Using seed recovery methods to determine causes of failed germination in native prairie species

Paige Leytem

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USING SEED RECOVERY METHODS TO DETERMINE CAUSES OF FAILED GERMINATION IN NATIVE PRAIRIE SPECIES

A Thesis Submitted
In Partial Fulfillment
Of the Requirements for the Designation
University Honors with Distinction

Paige Leytem
University of Northern Iowa
May 2014
Abstract

Prairie restorations are expensive and emergence rates as low as ten percent are often observed. This could be because seeds are exposed to dangers from microbial and fungal attack, as well as predation from granivores after planting. Our experiment aimed to determine the post-dispersal seed fates of four native prairie species after they had been planted in the soil and covered with an exclosure to limit vertebrate predation. It was performed in a prairie restoration on the University of Northern Iowa Campus in Cedar Falls, Iowa. I coated five sets of 100 seeds of each species (Elymus canadensis, Oligoneuron rigidum, Eryngium yuccifolium, and Desmodium canadense) with fluorescent Glogerm™ and planted them at a depth of five millimeters in four rows (one row of 100 seeds per species, per exclosure) inside five wire mesh exclosures. After five weeks, seedling emergence data was collected and the top layer of soil from each row was excavated from within the exclosures. Collected soil was examined under a UV lamp and recovered seeds were tested for viability. I hypothesized that a majority of the seeds planted would be recovered, and that most of those recovered would be viable. Seed fates differed among the four species and were identified as emerged in the field, died during emergence, viable, and non-viable. Only 10-27% of the seeds planted were accounted for after the first recovery date and 4-10% after the second, and the majority tested were non-viable. Out of those recovered, 39% emerged as seedlings with D. canadense and E. canadensis demonstrating the highest emergence rates of the four species. It is evident that finding seeds after planting is still an obstacle that must be overcome in order to better understand post-dispersal seed fates.
Acknowledgements

I would like to thank my thesis advisor, Dr. Laura Jackson, for all of her help as well as for welcoming me into her lab and allowing me to work on this project. I would also like to thank Dr. Mark Sherrard for serving on my committee. I want to thank Carmen Pellish for her tremendous help, guidance, and support throughout this project as she worked toward completing her Masters Thesis. I would also like to recognize Madelyn Espinoza-Cotton for her assistance in preliminary greenhouse experiments. A special thanks to Dave Williams and Greg Houseal of the Tallgrass Prairie Center for their willingness to aid in plant identification, use of the TPC greenhouse and any other equipment needs I had, as well as for managing the restoration site our experiment was performed on. I also want to acknowledge Ben Hoksch and Andrew Ridgway for their help in counting out seeds.

I would like to thank Jessica Moon for her continued support throughout the Honors Thesis process and for her help editing. I would also like to thank Mary Jo Halder of the UNI Writing Center for her assistance with editing.

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Chapter 1

Introduction

Native prairies provide outstanding ecological benefits to their surrounding plant, animal, and human communities. Some of these benefits include carbon sequestration, erosion control, and increased habitat for wildlife (Smith 2010). Prior to Euro-American settlement, a large portion of the Midwest and most of Iowa was covered with native prairie. However, by 1900 over 90% had been tilled up for agricultural purposes. Now less than 0.01% of Iowa’s tallgrass prairie remains (Smith 1998). To restore these prairies and reap their benefits once again it is necessary to plant new seed to replace what has been lost through the destruction of native prairie lands. Natural seed sources such as prairie remnants are scarce and can be hard to find; therefore, the commercial seed industry is often relied upon to meet demands for seed. However, seed mixes can cost anywhere from $750 per ha for a low diversity mix (fewer than 25 species) to over $6,000 per ha for a high diversity mix (70 plus species; Ion Exchange 2013).

On top of these high seed costs, prairie plantings are often unpredictable. Hundreds of seeds are planted per square meter, yet germination rates as low as 10% are often observed (Williams et al. 2007). The combination of high initial seed cost and low establishment makes for very expensive restorations. A seed or seedling’s fate can be categorized as pre-dispersal mortality, persistence in the soil, and post-emergence mortality (Chambers and MacMahon 1994; Clark and Wilson 2003). Relatively little previous research has been done with regard to the perplexing issue of post dispersal seed fates (Fenner and Thompson 2005). A
potential reason for this is that locating seeds after they have been planted is extremely difficult. Through this research, I hope to gain a better understanding of what happens to seeds in the soil, as well as contribute seedling recovery methods that I have developed to aid in future studies. It is my hope that the work done on this project will help chip away at the mysteries of post-dispersal seed fates so that future prairie restorations can prosper and reveal their worth as an essential and productive ecosystem.

Literature Review

Many factors can affect whether or not a seed will germinate. These include planting depth, temperature, altitude, soil type, water and light availability, seed age, and parental environment. In addition to these factors, seeds are also exposed to dangers from microbial attack, fungal attack, and granivory from both vertebrates and invertebrates (Fenner 2005). I examined several studies that addressed the various factors affecting post-dispersal seed fates and used methods similar to my own.

A study on an Ohio wet meadow restoration examined the effects of exclosures (cage-like devices designed to exclude predators) on seed predation by all bird and mammalian herbivores and granivores. They found that species richness and diversity was significantly greater inside the exclosures than outside the exclosures (Fraser and Madson 2008).

Clark and Wilson (2003) also employed exclosures to study post-dispersal seed fates. They studied four species commonly found in western Oregon prairies;
two native, *Bromus carinatus* var. *carinatus* and *Prunella vulgaris* var. *lanceolata*, and two non-native, *Cynosurus echinatus* and *Daucus carota*. The three seed fates they attempted to quantify were death, persistence as a seed, and establishment as a seedling. These fates were measured by recovering the seeds from the soil one year after planting and testing for viability. Emergence was measured in the field. Seed death by fungal attack was also assessed using a fungicide treatment. Overall seed death was measured by subtracting the sum of the seeds whose fates were known from the total number of planted seeds (Clark and Wilson 2003). The most common fate observed was seed loss to unmeasured causes (seeds were not located/accounted for). Fungal disease generally caused less than 10% of the death in each of the four species. Indirect evidence showed that invertebrate predation caused death in only one of the species. Survival as an established seedling was more common than persistence as a seed in three of the four species (Clark and Wilson 2003).

Van Mourik et al. (2005) determined that seeding density plays a role in relative death by fungal attack. Seeds were placed in mesh bags and planted in the soil so they could be recovered at a later date. The elevated density of seeds in the bag relative to the densities found in natural seed banks allows increased levels of pathogens, which can cause seed mortality. They found that decreasing the density of seeds inside the mesh bags greatly reduced seed loss due to soil pathogens.

Our research aimed to determine the post-dispersal fates of seeds as they persisted in the soil or emerged as seedlings. Seeds of four native prairie species (*Elymus canadensis, Oligoneuron rigidum, Eryngium yuccifolium*, and *Desmodium*)
canadense) were planted in the soil and covered with an exclosure to limit vertebrate predation.

**Experimental Design**

Species were selected to represent a range of common native prairie species and were planted within a real prairie restoration on the University of Northern Iowa campus in Cedar Falls, Iowa. Seeds were also coated in fluorescent Glogerm™ to aid in the recovery process. I selected *D. canadense, E. yuccifolium, E. canadensis,* and *O. rigidum* as my four species because they have a relatively large seed size, have short dormancy, are commonly found in restorations, and have easily identifiable seedlings (Williams 2010). Species were also chosen to represent each of the main functional groups: grass, forb non-legume, and forb legume. I chose to perform this experiment on a real prairie restoration instead of in the lab because restored prairie conditions would provide more realistic results of what is actually happening to newly planted prairie seeds than would lab conditions. I felt the results would be more practical and therefore more useful when considering the outcomes of prairie restorations and ways to improve them.

Through this research I hope to build on my knowledge of how to best recover seeds from the soil while determining the fates of the seeds we planted. I hope to identify useful methods of seed recovery to aid future researchers who are also interested in determining post dispersal seed fates. The knowledge gained from analyzing the fates of recovered seeds will potentially help lower the costs of prairie restorations in the future, by identifying leading mortality causes.
Hypotheses & Research Questions

I predicted two possible outcomes to the study: that all of the seeds I planted would be located and that viabilities would remain relatively constant, or that I would not recover all of the seeds and there would be losses attributed to unknown factors such as invertebrate predation. The questions guiding my research were: what are the most common post-dispersal seed fates? Is loss of viability a large contributor to low emergence rates? Is there a difference in fates among species? Can the fates of seeds in the soil be tracked?
Chapter 2

Methods

Seeds were purchased from Ion Exchange Inc. and stored in the refrigerator at 5°C prior to planting. The experiment was performed on a 0.607 ha prairie restoration site located on the University of Northern Iowa campus (42° 30’ 30” N; 92° 27’ 27” W) in Cedar Falls, IA on a small alluvial bench along the University branch of Dry Run Creek. The soils on the site were classified as a Saude-Urban land complex with zero to two percent slopes (NRCS, 2014). Prior to restoration, the site was dominated by Bromus inermis Leyss. (Smooth brome), Agropyron repens (L.) Gould (Quack grass), and Poa pratensis L. (Kentucky blue-grass). The site was sprayed with glyphosate on 17 May 2013 and burned on 3 June 2013 by Dave Williams of the Tallgrass Prairie Center to remove all vegetation. Several hours after burning, a mixture of prairie seed, excluding our four species, was planted at a depth of approximately 1 cm using a no-till grass drill operated by Dave Williams.

Prior to planting, I counted out seeds of each of the four species into twenty-five groups of 100 and placed them in labeled vials for a total of 100 vials and 10,000 individual seeds. Seeds were emptied from their vials onto paper plates in piles of 100. The seeds were then coated with orange fluorescent Glogerm™ using a plastic dropper (Glogerm 2014). I decided to use fluorescent Glogerm™ as a marker to make the seeds easier to recover. A previous study (Huisman 2010) tested the effects that application of Glogerm on seeds has on germination and viability rates and found that it does not have a significant affect. I also performed a pilot study prior to the start of the experiment to test two different types of Glogerm™, an
orange liquid and white powder form. I utilized *O. rigidum* as our test subject. Seeds were covered in both types of material and buried in potting soil for one to five weeks. While 85-100% of the seeds were located throughout the study between both types of Glogerm™, it was determined that the liquid Glogerm™ persisted on the seeds longer and made them easier to view in the soil because of its bright orange color.

After being coated with Glogerm™ the seeds were left in the lab to dry for four days. After a day of drying, the seeds were transferred to large absorbent paper towels to aid in drying (see Figure 1). When it was determined that the seeds were sufficiently dry (no longer sticking to one another), seeds were returned to their respective vials.

Figure 1: Seeds, in piles of 100, after they have been transferred onto paper towels to dry.
Exclosures were constructed using 0.64cm wire hardware cloth cut into 1.1m x 0.6m pieces. A 5cm x 5cm square was cut from each corner of the wire and edges were folded down and fastened with 10cm zip ties to create an exclosure measuring 1m long, 0.5m wide, and 0.05m deep as shown in figure 2.

Figure 2. One of the exclosures over an experimental plot used to limit vertebrate predation in the field.

On 7 June 2013, random numbers were generated in Microsoft Excel to create twenty-five coordinate points throughout the site for each exclosure. Stake wire flagging was used to temporarily mark these locations.

On 11 June 2013 grooves were created for each exclosure to be placed inside. They were traced with a triangular, hand-held (garden) trowel, using one of the wire exclosures as a template. Grooves 100 cm long were then dug approximately three cm deep and one cm wide using a splitting maul. Excess biomass was removed from each 1m x 0.5m plot prior to planting. Four mock drill lines were drawn
lengthwise inside each experimental plot using a hand held (garden) trowel at an approximate depth 1 cm to mark where seeds would be planted. Lines were drawn ten cm apart with a ten cm buffer separating the first and last row from the edge. One row of 100 seeds of each species was sown by hand into the drill lines each cage (figure 3). Each species was assigned a number (1=\textit{D. canadense}, 2=\textit{E. yuccifolium}, 3=\textit{E. canadensis}, 4=\textit{O. rididum}) so it could be randomly assigned a row in each cage.

Figure 3: Seeds marked with Glogerm™ being sewn by hand into their drill lines.

After the seeds were sprinkled into their respective drill lines, exclosures were placed over them and into their respective grooves. A total of six twelve-gauge wire anchors were placed into the ground, spaced evenly around the exclosure, to hold it in place. Two anchors were placed on each 1m side and one on each 0.5m side. Each plot was then labeled 1-25 with a metal tag.
On July 15th 2013 five experimental plots were randomly selected for the first data point in our study. The anchors were removed, exclosures were pulled up pulled, and the interior of each plot was weeded. The four rows of planted fluorescent seeds were then located. Seedlings in each of the four rows were identified, recorded, and removed. A hand held shovel/trowel was then used to remove the top layer of soil from each row. Soil was removed at a depth of approximately 5cm and a width of approximately 8cm along each row. It was then deposited into 4-L plastic Ziploc® bags, labeled with the recovery date and respective plot and row number (figure 4). Soil was stored in the refrigerator at 5°C for 1 – 7 days after collection.

Figure 4. Collecting soil samples in a plastic Ziploc® bag.
Soil samples were placed on paper plates and sorted through with tweezers and a scalpel to break up sod chunks. Ultraviolet lamps (UV) were used to locate marked seeds in the soil samples. Each bag of soil consisted of one row’s worth of soil and took approximately 0.5 h to analyze. Recovered seeds and seed-like fragments were placed in a vial labeled with the respective cage and row number, and returned to the refrigerator to await further analysis.

Each seed or fragment was carefully examined and placed in a seed fate category: intact (a whole seed), partially germinated (evidence of a root structure), an empty seed coat, or in the case of E. canadensis, empty floral structures or “chaff”. Intact seeds were then tested for viability using a tetrazolium chloride 1% test (TZ test). TZ tests were administered based on the testing protocols suggested by Patil and Dadlani (2011). The seeds from each vial were placed in a Petri dish and soaked in distilled water for one hour. They were then cut in half lengthwise, (except for O. rigidum because of its small size), submerged in TZ solution and placed in a drying oven at 35°C for 1 hour. Seeds were then examined under a microscope to determine if they were viable or not. A pink embryo indicated that the embryo was capable of respiration, and thus viable. Results were recorded and analyzed in an Excel spreadsheet where raw numbers were converted to percent of total recovered. Means and standard errors were calculated using excel and graphs were created using SigmaPlot 10. After the second sampling date, seeds of the same species were accidentally combined instead of being kept in separate Petri dishes for each experimental plot. This allowed means to be calculated but not standard error.
On 11 September 2013 an additional five sets of research plots were sampled, using the recovery processes outlined above. TZ tests were conducted for leftover seed from Ion Exchange Inc. that had not been planted, but instead were stored in the refrigerator at 5°C for 3 months. Three more seed recovery dates were scheduled through the remainder of 2013 and into the spring of 2014. However, due to low recovery rates in the September 2013 recovery, the 2014 dates were cancelled.
Chapter 3

Results

I recovered 5-46% of the seeds initially planted in an individual row over the first two sampling dates, meaning in each row of 100 seeds, between 5 and 46 of the seeds initially planted were recovered. Sixteen percent of seeds planted were accounted for from the experimental plots sampled by the second sampling date. Of those accounted for, only 39% fell in the emerged category.

For the first sampling date, 17 July 2013, 10.2% of *O. rigidum* seeds were accounted for as a recovered seed or germinated seedling (see Table 1). Relatively equal numbers of seeds or seedlings from the other three species were recovered at this date as well.

Table 1: Seed size in grams for each species and percent seeds accounted for (includes all fates) with the standard error across both sampling dates (N=5 replicates of 25). Standard errors for *E. canadensis* and *E. yuccifolium* were unable to be calculated for week 13 because of a human error during viability testing.

<table>
<thead>
<tr>
<th>Species</th>
<th>Seed Size (g)</th>
<th>July 17th (SE)</th>
<th>September 11th (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. canadense</em></td>
<td>0.0038</td>
<td>26.4 (7.4)</td>
<td>11.4 (3.5)</td>
</tr>
<tr>
<td><em>E. canadensis</em></td>
<td>0.0046</td>
<td>27.0 (8.1)</td>
<td>13.6 (-)</td>
</tr>
<tr>
<td><em>O. rigidum</em></td>
<td>0.0007</td>
<td>10.2 (1.5)</td>
<td>4.0 (1.5)</td>
</tr>
<tr>
<td><em>E. yuccifolium</em></td>
<td>0.0052</td>
<td>25.0 (8.6)</td>
<td>12.8 (-)</td>
</tr>
</tbody>
</table>

The recovery rate was lower on the second sampling date (11 September 2013) than the first. Percentages of recovered seeds were 11.4% *D. canadense*,
13.6% *E. canadensis*, 4.0% *O. rigidum*, and 12.8% *E. yuccifolium*. The average time spent recovering seeds from one bag of soil from the first sampling date was 0.5 h, but was reduced to approximately 0.33 h for the second sampling date as seed locating skills improved. Heavily compacted sod chunks in the samples made retrieving seeds difficult during both recovery dates.

Possible seed fates included emergence as a seedling in the field, partial germination in the soil/below the soil surface (discovered while analyzing soil samples), and viable or non-viable, as determined by TZ tests (see figure 5). At the first sampling, loss of viability was the most common seed fate for all species except *D. canadense*. Percentages are based on the total number of seeds of each species found, not the total number initially planted. The mean was calculated for 5 replicate plots, not the N.

The most common fate for *E. Canadensis* was non-viable, however, a large portion (26.7%) had germinated below the surface. Though no emerged *E. yuccifolium* seedlings were found, 1.6% of seeds accounted for were partially germinated and 22.4% were viable. All fates were present during the first sampling period for *O. rigidum* with the majority (39.2%) being non-viable. The most common fate for *D. canadense* was emergence, with 26.4% of the total seeds planted having emerged from the soil, and 100% of those accounted for having emerged. No seeds or partially germinated seeds were found for this species during the first sampling.
Figure 5: The mean and standard error of the proportion of seeds that successfully emerged as seedlings, partially germinated in or below the soil surface, and that were determined to be viable and non-viable for each species after the first recovery date, July 15, 2013. N= the total number of seeds of each individual species that were accounted for on the first sampling date. Means and standard errors are for five replicate lots of 100 seeds each.

At the second sampling, (see Figure 6), the most common fate for *D. canadense* was once again emergence, with 96% of the seeds accounted for having emerged. One partially germinated seed was also found in a recovered soil sample, as was one viable intact seed. The majority of *E. canadensis* seeds had germinated to some degree. Fifty-nine percent had emerged in the field and 21.5% had partially germinated in the soil. Only twenty *O. rigidum* seeds/seedlings were accounted for
during the second sampling, possibly due to the fact the *O. rigidum* seeds are so small (see table 1). Of those accounted for, 45.0% were partially germinated and 55.0% were non-viable. Of the *E. yuccifolium* seed accounted for, 90.6% was non-viable and 9.4% was viable.

Figure 6: The mean proportion of seeds that successfully emerged as seedlings, partially germinated in or below the soil surface, and that were determined to be viable and non-viable for each species after the first recovery date, September 11, 2013. N= the total number of seeds of each individual species that were accounted for the second sampling date. Means and standard errors are for five replicate lots of 100 seeds each. Standard error bars are missing on this graph do to a human error during sampling and testing.
The low viability rates observed in recovered seeds were much lower than the viabilities provided by Ion Exchange at the time of purchase (see table 2). The viability listed on the seed packet at the time of purchase was inconsistent with the UNI lab-determined viabilities of leftover seed that had been stored in the refrigerator at 5°C since purchase (tested in September 2013).

Table 2: Viability given by the commercial seed source (Ion Exchange Inc.) at the time of purchase in March 2013 determined through TZ and germination tests. Column 2 shows viability of unplanted seeds determined by TZ tests in the UNI lab in September 2013 after storage at 5°C (N=50).

<table>
<thead>
<tr>
<th>Species</th>
<th>Ion Exchange Viability (%) (Date Conducted)</th>
<th>In-Lab Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. canadensis</em></td>
<td>90 (3/6/13)</td>
<td>54</td>
</tr>
<tr>
<td><em>O. rigidum</em></td>
<td>94 (10/4/12)</td>
<td>0</td>
</tr>
<tr>
<td><em>D. canadense</em></td>
<td>99 (3/23/13)</td>
<td>86</td>
</tr>
<tr>
<td><em>E. yuccifolium</em></td>
<td>92 (1/9/13)</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 3 (below) shows the viability rates observed in recovered seed after both sampling dates. Viabilities given by the seed source for all four species were all above 90%; however, those determined in the UNI lab on refrigerated seed were quite lower, as were viabilities of planted seed. The most notable differences were with *E. canadensis* and *O. rigidum*. The seed source viability for *E. Canadensis* was 90%, however, only a 54% viability rate was observed in the lab. The seed source
listed 94% as the viability for *O. rigidum*, but we found no viable seeds during our tests.

Table 3: UNI lab determined viabilities of recovered intact seeds from both sampling dates. N=the number of seeds tested.

<table>
<thead>
<tr>
<th>Species</th>
<th>July 15th Viability (%)</th>
<th>September 11th Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. canadense</em></td>
<td>N/A (N=0)</td>
<td>100 (N=1)</td>
</tr>
<tr>
<td><em>E. canadensis</em></td>
<td>22.4 (N=85)</td>
<td>14.3 (N=14)</td>
</tr>
<tr>
<td><em>O. rigidum</em></td>
<td>33.3 (N=30)</td>
<td>0 (N=11)</td>
</tr>
<tr>
<td><em>E. yuccifolium</em></td>
<td>22.8 (N=123)</td>
<td>9.4 (N=64)</td>
</tr>
</tbody>
</table>
Chapter 4

Discussion

Despite selecting prairie species that have relatively large seeds and coating them with Glogerm™ to make them easier to see, I only recovered 652 of the 4,000 seeds (16.3%) initially planted (the remaining 6,000 were left in the ground for future sampling dates). This number is much lower than I expected. I speculated that invertebrates might have eaten some seeds, as there was evidence of ants and small beetles in and around the research plots. Others may have been destroyed by soil pathogens or been lost in the soil so that I was unable to locate them. Clark and Wilson (2003) found that seeds are more likely to survive as established seedlings than to persist in the soil as seeds. Their most common seed fate was death due to unmeasured factors. Only 2% of seeds recovered in their study were non-viable, however, they reasoned that some of the senesced seeds may have decayed and disappeared in the soil. I suspect that this was the fate of many of my seeds as well.

Flecks of Glogerm™ were abundant in the bags of soil analyzed, which suggests that it was coming off the seeds. This made them more difficult to find and greatly reduced recovery rates. By the second sampling date, seeds were even more difficult to find as they had been in the ground longer and were exposed to biological and elemental hazards for more time.

While the Glogerm™ was helpful in finding some seeds; it is evident that a more reliable method is needed in order to increase the fraction of seeds that are recovered. Other ideas for seed tracking devices that may be explored in this lab by
future students include radioactive tracers and brightly colored agricultural seed dyes.

Human error could also have played a role in the low recovery rates, as some of the seeds may have been difficult to see amongst the sod clumps and vegetation within the soil samples. One way of accounting for this would be to bury small beads in the soil and recover them in the same way as the prairie seeds. This would help determine recovery rates without the uncertainty of invertebrate predation, fungal attack, or decomposition. Another idea is to recover the seeds immediately after planting to determine my find rate.

Of the seeds I was able to recover, the majority were non-viable. This could indicate that there is a steep drop in viability immediately or shortly after seeds are planted. I did not anticipate that the seeds would lose viability so quickly, and based on viability rates provided by the seed source, only 8-10% of the seeds planted were expected to be non-viable. This suggests that rapid loss of viability could be a cause for low seedling emergence rates. However, TZ tests performed in the lab after the first sampling date indicated that the initial seed planted was less viable than expected based on the information provided by the seed source. This experience taught me that it is important to always test purchased seed independently before planting to ensure that the viabilities reported by the seed source are accurate. Performing both germination and TZ tests would be a good way to ensure the most reliable results.

Out of the total seeds planted for both sampling dates, 6.4% emerged, and 1.7% partially germinated. It is difficult to determine whether or not the partially
germinated seeds died during emergence or if they would have survived had we not disrupted the soil. One way to reduce this uncertainty would be to analyze soil samples immediately after removing them from the field. If partially germinated seedlings were included in the emerged category there would be a total emergence of 9.8%, which is consistent with the findings of other studies on prairie planting emergence. It is evident that in order to fully understand post-dispersal seed fates and why emergence is so low, better methods of locating seeds after planting are needed.

Since emergence of *O. rigidum* and *E. yuccifolium* was very low I took a closer look at the recommended germination conditions for each of the species I chose. If found that *D. canadense* and *E. candensis* should germinate upon sowing in a warm location without the need of pretreatment aside from cold dry storage (Prairie Moon 2014). These requirements were met within this restoration, which could explain why I saw emergence from these two species. *O. rigidum* and *E. yuccifolium* germinate best after a period of cold, moist stratification (Prairie Moon 2014). Very low emergence was observed in *O. rigidum* and no emergence in *E. yuccifolium*. This could be attributed to the fact that seeds were planted in late spring when temperatures remained consistently warm, eliminating any chance of natural cold stratification. According to the germination instructions given by Prairie Moon Nursery (2014), stratification is unnecessary if planting in the fall or using a seed drill. However, since my planting method only simulated a seed drill it may not have successfully broken dormancy and allowed for optimal germination conditions as a real seed drill would have. Therefore I feel that cold stratification of *O. rigidum* and
*E. yuccifolium* would have increased emergence rates and provided better results overall.

When this experiment began I expected to find most of the seeds that were planted since I was able to recover nearly all of the seeds from the greenhouse pilot studies. However, after the first recovery attempt it was evident that I would not be able to recover all of the seeds. I also expected the recovered seed viability to stay relatively constant. However, I was surprised by the drastic reduction in viability rates that were observed. Loss of viability was one of the most common post dispersal seed fates. I recognize that some of the seed was unviable when planted as indicated by my TZ tests, however, loss in viability was still observed even after accounting for lower starting viabilities. This suggests that loss of viability does indeed contribute to low emergence rates.

Though I successfully tracked the fates of about 16% of the seeds I planted, it is evident that better methods for tracking seeds are necessary in order to determine post dispersal seed fates. This research helped determine that fluorescent material is a useful tool for locating seeds in the soil; however, a material that will persist on the seeds for an extended period of time would improve results. In addition, many of the seeds were simply not found. One possible way to solve this problem would be to plant seeds in mesh bags. This method would ensure that seeds stay in one area and can be recovered from within the bag. Other methods, such as radioactive tracers, were not tested in my lab but it would be worthwhile for future research to look into different tracking devices.
Low emergence rates in prairie restorations are still a problem and therefore determining the reasons behind these low rates is important. I hope that future researchers will benefit from what I’ve learned through my experiment by always testing seed viabilities before planting, utilizing seed tracking aids (such as dye or tracers), employing controls (such as inert objects, immediate excavation and recovery), and choosing species that emerge readily without the need of stratification or special germination conditions.
Chapter 5

Summary

Determining post dispersal seed fates is an important step in the improvement of prairie restorations. However, few previous studies have examined below ground post dispersal seed fates. Through coating four species with fluorescent Glogerm™, planting them in the soil for a period of five to eleven weeks, and recovering them from the soil I was able to learn a great deal about the logistics of tracking seeds in the soil. Although recovery rates were low, I determined that one of the leading causes of low seedling emergence is loss of viability. Though predation by invertebrates and death by fungal attack were not measured in my study, they are also possible causes for low emergence and should be looked at more closely. It is my hope that future researchers continue to build on the progress I have made in the area of below ground post dispersal seed fates. More research is needed to ascertain the causes of low emergence rates in prairie restorations so that practitioners can pinpoint causes of seed loss and adjust their restoration approaches accordingly. This will allow them to have more successful and cost effective prairie restorations.
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