**The Evergreen State College**

**Graduate Program on the Environment**

### Thesis Prospectus

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**STUDENT AGREEMENT:**

**SIGNATURE: \_*Savannah Richard*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ DATE\_\_\_12/11/2020\_\_\_\_\_\_\_\_\_\_\_\_\_**

**FACULTY READER APPROVAL:**

**SIGNATURE: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ DATE\_\_**12/11/2020**\_\_\_\_\_**

**MES DIRECTOR APPROVAL:**

**SIGNATURE:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ DATE\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Provide the working title of your thesis.

The Impacts of Restoration Practices on Pollen Availability for Six South Puget Sound Prairie Plant Species

1. In 250 words or less, summarize the key background information needed to understand your research problem and question.

Due to plants immobile nature, sexual reproduction is reliant on pollen transference facilitated by abiotic and biotic factors. If plant populations are decreasing due to low reproductive success, it is possible that the distribution of pollen is being impacted. A reduction in pollen quantity or quality is referred to as pollen limitation (Ashman et al., 2004). The limitation of pollen can be caused by factors relating to ecological disruption or reproductive evolution. Ecological disruptions that can impact pollen include decreases in pollinator population or fragmentation of habitat. Plants that have evolved to be reproductively dependent on pollinators are intrinsically linked to their pollinator populations, especially specialist plant-pollinator associations (Knight et al., 2005).

The limitation of pollen can have negative implications for not only plant reproductive success but evolutionary impacts as well. Chronic pollen limitation can lead to plants selecting for self-pollination as a preferred reproductive strategy. This switch to self-pollination greatly reduces genetic complexity in plant populations and could potentially lead to plant communities being less evolutionarily adaptable. Although, plants that have evolved to be hermaphroditic or partially self-compatible have a much lower rate of pollen limitation. It is thought that these are reproductive strategies to avoid pollen limitation (Knight et al., 2005). Often times the ecological disruptions that lead to pollen limitation can be attributed to anthropogenic impacts on landscapes leading to habitat fragmentation or the reduction of pollinator communities.

1. State your research question(s).

Are South Puget Sound prairie plant species experiencing reduced reproductive success due to pollen limitation?

What if any restoration practices are associated with pollen limitation?

1. Situate your research problem within the relevant literature. What is the theoretical and/or practical framework of your research problem?

Pollen limitation research is often carried out using pollen supplementation studies based on multiple theories foundational to evolutionary biology. The optimality theory is based on Darwin’s theory of natural selection and is used in evolutionary biology modeling to determine fitness. Natural selection presumes that organisms are in constant competition to evolve adaptive traits or behaviors to maximize fitness. This maximization of fitness translates to the assumption that in an ideal environment, organisms will reproduce and function at an optimum rate. If this is not the case external pressures such as competition or environmental changes may be to blame (Parker and Smith 1990). For pollen limitation studies the optimality theory assumes that a plant existing in an optimal abiotic and biotic environment will not be any more reproductively successful when more pollen is introduced. If the plant has higher reproductive success when pollen is introduced this would indicate that an ecological disturbance is taking place which is limiting pollen (Knight et al. 2005).

Another important concept is the sexual selection theory. This theory states that female reproduction is limited by resources rather than access to males. This theory is more relevant to dioecious plants (separate male and female flowers) rather than monoecious or hermaphroditic plants capable of self-pollination. The assumption for dioecious plants is that male populations are not the limiting factor in reproductive success (Knight et al., 2005). If access to males is not the limiting factor in reproductive success, then we can assume other factors are responsible.

1. Explain the significance of this research problem. Why is this research important? What are the potential contributions of your work? How might your work advance scholarship?

If chronic pollen limitation is occurring in a plant species this can lead to reduction in quantity and quality of seed set and ultimately a decline in population. This can impact plant species diversity and associated insects and animal species that may rely on specific plants for nectar or larval hosts. Pollen limitation can also drive plant evolutionary patterns for reproductive strategies including changes in pollinator associations and self-compatibility. Pollen limitation can also be a symptom of environmental disruption such as habitat fragmentation or pollinator loss. These disruptions can be attributed to anthropogenic landscape changes and climate change impacts.

The historical range of South Puget Sound prairies ranged from Tacoma to Chehalis and was maintained through intermittent burning by Indigenous peoples. These prairie ecosystems have been reduced by 95% due to human development, shrub and tree encroachment, invasive plant colonization, grazing and a halt in maintenance through fire (Peter and Harrington 2014). Currently fragments of prairie exist in South Puget Sound and are actively managed through prescribed burns, invasive plant removal, shrub and tree removal and herbicide application. Native South Sound plant species are specially adapted to intermittent fire disturbances, but other ecological disturbances could greatly impact reproduction and establishment. The sites selected for this study are in varying stages of restoration and received different treatments. Sites that are less restored with more habitat fragmentation, invasive species and shrub encroachment are expected to have higher instances of pollen limitation among native prairie plant species. Restoration sites that have received regular prescribed burns, invasive and tree removal are though to have lower levels of pollen limitation due to environmental conditions matching evolutionary plant patterns.

The results from this study will contribute to South Puget Sound land managers knowledge of restoration practices and native plant reproduction and establishment. If pollen limitation is found to occur among certain plant species in specific conditions, future restoration practices can be changed to promote the growth and establishment of these plant species.

1. Summarize your study design[[1]](#footnote-1). If applicable, identify the key variables in your study. What is their relationship to each other? For example, which variables are you considering as independent (explanatory) and dependent (response)?

During the summer of 2020, six South Puget Sound prairie restoration sites managed by the Center for Natural Lands Management (CNLM) were selected for sampling. These sites included; Glacial Heritage, Cavness, Deschutes, Johnson Prairie, Tenalquot and Wolf Haven. For each restoration site six floral species were selected to sample; *Eriophyllum lanatum, Leucanthemum vulgare, Brodiaea coronaria, Hypericum perforatum, Plectritus congesta* and *Lupinus lepidus*. For the six plant species ten paired samples at each site were treated with hand pollination or left to be naturally pollinated. Measurements such as flower height, number of stalks and number of fruits will determine reproductive strategy and plant fitness. The seeds that were collected will be counted to quantify reproductive success based on seed set from the two treatment types.

The independent variable for this study is the pollination treatment (hand pollinated and naturally pollinated). Pollen limitation will be determined by reproductive success which will be measured by seed set quantity and viability. The dependent variables will be seed set and seed viability for each plant species for the two treatment types. The two treatments types will determine if pollen limitation is occurring for each species at each site.

1. Describe the data that will be the foundation of your thesis. Will you use existing data, or gather new data (or both)? Describe the process of acquiring or collecting data[[2]](#endnote-1).

I will be using data from an experiment conducted by Susan Waters during the summer of 2020. These data include plant fitness measurements such as species, plant height, number of fruits and fruit predation. Ten of each treatment type (hand pollinated and natural) for each of the six plant species at each restoration site. There is also restoration treatment data such as prescribed burns and herbicide applications, for the past 15 years.

Seeds were also collected for each plant species sample. I will count the seeds to quantify reproductive success and pollen limitation for each treatment type, site and plant species. I will also be germinating a subset of the seed to determine germination rates and the ratio of viable seed per seed set. I can do this using a germination chamber either at the Evergreen State College (TESC) laboratory or potentially at CNLM Shotwell’s Landing. Another option for determining seed viability would be to use TESC laboratory to create a tetrazolium stain solution. I will choose one of these methods to generate seed viability data that will determine sexual reproductive success along with seed set counts.

1. Summarize your methods of data analysis. If applicable, discuss specific techniques that you will use to understand the relationships between variables (e.g., interview coding, cost-benefit analysis, specific statistical analyses, spatial analysis) and the steps and tools (e.g., lab equipment, software) that you will take to complete your analyses.

For seed germination analysis I will either be using a germination chamber or create a tetrazolium stain in the TESC laboratory. For this I will need to contact the science support center and create a COVID safety plan. After my COVID safety plan has been approved I will be requesting lab space with a hood and chemicals to make the tetrazolium stain. The tetrazolium stain works by staining living tissue within the seed red after soaking for two days (França-Neto and Krzyzanowski, 2019). I can then count the stained red viable seeds and the non-stained unviable seeds to determine seed viability for each seed lot.

The primary metrics I will be using to quantify plant reproduction will be seed set and seed quality. I plan on comparing each of the six plant species individually across the six restoration sites. From this analysis I will be able to see if restoration site has a significant impact on pollen availability. I will then compare all six plant species reproduction rates against each other to test for significant variations among the co-flowering plant communities.

1. Address the ethical issues[[3]](#endnote-2) raised by your thesis work. Include issues such as risks to anyone involved in the research, as well as specific people or groups that might benefit from or be harmed by your thesis work, perhaps depending on your results. List any specific reviews you must complete first (e.g., Human Subjects Review or Animal Use Protocol Form).

The data collection for this study was conducted on historical indigenous land, previously inhabited by Chehalis, Squaxin and Nisqually tribes. Prairie ecosystems were historically managed by indigenous populations that regularly set fire to prairies and harvested fire adapted edible and medicinal plant species. The colonization by European settlers and displacement of indigenous tribal peoples caused the encroachment of forests and introduction of invasive plant species that now dominate previous prairie lands. These prairie lands are currently managed by the Center for Natural Lands Management and Joint Base Lewis-McCord. Current management strategies strive to replicate what indigenous populations traditionally did for thousands of years. The data I am using was not produced in concert with Indigenous tribal communities.

1. List specific research permits[[4]](#endnote-3) or permissions you need to obtain before you begin collecting data (e.g. landowner permissions, agency permits).

I will need permission from Susan Waters to use all relevant site and sample data as well as seeds collected. Because the field collection was already conducted, I do not need site permissions.

1. Reflect on how your positionality as a researcher could affect your results and how you will account for this in the research process[[5]](#endnote-4).

I identify as female in a male dominated scientific field. This is a common dynamic for female identifying individuals in STEM fields, which means I will be subject to implicit bias throughout my future career. I am fortunate enough to have female mentors in the biological sciences to advise and guide me along this process. In an attempt to balance the inequities in my field of study I will seek out female authored scientific research papers.

1. Provide at least a rough estimate of the costs associated with conducting your research.  Provide details about each budget item so that the breakdown of the final cost is clear.

If I choose to perform a seed viability test using tetrazolium, I can create this stain with 2,3,5-Triphenyl tetrazolium chloride and distilled water. 2,3,5-Triphenyl tetrazolium chloride is prices at $25.00 per 5 grams. Although I will not be able to calculate how much 2,3,5-Triphenyl tetrazolium chloride is needed until I know the total seed quantity to be tested.

1. Provide a detailed working outline of your thesis.
2. Introduction
   1. Thesis statement: Pollen limitation can lead to serious reproductive impacts, but the impacts and causes of pollen limitation cannot be generalized across plant communities and vary according to plant species life history and reproductive strategy.
3. Background Information: The Journey of Pollen
   1. Pollen availability and distribution
      1. Anderson and Minnaar 2020
   2. Pollen impact on plant reproduction
      1. Haig 1988
   3. Competition and facilitation
      1. Tur et al., 2016
      2. Delph 2019
   4. Conspecific and heterospecific pollen
      1. Arceo Gomez et al., 2016
   5. Floral evolution in response to pollinators
      1. Specialist and generalist
      2. Floral morphology
      3. Fenster et al., 2004
   6. The evolution of self-compatibility in dioecious plants
      1. Knight et al., 2004
      2. Ashman et al., 2005
4. Theoretical Framework
   1. Optimality theory
      1. Knight et al., 2004
      2. Ashman et al., 2005
   2. Sexual Selection Theory
      1. Knight et al., 2004
   3. Bateman’s Principle of Plant Reproduction
      1. Burd 1994
5. Pollen limitation and supplementation studies
   1. Quantifying plant reproductive success
      1. Using more measures than just seed set
   2. Pollen supplementation timing and frequency impact on reproduction success
      1. Ishii et al., 2001
   3. Over ovule production as a hedge betting strategy for a stochastic environment
      1. Knight et al., 2004
6. Implications of Pollen Limitation and Ecological causes
   1. Ecological and evolutionary impacts of pollen limitation
   2. Potential causes of pollen limitation
      1. Ecological disturbances, climate change
      2. Anthropogenic disturbances, habitat fragmentation
      3. Pollinator population decline
   3. Restoration solutions
7. Conclusion
8. Provide a specific work plan and a timeline for each of the major tasks in the work plan. Be as realistic as you can, even though you will probably need to alter this schedule as you complete the tasks. Remember that faculty readers take time to return your drafts and that the final polishing and formatting of your thesis for binding will take longer than you ever imagined.

![Timeline, bar chart

Description automatically generated]()

1. Who, beyond your MES faculty reader, will support your thesis? Indicate support both within and outside of Evergreen. Be specific about who they are and in what capacity they will support your thesis. If you are working with an outside agency or expert, be specific about their expectations for your data analysis or publication of results.

I am currently working with Sarah Hamman, who is my MES thesis reader as well as an Ecologist at the EcoStudies Institute. I’m also working with Susan Waters a pollination ecologist with Quamash EcoResearch. I previously worked with Susan Waters for pollen ecology internship for Fall 2019 and Winter 2020 quarters. Susan has the capacity to share previously collected data and collected seeds with me. She will also be available to assist me with statistical analysis. I will be sharing all results and data analysis from the project with Susan Waters.

1. List the 3-5 most important references you have used to identify the specific questions and context of your topic, help with issues of research design and analysis, and/or provide a basis for interpretation. For each annotated reference, explain how your project specifically connects to the source by extending, challenging, or responding to the conclusions, methods, or implications. For any other sources cited in this document provide a complete bibliographic citation.

Ashman, T.-L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., & Wilson, W. G. (2004). POLLEN LIMITATION OF PLANT REPRODUCTION: ECOLOGICAL AND EVOLUTIONARY CAUSES AND CONSEQUENCES. *Ecology*, *85*(9), 2408–2421. <https://doi.org/10.1890/03-8024>

These researchers analyze the current methods, process and theoretical framework used in pollen

limitation studies using pollen supplication. The main purpose of this research article is to determine if

current methods of analyzing seed set are an adequate measurement of pollen limitation. This study

concludes that site specific environmental factors and plant life history are important components in

determining pollen limitation. They also suggest that more research is needed on the plant community

level rather than the plant species level.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolution, and Systematics*, *35*(1), 375–403. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132347>

This paper reviews research regarding the determination of floral syndromes (specialized morphology)

is evolutionarily influenced by specialized pollinators. Researchers mention that specialist pollinator

plant relationships are more prone to pollen limitation when compared to generalist relationships.

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen Limitation of Plant Reproduction: Pattern and Process. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 467–497. <https://doi.org/10.1146/annurev.ecolsys.36.102403.115320>

This paper attempts to review theoretical framework of pollen limitation studies while situating pollen

limitation in a broad evolutionary context. Researchers propose that pollen supplication studies often

reveal pollen limitation for evolutionary reasons. They suggest that this could be due to pollen

limitation as an evolutionary strategy to adapt to a changing environment. This would prevent plants

from over producing flowers and fruits during times of limited pollination.

Work Cited

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1. [↑](#footnote-ref-1)
2. [↑](#endnote-ref-1)
3. [↑](#endnote-ref-2)
4. [↑](#endnote-ref-3)
5. [↑](#endnote-ref-4)