

Pollination biology and ecology of Willamette Valley prairies and Willamette daisy (*Erigeron decumbens*)



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Land Management, Northwest District

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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: A Halictid (*Lasioglossum* sp.) visiting Willamette Daisy (*Erigeron decumbens*) in the Willamette Valley. Photograph by J. Christina Mitchell.

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Pollination biology and ecology of Willamette Valley prairies and Willamette daisy (*Erigeron decumbens*)

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 4-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.

From 2019 to 2022, 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. In 2022, we conducted pollen supplementation experiments to determine the effect of pollinators on Willamette daisy seed set, and field observations to understand the role of insect pollinators and other flowering prairie plants on Willamette daisy population growth and survival.

Willamette daisy plants were grown out from the 2021 field crossing experiment and we found greater survivorship in distantly- and locally-crossed plants. Treatments had a large range in the number of leaves per plant, and the total number of leaves differed between the distantly and locally crossed treatments, but not between the distantly or locally crossed and the none and self-crossed treatments. Pollen supplementation increased Willamette daisy seed set 20.4% over open-pollinated controls; a similar trend, but bigger difference, compared to previous years. Willamette daisy relies on pollination for successful reproduction, and pollen limitation varies by site. At three of five sites, greater surrounding Willamette daisy floral density correlated with increased seed set. Pollinator communities of Willamette daisy were more similar at Ankeny Refuge, Fisher Butte, Finley Refuge, Jefferson Farm, Kingston Prairie, and Oxbow West, compared to Calapooia, Greenhill, and Speedway. 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.

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*, Nutt. [Asteraceae]; 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. Previous observations of pollinators on Willamette daisy were restricted to a few sites and a single season, but suggest a diversity of insects are visitors, including moths, butterflies, flies, and solitary bees (Jackson 1996). It is likely that these 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). 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.

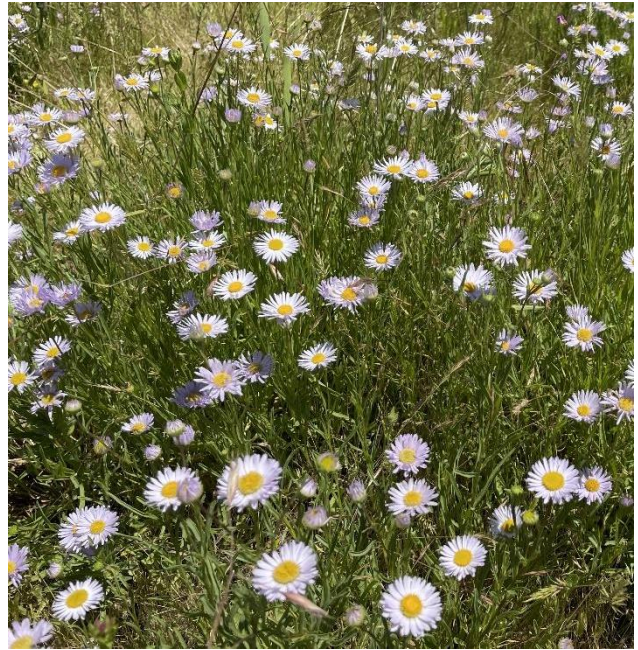


Figure 1. Patch of Willamette daisy (*Erigeron decumbens*) in a Willamette Valley prairie, OR. Photograph by J. Christina Mitchell.

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 2022, we conducted field work at Ankeny and Finley National Wildlife Refuges (US Fish and Wildlife Service, FWS), Fisher Butte (USACE), Greenhill and Oxbow West (BLM), and Jefferson Farm and Kingston Prairie (private; Figure 2). Between 2019 and 2021, we also conducted work at Calapooia (private) and Speedway (BLM).

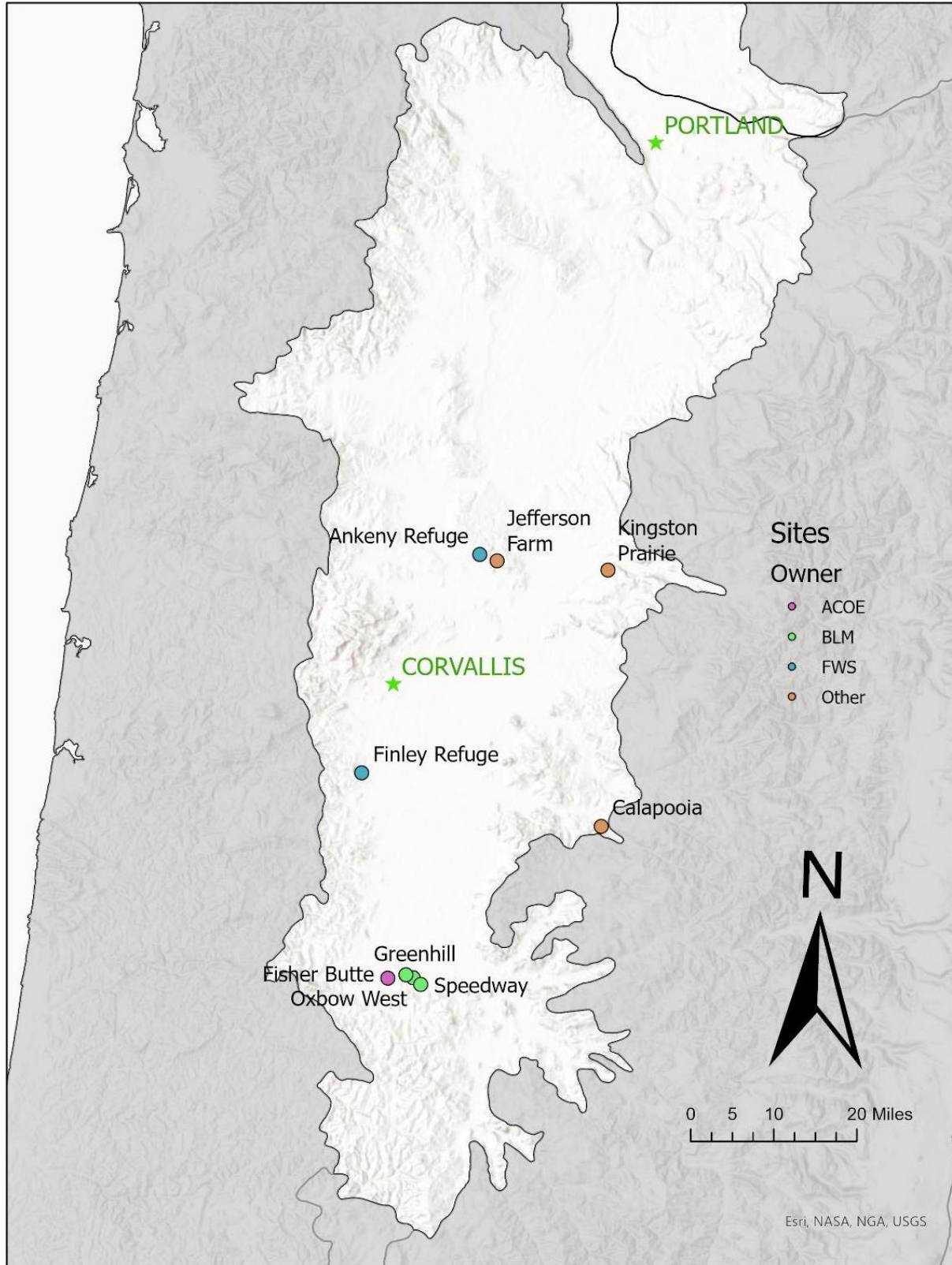


Figure 2. Location of sites used for pollinator research between 2019 and 2022, across the Willamette Valley Ecoregion, Oregon, USA.

2. GOALS AND OBJECTIVES

The goals of this project are to better understand the mating system and pollination ecology of Willamette daisy (*Erigeron decumbens*), and to document pollinators at prairie sites and pollinators' association with Willamette daisy.

Specific objectives include:

- 1) Determine whether the breeding system of Willamette daisy is autogamous, or affected by inbreeding or outbreeding depression;
- 2) Determine whether Willamette daisy seed production is pollinator-limited through a pollen supplementation experiment; and
- 3) Extensively sample the Willamette Valley prairie pollinator community, with a focus on those that visit Willamette daisy, but including flowering plants throughout the season.

3. METHODS

3.1. Project Activities

We performed research at nine sites since this project began in 2019 (Table 1). In 2022, we collected data from Ankeny Refuge, Finley Refuge, Fisher Butte, Greenhill, Jefferson Farm, Kingston Prairie, and Oxbow West. Additionally, we worked to integrate Oregon and Washington prairie datasets and collaborate on species and morphospecies identifications. We also prepared materials to be submitted to the Oregon State Arthropod Collection (OSAC) and developed and began testing regional pollinator keys. Details for experiments not conducted in 2022 can be found in previous reports (Kaye et al. 2022).

Table 1. Willamette Valley pollinator study sites and research activities, except the breeding biology (crossing) study.

Site	Owner	Pollen Supplementation				Willamette Daisy Hand-netting				Behavioral Observations				Network Sampling				Transect Sweeps & Pan Traps			
		2019	2020	2021	2022	2019	2020	2021	2022	2019	2020	2021	2022	2019	2020	2021	2022	2019	2020	2021	2022
Ankeny Refuge	USFWS					X	X	X	X	X					X ³	X	X		X		
Calapooia	private		X			X	X												X		
Finley Refuge	USFWS	X	X	X	X	X	X	X	X	X					X ³	X	X		X		
Fisher Butte	USACE	X ¹	X	X	X	X	X	X	X	X					X ²	X ²	X	X		X	
Greenhill	BLM							X	X							X	X				
Jefferson Farm	private	X	X	X	X	X	X	X	X	X					X ²	X ²	X	X		X	
Kingston Prairie	Greenbelt Land Trust	X	X	X	X	X	X			X											
Oxbow West	BLM	X	X	X	X	X	X	X	X	X					X ²	X ²	X	X		X	
Speedway	BLM					X	X			X											

X¹ indicates self-fertilization (caging) was also performed at this site in this year.

X² indicates data were collected and funded jointly by the Center for Natural Lands Management and IAE (Waters 2021).

X³ 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 containers 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. The following measurements were taken for each surviving plant on July 8, 2022 (Figure 3): length of longest leaf (cm), total number of leaves, whether the plant was flowering, and if so, the length of flowering stalk(s), total number of open flowers, and estimated proportion of open to closed or developing buds. We plan to measure all surviving plants again in July 2023.



Figure 3. Willamette daisy plants from 2021 field crossing experiment, prior to measurement in July 2022. Photograph by J. Christina Mitchell.

Analyses

The no-pollination and self-pollination treatments had low survivorship (N = 14) 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 171 plants measured, we used 25 representatives from the distant cross, 25 representatives from the local cross, and 10 representatives from the none and self-crosses to compare treatments. We quantified plant growth using the total number of leaves 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

Willamette daisy flower heads are a capitula of 100-200 individual florets, each capable of using a ‘plunger’ mechanism to push dehisced pollen up with the growing stigma, which then becomes receptive. The florets open serially, from the outside of the capitula towards the center, over a period of 5-10 days depending on site conditions (Kaye et al. 2022). We conducted a pollen supplementation experiment to determine whether Willamette daisy is pollen limited.

In 2022, we conducted pollen supplementation experiments at Finley Refuge, Fisher Butte, Jefferson Farm, Kingston Prairie, and Oxbow West (Table 1). At each site, five sets of four plants (quartets) were randomly selected. For each plant, two flower heads (capitula) per plant were randomly selected and were either given pollen supplementation or left as unmanipulated open pollinated controls, resulting in 20 plants and 40 capitula per site. To reduce the likelihood of an incompatible donor, we collected pollen from plants separated by at least 3 m in each quartet in an alternating pattern (Figure 4b), and each recipient capitulum received pollen from three other plants. In June 2022, we provided pollen to nearly all florets in the capitulum by conducting three supplementation events, when capitula were ~30% open, ~60% open, and ~90% open. In 2022, supplementation events occurred on days two, four, and seven of each flower head’s anthesis. We used ‘bee brushes’ to simulate natural pollination; we defrosted, washed, and dried honeybees (*Apis mellifera*), then glued them belly side out to the end of toothpicks. To collect and deposit pollen, bee brushes were lightly dragged over multiple flower heads in a forward

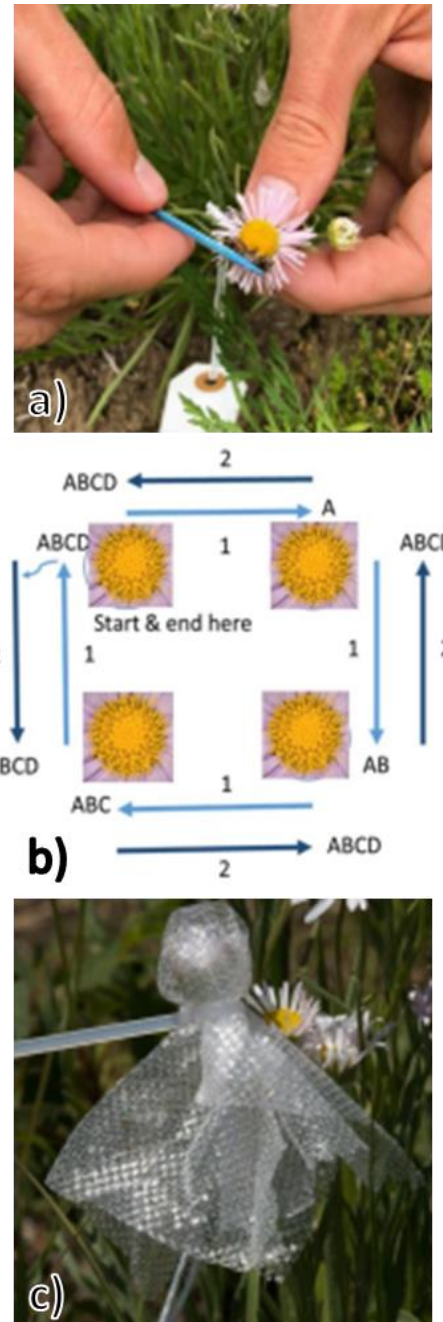


Figure 4. Depictions of (a) a bee stick in use, (b) the experimental pollination design, and (c) a bagged flower head.

and reverse sequence (Figure 4a). Bee brushes were discarded after each use. After hand pollination, all individual flower heads were covered with breathable plastic material and tied below the flower head to prevent dispersal loss of seeds (Figure 4c) and retrieved after three weeks. Seeds of each capitula were counted and categorized as either ‘filled’ or ‘unfilled’ by visual inspection with a dissecting scope.

Surrounding Willamette Daisy Density

In 2022, we added an additional study element by measuring surrounding Willamette daisy floral density to assess whether baseline pollination was density dependent. At each site, we measured 4-m outwards from the center of each experimental plant (A, B, C, D) in multiple directions and created a 4-m radius sampling circle. Within this sampling circle, we counted all open Willamette daisy flowers.

Analyses

We tested for an effect of pollen supplementation on seed set across all sites with linear mixed-model regression (R Core Team 2022). For each site and year (2019-2022), we assessed the change in seed set with paired t-tests. In 2022, we also determined whether the density of surrounding Willamette daisy flowers influenced Willamette daisy seed set. We correlated the number of filled seeds, from supplemented and non-supplemented flowers, with surrounding Willamette daisy floral density, because we expected increased floral density would increase seed set in naturally pollinated ‘control’ flowers as well as supplemented ‘experimental’ flower. We square root-transformed data to improve normality.

3.4. Prairie Plant and Pollinator Community Sampling

Willamette Daisy Pollinator Observations

In 2022, we continued efforts to document potential pollinators of Willamette daisy (Table 1), using hand-netting techniques on patches of Willamette daisy in sites where it occurs. We observed pollinators on Willamette daisy patches at Ankeny Refuge, Finley Refuge, Fisher Butte, Jefferson Farm, and Oxbow West. As the cool and wet spring seemed to delay phenology in 2022, we began Willamette daisy observations and pollinator sampling on June 21st, and continued sampling until July 7th. Time spent hand-netting was split into 30–60-minute periods across sites and over multiple days to avoid weather bias. We attempted to sample each site for a total of six hours. Insects were only netted if they were observed to visit reproductive parts (anthers, stigma) of the Willamette daisy flower head (Figure 5c).

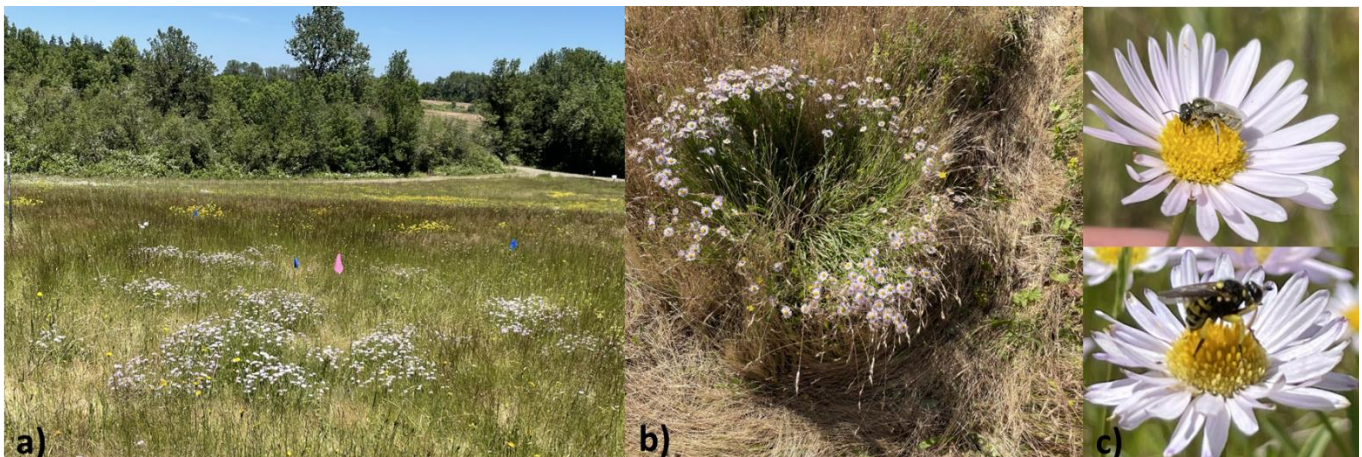


Figure 5. Examples of a Willamette daisy (a) patch, (b) plant with worn observation path, and (c) visitors.

Plant-pollinator Networks

In 2022, we continued efforts to quantify the prairie pollinator community using plant-pollinator network surveys (Waters 2021), as studying Willamette daisy pollinators in isolation neglects the broader ecological context. We performed floral and pollinator network surveys at Ankeny Refuge, Finley Refuge, Fisher Butte, Greenhill, Jefferson Farm, and Oxbow West. In 2022, we began sampling prairies on May 15th and ended sampling efforts on July 13th. We performed five sampling events at all sites, except for Greenhill, where scheduling delays accommodated only three sampling events. 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 Willamette daisy pollinators throughout the season. These species lists were then used to target observations and hand-netting efforts of pollinators throughout the flowering season. Similar to the Willamette daisy pollinator observations, 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.

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). 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 *Ceratina* sp. and *Lasioglossum* sp., with other keys in active development.

Analyses

To compare Willamette daisy pollinator communities among prairie sites, we created a similarity matrix of pollinator species captured at each site, summed across years (2019-2022). To visualize relationships between each site's Willamette daisy pollinator community, we conducted nonmetric multidimensional scaling (NMDS) using the metaMDS function in the package vegan with the Bray–Curtis distance measure and random starting configuration (Oksanen et al. 2020). NMDS allows for visual comparison of community composition and determination of which communities are most, or least, similar to one another.

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 (2019-2022). 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). By 2022, there was some variation in survivorship; there were 81 'distant' plants, 76 'local' plants, nine 'self' plants, and five 'none' plants. Out of 171 measured plants measured, nine were flowering in July 2022. Of those nine, two were distantly crossed, six were locally crossed, and one was self-crossed.

The total number of leaves differed between the distantly and locally crossed treatments ($p = 0.007$), but not between the distantly or locally crossed and the none and self-crossed treatments ($p = 0.140$ and $p = 0.892$ respectively; Figure 6). Each treatment had a large range in the number of leaves per plant. Distantly crossed plants ranged from 19-144 leaves per plant (mean 66.1), locally crossed plants ranged from 20-111 leaves per plant (mean 52.3), and no cross or self-crossed plants ranged from 30-86 leaves per plant (mean 56.9).

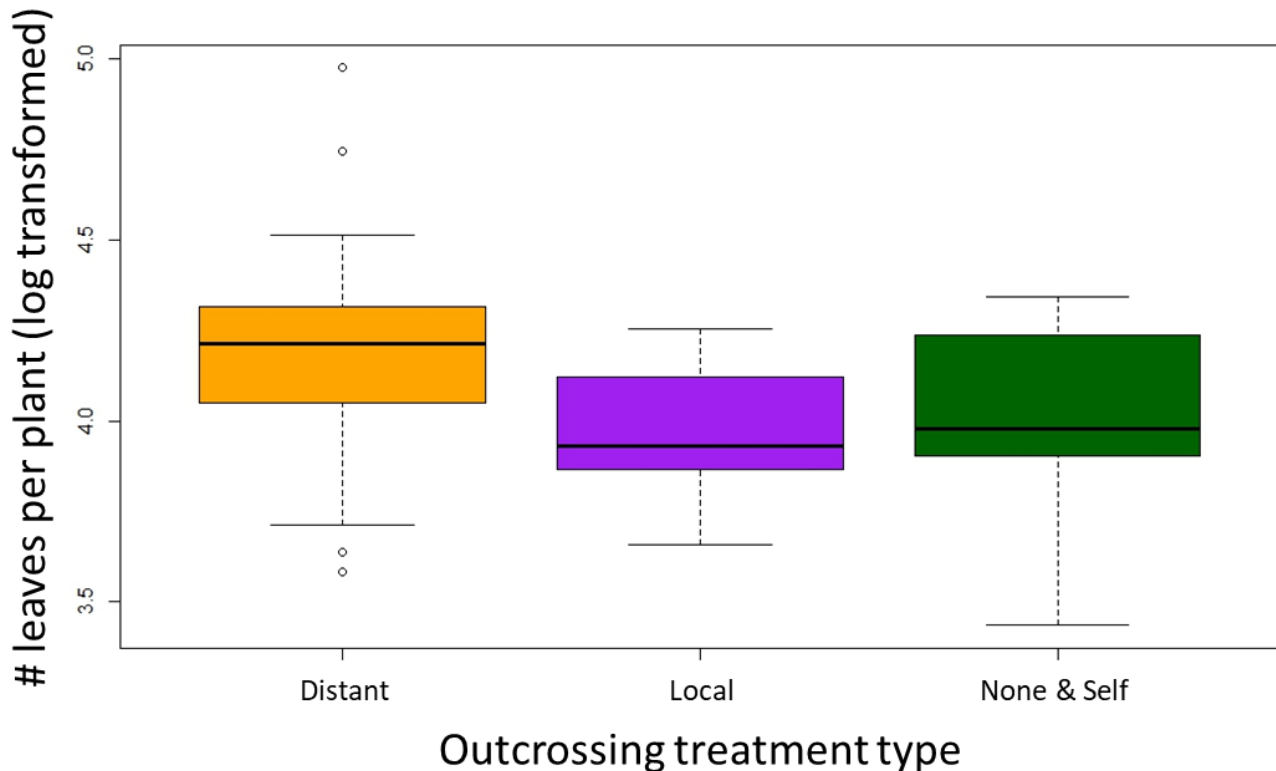


Figure 6. Mean number of leaves, log transformed, per type of outcrossing treatment: distantly crossed (left, orange, $N = 25$); locally crossed (middle, purple, $N = 25$); and none and self-crossed plants (right, green, $N = 10$).

4.2. Pollen Supplementation Experiments

Based on a linear regression with site and year as nested random effects, pollen supplementation increased seed set. Supplementation resulted in 6.5% increased seed set in 2019, 6.3% increased seed set in 2020, 13.2% increased seed set in 2021, and 20.4% increased seed set in 2022. Consistent with previous reports (Kaye et al. 2022), the amount of increase in seed set varied by site and year (Figure 7).

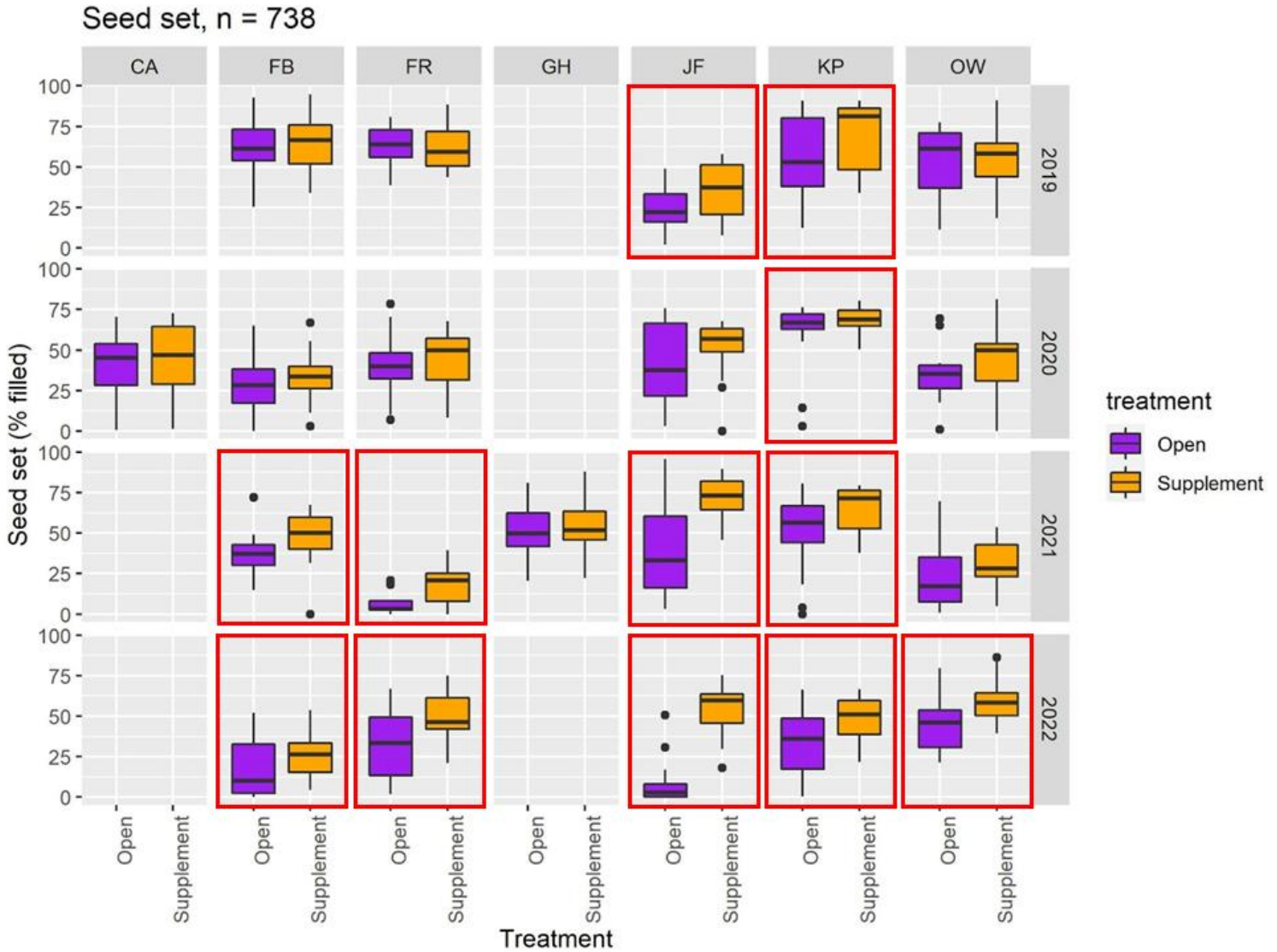


Figure 7. Seed set (the proportion of filled seeds) at each site and year. Results from open-pollinated flowers are shown in purple and supplemented, treatment flowers are shown in orange. Treatment plants received two doses of pollen supplementation in 2019-2020 and three doses in 2021-2022. Red boxes outline specific sites and years where pollen supplementation significantly ($\alpha < 0.05$) increased seed set. Sites included Calapooia (CA), Fisher Butte (FB), Finley Refuge (FR), Greenhill (GH), Jefferson Farm (JF), Kingston Prairie (KP), and Oxbow West (OW).

Surrounding Willamette Daisy Density

In 2022, we compared the total number of Willamette daisy flowers within a 4-m radius to the number of filled seeds in each experimental flower from the pollen supplementation experiment. Most experimental plants were surrounded by < 500 Willamette daisy flowers, though some were surrounded by 2000 flowers or more. At Fisher Butte ($p = 0.00001$, $r^2 = 0.41$), Finley Refuge ($p = 0.01$, $r^2 = 0.16$), and Kingston Prairie ($p = 0.02$, $r^2 = 0.14$), the amount of Willamette daisy filled seeds increased with increased surrounding floral density (Figure 8).

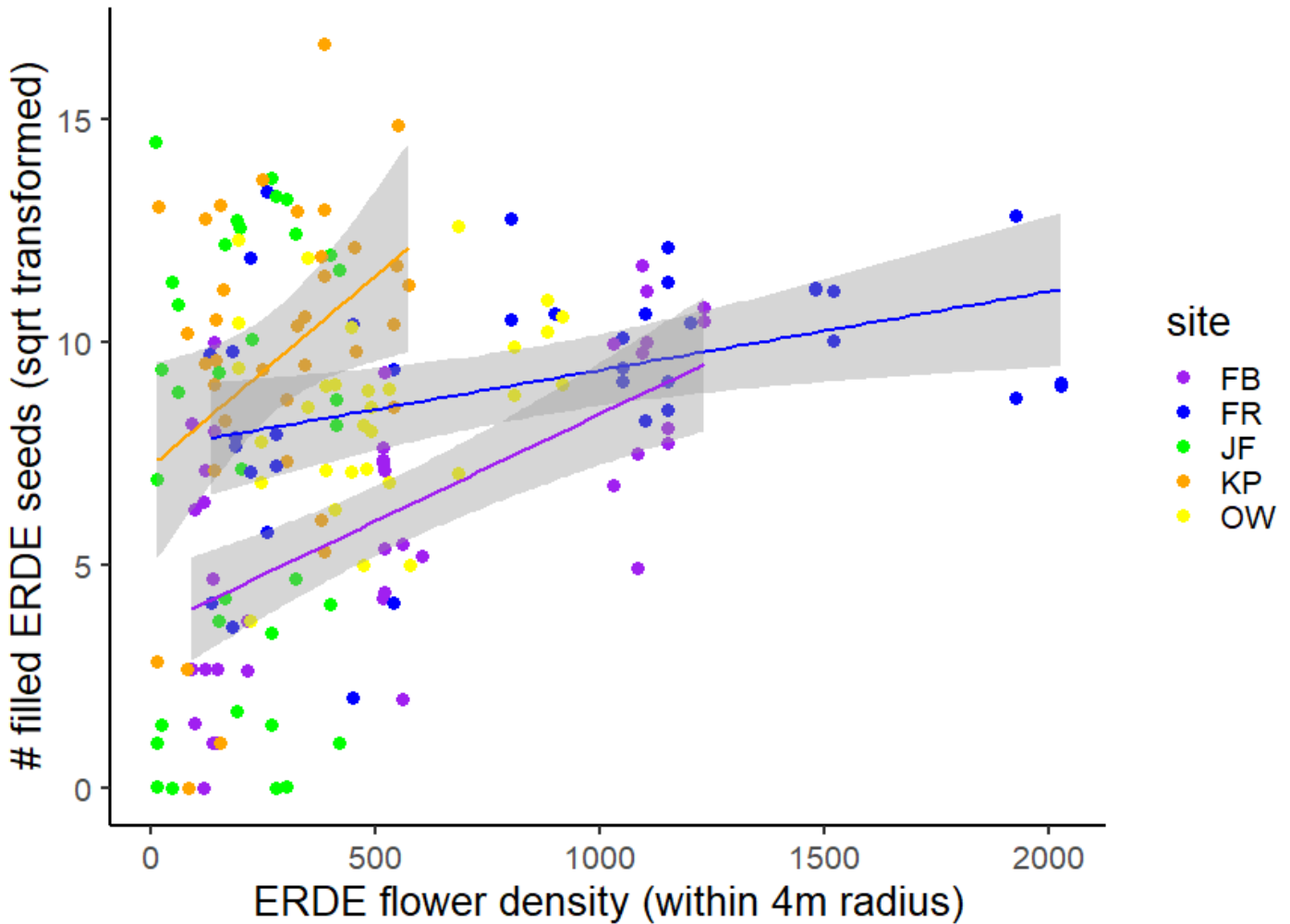


Figure 8. Density of Willamette daisy (ERDE) flowers within a 4-m radius of pollen supplemented plants compared to the number of filled seeds (square root-transformed). Sites included Fisher Butte (FB), Finley Refuge (FR), Jefferson Farm (JF), Kingston Prairie (KP), and Oxbow West (OW). Gray shading represents 95% confidence intervals and included only for significant relationships.

4.3. Prairie Plant and Pollinator Community Sampling

Willamette Daisy Pollinator Observations

Across years, there is variation in the similarity of Willamette daisy pollinator communities among sites (Figure 9). NMDS results (stress = 0.11) indicate that Ankeny Refuge, Finley Refuge, Fisher Butte, Jefferson Farm, Kingston Prairie, and Oxbow West are more similar to each other than Calapooia, Greenhill, and Speedway. Between 2019 and 2022, we captured 2713 insects visiting Willamette daisy flowers. The top six most abundant groups accounted for 51.8% of all insects captured, and by abundance, were syrphid flies (*Toxomerus marginatus*, *Sphaerophoria sulphuripes*, and *Eupeodes fumipennis*, N = 875), *Lasioglossum* sp. (N = 271), and *Halictus ligatus* (N = 258; Appendix A).

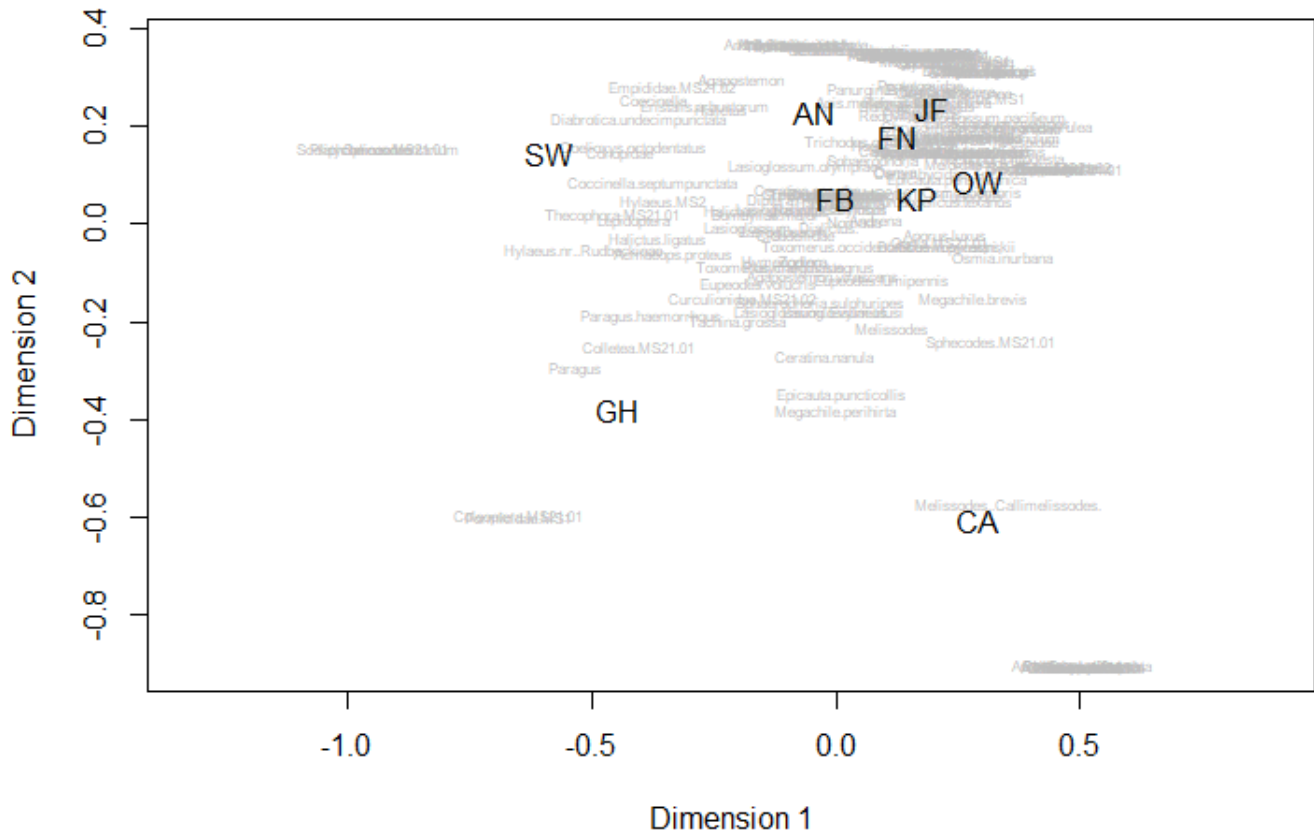


Figure 9. Nonmetric multidimensional scaling of Willamette daisy pollinator communities at each site, across years (2019-2022). Sites included Ankeny Refuge (AN), Calapooia (CA), Fisher Butte (FB), Finley Refuge (FN), Greenhill (GH), Jefferson Farm (JF), Kingston Prairie (KP), Oxbow West (OW), and Speedway (SW).

Plant-pollinator Networks

In 2022, we observed a total of 65 plant species and 1 172 pollinator visits across six sites. Finley Refuge (300), Fisher Butte (256), and Ankeny Refuge (216), had more pollinator visits than Greenhill (125), Oxbow West (126), and Jefferson Farm (149). We identified captured pollinators to 83 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 2022 collection, as we are in the process of 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 2022. 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. Red lines represent bee species, blue lines represent flies, green lines represent wasps, gold lines represent beetles, and gray lines represent other taxa.

Complexity can vary greatly depending on the amount of time observed; these network diagrams show complexity over a 4-year period, compared to network diagrams representing a single year of complexity (Kaye et al. 2022). Compared to observations from a single year (2021), network diagrams representing Fisher Butte and Oxbow West's 2019-2022 plant-pollinator network were much more complex and demonstrated the large influence of flies, particularly *Toxomerus sp.*, at these sites (Figure 10). Even observed over a longer period of time, Ankeny Refuge remains more complex than Finley Refuge. The plant-pollinator network at Ankeny Refuge also has a larger component of bees, compared to the majority of syrphid flies found in the plant-pollinator network at Finley Refuge (Figure 11). In 2021, the plant-pollinator network at Greenhill looked similar in structure to the network at Jefferson Farm, though it had a greater diversity of pollinators (Kaye et al. 2022). When considering 2019-2022 plant-pollinator networks, however, Jefferson Farm had a much more complex and diverse network compared to Greenhill (Figure 12). It is important to note that the Greenhill network is based on two years of data instead of the four collected from Jefferson Farm, but a difference remains.

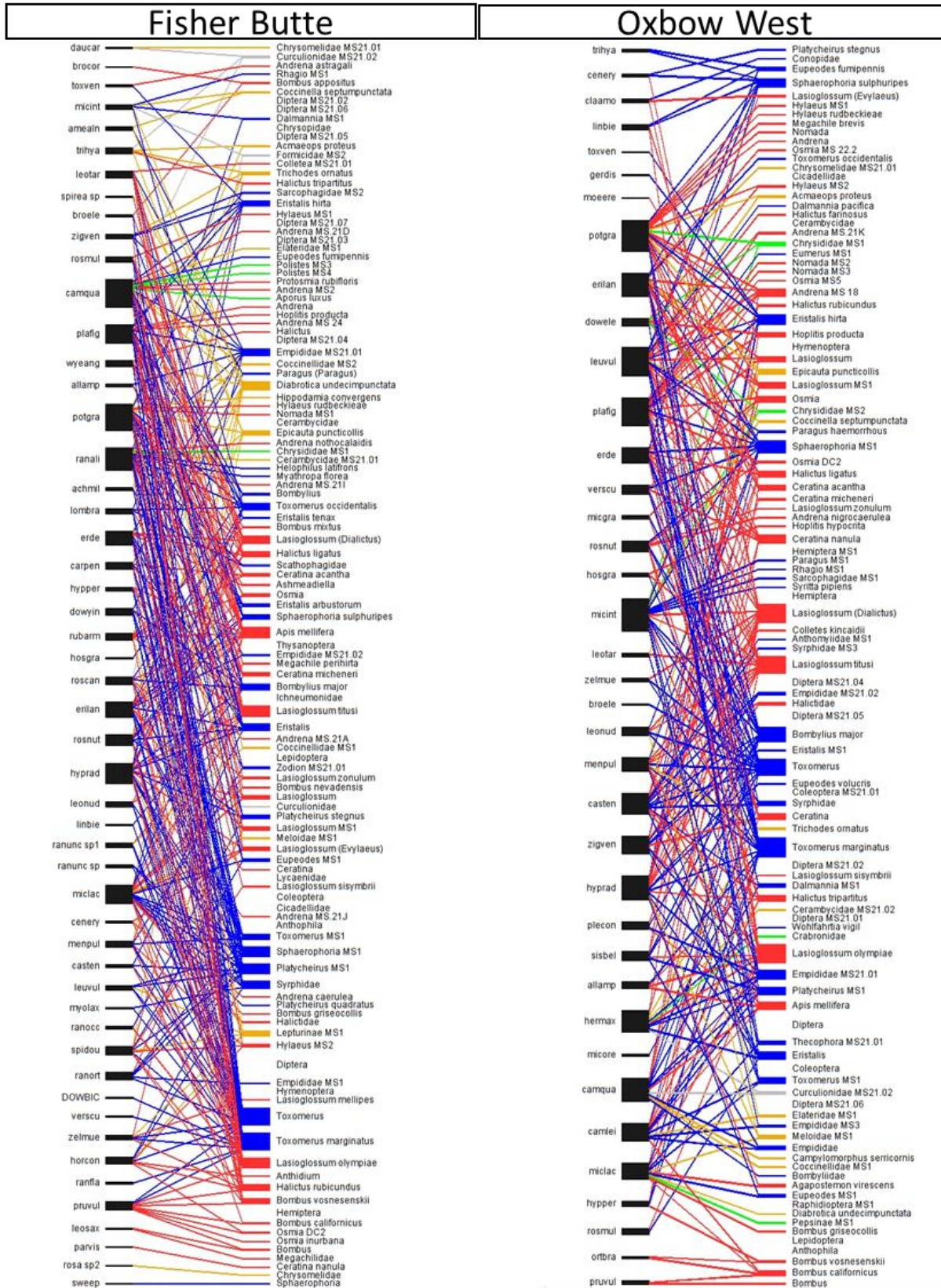


Figure 10. Plant-pollinator networks from 2019 to 2022 for Fisher Butte and Oxbow West. Red = bees, blue = flies, green = wasps, gold = beetles, and gray = other taxa.

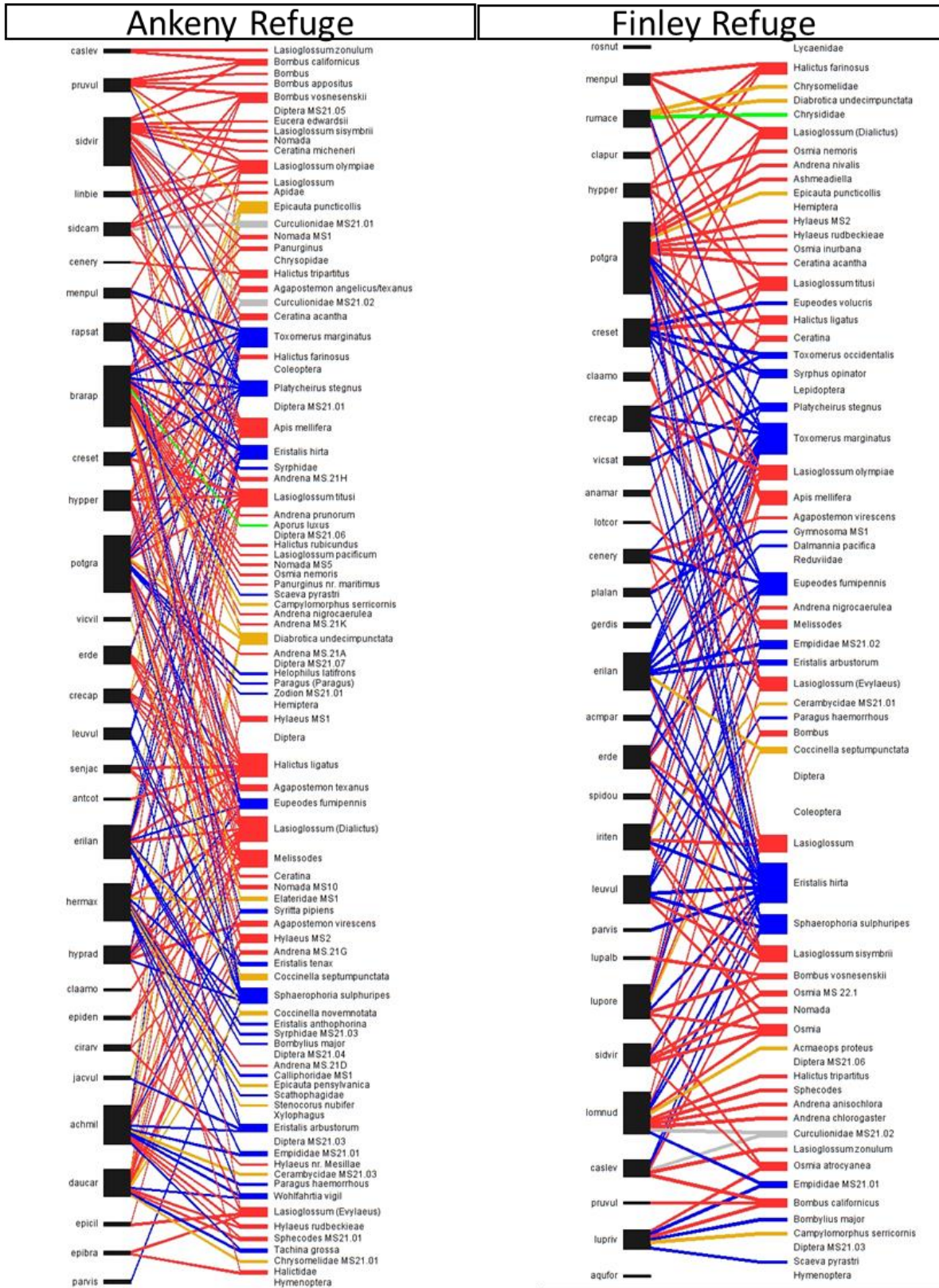


Figure 11. Plant-pollinator networks from 2019 to 2022 for Ankeny Refuge and Finley Refuge. Red = bees, blue = flies, green = wasps, gold = beetles, and gray = other taxa.

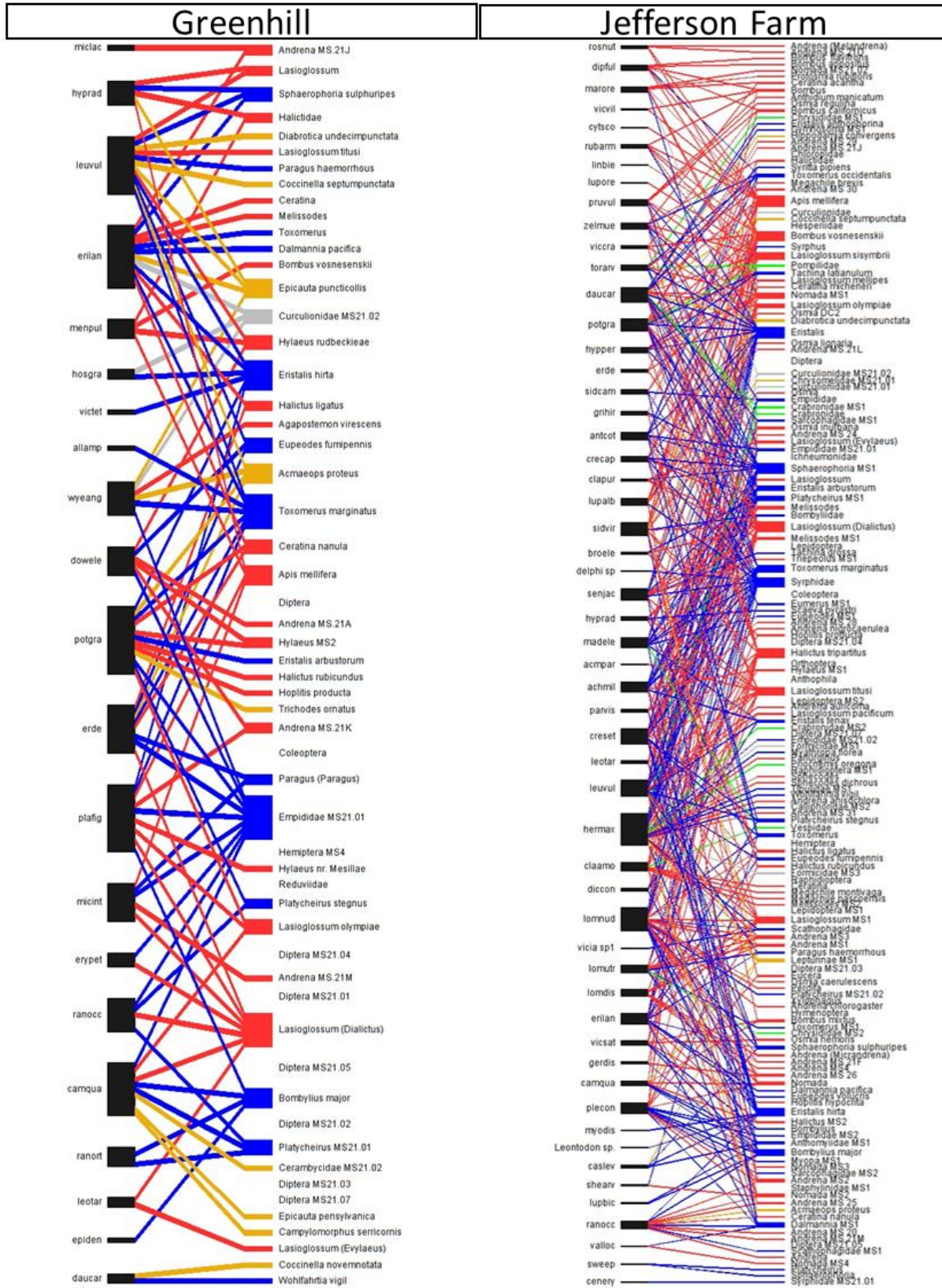


Figure 12. Plant-pollinator networks from 2019 to 2022 for Greenhill and Jefferson Farm. Red = bees, blue = flies, green = wasps, gold = beetles, and grey = other taxa.

5. DISCUSSION

Work conducted over the last four 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. This may indicate that while seed set is greatly reduced without dispersed pollen, plants from those resulting seeds can experience similar growth. Most plants measured in 2022 were not yet reproductive, and measurements in 2023 may further elucidate whether this characteristic varies between treatments or not.

Our results show a clear trend that Willamette daisy is highly dependent on pollinators for seed set and successful reproduction. The difference in seed set between pollen-supplemented and open-pollinated Willamette daisy capitula was greater in 2022 (20.4%) compared to previous years (13.2% in 2021, 6.3% in 2020, and 6.5% in 2019). In 2022, we were also able to determine an influence of available Willamette daisy pollen, quantified by floral density, on the success of seed set. These results suggest management activities that maximize the abundance of effective pollinators and increase the density of Willamette daisy patches or plantings will benefit Willamette daisy survival.

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. We documented 51 identified taxa and 1155 individuals of bee species, and 38 identified taxa and 1342 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 pollinator visitors indicate 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. 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 and depends on pollinators for successful reproduction.
- Willamette daisy seed set can increase with greater surrounding Willamette daisy floral density.
- Establishing functioning pollinator communities for Willamette daisy 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.

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APPENDIX A. IDENTIFICATIONS AND ABUNDANCES OF WILLAMETTE DAISY POLLINATORS

Identification of Willamette daisy visitor	Total abundance (2019-2022)
<i>Acmaeops proteus</i>	14
Acrididae	10
<i>Agapostemon angelicus/texanus</i>	21
<i>Agapostemon virescens</i>	8
<i>Andrena</i> sp.	42
<i>Andrena angustitarsata</i>	2
<i>Andrena nigrocaerulea</i>	1
<i>Anthidium</i> sp.	3
<i>Anthidium utahense</i>	3
<i>Apis mellifera</i>	8
<i>Aporus luxus</i>	11
<i>Ashmeadiella</i>	6
<i>Bombus</i> sp.	1
<i>Bombus vosnesenskii</i>	2
<i>Bombylius</i> sp.	1
<i>Bombylius major</i>	24
<i>Campylomorpha serricornis</i>	2
<i>Ceratina</i> sp.	19
<i>Ceratina acantha</i>	30
<i>Ceratina micheneri</i>	1
<i>Ceratina nanula</i>	48
Chrysididae	2
<i>Chrysolina hyperici</i>	4
Chrysomelidae	8
Cicadellidae	9
<i>Coccinella septempunctata</i>	21
<i>Coelioxys</i> sp.	1
<i>Coelioxys octodentatus</i>	6
<i>Coelioxys rufitarsis</i>	1
Coleoptera	6
Colletidae	3
Conopidae	4
Crabronidae	2
Curculionidae	13
<i>Dalmannia pacifica</i>	3
<i>Diabrotica undecimpunctata</i>	5
Diptera	83
Empididae	25

Epicauta puncticollis	30
Eristalis arbustorum	10
Eristalis hirta	10
Eristalis stipator	1
Eristalis tenax	3
Eucera edwardsii	1
Eumerus sp.	1
Eupeodes sp.	3
Eupeodes fumipennis	186
Eupeodes volucris	4
Formicidae	1
Gonia sp.	13
Goniini	2
Halictidae	9
Halictus farinosus	13
Halictus ligatus	258
Halictus rubicundus	19
Halictus tripartitus	37
Harmonia axyridis	1
Hemiptera	21
Hoplitis producta	7
Hylaeus sp.	17
Hylaeus nr. Rudbeckieae	22
Hymenoptera	15
Ichneumonidae	2
Lasioglossum sp.	96
Lasioglossum (Dialictus)	175
Lasioglossum (Evylaeus)	24
Lasioglossum olympiae	43
Lasioglossum pacificum	6
Lasioglossum sisymbrii	19
Lasioglossum titusi	40
Lasioglossum zonulum	3
Lepidoptera	13
Megachile brevis	26
Megachile montivaga	1
Megachile pascoensis	1
Megachile perihirta	16
Megachile pugnata	2
Melissodes sp.	31
Melissodes (Callimelissodes)	3
Nomada sp.	39
Odonata	1
Orthoptera	3

Osmia sp.	16
Osmia atrocyanea	1
Osmia cara	1
Osmia inurbana	8
Osmia nemoris	13
Paragus sp.	14
Paragus haemorrhous	18
Pentatomidae	2
Platycheirus sp.	38
Platycheirus stegnus	82
Pollenia pediculata	1
Pollenia rudis	1
Raphidioptera	2
Reduviidae	11
Scaeva pyrastris	9
Sceliphron caementarium	1
Sphaerophoria sp.	7
Sphaerophoria sulphuripes	241
Sphecodes sp.	1
Stelis sp.	1
Syrpitta pipiens	1
Syrphidae	2
Syrphus opinator	3
Syrphus torvus	1
Tachina grossa	5
Tachinidae	2
Thecophora modesta	2
Thecophora sp.	9
Thecophora propinqua	1
Toxomerus marginatus	448
Toxomerus occidentalis	77
Toxonagria sp.	1
Trichodes ornatus	1
Vespidae	2
Wohlfahrtia virgil	2
Zodion sp.	7