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The effect of cold stratification and perigynia removal on the germination of three prairie carex species

Christina M. Boeck Crew
University of Northern Iowa

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THE EFFECT OF COLD STRATIFICATION AND PERIGYNIA REMOVAL ON THE GERMINATION OF THREE PRAIRIE CAREX SPECIES

An Abstract of a Thesis

Submitted

in Partial Fulfillment

of the Requirements for the Degree

Master of Science

Christina M. Boeck Crew

University of Northern Iowa

July 2019

ABSTRACT

Sedges (*Carex spp.* Linneaus. [Cyperaceae]) are a major component of Midwestern U.S. tallgrass prairie and prairie wetland plant communities, yet they are often lacking in restoration projects because they can be difficult to germinate. Cold-wet stratification and perigynia removal have been shown to increase germination in some *Carex* species. The germination response of 3 native species, plains oval or shortbeak sedge (*C. brevior* (Dewey) Mack.), field oval or troublesome sedge (*C. molesta* Mack.), and prairie or Bicknell's sedge (*C. bicknellii* Britton.), was tested to cold-wet stratification (28d) and perigynia removal in stored seed (10 months). Seed treatments included nonstratified with perigynia intact (control), nonstratified with perigynia removed, stratified with perigynia intact, and stratified with perigynia removed. Four replicates of 100 seeds treatment were held under a diurnal temperature regime 30/15 °C (86/59 °F) with 12 hours of light for 84 days. Fresh seed was also tested for *C. brevior* and *C. molesta.* Stratification increased total germination in fresh *C. molesta* and stored *C. brevior* seeds and marginal increased total germination in stored *C. molesta* and fresh *C. bicknellii* seeds. Stratification also increased germination rate in all species and seed states. Perigynia removal increased total germination in fresh *C. bicknellii* and stored *C. brevior* seeds and marginally in stored *C. molesta* seeds. Perigynia removal also increased germination rate in *C. brevior*. Combining stratification with perigynia removal did not provide any additional advantage and actually resulted in a decrease in total germination in fresh *C.*

brevior seeds. *C. molesta* germinated well, to at least 91.3%, without treatment. Generally speaking, stratification overall increased germination in all species most effectively, but perigynia removal may provide some benefits if stratification is not available.

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This Study by: Christina M. Boeck Crew

Entitled: The Effect of Cold Stratification and Perigynia Removal on the Germination of Three Prairie *Carex* Species

has been approved as meeting the thesis requirement for the

Degree of Master of Science

Date	Dr. Mark C. Myers, Chair, Thesis Committee
Date	Dr. Kenneth J. Elgersma, Thesis Committee Member
Date	Dr. Mark E. Sherrard, Thesis Committee Member
Date	Dr. Daryl D. Smith, Thesis Committee Additional Member
Date	Gregory A. Houseal, Thesis Committee Additional Member
Date	Dr. Jennifer Waldron, Dean, Graduate College

DEDICATION

This thesis to my family. Thank you.

ACKNOWLEDGEMENTS

Funding for this project was provided by the 2010 Wetlands II Project (5569) through the Iowa Natural Resource Conservation Service (NRCS) of the United States Department of Agriculture (USDA) and by the Tallgrass Prairie Center at the University of Northern Iowa. I would like to sincerely thank Dr. Mark Myers, Dr. Kenneth Elgersma, Dr. Mark Sherrard, Dr. Daryl Smith, Greg Houseal, and all the staff at the Tallgrass Prairie Center. Thank you for all of your patience, help, and guidance. It has been an honor to have the opportunity to work with you all.

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CHAPTER 1

LITERATURE REVIEW

Sedges (*Carex spp.* Linneaus. [Cyperaceae]) are a major component of tallgrass prairie and prairie wetland plant communities in the U.S. Midwest, frequently accounting for up to 25% of above ground biomass in tallgrass prairies (Coppedge et al. 1998) and comprising 25-30% of total cover in prairie wetlands (Aronson and Galatowitsch 2008; Ashworth 1997). In tallgrass prairies, sedges often account for a larger proportion of total species richness than perennial grasses (Coppedge et al. 1998). Despite their ecological importance, sedges are often neglected in tallgrass prairie and prairie wetland restoration plantings. The commercial availability of *Carex* seed can be a limiting factor. Propagation methods for *Carex* species, including protocols for seed production, harvest, and cleaning, have historically lagged behind those of native prairie grasses and forbs (Houseal and Smith 2010). Even when included in restoration plantings, sedges can be difficult to germinate and establish due to an incomplete understanding of their dormancy and germination requirements, sensitivity to local site conditions, and competitive interactions with other plant species (Hipp et al. 2008; Iannone and Galatowitsch 2008; Schütz 1997).

Failure to account for and include *Carex* species in tallgrass prairie and prairie wetland restorations may reduce the probability of successful restoration outcomes. Recently established native prairie reconstructions are especially vulnerable to invasion by exotic species (Zedler and Kercher 2004), and excluding *Carex* spp. from native plantings may increase the likelihood of invasion by exotic cool season grasses such as reed canary grass (*Palmaris arundinacea* L. [Poaceae]) and smooth brome (*Bromus inermis* Leyss. [Poaceae]) (Budelsky and Galatowitsch 2000; Kettenring and Galatowitsch 2007b). Iannone and Galatowitsch (2008) found that rapid establishment of native plants can slow or prevent invasion of weedy species.

Rapid establishment of *Carex* species with physiological dormancy can only happen if the seed sown is nondormant and ready to germinate (Kettenring and Galatowitsch 2011). Seed dormancy promotes longer-lived seed and species persistence in soil seed banks; however, it can present challenges in prairie restoration by hindering germination and subsequent plant establishment. At maturity and dispersal from the parent plant, sedge seeds are conditionally dormant (Kettenring and Galatowitsch 2007a; Schütz 1997; Schütz and Rave 1999). Conditional seed dormancy allows germination only under a specific set of environmental signals, protecting the seed from germinating during conditions unfavorable to survival, such as during winter, drought or flood conditions, or under excessive leaf canopy shade (Baskin and Baskin 1998). The change from dormancy to nondormancy is not abrupt. Under favorable conditions, the range of conditions conditionally dormant seed are able to germinate under widens until the seeds are nondormant (Baskin and Baskin 1985; Kettenring and Galatowitsch 2007a; Schütz 2000.) When seeds are nondormant, they germinate over the widest range of conditions possible within the species' genetic and epigenetic parameters (Baskin and Baskin 1985).

Temperature (Baskin et al. 1996; Hoag et al. 2001; Schütz and Rave 1999; Schütz 2000) and light (Baskin et al. 1996) are important environmental variables regulating dormancy in Carex seed, but their effects may vary among species. Schütz and Rave (1999) saw high germination rates at 30°C (86°F) in many sedge species and noted the importance of a fluctuating temperature regime. Kettenring and Galatowitsch (2007a) showed that an optimal temperature regime for germination of conditionally dormant and non-dormant *Carex* seed is diurnal 27/15°C (80.6/59°F). A light regime, with light during hours of higher temperature and dark during hours of lower temperature to simulate natural conditions has, been shown to increase germination in some *Carex* species (Schütz and Rave 1999). In native tallgrass prairie and prairie wetland restoration, it is desirable to break conditional dormancy of sedge seeds immediately prior to sowing as means to improve seed germination and enhance *Carex* establishment.

A number of pre-sowing seed treatments have been demonstrated to increase germination in *Carex* species. Cold-wet stratification breaks dormancy and increases germination for many *Carex* species by widening the required temperature range needed for germination and improving germination rates (Budelsky and Galatowitsch 1999; Hoag et al. 2001; Kettenring and Galatowitsch 2007b; Schütz and Rave 1999). For example, Schütz and Rave found that cold-wet stratification at 4°C (39.2°F) for 6 months (1999) increased germination in 28 of 32 *Carex* species tested. Hoag et al. (2001) increased germination in Nebraska sedge (*C. nebrascensis* Dewey.) with cold-wet stratification for 32 days at 3°C (37.4°F). Stratification also hastens germination in several species (Hough-Snee and Cooper 2011; Hoag et al. 2001; McGinnis and Meyer 2011).

One characteristic of a true sedge is that the seed or achene is enclosed in sack-like structures called a perigynium. Perigynia removal has been shown to increase germination in some *Carex* species, including Nebraska sedge (*C. nebrascensis* Dewey.) (Hoag et al. 2001; McGinnis and Meyer 2011), awlfruit sedge (*C. stipata* Muhl. Ex Willd.) (Hough-Snee and Cooper 2011), yellowfruit sedge (*C. annectens* (E.P. Bicknell) E.P. Bicknell.) and bottlebrush sedge (*C. hystericina* Muhl. Ex Wiild.) (Nelson et al. 2018). *Carex* perigynia can be removed using a variety of methods. Hough-Snee and Cooper (2011) used a razor knife to cut each perigynium away individually, while Hoag et al. (2001) used forceps or sandpaper to remove them. A commercially available brush machine may also be used (Houseal and Smith 2010).

The effects of stratification and perigynia removal on the germination of *Carex* species common in Midwestern US prairies: plains oval or shortbeak sedge (*C. brevior* (Dewey) Mack.), field oval or troublesome sedge (*C. molesta* Mack.), and prairie or Bicknell’s sedge (*C. bicknellii* Britton.) was studied. *Carex brevior*, *C. molesta*, and *C. bicknelli* are all common sedges in high-quality Midwestern tallgrass prairies remnants and prairie wetlands (Hipp et al. 2008; Houseal and Smith 2010). *Carex brevior* and *C. molesta* are considered facultative hydrophytes, occurring in both wetlands and non-wetland prairies; *C. bicknelli* is classified as a facultative upland species (USDA NRCS 2019). All 3 species belong to section *Ovales* (Kunth.), with vegetative culms, gynecandrous spikes, and perigynia bearing marginal, epidermal wings (Hipp et al. 2006), and all flower from April to June and fruit from June to July (Flora of North America 2008; Hipp et al. 2008; Mohlenbrock 1999). The objective of this study was to determine whether cold-wet stratification, perigynia removal, or their combination promote earlier germination or increased total germination of these 3 prairie wetland sedges. The combined effects of seed stratification and perigynia removal on the germination of these species have not yet been reported in the literature.

CHAPTER 2

METHODS AND MATERIALS

Objective

The objective of this study was to test the effect of perigynia removal and cold-wet stratification on the germination of *C. brevior*, *C. molesta, and C. bicknellii*.

Seed Source and Pretreatment

Native seed originating from remnant prairies in Northeast Iowa was provided by the Tallgrass Prairie Center’s Natural Selections Program at the University of Northern Iowa, Cedar Falls, Iowa. It had been propagated in field plots at the Tallgrass Prairie Center (42.51052° N, 92.47560° W). Soils of the propagation plots consisted of 391B Clyde-Floyd complex, 1-4% slope and 83B Kenyon loam, 2-5% slope (NRCS 2013).

Ripe seed was combine-harvested in early July 2011 into a fabric collection bag, air-dried, rough-cleaned, and air-screen-cleaned twice (Westrup air-screen cleaner, type LA-LS, No. LALS-0106) (Table 1: Initial Air-Screen Cleaner Settings) following Houseal (2007) with adjustments based on trial and error (Houseal, personal communication). The machine was cleaned prior to use and between species to eliminate the possibility of contamination.

Studies of the effects of stratification and perigynia removal on *Carex* germination have used both freshly harvested (Hough-Snee and Cooper 2011; McGinnis and Meyer 2011; Schütz and Rave 1999) and stored seed (Hoag et al.

2001; Jones et al. 2004). Stored seed is most commonly used in prairie restoration projects (Jones et al. 2004), but fresh seed may also be used in some circumstances. Germination trials were conducted using fresh seed for all 3 species (*C. brevior*, *C. molesta*, and *C. bicknelli*). To determine if effects of the seed treatments varied with the age of the seed, *C. brevior* and *C. molesta* germination trials were repeated using stored seed. Stored seed was harvested in late June 2010 and prepared as described above, then stored in refrigerated zip-lock bags, as typically done in restoration, for 10 months. Only fresh seed was tested for *C. bicknellii* due to limited seed availability.

Table 1. Initial Air-Screen Cleaner Settings

	Westrup Air-Screen Cleaner (type LA-LS, No. LALS-0106)					
	Screens (mm), round			Air-flow Valves		
	Scalping		Sifting	Cyclone	Damper	Final
Species	Top	Middle	Bottom	#1	#2	#3
C. bicknellii	4	3.8	0.9	3	1	1
C. brevior	3.4	3	1	3	1	1.25
C. molesta	3.4	3.2	1	3	1	1.25

Experimental Design

Germination trials employed a 2 × 2 factorial design with stratification (non-stratified, NoS or stratified, S) and perigynia removal (perigynium intact, P or perigynium removed, NoP) as factors. Each treatment group was replicated 4 times for each species of each seed type (fresh or stored).

To remove the perigynia, seed was passed twice through a Westrup brush machine (type LA-H. No. LAH-0105) (Table 2) (Houseal 2007). Once the perigynia were removed, the seed was passed through the air-screen cleaner to remove any loose perigynia material (Table 3). The machines were thoroughly cleaned prior to use and between species. The seeds were haphazardly examined under magnification to ensure no visible damage to the seeds occurred during perigynia removal.

To stratify seed, seed from each perigynia treatment for each species was placed between pieces of distilled water-moistened 10.2 x 10.2 cm blue blotter paper in 11 x 11 x 3.5 cm clear polystyrene containers (Hoffman Manufacturing, model 156C) with tight-fitting lids for 28 days at 7-7.5°C and 37-41% RH.

Table 2. Brush machine settings

Westrup brush machine (type LA-H. No. LAH-0105)			
Brush	RPM	drum screen	gate opening
medium	6.5	#10 (2mm)	6.35 mm

Table 3. Air-screen cleaner settings after perigynia removal

	Westrup Air-Screen Cleaner (type LA-LS, No. LALS-0106)					
	Screens (mm), round			Air-flow Valves		
	Scalping		Sifting	Cyclone	Damper	Final
Species	Top	Middle	Bottom	#1	#2	#3
C. bicknellii	2.6	1.6	0.9	3	1.5	1
C. brevior	2.6	1.8	1.1	3	1.5	1
C. molesta	2.6	1.6	1	3	1.5	1

Germination Trials

One hundred seeds were hand-selected for each of the 4 replicates of each treatment group for each species. Seeds were examined to ensure that the perigynia was completely intact or removed, as required for each group. Seeds in each replicate were evenly spaced in 10 rows of 10 on blotter paper moistened with distilled water in the bottom of a clear polystyrene container with a tight fitting lid. The boxes were haphazardly placed in a Percival Scientific Intellus germination chamber (model 130L) set to 30/15 °C (86/59 °F) with 12 hours of light and 12 hours of dark per day. The fresh seed germination trials began on September 1, 2011 and the stored seed trials began on April 19, 2011.

Germination was defined as the presence of a radicle and a coleoptile, visible with the naked eye (Schütz and Rave 1999). Seeds were checked for germination and germinants were removed every day for 30 days in fresh seed and 24 days in stored seed, then every other day through day 48 in fresh seed and day 42 in stored seed until germination rates slowed, after which they were checked every 6 days, through 84 days. At the end of the trial, any nongerminated seed that was identified as soft or empty by gently pinching with forceps was excluded from germination calculations (Baskin and Baskin 1998; Schütz and Rave 1999), amounting to no more than 5 seeds removed from each replicate.

Statistical Analysis

Germination was calculated as the percent of all seeds germinated at 6 day intervals over the 84 day study period. Calculations were based on 100 seeds per treatment replicate, corrected for seed viability. Prior to analysis, all germination trial data was assessed for normality and homogeneity of variance. When data violated ANOVA assumptions, a square root transformation was applied. The data presented in figures are untransformed.

Total germination is defined as the percentage of seeds that successfully germinated at the end of each 84 day germination trial. Differences in total germination among treatment groups were analyzed using Analysis of Variance (ANOVA) followed by Tukey’s HSD pairwise comparisons of means. A generalized linear model incorporating seed type was also used to compare day 84 total germination for *C. brevior* and *C. molesta*. Variation in germination rate were analyzed using repeated measures ANOVA. All statistical analyses were conducted using R (R Development Core Team 2019). Significance was defined as $P < 0.05$ and marginally significant as $0.05 < P < 0.10$.

CHAPTER 3

RESULTS

Carex brevior

Fresh Seed

Total germination at day 84 ranged from 81.8% for untreated seed to 89.9% for nonstratified seed with perigynia removed (Fig. 1). Neither stratification nor perigynia removal significantly increased total germination (Table A1: ANOVA Total Germination of *C. brevior* Fresh Seed); however, there was a significant interaction. Perigynia removal increased germination in nonstratified seed and decreased germination in stratified seed. While the seed treatments did not affect total germination, both significantly increased germination rate, promoting earlier germination compared to untreated seed (Table A2. RM ANOVA of *C. brevior* Fresh Seed). Untreated (control) seed reached 50% germination by day 20, while treated seed reached it by day 10-16 (B1. Fresh Seed Trial Raw Data).

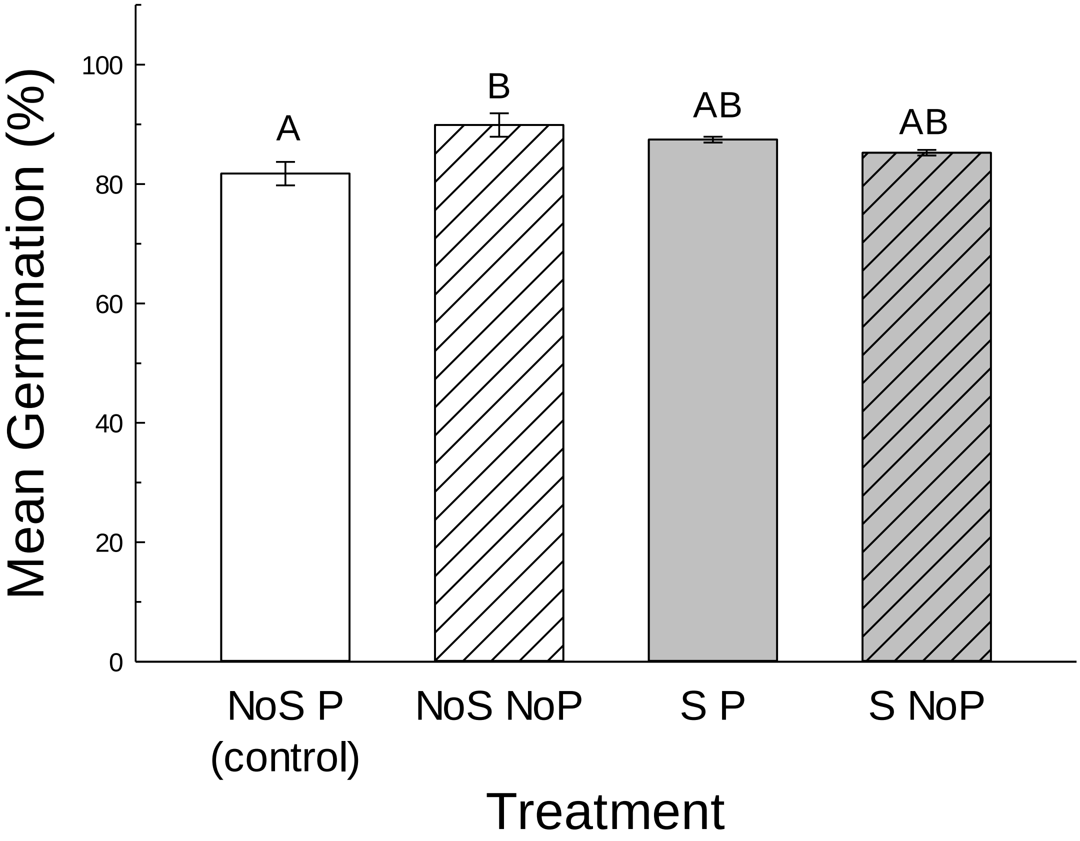

Figure 1. *Carex brevior* Fresh Seed Total Germination

Total day 84 mean germination (%) (±SE) of fresh *C. brevior* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Means with different letters are significantly different (Tukey's HSD). Neither stratification nor perigynia removal affected total germination and there was an interaction (ANOVA, $F_{1,12}$=7.6, p=0.017) and combining stratification and perigynia removal decreased germination by 2.2-4.6% compared to either treatment alone.

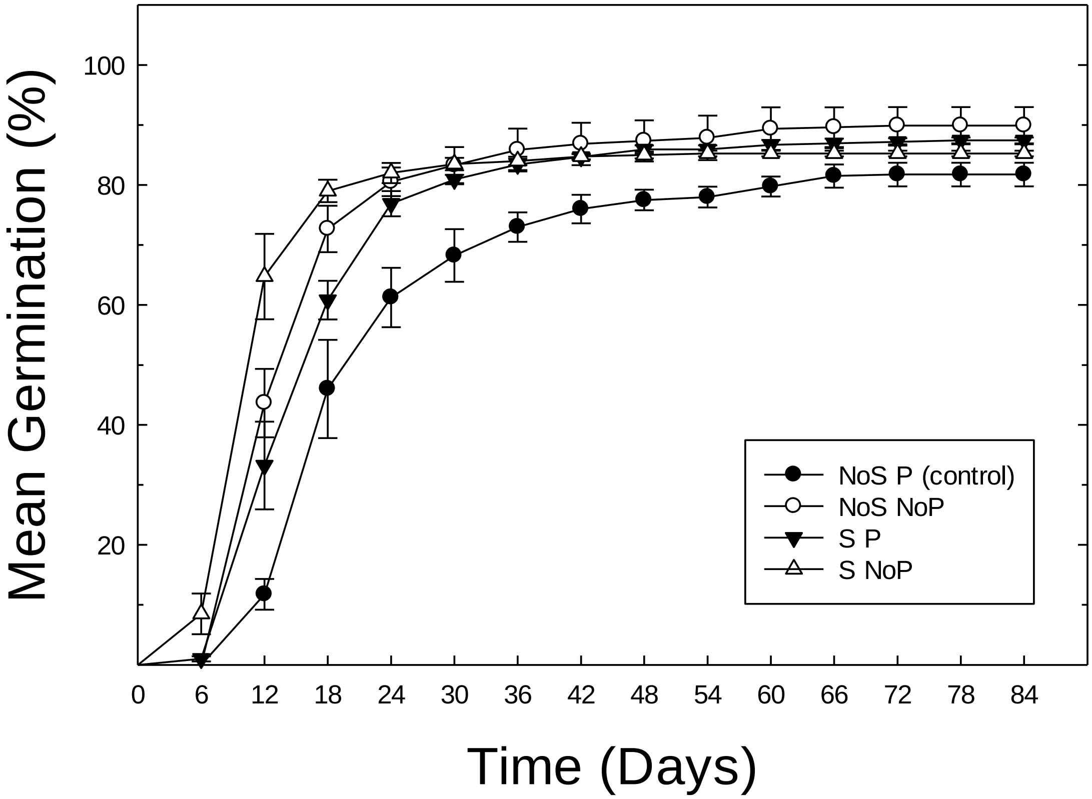

Figure 2. *Carex brevior* Fresh Seed Germination Rate

Rate of mean germination (%) (±SE) of fresh C. brevior seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Stratification (RM-ANOVA, $F_{12, 684}$=4.8, p=0.029 and perigynia removal ($F_{12, 684}$=4.1, p=0.043) increased germination rate.

Stored Seed

Total germination of untreated stored seed was approximately 25.5% less than in fresh seed (Table A3. General Linear Model ANOVA Total Day 84 Germination *C. brevior* Fresh and Stored Seed) and ranged from 56.4% for untreated seed to 79.3% for stratified seed with perigynia removed (Fig. 3). Overall, both stratification and perigynia removal significantly increased total germination; however, there was a significant interaction, with stratification increasing germination only in seeds with perigynia intact and not with perigynia removed (Table A4. ANOVA Total Germination of *C. brevior* Stored Seed). Perigynia removal increased total germination by 22.5% in nonstratified seed and had little effect on stratified seed. Stratification increased total germination by 21.4% when the perigynia was intact and had no effect on germination rates of seed with the perigynia removed. The combination of stratification and perigynia removal did not significantly increase germination compared to either treatment alone.

In addition to affecting total germination, all seed treatments increased germination rates (Fig. 4; Table A5. RM ANOVA of *C. brevior* Stored Seed). Stratification had a stronger effect on germination rate than perigynia removal and promoted earlier germination, with untreated (control) seed reaching 50% germination by day 54 and treated seed reaching it by day 10-28 (B2. Stored Seed Trial Raw Data). Generally, fresh *C. brevior* seed germinated more rapidly than stored seed (Fig 4; Fig 2).

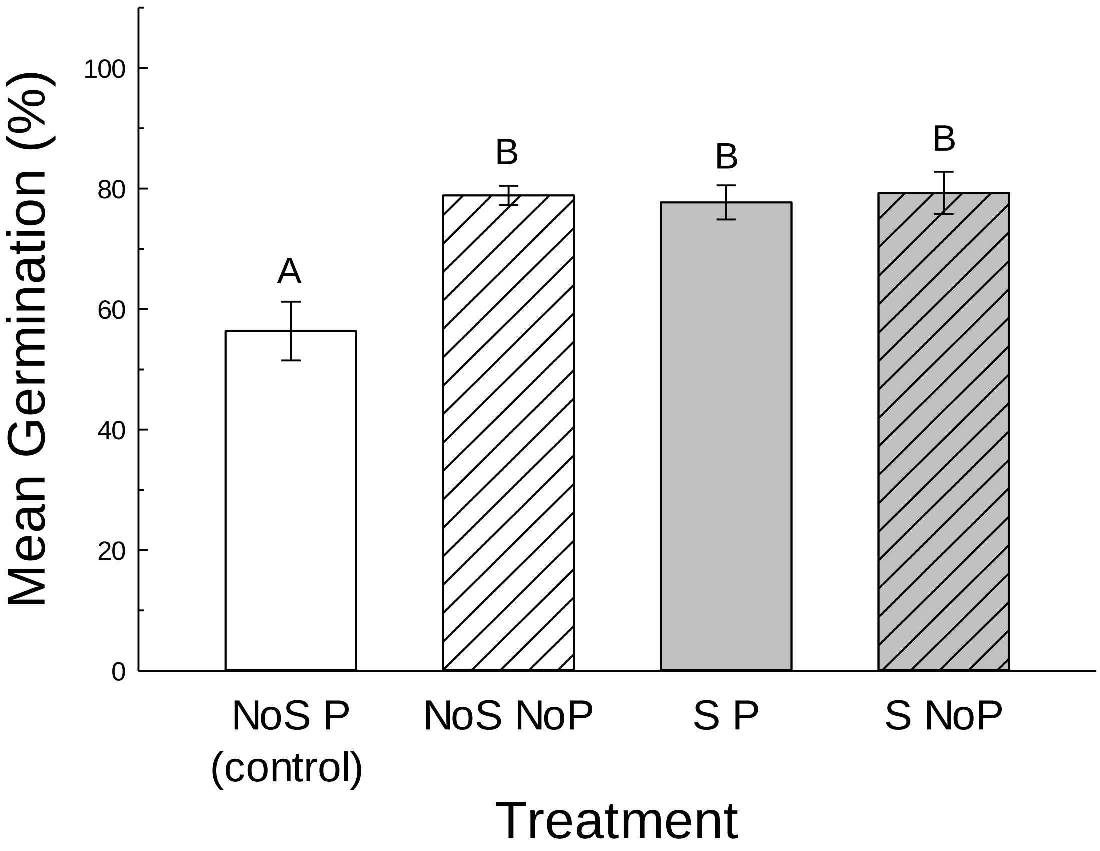

Figure 3. *Carex brevior* Stored Seed Total Germination

Total day 84 mean germination (%) (±SE) of stored *C. brevior* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Means with different letters are significantly different (Tukey's HSD). Stratification increased total germination by 21.4% (ANOVA, $F_{1,12}$=10.2, p=0.008), perigynia removal increased it by 22.5% ($F_{1,12}$=12.4, p=0.004) and there was an interaction ($F_{1,12}$=9.4, p=0.010) in that the treatments only increased total germination when they were not combined.

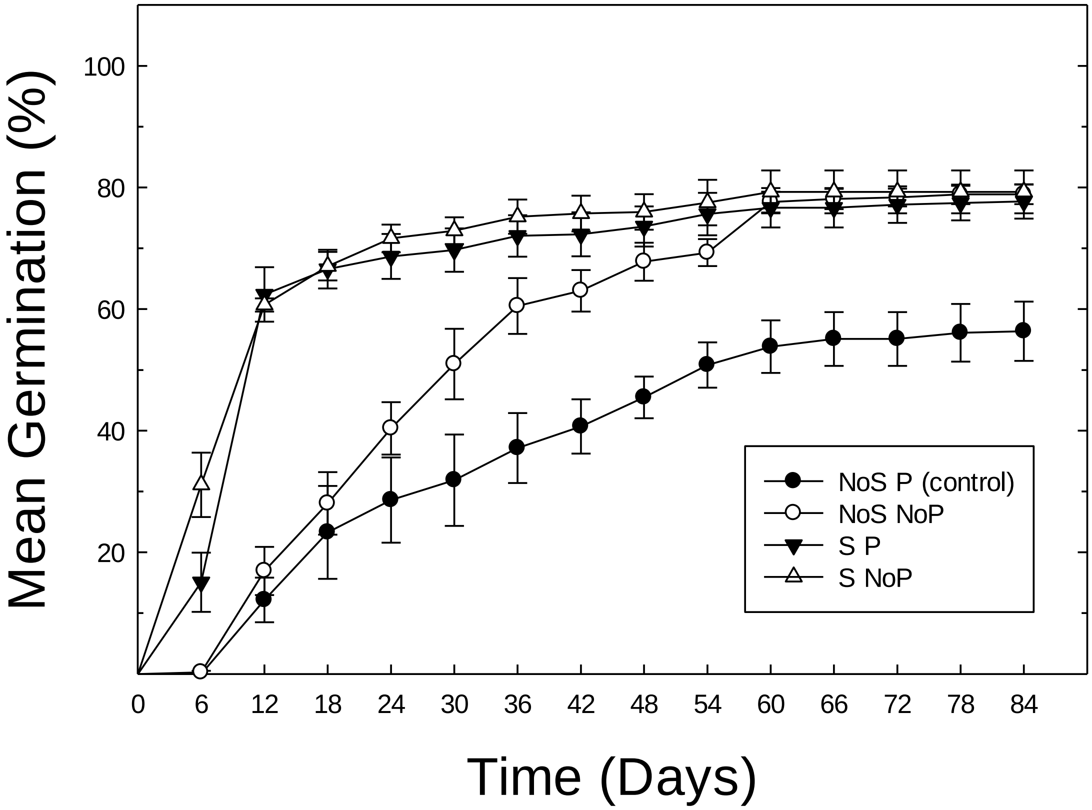

Figure 4. *Carex brevior* Stored Seed Germination Rate

Rate of mean germination (%) (±SE) of stored *C. brevior* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Stratification (RM-ANOVA, $F_{12, 604}$=71.2, p=<0.001 and perigynia removal ($F_{12, 604}$=14.1, p=<0.001) increased germination rate, and there was an interaction ($F_{12, 604}$=20.8, p=<0.001).

Carex molesta

Fresh Seed

Total germination ranged from 91.3% for untreated seed to 97.8% for stratified seed with perigynia removed (Fig. 5). Stratification significantly increased total germination. Neither perigynia removal nor the combination significantly increased total germination, and there was no interaction (Table A6. ANOVA Total Germination of *C. molesta* Fresh Seed).

Stratification significantly promoted earlier germination (Fig. 8; Table A7. RM ANOVA of *C. molesta* Fresh Seed). Stratification had a stronger effect on germination rate than perigynia removal.

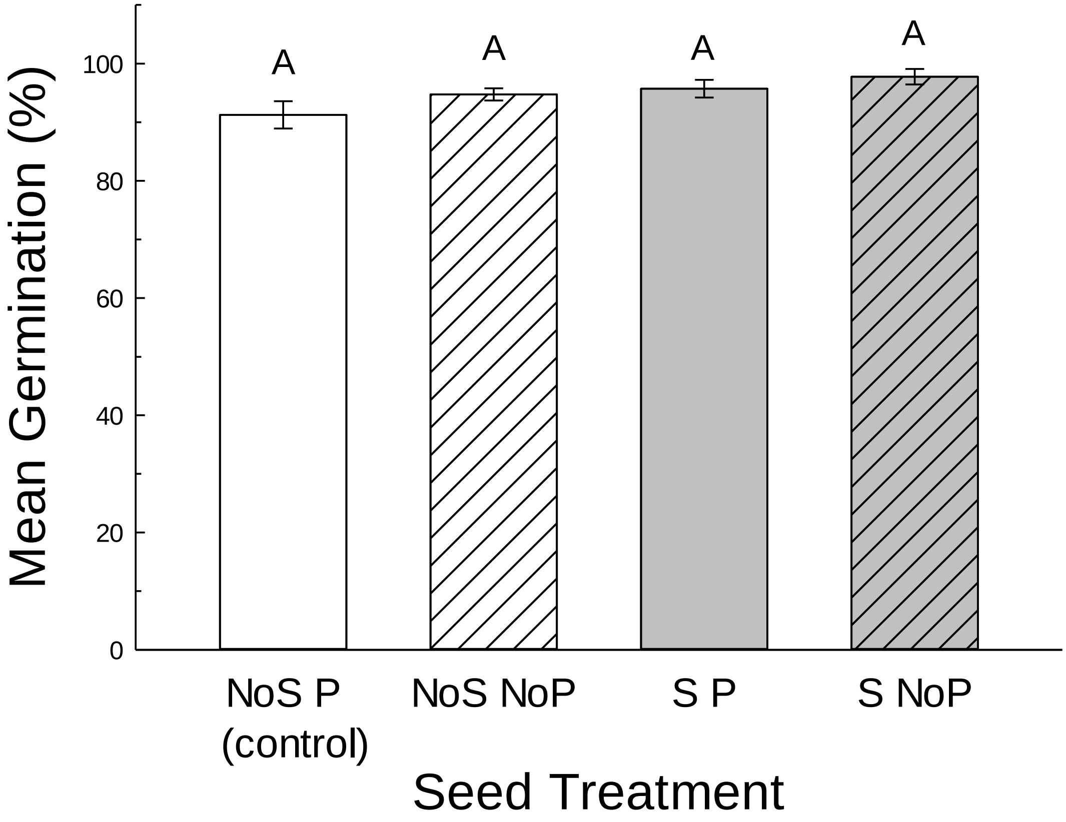

Figure 5. *Carex molesta* Fresh Seed Total Germination

Total day 84 mean germination (%) (±SE) of fresh *C. molesta* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Means with different letters are significantly different (Tukey's HSD). Stratification increased total germination by 4.5% (ANOVA, $F_{1,12}$=5.3, p=0.040).

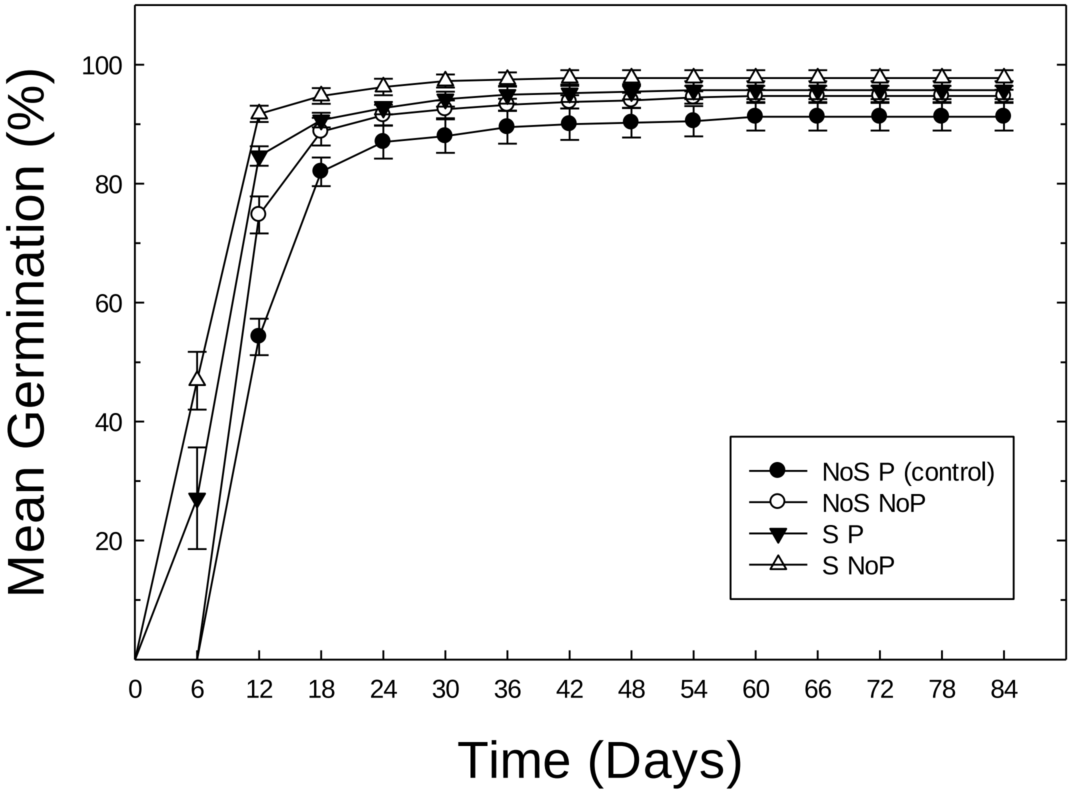

Figure 6. *Carex molesta* Fresh Seed Germination Rate

Rate of mean germination (%) (±SE) of fresh *C. molesta* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Stratification (RM-ANOVA, $F_{12, 684}$=7.5, p=0.006) increased germination rate.

Stored Seed

Total germination ranged from 93% for untreated seed to 98.7% for stratified seed with perigynia removed (Fig. 7). Neither stratification nor perigynia removal significantly increased total germination, and there was no interaction (Table A8. ANOVA Total Germination of *C. molesta* Stored Seed).

Stratification promoted earlier germination (Fig. 8; Table A9. RM ANOVA of *C. molesta* Stored Seed) and had a stronger effect on germination rate than perigynia removal.

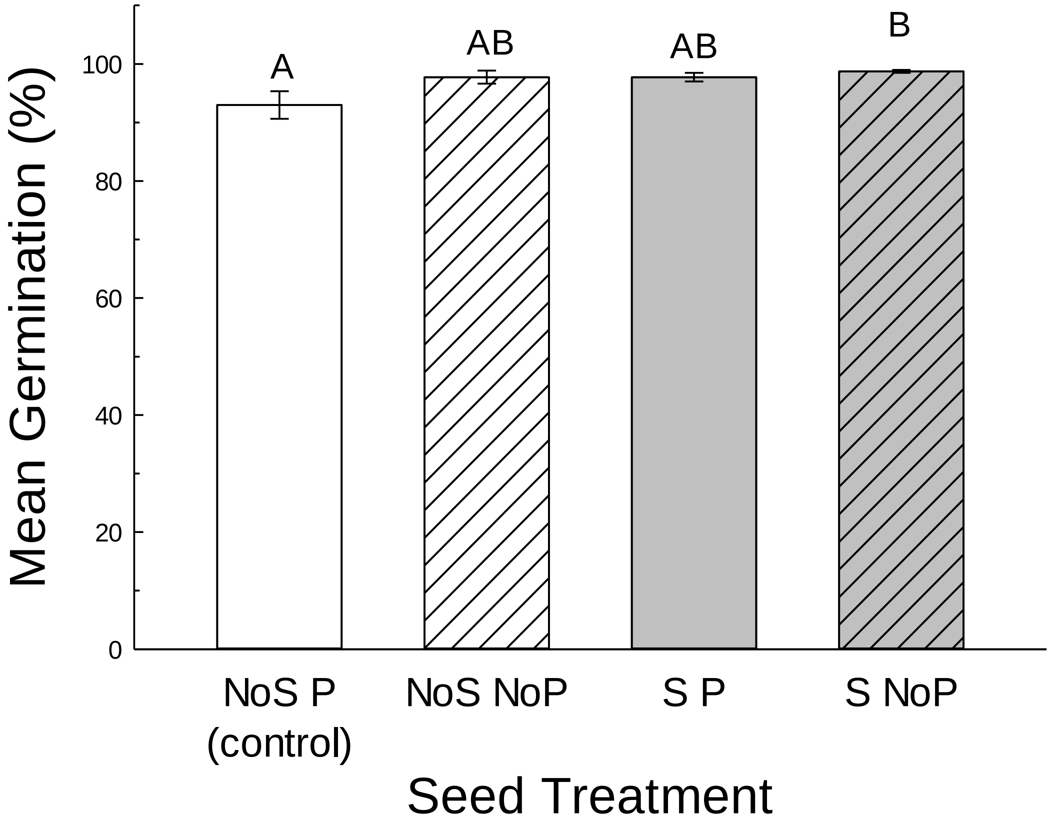

Figure 7. *Carex molesta* Stored Seed Total Germination

Total day 84 mean germination (%) (±SE) of stored *C. molesta* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Means with different letters are significantly different (Tukey's HSD). Stratification and perigynia removal marginally increased total germination by 4.8% and 4.6% respectively (ANOVA, $F_{1,12}$=4.5, p=0.056).

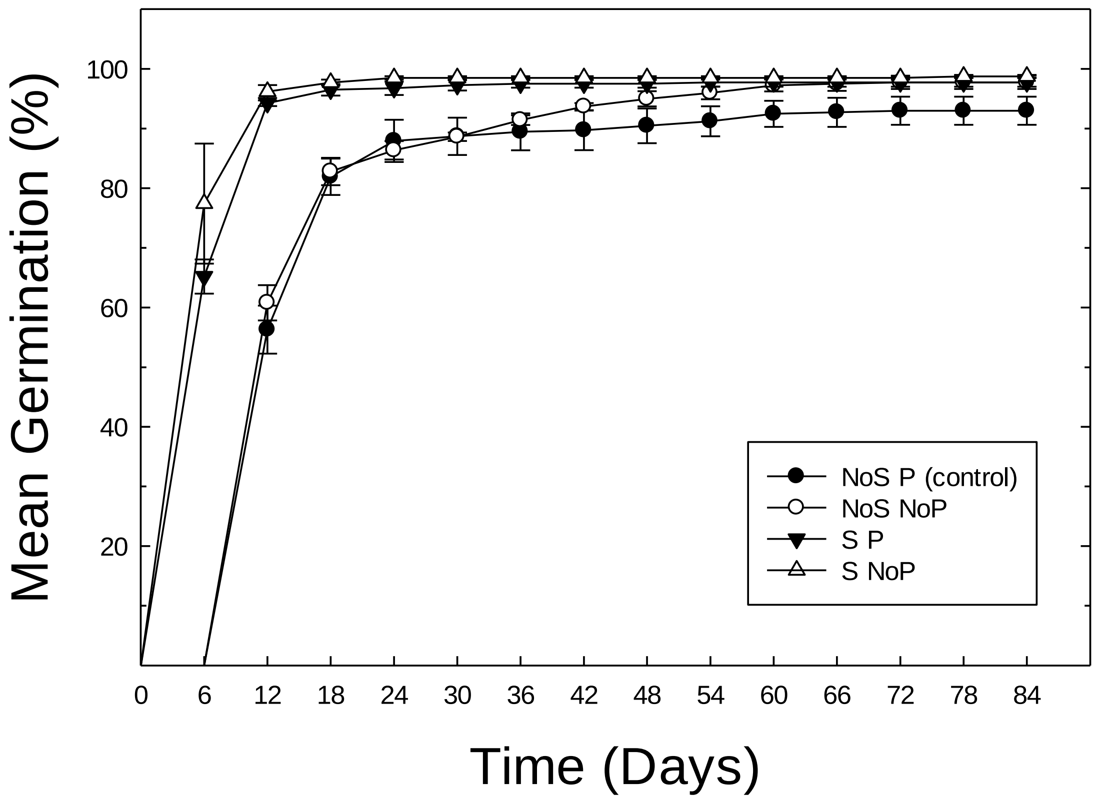

Figure 8. *Carex molesta* Stored Seed Germination Rate

Rate of mean germination (%) (±SE) of stored *C. molesta* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Stratification (RM-ANOVA, $F_{12, 604}$=77.9, p=<0.001) increased germination rate.

Carex bicknellii

Fresh Seed

Total germination ranged from 63% for untreated seed to 85.7% for stratified seed with perigynia removed (Fig. 9). Perigynia removal significantly increased total germination, while stratification increased it marginally (Table A11. ANOVA Total Germination of *C. bicknellii* Fresh Seed). The combination of stratification and perigynia removal did not significantly increase germination compared to either treatment alone and there was no interaction.

While all seed treatments promoted earlier germination and increased germination rates (Fig. 10; Table A12. RM ANOVA of *C. bicknellii* Fresh Seed), stratification had a stronger effect on germination rate than perigynia removal.

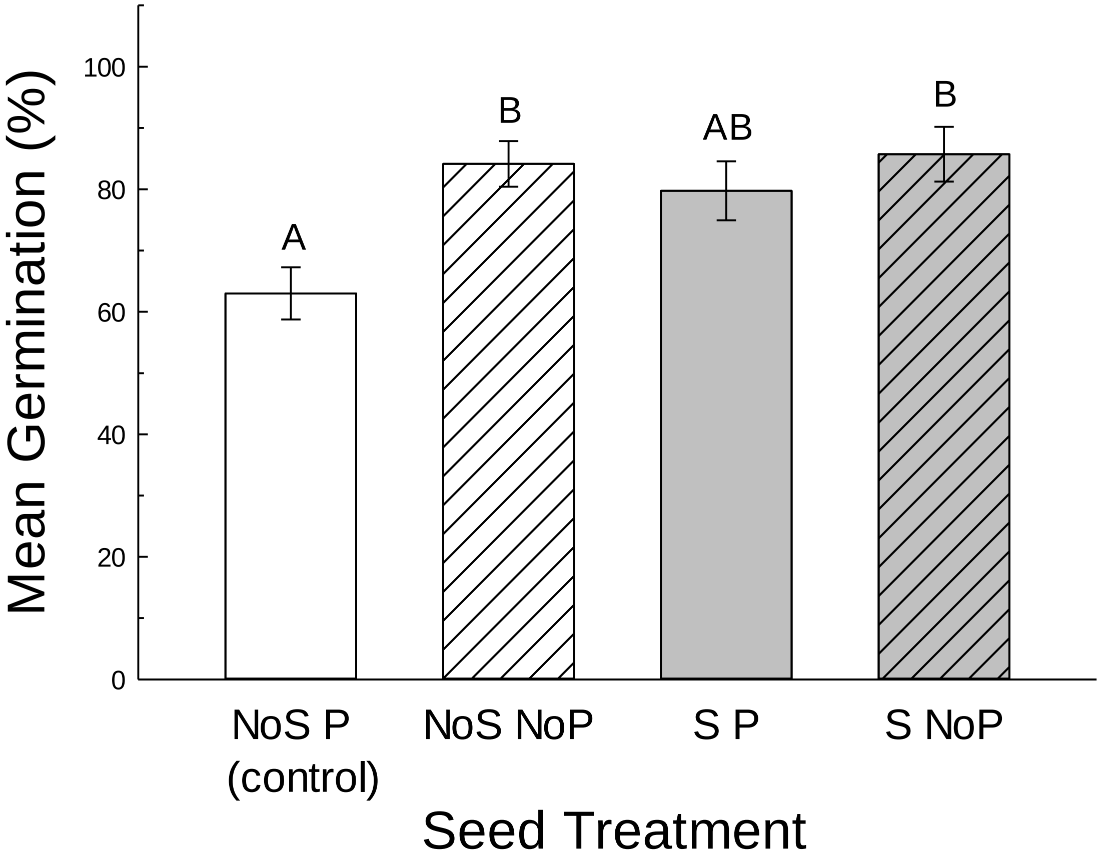

Figure 9. *Carex bicknellii* Fresh Seed Total Germination

Total day 84 mean germination (%) (±SE) of fresh *C. bicknellii* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Means with different letters are significantly different (Tukey's HSD). Perigynia removal increased total germination by 21.1% (ANOVA, $F_{1,12}$=9.8, p=0.009). Stratification marginally increased total germination by 16.7% (ANOVA, $F_{1,12}$=4.5, p=0.056).

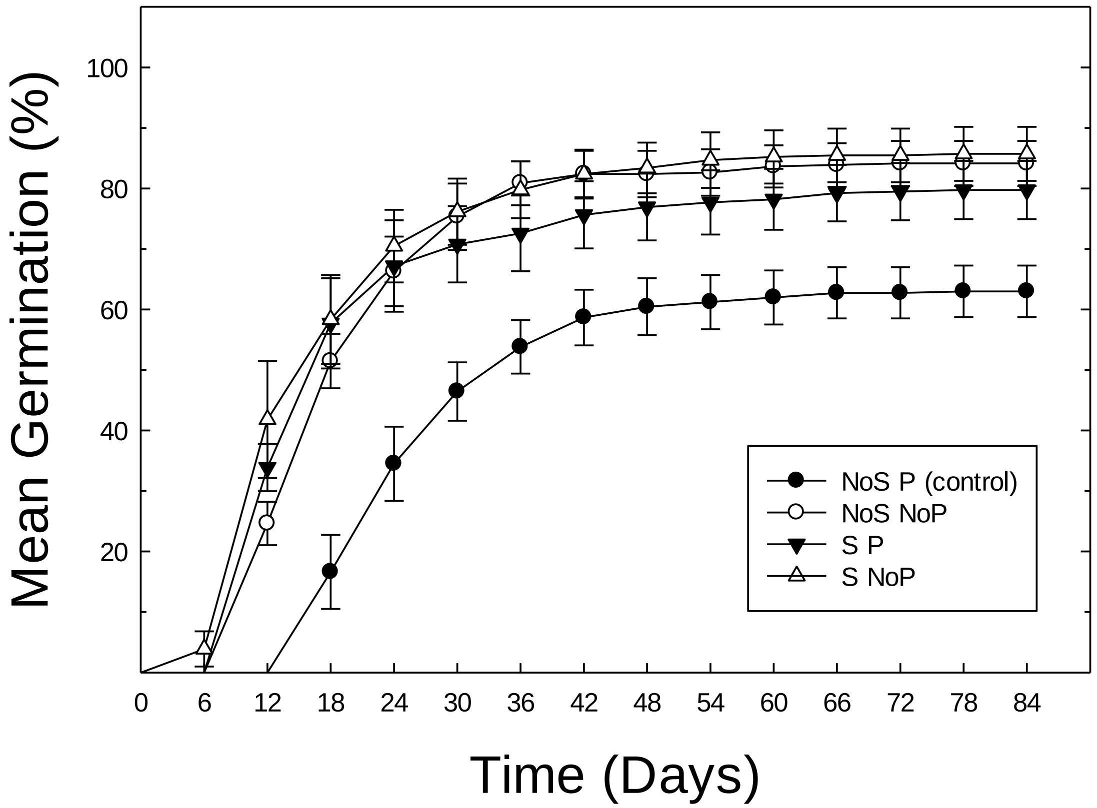

Figure 10. *Carex bicknellii* Fresh Seed Germination Rate

Rate of mean germination (%) (±SE) of fresh *C. bicknellii* seed following stratification and perigynia removal (NoS: not stratified, S: stratified, P: perigynia intact, NoP: perigynia removed). Stratification (RM-ANOVA, $F_{12, 684}$=5.5, p=0.020) increased germination rate.

CHAPTER 4

DISCUSSION

Both seed treatments, cold-wet stratification and perigynia removal, tended to increase *Carex* total germination and germination rate. In general, the effects of stratification were greater than perigynia removal. Stratification increased day 84 total germination in four (stored *C. brevior*, fresh and stored *C. molesta,* and fresh *C. bicknellii)* of the five species × seed type combinations and increased germination rate in all 3 species in both seed states.

Stratification had the most impact on germination rate and timing within the first 18 days. In stored *C. brevior*, by day 6, stratification yielded at least 14.8% more germination than perigynia removal or the control and by day 12 it was by at least 48.5%. In fresh *C. brevior*, stratification yielded 21.4% more germination at day 12 over the control. In stored *C. molesta*, the stratified treatments yielded at least 65.2% more germination at day 6 and 33.4% at day 12. In fresh *C. molesta*, the stratified treatments yielded 27.1% more germination at day 6 and 9.9% by day 12.

Perigynia removal increased day 84 total germination in 3 (stored *C. brevior* and *C. molesta*, and fresh *C. bicknellii*) of the 5 species × seed type combinations and increased germination rate only in fresh and stored *C. brevior*. Perigynia removal did not affect germination rate in *C. molesta* or *C. bicknellii*.

Perigynia removal had the most impact on *C. brevior* germination rate initially in fresh seed, while it had a more gradual impact in stored seed. In fresh *C. brevior*, perigynia removal yielded 31.8% more germination than the control at day 12 and 26.7% more than the control and 11.9 % more than stratification alone at day 18. In stored *C. brevior*, the rate with perigynia removed became different than the control at day 24 by 11.8%, though it remained significantly less than the rate of the stratified treatments through day 42.

Gains in total germination and germination rate provided by seed treatments are important because they increase the probability of *Carex* spp. becoming established and competing against invasive species. Invasives, like reed canary grass, are a major threat to native plant establishment in restoration plantings (Kettenring and Galatowitsch 2007b). Reed canary grass can go from being nonexistent to dominant in a restoration within a few year years (Galatowitsch and van der Valk 1996; Green and Galatowitsch 2001; Mulhouse and Galatowitsch 2003; Wetzel and van der Valk (1998) found that shoot height of upright sedge (*Carex stricta* Lam.) was reduced by the presence of reed canary grass, and Budelsky and Galatowitsch (2000) showed that it prevented the establishment of hairy sedge (*Carex lacustris* Stued). Without active efforts to ensure rapid native establishment, invasive species like reed canary grass can quickly take over a site and prevent *Carex* establishment (Kettenring and Galatowitsch 2007a) due to germination light requirements (Kettenring et al. 2006). Seed treatments like stratification and perigynia removal that increase germination rate are important in ensuring rapid *Carex* spp. establishment. Kettenring and Galatowitsch (2011) found that most of the *Carex* emergence that was going to happen in restoration plantings occurred within the first year, further highlighting the importance of optimizing germination. Additionally, reducing the time between sowing and germination, minimized seed hazards like seed predation, seed loss and burial due to flooding, and loss of viability (Budelsky and Galatowitsch 1999; Jones et al. 2004; van der Valk et al. 1999) .Earlier, more rapid germination and higher germination rates increase the probability of *Carex* establishment and help block light needed for invasive seed germination.

There was no evidence for additive effects of the seed treatments. Combining stratification and perigynia removal did not increase total germination over either treatment alone for any species or seed state, and it actually decreased total germination in fresh *C. brevior* by ~2-5%*.* The combined seed treatments also did not provide any additional advantage in increasing germination rate, with the slight exception of mildly increasing germination rate over days 6-18 in stored and fresh *C. brevior* and fresh *C. molesta*. Given these results, applying both treatments to *Carex* seed is not recommended.

Stratifying *C. brevior* seed is recommended. Stratification increased total germination of stored seed, and while no treatment increased total germination in fresh *C. brevior* seed, stratification increased germination rate to a greater degree than perigynia removal. If stratification is not feasible, perigynia removal would potentially provide increased germination over no treatment at all.

Given a choice between stratification and perigynia removal, stratifying *C. molesta* seed is also recommended. Stratification hastened germination and increased germination rate; however, even untreated *C. molesta* had very high germination rates (>90%), so the proportional benefits of seed treatment were less pronounced in this species.

Though stratification is widely accepted as protocol for increasing germination in *Carex* spp., these findings suggest that perigynia removal should be considered for fresh *C. bicknelli* seed. Increases in total germination and germination rate were slightly greater for perigynia removal than for stratification in this species. If perigynia removal is not feasible, stratification would provide increased germination over no treatment at all.

Overall stratification is the best option to increase germination in most instances. Compared to perigynia removal, germination rate was accelerated the most by stratification in both seed states. Increasing germination timing and rate, especially in the first few weeks after sowing, provides an important competitive advantage in the field setting, with limited resources and among competition. Stratification allows for a maximization of seed supplies and limited budgets (Kettenring and Galatowitsch 2007b) and does not require any special equipment. Natural stratification by, means of fall sowing, however, is not recommended due to seed loss from predation, flooding and burial (Janes et al. 2004).

CHAPTER 5

CONCLUSION

In summary, stratification overall increased germination in all species the most, and the effects of perigynia removal were most notable in stored *C. brevior* and fresh *C. bicknellii.* Considering the advantage of earlier and rapid germination as well as increasing total germination and ease of treatment, stratification is recommended for *C. brevior*. If stratification is not feasible, perigynia removal may be beneficial. Stratification may not be necessary in *C. molesta* due to overall fast germination and high total germination, though it did provide earlier germination that would likely be beneficial. Perigynia removal may provide the largest advantage in fresh *C. bicknellii*, but stratification would also provide an advantage. Combining stratification and perigynia removal is not advised for these species.

Further studies looking at the effects in stored *C. bicknellii* seed, in seed stored for longer periods of time, and in other *Carex* species that are more difficult to germinate as well as the effects in a field setting would be beneficial.

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APPENDIX

ANOVA TABLES

Table A1. ANOVA Total Germination of C. brevior Fresh Seed

Source	Type III SS	*df*	Mean Squares	*F*	*p*
Stratification	1.108	1	1.108	0.079	0.783
Perigynia Removal	35.373	1	35.373	2.536	0.137
Strat. x Perig.	106.657	1	106.657	7.647	0.017
Error	167.367	12	13.947		

Table A2. RM ANOVA of *C. brevior* Fresh Seed

	error*df*	*F*	*p*
Intercept	684	4847	<.0001
Time	684	626	<.0001
Stratification	12	16.5	0.0016
Perigynia Removal	12	25.2	0.0003
Time x Stratification	684	4.81	0.0286
Time x Perigynia Removal	684	4.13	0.0426
Strat. x Perig.	12	1.2	0.2950
Time x Strat. x Perig.	684	0.43	0.5112

Table A3. General Linear Model ANOVA Total Day 84 Germination *C. brevior* Fresh and Stored Seed

	df	Sum Squared	Mean Squared	*F*	*p*
Trial	1	1357.8	1357.8	44.841	<.0001
Stratification	1	26046	260.46	8.6015	0.0073
Perigynia Removal	1	164.32	164.32	5.4268	0.0286
Trial x Strat.	1	214.67	214.67	7.0892	0.0136
Trial x Perig.	1	450.76	450.76	14.886	0.0008
Strat. x Perig.	1	56.21	56.21	1.8564	0.1857
Trail x Strat. x Perig.	1	488.51	488.51	16.133	0.0005
Residuals	24	726.73	3028		

Table A4. ANOVA Total Germination of *C. brevior* Stored Seed

Source	Type III SS	*df*	Mean Squares	*F*	*p*
Stratification	473.933	1	473.933	10.166	0.008
Perigynia Removal	579.606	1	579.606	12.433	0.004
Strat. x Perig.	438.065	1	438.065	9.397	0.010
Error	559.438	12	46.62		

Table A5. RM ANOVA of *C. brevior* Stored Seed

	error*df*	*F*	*p*
Intercept	604	851	<.0001
Time	604	1140	<.0001
Stratification	12	77.8	<.0001
Perigynia Removal	12	4.97	0.0456
Time x Stratification	604	71.2	<.0001
Time x Perigynia Removal	604	14.1	0.0002
Strat. x Perig.	12	1.83	0.2010
Time x Strat. x Perig.	604	20.8	<.0001

Table A6. ANOVA Total Germination of *C. molesta* Fresh Seed

Source	Type III SS	*df*	Mean Squares	*F*	*p*
Stratification	55.614	1	55.614	5.316	0.040
Perigynia Removal	30.719	1	30.719	2.936	0.112
Strat. x Perig.	2.124	1	2.124	0.203	0.660
Error	125.543	12	10.462		

Table A7. RM ANOVA of *C. molesta* Fresh Seed

	error*df*	*F*	*p*
Intercept	684	5361	<.0001
Time	684	340	<.0001
Stratification	12	18.8	0.0010
Perigynia Removal	12	3.36	0.0915
Time x Stratification	684	7.51	0.0063
Time x Perigynia Removal	684	0.64	0.4230
Strat. x Perig.	12	0.14	0.7111
Time x Strat. x Perig.	684	0.01	0.9417

Table A8. ANOVA Total Germination of *C. molesta* Stored *S*eed

Source	Type III SS	*df*	Mean Squares	*F*	*p*
Stratification	33.091	1	33.091	4.469	0.056
Perigynia Removal	33.091	1	33.091	4.469	0.056
Strat. x Perig.	14.307	1	14.307	1.932	0.190
Error	88.865	12	7.4050		

Table A9. RM ANOVA of *C. molesta* Stored Seed

	error*df*	*F*	*p*
Intercept	604	10462	<.0001
Time	604	306	<.0001
Stratification	12	165	<.0001
Perigynia Removal	12	2.63	0.1305
Time x Stratification	604	77.9	<.0001
Time x Perigynia Removal	604	0.1	0.7573
Strat. x Perig.	12	0	0.9524
Time x Strat. x Perig.	604	0.44	0.5064

Table A10. General Linear Model ANOVA Total Day 84 Germination *C. molesta* Fresh and Stored Seed

	df	Sum Squared	Mean Squared	*F*	*p*
Trial	1	29.873	29.873	3.3437	0.0799
Stratification	1	87.241	87.241	9.7649	0.0046
Perigynia Removal	1	0.022	0.022	0.0024	0.961
Trial x Strat.	1	1.453	1.453	0.1626	0.6904
Trial x Perig.	1	63.791	63.791	7.1402	0.0133
Strat. x Perig.	1	2.706	2.706	0.3028	0.5872
Trail x Strat. x Perig.	1	13.728	13.728	1.5365	0.2271
Residuals	24	214.42	8.934		

Table A11. ANOVA Total Germination of *C. bicknellii* Fresh Seed

Source	Type III SS	*df*	Mean Squares	*F*	*p*
Stratification	335.348	1	335.348	4.479	0.056
Perigynia Removal	735.088	1	735.088	9.817	0.009
Strat. x Perig.	229.598	1	229.598	3.066	0.105
Error	898.526	12	74.877		

Table A12. RM ANOVA of *C. bicknellii* Fresh Seed

	error*df*	*F*	*p*
Intercept	684	1638	<.0001
Time	684	859	<.0001
Stratification	12	21.7	0.0006
Perigynia Removal	12	16.9	0.0014
Time x Stratification	684	5.47	0.0196
Time x Perigynia Removal	684	0.26	0.6109
Strat. x Perig.	12	6.12	0.0293
Time x Strat. x Perig.	684	0.03	0.8650