Master's thesis

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Norwegian University of Science and Technology
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Department of Biology

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## Abstract:
In the wake of dwindling pollinator populations, we need to look for ways to keep the pollinators from disappearing. One way of doing this could be to restore landscapes that house plant-communities that experience pollen limitation. This occurs when a plant does not receive enough pollen to fertilize each ovule. For animal pollinated plants, this occurs when there are too few pollinators. We compared the degree of pollen limitation in the mainly bumblebee-pollinated species *Campanula rotundifolia* between an abandoned semi-natural grassland and an intact semi-natural grassland. In both sites, we compared seed production between hand-pollinated and open-pollinated flowers to test whether pollen limitation is more severe in the abandoned site. We show that the plants in the abandoned site are under heavy pollen limitation compared to the intact site, though these results should be considered with caution due to the limited sample size. We found no difference in the size of the seeds produced between the different treatments, suggesting that pollen competition has possibly little effect on seed quality.

## Sammendrag:

Populasjoner av pollinatorer forsvinner, og vi trenger nye mater å hindre dem i å forsvinne. En måte å gjøre dette på kan være å restaurere landskap med plantepopulasjoner som opplever pollen-begrensning. Dette skjer når en plante ikke mottar nok pollen til å befrukte hver eggcelle. For planter som pollineres av dyr forekommer dette når det er for få pollinatorer. Vi sammenlignet graden av pollen begrensning i den hovedsakelig humle-pollinerte arten *Campanula rotundifolia* mellom en forlatt semi-naturlig eng og en intakt semi-naturlig eng. I begge lokasjonene sammenlignet vi frøproduksjonen mellom håndpollinerte og åpen-pollinerte blomster for å teste om pollenbegrensningen var mer alvorlig i den forlatte engen. Vi viser at planter i den forlatte engen opplever kraftig pollen begrensning sammenlignet med den intakte engen, selv om resultatene burde vurderes med forsiktighet på grunn av den begrensede prøvestørrelsen. Vi fant ingen forskjell i størrelsen på frøene produsert i de ulike behandlingene, som tyder på at pollen konkurranse muligens har lite effekt på frø-kvalitet.

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## Introduction:

We are facing a pollinator crisis. Populations of pollinating insects all over the world are diminishing at a worrying pace (Marshman et. al, 2019, Obeso & Herrera, 2018). There are many reasons for this decline, including the use of pesticides, our use of monocultures, global climate change and habitat loss (Potts et al. 2010). It is estimated that between 70 and 85% of our agricultural plants are to some extent dependent on animal pollination (Kluser & Peduzzi, 2007). If nothing is done to stop the decline of pollinators, this crisis could have large economic implications, and could generate problems with feeding humanity. Studies are therefore being conducted around the world to find ways to improve the state of communities of pollinating insects, the plants they pollinate, and the ecosystems that favor pollinator biodiversity. (Kluser & Peduzzi, 2007).

Because habitat loss is a major contributor to pollinator declines (Brown and Paxton 2009; Winfree et al. 2009), habitat restoration has become an increasingly important method for augmenting the diversity of pollinating insects (Holl et.al, 2003). Many pollinator species have specific requirements that must be met for populations to be stable, and ecological restoration is a way of restoring destroyed or otherwise rare habitats back to the conditions that meet these requirements. The method has been used in a variety of landscapes, including wetlands (Holl et al, 2003) and forests (Lamb et al., 2012). Specifically, when it comes to pollinating insects, restoration of grasslands and semi-natural agricultural landscapes has been especially important in safeguarding pollinator diversity (Kremen & M'Gonigle, 2015, Rotches-Ribalta et al, 2018).

Semi-natural grasslands can be defined as a community of native grasses and herbs that have been created by regular grazing or mowing, but have not been artificially fertilized, plowed or exposed to the growing of commercial seeds (Hovstad et al. 2018; Bruinenberg et.al, 2002). These grasslands are important habitats for a wide range of pollinator species, but are threatened and disappearing from agricultural landscapes (Hovstad et al. 2018; Johansen et al. 2022). The main threat seems to be abandonment and change in use, and it is suggested that climate change could affect the rate of which semi-natural grasslands change once they are abandoned (Hovstad et al. 2018). A study in Sweden showed that restoring semi-natural pastures resulted in increased diversity in pollinating insects (Noreika et al. 2019). This was in large due to the high number of flowering plant species that preferred the kind of landscape conditions found within these semi-natural pastures. The study also suggests that the plant-pollinator network can be restored in relatively isolated areas within 10-20 years. (Noreika et al. 2019).

An increase in pollinator species diversity and abundance through restoration actions are expected to improve pollination and provide reproductive benefits to insect pollinated plants. The intensity of interactions between flowering plants and their pollinators (number of visits to the flower) can affect pollen limitation and the degree of pollen competition. Plants experience pollen limitation when there are too few pollinators to efficiently spread pollen among individuals from the population and provide sufficient pollen to the stigma. This decreases the number of ovules fertilized per plant, and thus the number of seeds produced. In contrast, when pollen load exceeds the amount necessary to fertilize all the ovules, plants may experience pollen competition. If the ability to fertilize an ovule is correlated with the genetic quality of the pollen, we expect the seeds produced under pollen competition to be of better quality (Hildesheim et al., 2019). In addition, as a plant is often visited several times by different pollen donors, the competition may increase, as pollen now comes from different individuals. This can generate selective pressures for traits that increase chances of successful fertilization, and on the pollen's ability to compete with other pollen, such as the time it takes the pollen to fertilize the flower (Pélabon et al., 2015; Lankinen et.al, 2016). Several factors can affect the degree to which plants experience pollen limitation or pollen competition, for example altitude (Maad et al., 2013) or, as we examine in this study, the biotype.

Animal pollinated plants often evolve floral traits that make them more appealing to their most common pollinator (Maad et al. 2013). Traits like flower size, number, scent and color, help to attract pollinators from a distance, while traits like nectar and resin are used as a reward for pollinators once they land on the flower. The shape of the flower may then influence the probability of the pollinator coming into contact with the parts of the flower that ensure pollen transfer to and from the pollinator (Armbruster, 2014). For *C. rotundifolia*, some of the most common pollinators are bumblebees (Maad et al. 2013; Fenster, et.al, 2004) When pollen limitation is high, we expect stronger selection for traits that attract pollinators such as petal size or flower number (Maad et al., 2013; Sletvold and Ågren 2014; Albertsen et al. 2020).

With a limited amount of resources available, organisms are often facing trade-offs among traits that can increase their fitness. A very common example is the example of size of offspring versus number of offspring (Smith & Fretwell, 1974). While both traits provide a benefit to the offspring, the limited amount of resources available to the mother means that the increase in one trait must come at the expense of the other, creating a trade-off. For plants, this translates to the trade-off between seed size and seed number (Paul-Victor and Turnbull, 2009). If plants produce small seeds, they often produce many at a time, with each individual seed having a small chance of survival and germination. Plants with big seeds give each seed a better chance of germination and survival, but generally produce fewer of them. While it is more common, and often easier, to examine this trade-off when comparing different species, studies have suggested that the seed size/number trade-off can vary within populations of the same species as well (e.g. Brancalion & Rodrigues , 2014).

Landscape-restoration is a technique that affects many species, but to better understand the impacts of restoration on the reproductive success of insect pollinated species, we need to investigate the species level effect. We have chosen *Campanula rotundifolia*, the common harebell, for this analysis. The species is insect pollinated, mainly by bumblebees, and is therefore sensitive to changes in the pollinator community (Grindeland, 2023). In addition, *C. rotundifolia* can produce many seeds at a time, over 100 per flower, and can therefore effectively show the difference between a community with or without pollen limitation. Finally, *C. rotundifolia* is self-incompatible, meaning it will not fertilize itself, and is therefore very sensitive to changes in the pollen community. While other studies earlier have examined the pollinator communities more closely (e.g Heggøy, 2021; Straume, 2022), we hope to shed a light on how improving the conditions for pollinators through restoration, will impact ecosystem functioning, i.e. pollination. The results of this study can then be compared to similar studies with other species to more clearly show the effects of restoring abandoned grasslands.

The goal of this study was to understand more about how the semi-natural grassland ecosystem functions, and how this functioning changes with the restoration process. By comparing one intact semi-natural grassland with an abandoned one, we hope to learn more about the interactions between pollinators and plants in this type of habitat, with *C. rotundifolia* as the example species. The data from this study will then be used in a multi-year restoration project to examine the effects of restoring an abandoned semi-natural grassland back into an intact one.

# Methods:

## Sites

The sites were selected based on several criteria. The abandoned site needed to be extensively managed in the past (grazed and/or mown, and not fertilized, plowed or sown), while at the same time being abandoned long enough for the plant species composition has become dominated with graminiods and species common in forested habitats (e.g. *Germanium sylvaticum, Anemone nemorosa and Oxalis acetosella).* In addition, the abandoned site needed to include some typical flowering plants usually found in semi-natural grasslands, such as *C. rotundifolia, Anthoxanthum odoratum and Stellaria graminea*. The intact site had to have similar environmental conditions (altitude, soil, etc.,). This site also had to have a long history of proper management in accordance with government regulations (Landbruksdirektoratet, 2021). This included the removal of hay using light weight machinery, no plowing, no fertilizing and no sowing of commercial seeds. In addition, the sites had to be located more than 1 km apart from each other. The site containing the abandoned semi-natural grassland selected for the project is at Steinkjer (63.934261, 11.436819, Figure 1) and the intact site is at Ingstadnes (63.443193, 11.202662, Figure 2). The first site is an abandoned semi-natural grassland that is in the later stages of succession and contains a large portion of forest plant species, trees and shrubs. The area is about 2500 $m^2$, and around 70 meters above sea level. The second site is on an intact semi-natural grassland next to a field, on an incline. The total area is about 3200 $m^2$, and 30 meters above sea level. There is no trees or large shrubs at this site, and the site is dominated by common semi-natural grasslands species. The flowers we measured were selected from individuals that were at least a couple meters inside the grassland, to minimize the chances of being impacted by other biomes or roads.

The fieldwork for this study has been performed between the 11$^{th}$ of July and the 13$^{th}$ of August in 2022.


*Figure 1: The abandoned site at steinkjer*


*Figure 2: The intact site at Ingstadnes*

### Plant species

*C. rotundifolia* is a perennial species of flowering plant (Stevens et. al 2012). It can be found across Norway in a variety of environments, but it thrives best in grasslands and grazing fields (Grindeland, 2023). *C. rotundifolia* have sequential hermaphroditical flowers, meaning the male organs (the anther) develops first and releases the pollen before the female organs (the stigma) develop and become receptive. (Stevens et. al 2012). This minimizes the chances of self-pollination, although harebells are mostly self-incompatible. Flowers are usually pollinated by bees, including some specialized species of bumblebee. Other pollinators, like hoverflies or flies, have also been observed visiting this species from time to time, although these visits have a smaller chance of resulting in pollination (Grindeland, 2023).

### Pollinator community.

The pollinator community in the two sites was also sampled in each site. This was done by making a 50 × 1 meter transect in both sites, at least two meters from the edge of the site to avoid edge effects. The transect was observed for two 15-minute periods with a 10-minute break in between. During these observations, every pollinator that was seen landing on a flower was recorded and placed in one of 5 categories: Bumblebee, solitary bee, fly, hoverfly and butterfly. This process was repeated twice per site. The transect in the abandoned site was observed on July 12$^{th}$ and August 12$^{th}$, whereas the transect in the intact grassland was observed on July 19$^{th}$ and August 13$^{th}$. We made sure these were days where *C. rotundifolia* were flowering, so that we knewThe weather conditions were similar on these days to avoid confounding factors from weather or temperature.

### Experimental design

In each site, individual plants were measured in pairs, where one flower on one plant was hand pollinated and one flower on the other plant remained open pollinated. The samples were selected from individuals that matched the following requirements: 1) each plant had to have a flower that was sexually mature in the female stage, 2) the paired individuals receiving different treatments had to be from different individuals but within 2 meters, and 3) if possible, each plant in the pair should have similar flower size (corolla width and tube diameter), number of flowers, height and flower stage. We tested for pollen limitation by comparing the total number of the seeds collected from each plant and examined pollen competition by comparing the individual weight of the seeds. Comparisons were made both between treatments and between sites.

## Measurements

Following Maad et al. (2013) we measured flower-height above ground (in cm), shoot length (in cm), number of flowers, number of fruits, number of buds, corolla width (CW) (in mm), tube diameter (TD) (in mm), non-orthogonal flower length (NOFL) (in mm), style length (in mm) and flower stage. Once collected, number of seeds and seed weight were also measured. The height of the flower above ground was measured by measuring the distance in cm from the base of the flower that was measured and a straight line down to the ground. Shoot length was the full length of the shoot from the ground up (not including the corolla). The shoot was carefully "stretched out" to get the full length. The number of flowers, buds and fruits were counted. CW was measured with electronic calipers by measuring the distance from the tip of one petal to a point on the opposite side of the flower, right between two petals (figure 3). TD was also measured with calipers as the diameter of the tube at the point where the petals start to split. NOFL was defined and measured as the distance between the tip of a petal and the base of the flower. As it would not be possible to fit a ruler into the flower, SL was measured by putting the tip of a zip-tag down into the flower and noting how far up from the tip on the zip-tag the style reached, and then measuring the distance from the tip of the zip-tag and up to that point. Flower stage was divided into two categories: 1, where anthers were opened and 2, where the style has split, and the stigma is exposed. In addition to these traits, the individual flowers that were measured were marked with zip-tags, and given a patch identity, flower identity and treatment. The zip-tags were marked with lines of different colors to mark this combined identity. A bamboo pole was also planted next to each sampled pair to help finding the flowers again for seed collection, and approximate distance and relative direction from the pole was noted. Finally, the flowers designated "hand pollinated" were pollinated using the style of two flowers from two separate plants, collected from the same site but from individuals at least 5 m away from the sample, to minimize chance of relatedness. After 4 weeks the fruits from the measured flowers were bagged to capture the released seeds when the fruit was mature. The bagged fruits were collected after 6-8 weeks to ensure that the seeds were fully developed. In addition, other fruits that grew on the sampled plants were also collected, even though the flower traits for these fruits were measured when they were flowers.

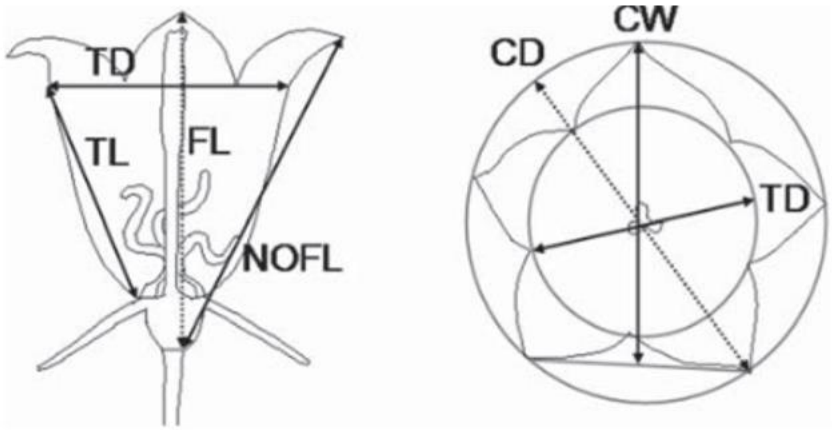


*Figure 3: Figure from Maad et. al, 2013, illustrating the different measurements taken of* C. rotundifolia*. CW=corolla width, TD=tube diameter, NOFL= non-orthogonal flower length. CD, TL and FL were not measured in this study.*

### Seed measurements

After the fruits were collected, they were taken to the lab. There, seeds were extracted, weighed, and photographed. When weighed, the seeds from each flower were placed in a petri dish and weighed to the nearest milligram. The seeds were then dispersed on a sheet of paper and photographed, using a ruler as a reference point (figure 4). A toothpick was used to gently move around seeds that were too close. This was to enable counting them using digital software. All the seeds from the collected measured flowers were counted. In addition, the seeds from some of the “extra” fruits that were collected were measured the same way. Of these, the seeds from all the extra fruits from the abandoned site, and the seeds from a random selection of extra fruits from the intact site (excluding individuals that were already represented 4 times), were counted.

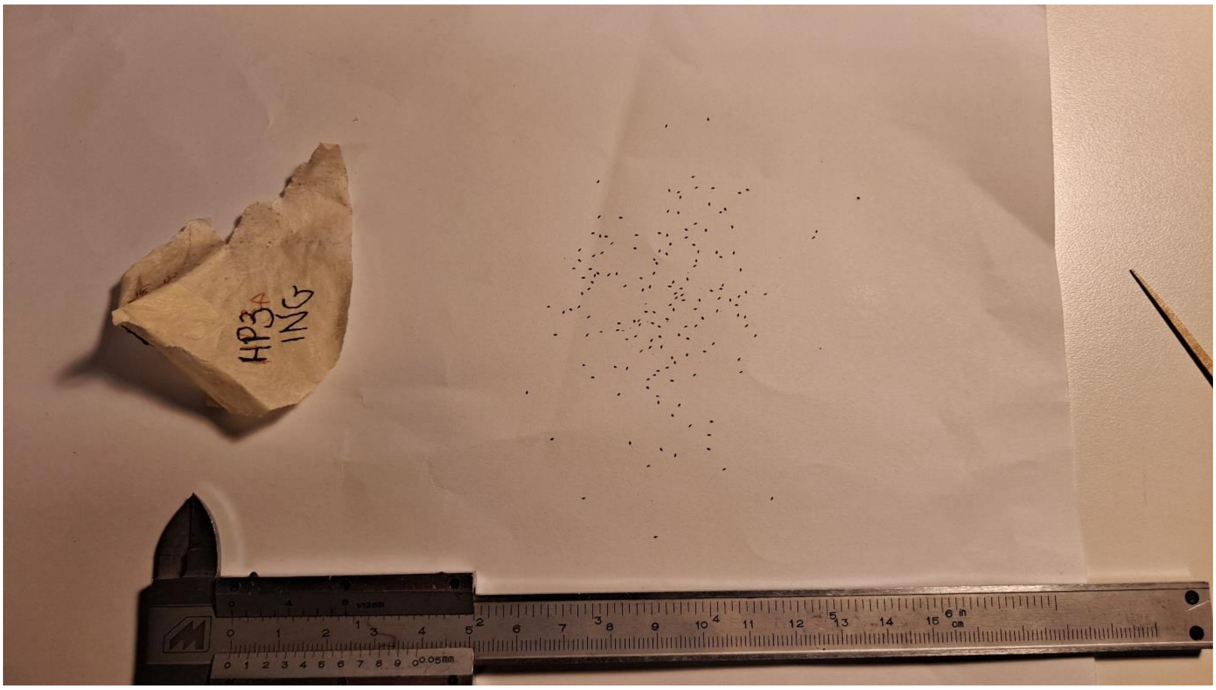


*Figure 4: An example of the seed-counting setup used for ImageJ. The note is used to identify which sample this is (in this case, the 3rd hand-pollinated from the intact site, Ingstadnes), and the calipers are used as a refference point for size.*

The software chosen to count the seeds was ImageJ. Photos were converted to 8-bit before the seeds were identified by the program (Rashid Al-Yahyai, 2020). Then, any particle from a size of 0.001 cm and more was counted. The size requirement was chosen to exclude other particles, like pieces of the fruit's shell that followed when the seeds were extracted.

## Statistical analyses

The data were analyzed in R 4.2.1. To start the analysis, I made a mixed effect linear model examining the effects of location and treatment on seed number. This was to test the hypothesis that the flowers in the open treatment in the abandoned site would be more pollen limited than the flowers in the open treatment in the intact site. If this was the case, we should observe an interaction between the two variables. To make sure this model was correct, I compared it to other similar mixed effect linear models (one without the interaction effect, one with each of the variables alone and one without any variables) using an Akaike information criterion (AIC). Each model also used patch as a random effect to account for the flowers in each treatment that were "paired up".

I tested whether the two populations differed in flower size using linear models where the response variables were the different floral traits, and the predictor variable was location. Each individual trait (CW, TD, NOFL, SL) received its own model. Patch was treated as a random factor. In these models, we only took into account the seeds from flowers we had measured the traits for, and not the seeds from the "extra" fruits. Furthermore, we tested whether there were differences in the number of flowers, buds and fruits produced depending on the location. For this test, 4 linear models were created, using fruits, buds, flowers and a combination of all of them as response, location as predictor variable and patch as random factor. Here as well, as the "extra" fruits were from the same plants as the original measured flowers, they were not included, so that each plant was only counted once. In addition, an outlier with many more buds than the rest (n=124) was removed from the model testing for number of buds. Finally, to test whether there was a trade-off between seed mass and seed number, a linear model was created using the average weight per seed as predictor, and the number of seeds as variable, as usual with patch as a random factor. In this model, three flowers produced seeds whose mass was below the precision of the balance were treated as outliers and removed.

## Results:

### Plants and pollinators:

*C, rotundifolia* were less abundant in the abandoned grassland than in the intact one, and we were only able to sample a total of 9 pairs (18 individual plants) in the abandoned site. This was all the plants that met the requirements we set beforehand. Unfortunately, when we collected the seeds, several fruits had been eaten by slugs, and so, only 10 fruits from the originally measured flowers were collected and measured (5 from the hand-pollinated treatment, and 5 from the open-pollinated treatment, 2 pairs). For the intact grassland, 20 pairs, (40 individual plants) were sampled, and all but 2 fruits were collected (not a pair). Additionally, 13 "extra" fruits from the abandoned site, and 19 from the intact site ended up being used in the study. These were added to the dataset as "open-pollinated flowers".

Plants sampled from the abandoned grassland had on average 1.3 (± 0.29) flowers, whereas plants from the intact grassland had an average of 2.20 (± 0.19) flowers at the time of the sampling. None of the plants from the abandoned site had fruits at the time of measurement, but they had on average 9.89 (± 3.72) buds. One plant had a much larger bud-count than the others (n=124), and if we remove this individual, the average number of buds becomes 3.17 (±0.44). The plants from the intact site had on average 0.55 (± 0.079) fruits and 0.9 (± 0.29 without outlier, ± 2.50 with outlier) buds at the time of measurement. All of these traits were statistically different between locations (flower number; $F_{1:56}=6.19$, $p=0.016$, bud number excluding outlier; $F_{1:55}=18.88$, $p>0.01$). However, adding together the fruits, flowers and buds for each location, the abandoned site had an average of 4.35 (±0.61) flowers, while the intact site had 3.65 (±0.40) flowers which is not significantly different ($F_{1:55}=0.94$, $p=0.34$). This means that although the plants in the two sets differ in the number of flowers, buds and fruits at the time of sampling, they did not differ in the total number of flowers produced.

When we compared floral traits between the two populations, we found that plants from the abandoned grassland had an average CW of 19.28 (±0.79) mm, while plants from the intact grassland had an average of CW of 19.02 (±0.51) mm. Plants from the abandoned site had an average TD of 11.19 (±0.34) mm, whereas plants from the intact site had an average TD of 12.46 (±0.23) mm. The average NOFL of the plants in the abandoned site is 22.43 (±0.53) mm, and in the intact site the average NOFL was 23.35 (±0.36) mm. Finally, the average SL for plants in the abandoned site was 14.29 (±0.55) mm, and for plants in the intact site the SL was 15.92 (±0.37). Plants from the abandoned site had significantly smaller TD ($F_{1:56}=9.45$, $p>0.01$) and SL ($F_{1:56}=6.20$, $p=0.016$) than the plants from the intact site. The CW ($F_{1:56}=0.079$, $p=0.78$) and NOFL ($F_{1:56}=2.05$, $p=0.16$) were not statistically different between the sites.

We observed far more pollinators at the intact grassland site than at the abandoned site, both in number and diversity (see table 1). The exception were flies which were more abundant in the first survey at the abandoned site than at the intact site. Solitary bees and bumblebees were only observed at the intact site, and the bumblebees were only observed in the first study. There were no observed butterflies.

*Table 1: Table showing the number of observations of the different pollinator groups in the different sites for each survey.*

| Pollinator | Abandoned 1 | Abandoned 2 | Intact 1 | Intact 2 |
|---|---|---|---|---|
| Hoverfly | 5 | 1 | 7 | 19 |
| Fly | 54 | 0 | 48 | 19 |
| Solitary bee | 0 | 0 | 1 | 4 |
| Bumblebee | 0 | 0 | 12 | 0 |
| Butterfly | 0 | 0 | 0 | 0 |

### Pollination experiment

We observed a large difference in number of seeds produced in the open-pollinated flowers from the respective sites. In the best-fitting model, the number of seeds produced in the open treatment in the abandoned grassland was statistically different from the other treatments and sites (difference in AIC from second best model=16.32, table A1). This model included both location and treatment as variables, and the interaction between them, indicating that the difference in seed number between hand-pollinated and open-pollinated flowers differed among sites (figure 5). Open-pollinated flowers from the abandoned grassland produced an average of 38.35 (±17.42) seeds per flower, whereas the flowers from the intact grassland produced on average 136.99 (±11.78) seeds per flower. In comparison, hand pollinated flowers produced on average 164.48 (±29.83) seeds per flower in the abandoned grassland, and 148.10 (±14.97) seeds per flower in the intact grassland.  The difference between the treatments in the intact grassland, as well as the difference between the hand-pollinated flowers from the different sites was not statistically significant.

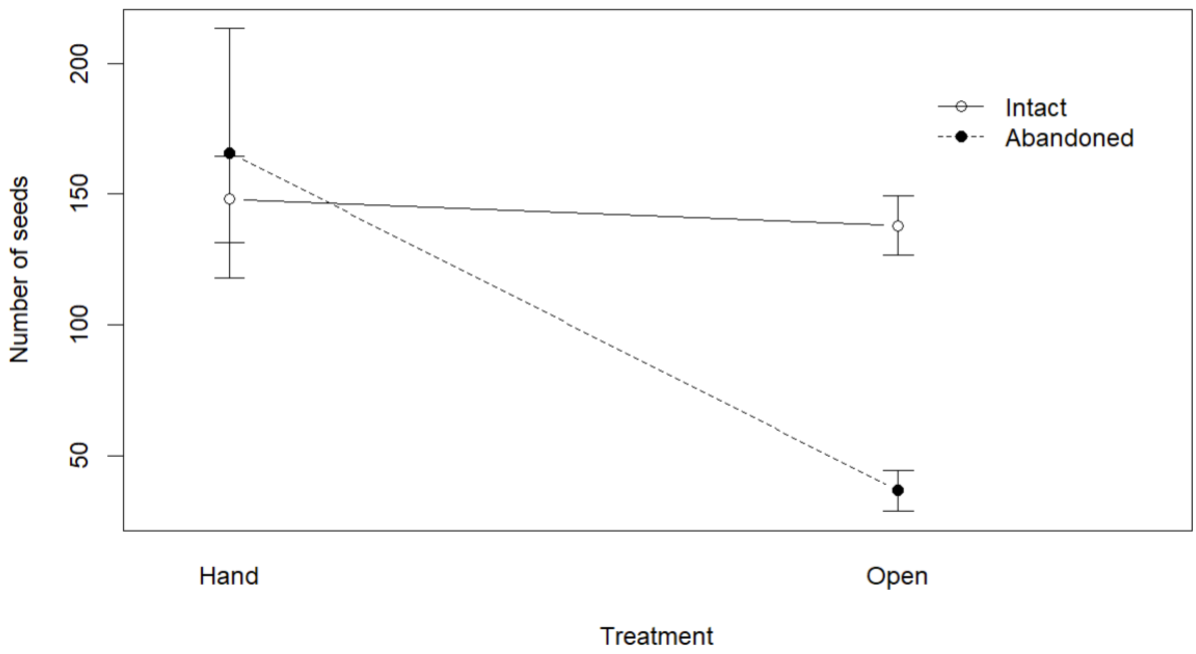


*Figure 5: Graph showing the average number of seeds in each treatment. The black dots show data from the abandoned site (Steinkjer) while the white spots show the data from the intact site (Ingstadnes)*

## Variation in seed size

We compared individual seed weights between sites and treatments to test whether there was a difference in seed quality among sites. We did not find any relationship between individual seed mass and seed number among flowers in either population ($F_{1:72}=0.088$, $p=0.77$) or treatment ($F_{1:72}=0.075$, $p=0.79$) (figure 6). Additionally, we did not find any significant difference in the quality of the seeds among sites. In the abandoned site, the average seed weight for the open-pollinated treatment was 0.054 (±0.0032) mg, and for the hand-pollinated it was 0.058 (±0.0067) mg. In the intact site, the average seed weight for the open-pollinated treatment was 0.056 (±0.0022) mg, and 0.056 (±0.0031) mg for the hand-pollinated treatment.

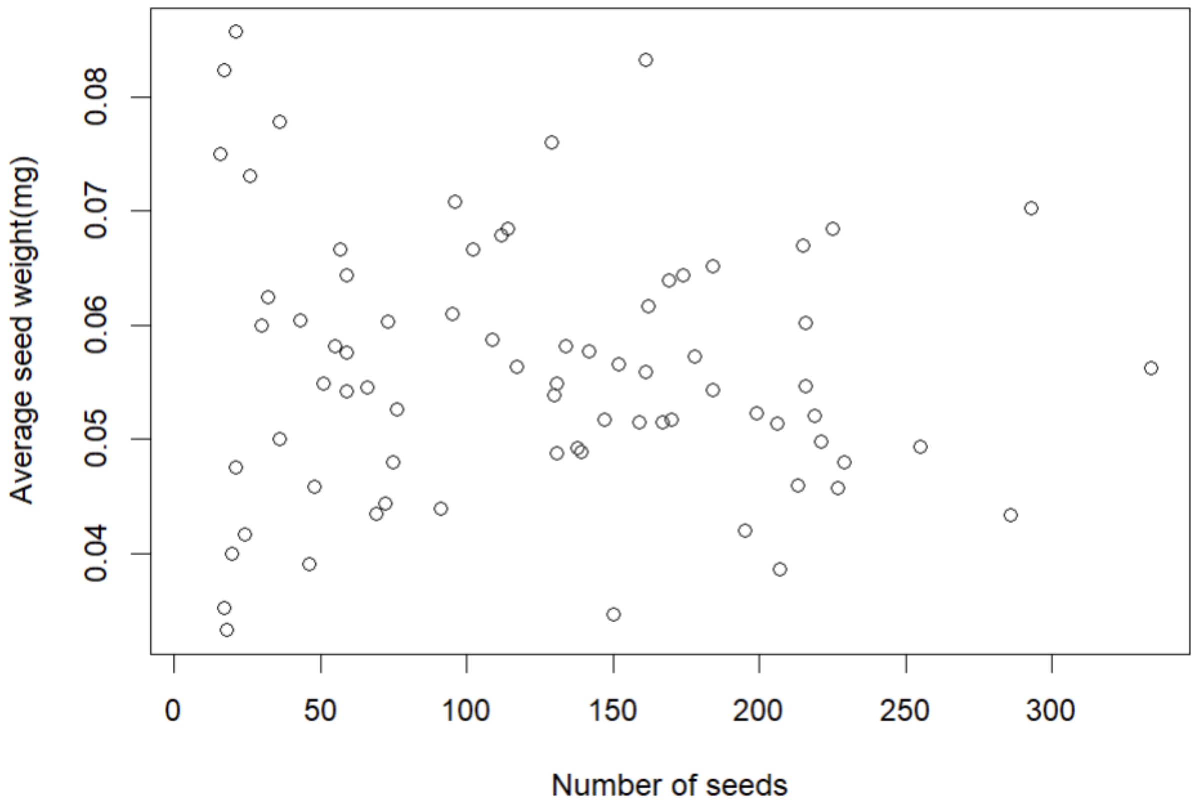


*Figure 6: Plot showing relationship between Number of seeds (x-axis) and weight per seed in g (y-axis). There is no significant interaction between the number and size of the seeds produced.*

## Discussion:

The goal of the study was to examine the pollinator-flower interaction of *C. rotundifolia* and it's pollinators by comparing an abandoned semi-natural grassland with an intact one. Flowers from the abandoned site in the open-pollination treatment produced significantly less seeds than flowers from the hand-pollinated treatment, and both treatments in the intact site. Additionally, there was no statistically significant difference in seed production between the flowers from the hand-pollinated treatments in either landscape. This suggests that the lower seed production is due to pollen limitation, and not another factor varying between the two landscapes. The results suggest that flowers from the abandoned grassland suffer from pollen limitation compared to the flowers from the intact grassland.

When it comes to the size and shape of the flower, the flowers from the intact grassland had significantly wider tube diameters and longer style lengths. This matches up with the pollinator data we found, as larger pollinators like bumblebees would require wider flowers to be able to enter, and longer styles to deposit the pollen. In addition, while not large enough to register as significantly different, the difference in non-orthogonal flower length should also be mentioned, as the averages from the abandoned and intact sites differed by over a millimeter, similar to the TD and SL. This also suggests an adaption to larger pollinators.

We did not detect significant trade-off between the number of seeds produced and the individual weight of the seeds. In other words, we did not find any indication that any pollen-abundance or competition resulted in higher seed quality as defined by the mass of the seed. This could suggest that *C. rotundifolia* prioritizes the number of seeds over the individual size, and when faced with pollen limitation, still allocates its resources to produce as many seeds as possible, rather than improving the odds of each seed. Other measures of seed quality that we did not test for could still be present, but this should be examined in another study.

This result also suggests that the intact site does not suffer from pollen competition. Because we found more pollinators in the intact site, we could assume that the chance of pollen competition is higher here. But as we could not find any difference in seed quality between treatments or sites, competition seems unlikely.

While the sampled plants in the intact grassland on average had more sexually mature flowers and fruits than the abandoned site at the time of measurement, the plants in the abandoned site had more buds. Combining these three counts showed there was no statistically significant difference in total flowers produced in the two sites. As we tried to choose plants similar to the ones in the abandoned site, this suggests we can rule out number of flowers as a cause for any of the effects we observed.

We obtained a crude evaluation of the population “fitness” by calculating the average individual reproductive output as the product of the average total number of flowers with the average number of seeds per flower from the open treatment. In the abandoned site, each individual produces an average of 144.96 seeds while individuals from the intact site produced an average of 500.01 seeds. This does not take into account predation however. This does however seem to coincide with the earlier results of this study, indicating that the abandoned site is more pollen limited than the intact site. Thus, individuals in the intact grassland produce almost 4-5 times more seeds than in the abandoned site.

One thing worth noting is the difference in sample size between the two sites. Not only was it difficult to find suitable flowers in the abandoned site, but many of the sampled flowers were eaten before their fruits could be collected. As mentioned earlier, the data does suggest that the difference in seed production is due to pollen limitation, and thus by extension, the difficulty in finding suitable samples from the abandoned grassland could be due to pollen limitation as well. Despite the low sample size, the results are a good indicator of the state of the population, as we sampled 100% of the population that was flowering at the time. Other causes for this difficulty could be the aforementioned predators that ate the fruits before they could be collected, less sunlight than the intact grassland, worse soil quality, or any other difference in the landscape that we did not consider.

While the data points towards pollen limitation for this experiment, further research is suggested to strengthen this conclusion and to examine to which degree this is correct for other semi-natural grasslands. The data in this thesis is from the first-year study about pollen availability in abandoned and intact semi-natural grasslands, and the experiment will thus continue with more data, both from the same sites we have examined in this study, as well as new sites in different parts of the country. Additionally, the abandoned site will go through a restoration process, and the data from this year will therefore prove useful when examining how this affects the pollen limitation. In addition to the continuation of this specific study, it would be beneficial for research on the subject if other research groups performed similar studies, either in different parts of the country, in other environments, or with another study species. In future research, comparing other sites than the two we have looked at will provide valuable data that can then be compared to our data to examine whether our findings coincides with semi-natural grasslands in general. It could also be interesting to look more closely at the pollinators themselves, and run a more in-depth observation or experiment on the pollinator community, to compare those results with those from this study.

## Conclusion:

The study seems to point towards the abandoned site being pollen limited in comparison to the intact site. Both *C. rotundifolia* and the pollinators themselves were less abundant in the abandoned site. Each flower in the open treatment produced significantly less seeds than the flowers in the hand pollinated treatment and the flowers in both treatments in the intact site. In addition, there did not seem to be a difference in seed quality, despite the difference in number. The size and shape of the flower also seemed to be more attractive to larger pollinators in the intact site. While it seems likely that the flowers in the abandoned site are pollen limited, there could be other factors that make them less successful than the flowers in the intact site. The plants in the abandoned site were more victim to predators, and the presence of taller plants and trees could result in less sunlight reaching the plant. It is also too soon to say that abandoned semi-natural grasslands in general are pollen limited, or that intact semi-natural grasslands are not pollen limited, as this study is small in scope. The data in this thesis could however be used as a basis for further research on the subject. Hopefully the results of this and similar study shows that semi-natural grasslands should be preserved to a better degree than they are now, as they seem to provide a good environment for many pollinators.

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# Appendix:

*Table A1: Table showing the results of the AIC test performed in R to find the factors that affect number of seeds produced. In all models, patch is treated as a random factor.*

| Model | Number of parameters | Relative AIC |
|---|---|---|
| Treatment*location | 6 | 0 |
| Treatment + location | 5 | 16.32 |
| Treatment | 4 | 32.55 |
| Location | 4 | 26.88 |
| Only random effects | 3 | 44.50 |

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