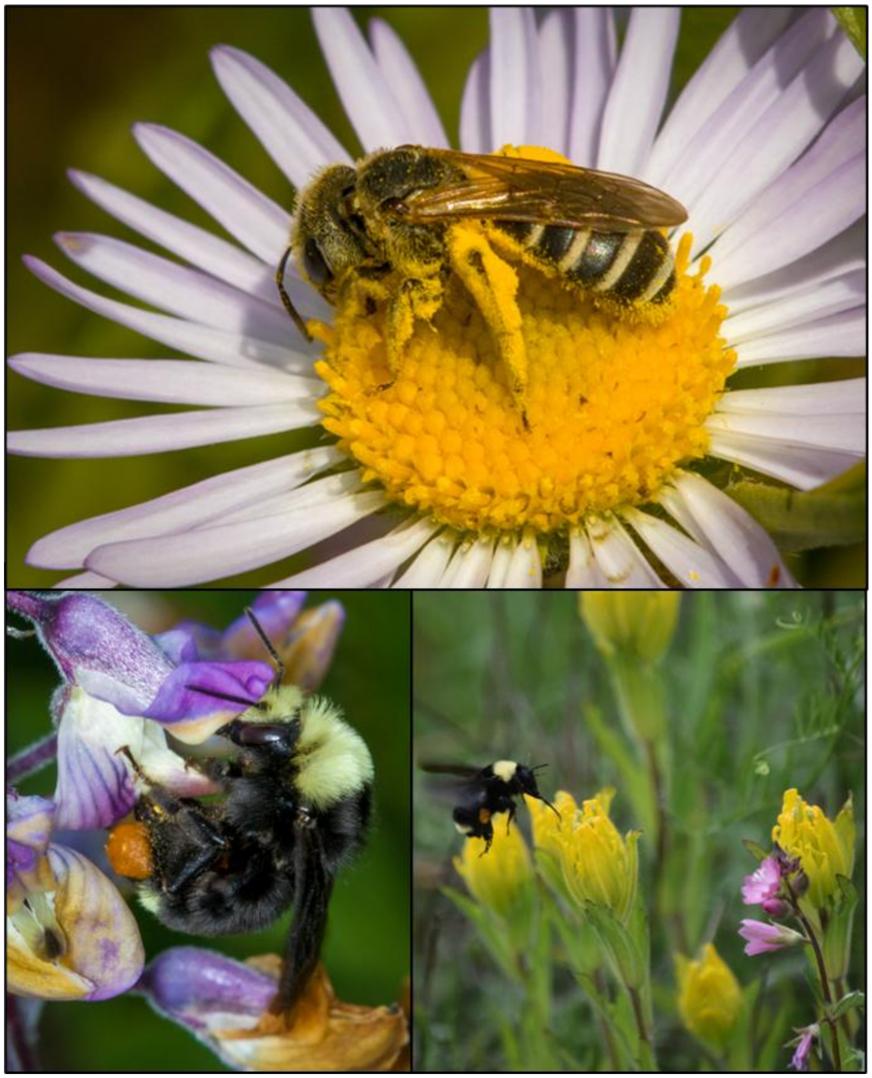
Pollination biology and ecology of Willamette Valley prairies and rare plant species



March 2025

Progress Report to the US Army Corps of Engineers, Portland District

Report prepared by J. Christina Mitchell, Tom Kaye, and Scott Harris

Institute for Applied Ecology

PREFACE

IAE is a non-profit organization whose mission is the conservation of native ecosystems through restoration, research, and education. IAE provides services to public and private agencies and individuals through development and communication of information on ecosystems, species, and effective management strategies. Restoration of habitats, with a concentration on rare and invasive species, is a primary focus. IAE conducts its work through partnerships with a diverse group of agencies, organizations, and the private sector. IAE aims to link its community with native habitats through education and outreach.

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Cover photographs: Top: Sweat bee (*Halictus sp.*) on Willamette daisy (*Erigeron decumbens*). Bottom left: Bumble bee (*Bombus vosnesenskii*) on Kincaid's lupine (*Lupinus oreganus*). Bottom right: Bumble bee (*Bombus californicus*) on golden paintbrush (*Castilleja levisecta*). Photographs by David Cappaert (top and bottom left) and Owen Cass (bottom right). All other pictures taken by the authors and IAE staff.

SUGGESTED CITATION

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EXECUTIVE SUMMARY

Global losses in biodiversity highlight the diverse, and often dependent connections between species. The conservation of threatened and endangered plants must also consider management of associated plant and arthropod communities, including pollinators. To better understand the role of pollinator communities for the conservation of rare plant species, we conducted a 5-year study of endangered Willamette daisy (*Erigeron decumbens*) and began similar studies of recently delisted golden paintbrush (*Castilleja levisecta*) and threatened Kincaid's lupine (*Lupinus oreganus*), all perennial forbs endemic to the Willamette Valley, Oregon.

Between 2019 and 2024, the Institute for Applied Ecology (IAE) partnered with the US Army Corps of Engineers (USACE) to better understand Willamette daisy pollination biology and Willamette Valley pollinator ecology. Our results showed that Willamette daisy is dependent on pollinators to persist, and that Willamette daisy populations would be more successful with increased pollination services. We continued this research to expand our knowledge of pollinator biology and pollinator ecology on golden paintbrush and Kincaid's lupine. In 2024, we used methods trialed in 2023 to conduct a pollen supplementation experiment on golden paintbrush and Kincaid's lupine at four sites across the Willamette Valley. This research will help to understand the effects of pollinators on golden paintbrush and Kincaid's lupine seed set and with corresponding field observations, understand the role of insect pollinators and other flowering prairie plants on golden paintbrush and Kincaid's lupine population growth and survival. After one year of sampling our results demonstrate a different pollinator community, dominated by two different bumble bee species, visit golden paintbrush and Kincaid's lupine compared to Willamette daisy. We also saw varying responses of seed set to pollen supplementation efforts and increased surrounding floral density. Plant-pollinator networks varied by site and indicate that insects visiting these rare plants are members of pollinator communities that rely on a wide range of co-occurring plant and insect species. Conservation of rare plant populations hinges on supporting a thriving and diverse network of insect pollinators and flowering plants in the prairie ecosystem.

1. INTRODUCTION

Conservation of endangered plants relies on successful habitat management, which includes the protection of pollinators that facilitate sexual reproduction of most forbs. Understanding relationships between plants and pollinators may provide key insight to recovering threatened and endangered species. Through experimental approaches, the degree to which a plant may rely on arthropods for pollination, and ultimately fertilization, can be determined. Through observational studies and other techniques, important pollinator species can be identified, and environmental factors associated with those populations can be considered in management decisions. With this knowledge, land managers can address the broader ecological determinants of species recovery.

Golden paintbrush (*Castilleja levisecta*), Kincaid's lupine (*Lupinus oreganus*), and Willamette daisy (*Erigeron decumbens*) are endemic species to the Willamette Valley in Oregon (Figure 1). Golden paintbrush was federally delisted as threatened in 2023 but remains Oregon state listed as endangered (USFWS 2023). Kincaid's lupine and Willamette daisy are listed by the US Fish and Wildlife Service and Oregon Department of Agriculture as threatened and endangered species, respectively (USFWS 2016). In previous research, we have observed that very small populations of Willamette daisy produce few seeds, which is a serious concern for the conservation of this species (Thorpe and Kaye 2011) and suggests pollinator conservation could be crucial for population viability. Observations of pollinators on Willamette daisy suggest a diversity of insects are visitors, including beetles, butterflies, flies, and native bees (Jackson 1996, Mitchell et al. 2023). Evidence suggests insects contribute to Willamette daisy reproduction, and that they in turn depend on well-managed prairie habitat to support their full, and varied, life cycles (Wojcik et al. 2018, Mitchell et al. 2024).

Figure 1. Willamette Valley endemic plants: golden paintbrush, Kincaid's lupine, and Willamette daisy.

As rare plants of sparse distribution, golden paintbrush, Kincaid's lupine, and Willamette daisy face two factors that might limit effective pollination: 1) pollen delivery may be low, because pollinator abundance is often less in small habitat fragments; and 2) 'stigma contamination'. Stigma contamination can occur if few individuals of the target plant species are dispersed among abundant other species, and generalist pollinators that carry multiple pollen species block adhesion of golden paintbrush, Kincaid's lupine, or Willamette daisy pollen to stigmas with an alternative species' pollen grain. Understanding which pollinators are most important to these rare plant species, with the goal of improving reproduction, is key to managing these threatened and endangered populations.

To address these concerns, IAE partnered with the US Army Corps of Engineers (USACE), Portland District to better understand the breeding biology and pollination ecology of golden paintbrush, Kincaid's lupine, and Willamette daisy in remnant and restored prairies throughout the Willamette Valley. In 2024, we conducted field work at Ankeny and Finley National Wildlife Refuges (US Fish and Wildlife Service; USFWS), Big Spires, Dorena East Wildlife Area, and Fisher Butte (USACE), Greenhill (BLM), and Herbert Farm Natural Area (City of Corvallis; Figure 2). Between 2019 and 2023, we also conducted work at Calapooia and Jefferson Farm (private), Kingston Prairie (Greenbelt Land Trust), and Speedway and Oxbow West (BLM).

This report describes research activities focused on golden paintbrush, Kincaid's lupine, and Willamette daisy and plant-pollinator networks in multiple prairies throughout the Willamette Valley. Using the successful field and statistical methods we developed for the Willamette daisy studies, we trialed field protocols in 2023 (Mitchell et al. 2024) and conducted pollen supplementation experiments and pollinator surveys in 2024 to address the same research questions for golden paintbrush and Kincaid's lupine. Specific details of golden paintbrush and Kincaid's lupine pollination ecology and pollinator communities are not well known. Both species are often visited by bumble bee species, and we expect this genus (*Bombus sp.*) plays a large role in these species' pollination.

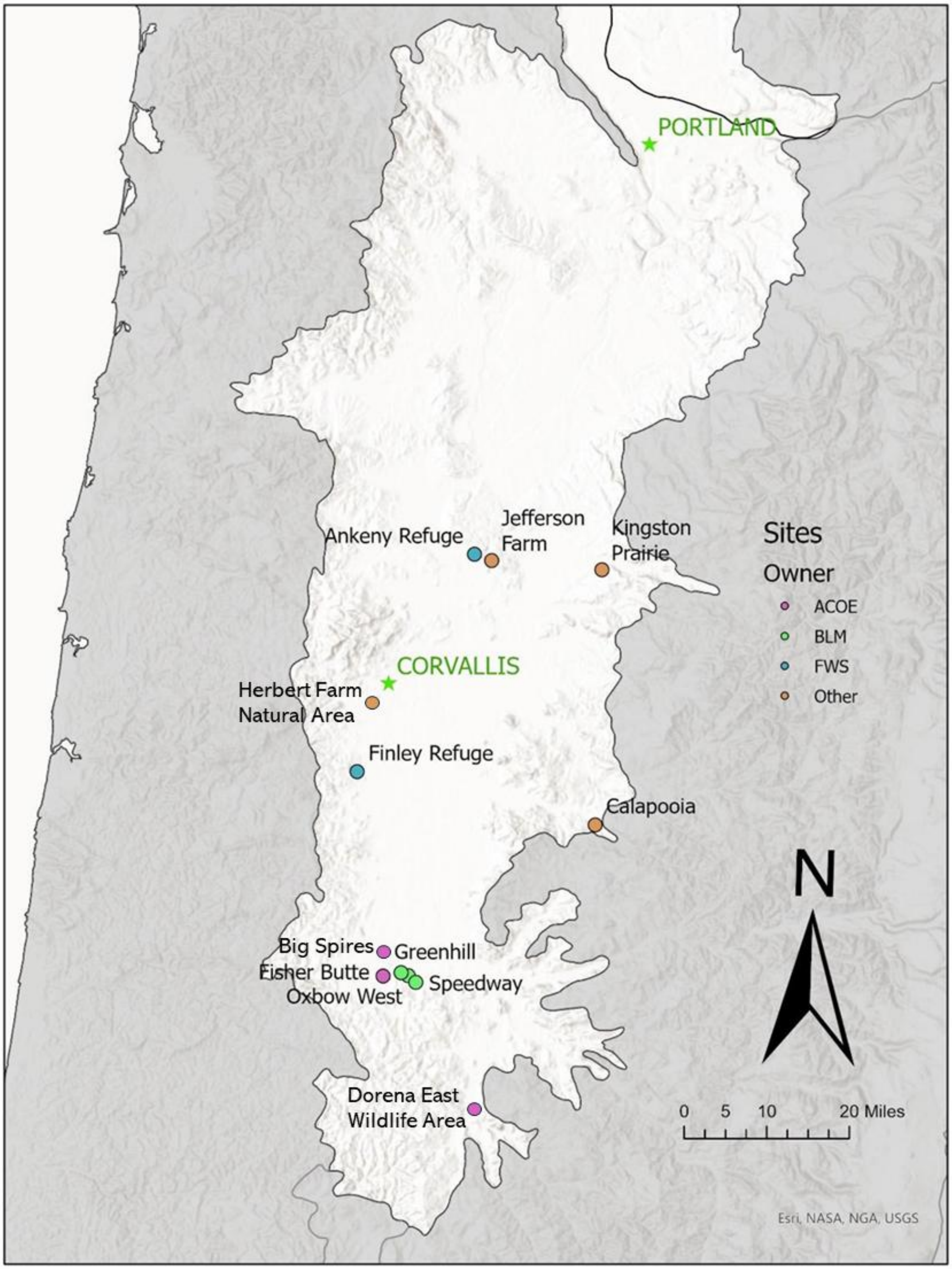

Figure 2. Location of remnant and restored prairie sites used for pollinator research between 2019 and 2024, across the Willamette Valley Ecoregion, Oregon, USA. USFWS is abbreviated as FWS in the figure legend.

2. GOALS AND OBJECTIVES

The goals of this project are to better understand the pollinator and associated plant community in Willamette Valley prairies, specifically including the pollination ecology of rare plants like golden paintbrush, Kincaid's lupine, and Willamette daisy.

Specific objectives include:

1) Use insights from Willamette daisy field trials to conduct pollen supplementation experiments and accompanying pollinator surveys for golden paintbrush and Kincaid's lupine;

2) Extensively sample the Willamette Valley prairie pollinator community from plants flowering throughout the season; and

3) Analyze collected data to assess variation in prairie plant-pollinator networks across space and time.

3. METHODS

3.1. Project Activities

We have performed research at 12 sites since this project began in 2019, and at seven sites in 2024 (Table 1, Mitchell et al. 2024). In 2024, we collected data from Ankeny National Wildlife Refuge, Big Spires, Dorena East Wildlife Area, Finley National Wildlife Refuge, Fisher Butte, Greenhill, and Herbert Farm Natural Area. We presented this research at regional conferences and are compiling a subset of our insect collection to submit for further genetic testing and another for submission to the Oregon State Arthropod Collection (OSAC). We continue to refine our collection determinations through improved identification resources for our region via collaborations with David Cappaert, OSAC, and the Oregon Bee Atlas.

Table 1. Willamette Valley pollinator study sites and select research activities. Previous reports describe additional sites and activities prior to 2024.

		Golden paintbrush pollen supplementation		Kincaid's lupine pollen supplementation		Golden paintbrush hand-netting		Kincaid's lupine hand-netting		Prairie plant/pollinator network sampling					
Site	**Owner**	**2023**	**2024**	**2023**	**2024**	**2023**	**2024**	**2023**	**2024**	**2019**	**2020**	**2021**	**2022**	**2023**	**2024**
Ankeny National Wildlife Refuge	USFWS		X				X				X[2]	X	X	X	X
Big Spires	USACE		X		X		X		X						
Dorena East Wildlife Area	USACE													X	X
Finley National Wildlife Refuge	USFWS		X		X		X		X		X[2]	X	X	X	X
Fisher Butte	USACE									X[1]	X[1]	X	X	X	X
Greenhill	BLM											X	X	X	X
Herbert Farm Natural Area	City of Corvallis	X	X	X	X		X		X						

X[1] indicates data were collected and funded jointly by the Center for Natural Lands Management and IAE (Waters 2021).
X[2] indicates data were collected and funded jointly by Quamash EcoResearch and IAE.

3.2. Pollen Supplementation Experiments

<u>Pollen Supplementation Experiments</u>

We used previously described methods designed for Willamette daisy (Mitchell et al. 2023) to develop and trial methods to conduct similar pollen supplementation experiments with golden paintbrush and Kincaid's lupine (Mitchell et al. 2024). In 2024, we expanded on previous efforts and conducted pollen supplementation experiments and associated pollinator observations for golden paintbrush at Ankeny National Wildlife Refuge, Big Spires, Finley National Wildlife Refuge, and Herbert Farm Natural Area, and for Kincaid's lupine at Big Spires, Finley National Wildlife Refuge, and Herbert Farm Natural Area.

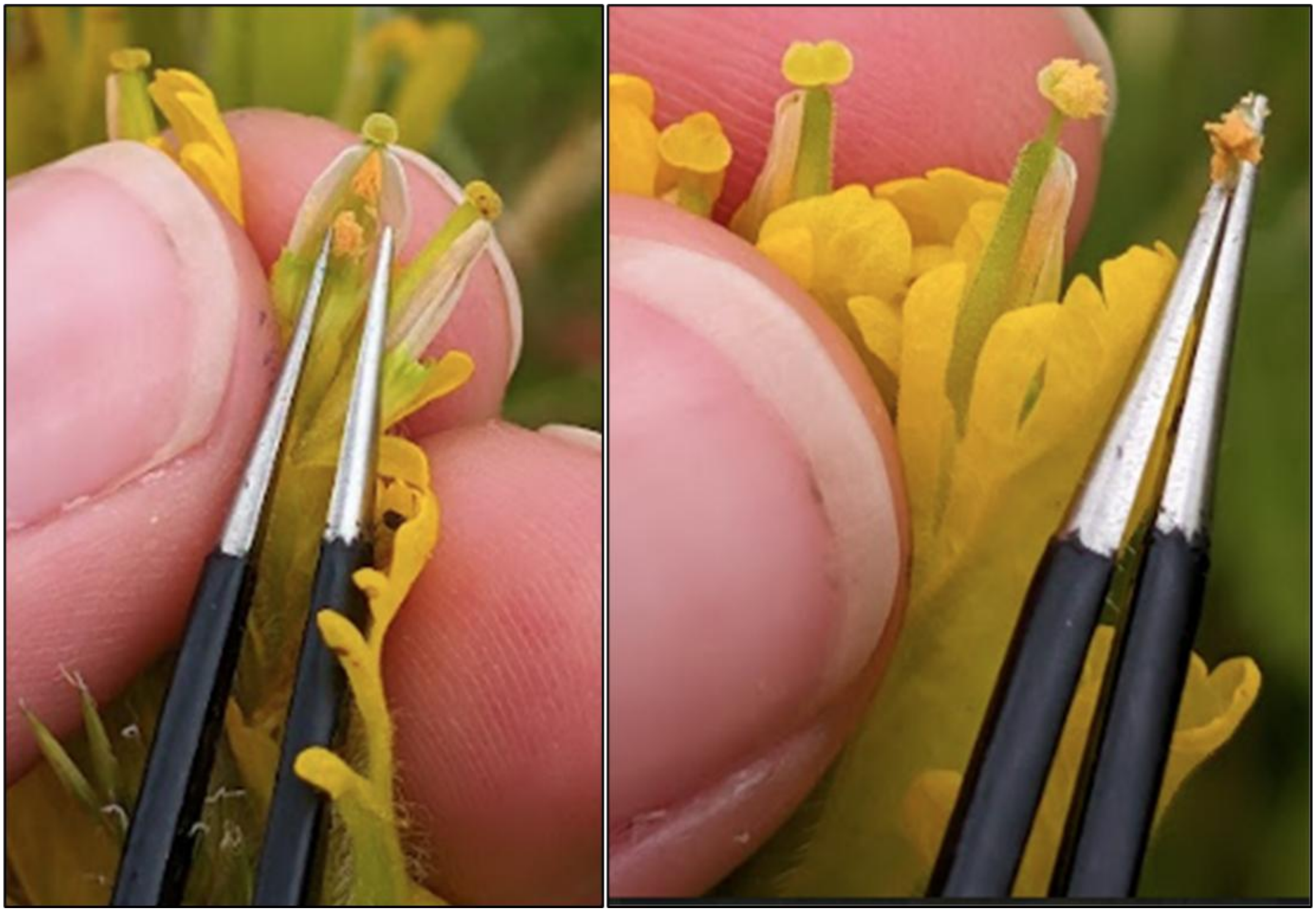

Figure 3. Example of pollen supplementation on golden paintbrush flower; collecting dehiscing anthers from one flower and moving them to touch the receptive stigmas on the experimental flower.

For golden paintbrush, we marked the start of the pollen supplementation trial on each experimental flowering stalk by tying a pipe cleaner below the lowest (oldest) flower with receptive stigmas. The control stalks were not manipulated and left open to natural pollination. The treatment stalks were manipulated to supplement naturally occurring pollination. This process was completed by removing dehiscing anthers from separate plants and rubbing the available pollen on receptive stigmas on the treatment stalks (Figure 3). On each day of supplementation, this process was repeated for all open flowers along the flowering stalk. This supplementation process was repeated every two to three days for a total of five to eight supplementation events per site. Experimental plants at Herbert Farm Natural Area were set up a bit later than other sites and therefore had less supplementation events because the water level of Muddy Creek was too high to cross into the west field early in the Spring. At the end of the supplementation trial, another pipe cleaner was tied above the last manipulated flower and all stalks were collected once seed capsules were mature (on July 10th 2024). All seeds from the seed capsules between the pipe cleaner markers were counted and categorized as either 'filled' or 'unfilled' by visual inspection with a dissecting scope.

Figure 4. Example of pollen supplementation on Kincaid's lupine flower; collecting pollen from dehiscing anthers inside the keel of one flower and touching it to the receptive stigmas of the experimental flower.

For Kincaid's lupine, we marked the start of the pollen supplementation trial on each experimental flowering stalk by tying a pipe cleaner below the lowest (oldest) flower with receptive stigmas. The control stalks were not manipulated and left open to natural pollination. The treatment stalks were manipulated to supplement naturally occurring pollination. This process was completed by exuding pollen out of the keel of flowers on a separate plant and collecting the pollen on the back of a black spoon (Figure 4). That pollen was then brought to the experimental flower and touched to the receptive stigma, which was exposed by actuating the 'piston' mechanism of the flower. On each day of supplementation, this process was repeated for all open flowers along the flowering stalk. This supplementation process was repeated every two to three days for a total of six supplementation events per site. At the end of the supplementation trial, another pipe cleaner was tied above the last manipulated flower and any unopened flowers above this point were broken off. Experimental flowering stalks were covered with breathable fabric and secured below the marked flowers to prevent dispersal loss of seeds; all stalks were collected once seed pods were mature (on July 9th or 10th 2024). All seeds from the seed pods between the pipe cleaner markers were counted and categorized as either 'filled' or 'unfilled' by visual inspection with a dissecting scope.

Surrounding Floral Density

Similar to our work with Willamette daisy (Mitchell et al. 2023), we quantified the surrounding floral density to assess whether baseline pollination was density dependent. For golden paintbrush, we measured 4-m outwards from the center of each experimental plant to create a 4-m radius circular sampling area and counted all golden paintbrush flowering stalks within that circle. We followed the same protocol for the Kincaid's lupine experimental plants, counting all Kincaid's lupine racemes within a 4-m radius circular sampling area around each experimental plant.

Analyses
We tested for an effect of pollen supplementation on seed set for golden paintbrush and Kincaid's lupine across all sites sampled using linear mixed-model regression with site specified as a random effect (R Core Team 2024). For each species and site, we assessed the change in seed set with paired t-tests. We also determined whether the density of surrounding golden paintbrush or Kincaid's lupine flowers influenced seed set. We correlated the number of filled seeds, from supplemented and non-supplemented flowers, with surrounding golden paintbrush or Kincaid's lupine floral density. We expected that increased floral density would increase seed set in naturally pollinated 'control' flowers as well as supplemented 'experimental' flowers. This expectation assumes that increased floral density would increase pollinator visitation to that patch.

3.3. Prairie Plant and Pollinator Community Sampling

Plant-pollinator Networks
In 2024, we continued efforts to quantify the prairie pollinator community using plant-pollinator network surveys (Figure 5; Waters 2021), as studying rare plant pollinators in isolation neglects the broader ecological context. We performed floral and pollinator network surveys at Ankeny National Wildlife Refuge, Dorena East Wildlife Area (DEWA), Finley National Wildlife Refuge, Fisher Butte, and Greenhill.

Figure 5. Examples of a transect marked with flags, crew surveying quadrat for flowering plants, and surveying a floral patch for visiting pollinators.

In 2024, we began sampling prairies on May 14th and ended sampling efforts on July 16th. We performed five complete sampling events at each site surveyed. Transects were sampled throughout the survey period to determine estimated abundance and spatial extent of flowering units on plant species in flower. These data provided an assessment of which plant species may provide floral resources for rare plant pollinators throughout the season. These species lists were then used to target observations and hand-netting efforts of pollinators throughout the flowering season. Insects were only netted if they were observed to visit reproductive parts (anthers, stigma) of the target flowering plant. This information can help determine which plant species provide floral resources to pollinator species visiting Willamette daisy and other rare plants.

Golden Paintbrush and Kincaid's Lupine Pollinator Observations

In 2024, we continued efforts to document potential pollinators of rare plant species using hand-netting techniques on patches of golden paintbrush and Kincaid's lupine in sites where these species occur. We observed pollinators on golden paintbrush and Kincaid's lupine patches at Ankeny National Wildlife Refuge, Big Spires, Finley National Wildlife Refuge, and Herbert Farm Natural Area. Time spent hand-netting was split into 15–45-minute periods across sites and over multiple days to avoid weather or phenological biases. We attempted to sample each site for a total of three hours per plant species. Insects were only netted if they were observed to visit reproductive parts (anthers, stigma) of the target flowers. For our results, we also included pollinators observed on golden paintbrush and Kincaid's lupine during 2019-2024 network surveys.

Insect Identification

Following curation, insects were identified to species if possible (most bees and syrphid flies), genus (unidentifiable bees and syrphids), or left at family or order (most beetles, non-syrphid flies, other taxa). Some similar insects were identified to morphospecies, and some specimens were genetically barcoded to improve species-level identification. Throughout our work on this project, we have accumulated a variety of taxon-specific resources to identify regional species (Kaye et al. 2022, Mitchell et al. 2024). We often consult taxonomic experts and use keys in development, as regional keys are typically lacking or nonexistent. David Cappaert has developed keys for regional species in the family Andreninae and the genera *Ceratina sp.* and *Lasioglossum sp.*, with other keys in development. In addition, David created a web-based glossary guide that provides high-quality pictures as examples of the numerous insect taxonomy terms used in pollinator identification.

Analyses

To compare flowering plant pollinator communities among prairie sites, we created network diagrams of plant and insect associations at each site using package bipartite in R (Dormann et al. 2008), summed across years (2019-2024). These plant-pollinator networks were made by relating the abundances of plant species at each site to the abundances of pollinator species that visited that plant species at each site.

4. RESULTS

4.1. Pollen Supplementation Experiments

Pollen Supplementation Experiments

We conducted the golden paintbrush pollen supplementation experiment on five plants (each plant with a treatment and control flowering stalk) at each site surveyed (N = 4). Following the supplementation experiment and prior to collecting the flowering stalks for seed counting, many of our experimental flowering stalks were damaged by herbivores or other means. As a result, many flowering stalks were missing and these data could not be included in analyses. Since we needed paired data for analyses, we could not include any data collected from Ankeny National Wildlife Refuge but we could include five plants worth of data from Big Spires, three plants of data from Finley National Wildlife Refuge, and one plant of data from Herbert Farm Natural Area.

The Kincaid's lupine pollen supplementation experiment was more time intensive than the golden paintbrush experiment, so we reduced our sample size to accommodate our time constraints. We conducted the Kincaid's lupine pollen supplementation experiment on three plants at Big Spires with all data used in analyses, on four plants at Finley National Wildlife Refuge with three plants worth used in analyses, and on five plants at Herbert Farm Natural Area with all data used in analyses.

Our sample size for 2024 was limited, but we did not find a consistent response of pollen supplementation increasing seed set for golden paintbrush (paired data from nine experimental plants across three sites) or Kincaid's lupine (paired data from 11 experimental plants across three sites). The data displayed in Figure 6 show all sites pooled together for simplicity. We found limited differences (Table 2) between open and supplemented treatments across all sites, or when sites were analyzed separately, for either golden paintbrush or Kincaid's lupine.

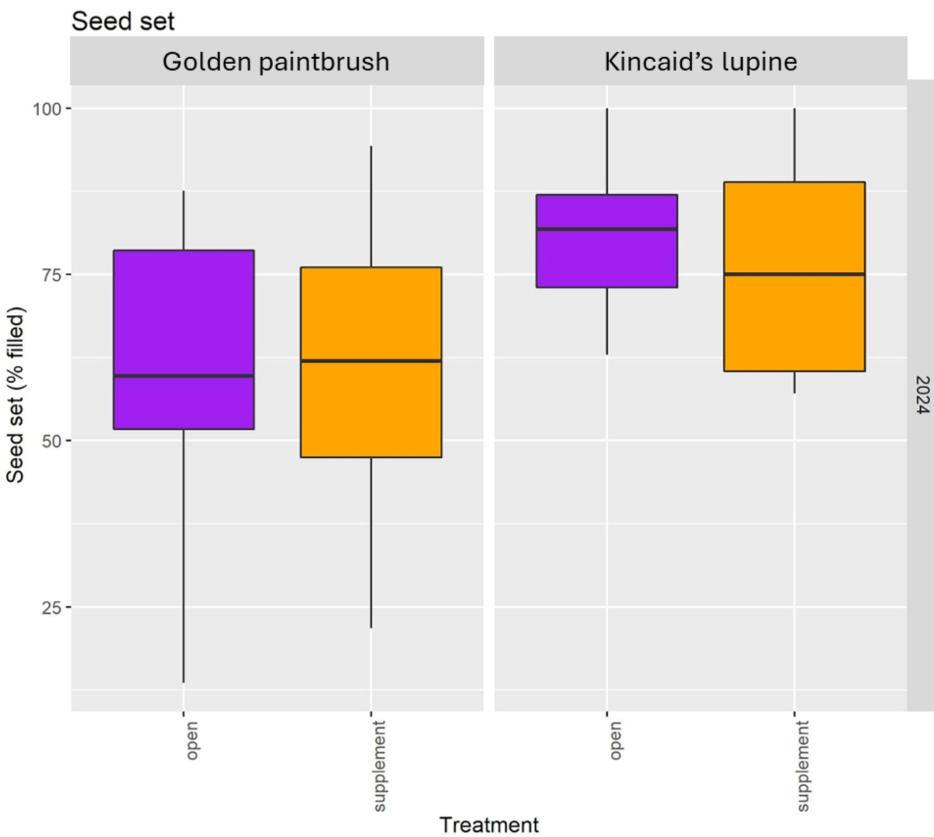

Figure 6. Seed set (the proportion of filled seeds) pooled across all sites sampled, for each species studied (golden paintbrush N = 9, Kincaid's lupine N = 11). Results from open-pollinated flowers are shown in purple and pollen supplemented flowers are shown in orange.

Table 2. Statistical results between open and supplemented data (comparing differences in the proportion of filled seeds) for golden paintbrush and Kincaid's lupine, at each site analyzed.

Species	Site	Sample size (for analyzes)	P-value (alpha = 0.05)
Golden paintbrush	Big Spires	5	0.472
Golden paintbrush	Finley National Wildlife Refuge	3	*0.070*
Golden paintbrush	Herbert Farm Natural Area	1	0.973
Kincaid's lupine	Big Spires	3	0.462
Kincaid's lupine	Finley National Wildlife Refuge	3	0.934
Kincaid's lupine	Herbert Farm Natural Area	5	0.523

Surrounding Floral Density

We found different results for golden paintbrush (N = 9) and Kincaid's lupine (N = 11) when comparing the total number of flowering stalks within a 4-m radius to the number of filled seeds in each pollen supplementation experimental flowering stalk. Filled seed of golden paintbrush increased with increased surrounding floral density at Finley National Wildlife Refuge (p = 0.04), but decreased with increased surrounding floral density at Big Spires (p = 0.02; Figure 7). There was no significant interaction (alpha = 0.05) between the number of filled seed and surrounding floral density at Herbert Farm Natural Area. The amount of Kincaid's lupine filled seed had limited correlations with surrounding floral density at Big Spires, Finley National Wildlife Refuge, or Herbert Farm Natural Area (Table 3).

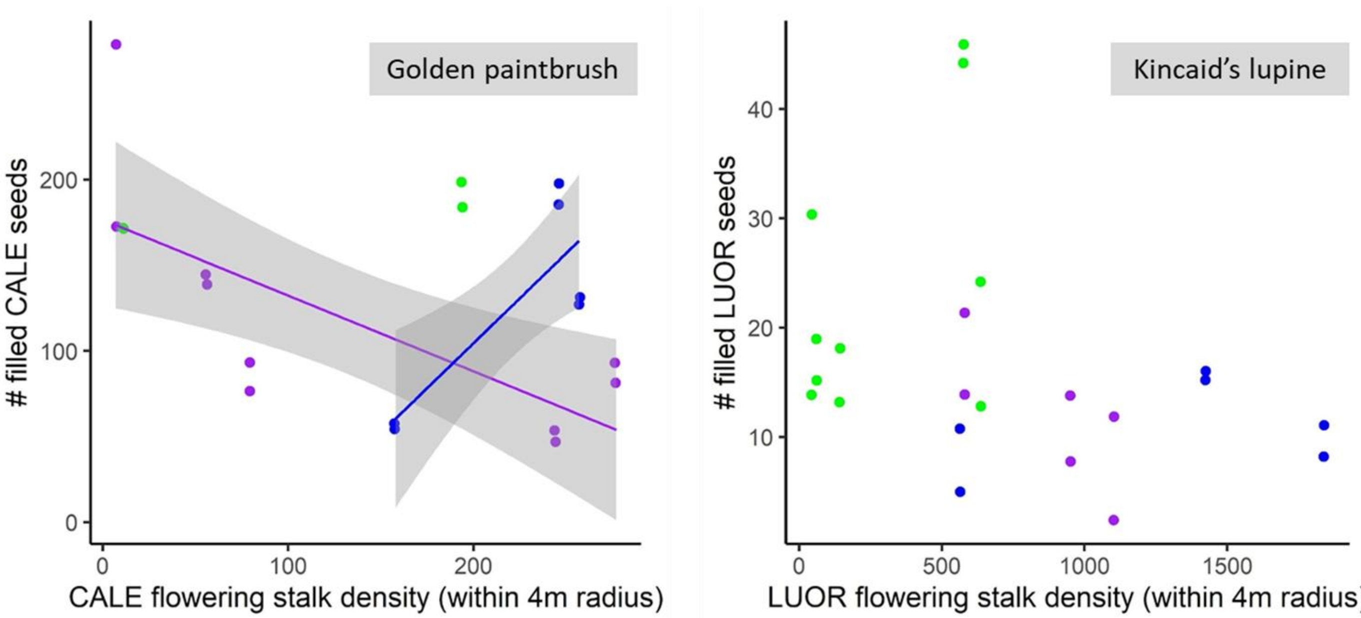

Figure 7. Density of golden paintbrush (left) and Kincaid's lupine (right) flowering stalks within a 4-m radius of pollen supplemented plants compared to the number of filled seeds. Sites included Big Spires (purple), Finley National Wildlife Refuge (blue), and Herbert Farm Natural Area (green). Gray shading represents 95% confidence intervals and is included only for significant relationships (alpha = 0.05).

Table 3. Statistical results between surrounding floral density and number of filled seed for golden paintbrush and Kincaid's lupine, at each site analyzed.

Species	Site	Sample size (for analyzes)	P-value (alpha = 0.05)
Golden paintbrush	Big Spires	5	**0.018**
Golden paintbrush	Finley National Wildlife Refuge	3	**0.038**
Golden paintbrush	Herbert Farm Natural Area	1	0.113
Kincaid's lupine	Big Spires	3	*0.095*
Kincaid's lupine	Finley National Wildlife Refuge	3	0.531
Kincaid's lupine	Herbert Farm Natural Area	5	0.157

4.2. Prairie Plant and Pollinator Community Sampling

Plant-pollinator Networks

In 2024, we observed a total of 90 plant species and 1709 pollinator observations across five sites. Ankeny National Wildlife Refuge (421), Dorena East Wildlife Area (338), Finley National Wildlife Refuge (348), and Greenhill (365), had more pollinator visits than Fisher Butte (237). We identified captured pollinators (mostly bees, other insect determinations not yet complete) to 53 levels of identification; we identified most specimens to species, but others were left at genus or suborder level pending availability of more developed keys. We were conservative with our designation of morphospecies in the 2024 collection, as we continue making morphospecies determinations consistent across the Oregon and Washington Prairie Pollinator Collections, in perpetuity from 2019.

We created network diagrams for each site representing plant-pollinator networks sampled between 2019 and 2024. Network diagrams display plants codes on the left (Kaye et al. 2022: Appendix B) and insect species on the right. Observations of insect species contacting the reproductive parts of flowering plant species are represented with lines, the thicker the line, the more interactions between plant and insect species. Complexity can vary greatly depending on the amount of time observed; these network diagrams show complexity over a 6-year period, compared to network diagrams representing a single year of complexity (Kaye et al. 2022). Even when compared to network diagrams over a 5-year period (Mitchell et al. 2024), these 6-year network diagrams show increased complexity throughout the network. Ankeny and Finley National Wildlife Refuges (Figure 8) have five years of plant-pollinator community sampling and Fisher Butte (Figure 9) has six years of plant-pollinator community sampling, and these are the most complex networks. Greenhill (Figure 9) and Dorena East Wildlife Area (Figure 10) have four and two years of plant-pollinator community sampling respectively, and have less complex networks. Dorena East Wildlife Area was sampled for the second time in 2024, and has a surprising amount of complexity for just two years of data. It is important to note that this network diagram only represents most of the captured and identified bee species, as the non-bee identifications are still in process.

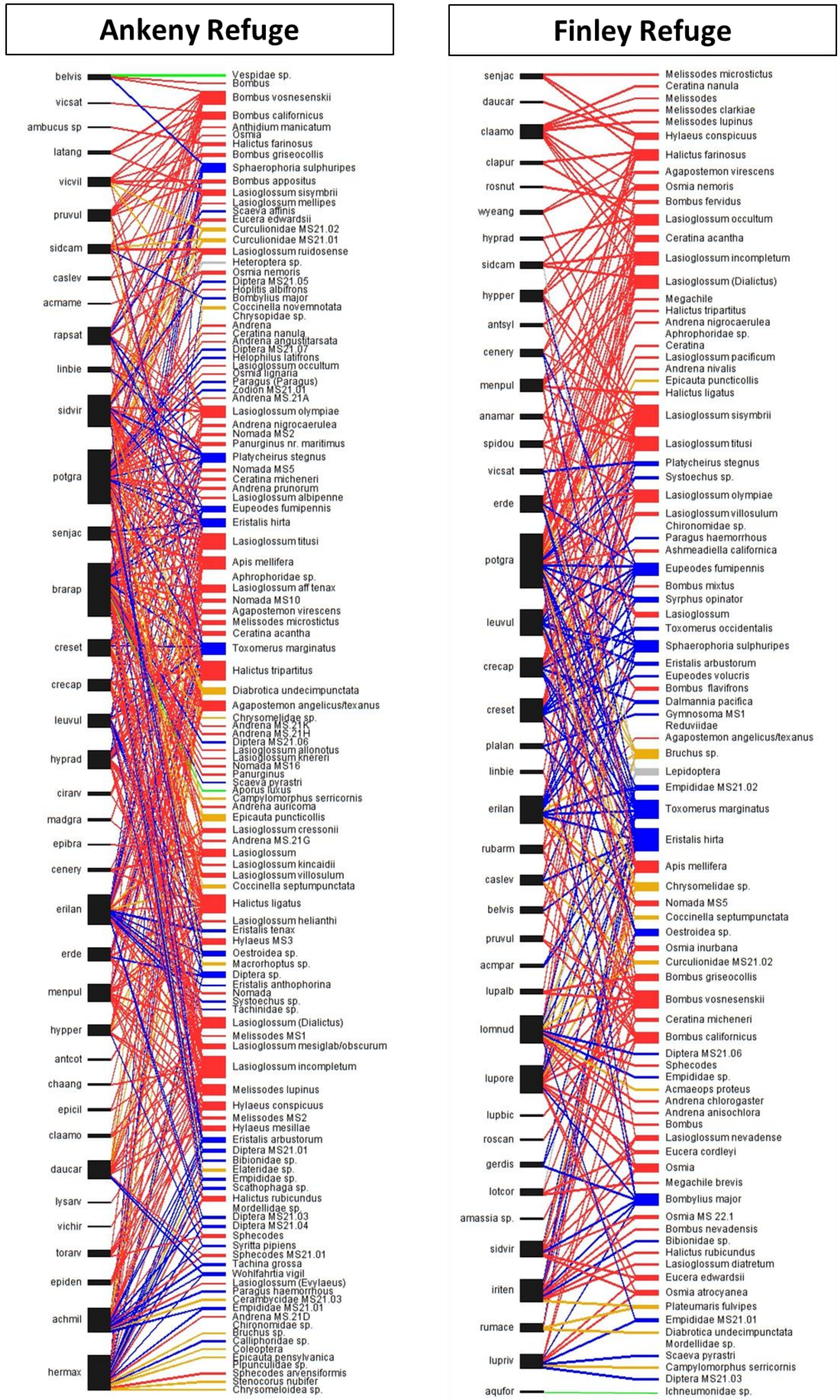

Figure 8. Plant-pollinator networks from 2020 to 2024 for Ankeny and Finley National Wildlife Refuges.

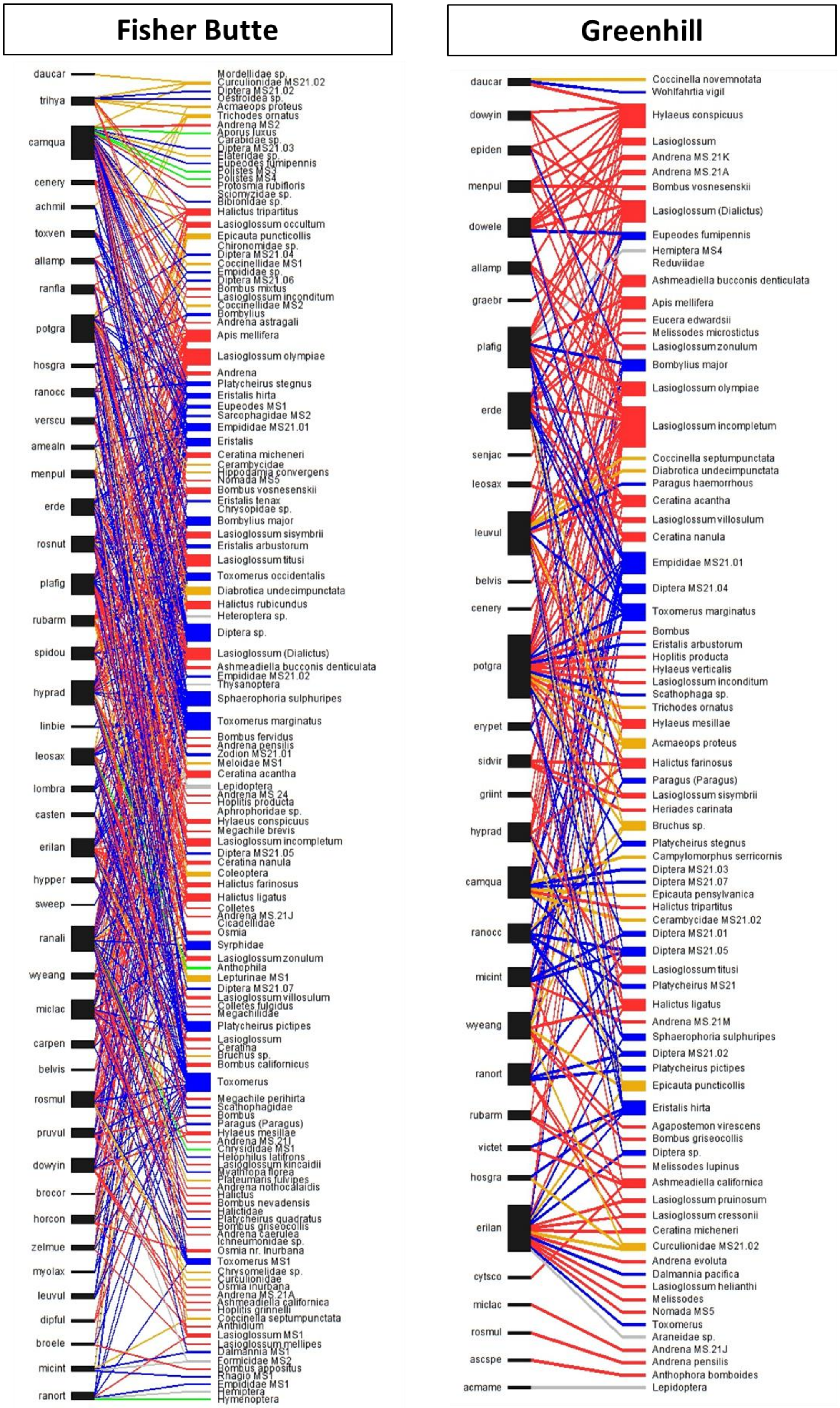

Figure 9. Plant-pollinator networks from 2019 to 2024 for Fisher Butte and 2021 to 2024 for Greenhill.

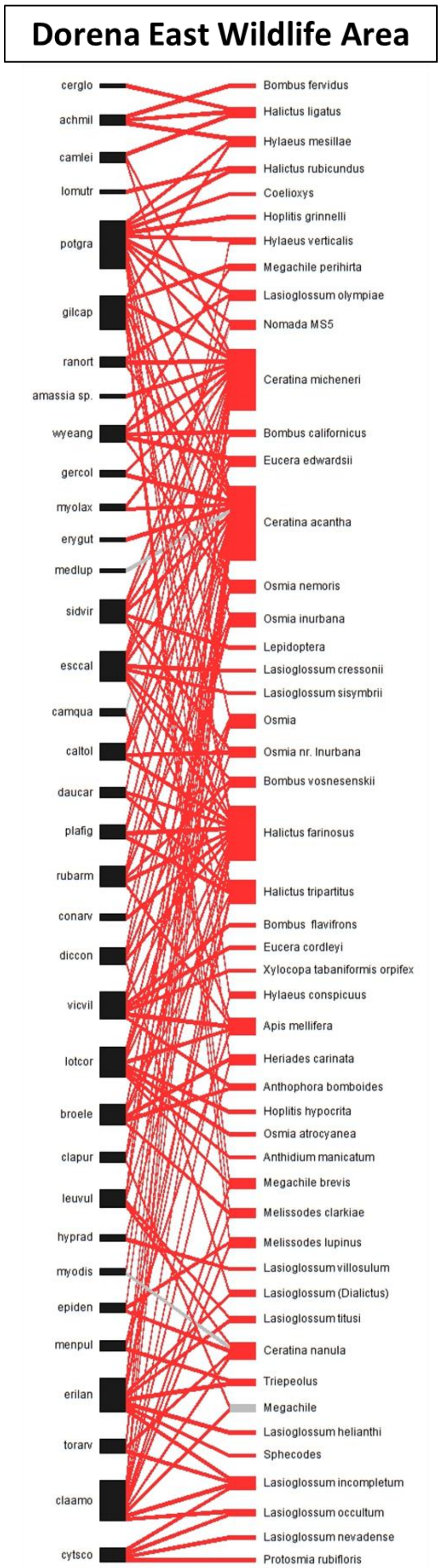

Figure 10. Plant-pollinator networks from 2023 to 2024 for Dorena East Wildlife Area.

<u>*Golden Paintbrush and Kincaid's Lupine Pollinator Observations*</u>

Between 2019 and 2024, we captured and identified 162 insects visiting flowers of golden paintbrush (Appendix A). These insects represent 13 different categories of identification, whether of species, genera, or family determination. One species, *Bombus californicus* (bumble bee, 135 captures) represented 83.3% of all insects captured off golden paintbrush. *Eristalis hirta* (fly, 11 captures) was the next most commonly caught insect off golden paintbrush and represented 6.8% of all captures. We captured a total of 143 bees, 14 flies, and five beetles of different species off golden paintbrush.

Between 2020 and 2024, we captured and identified 1181 insects visiting flowers of Kincaid's lupine (Appendix B). These insects represent 40 different categories of identification, whether of species, genera, family, or order determination. One species, *Bombus vosnesenskii* (bumble bee, 903 captures) represented 76.5% of all insects captured off Kincaid's lupine. *Bombus mixtus* (bumble bee, 72 captures) was the next most commonly caught insect off Kincaid's lupine and represented 6.1% of all captures. We captured a total of 1169 bees, eight flies, three beetles, and one butterfly of different species off Kincaid's lupine.

5. DISCUSSION

After one year of sampling, pollen supplementation trials for golden paintbrush and Kincaid's lupine differed from previous Willamette daisy results (Mitchell et al. 2024). Our supplementation treatments did not consistently result in higher seed set compared to flowers exposed to background pollination rates. Our results are limited by our small sample size, including the loss of paired data due to damage in the field, but may suggest these species are not as pollen limited as Willamette daisy or our additional pollen does not contribute to increased seed set. Alternatively, our supplementation protocols may not effectively mimic the long-tongued bees (i.e. bumble bees) that pollinate these plants, possibly resulting in inadequate pollination. We will repeat this experiment in 2025 with an increased sample size and attempts to reduce damage to experimental flowering stalks in the field, in efforts to improve the strength of evidence.

Also different than previous Willamette daisy results (Mitchell et al. 2024), increased surrounding floral density did not have a clear, positive effect of increased filled seed in golden paintbrush or

Kincaid's lupine. Though our sample size was low, we saw variation in responses between these two plant species and among the sites we sampled. It seems reasonable to think that the number of filled seed would increase as available pollen increases, quantified by increased surrounding floral density, and we are unsure why golden paintbrush filled seed would decrease with increased surrounding floral density at Big Spires. We do not think these results are an indication of inbreeding depression, because the genetic diversity of seed and plugs outplanted at Big Spires was quite high.

After one year of targeted sampling, pollinator communities of golden paintbrush and Kincaid's lupine are quite different than those found visiting Willamette daisy (Mitchell et al. 2024). In general, we found much lower diversity on these species compared to Willamette daisy. Bumble bee species were the dominant visitor for golden paintbrush and Kincaid's lupine, though interestingly, not the same bumble bee species though these plants co-occur. This indicates the flower morphology or other cues from these plant species are affecting the attractiveness or resource acquisition to different bumble bee species. Additionally, there were much less non-bee visitors to golden paintbrush and Kincaid's lupine than we found for Willamette daisy.

Plant-pollinator community networks showed large variation by site and season over a six-year period. Plant-pollinator networks can vary over time as plant and insect species emigrate or are extirpated from an area, immigrate or are introduced to an area, or factors change, including resource availability, affecting species' interactions and mutualisms. Therefore, to effectively manage rare plants and their associated pollinators, long-term studies over multiple sites are needed to make inference across the ecoregion. The conservation implication is that we need to conserve many examples of prairies to conserve a wide diversity of plants and pollinators.

The collection and dataset created by this project represent the most comprehensive information on plant-pollinator networks in prairies of the Pacific Northwest. We will continue to organize and publish a reference collection with the Oregon State Arthropod Collection, and to create and contribute to regional keys. Future research should investigate the factors that drive diversity, and spatial and temporal variation in pollinator communities. These methodologies can be used across Willamette Valley prairies to assess the effectiveness of management treatments on species of conservation concern. A holistic understanding of species' pollinator communities, how the surrounding plant community affects and supports these pollinators, and how to restore and manage prairie habitat to best support pollinators will be crucial for long term conservation of endangered prairie plant species.

6. CONCLUSIONS

In summary,

- Golden paintbrush and Kincaid's lupine have different responses to pollen supplementation and surrounding floral density than Willamette daisy after one year of sampling.
- Golden paintbrush and Kincaid's lupine are most commonly visited by bumble bees, but not by the same bumble bee species even though these plants co-occur.

- Establishing functioning pollinator communities for rare plant survival depends on providing sufficient seasonal resources for the entire prairie pollinator community, through establishment of a resilient prairie plant community.
- High variation exists among prairie plant-pollinator communities throughout sites and across years. This reinforces the need for repeated surveys to distinguish true population and community trends from annual fluctuations.

7. REFERENCES

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APPENDIX A. 2024 GOLDEN PAINTBRUSH VISITORS

Table A4. All insects captured on golden paintbrush (C*astilleja levisecta*) and identified to the lowest possible denomination. Represented taxa were collected from the golden paintbrush observations that were coupled with the pollen supplementation experiment and from the network observations.

Golden paintbrush insect visitors (2019-2024)	
Species	**Number of insects caught**
Anthomyiidae MS1	1
Bombus vosnesenskii	1
Halictus farinosus	1
Lasioglossum ruidosense	1
Lasioglossum sisymbrii	1
Lepturinae MS1	1
Staphylinidae MS1	1
Bombus nevadensis	2
Lasioglossum titusi	2
Sphaerophoria sulphuripes	2
Curculionidae MS21.02	3
Eristalis hirta	11
Bombus californicus	135

APPENDIX B. 2024 KINCAID'S LUPINE VISITORS

Table A5. All insects captured on Kincaid's lupine (*Lupinus oreganus*) and identified to the lowest possible denomination. Represented taxa were collected from the Kincaid's lupine observations that were coupled with the pollen supplementation experiment and from the network observations.

Golden paintbrush insect visitors (2019-2024)	
Species	**Number of insects caught**
Agapostemon angelicus/texanus	1
Agapostemon virescens	1
Andrena miranda	1
Bombus caliginosus	1
Hoplitis hypocrita	1
Lasioglossum occultum	1
Lasioglossum olympiae	1
Lasioglossum pacificum	1
Megachile sp.	1
Osmia inurbana	1
Osmia lignaria	1
Bombyliidae sp.	1
Bombylius major	1
Empididae MS21.02	1
Eupeodes fumipennis	1
Coccinella septumpunctata	1
Lepidoptera	1
Andrena angustitarsata	2
Halictus tripartitus	2
Lasioglossum sisymbrii	2
Lasioglossum zonulum	2
Osmia mini	2
Bruchus sp.	2
Andrena sp.	3
Bombus nevadensis	3
Bombus sp.	4
Bombus griseocollis	4
Eristalis hirta	4
Eucera edwardsii	6

Bombus melanopygus	7
Eucera cordleyi	8
Osmia sp.	8
Osmia cyanella	9
Osmia cf albolateralis	10
Apis mellifera	14
Bombus californicus	29
Eucera sp.	30
Osmia atrocyanea	38
Bombus mixtus	72
Bombus vosnesenskii	903