THESIS

GREEN ROOF EFFECTS ON FLORAL PHENOLOGY AND FLORAL NECTAR RESOURCES

Submitted by

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ABSTRACT

GREEN ROOF EFFECTS ON PLANT GROWTH, FLORAL PHENOLOGY, AND FLORAL NECTAR RESOURCES

This study investigates the potential for green roofs to support pollinator diversity and abundance in urban ecosystems through the altered floral phenology and floral abundance of plants. I compare floral phenology and the floral abundance of green roof plants to plants grown at grade on the Front Range in Fort Collins, Colorado, and how these changes may affect pollinator biodiversity in urban ecosystems. I employed an independent block design, with one green roof and one ground-level garden, approximately 120 meters apart, with replicate plants of 4 species at each garden. I found the abundance of flowers to be variable, depending on the plant species. However, all species of plants tested bloomed earlier when grown on the green roof than when grown at grade. Pollinator abundance and diversity was low at both study locations.

Nectar quantity and quality are diverse across a landscape and affect the health and behavior of some pollinators. I evaluated nectar volume and nectar sugar concentration between plant replicates grown on a green roof and grown at grade. Volume was measured *in situ* and sugar concentration was measured both in situ using a refractometer and, in a laboratory, using UPLC-RI. We found that there was no clear difference between nectar volumes of plants grown on the green roof and at grade while nectar sugar concentrations were generally higher in green roof plants.

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1.1 Introduction

Urban ecosystems rely on pollination to produce fruits, vegetables, and seeds for both human and wildlife consumption. However, urbanization alters plant-pollinator interactions in ways that may be detrimental (Potts et al. 2016; Ollerton 2011). North America, and the rest of the globe, are experiencing unprecedented insect decline across many taxa (Potts et al. 2010). This loss of abundance and diversity has far-reaching implications for food systems and ecological systems. One particularly diverse and important group of pollinators is bees. There are approximately 20,000 different bee species worldwide. It is estimated that Colorado's diverse landscape is home to nearly 950 known native bee species (Scott et al. 2011). Green infrastructure technologies, such as green roofs, may play an important role in protecting pollinator diversity and abundance in urban areas from habitat destruction and degradation.

Pollinator decline is caused by a confluence of known and unknown factors. Pesticide use in ornamental and food crop production combined with climate change are likely major factors negatively impacting pollinators globally (Potts et al. 2010). Warming temperatures alter both the pollinator lifecycle and the lifecycle of the plants on which they rely (Bartomeus 2011). Additionally, changes in land cover have become an obstacle for pollinators. Native landscapes across the globe have been converted into farmland and urban and peri-urban systems, which fragment and degrade the remaining native habitats. Currently, over 55% of the world's population lives in cities and that percentage is expected to grow to 68% by the year 2050 (UN 2018). Approximately 90% of the US population is projected to live in urban settings by the year

2050 (UN 2018). However, with the implementation of green infrastructure, the ecological burden of land conversion to urban and suburban systems may be lessened and provide a path for people to exist in concert with a variety of plants and pollinators that are native to the region.

Green urban infrastructure inhabits a variety of forms, typically at grade (ground level). Green infrastructure implemented during city planning is most often designed to address stormwater challenges or to provide green amenity space for residents (Grabowski 2022). In addition to stormwater solutions, green infrastructures, such as parks, bioswales, green roofs, rain gardens, and even turfgrass provide direct and indirect benefits to humans. These benefits include noise reduction, urban heat island mitigation, recreational space, and much more (Parker 2019). Habitat provisioning is another benefit provided by green urban infrastructure, but the efficacy of that habitat is dependent on the quality, density, and patch size of the green infrastructure (Cameron 2012; Parker 2019).

Green roofs provide habitat for a variety of fauna, including birds and arthropods, by providing plant material necessary for nesting, resting, feeding, and breeding. It has been anecdotally noted by green roofing practitioners that green roof soilless substrates often reach higher diurnal temperatures than garden analogs at grade. Green roofs will generally have higher sun and wind exposure and lower water-holding capacity than ground-level gardens. Due to the unique growing environment on green roofs, the flowering phenology of some flowering plant species is likely to be altered. Because of these warmer, drier conditions on the green roof, some species of plants are likely to flower earlier in the season (Fitter 2002; Song 2013). The warmer root zone temperatures may play a role in the mobilization of stored carbohydrates from the roots into the

shoots for growth, flowering, and seed production (Greer et al. 2006; Loescher et al. 1990). Green roofs provide unique habitat and forage opportunities for urban pollinators that would otherwise be unavailable, but more research on the effects of the green roof environment is needed to manage pollinator health and biodiversity more accurately in urban ecosystems.

Green roofs provide unique habitat opportunities for flora and fauna in urban ecosystems (Oberndorfer et al. 2007) that are not available on other roofing options. The earlier bloom times have potential synergies with urban pollinator conservation. Some bee species have altered phenology, emerging earlier in the season, likely related to global temperature increases (Bartomeus 2011). I hypothesize that the expected higher diurnal temperatures of green roofs relative to garden analogs at-grade will alter the floral phenology of some flowering plant species (San Wai and Newman 1992), potentially leading to green roofs providing early-season foraging options for pollinators. In this chapter, I ask how a green roof environment, compared to a garden environment at grade, impacts floral phenology, flower abundance, and plant health metrics.

1.2 Materials and Methods

1.2.1 Study Location.

This experimental study was conducted on the front range of the Rocky Mountains in Fort Collins, Colorado, on the campus of Colorado State University. Fort Collins, Colorado, is nestled between the Great Plains and the front range of the Rocky Mountains and experiences a temperate climate with warm summers and cold winters with about 500 mm of annual

precipitation. The study site is in an urban environment and immediately surrounded by intensely managed green space and an urbanized landscape.

The green roof used in this study is located above the second floor of the Nutrien Agricultural Sciences Building (40.5732, -105.0808) on the campus of Colorado State University. The green roof's construction was completed in March 2022. The green roof is approximately 65 m^2. It is an intensive-style green roof with tapered depth. The depth ranges from approximately 30 cm in the shallowest section to 60 cm in the deepest section. The growing substrate is composed primarily of 60% expanded shale, 20% high-quality compost, 10% vermiculite, and 10% peat moss by volume. No additional substrate amendments were made during the study.

The ground-level study site (40.5730, -105.0825; is located approximately 137 m west of Nutrien Agricultural Science Building's green roof. In March 2022, turfgrass was removed from the location to prepare the site for the research plants. Additionally, the native soils were amended with perlite and compost before planting. A 1.5 cm layer of compost and substrate was applied to the plot and then incorporated into the upper 12 cm of the soil surface.

All plots were irrigated for 10 minutes, three days a week. Blocks 4, 5, and 6 were blocked by a supplemental irrigation regime. This supplemental irrigation was in addition to the regularly scheduled irrigation that all six of the blocks received. Block 4 received a 15-minute supplemental irrigation event every Monday and block 5 received a five-minute supplemental irrigation event every Monday. Block 6 did not receive any additional irrigation.

1.2.2 Plant Material.

Several plant species, both native and non-native to the Colorado Front Range, were selected for this study (Table 1). Plant selection was evaluated on multiple criteria. Plant species needed to be able to tolerate the different growing conditions of the green roof and at-grade sites. Because both study sites were constructed in the spring of 2022, we selected species that we expected to bloom in the same growing season that they were planted. Additionally, we selected species with corollas that were conducive to nectar sampling. Finally, we were limited by what species were commercially available. *Asclepias incarnata, Ipomopsis aggregata,* and *Oenothera speciosa* were purchased from High Plains Environmental Center (Loveland, Colorado, USA). *Allium* 'Millennium' was purchased from Arbor Valley (Fort Collins, Colorado, USA). All plant replicates in this study on the green roof and at-grade were planted on April 15, 2022. Six other species were planted on the green roof and at-grade but did not bloom during the study period.

Table 1. Plant common names, scientific names, and family names that were used in this study.

Common Name	**Scientific Name**	**Family**
Millennium Chives	*Allium* 'Millennium'	Amaryllidaceae
Swamp Milkweed	*Asclepias incarnata*	Apocynaceae
Scarlet Gilia	*Ipomopsis aggregata*	Polemoniaceae
Evening Primrose	*Oenothera speciosa*	Onagraceae

1.2.3 Experimental Design.

An independently replicated, blocked design was used in this experiment. Blocks 1, 2, and 3 were located on the green roof and blocked by depth. There was 30 cm between each of the three blocks in the green roof site and in the at-grade site. Blocks 4, 5, and 6 were in the site at-grade and blocked by supplemental irrigation. Each block was 3 m long by 1.5 m wide. Plant species

replicates were randomly arranged in each row of a 5x10 array so that there were 5 replicates per plant species per block for a total of 15 replicates per species on the green roof and 15 replicates per species at-grade.

The shallowest point in block 1 was 380 cm, and its deepest point was 455 cm, averaging 417.5 cm across the block. The shallowest point in block 2 was 495 cm, and its deepest point was 540 cm, averaging 517.5 cm across the block. The shallowest point in block 3 was 555 cm, and its deepest point was 565 cm, averaging 560 cm across the block.

Blocks 4, 5, and 6 were blocked by a supplemental irrigation regime. This supplemental irrigation was in addition to the regularly scheduled irrigation that all six of the blocks received. Block 4 received a 15-minute supplemental irrigation event every Monday and block 5 received a five-minute supplemental irrigation event every Monday. Block 6 did not receive any additional irrigation.

Substrate volumetric water content, substrate temperature, and solar radiation were measured at the green roof and grade sites using HOBO weather stations (H21-USB, Onset Computer Corporation; Bourne, MA, USA; Table 2). The substrate volumetric water content was measured for each block using a HOBO moisture sensor (S-SMC-M005, Onset Computer Corporation; Bourne, MA, USA) that was buried 11 cm below the surface of the substrate in the center of each block. The substrate temperature was measured for each block using a HOBO temperature sensor (S-TMB-M006, Onset Computer Corporation; Bourne, MA, USA) that was buried 11 cm below the surface of the substrate in the center of each block. On the green roof, one solar radiation

sensor (A-LIB-M003, Onset Computer Corporation; Bourne, MA, USA) was placed equidistantly between block 1 and block 2, and the second sensor was placed equidistantly between block 2 and block 3. At the at-grade site, one solar radiation sensor was placed equidistantly between blocks 4 and 5, and the second solar radiation sensor was placed equidistantly between blocks 5 and 6.

Table 2. The minimum, maximum, and mean temperatures at each site location over the growing season and the average solar radiation and moisture content over the growing season.

Environmental Data	May		June		July		August		September	
	GR	AG	GR	AG	GR	AG	GR	AG	GR	AG
Min. temp. (°C)	13	11.1	18	14.7	18.5	18.5	23.9	18.2	16.4	15
Max. temp. (°C)	16.8	14.2	37.3	21	37.5	23	40.3	22.2	36.8	19.8
Mean temp. (°C)	20.3	14	27.5	18.5	30	25.8	31.3	22.5	27.4	17.9
Solar Radiation	226.2	148.8	269.4	149.2	237	113.6	241.2	83.7	210.1	176.1
Moisture	0.15	0.34	0.14	0.36	0.12	0.37	0.09	0.33	0.06	0.26

1.2.4 Flower Surveys.

Flower count surveys were conducted on a weekly basis during bloom time. Flower counts began during the week of the first bloom for a species and ended either when the species produced no new flowers for the season or when the irrigation was stopped on 9/1/2022. Flowers were only counted if the reproductive structures were intact. The same flower may have been counted over multiple weeks. For *Allium* 'Millennium' and *Asclepias incarnata* the number of umbels that contained flowers with intact reproductive structures was counted instead of individual flowers. No flower count data was collected for *Oenothera speciosa* because Japanese beetles destroyed the buds prior to flower development.

1.2.5 Plant Size Measurements.

To assess plant health, height, and width measurements were recorded once in week 6 and again in week 19 to create a plant size index for each of the replicates (Figure 3). Height measurements were taken from the surface of the substrate to the tallest apical meristem. In the case of *Allium* 'Millennium', height measurements were taken from the substrate surface to the apex of the tallest leaf. The first width measurement was taken at the widest section of the plant, 10 cm above the surface of the substrate. The second width measurement was taken perpendicular to the first, widest measurement. A plant size index was calculated by averaging the values for both widths and heights.

At the end of the growing season (9/19/22 for *Allium* 'Millennium' and *Oenothera speciosa* 10/7/23 for *Ipomopsis aggregata,* 10/12/22 for *Asclepias incarnata*), the above-ground biomass was harvested, and replicates were individually bagged in labeled brown paper bags, dried, and weighed for each plant species. *Allium* 'Millennium', *Asclepias incarnata,* and *Ipomopsis aggregata* were dried in a drying oven for 48 hours at 105 degrees Celsius. *Oenothera speciosa* was dried at 70 degrees Celsius for 72 hours.

The plant relative chlorophyll concentration measurements were made for *Allium* 'Millennium,' *Asclepias incarnata,* and *Oenothera speciosa* using atLEAF CHL Plus Handheld Chlorophyll Meter (FT Green LLC, Wilmington, Delaware, USA). I did not measure chlorophyll for *Ipomopsis aggregata* due to the shape of the chlorophyll meter and the lanceolate structure of the *I.* aggregata leaf. All measurements were made between 9:00 am and 11:00 am on sunny

mornings to avoid the effects of the time of day and the angle of the sun on the measurements. To take the measurement, the leaf apex was placed into the handheld meter and the leaf midrib was adjusted to be in the center of the light sensor.

1.2.6 Pollinator Observations.

Following the protocol developed by Mason et al. (2018) each species replicate was observed for two minutes between 9:00 am and 11:00 am once every week during their flowering period. Per Mason et al. (2018), pollinators were identified to morphospecies during the *in situ* observations. Visiting pollinators were counted if they contacted floral reproductive structures during the two-minute observational window (Mason et al. 2018). A plant species replicate was only observed if it had at least one flower with intact floral reproductive structures.

Five blue vane traps (BanfieldBio Inc., Seattle, Washington, USA) were set up along a transect from the plots at-grade to the green roof, with one trap in the center of plots at-grade, two along the transect, one in the center of the green roof, and on the non-green roof of the Plant Science Building at Colorado State University in Fort Collins, Colorado. The trap on the non-green roof was approximately 13 meters north of the plots at-grade. The traps were placed in their respective locations from 6/8/22-6/10/22 and 7/18/22-7/20/22 from 9 am to 3:30 pm each day. Samples were collected from the trap at the end of each sampling day and stored in vials filled with 70% isopropyl alcohol. The blue vane traps were filled with a one-to-ten ratio of Dawn Dish Soap to water. Samples collected from the blue traps were identified to morphospecies groups described by Mason et al. (2018).

1.2.7 Statistical Analysis.

All statistical analyses were conducted in RStudio, version 1.41717 (RStudio, Inc., Boston, Massachusetts, USA). Using blocks as independent replicates, a comparison between the green roof and at-grade sites was conducted. Flower count data were analyzed using a general linear mixed effects model site-by-time interactions as the fixed effects and block as the random effect, and a Poisson Distribution was assumed to model the count data. Week was calculated as a categorical variable in this model.

A linear mixed-effects model was used to analyze above-ground biomass and relative chlorophyll content. Each block was considered an independent replicate used to make a direct comparison between the green roof and at-grade site locations. Site location (green roof or at-grade), week, and site-by-week interactions were the fixed effects in the model, and block and replicate within the block were the random effects for the model.

1.3 Results and Discussion

1.3.1 Floral Phenology and Abundance.

The results of the general linear mixed-effects regression model analysis showed that floral phenology was accelerated on the green roof for Allium 'Millennium' and *Ipomopsis aggregata* (Figure 1 and Figure 2)**.** *Allium* 'Millennium' green roof replicates had significantly more flowers than the replicates at grade starting at week 9 ($P < 0.05$, Table 3). At week 15, the *Allium* 'Millennium' grown at grade had higher flower counts than those grown on the green roof ($P < 0.05$) the plants that were grown at grade. *Ipomopsis aggregata* grown on the green roof had

significantly more flowers beginning at week 13 ($P < 0.05$, Table 4) until week 17 ($P > 0.05$), when both the plants on the green roof and the plants at grade began to senesce. *Oenothera speciosa* green roof replicates began blooming two weeks earlier than the replicates at-grade, and *Asclepias incarnata* green roof replicates began blooming four or five weeks earlier than the replicates at-grade (Table 5).

Additionally, over 73% of the green roof replicates for *Asclepias incarnata* replicates bloomed during peak bloom and 13% of the replicates at grade bloomed during peak bloom at weeks 13 and 17 respectively. *Allium* 'Millennium' green roof replicates had the highest floral density between weeks 11 and week 13 and the replicates at-grade had the highest floral density between weeks 15 and 17. For *Allium* 'Millennium', the green roof replicates reached peak bloom three weeks before the replicates at-grade and the peak bloom for the replicates on the green roof was less than the replicates at-grade. *Ipomopsis aggregata* green roof replicates reached peak bloom two or three weeks earlier than the replicates at-grade and the green roof replicates reached a higher flower during peak bloom than the replicates at-grade.

Earlier bloom times have potential synergies with urban pollinator conservation. Some bee species have been found to emerge earlier in the season, likely related to global temperature increases and the urban heat island effect (Bartomeus 2011). Green roof plant species blooming earlier in the season than their ground-level counterparts may provide urban foraging opportunities that would otherwise not exist. An emerging phenomenon exists, the plant-pollinator phenological gap, which describes asynchronies forming between pollinators and their associated plant hosts. Linked to climate change and other warming effects, pollinators and

plants emerge and bloom earlier in the season, but not always at the same rates, causing a lack of pollinators for sexual plant reproduction or a lack of forage opportunities for early emerging bees (Kudo and Ida 2013). By leveraging the altered bloom times of green-roof plants of affected species, we may be able to narrow the phenological gap between plants and pollinators to better conserve and manage pollinator abundance and diversity in urban ecosystems.

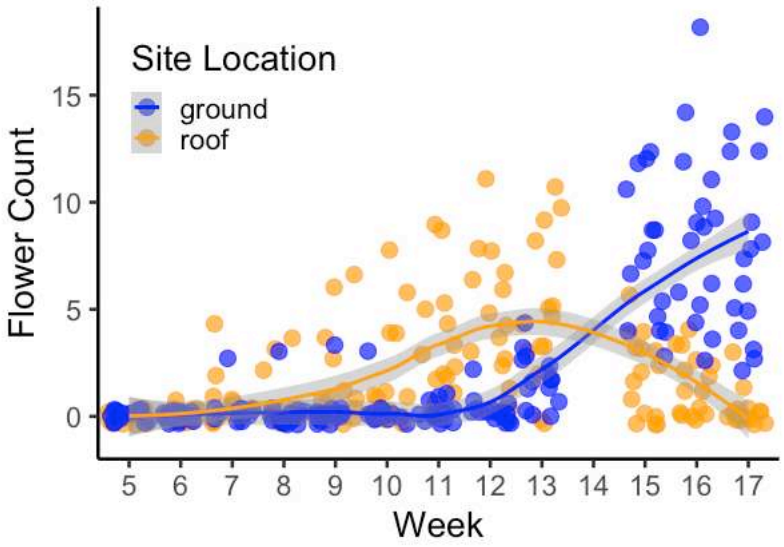

Figure 1. Floral phenology and abundance for *Allium* 'Millennium' beginning at week 5 (6/5/22) and ending at week 17 (9/3/22). A smooth curve was fitted using locally estimated parametric smoothing to visualize the relationship between plants on the ground and roof.

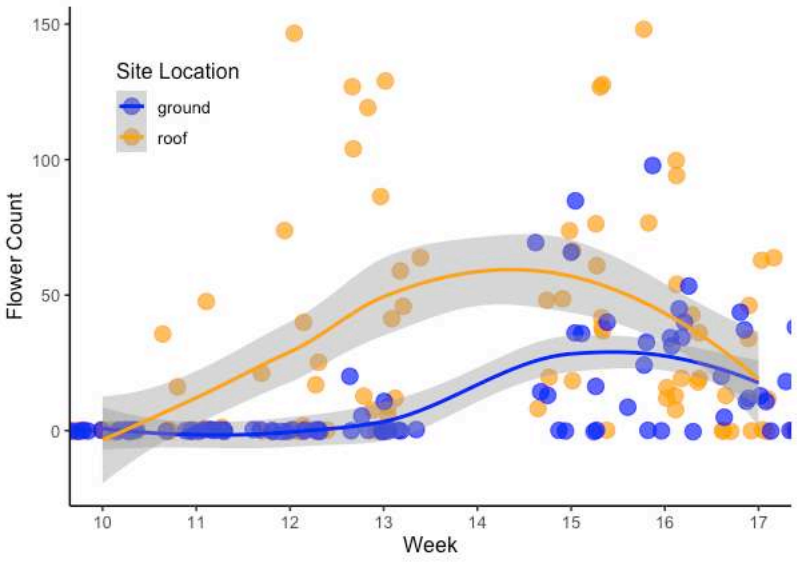

Figure 2. Floral phenology and flower abundance for *Ipomopsis aggregata* beginning at week 10 (7/10/22) and ending at week 17 (9/3/22). A smooth curve was fitted using locally estimated parametric smoothing to visualize the relationship between plants on the ground and roof.

Table 3. The standard error, z-scores, and p-values generated from the model depicting site-by-site comparisons the floral phenology for *Allium* 'Millennium' at each week.

Model Output for Ground - Roof Contrast of Allium 'Millennium'			
Week Number	Standard Error	Z Score	P-Value
Week 5	8917.93	0	1
Week 6	6226.076	-0.003	0.9978
Week 7	0.686	-1.535	0.1247
Week 8	0.686	-1.535	0.1247
Week 9	0.633	-3.214	0.0013
Week 10	0.63	-3.353	0.0008
Week 11	0.616	-4.426	< 0.0001
Week 12	0.538	-5.178	< 0.0001
Week 13	0.276	-3.671	0.0002
Week 15	0.258	5.28	< 0.0001
Week 16	0.283	6.558	< 0.0001
Week 17	0.533	6.319	< 0.0001

Table 4. The standard error, z-scores, and p-values generated from the model depicting site-by-site comparisons the floral phenology for *Ipomopsis aggregata* at each week.

Model Output for Ground - Roof Contrast of *Ipomopsis aggregata*			
Week Number	Standard Error	Z Score	P-Value
Week 10	1879.91	0	0.9998
Week 11	1273.01	-0.015	0.9878
Week 12	1273.49	-0.016	0.9871
Week 13	0.51	-7.18	< 0.0001
Week 15	0.484	-2.638	0.0083
Week 16	0.484	-2.15	0.0316
Week 17	0.49	-1.438	0.1504

Table 5. The percentage of green roof replicates and at-grade replicates in bloom each week for each species, beginning at week five (6/5/22) and ending at week 17 (9/3/22). Data were not collected in week 14.

Percentage of Plant Replicates in Flower by Species and Site Location									
	Genus species	*Allium* 'Millennium'		*Oenothera speciosa*		*Asclepias incarnata*		*Ipomopsis aggregata*	
	Site Location	GR	AG	GR	AG	GR	AG	GR	AG
Month	Week #								
June	5	0	0	0	0	0	0	0	0
	6	7	0	0	0	0	0	0	0
	7	33	7	0	0	0	0	0	0
	8	20	7	0	0	0	0	0	0
July	9	53	7	13	0	0	0	0	0
	10	53	7	80	0	13	0	0	0
	11	87	20	53	33	40	0	33	0
	12	87	20	100	100	47	0	53	0
August	13	87	80	73	93	73	0	93	23
	14	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	15	67	100	67	27	67	7	93	69
	16	60	100	67	7	67	7	93	77
September	17	13	100	13	13	13	13	47	77

1.3.2 Plant Health.

Plant size indices were calculated as a measure of relative plant health and cover at week 6 and week 19 (Figure 3). By week 6 after planting, all three species *Allium* 'Millennium', *Asclepias incarnata*, and *Ipomopsis aggregata* were smaller at grade than on the green roof. However, by

week 19, all three species had larger plants at grade than on the green roof, although not always by much.

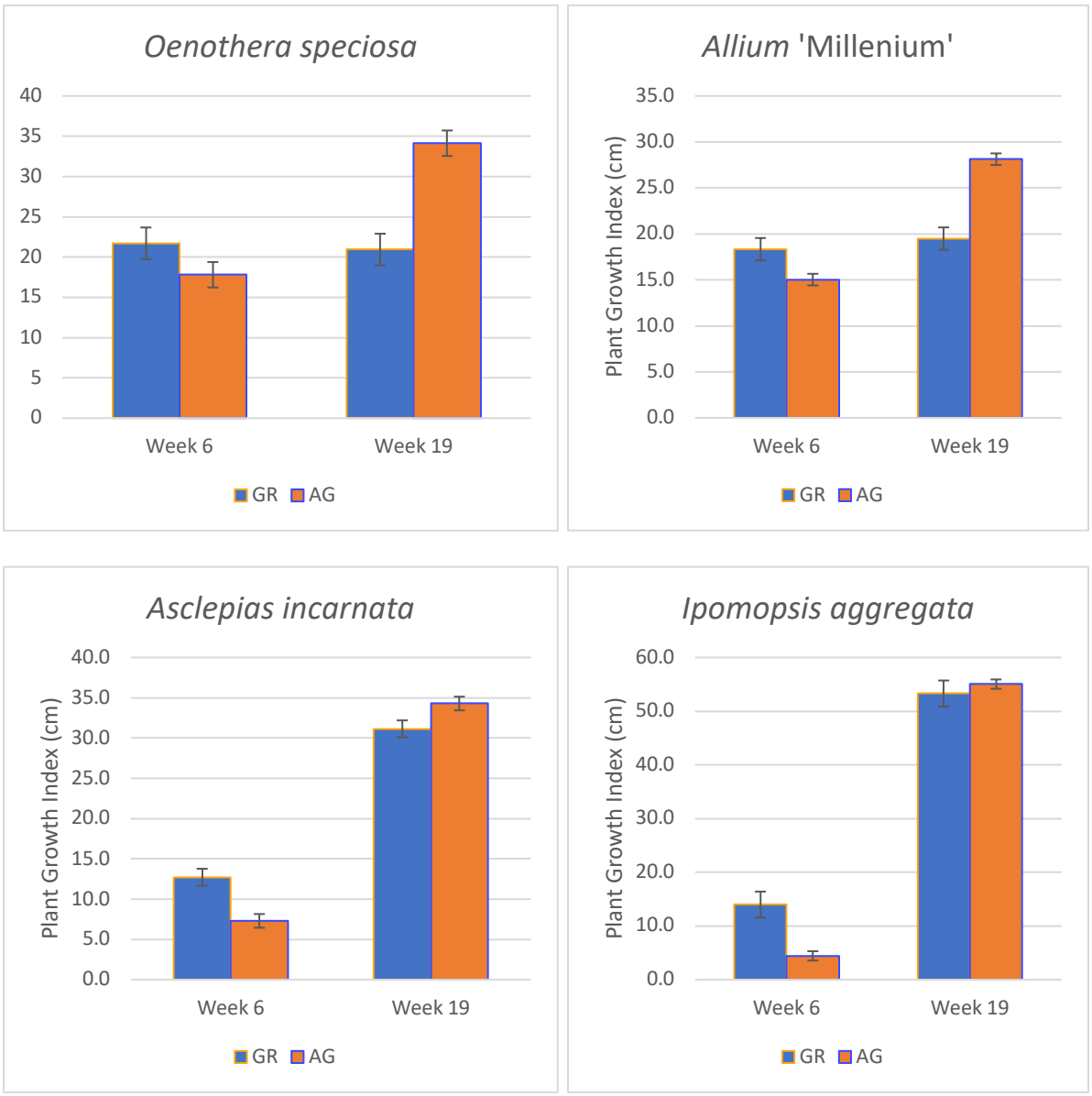

Figure 3. Plant sizes (cm) for *Allium* 'Millennium', *Asclepias incarnata, Ipomopsis aggregata,* and *Oenothera speciosa* at grade and on the green roof at weeks 6 and 19.

There were no significant differences in the average weight of the above-ground biomass of replicates grown on the green roof and replicates grown at-grade for *Allium* 'Millennium', *Asclepias incarnata,* and *Oenothera speciosa* (Figure 4). *Ipomopsis aggregata* replicates had more aboveground biomass on the green roof than the replicates at-grade (Figure 5). There were significant differences between the dry, aboveground biomass for *Ipomopsis aggregata* green roof replicates and replicates at-grade ($P < 0.001$). Though not statistically significant, *Oenothera speciosa* replicates at-grade generally had more aboveground biomass in the replicates at-grade than the green roof replicates. These data suggest that plant size response to green roof conditions is variable between plant species.

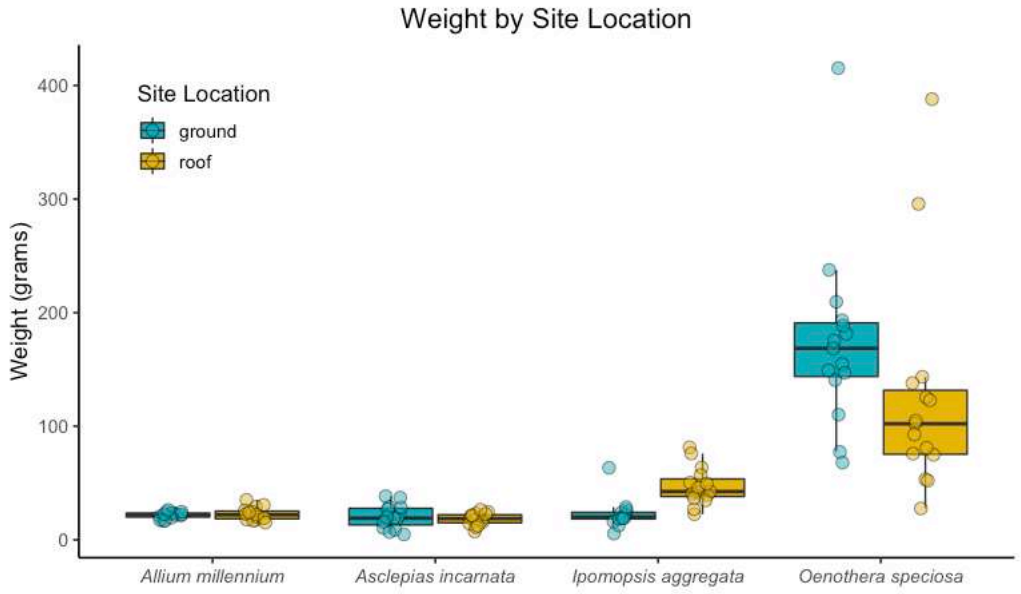

Figure 4. Dry weight (above-ground biomass) in grams, for each plant species on the green roof and at grade

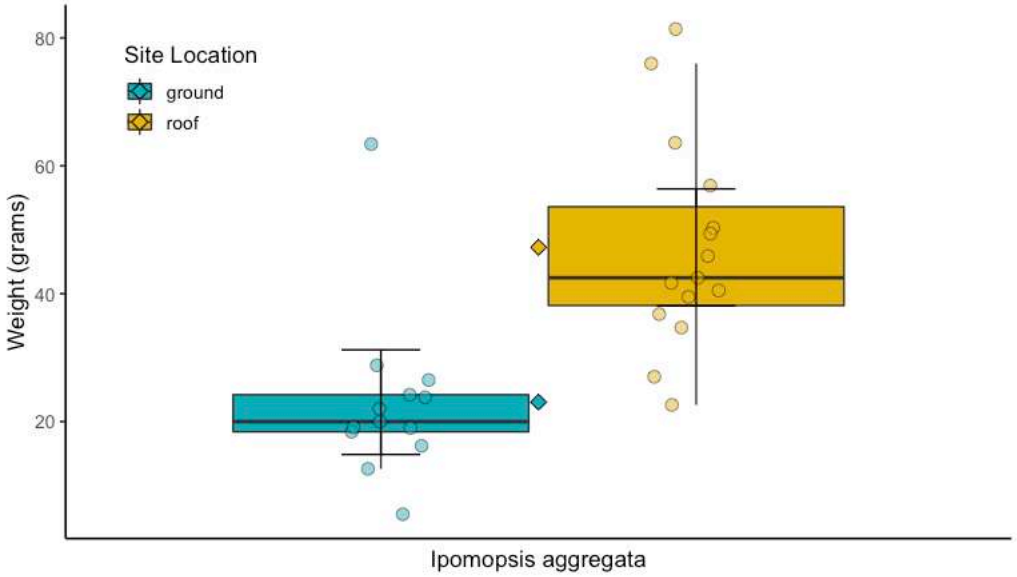

Figure 5. Dry weight (above-ground biomass) in grams, for *Ipomopsis aggregata* on the green roof and at grade. The diamonds represent the mean calculated by the model.

The relative chlorophyll content of *Allium* 'Millennium' was not significantly different between replicates grown on the green roof and replicates grown at-grade (Figure 6). The average relative chlorophyll content of *Asclepias incarnata* and *Oenothera speciosa* was lower in replicates grown at-grade than replicates grown on the green roof (Figure 6). Chlorophyll measurements were not made on *Ipomopsis aggregata* because of the shape of the leaf and limitations of the atLeaf chlorophyll meter.

Chlorophyll content is used as a measure for plant health and stress (Pavlovik et al. 2015), which provides context for plant flowering. The relative chlorophyll content provides a metric for assessing plant health in the environment. For *Allium* 'Millennium' and *Oenothera speciosa*, there was no obvious difference and both species flowered profusely in the green roof plots and the plots at grade. However, *Asclepias incarnata* had significantly lower relative chlorophyll

content at grade than on the green roof, and most of the plants at grade did not bloom, despite there being no significant difference in the average weight of above ground biomass in the two locations. This suggests that *Asclepias incarnata* was able to generate more resources for floral production on the green roof than at grade in a single season.

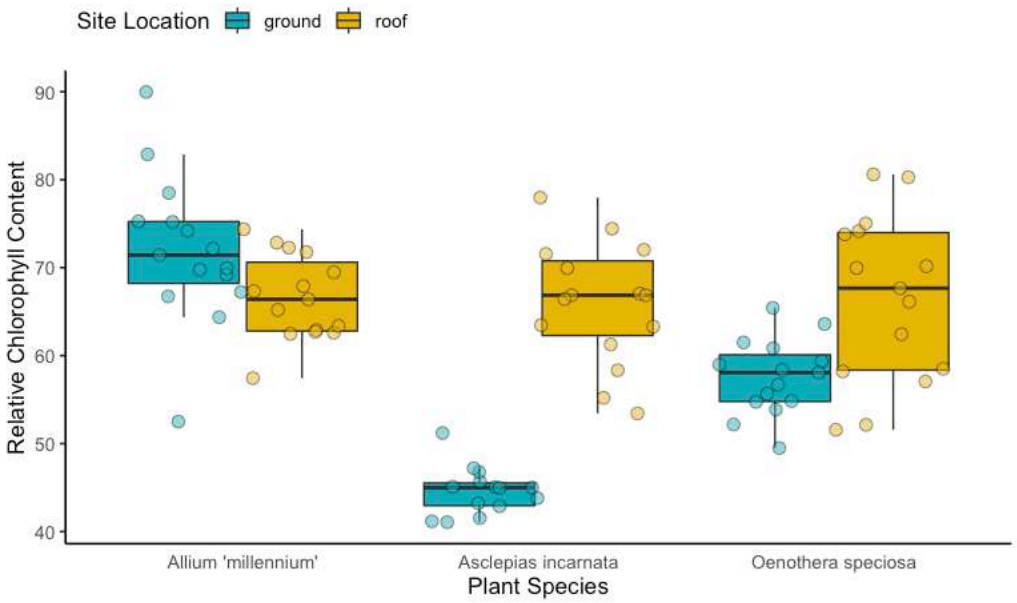

Figure 6. The relative chlorophyll content of each plant species grown on the green roof (GR) and at-grade (AG). The teal boxplots represent replicates grown at-grade and the yellow boxplots represent replicates grown on the green roof.

1.3.3 Pollinator Observations.

Bee diversity and abundance were low for the *in situ* observations conducted at sites on the green roof and at-grade (Table 6). No individuals belonging to the morphospecies group hairy leg bee were observed. Additionally, the traps did not collect any green metallic bees, cuckoo bees, or hairy belly bees (Table 7). Cuckoo bees and striped sweat bees were only observed on the green roof and bumblebees were only observed at-grade. Green metallic bees were more commonly observed at-grade than on the green roof. Striped sweat bees were observed on the green roof, but not at-grade. However, they were present in one of the transect traps and in the trap on the

bare roof. Tiny dark bees were only observed on the green roof but were found in both traps along the transect at-grade. Hairy leg bees were not observed on the green roof or at-grade but were present in all traps except for the trap on the green roof. Cuckoo bees were only observed on the green roof and were not present in any of the traps. Other groups including Coleoptera, Diptera, and Lepidoptera were observed in both locations and found in all traps except for the trap on the bare roof. The green roof had notably more observations belonging to the green roof and the green roof trap had the most individuals belonging to the "other" category.

The methods used for pollinator observations may have contributed to the low abundance and diversity of bee observations. Anecdotally, I often observed bees on plants that I was not actively observing. Therefore, I was unable to count them among our samples. Additionally, only observing between 9 am and 11 am may have limited what morphospecies we were able to observe. In a follow-up study, it would be worth incorporating an additional sample method and additional time frames for sampling to allow for a more complete sampling of the abundance and diversity of bees present at each location.

Table 6. The aggregated *in situ* observations following the protocol developed by Mason et al. (2018) the green roof sites (GR) and the sites at-grade (AG).

Honeybee		Bumble bee		Green Metallic Bee		Hairy Belly Bee		Striped Sweat Bee		Tiny Dark Bee		Hairy Leg Bee		Cuckoo Bee		Other	
AG	**GR**	**AG**	**GR**	**AG**	**GR**	**AG**	**GR**	**AG**	**GR**	**AG**	**GR**	**AG**	**GR**	**AG**	**GR**	**AG**	**GR**
4	**7**	**1**	**0**	**8**	**1**	**0**	**1**	**0**	**3**	**0**	**1**	**0**	**0**	**0**	**9**	**5**	**24**

Table 7. The bee diversity and abundance data where a.) corresponds to one trap along a transect between the site at-grade and the green roof site, b.) corresponds to the second trap along the transect, c.) corresponds to the trap on the non-green roof, d.) corresponds to the trap on the green roof, and e.) corresponds to the trap in the plot at-grade.

	Honeybee	**Bumble bee**	**Green Metallic Bee**	**Hairy Belly Bee**	**Striped Sweat Bee**	**Tiny Dark Bee**	**Hairy Leg Bee**	**Cuckoo Bee**	**Other**
a.	4	1	0	0	1	1	6	0	3
b.	2	0	0	0	0	1	1	0	1
c.	0	0	0	0	1	0	2	0	0
d.	2	0	0	0	1	0	0	0	7
e.	0	0	0	0	0	0	8	0	2

1.4 Conclusion

For all species tested, plant replicates bloomed earlier on the green roof than at-grade. Flower count response was variable among species between replicates grown on the green roof and at-grade. Aboveground biomass and chlorophyll content were also variable among species between replicates grown on the green roof and at-grade; however, the trends showed that aside from *Allium* 'Millennium', relative chlorophyll content was lower at-grade. Generally, aboveground biomass was not affected by site location. However, *Ipomopsis aggregata* had significantly more aboveground biomass in replicates grown on the green roof than those grown at-grade. Pollinator visitation was low on the green roof and at-grade, which is expected in an urban setting. Bee diversity and visitation were similar between the two sites. The green roof had notably more non-bee visitations than the site at-grade.

CHAPTER 2. GREEN ROOF EFFECTS ON FLORAL NECTAR RESOURCES OF GREEN ROOF PLANTS

2.1 Introduction

Habitat loss and fragmentation due to urbanization affect species richness, abundance, and plant-pollinator interactions of bee populations (Geslin 2013; Swenson 2000; Xiao 2016). To what extent urbanization affects pollinator diversity is dependent on local habitat quality and the severity of degradation in the surrounding area (Bates 2011). Local habitat quality for pollinators is in part a function of forage availability and quality, both of which can affect pollinator health and behavior.

Forage quality impacts pollinator behavior. Honeybees will alter their foraging patterns and behaviors according to the quality and quantity of forage options in the surrounding area. Colonies will adjust their foraging behavior based on the abundance of forage options. If there is an abundance of forage, honeybees will target forage patches with higher sugar concentrations, likely meaning these patches have added value for pollinators (Seeley 1986). In addition to honeybees, some species of bumblebees also alter foraging patterns and behavior based on the quality of forage patches (Cartar 2004; Cibula and Zimmerman 1987).

However, forage quality is not just a measurement of nectar volume. The amount of sugar per flower is an important metric to consider when measuring forage quality and relating it to pollinator visitation (Fowler et al. 2016). Additionally, nectar sugar concentrations may play a role in the suite of pollinators associated with a plant species. However, there is a conflicting

argument that phylogeny is the main driver of these pollinator syndromes and that these sugar ratios are not a direct result of pollinator syndromes (Nicolson 2022).

As reported in Chapter 1 (2023; Table 2), the green roof environment experiences higher temperatures and much lower moisture availability, simulating water-limited conditions when compared to the plots at-grade. Nectar volume and sugar concentrations have variable responses to water-limited conditions, depending on the plant species (Deschamps 2021). In *Epilobium angustifolium,* drought conditions had a significant negative effect on nectar volume and no effect on nectar sugar concentrations (Carroll 2001).

Floral nectar resources have been shown to react differently during water-limited conditions, depending on the plant species. Pollinator health and behavior can be affected by nectar volume and nectar sugar concentrations. It is important to understand how the green roof environment impacts floral nectar resources in order to better manage pollinators in urban ecosystems. With the water-limited nature of green roofs in mind, relative to garden conditions at grade, I ask how green roof conditions impact nectar volume and sucrose, glucose, and fructose concentrations in *Ipomopsis aggregata.*

2.2 Materials and Methods

2.2.1 Location and Plant Material.

The site locations were the same locations described in Ruszkowski and Bousselot (2023). Both the green roof and at-grade sites were located on Colorado State University's Campus in Fort Collins, Colorado. The plant material used in the nectar study was a subset of the plant material

used in Ruszkowski and Bousselot (2023). *Ipomopsis aggregata* was the primary species used in this study. Some nectar was extracted from *Oenothera speciosa,* prior to the bud and flower herbivory of the Japanese beetle. Thus, I only present volume data on *Oenothera speciosa.*

2.2.2 Nectar Extraction, Volume Measurements, and *In Situ* Sucrose Measurements.

To ensure that nectar was not extracted by pollinators prior to sampling, pollinator mesh exclusion bags were placed over freshly opened flowers between 12 and 24 hours prior to sampling. Following the pollinator exclusionary period, nectar was sampled using 5-microliter capillary tubes (VWR MICROPIPET DISPOS 5UL, VWR INTERNATIONAL, Radnor, Pennsylvania, USA, and Capillary Tube, Sigma-Aldrich, St. Louis, Missouri, USA). The capillary tube was inserted into the flower corolla until the base of the tube came into contact with the nectary, using capillary action to draw the nectar into the tube. The capillary tube was rotated in the nectary until no more nectar was drawn into the tube (Morrant et al. 2009).

Volumetric measurements were conducted by using a ruler to measure how far the nectar traveled up the tube and using that measurement to calculate the volume where the inner radius of the capillary tube was 0.17 mm (Sigma-Aldrich) or 0.25 mm (VWR). *In situ* sucrose concentration measurements were conducted using an Eclipse Handheld Refractometer (Brix 50 Low Volume, Bellingham + Stanley, Tunbridge Wells, Kent). After volumetric measurements were made, if the sample was not stored for UPLC-RI analysis, the nectar was expelled onto the sample plate of the refractometer and the reading was recorded.

2.2.3 UPLC-RI Analysis for Nectar Sugar Analysis of Glucose, Sucrose, and Fructose.

Nectar samples were stored at -80°C until the time of sample preparation. Samples were prepared by diluting with 50 µL LC grade acetonitrile: LC grade water (75:25, v/v) and further diluted by a factor of two with LC grade acetonitrile: LC grade water (75:25, v/v). Ten microliters of diluted flower nectar were injected into an LC300 UHPLC system equipped with an LC300 solvent delivery pump (20-µL sample loop, µL pickup injection mode) (PerkinElmer, Shelton, CT). An Epic Amine HD column (4.6 x 100 mm, 5.8 µm; PerkinElmer) was used for the chromatographic separation of glucose, fructose, sucrose, and maltose. Peaks were identified by comparing retention times with authentic sugar standards. The column was maintained at 35°C. Separation was achieved in isocratic mode (9 mins). The mobile phase consisted of LC-grade acetonitrile: LC-grade water (75:25, v/v). The flow rate was set to 1.5 mL/min. Samples were held at 6 °C in the autosampler. Detection was performed on a PerkinElmer LC 300 refractive index (RI) detector. The RI cell was maintained at 35°C with a sampling rate of 5 Hz and positive polarity.

2.2.4 Data Analysis

A linear mixed-effects model was used to compare nectar volume, *in situ* sucrose measurements, glucose, sucrose, and fructose measurements between replicates grown on the green roof and replicates grown at-grade. A block design was implemented, described in the methods of Ruszkowski and Bousselot (2023). Each block was considered an independent replicate used to make a direct comparison between the green roof and at grade site locations. Site location (green roof or at grade), week, and site-by-week interactions were the fixed effects in the model, and block and replicate within the block were the random effects for the model.

Simplicity Chrom software (version 1.6 PerkinElmer, PerkinElmer, Inc., Waltham, Massachusetts, USA) was used to detect and integrate peak areas and to calculate linear regression of analytical standards used for quantification. A seven-point calibration curve was prepared in 75:25 acetonitrile from four authentic sugar standards and ranged from 156.2 µg/mL - 10000 µg/mL. The corresponding linear regression equation was used for quantification (µg/mL) for each analyte, which was then adjusted for the precise volume of nectar (mg/mL). The limit of detection (LOD) was calculated as 3 times the standard deviation of the blank divided by the slope of the calibration curve. Likewise, the limit of quantitation (LOQ) was calculated as 10 times the standard deviation of the blank divided by the slope of the calibration curve.

2.3 Results and Discussion

2.3.1 Nectar Volume.

Oenothera speciosa and *Ipomopsis aggregata* did not differ significantly in nectar volume between replicates grown on the green roof and replicates grown at grade ($P > 0.05$, Figure 7). However, for both species, nectar volumes were observed to be slightly lower on the green roof than at grade. This is likely caused by the more water-limited conditions of the green roof environment and is corroborated by the finding that *Epilobium angustifolium* in drought conditions had a significant negative effect on nectar volume and no effect on nectar sugar concentrations (Carroll 2001). Nectar volume was generally lower and had more fluctuation in

Ipomopsis aggregata replicates grown on the green roof than the replicates grown at grade (Figure 8).

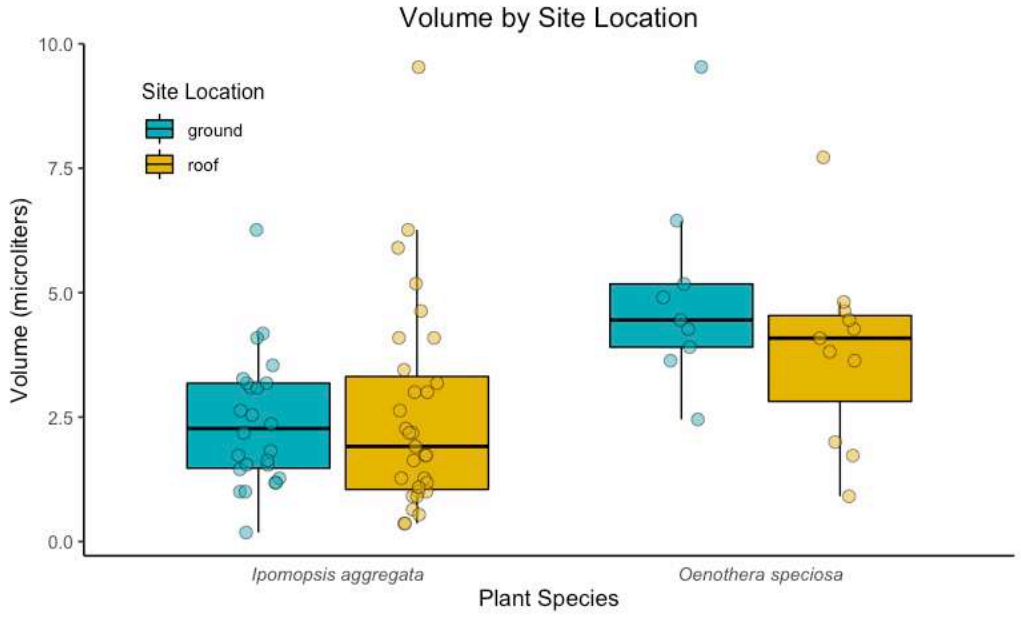

Figure 7. The nectar volume, in microliters, for *Ipomopsis aggregata and Oenothera speciosa.*

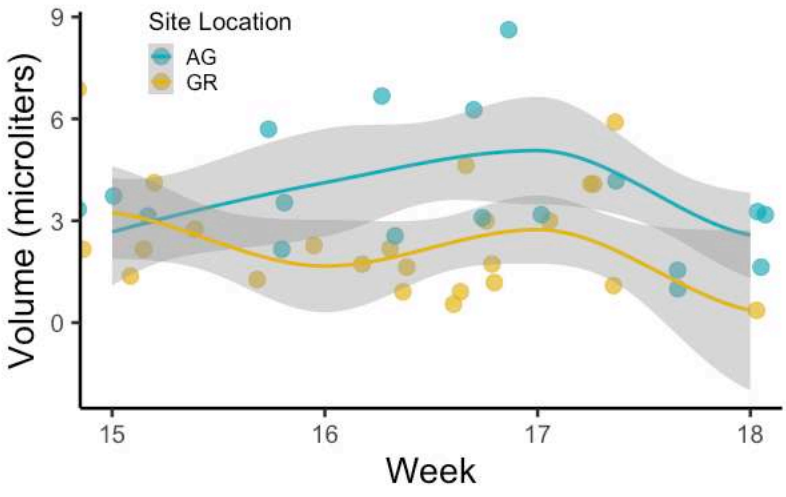

Figure 8. The nectar volume for *Ipomopsis aggregata* from week 15 to week 18. A smooth curve was fitted using locally estimated parametric smoothing to visualize the relationship between plants on the ground and roof.

2.3.2 *In Situ* Sucrose Concentrations.

Sucrose concentrations made *in situ*, using the Brix Refractometer, were higher in *Ipomopsis aggregata* replicates grown on the green roof than the replicates grown at grade ($P < 0.05$, Figure 9). This may be tied to the water-limited conditions of the green roof as this response is likely dependent on plant species (Deschamps 2021). These results have implications for honeybee and bumblebee health and foraging behavior (Cartar 2004; Cibula and Zimmerman 1987; Seeley 1986). If green roofs are providing a stable source of high-quality nectar, pollinators such as honeybees and bumblebees may not need to forage outside of city limits to find high-quality forage patches, if the green roof is accessible to these pollinators.

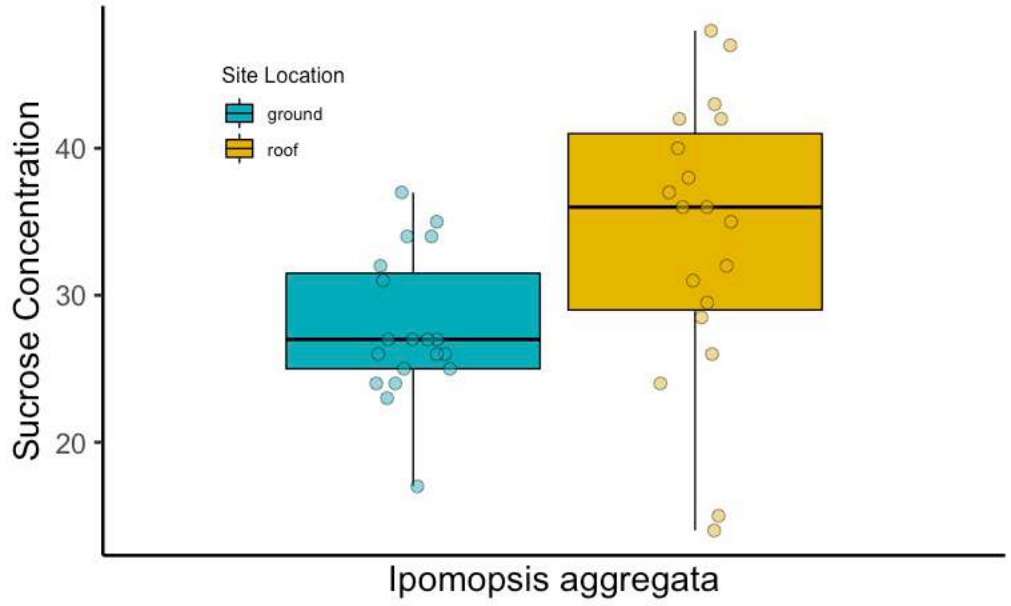

Figure 9. The sucrose concentrations of *Ipomopsis aggregata* where measurements were made *in situ,* using a refractometer.

2.3.3 Glucose, Sucrose, and Fructose Concentrations.

All three sugars were found in higher concentrations in replicates on the green roof than at grade (Figure. 10). That difference was statistically clear for sucrose and fructose ($P < 0.05$) but not for glucose ($P > 0.05$). Fructose and sucrose had higher concentrations in nectar from replicates on the green roof than the replicates that were grown at grade ($P < 0.05$ and $P < 0.01$, respectively). The variation in sucrose and glucose were similar between the green roof and the ground. However, the green roof had higher variation in fructose concentrations than at grade.

Carrol et al. 2001 found in a controlled greenhouse study of *Epilobium angustifolium* that nectar volume was affected by water stress, but sugar concentrations were not significantly affected. I found that volumes were not significantly different between the green roof and the ground, but sugar concentrations were generally higher on the green roof, which is more water limited. Variations in the ratios and concentrations of these three sugars may play a role in the health of associated pollinator syndromes (Fowler et al. 2016; Nicolson 2022). Fowler et al. (2016) found that there was a positive relationship between pollinator visitation and nectar sugar concentrations. If green roofs are minimally negatively impacting nectar volume and significantly increasing nectar sugar concentrations, green roofs may provide unique, high-quality forage opportunities for pollinators that may otherwise be unavailable in urban ecosystems.

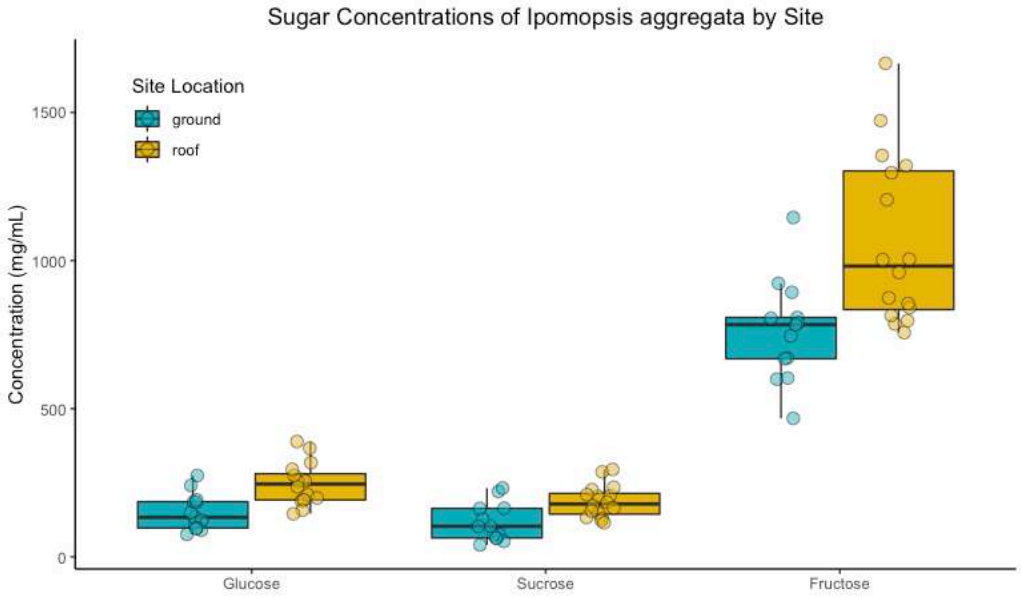

Figure 10. The concentrations of glucose, sucrose, and fructose in *Ipomopsis aggregata* grown on the green roof and at grade.

Conclusion

Nectar volumes in *Ipomopsis aggregata* and *Oenothera speciosa* were not significantly different between the replicates grown on the green roof or the replicates grown at grade for either species. Despite not being statistically significant, nectar volumes for both species were observed to be slightly less for the replicates grown on the green roof. *Ipomopsis aggregata* sucrose concentrations were higher for both the *in situ* measurements using the refractometer and for the lab analyses for replicates grown on the green roof. Additionally, fructose concentrations were higher for *Ipomopsis aggregata* replicates grown on the green roof. Finally, there was no statistical difference in glucose concentrations between *Ipomopsis aggregata* replicates grown on the green roof when compared to the replicates at grade. Generally, *Ipomopsis aggregata replicates* had higher nectar sugar concentrations in replicates grown on the green roof while nectar volume was mostly unaffected by the site location.

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