate school.

Finally, I would like to thank my friends and colleagues at the South Carolina Botanical Garden, not only for their support, but also for allowing me to go to work on a graduate degree while still working at the Garden.

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CHAPTER ONE

GERMINATION TRENDS AND DORMANCY REQUIREMENTS IN *Schwalbea americana* (L.), AN ENDANGERED HEMIPARASITE

Introduction

Prior to European settlement, longleaf pine (*Pinus palustris* Mill.) dominated the Southeastern Coastal Plain of North America from Virginia to Texas (Landers et al., 1995; Frost, 1993). Throughout the centuries following European settlement, as technology progressed and the population grew, the once-ubiquitous longleaf pine was increasingly exploited as a natural resource. This exploitation, along with fire suppression and the clearing of land for agriculture and development, reduced the longleaf pine ecosystem by the 1990s to only approximately 3% of its pre-colonial range (Frost, 1993). Fortunately, in the past several decades there have been a growing number of conservation and research efforts aimed at conserving the remnants of the longleaf pine ecosystem, as well as re-establishing it in areas where it historically occurred.

The longleaf pine ecosystem encompasses 23 plant communities found in sites ranging from xeric to seasonally wet and is home to some of the highest vascular plant diversity found outside of the tropics, including many rare and endemic taxa (Peet, 2006). Of these plant communities, the most emblematic ones would arguably be the savannas and flatwoods found on the Southeastern coastal plain. These landscapes are found in seasonally wet locations, have widely spaced trees, and have a diverse herbaceous layer that is dominated by grasses and their allies. Shrubs are generally scarce, though their abundance can depend on the occurrence and frequency of past fires (Peet, 2006). These

fire events are critical to maintaining the rich herbaceous layer, as the herbaceous, fire adapted species carry the flames quickly, controlling the fire-intolerant shrubs that would otherwise outcompete them (Brockaway & Lewis, 1997; Walker & Silletti, 2006; Cox et al., 2004). As such, fire suppression is one of the most significant threats to the herbaceous layer in longleaf pine systems.

Among the plants found in the longleaf pine savanna herbaceous layer is American chaffseed (*Schwalbea americana* L.; hereafter *Schwalbea*), a hemiparasite that was listed as an endangered species due to the reduction of its once broad range to only 72 known locations in five states (USFWS, 1995). As of 2019 South Carolina was the state with the most extant populations of *Schwalbea* with 18 known occurrences, compared to the 42 extant populations in 1995 (USFWS, 1995, 2019). *Schwalbea* is a disturbance-dependent perennial forb that occurs in open, ecotonal, fire-maintained habitats (Glitzenstein et al., 2016). In South Carolina, for example, the persistence of *Schwalbea* populations is largely attributed to frequent burning of private quail hunting plantations, with isolated populations also found on public lands (USFWS, 1995). *Schwalbea* has been the focus of many conservation and reintroduction efforts since it was federally listed in the early 1990s, and while some of these efforts were considered successful, the plant's low rates of *in situ* recruitment remain a cause for concern. Studies have been conducted observing the effects of burn seasonality and recruitment in associated longleaf pine savanna species, namely wiregrass (*Aristida beyrichiana, A. stricta*). *A. beyrichiana* has been found to require growing season burns to stimulate optimal seed production and germination, followed by a period of 1 to 2 years without a burn to ensure seedling

survival (Mulligan et al., 2002). Considering the life history of wiregrass, which has been documented as a frequent co-occurring species alongside *Schwalbea*, it logically follows that *Schwalbea*'s life history could closely mirror that of wiregrass.

Previous studies have reported that in a greenhouse setting *Schwalbea* can achieve germination rates exceeding 70% (Glitzenstein et al., 2016) and 90% (Obee & Cartica, 1997) after a month-long moist stratification. The term stratification refers to the treatment of seeds to simulate natural conditions that prepare them to germinate. These high germination rates are contrasted by low recruitment in the field (Kirkman et al., 1998). It has been suggested that there may be a morphological seed dormancy (MD) preventing the seed from germinating until the embryo has matured following dispersal (Punsalan, A. 2019, February 21. Phone interview.; Punsalan et al., 2016). This is supported by some studies which found that *Schwalbea* can germinate well without a moist stratification treatment (Gustafson et al., 2017). Contrarily, many other studies have demonstrated a cold, moist stratification treatment to be critical to high germination rates (Glitzenstein et al., 2016; Obee & Cartica, 1997). Moist stratification is a common trigger to overcome physiological seed dormancy (PD), suggesting that there may be physiological (i.e., hormonal) barrier to *Schwalbea* germination. In PD, either the absence of a germination-promoting hormone, or the presence of a hormonal inhibitor. These hormonal factors have been observed in some species to change based on environmental conditions after dispersal (Skubacz & Daszkowska-Golec, 2017). For instance, PD can deepen or weaken in response to environmental cues perceived by seeds both before and after dispersal, thereby changing the required conditions to achieve germination. In other

cases, both an immature embryo and hormonal barriers work together in what is called morphophysiological seed dormancy (MPD), which would require a period of embryo maturation as well as a moist stratification to germinate (Baskin & Baskin, 2021; Finch-Savage & Leubner-Metzger, 2006).

Schwalbea has recently been reassigned to the Orobanchaceae family from the closely-related Scrophulariaceae (McNeal et al., 2013), and as such, germination requirements for other Orobanchaceae species will be used to propose important factors affecting *Schwalbea* germination, and to serve as a comparison. The closest extant relatives to *Schwalbea* are the woodland holoparasite species *Epifagus virginiana* (L.) W.P.C. Barton, and *Conopholis americana* (L.) Wallr. Both related species occur throughout Schwalbea's present, and historic, range. Prior research has demonstrated that *Epifagus* breaks dormancy under dry, cold storage conditions in sealed containers that suggest only MD is at play (Baskin & Baskin, 2014a). Similarly, *Conopholis americana* is known to disperse seed with undifferentiated embryos, which would have to grow before germination (Percival, 1931), indicating there is MD or MPD involved. *Conopolis* and *Schwalbea* both can have variable bloom times, usually in the spring, but external factors can lead them to extend their flowering season into the summer. Considering that these plants can flower and fruit around the same time of year, it is likely that they would experience similar environmental conditions that may influence germination. Similarly, *Epifagus* blooms considerably later in the year but could also set fruit around the time that a late-flowering *Schwalbea* would. While there are similarities in these species' native ranges, there are perhaps more significant differences. For example, *Epifagus* and

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Conopolis are holoparasites, while *Schwalbea* is a hemiparasite; morphological dormancy has yet to be recorded in hemiparasitic Orobanchaceae species (Baskin & Baskin, 2014b). Perhaps a more important difference is the species' preferred habitats, with *Schwalbea* requiring frequent fire disturbance and open savanna site, and the remaining two species preferring woodlands with little or no disturbance. In considering these factors, we believe that the most important connection between these species is the similarities in seasonal changes which could serve as environmental cues affecting seed germination.

Related species that are perhaps more analogous to *Schwalbea* than its Southeastern US relatives are all native to the Asian continent. Most notable among these species is *Siphonostegia chinensis* Benth., which is the closest living relative to *Schwalbea* (Schneeweiss, 2013). *Siphonostegia* is also a root hemiparasite that inhabits grasslands and rocky mountainsides, though it is an annual herb, unlike *Schwalbea*, which is a perennial (Fan et al., 2019). Recent research has shown that *Siphonostegia* seeds are responsive to temperature and light during germination, though the focus was not specifically on seed dormancy. Fan et al. (2019) observed that under different combinations of environmental factors, i.e., temperature, drought, and light, there were different germination "peaks" that appeared to not be specifically tied to any single factor. In the aforementioned study it is clear that *Siphonostegia* under conditions that are not analogous to those found in longleaf pine savannas. It is possible that there could be similar patterns found in Schwalbea germination, especially considering the role that fire disturbance plays in longleaf pine ecosystems. In considering the germination ecologies of multiple species which bear either a taxonomic and/or a geographical relationship to

Schwalbea, we believe we will begin to form a better understanding of this endangered plant's germination behavior and requirements.

The purpose of this study is to serve as the first step in filling in the gaps in the current knowledge of the life history and ecology of *Schwalbea* to inform current and future efforts to re-establish this species throughout its historical range. Through this research we are hoping to identify the critical requirements for successful *in situ* recruitment of this plant. In this portion of the study, we conducted an experiment to detect signs of MD, specifically the effect of dispersal time after fruit dehiscence (i.e. the beginning of seed dispersal), and the role of PD, requiring a moist stratification period to germinate. When MD is at play there should be an increase in germination as the seeds are allowed to continue to ripen after dispersal begins. When PD is involved, there is an increase in germination when favorable growing conditions follow a period of unfavorable conditions that persisted for a sufficient period of time. We predicted that there will be a gradual decline in total germination among non-stratified seeds as the period between ripening and dispersal increases. Simultaneously we suspected there would be a gradual increase of stratified seed germination over the same period. We also predicted that mean germination time will decrease as time between ripening and dispersal increases. This faster germination response should translate to greater germination index values for that same period.

Methods

    In August 2019 seeds were collected from *Schwalbea* plants being safeguarded at the South Carolina Botanical Garden in Clemson, SC. These parent plants were grown from seed collected from the Francis Marion National Forest at a location known as the "ballfield site" (33.042394, -79.592796). All seeds were collected from brown, dry capsules on plants which were dying back, just as the capsules were beginning to dehisce. The seeds were removed from the capsules and collected in an envelope of wax paper where they were thoroughly mixed to ensure homogeneity in various treatments. Half of the seeds were set aside, and the rest were stored at room temperature (Obee & Cartica, 1997) in the wax paper envelope. Of the seeds set aside, 40 were placed on growing media (sterilized using an autoclave) in 4 petri dishes. The growing media used was the standard potting mix, consisting of 50% sphagnum peat, 30% medium grade horticultural perlite, and 20% vermiculite (Glitzenstein et al., 2016). The seeds were arranged in a triangular pattern with 4 seeds spaced evenly on the bottom row, 3 seeds on the second row, 2 seeds on the third, and 1 seed on the top row.

    The petri dishes were placed in a growth chamber at 25°C and 70% humidity. The dishes were checked every other day to monitor moisture levels and to check for new germinants. Germination was recorded when the radicle was first visibly emerging from the seed. The number of total germinants was recorded on this two-day schedule. Similarly, 40 seeds of those that were set aside were placed in petri dishes, prepared identically with the same sterilized, standard media. These petri dishes were then covered with Parafilm laboratory film to exclude potential fungal, bacterial, or weed

contamination, and were refrigerated at 4°C (Obee & Cartica, 1997) for 28 days

(Glitzenstein, 2016) as a cold, moist stratification. Following this 28-day period, the

seeds were placed in the growth chamber under the same conditions and monitoring

schedule for an additional 28 days. These treatments were repeated with new sets of

seeds from the wax paper envelope every two weeks over a period of 16 weeks. Seeds for

all treatments during this experiment were taken at random from the same initial

collection of seeds that were stored in the growth chamber. Following the 28-day

observation period for each dish, the seedlings were each transplanted into individual

containers and grown out for later research use (Figure 1.1).

Figure 1.1 Biweekly treatment setup and progression. This series of steps is for a single treatment week and was repeated at the beginning of each treatment week. Seeds were sown from the packet of stored seeds in the growth chamber. Cold stratification (CS) treatments were refrigerated at 4°C before being placed in the growth chamber. Fully stratified CS treatments and not stratified (NS) treatments were placed in a growth chamber at 25°C and 70% humidity.
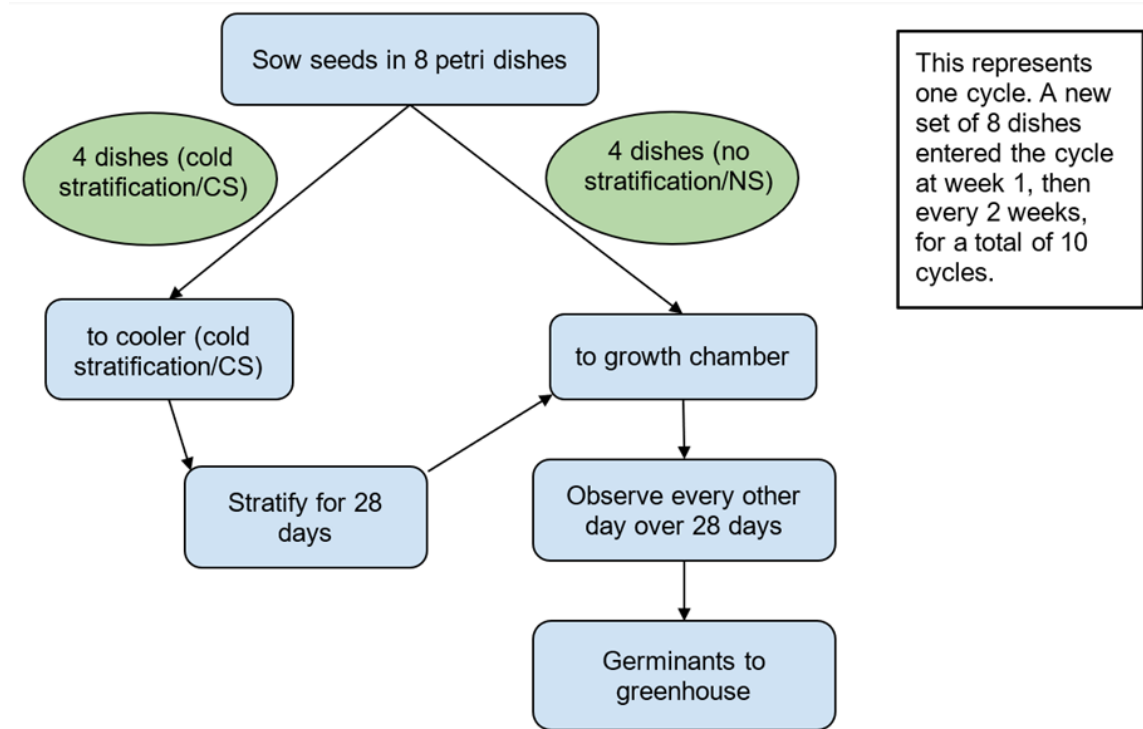
Calculations and Analyses

The Mean Germination Time (MGT) was calculated using the following formula:

$$MGT = \frac{\sum Dn}{n}$$

(1)

where Dn represents the days until observed germination and n represents the total number of germinants observed (Javaid et al., 2018). Germination Index (GI) is a tool

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that helps to characterize the magnitude of a group of seeds' germination response. GI was calculated using the following formula as described by Kader (2005):

$$GI = (14 \times n1) + (13 \times n2) + \cdots + (1 \times n14) \qquad (2)$$

where $n1, n2, \ldots n14$ are the number of germinated seeds recorded on observation days 1 through 14. The multipliers 14 through 1 are the weights given to the germinants, decreasing by 1 at each subsequent observation. Fourteen is the upper limit of the weights because observations were made every other day for 28 days for a total of 14 observations per petri dish.

Statistical analyses were performed using JMP Pro v. 16. The independent variables considered in analysis were treatment week and stratification treatment, and the dependent variables that were used for analysis consisted of the germination totals, the MGT, and the GI for each of the stratified and unstratified biweekly treatments. When calculating MGT, seeds that did not germinate were excluded from analysis. The data were tested for normality using q-q plots revealing normal distribution, and a Box-Cox test. The Box-Cox test returned a value of λ=1.25, so no transformation was needed (Hiesl, 2016). The data were analyzed using 2-way ANOVA, using stratification and treatment week as the categories to compare germination responses of seeds sown in different treatment weeks and under different stratification levels. Following this, Tukey's Honest Significant Difference test for post-hoc pairwise comparisons. Analyses assumed a 95% confidence interval (α=.05).

Results

Between the two stratification treatments there was a significant increase in germination in the cold stratification treatments as compared to those which were not stratified ($6.1 \pm .24$ vs. $1.73 \pm .24$; $F=171.09$; $P<.001$; Figure 1.2). When the cold stratified and non-stratified treatments are separated by treatment week, it is clear that in most treatment week pairs germination response was significantly greater following stratification.

Treatment week had a significant effect on the germination totals through the experiment ($F=6.37$; $P<.001$; Figure 1.2). When displayed on a chart with boxplots for each stratification and treatment week combination, the significant treatment effects were clearly restricted to the stratified treatments (Figure 1.2). Germination totals peaked between weeks 5 and 9 in the stratified treatments. These weeks had the highest mean total germinated ($8.75 \pm .75$ and $9 \pm .75$ respectively; Figure 1.2). Week 7 had lower germination totals than the week preceding of the week following, likely due to some of the dishes having fungal contamination, which may have caused lower germination totals. At weeks 15 and 17 there was a steep decrease in germination totals within the stratified seeds. Treatment week had no significant impact on germination rates within the non-stratified treatments.

Figure 1.2 Shown are the average number of germinants for cold stratified (CS) treatments (blue) and not stratified (NS) treatments (red), separated by treatment week Treatment weeks are the amount of time exposed to dry warm conditions before sowing and initiating stratification treatments. There is a significant increase in germination (F=171.09, P<.001) following stratification, and a significant effect of treatment week (F=6.37, P<.001) on total germinants as the seeds were exposed to warm temperatures before being stratified. Germination peaked at weeks 5 and 9 in the CS treatments, and sharply declined following week 13 in the CS treatments.
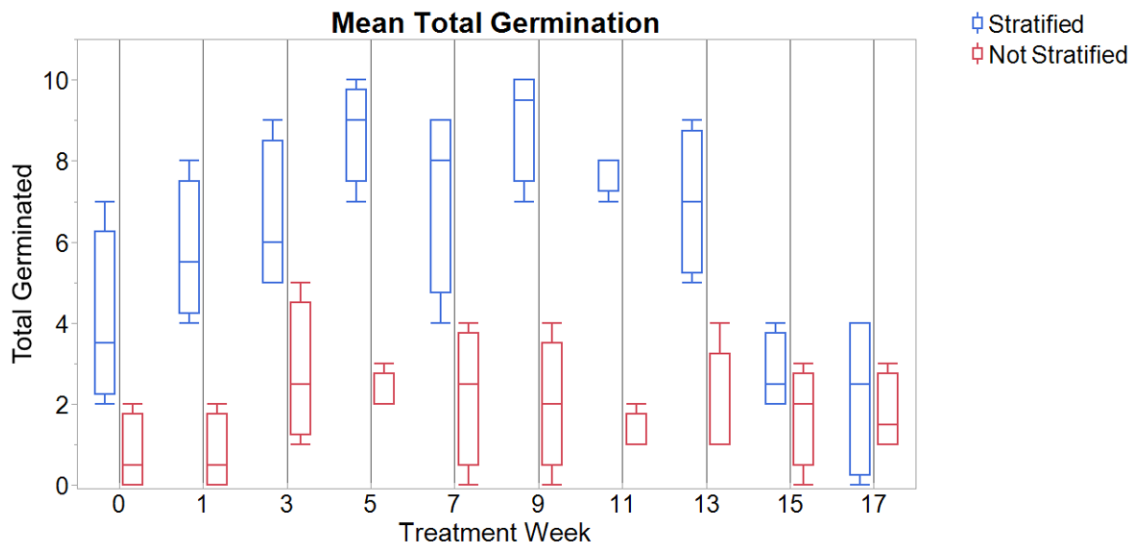
Analysis of the stratification and treatment week variables showed that stratification resulted in the fastest germination (F=95.82; P<.001). Stratification significantly decreased MGT for treatment pairs from weeks 1 through 11 (CS+1 to CS+11 vs NS+1 to NS+11), and around week 13 the treatment effects weakened and became insignificant (Figure 1.3). In comparisons within the cold, moist stratification treatment, there was also a significant treatment effect (F=8.38; P<.001) of treatment week on MGT with more days between sowing and germination at the end of the experiment (CS+13 to CS+17) (Figure 1.3). Again, when these same comparisons were made for the non-stratified treatments no significant differences were detected (F=1.38; P=.25). The fastest

germination response (i.e. lowest MGT) occurred at weeks 5 and 9, before it slowed

beginning at week 13 (Figure 1.3).

Figure 1.3 Shown are the Mean Germination Time (MGT) for cold stratified (CS) treatments (blue) and not stratified (NS) treatments (red), separated by treatment week. Treatment weeks are the amount of time exposed to dry warm conditions before sowing and initiating stratification treatments. CS treatments as a whole were significantly faster to germinate than NS treatments (F=95.82; P<.001). Additionally, treatment week had a significant effect on MGT (F=8.38; P<.001) as MGT decreased between weeks 1 to 3 in the NS treatments, and increased in the CS treatments during weeks 15 and 17.
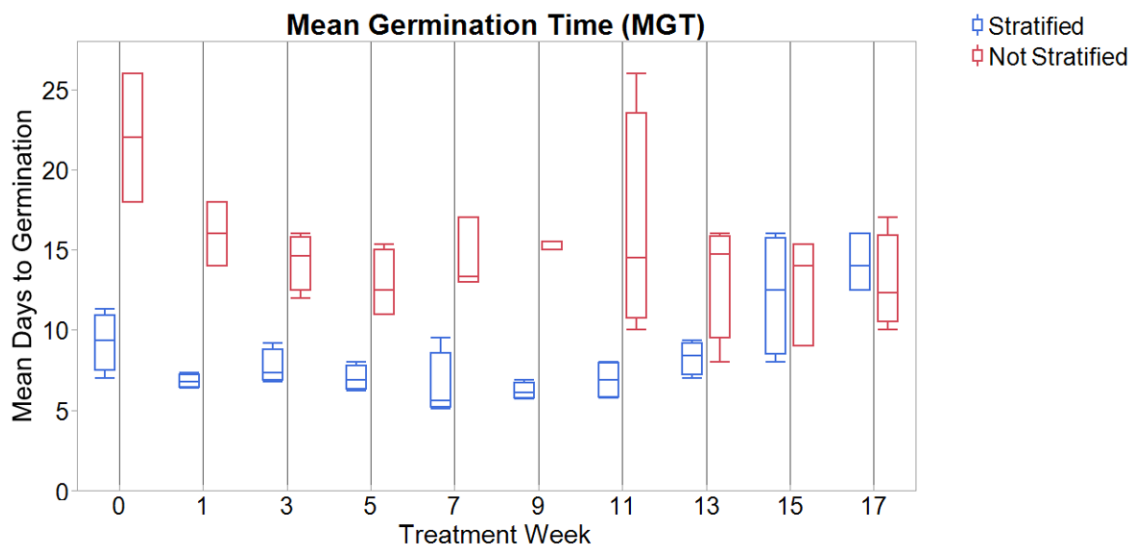
Further exploration of the treatment effects using Germination Index (GI) calculations

revealed a strong response to stratification (CS) and after-ripening (i.e., Treatment Week)

treatments (Figure 1.4). As with previous analyses, significant differences were observed

between stratified (CS) and non-stratified (NS) seeds (F=311.18; P<.001). Treatment

week had a significant impact on GI (F=11.58; P<.001). In comparison to the NS

treatments, all the CS treatments had significantly higher GI values with the exception of

CS+0, CS+15, and CS+17. GI values increased at a moderate rate, then peaked between

CS+5 and CS+11 before declining steeply through CS+17.

Figure 1.4 Shown are the germination index (GI) values for cold stratified (CS) treatments (blue) and not stratified (NS) treatments (red), separated by treatment week Treatment weeks are the amount of time exposed to dry warm conditions before sowing and initiating stratification treatments. CS treatments as a whole had significantly higher GI values (i.e. more and faster germination) than NS treatments ($F=311.18$, $P<.001$). Additionally, treatment week had a significant effect on GI ($F=11.58$, $P<.001$) as GI values peaked in the CS treatments around week 9 and sharply decreased following week 13.
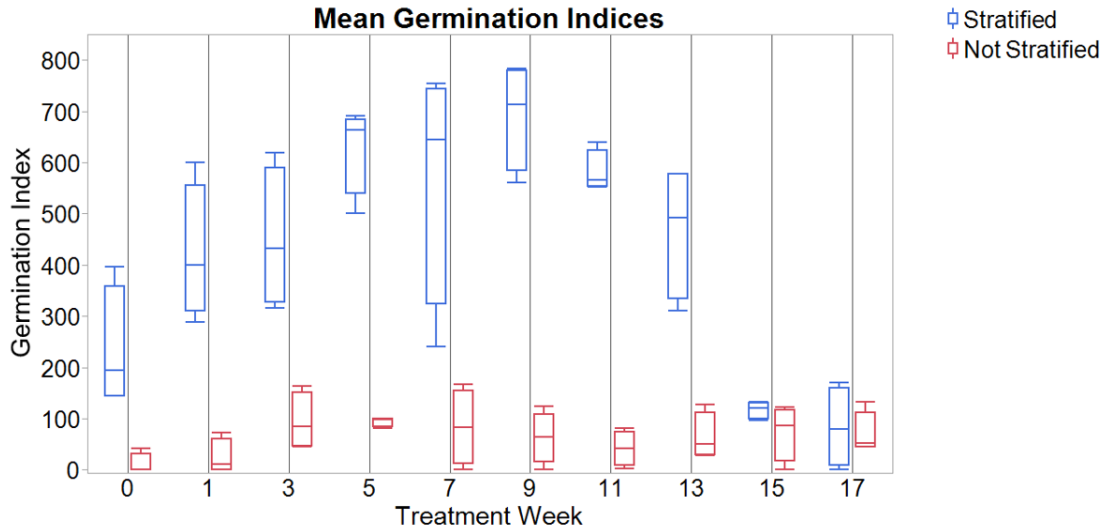
Discussion

The findings of this study are in many ways consistent with those of prior studies of

geographically separate *Schwalbea* populations which have found that a cold, moist

stratification period greatly enhances the germination rates of *Schwalbea* seeds, as

compared to those that have received no cold treatment. This is a commonly encountered

form of seed dormancy that helps to ensure that seeds germinate under favorable

conditions (Finklestein et al., 2008). Such a requirement makes a strong case for the

presence of PD preventing germination (Finch-Savage & Luebner-Metzger, 2006; Baskin

& Baskin, 2004, 2021). An interesting trend observed in this study was that average time

for germination to occur initially decreased slightly, and germination rates increased

along with the amount of time between dispersal and stratification, reaching shortest

germination time and highest germination rates around weeks 7 and 9. Following week

11 there was an abrupt change and a significant difference between weeks 7 and 9, and

weeks 15 and 17. Seeds sown at weeks 15 and 17 germinated at the same or lower rates

as those sown at weeks 0 and 1, and were as slow, if not slower, to germinate.

These results, when considering that stratification significantly increased germination

regardless of the treatment week, suggest that there may be more at play than a simple

non-deep physiological dormancy; potentially an "after-ripening" requirement. Such a

requirement has been described for other members of the Orobanchaceae, consisting of a

six to eight week period in warm temperatures before germination (Baskin & Baskin,

2014a). Indeed, there are many potential causes for this change, and further study will be

required to sufficiently describe them. A plausible explanation for this is that multiple

dormancy requirements are acting concurrently which must both be met for *Schwalbea* to

germinate at optimal rates. Most obviously there is a physiological dormancy which

allows the seeds to emerge at the appropriate time, when environmental conditions are

more likely to favor seedling survival (i.e. spring). The second of these two requirements

is more elusive and will require further study to properly describe it. One possibility is

that it is an additional physiological dormancy, potentially one that requires warm conditions for a period of time, followed by a cold period to truly neutralize any hormones or other compound that may interfere with germination. In temperate climates, more complex temperature fluctuations are necessary to break seed dormancy (Penfield, 2017). Another possibility is that there is a morphological component to the seed dormancy. In such a case the seed would be underdeveloped at the time of dispersal and would therefore require a period of post-dispersal maturation before the seed can germinate (Finch-Savage & Luebner-Metzger, 2006; Baskin & Baskin, 2004, 2021). However, Penfield (2017) reports that there is debate about whether dry after ripening effects observed in seeds stored in dry conditions is an actual adaptation that is relevant beyond laboratory or greenhouse settings, or if it is simply a result of storage techniques that keep stored seeds much drier than what would naturally occur in situ. Regardless of the relevance of dry after ripening to field conditions, understanding its impact on germination in a greenhouse setting will aid in more effective propagation for safeguarding and reintroduction efforts. A third potential factor could be an induced dormancy triggered by environmental conditions that are unfavorable for successful germination (e.g. extended storage period). In such a case, a more intense stratification treatment (i.e. longer or colder) may be necessary to break dormancy. Finally, it is worth considering that the seeds may have gradually lost viability throughout the experiment. To simulate the environmental conditions that the undispersed seeds would experience in the field, the seeds were stored in the growth chamber where they were exposed to

temperature fluctuations. Temperature fluctuations and are known to affect seed dormancy and germination, so they cannot be ruled out as factors (Penfield, 2017).

Future studies of the germination patterns of non-stratified *Schwalbea* seeds may be worthwhile, particularly to elaborate upon findings presented in this paper. A germination study similar to this one, but with a larger sample size (to lessen the impacts of contamination, and an additional level of cold stratification or similar PD-breaking treatment may yield interesting results. To expand upon the potential presence of an "after-ripening" requirement, further studies should be conducted. To truly characterize the nature *Schwalbea*'s germination requirements, anatomical studies, hormonal bioassays, more extensive and in-depth germination studies, must be conducted (Baskin & Baskin, 2004, 2021)

These findings, in the context of burn seasonality and the life history of *Schwalbea*, may be a significant finding in understanding and implementing optimal management strategies. Understanding the environmental conditions that contribute to successful germination will help us fit *Schwalbea*'s reproduction into a broader ecological context, which, in turn, may be used to inform management practices and conservation and reintroduction efforts. Of particular significance, we believe, is the intersection of this plant's reproductive ecology and burn seasonality. *Schwalbea* is known to have a strong flowering response to fires which may occur in either the dormant and growing seasons (Norden & Kirkman, 2004). Future research to understand how burn seasonality may affect flowering and seed dispersal time could be very impactful, especially in conjunction with this study's findings. For example, the seasonality of a burn could alter

the time of flowering and, therefore, seed dispersal. One such case is *Pityopsis graminifolia* (Michx) Nutt., a preferred host of *Schwalbea* (Glitzenstein et al., 2016), is known to have a strong flowering response to burn events, and is able to reproduce with significantly more success following early growing season burns rather than late growing season or dormant season burns (Brewer & Platt, 1994) Then placed in the context of seasonal temperatures and precipitation, bloom and seed dispersal times could determine the environmental conditions that seeds experience and could impact their dormancy breaking requirements. Investigating this topic will require further observation of longer-term germination trends, ideally with a larger sample size. A fuller understanding of the decline in germination rate and increase in time to germinate would be critical to evaluating this in the future.

CHAPTER TWO

IMPACTS OF HOST AND WATER AVAILABILITY ON THE DEVELOPMENT OF
AMERICAN CHAFFSEED, *Schwalbea americana* L., FOLLOWING GERMINATION

Introduction

Parasitism is a natural phenomenon that can be found in all biological kingdoms,
including the kingdom Plantae, wherein it is known to have evolved independently and
recurrently (Yoshida et al., 2016; Westwood et al., 2010). Some plants, such as *Hypopytis
monotropa* (Ericaceae) and various achlorophyllous orchids, are mycoheterotrophic,
meaning they parasitize mycorrhizal fungi (Merckx, 2013). On the other hand, true plant-
on-plant parasitic species (here referred to as plant parasites or parasitic plants) parasitize
other plants directly. While all plant parasites attach to their hosts through structures
called haustoria (Merckx, 2013; Yoshida et al., 2016), among the various species of plant
parasites there are several different methods by which they may parasitize another plant.
Some parasitic plant species are specialized to attach to the roots of a host plant, and so
are referred to as root parasites, while on the other hand, stem parasites attach to the stem
of a host plants (Twyford, 2018; Těšitel, 2016; Yoshida et al., 2016). Some parasitic
plants, called holoparasites, depend entirely on the host plant and are unable to
photosynthesize on their own, while alternatively, hemiparasitic plants can
photosynthesize on their own, and so they are only partially dependent on their host plant
(Twyford, 2018; Těšitel, 2016; Yoshida et al., 2016). Hemiparasitic plants may also be
obligate parasites, which require a host to survive and to fully complete their life cycle, or
they may be facultative parasites which derive a benefit from the host species, while not

necessarily requiring a host to survive or complete their life cycle (Twyford, 2018; Těšitel, 2016).

The Orobanchaceae is a family of plants that almost exclusively consists of root parasites ranging from common agricultural weeds to federally protected species (Brun et al., 2021; USFWS, 2019; Matthies, 2017). As such, members of this family are excellent model taxa for researchers seeking to further our understanding of parasitism, and have been the focus of many studies in recent years (Brun et al, 2021; Korell et al., 2020; Twyford, 2018; ; Těšitel, 2016; Těšitel et al., 2015). Nonetheless, many gaps remain in our understanding of plant parasites. These areas include parasite-host interactions such as host quality and parasite resistance, resource allocation and use within the parasite, broader ecological impacts of parasitism, and the impacts of abiotic factors on the parasitic plants themselves.

As with all other plants, parasitic plants can be significantly influenced by both biotic and abiotic factors in their environment. Drought, salinity, and heavy metal pollution can all have varying effects on plant parasites (Zargochev et al., 2021). The effects of these factors appear to be largely dependent on the plant species themselves and would likely have to be considered on a case-by-case basis due to the variability of tolerance between different species. It is known that moisture and nutrient availability can indirectly affect plant parasites by affecting the health of the host plant. For example, such an interaction is known between *Rhinanthus* and its host plant wherein the reduction in competitive pressure from the host species during drought created a net benefit for the *Rhinanthus* and vice versa (Těšitel et al., 2015).

Additionally, parasitic plants are also susceptible to similarly noteworthy impacts from biotic interactions with their host plants. Some parasitic plants are very specific in their preferred host such as *Epifagus virginiana* and its obligate host, *Fagus grandifolia* (Tsai & Manos, 2010), while others are more generalist in nature, such as *Melampyrum arvense* which parasitizes many widely-distributed Eurasian native species including *Linum usitatissimum* L., *Lolium perenne* L., and *Medicago sativa* L. (Matthies, 2017). Generalist parasitic plants may still have preferred hosts while still being able to parasitize other less "ideal" hosts. *Melampyrum arvense* demonstrates a distinct benefit from attaching to certain leguminous host species that was not observed to the same degree with hosts in other functional groups (Matthies, 2017). Notably, however, the same publication did mention that in general, plants originating from more nutrient rich environments were good hosts, even if they were in other functional groups (i.e. grasses or non-leguminous forbs) (Matthies, 2017). Another factor impacting host suitability is the ability of the host species to respond to the haustorial attachment of parasitic species. In some cases, host plants may be able to block or severely limit a parasite's haustorial connection, rendering it useless, or they may produce cytotoxic compounds to damage the parasite itself (Albert et al., 2021). Conversely, some parasitic plants can evade or suppress host species immune responses to ensure a successful connection to their host (Albert et al., 2021). In some cases, pathogens may even be transferred between the host and parasite via the haustoria (Zargochev et al., 2021).

Host plant suitability can also vary due to the health status of the host. Watson (2009) identified a phenomenon wherein the limited distribution of a parasitic plant

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within the greater distribution of a suitable host could be explained by the access of the host plant to resources, with hosts with greater access being more suitable to parasitic plants. While this may seem to contradict the previously mentioned findings of Těšitel et al (2015), these phenomena are not mutually exclusive and could even be expected when considering the diversity of parasitic plants and their global distribution. Host plant suitability can also affect the resource use and allocation of the parasitic plant itself. In the annual root hemiparasite *Melampyrum arvense*, a preferred host that was linked to greater nutrient availability resulted in significantly greater shoot growth before flowering, which decreased along with the suitability of the host species. For example, *M. arvense* did not even flower when paired with the least suitable host (Matthies, 2017). These findings, however, are specific to this single parasitic species, and may differ for other species, especially with different life histories, geographic, or taxonomic origins.

Some parasitic plants can cause severe damage to important agricultural crops and can greatly reduce crop yield. In areas with *Striga* infestations, yields can be reduced by 15-20% on average with localized areas of total crop failure (Rubiales et al., 2019). This can cause billions of US dollars in losses, as well as harm the welfare of local populations and subsistence farmers. In stark contrast to crop losses, some species may be a lucrative crop themselves. In India, for example, sandalwood (*Santalum album*) nearly went extinct, due partially to overharvesting of wild trees. Today, however, some landowners are cultivating it for harvest, despite the challenges arising form its slow growth and hemiparasitic nature (Srikantaprasad et al, 2022).

Similar to how *Striga* species can cause severe crop losses, parasitic plants can act as "ecosystem engineers" by suppressing populations of host plants in such a way that allows for greater biodiversity by allowing other species to establish in the gaps left by the normally aggressive host species (Twyford, 2018). Such plants can be very valuable in restoration efforts where they are native. Parasitic plants are sometimes even rare or endangered themselves, and so may be the focus of conservation efforts. For example, *Melampyrum arvense* is a once common annual root hemiparasite that has become threatened due to exclusion from agricultural seed (Matthies, 2017). A similar example would be *Schwalbea americana* which has been the focus of conservation efforts for decades (USFWS, 2019). While *S. americana* is very similar to *Melampyrum arvense*, there are some key differences that are well worth investigating and will be the focus of this study. American chaffseed, *Schwalbea americana* L., (hereafter *Schwalbea*) is an endangered Orobanchaceae species native to the eastern United States which has a broad historical distribution that today has been greatly reduced in size due to human activities since European colonization. Presently this species occurs mostly in southern states in fire-maintained longleaf pine (*Pinus palustris* Mill.) habitats. Like longleaf pine, *Schwalbea* is known to inhabit a wide variety of habitats and is known to associate with an assortment of plants occurring from subxeric to hydric conditions, though it is most frequently found in ecotones between wet savannas and flatwoods (USFWS, 2019). Like the other members of the Orobanchaceae family, *Schwalbea* is a root hemiparasite that is able to parasitize a wide variety of unrelated host species (Helton et al., 2000). Unlike many of its close relatives, however, it has been observed that the presence of a host is

not necessary for *Schwalbea* to germinate or survive (Helton et al., 2000; Gustafson et al., 2017). Such a characteristic might lead one to believe that *Schwalbea* is a prolific plant that is able to thrive in many different situations, however, the reality is quite the opposite. *Schwalbea* has been the focus of a variety of repeated studies that aim to understand the plant's life history and ecology, as well as conservation efforts to reintroduce and reestablish it in portions of its historical range (USFWS, 2019). Despite this, many of these efforts have yielded mixed results with few significant successes (Glitzenstein et al., 2016; Kirkman et al., 1998 ; Obee & Cartica, 1997).

In Chapter 1 we observed that seed germination for *Schwalbea* occurred at high rates after a moist stratification at 4°C for a period of 28 days when given ample moisture in a growth chamber. These high germination rates are sharply contrasted by field experiments and monitoring projects which have reported relatively low germination and recruitment numbers (Kirkman et al., 1998; Kelly et al., 2022). Given that *Schwalbea* is a plant that can be found living in a variety of conditions and the apparently facultative nature of its relationship with host species, it is likely that a connection to a host is a "survival enhancer" that allows small seedlings and individuals in stressful conditions to compete and survive. Specifically, we believe that the presence of a suitable host may result in more vigorous seedlings that can survive fluctuations in and limited amounts of critical resources (i.e., moisture). In a greenhouse environment it has been shown that a healthier host plant (i.e., *Pityopsis gramninifolia*) generally leads to a healthier attached *Schwalbea,* but that there is no significant host effect on *Schwalbea* that are grown in lower light conditions (Fuller et al., 2016). Such results point to the role of the host plant

being a source of mostly water and soil nutrients rather than photosynthate.

Understanding and characterizing this relationship between *Schwalbea* and its host plant

could be a factor that contributes to the individual's fitness and vigor in future growing

seasons. Through this study we hope to identify the potential benefits of parasitism in

first year *Schwalbea* seedlings, especially in relation to moisture availability. There

certainly are many topics that need further study regarding parasitic plants themselves,

and it is important to continue learning about these plants, not only for the sake of better

understanding the plants themselves, but also in the context of the significant impacts that

these plants can have on their ecosystems and agriculture.

Methods

Schwalbea seeds were collected from safeguarded plants at the South Carolina

Botanical Garden in late July of 2021, just as capsules began dehiscence and were

stratified for 28 days in a cooler set to 4°C (Me, 2019). In total, 32 metal trays were used

as growing containers for this experiment. Of these, 16 metal trays were prepared with

drainage holes in the bottom to allow free drainage of water. The remaining 16 trays were

prepared with 2 drainage holes on each side (8 total holes) that were located 1.5 cm

above the bottom of the tray to create a small reservoir of water at the bottom of the tray.

All trays were filled to the same level with identical soil mixtures consisting of 30% fine

sand, 30% river sand (coarse), and 40% peat. Soils and trays were autoclaved to ensure

that no unwanted seeds would germinate in the trays. Half of the trays of each soil

moisture treatment were planted with *Pityopsis graminifolia* (Michx.) Nutt. as a host

plant for the *Schwalbea. Pityopsis* was chosen because it is a known preferred host

species across the native range of *Schwalbea* (Helton et al., 2000; Glitzenstein et al.,

2016; Kelly and Denhoff, 2022). The resulting treatment combinations consisted of

reservoir + host (RH), free draining + host (FH), reservoir + no host (RN), and free

draining + no host (FN) (Figure 2.1).

Figure 2.1 Variables were combined in a factorial design using host presence and
moisture level as the factors with 2 levels each: host, no host, reservoir, and free draining.

	Host	No Host
Reservoir	Reservoir + Host (RH)	Reservoir + No Host (RN)
Free Draining	Free Draining + Host (FH)	Free Draining + No Host (FN)

All trays were then fertilized with organic fish emulsion at a rate of 1 tbsp/gal, and

the *Pityopsis* host plants were allowed to establish for 28 days as the *Schwalbea* seeds

were stratifying per the methods described in Chapter 1. Fish emulsion was used because

it is an organic fertilizer that is less likely to burn sensitive plants than synthetic salt-

based fertilizer, and has been successfully used with *Schwalbea* in the past (Gustafson et

al., 2017). The intent of adding fertilizer was not to nutritionally support the *Schwalbea*

but to ensure that the *Pityopsis* host plants were established and healthy. Each tray was

divided into 20 sections (5 rows of 4 sections) with a toothpick placed near the center of each section. The stratified seeds were then sowed by placing 5 individual seeds around each toothpick using moistened tweezers. Moistened tweezers were used so that the seeds could be picked up using the surface tension of the water rather than pinching the seeds and risking mechanical damage. Trays were placed randomly on a greenhouse bench in 8 rows of 4 trays. In every row there was one of each treatment combination, but the order within the row was randomly generated. All trays received identical overhead watering via misting sprinklers that ran for 45 minutes every other day for the free draining trays to cycle between saturated to nearly dry between watering. This wet-dry cycle was maintained throughout the experiment by adjusting the irrigation timing for seasonal changes due to shortening days and less evaporation or transpiration. Ultimately the irrigation was adjusted down to running for 45 minutes every 4 days by the end of the experiment in late January.

At the end of the experiment each tray was carefully disassembled as the seedlings were counted. A small stream of water was used to rinse away soil to expose host roots. Leaf pairs were then counted and recorded, as well as the presence or absence of buds. Haustorial connections were recorded if they were visible, but were not included in analysis. Each seedling was labeled and set aside to dry between 2 paper towels. The labeled seedlings were then taken to a drying oven set to 70°C and allowed to dry for 4 days until the plants were completely dry. Dried specimens were then separated into root samples and shoot samples which were weighed separately to the nearest 0.1 mg.

Proportion of total biomass was calculated for all root and shoot samples by dividing the root or shoot biomass by the total biomass of the sample.

Statistical Analyses

Data analyses were conducted using JMP Pro v. 16 (SAS Institute Inc., 2021) comparing the effect of soil moisture (i.e., reservoir vs free draining), host presence, and their interactions, on *Schwalbea*'s above-ground biomass, below-ground biomass, percentages of above- and below-ground biomass, and dormant bud presence. All data were tested for normality using a Shapiro-Wilk test. Data with non-normal distribution were transformed using a standard Box-Cox transformation (Table 2.1) (Box & Cox, 1964). The data were analyzed as a two-way ANOVA in JMP, and tray number was included as a random effect nested in moisture for the analyses. This was done in case tray placement on the bench could impact evaporation rates and was done in addition to placing the trays in the center of the bench close to the irrigation system where the irrigation water is most evenly distributed. Treatment effects were considered statistically significant at $\alpha = 0.05$.

**Table 2.1** Variables and Box-Cox transformation formulae used prior to analyses were generated in JMP Pro 16.

| Variable	Transformation Used
Total Biomass	$\dfrac{(Total\ Biomass^{0.153} - 1)}{0.5267075047}$
Shoot Biomass	$\dfrac{(Shoot\ Biomass^{0.187} - 1)}{1.1480015446}$
Root Biomass	$\dfrac{(Root\ Biomass^{0.085} - 1)}{1.8443358933}$
Shoot Percent Biomass	$\dfrac{(Shoot\ Percent\ Biomass^{0.822} - 1)}{0.9420143051}$
Root Percent Biomass	$\dfrac{(Root\ Percent\ Biomass^{0.514} - 1)}{1.2920553149}$

Results

The seeds which were sown in this experiment had a low germination rate of only 9% (288 germinants of 3200 seeds), but there was no appreciable difference ($P=0.29$) in living germinants across the different treatments by the end of the observation period. In the reservoir treatments the *Schwalbea* seedlings had greater overall biomass ($-0.106 \pm .055$ vs $-0.323 \pm .047$; $P=.012$; Figure 2.2) due to greater root biomass ($-0.085 \pm .012$ vs $-0.131 \pm .010$; $P=.013$; Figure 2.4). Similarly, the trays with *Pityopsis* present as a host plant had *Schwalbea* seedlings that had greater total biomass ($-0.098 \pm .050$) compared to trays without hosts (-0.363 ± 0.052; $P=.0007$; Figure 2.2) which extended to greater shoot biomass ($-0.166 \pm .023$ vs -0.261 ± 0.024; $P=.0056$; Figure 2.3), root biomass (-

0.084 ± .011 vs -0.139 ± .012; *P*=.0027; Figure 2.4), and dormant bud formation (.317 ±

.032 vs .085 ± .034; *P*<.0001; Figure 2.5).

Figure 2.2 Two-way ANOVA of total biomass means indicated a significant effect of both moisture ($F=6.3444$, $P=.0123$) and host presence ($F=11.642$, $P=.0007$) on the total biomass of seedlings. Treatments included 2 levels of moisture (free draining and reservoir) and 2 levels of host presence (host and no host). *Schwalbea* seedlings with a reservoir and/or a host had significantly greater total biomass than their free draining and/or no host counterparts.
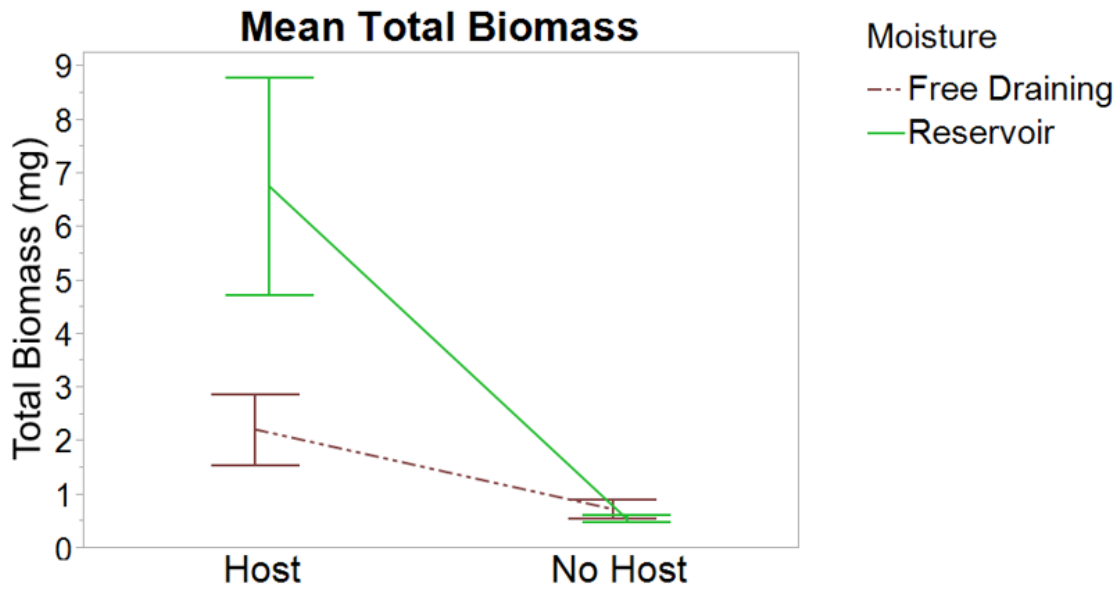
Figure 2.3 Two-way ANOVA of shoot biomass means indicated a significant effect of moisture ($F=3.9713$, $P=.0472$) and host presence ($F=7.8009$, $P=.0056$) on the shoot biomass of seedlings. Treatments included 2 levels of moisture (free draining and reservoir) and 2 levels of host presence (host and no host). *Schwalbea* seedlings with a reservoir and/or host had significantly greater total biomass than their no reservoir and/or no host counterparts.
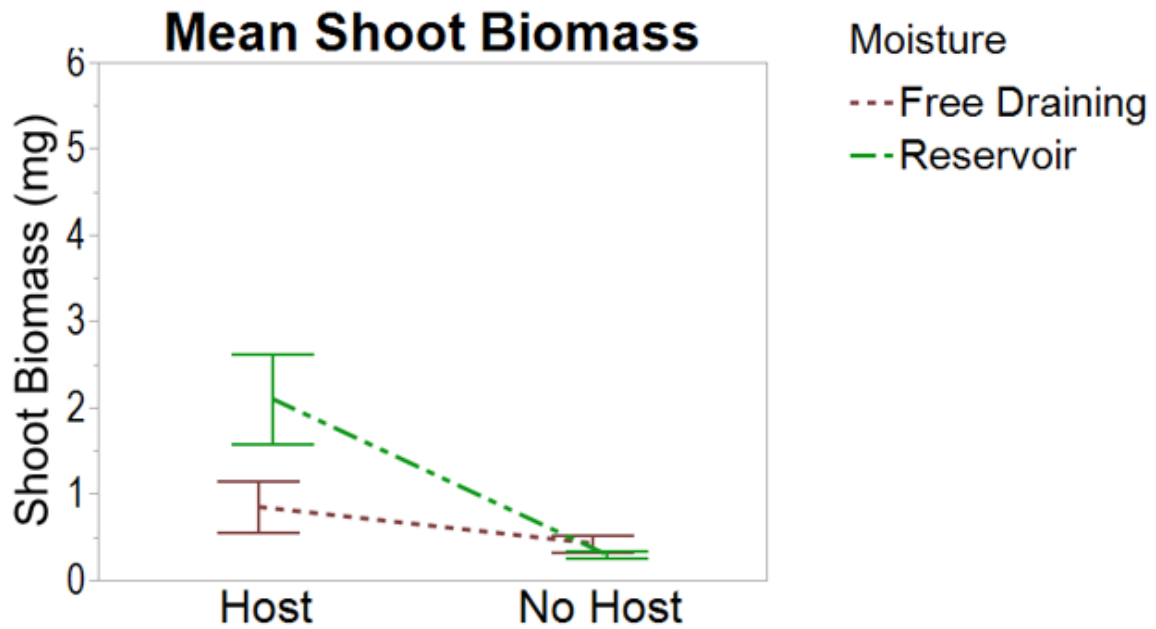
Figure 2.4: Two-way ANOVA of root biomass means indicated a significant effect of both moisture ($F=6.2190$, $P=.0132$) and host presence ($F=9.1528$, $P=.0027$) on the shoot biomass of seedlings. Treatments included 2 levels of moisture (free draining and reservoir) and 2 levels of host presence (host and no host). *Schwalbea* seedlings with a reservoir and/or a host had significantly greater total biomass than their free draining and/or no host counterparts.
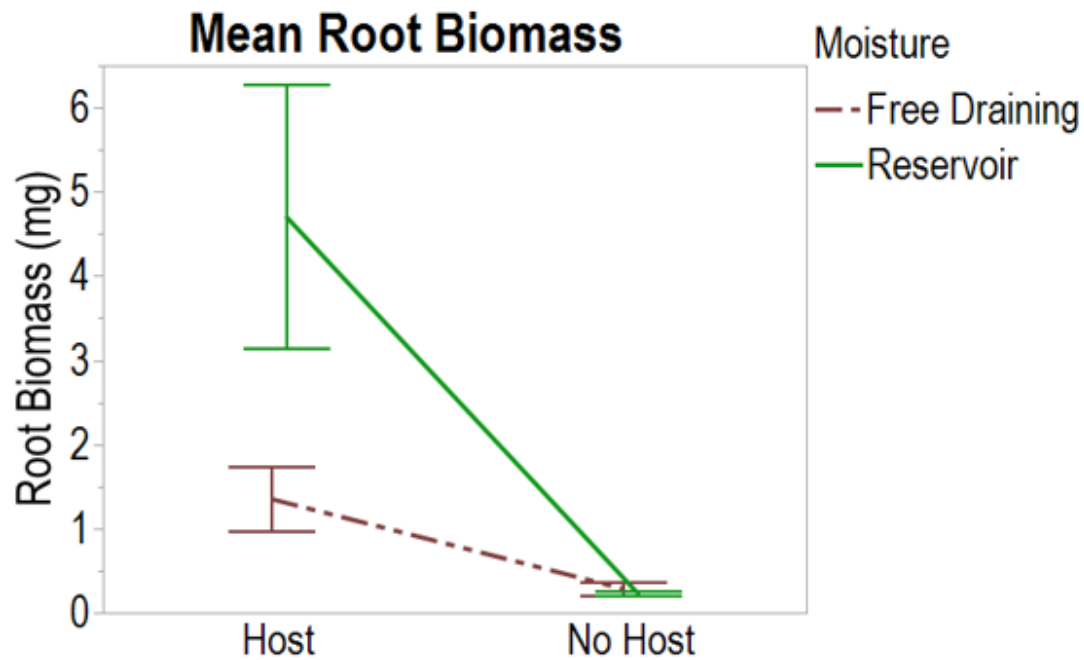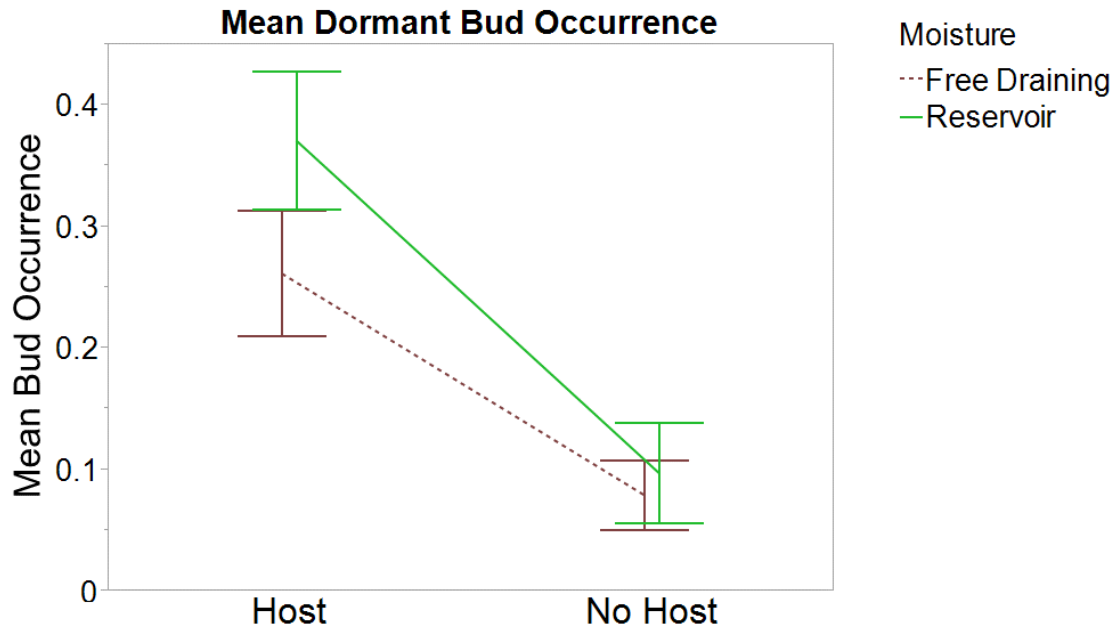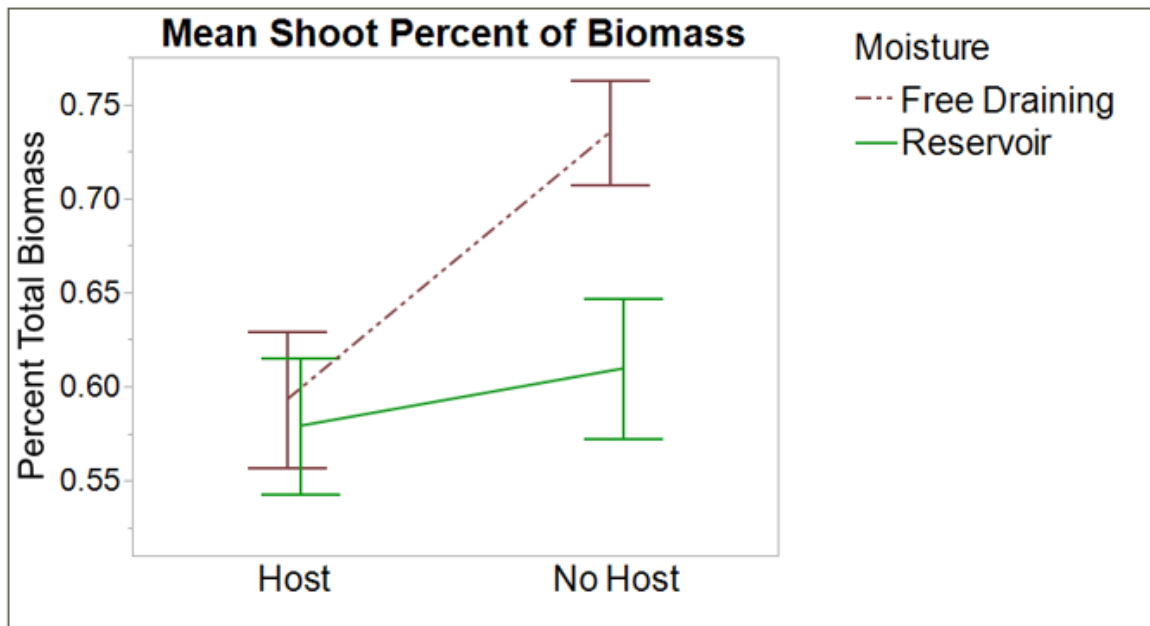
Figure 2.5 Two-way ANOVA of bud occurrence means indicated a significant effect of host presence (*P*<.0001) on the total biomass of seedlings. Treatments included 2 levels of moisture (free draining and reservoir) and 2 levels of host presence (host and no host). *Schwalbea* seedlings with a host had significantly greater total biomass than their no host counterparts.

Shoots constituted a significantly greater percentage of biomass in the free draining treatments than in the reservoir treatments (-0.310 ± .022 vs -0.387 ± .026; *P*=.0483; Figure 2.6). For the host treatments shoots made up a significantly smaller percentage of the total biomass than in the non-host treatments (-0.393 ± .024 vs -0.292 ± .025; *P*=.0135; Figure 2.6). When considering the roots as a percent of total biomass there is a significant increase in root biomass proportions in the presence of a host plant (-0.264 ± .021 vs -0.337 ± .022; *P*=.021; Figure 2.7), and no significant difference between moisture levels. In neither one of the two biomass proportion analyses was there an interaction between moisture and host availability (*P*=.109), however a Tukey test

demonstrated that the free draining, no host treatments (FN) had significantly greater

proportions of shoot biomass (and therefore lower proportions of root biomass) than in

either of the host treatments (FH and RH).

Figure 2.6 Two-way ANOVA of shoot percent of total biomass means indicated a significant effect of moisture (F=3.9343, P=.0483) and host presence (F=6.1799, P=.0135) on the shoot percent of total biomass of seedlings. Treatments included 2 levels of moisture (free draining and reservoir) and 2 levels of host presence (host and no host). *Schwalbea* seedlings with a reservoir and/or a host had significantly less shoot percent of total biomass than their free draining and no host counterparts.

Figure 2.7 Two-way ANOVA of root percent of total biomass means indicated a significant effect of host presence ($F=5.3963$, $P=.0209$) on the root percent of total biomass of seedlings. Treatments included 2 levels of moisture (free draining and reservoir) and 2 levels of host presence (host and no host). *Schwalbea* seedlings with a reservoir and/or a host had significantly greater root percent of total biomass than their no host counterparts.
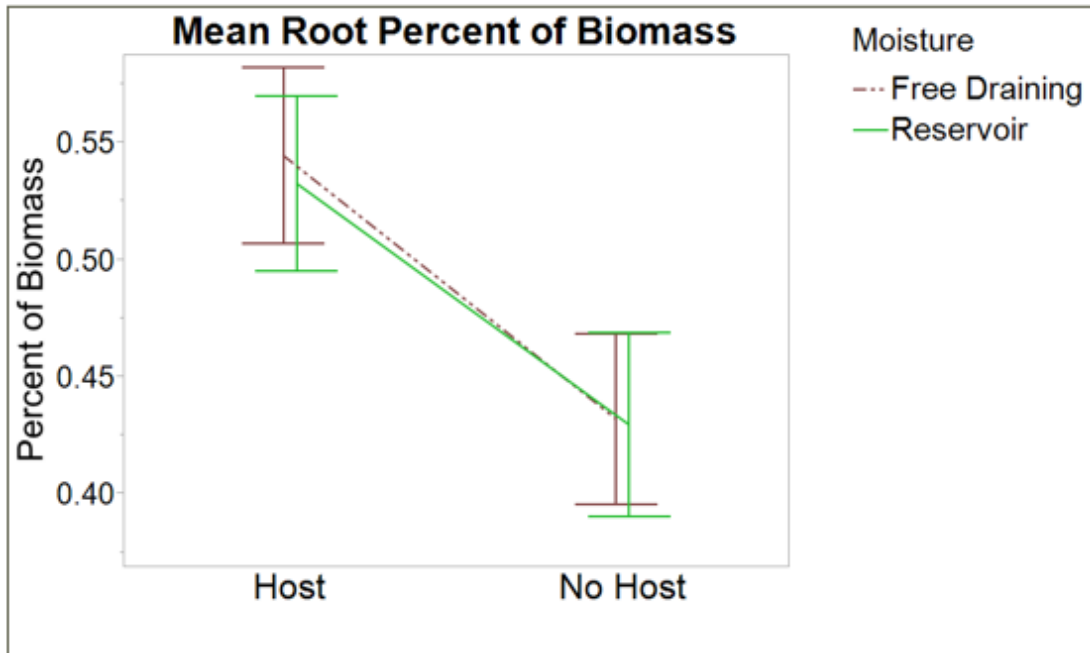
Discussion

While these previously mentioned results are not surprising, they do help to confirm what was suspected: that *Schwalbea* benefits from host presence and abundant moisture, i.e., greater resource availability.

While many plants that are capable of parasitizing another require a host for survival and reproduction, this is not necessarily the case for *Schwalbea americana* (Helton et al, 2000, Gustafson et al, 2017). This species is capable of surviving on its own but it does not reliably survive in reintroduction efforts (Fuller et al, 2016). Despite this plant's

ability to survive on its own, an appropriate host could enhance *Schwalbea*'s fitness and long-term survival in sites with high levels of competition.

Schwalbea seedlings that were exposed to higher levels of moisture had significantly greater biomass, including root, shoot, and total, than those grown in drier treatments. This was a predicted response to elevated moisture because *Schwalbea* is known to have an affinity for hydric, ecotonal sites. In both the reservoir and free draining treatments, the presence of a host resulted in plants with greater biomass and bud formation than in either of the corresponding non-host treatments. Not only was there more total biomass, but the presence of a host plant led to the root biomass comprising a significantly larger proportion of the total biomass, and there was a significant increase in the occurrence of dormant buds at the root crown. These effects were the greatest in the reservoir treatments but were still significant in the free draining treatments as well. These results suggest that the presence of a host plant influences carbon allocation patterns within *Schwalbea*. Thickened root systems with significantly greater root biomass, along with a greater incidence of dormant buds point to and energy storage strategy in the presence of a host. *Schwalbea*, it has been suggested, is likely a xylem-feeding hemiparasite, which would limit its uptake of photosynthate from the host plant and would still require substantial shoot tissue to produce photosynthate for storage (Fuller et al., 2016; Těšitel et al., 2015). Considering that *Schwalbea* is an herbaceous perennial species, there is a clear need for energy storage for winter dormancy, so root biomass is critical for the individual's survival. While this experiment did not extend through the following growing season, the formation of buds at the root crown suggest that the plant is capable

of regenerating either after dormancy or the early removal of the shoot through external factors such as fire, herbivory, or mowing.

Resource allocation may be a key purpose of *Schwalbea*'s parasitism and one of the important benefits of host presence. Considering the facultative nature of its hemiparasitism, *Schwalbea* may only need a host plant under certain circumstances, while being perfectly fine on its own in others. It has been observed that *Schwalbea* does not need a host to be able to germinate, and in greenhouse and nursery conditions is capable of surviving without a host present (Helton et al., 2000; Gustafson et al., 2017). With this being the case, it is possible that this hemiparasitic relationship could be a survival strategy for high competition environments. In the presence of host plants, *Schwalbea* on average developed more extensive root systems with portions of the root system appearing thickened like storage organs, as well as prominent dormant buds at the root crown just below the soil surface (Figure A-2). Notably, there was no such correlation with the percentage of shoot biomass of the total biomass. In fact, there was only significantly more percent shoot biomass with no host and lower moisture. This could indicate that the presence of a host alters the resource allocation strategy of the *Schwalbea* depending on host presence and resource availability. Alternatively, this increase in shoot biomass in the treatment without a host or reservoir could be the effects of residual seed dormancy that was not fully broken during stratification. In some cases of epicotyl physiological dormancy there may be regions of the embryo that break dormancy faster than others which require longer stratification (Baskin & Baskin, 2014a). This may not have been as important a factor in host treatments where natural plant

hormones that assist in breaking dormancy may have been accessible to the *Schwalbea* seeds due to their proximity to the host plant. Such a hypothesis could be tested in the future using different degrees of stratification treatment on seeds sown with or without a host, alongside testing the soil for hormonal exudates from the host plants.

Sites with elevated moisture levels can be densely vegetated with high competition between plants. Under these circumstances, the access to water is no longer the limiting factor; rather, soil nutrients and sunlight, along with the resulting photosynthate would be the most limiting resources. In such a case a plant parasite may benefit more in the long term by prioritizing the accumulation of the carbohydrates and mineral nutrients from the host plant. This may allow the *Schwalbea* to have a competitive advantage in the following season, allowing it to quickly emerge from dormancy, flower, and fruit before the surrounding vegetation can catch up and obscure the flowers from pollinators or the seeds from breezes that would disperse them. Moreover, *Schwalbea* invested more in shoot growth under host-free conditions with fewer resources and competition. In this case there were no hosts available, so the *Schwalbea* seedlings had to produce their own photosynthate to survive. These plants were overall smaller, but with more of their biomass in shoots with very minimal root growth. These plants in general did not have root systems that were as developed, and there were comparatively few individuals with dormant buds present (Figure A-1). Plants such as these would be unlikely to survive long term and the population would likely require some individuals with a connection to a host to ensure sufficient recruitment.

While there was no significant difference in the total number of living *Schwalbea* at the end of the experiment, there could be longer-term treatment effects that were simply not able to be observed under the scope of this experiment. The plants that were grown without a host plant present generally did not produce large root systems, while those which were grown with hosts generally had larger, thicker roots (i.e., greater root biomass), and frequently had large dormant buds below the soil. These individuals could be more likely to survive dormancy or a disturbance such as a fire and could have a competitive advantage with the stored energy. This stored energy could potentially help the plant in multiple ways. Firstly, energy stores could support a more vigorous growth response in the growing season that would allow the plant to grow rapidly and compete with neighboring plants for sunlight and visibility to pollinators and wind currents for seed dispersal. Secondly, stored energy may help support flower production and fruit set, resulting in more seed with greater viability. *Schwalbea*, like many perennials, likely relies on stored energy to support the new season's growth, flowers, and fruit, especially with the potential for high competition and frequent disturbance (i.e. fire). Additionally, stored energy could function as insurance that the plant can recover from other more stochastic disturbances such as predation, mechanical damage, or environmental extremes.

Further studies will need to be conducted to truly characterize the role of the hemiparasitic relationship that *Schwalbea* has with other plants. *Schwalbea* seedlings demonstrated an amplified host plant effect when in more hydric conditions as compared to those in drier soil conditions. While not essential to the plants' short-term survival, the

presence of a suitable host plant may influence resource allocation with potential longer-term implication for seedlings' survival, vigor, and fitness. These findings may help with future research to determine what are the main factors that will impact the success of future reintroduction efforts.

APPENDIX

<u>*Schwalbea*</u> <u>Anatomy and Development</u>

Figure A-1: The root systems of various *Schwalbea* seedlings ranged from thread-like with no buds (left) to thickened with dormant buds present (right).

Figure A-2: Thickened root system with prominent buds were not in proportion to the shoots produced.

Figure A-3: *Schwalbea* with a haustorial connection to the root of a *Pityopsis* host plant.

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