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

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Progress Report to the US Army Corps of Engineers, Portland District & the Bureau of Land Management, Northwest District

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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 photograph: Bumble bee (likely *Bombus fervidus californicus*) nectaring on golden paintbrush (*Castilleja levisecta*) at Herbert Farm. Photograph by Owen Cass.

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EXECUTIVE SUMMARY

Global losses in biodiversity highlight the diverse, and often dependent connections between species. The conservation of 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 plants, we conducted a 5-year study of Willamette daisy (*Erigeron decumbens*, Asteraceae), a perennial forb endemic to the Willamette Valley, Oregon, and listed as endangered under the U.S. Endangered Species Act.

Between 2019 and 2023, the Institute for Applied Ecology (IAE) partnered with the US Army Corps of Engineers (USACE) and the Bureau of Land Management (BLM) to better understand Willamette daisy pollination biology and Willamette Valley pollinator ecology. We conducted pollen supplementation experiments between 2019 and 2022 to determine the effect of pollinators on Willamette daisy seed set, and corresponding field observations to understand the role of insect pollinators and other flowering prairie plants on Willamette daisy population growth and survival.

Surviving Willamette daisy plants from the 2021 field crossing experiment showed that while none and self-pollen supplemented plants did not produce as much viable seed when compared to distant- or local-pollen supplemented plants, seeds that did germinate grew to a similar size and reproductive potential. Insects visiting Willamette daisy included several species of Hymenoptera (mostly Halictidae including *Halictus sp.*, *Lasioglossum sp.* (Dialictus), *Andrena sp.*, with some examples of Megachilidae and Apidae), and Diptera (mostly Syrphidae, especially *Toxomerus sp.* and *Sphaerophoria sp.*). Pollinator communities differed significantly from site to site and reveal that insects visiting Willamette daisy are members of networks that rely on a wide range of co-occurring plant and insect species. Conservation of Willamette daisy populations hinges on supporting a thriving and diverse network of insect pollinators and flowering plants in the prairie ecosystem. Our work with endangered Willamette daisy highlights the need for pollinators to improve the species' reproduction, and the need for management to promote native plants and a diverse pollinator community. To continue this work, we will conduct similar pollinator ecology studies focused on Kincaid's lupine (*Lupinus oreganus*) and golden paintbrush (*Castilleja levisecta*).

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 rare 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.

Willamette daisy (*Erigeron decumbens*, Figure 1) is endemic to grasslands in the Willamette Valley of Oregon and is listed by the US Fish and Wildlife Service and Oregon Department of Agriculture as an endangered species (U.S. Fish & Wildlife Service 2016). 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. 2023).

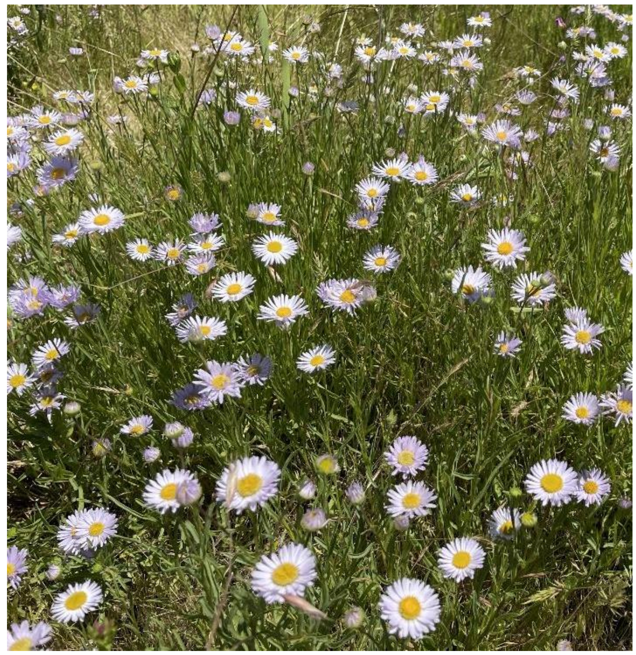

Figure 1. Patch of Willamette daisy (*Erigeron decumbens*) in a Willamette Valley prairie, OR. Photograph by J. Christina Mitchell.

As a rare plant of sparse distribution, Willamette daisy faces 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' can occur if few Willamette daisy individuals are dispersed among abundant other species, and generalist pollinators, that carry multiple pollen species, block adhesion of Willamette daisy pollen to stigma with an alternative pollen grain. Understanding which pollinators are most important to Willamette daisy, with the goal of improving reproduction, is also key to managing these endangered populations.

To address these concerns, IAE partnered with the US Army Corps of Engineers (USACE), Portland District and the Bureau of Land Management (BLM), Northwest District to better understand the breeding biology and pollination ecology of Willamette daisy in remnant and restored prairies throughout the Willamette Valley. In 2023, we conducted field work at Ankeny and Finley National Wildlife Refuges (US Fish and Wildlife Service, FWS), Fisher Butte and Dorena East Wildlife Area (USACE), Greenhill and Oxbow West (BLM), and Jefferson Farm (private; Figure 2). Between 2019 and 2022, we also conducted work at Calapooia, Kingston Prairie (private), and Speedway (BLM).

This report describes research activities that were mostly focused on Willamette daisy and plant-pollinator networks in multiple prairies throughout the Willamette Valley, Oregon. Using the successful field and statistical methods we developed for the Willamette daisy studies, we initiated field trials in 2023 to address the same research questions for golden paintbrush (*Castilleja levisecta*) and Kincaid's lupine (*Lupinus oreganus*). Our final results for Willamette daisy are presented in this report and the study is now transitioning to focus on paintbrush and lupine. BLM funding supported the Willamette daisy studies only, while USACE funding has supported studies of all three species.

Golden paintbrush was delisted from federally-threatened in 2023 and removed from the Endangered Species Act (USFWS 2023) following recovery efforts. Kincaid's lupine is state and federally listed as a threatened species by the Oregon Department of Agriculture and the U.S. Fish and Wildlife Service. 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.

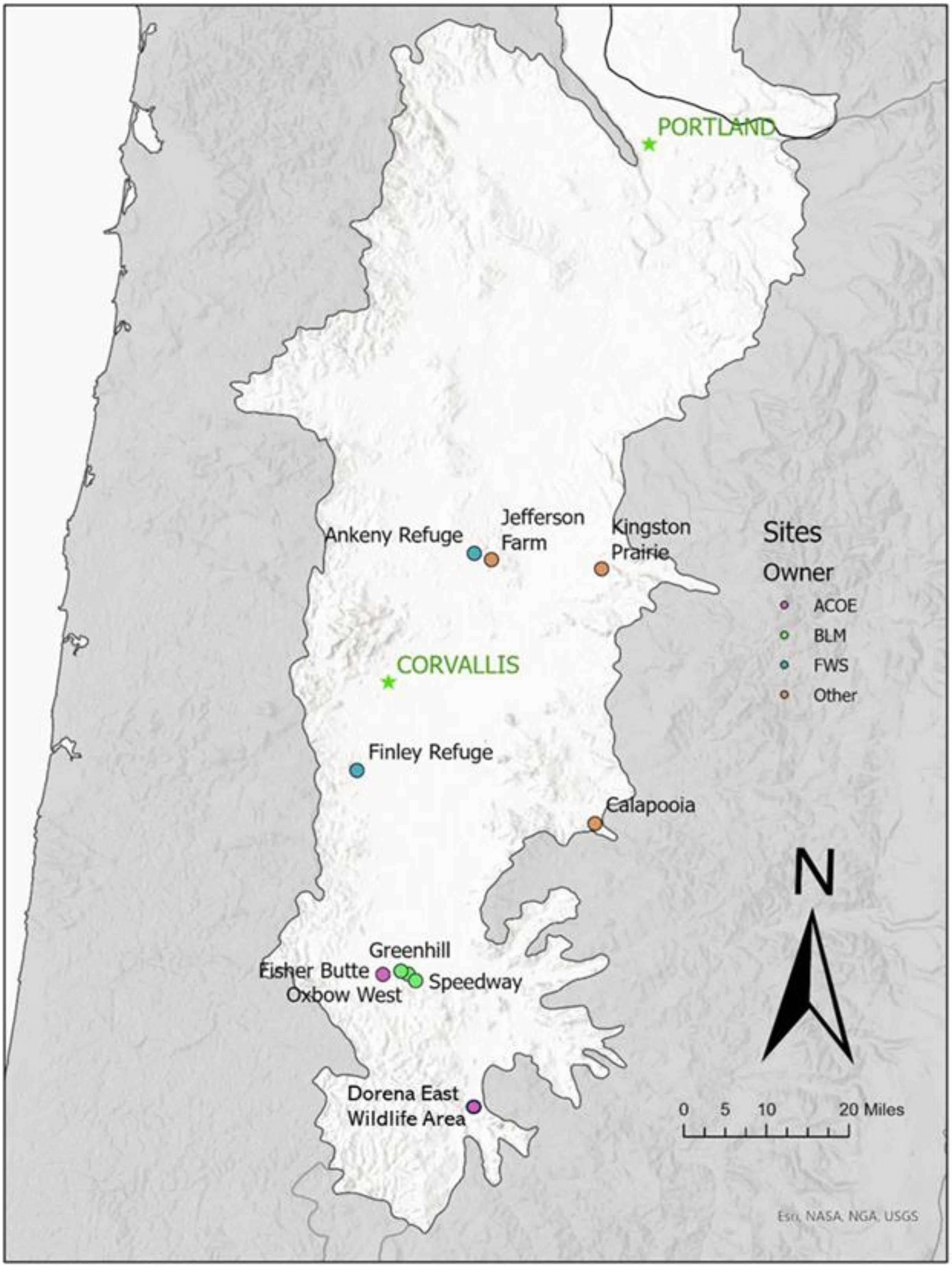

Figure 2. Location of remnant and restored prairie sites used for pollinator research between 2019 and 2023, across the Willamette Valley Ecoregion, Oregon, USA.

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 mating system and pollination ecology of rare plants like Willamette daisy (*Erigeron decumbens*).

Specific objectives include:

1) Determine whether the breeding system of Willamette daisy is autogamous, or affected by inbreeding or outbreeding depression;
2) Follow methods used for Willamette daisy pollen supplementation experiments to conduct field trials and refine methods for Kincaid's lupine and golden paintbrush pollen supplementation experiments; and
3) Extensively sample the Willamette Valley prairie pollinator community from plants flowering throughout the season.

3. METHODS

3.1. Project Activities

We performed research at ten sites since this project began in 2019 (Table 1, Kaye et al. 2022). In 2023, we collected data from Ankeny Refuge, Dorena East Wildlife Area, Finley Refuge, Fisher Butte, Greenhill, Jefferson Farm, and Oxbow West. We presented this research at a national and regional conference, 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). David Cappaert used our collection and others, including OSAC, to further refine and expand regional pollinator keys and associated identification resources.

Table 1. Willamette Valley pollinator study sites and select research activities.

		Willamette daisy pollen supplementation					Accompanying Willamette daisy hand-netting					Prairie plant/pollinator network sampling				
Site	**Owner**	**2019**	**2020**	**2021**	**2022**	**2023**	**2019**	**2020**	**2021**	**2022**	**2023**	**2019**	**2020**	**2021**	**2022**	**2023**
Ankeny Refuge	USFWS						X	X	X	X			X[2]	X	X	X
Calapooia	private		X				X	X								
Dorena East Wildlife Area	USFWS															X
Finley Refuge	USFWS	X	X	X	X		X	X	X	X			X[2]	X	X	X
Fisher Butte	USACE	X	X	X	X		X	X	X	X		X[1]	X[1]	X	X	X
Greenhill	BLM								X	X				X	X	X
Jefferson Farm	private	X	X	X	X		X	X	X	X		X[1]	X[1]	X	X	X
Kingston Prairie	Greenbelt Land Trust	X	X	X	X		X	X								
Oxbow West	BLM	X	X	X	X		X	X	X	X		X[1]	X[1]	X	X	X
Speedway	BLM						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. Breeding Biology Experiments

Experiments designed to better understand the breeding biology of Willamette daisy began in 2019 and continued into 2022 (see Kaye et al. 2022 for details). Controlled, field crosses were conducted at Fisher Butte in 2021 and included Jefferson Farm as a pollen source (Kaye et al. 2022). To document the mating system of the species, we performed four treatments of controlled crosses:

1. No pollination. A test for autogamy.
2. Self. A test for self-compatibility.
3. Local outcrossing. Cross to and from an individual at Fisher Butte (this cross type served as a reference or control)
4. Distant outcrossing. Cross from an individual at Jefferson Farm to an individual at Fisher Butte (as a test for outbreeding depression or heterosis).

In 2021, seeds (achenes) of each crossed capitula were counted and categorized as either 'filled' or 'unfilled' by visual inspection with a dissecting scope. Filled seeds are more likely viable, and are more opaque, rounded, and resistant to pressure. Unfilled seeds are not viable, and tend to be more translucent, flatter, and easier to depress. Filled seeds were then tested in a germination experiment. In March 2022, successful germinants were planted in conetainers and grown in the greenhouse. We assessed each successive stage of plant growth for each treatment: the proportion of filled seeds, the proportion of filled seeds that successfully germinated, and the proportion of germinants that grew to seedlings. In May 2022, surviving plants were transferred to 1-gallon pots and moved outside. We measured growth metrics from surviving plants on July 8, 2022 (Figure 3a), and again on June 27, June 28, and July 3, 2023 (Figure 3b). Growth metrics included: length of longest leaf (cm), total number of leaves, whether the plant was flowering and if so, the number of flowering stalks, total number of open flowers, and estimated proportion of open to closed or developing buds.

Figure 3. Willamette daisy plants from 2021 field crossing experiment, a) prior to measurement in July 2022 and b) late June early July 2023. Photographs by J. Christina Mitchell and Mara Friddle.

Analyses

The no-pollination and self-pollination treatments had low survivorship (N = 9) so we combined them into one category, none and self, and compared plant growth to the distant and local crosses. The initial field crossing experiment was set up so that multiple seeds from one experimental flower may have germinated, resulting in more than one plant representative of that experimental unit. To account for instances where more than one plant existed for a single experimental unit, these data were averaged. Of the 163 plants measured, we used 25 representatives from the distant cross, 25 representatives from the local cross, and 9 representatives from the none and self-crosses to compare treatments. We quantified plant growth using the total number of leaves per plant and quantified potential reproductive output using the total number of flowering stalks per plant and the number of flowers per plant, and log-transformed these values to improve normality. We compared the range and mean of these three categories using visual methods and determined statistical significance using an ANOVA test and Tukey Honest Significant Differences in R (R Core Team 2022).

3.3. Pollen Supplementation Experiments

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. This work was funded through the USACE. In 2023, weather seemingly caused atypical phenology for plants in our area, and golden paintbrush and Kincaid's lupine flowered and senesced much earlier than recent years. We conducted a limited trail for pollen supplementation techniques and data collection on these two species at Herbert Farm Natural Area. Most plants and flowers had senesced by the time we conducted our trial, so we did not collect accompanying floral density data or pollinator observations, but plan to for the 2024 experiments at other sites.

Figure 4. Example of conducting pollen supplementation trials with a) golden paintbrush (*Castilleja levisecta*) and b) Kincaid's lupine (*Lupinus oreganus*).

For golden paintbrush, we marked the start of the pollen supplementation trial on each experimental flowering stalk by tying a string 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 4a). This process was repeated every two days for a total of five supplementation events. At the end of the supplementation trial, another string was tied above the last manipulated flower and all stalks were collected once seed capsules were mature (on July 20th 2023). Seeds of each capitula were counted and categorized as either 'filled' or 'unfilled' by visual inspection with a dissecting scope.

For Kincaid's lupine, we marked the start of the pollen supplementation trial on each experimental flowering stalk by tying a string 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 in two ways. First, by activating the 'piston' mechanism of the flower structure and second, by vibrating with an electric toothbrush. This process was repeated every two days for a total of seven supplementation events. At the end of the supplementation trial, another string was tied above the last manipulated flower. Experimental flowering stalks were covered with breathable plastic material and tied below the marked flower to prevent dispersal loss of seeds; all stalks were collected once seed pods were mature (on July 20th 2023). Seeds of each capitula were counted and categorized as either 'filled' or 'unfilled' by visual inspection with a dissecting scope.

3.4. Prairie Plant and Pollinator Community Sampling

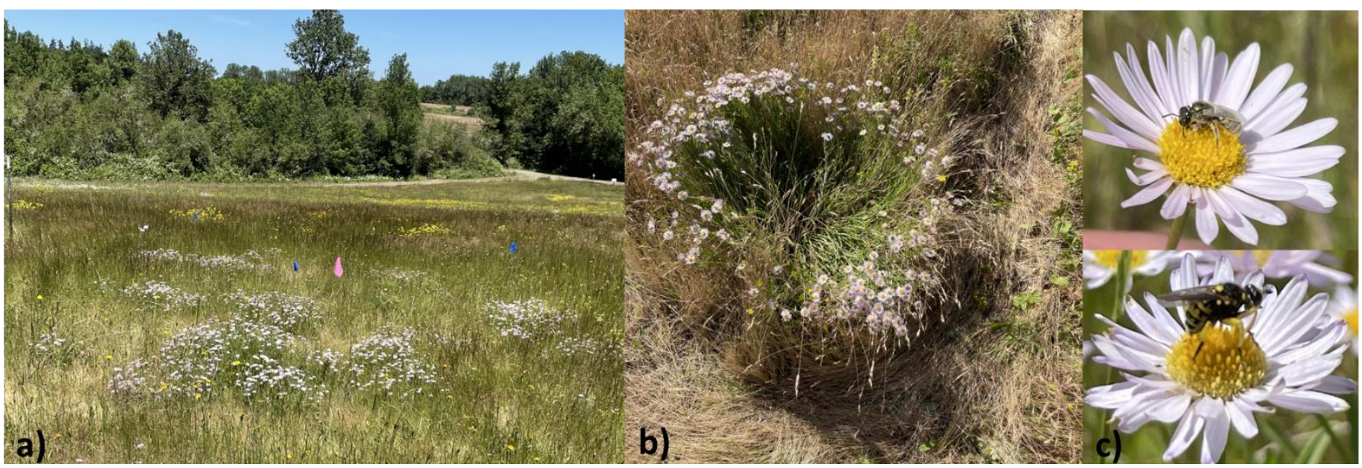

Figure 5. Examples of a Willamette daisy (a) patch, (b) plant with worn observation path, and (c) visitors.

Plant-pollinator Networks

In 2023, we continued efforts to quantify the prairie pollinator community using plant-pollinator network surveys (Waters 2021), as studying rare plant pollinators in isolation neglects the broader ecological context (Figure 5). We performed floral and pollinator network surveys at Ankeny Refuge, Dorena East Wildlife Area (DEWA), Finley Refuge, Fisher Butte, Greenhill, Jefferson Farm, and Oxbow West. In 2023, we began sampling prairies on May 3rd and ended sampling efforts on July 7th. We performed five sampling events at Dorena East Wildlife Area, Finley Refuge, Greenhill, and Oxbow West. We performed six sampling events at Ankeny Refuge, Fisher Butte, and Jefferson Farm. During each sampling period, transects were sampled 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.

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. 2023). We often consult taxonomic experts and use keys in development, as regional keys are typically lacking or nonexistent. David Cappaert has developed robust keys for regional species in the family Andreninae and the genera *Ceratina sp.* and *Lasioglossum sp.*, with other keys in active development. In addition, David is finalizing 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 (2019-2023). 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. Breeding Biology Experiments

Results from the 2021 field crossing experiment found greater seed set with distantly- and locally-crossed Willamette daisy flowers (~50% seed set) compared to no-pollination and self-pollination treatments (<5%). Following propagation of the filled seeds, there was no evidence that germination differed between treatments (p = 0.532), and mean seed germinability ranged from 55-64% across treatments (Kaye et al. 2022). We measured 171 surviving plants in 2022, 157 'local' and 'distant' plants, and 14 'self' and 'none' plants (Mitchell et al. 2023). Nine plants were flowering in July 2022, two were distantly crossed, six were locally crossed, and one was self-crossed. By 2023, there were 161 surviving plants and 85% were flowering in July. We measured 148 'local' and 'distant' plants, and 13 'self' and 'none' plants (Table 2).

Table 2. Summary of germinated seeds and survival of Willamette daisy plants from 2021 to 2023.

Experimental Treatment	2021: total seeds started	2021: total seeds germinated	2022: total seedlings placed in conetainers	2022: total plants in pots	2023: total plants in pots
Distant	640	362	176	81	78
Local	595	301	169	76	70
None	118	64	47	5	4
Self	133	46	27	9	9

Between the three categories of distant, local, and none and self, there was no significant difference when comparing the number of leaves, flowering stalks, or flowers per plant (log transformed to improve normality, Figure 6). Overall, there was more variation in the none and self category, but there were only nine representative plants for this category, compared to the 25 of the other two categories. Distantly crossed plants ranged from 9-398 leaves per plant (mean 112), 0-297 flowering stalks per plant (mean 109), and 0-634 flowers per plant (mean 196). Locally crossed plants ranged from 6-325 leaves per plant (mean 87), 0-384 flowering stalks per plant (mean 151), and 0-789 flowers per plant (mean 260). No cross or self-crossed plants ranged from 18-533 leaves per plant (mean 142), 5-372 flowering stalks per plant (mean 110), and 19-500 flowers per plant (mean 161).

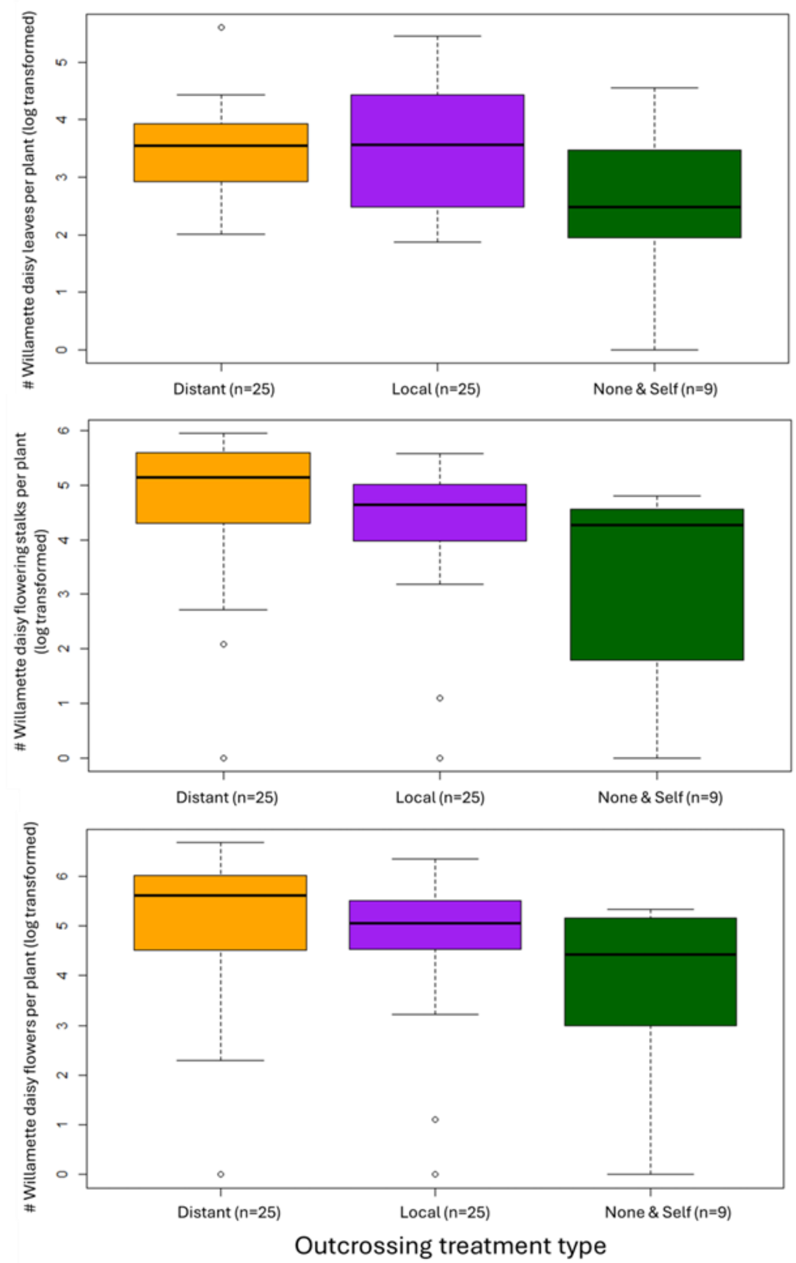

Figure 6. Mean number of log transformed leaves, flowering stalks, and flowers, per type of outcrossing treatment: distant (orange), local (purple), and none and self (green).

4.2. Pollen Supplementation Experiments

Phenologically, the golden paintbrush and Kincaid's lupine flowering period ended earlier than recent years and we were only able to conduct a small field trail for testing pollen supplementation methods. We trialed methods on five golden paintbrush plants and two Kincaid's lupine plants. Methods adapted to golden paintbrush worked well, and while the sample size was not large enough to calculate statistics, pollen supplemented flowers had greater seed set than control flowers (Figure 7). Methods adapted to Kincaid's lupine will be improved before implementing methods in 2024, as pollen supplemented flowers had reduced seed set compared to control flowers (Figure 7).

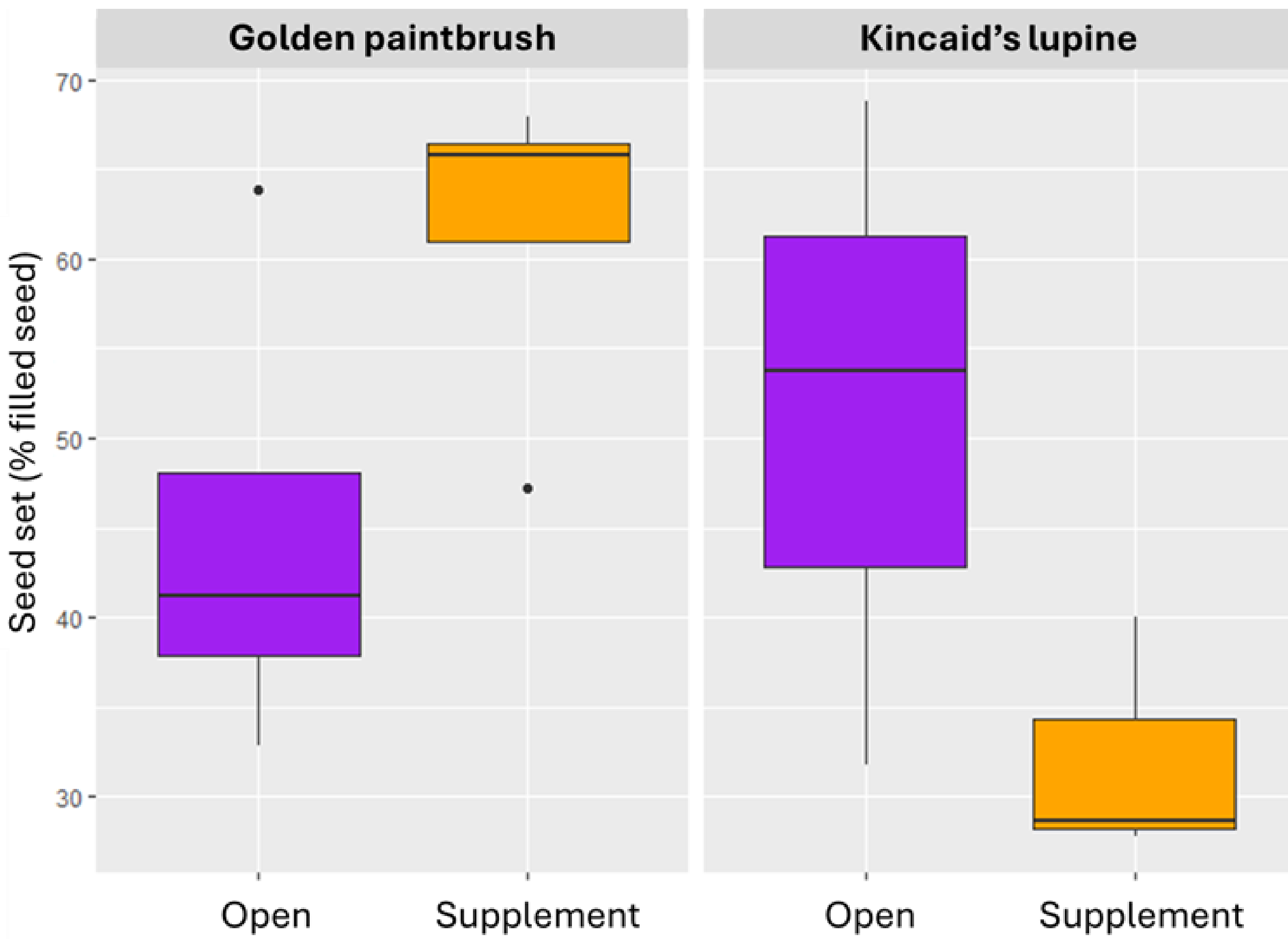

Figure 7. Seed set (the proportion of filled seeds) for open pollinated (purple) and pollen supplemented (orange) golden paintbrush and Kincaid's lupine flowers at Herbert Farm Natural Area.

4.3. Prairie Plant and Pollinator Community Sampling

Plant-pollinator Networks

In 2023, we observed a total of 97 plant species and 1113 pollinator visits across seven sites. Ankeny Refuge (282), Dorena East Wildlife Area (225), and Greenhill (187), had more pollinator visits than Finley Refuge (143), Oxbow West (103), Fisher Butte (93), and Jefferson Farm (80). We identified captured pollinators to 86 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 2023 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 2023. 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 5-year period, compared to network diagrams representing a single year of complexity (Kaye et al. 2022). Even when compared to network diagrams over a 4-year period (Mitchell et al. 2023), these 5-year network diagrams show increased complexity throughout the network. Fisher Butte, Jefferson Farm (Figure 8), and Oxbow West (Figure 9) each have five years of plant-pollinator community sampling and are also the most complex networks. Ankeny Refuge and Finley Refuge (Figure 10) each have four years of plant-pollinator community sampling, while Greenhill (Figure 9) has three years of sampling, and these three sites are more variable in the complexity of their networks. Dorena East Wildlife Area (DEWA, Figure 11) was sampled for the first time in 2023, and has a surprising amount of complexity for just one year 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.

Willamette daisy observations

Between 2019 and 2023, we captured and identified 2890 insects visiting flowers of Willamette Daisy (Appendix 1). These insects represent 171 different categories of identification, whether of species, genera, family, or morphospecies determination. Five identification categories represented 47.9% of all observed Willamette daisy visitations: *Toxomerus marginatus* (fly, 464 individuals captured), *Halictus ligatus* (bee, 287 individuals captured), Sphaerophoria sulphuripes (fly, 243 individuals captured), Eupeodes fumipennis (fly, 197 individuals captured), and *Lasioglossum sp.* (bee, 193 individuals captured). Additionally, 48 identification categories were represented by 10-100 individuals, and 118 identification categories were represented by less than 10 individuals across five years.

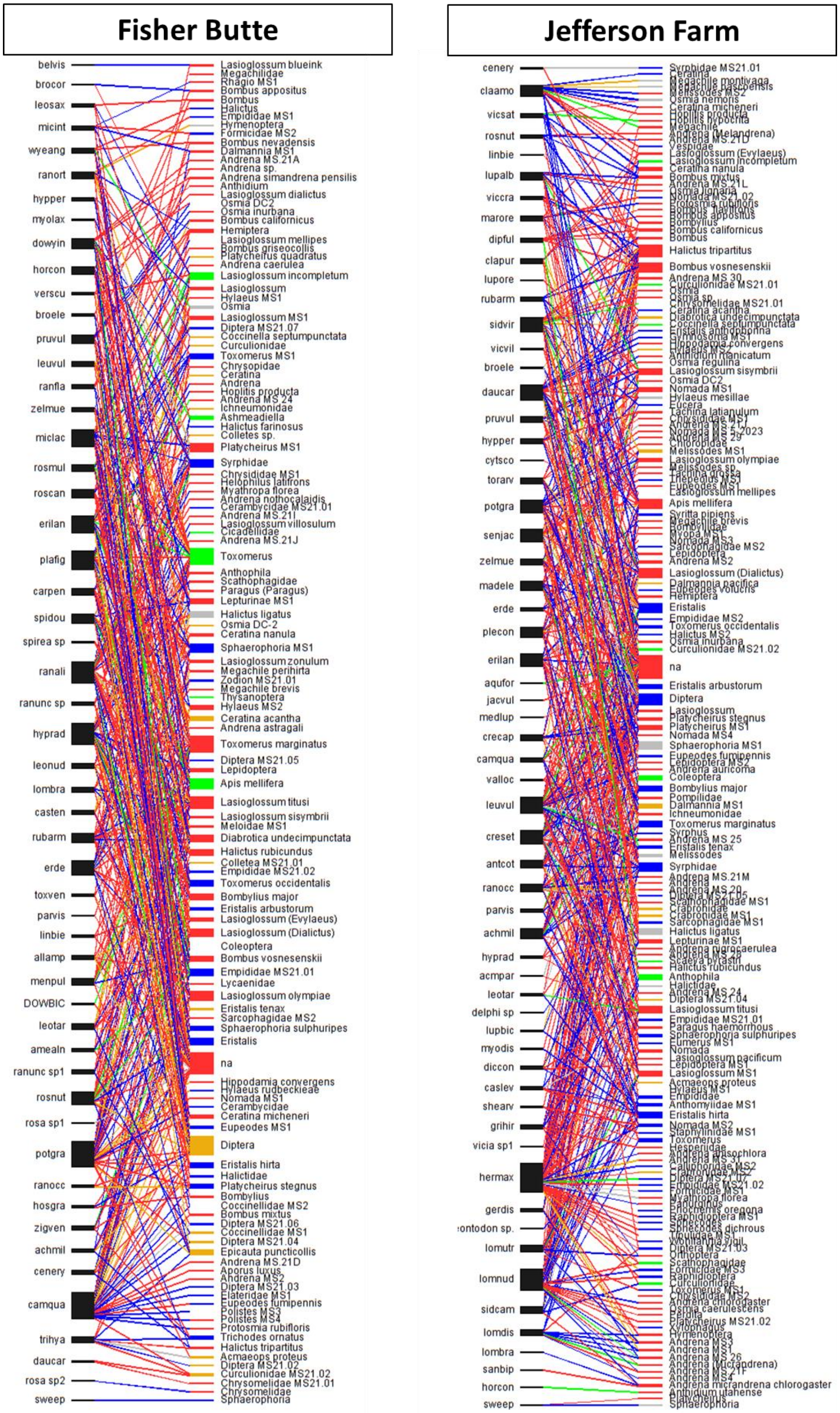

Figure 8. Plant-pollinator networks from 2019 to 2023 for Fisher Butte and Jefferson Farm.

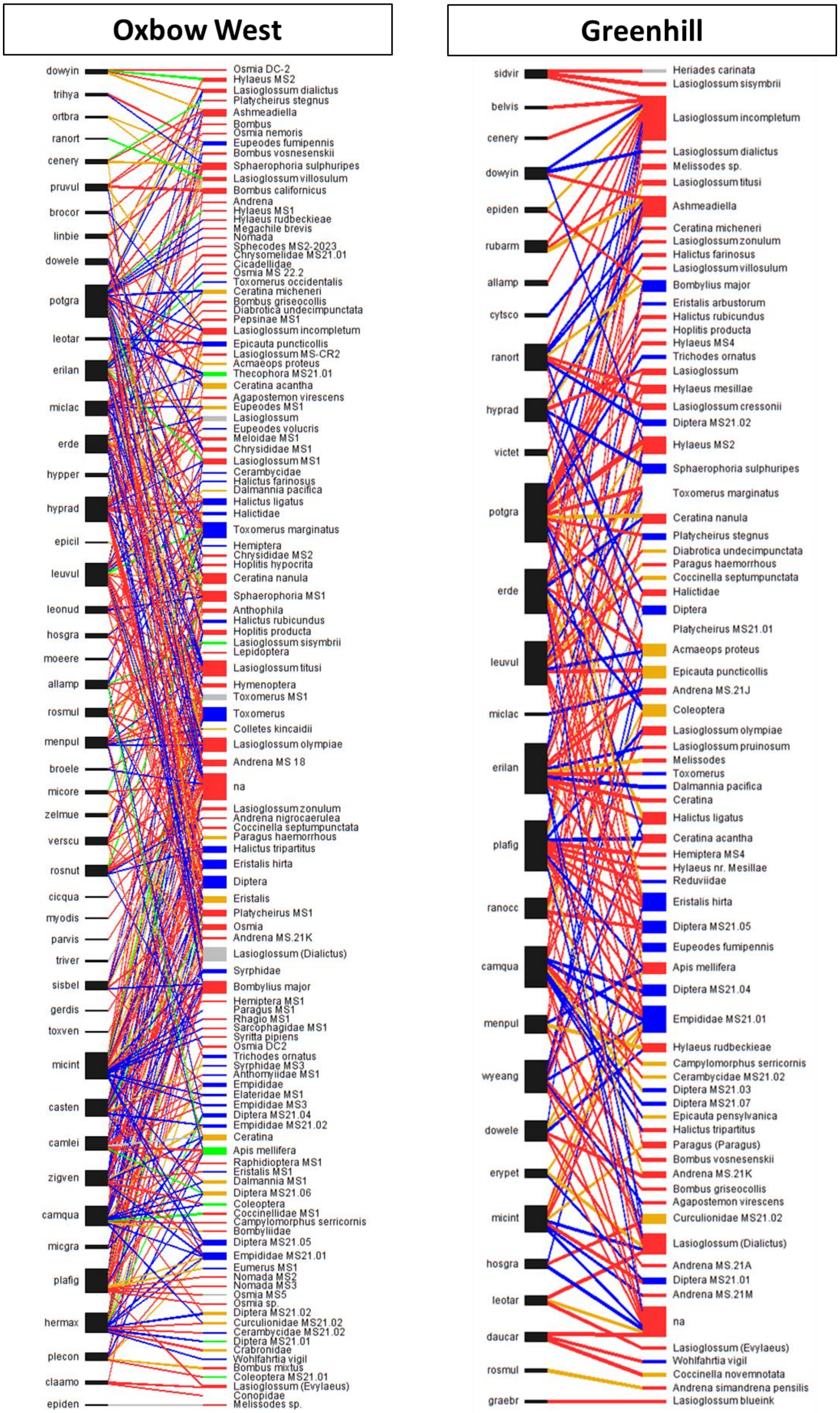

Figure 9. Plant-pollinator networks from 2019 to 2023 for Oxbow West and Greenhill.

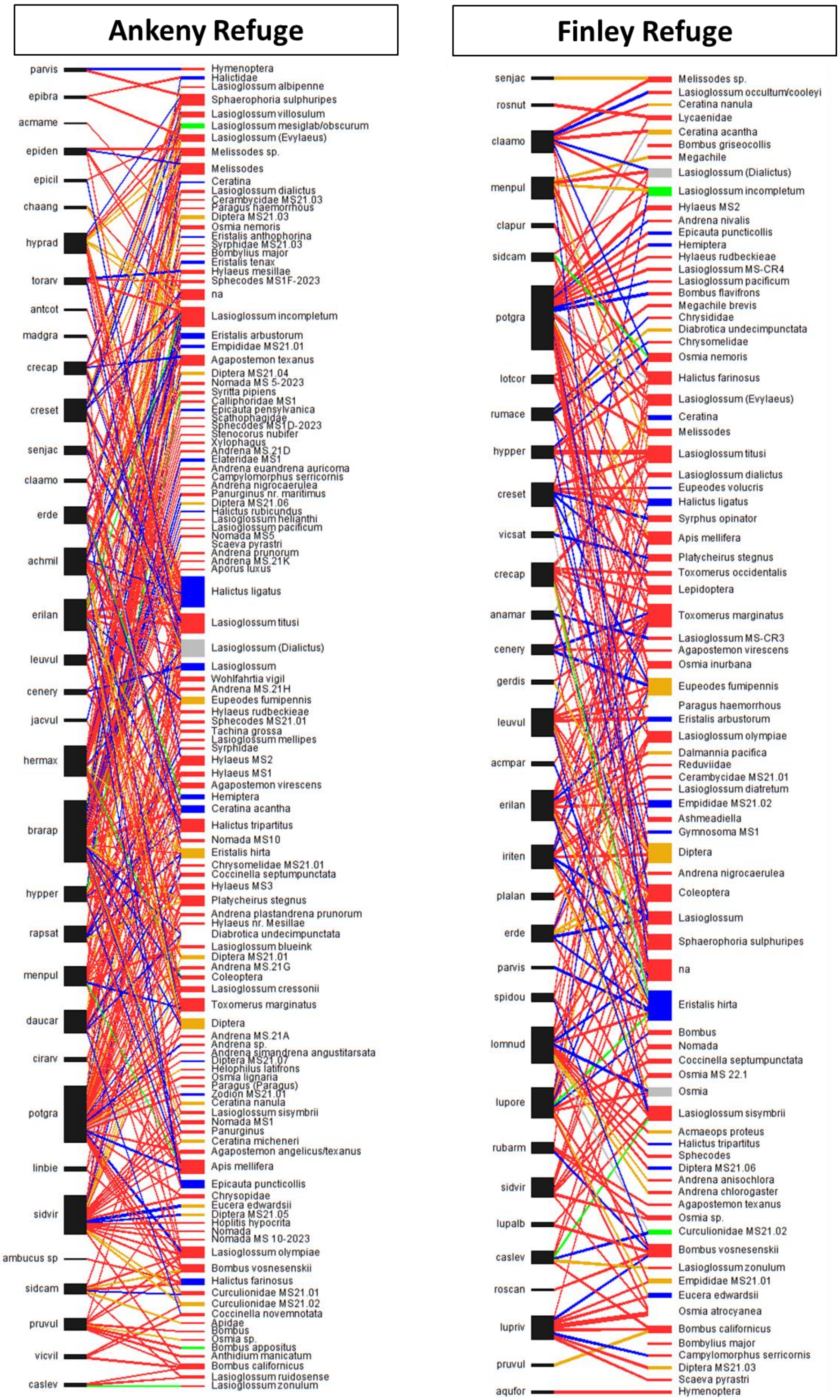

Figure 10. Plant-pollinator networks from 2019 to 2023 for Ankeny Refuge and Finley Refuge.

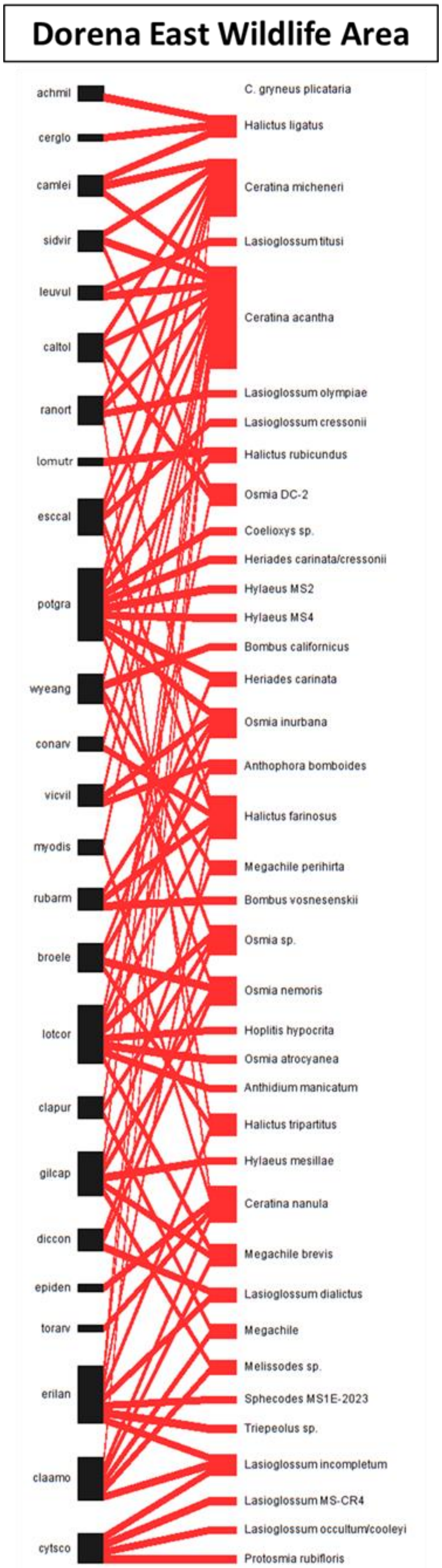

Figure 11. Plant-pollinator network from 2023 for Dorena East Wildlife Area (only bees were identified).

5. DISCUSSION

Work conducted over the last five years demonstrate Willamette daisy is self-incompatible, reliant on pollen from other plants for effective reproduction, which prevents inbreeding and promotes outbreeding (Kaye et al. 2022). Initial results from the 2021 field crossing experiment illustrate this through reduced seed set in no or self-pollination treatments compared to distantly- and locally-crossed pollination treatments. However, there was no difference in germination between the treatments. In 2022, plants grown from experimental seed also showed no difference between treatments in the number of leaves per plant (Mitchell et al. 2023). This may indicate that while seed set is greatly reduced without dispersed pollen, plants from those resulting seeds can experience similar growth. This hypothesis was further supported in 2023 by plants from all treatments experiencing similar reproductive potential, as measured through similar numbers of leaves, flowering stalks, and flowers. So, while none and self-pollinated plants were able to reach similar fitness after two years, distant and locally crossed plants produced much more viable seed that could in turn become surviving plants.

Pollen supplementation trials for golden paintbrush and Kincaid's lupine had mixed results. We believe our trialed methods for golden paintbrush were successful, as our data showed a difference in seed set between the pollen-supplemented and open-pollinated flowers. We used results from our Kincaid's lupine trial to improve methods for the 2024 field season, and believe our 2023 results indicate ineffective pollen transfer using hand manipulation of the flower's piston mechanism and the electric toothbrush. We were also limited by only having a few plants still in flower at the time of the trial, which could have resulted in supplementing with incompatible pollen.

Our results show a clear trend that Willamette daisy is highly dependent on pollinators for seed set and successful reproduction (Mitchell et al. 2023). Over time, we have determined that Willamette daisy pollinator communities varied by site and season. The most abundant taxa to visit Willamette daisy across multiple populations were flies, bees, beetles, wasps, bugs, and butterflies. The most abundant bee species were *Halictus ligatus* and *Lasioglossum sp.* and the most abundant flies were syrphids. During five years of sampling Willamette daisy, We documented 80 separate taxa and 1254 individuals of bee species, and 45 identified taxa and 1401 individuals of fly species. While we captured more flies than bees visiting Willamette daisy, flies are likely not as effective of pollinators, and therefore may not be as important for reproduction. These findings, along with Willamette daisy pollinators' high constancy (Kaye et al. 2022), suggest visitation by bee pollinators can result in quality pollination and increased seed set. The large amount of variation we observed in Willamette daisy pollinator visitors indicates that any single site and season are not representative of all prairies in the Willamette Valley. This result is consistent with our plant-pollinator community networks, showing large variation by site and season over a five-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 Willamette daisy and 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 a 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:

- Willamette daisy is pollen limited, depends on pollinators for successful reproduction, and can experience increased seed set with greater surrounding Willamette daisy floral density.
- Similar experiments conducted for golden paintbrush and Kincaid's lupine may elucidate similar dependencies on prairie pollinator communities.
- 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 sties 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. WILLAMETTE DAISY VISITORS, 2019-2023

Table A3. All insects captured on Willamette daisy (*Erigeron decumbens*) and identified to the lowest possible denomination. Represented taxa were collected from the Willamette daisy observations that were coupled with the four-year pollen supplementation experiment and from the network observations.

Identification Category	Number of Specimens
Acmaeops proteus	15
Acrididae	10
Agapostemon angelicus/texanus	24
Agapostemon texanus	2
Agapostemon virescens	9
Andrena sp.	3
Andrena angustitarsata	2
Andrena MS 1	5
Andrena MS 2	1
Andrena MS 3	32
Andrena MS 4	1
Andrena MS 5	1
Andrena nigrocaerulea	2
Anthidium sp.	3
Anthidium utahense	3
Anthophila (unidentified bee)	1
Aphrophoridae	13
Apis mellifera	8
Aporus luxus	11
Ashmeadiella sp.	15
Bombus vosnesenskii	3
Bombylius sp.	1
Bombylius major	34
Bruchus sp.	4
Campylomorphus serricornis	2
Cephidae	1
Ceratina sp.	4
Ceratina acantha	32
Ceratina micheneri	3
Ceratina nanula	69
Chelonus sp.	1
Chrysididae	2
Chrysolina hyperici	4
Chrysomelidae	2
Cicadellidae	9
Cleptes sp.	1
Coccinella septumpunctata	21
Coelioxys sp.	1
Coelioxys octodentatus	6
Coelioxys rufitarsis	1
Coenagrionidae sp.	1
Coleoptera MS	2
Colletea MS	3
Conopidae sp.	5
Crabronidae	2
Curculionidae MS	13
Cynipoidea sp.	1
Dalmannia MS	1
Dalmannia pacifica	4
Diabrotica undecimpunctata	5
Diptera MS1	9
Diptera MS2	4
Diptera MS3	7
Diptera MS4	14
Diptera MS5	3
Diptera MS6	1

Identification Category	Number of Specimens
Diptera sp.	28
Empididae MS1	11
Empididae MS2	17
Empididae sp.	1
Epicauta puncticollis	31
Eristalis sp.	2
Eristalis arbustorum	10
Eristalis hirta	17
Eristalis stipator	1
Eristalis tenax	3
Eucera edwardsii	2
Eumerus MS	1
Eupeodes sp.	3
Eupeodes fumipennis	197
Eupeodes volucris	4
Formicidae sp.	4
Goniini	15
Halictus confusus	3
Halictus farinosus	13
Halictus ligatus	287
Halictus rubicundus	14
Halictus tripartitus	39
Harmonia axyridis	1
Hemiptera MS	1
Heteroptera sp.	7
Hoplitis producta	7
Hylaeus MS	23
Hylaeus nr. Rudbeckieae	17
Hymenoptera	7
Ichneumonidae sp.	2
Lasioglossum	58
Lasioglossum (Dialictus)	193
Lasioglossum (Evylaeus)	19
Lasioglossum albipenne	4
Lasioglossum cressonii	1
Lasioglossum hyalinum	1
Lasioglossum incompletum	12
Lasioglossum inconditum	1
Lasioglossum mesiglab/obscurum	2
Lasioglossum MS1	1
Lasioglossum MS2	5
Lasioglossum MS3	7
Lasioglossum MS4	1
Lasioglossum MS5	4
Lasioglossum MS6	4
Lasioglossum MS7	2
Lasioglossum occultum/cooleyi	3
Lasioglossum olympiae	46
Lasioglossum pacificum	6
Lasioglossum pruinosum	1
Lasioglossum ruidosense	3
Lasioglossum sisymbrii	19
Lasioglossum titusi	42
Lasioglossum zonulum	2
Lepidoptera	18
Macrorhoptus sp.	1
Megachile brevis	27

Identification Category	Number of Specimens
Megachile montivaga	1
Megachile pascoensis	1
Megachile perihirta	16
Megachile pugnata	2
Melissodes sp.	38
Melissodes (Callimelissodes)	3
Meloidae	3
Nomada sp.	11
Nomada MS1	20
Nomada MS2	1
Nomada MS3	8
Nomada MS4	2
Oestroidea sp.	5
Osmia sp.	13
Osmia atrocyanea	1
Osmia cara	1
Osmia MS2	1
Osmia inurbana	8
Osmia MS	1
Osmia nemoris	14
Paragus sp.	15
Paragus haemorrhous	18
Pentatomidae sp.	3
Plateumaris fulvipes	7
Platycheirus sp.	38
Platycheirus stegnus	82
Pollenia pediculata	1
Pollenia rudis	1
Raphidioptera MS1	2
Reduviidae	11
Scaeva affinis	1
Scaeva pyrastri	9
Sceliphron caementarium	1
Sciomyzidae sp.	1
Sphaerophoria sp.	11
Sphaerophoria sulphuripes	243
Sphecodes sp.	1
Stelis sp.	1
Syritta pipiens	1
Syrphidae	2
Syrphus opinator	3
Syrphus torvus	1
Systoechus sp.	2
Tachina grossa	5
Tachinidae sp.	2
Tettigoniidae sp.	3
Thecophora modesta	2
Thecophora sp.	11
Thecophora propinqua	1
Thysanoptera	10
Toxomerus sp.	7
Toxomerus marginatus	464
Toxomerus occidentalis	77
Toxonagria sp.	1
Trichodes ornatus	1
Vespidae sp.	2
Wohlfahrtia virgil	2
Zodion sp.	9