1. **Introduction**
   1. Explain “dormancy release”
      1. Only winter stratification controls dormancy release
   2. Explain “germination requirements”
      1. Many things can control germination
      2. Here, I chose to look only at germination temperature
      3. Temperatures were appropriate for Western Washington
   3. Questions: *Do CAIN and CATU have a dormancy requirement? What temperature regime (season) promotes the highest germination percentage, rate, and uniformity?*

**INTRODUCTION**

Seeds are reproductive units … Seeds are relatively resilient structures, often able to endure more extreme temperatures and certainly more extreme drought than their adult counterparts. Seedlings, on the other hand, are extremely vulnerable. Without a well-established root system and energy stored in hardened leaf and stem tissues, seedlings are especially susceptible to drought, fungal infection, extreme temperatures, and herbivory. Germination is risky business, and waiting patiently until the time is right increases the chances that a seedling will survive the establishment phase, after which the likelihood of survival greatly increases. The ability to delay germination until the appropriate season, called seed dormancy, is one adaptation that deals with this challenge, and is an important strategy for many plants.

Seed dormancy describes the physiological prevention of germination, even in the presence of ideal conditions of moisture and temperature. The release of dormancy is accomplished through exposure to the cold, wet conditions which characterize our Western Washington winters. During winter exposure or “stratification”, a simulated winter season we can create in a nursery environment, seeds undergo a physiological change which gives the seed the potential to germinate. This physiological change is often temporary, and dormancy returns after a specified period of time or when the temperature rises above a certain threshold. In effect, the release of dormancy opens up a window of time during which germination is able to occur. This typically lasts through the spring season. This clever mechanism prevents seeds from germinating in the fall, when temperatures and day length are very similar to spring, but when winter will soon follow. For species that do not rapidly establish, being able to distinguish between spring and fall provides a distinct advantage.

The release of dormancy opens up a window of opportunity for germination, but does not guarantee that germination will occur. For most species, other conditions must also be met. The most basic of those requirements is moisture. Moisture is required for the germination of any seed. Imbibed seeds are metabolically active and able to detect cues in their environment. Water is required for the expansion of the radicle, which breaks through the seed coat and initiates germination.

Another prerequisite for germination is that temperatures are within an acceptable range.

The first step to identifying a safe time to germinate lies in identifying the correct season. Summers in Western Washington are warm and dry, and winters are cool and wet, with occasional freezing temperatures. Depending on the strategy of a species, spring germination might be preferred, allowing more growth to occur before winter. Depending on the species, habitat, and region, summer might be a better

Germination cues can also stimulate germination in a seed, but only after dormancy has been relieved through winter stratification.

or the presence of a disturbance (which would give a seedling a higher chance of survival in areas where resources are scarce).

It is helpful to think of seed dormancy and germination as two separate layers. First is dormancy, which prevents the seed from germinating in favorable conditions during an unfavorable season. By waiting until the preferred season, survival of the seedling in greatly increased. Under that layer of dormancy is the ability to detect an opportunity for germination…

In the context of propagation, seed dormancy can pose a real challenge. Incorporating any species into a restoration program requires producing large quantities of plants or seeds, and non-uniform germination can limit genetic diversity in the resulting crop. To facilitate propagation of these sedges, a number of seed treatments and germination conditions are being tested. This includes removing the perigynia (a protective structure that surrounds the seed), soaking in smoke water, and pinpointing the ideal length of time in winter stratification and germination temperature range. The focus is on methods that can be used operationally by conservation nurseries and restoration practitioners.

Most temperate sedges are released from dormancy by a cold/wet stratification period (Schutz, 2000). Determining whether stratification releases CAIN and CATU from dormancy, and the ideal length of time in stratification, will be beneficial to propagators; insufficient stratification could result in a failure to meet dormancy requirements, and longer than necessary stratification delays propagation schedules and reduces the amount of time available for after-ripening prior to stratification.

By comparing seeds that have been stratified for different lengths of time, we may see evidence of the presence or absence of conditional dormancy. Conditional dormancy would be indicated by the differing ability of seeds to germinate in three distinct temperature regimes as a result of their respective stratification treatment (Hartmann, Kester, Davies, & Geneve, 2011). Preliminary testing conducted by the Bakker lab suggests that this could be the case, as seeds germinated at a much higher percentage in spring temperatures with zero and one month of winter stratification, when compared to germination in summer temperatures. If results show that a longer stratification period allows germination to occur over a wider range of conditions, propagators could use stratification to gain the flexibility to germinate seeds in any environment that falls within this range of temperatures.

Alternatively, seeds exposed to each stratification duration may show the same preference for a particular temperature regime. For many sedges, fluctuating temperatures with a daily maximum between 20 and 25°C and a minimum of 10°C are ideal for germination (Schutz, 2000). Optimal germination temperatures are unknown for CAIN and CATU, so seeds will be placed in three different temperature regimes, representing spring, summer, and an intermediate “spring in a greenhouse”, to see whether CAIN or CATU prefer any of these temperature ranges for germination. If it is found that seeds prefer warmer temperatures, a greenhouse or cold frame could help propagators achieve these higher temperatures and germination rates in a production setting.

From this experiment I hope to learn which stratification duration results in the highest percentage of germination. Seeds may show a preference for a particular germination condition regardless of stratification regime, or they may display a pattern suggesting conditional dormancy. The timing and uniformity of germination may also be affected by the treatment conditions. Time to 50% germination will be calculated for each treatment, and curves that visualize the percentage and uniformity of germination will also be created. Together, these results will inform propagators of the combination of stratification treatment and propagation environment which should result in the highest and most uniform germination.

**MATERIALS**

Two accessions of *Carex inops* ssp*. inops* (CAIN) and one accession of *Carex tumulicola* (CATU) were evaluated in this trial. The South Sound Prairie Conservation Nursery provided 20 g of CAIN seed (362 seeds/g, 96.1% pure), which was cultivated and harvested from the Violet Prairie Seed Farm (Tenino, WA) on May 15th, 2015. This seed lot will be referred to as VP-CAIN, and is the primary seed lot tested throughout this experiment. 9.7 g of CAIN seed (318 seeds/g, 97.8% pure) were wild-collected on June 15th, 2015 from Tenalquot Prairie (Rainier, WA). This seed lot, referred to as TQ-CAIN, was only used in this trial due to the limited quantity of seed. 31.2g of CATU seed (681 seeds/g, 97.7% pure) was wild-collected from Naas Prairie on Whidbey Island (Coupeville, WA) on September 13th, 2015. CATU seed heads were threshed with a food processor and chaff was removed with a series of soil sieves. Seeds of both species were stored at room temperature. A summary of these three seed accessions and the experimental codes which will be used to refer to them from this point forward, is presented below for reference (Table 1).

Table 1. Summary of seed accessions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Source** | **Type** | **Code** |
| *Carex inops* ssp. *inops* | Violet Prairie Seed Farm (Tenino, WA) | Cultivated | VP-CAIN |
| *Carex inops* ssp*. inops* | Tenalquot Prairie (Rainier, WA) | Wild | TQ-CAIN |
| *Carex tumulicola* | Naas Prairie (Coupeville, WA) | Wild | CATU |

**METHODS**

Work began in September 2015, 19 weeks after collection for VP-CAIN, 20 weeks after collection for TQ-CAIN, and 4 weeks after collection for CATU. Seeds were first assessed for water-permeability by weighing before and after soaking for 24 hours; all seed imbibed water readily without scarification. 200 seeds from each accession were then assessed using the Tetrazolium (TZ) test according to the protocol described in the Tetrazolium Testing Handbook (Peters, 2000), to establish a baseline for seed viability.

Roughly 3200 seeds from each accession were prepared for testing by imbibing in aerated DI water for 24 hours. All seeds were then rinsed in a 10% concentrated bleach solution (1:10 bleach: DI water) for one minute, followed with a DI water rinse, and blotted dry. Bleaching seeds reduces the impact of surface molds, which can thrive in humid incubation chambers, on seed viability and germination rates (J. Bakker, personal communication, August 2, 2015).

Prepared seeds from each accession were randomly divided into 16 treatment groups, with 200 seeds per group. Each treatment group was then divided evenly between 4 petri dishes (size, material, Carolina Biological Supply), with 50 seeds per dish. Seeds were placed on two sheets of filter paper (9cm, qualitative, Carolina Biological Supply) moistened with DI water, covered with a lid, and placed in the winter, spring, intermediate, or summer incubator (\_\_\_ type and manufacturer of incubators). Table 2 describes the incubation sequence and duration for each treatment group. This setup was repeated for each of the three seed accessions.

*Table 2*. Duration and conditions experienced by each treatment group as they moved through germination incubators. Incubator conditions: Winter: 5°C (41°F) and light for 10 hours; 2°C (36°F) and dark for 14 hours; Spring: 15°C (59°F) and light for 12 hours; 8°C (46°F) and dark for 12 hours; Intermediate: 19°C (66°F) and light for 12 hours; 11°C (52°F) and dark for 12 hours; Summer: 24°C (74°F) and light for 14 hours; 14°C (57°F) and dark for 10 hours.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Weeks | | | | | | |
| Treatment # | 0-4 | 5-8 | 9-12 | 13-16 | 17-20 | 21-24 | 25-28 |
| 1 | winter | | | | | | |
| 2 | spring | | | | | | |
| 3 | intermediate | | | | | | |
| 4 | summer | | | | | | |
| 5 | winter | spring | | |  |  |  |
| 6 | winter | intermediate | | |  |  |  |
| 7 | winter | summer | | |  |  |  |
| 8 | winter | | spring | | |  |  |
| 9 | winter | | intermediate | | |  |  |
| 10 | winter | | summer | | |  |  |
| 11 | winter | | | spring | | |  |
| 12 | winter | | | intermediate | | |  |
| 13 | winter | | | summer | | |  |
| 14 | winter | | | | spring | | |
| 15 | winter | | | | intermediate | | |
| 16 | winter | | | | summer | | |

Seeds were checked every 2-3 days for the duration of the experiment. Germinants were tallied and removed, and DI water was added when necessary. Germination was defined as emergence of both a root and a leaf. Although no minimum length was specified, both root and leaf had to be visible and identifiable, which usually occurred when they were at least 1 mm long. Seeds that had only one or the other, or for which root and leaf were too small to be identified, remained in the dish until the next day that seeds were checked.

Each treatment group was TZ tested at the end of its incubation sequence to determine the quantity of viable seeds remaining. Seeds were cut longitudinally, through the embryo, and incubated in 1% TZ at 30-35C for 48 hours. Only one half of each seed was evaluated.

1. **Results**

Table 3 describes the final germination percentage of each accession, for each combination of winter stratification and germination temperature. Percentages were calculated by dividing the number of germinants by the total number of seeds tested.

Table 3. Final germination percentage for each combination of months of winter stratification and follow-up incubation temperature regime.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **VP-CAIN** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **TQ-CAIN** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **CATU** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

The amount of time it takes for germination to occur is a characteristic of the germination pattern that can be important for nursery personnel and restoration professionals to be aware of. This effect is described as germination rate, and was assessed by measuring the number of days it took to reach 50% of the final germination percentage, with day zero being the day seeds were moved out of winter stratification and into the germination incubator. For all of the controls, which never moved incubators, day zero is the day the experiment began. Germination rate is displayed in Table 4.

Table 4. Germination rate

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **VP-CAIN** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **TQ-CAIN** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **CATU** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

Uniformity describes the amount of time, in days, from the beginning to the end of active germination. Here, this was calculated by subtracting the number of days it took for the first germinant to be recorded from the number of days on which the last germinant was recorded. –or- Here, this was calculated by identifying the day on which 95% of final germination had occurred, and subtracting that by the day on which 5% of germination had occurred. This date range is meaningful to nursery personnel because seedlings of different ages will require different watering regimes, different amounts and composition of fertilizer, and different growing environments. Treatments which can narrow this rate range would increase efficiency and decrease the time and attention required to produce a crop. Results are displayed in Table 5.

Table . Uniformity

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **VP-CAIN** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **TQ-CAIN** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **CATU** | 0 | 1 | 2 | 3 | 4 | 7 |
| spring |  |  |  |  |  |  |
| intermediate |  |  |  |  |  |  |
| summer |  |  |  |  |  |  |
| winter |  |  |  |  |  |  |

Germination rate and uniformity are most significantly affected by winter stratification and germination temperature for CATU. These differences are visualized in the germination curves in Figure 1.

XXXX

Figure . Matrix of germination curves for CATU. Months 0-4. Include Winter? Change middle incubator to INT.

For both accessions of CAIN, germination rate and uniformity were \_\_\_ affected by either winter stratification or germination temperature.

For any germination study, it is useful to assess seed lot viability before and after a treatment. Initial viability as determined by TZ testing, and the highest germination percentage recorded for each accession are displayed in Table 4.

Table . Initial TZ results vs. highest germination percentage.

|  |  |  |
| --- | --- | --- |
| Accession | Seed lot viability | Highest germination percentage |
|  |  |  |
|  |  |  |
|  |  |  |

Did germination temperature affect seed viability? Look at controls only? Or group all lots with the same germ temp? Include winter control?

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Accession | SPR | INT | SUM | WIN |
| VP-CAIN |  |  |  |  |
| TQ-CAIN |  |  |  |  |
| CATU |  |  |  |  |

Did length of time in winter stratification affect seed viability? Look at SPR germ temp ones only? Or group all? Include winter control?

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Accession | 0 | 1 | 2 | 3 | 4 | 7 |
| VP-CAIN |  |  |  |  |  |  |
| TQ-CAIN |  |  |  |  |  |  |
| CATU |  |  |  |  |  |  |

It seems like it might make the most sense to look at these using statistical methods, since I’m interested in separating the two factors.

1. **Discussion**
   1. CAIN
      1. Highest germination percentage achieved in the absence of winter stratification
      2. Germination percentage is low in summer temps regardless of time in winter
      3. Germination rate and uniformity are poor overall
      4. Suggests CAIN germinates in fall, no dormancy
   2. CATU
      1. 40% germination achieved with 1-3 months of winter stratification. CATU seeds have a dormancy requirement.
      2. Germination percentage is low in summer temps regardless of time in winter
      3. Germ rate and uniformity are optimized with 2 months of winter stratification
         1. Explain why this matters
         2. Starts to get at vigor- Germination percentage doesn’t

tell the whole story…

* 1. Talk about TZ results?
  2. Testing these two elements helped me establish baseline propagation information for CAIN and CATU, but there was room for improvement…

Germination cues- signal an opportunity/disturbance- go into this in the smoke water section…

Possible cues

Can only be acted upon if seed is not dormant