VARIATION IN POLLINATOR VISITATON AMONG CULTIVARS OF MARIGOLD, PORTULACA, AND BIDENS

By

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ABSTRACT

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The decline in pollinator populations has been documented worldwide in recent years with many researchers focusing on the matter. Many people are interested in ornamental annuals they can plant in their garden to better support pollinators and have many varieties and cultivars to choose from. However, there is a lack of research behind what specific cultivars of ornamental annuals are attractive to pollinators. We sampled pollinators visiting six cultivars of *Tagetes* spp. (marigold), eight cultivars of *Portulaca* spp. and ten cultivars *Bidens* spp. separately for two years to evaluate each cultivars relative pollinator attractiveness among each annual. Pollinators collected were categorized into four groups, Apis mellifera, Bombus impatiens, Wild Bees and Syrphids, to show the proportion of different pollinator visitors to each cultivar. There was significant variation among individual cultivar pollinator visitation rates found for each annual, with some cultivars having over double the visitation rate of others. We also evaluated nectar production and nectar quality of two selected *Portulaca* spp. and *Bidens* spp. cultivars based on first-year pollinator visitation data collected as a means of showing a possible reason for varying pollinator visitation among cultivars. Our results show clear pollinator preferences for certain cultivars, and that nectar production and nectar quality may be a driving influence. This research will better inform entomologists, horticulturalists, growers, and educators which of the selected cultivars of *Tagetes* spp. (marigold), *Portulaca* spp. and *Bidens* spp. have higher pollinator visitation, and that cultivar differences should be considered when labeling a plant as 'pollinatorfriendly'.

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INTRODUCTION

Evidence of pollinator decline throughout the world in both wild and managed bee populations has recently gained more attention, with pesticides, habitat loss, diseases and parasites among the top reasons for the decline (Potts et al. 2010, Ollerton et al. 2014, Goulson et al. 2015). In 2011, it was estimated that wild bumble bee populations had declined drastically in the United States in the last 20 years, with the four most impacted species declining from 70% to 96% (Cameron et. al 2011). While the number of managed honey bee colonies is increasing in the United States, the proportion of colonies lost quarterly each year still range from 9% to 16% (USDA-NASS 2022). To address these declines, strategies to alleviate pressure on pollinators are being studied and implemented in different ways throughout the United States. One example is the Environmental Protection Agency establishing a pollinator protection initiative that funds projects and provides a strategic plan for pollinator protection (EPA 2021). An important part of this plan is to increase public awareness of how pollinators are essential for fruit and vegetable production, and for pollination of wildflowers, shrubs, and trees (EPA 2021). In addition, organizations like the Xerces Society have increased efforts to provide the public with information on planting pollinator-friendly gardens to support native pollinators (Mader et al. 2011).

Over 80% of people in the United States live in urbanized areas with more choosing to move to urbanized areas every year (U.S. Census Bureau 2012). Worldwide, the number of people living in urban areas is also expected to increase from 55% as of 2018 to 68% by 2050 (United Nations 2019). Access to floral resources for pollinators in heavily or moderately urbanized areas has been shown to be beneficial for bee taxa, whereas floral resources for many other pollinator taxa may be more important in rural areas (Daniels et al. 2020, Theodorou et al.

2020). With the rising interest in pollinator conservation, more people want to know how they can play a role by purchasing flowers that support pollinators (Campbell et al. 2017, Wignall et al. 2019). Annual flowers make up a large portion of ornamental plants sold at garden centers and greenhouses across the United States (USDA-NASS 2019) and some are frequently visited by pollinators (Lowenstein et al. 2019). According to the 2019 Census of Horticulture Specialties, \$3.44 billion of annual bedding/garden and potted flowering plants were sold that year alone (USDA NASS 2019). Annual flowers are appealing to the average gardener because they are bred to bloom continuously throughout the growing season with vibrant colors (Wilde et al. 2015). However, consumers that purchase annual flowers at garden centers may be unaware of what flowers are good for supporting pollinators.

Garden centers and greenhouse growers have taken note of the public's interest in choosing annuals that support pollinators, and have begun placing 'pollinator-friendly' labels on plants (Khachatryan et al. 2017). Surveys of consumers show that many people are willing to seek out and pay more for plants being sold and labeled as 'pollinator-friendly' (Wignall et al. 2019, Campbell and Steele 2020). However, some plants labeled as 'pollinator-friendly' have been shown to have low visitation by pollinators (Garbuzov and Ratnieks 2014A, Garbuzov et al. 2017). Due to the lack of guidelines for labeling plants as 'pollinator-friendly', consumer choices may be misled when purchasing plants.

Floral rewards produced by various cultivars could be the reason for the discrepancy seen in pollinator visitation. During the breeding process, the selection for annual cultivars is based on vigor, growth habit, floral display, and floral abundance (Horn 2002, Guo and Warner 2020). Nectar quality and availability are a main driver of floral visitation for pollinators such as bumble bees (Somme et al. 2015) and syrphid flies (Van Rijn and Wäckers 2016). Nectar sugar

concentrations have also been shown to vary among plant taxa in agricultural and nonagricultural areas (Pamminger et al. 2019), as well as in urban gardens (Tew et al. 2022). When plants are selected for breeding, floral traits like nectar are usually ignored in favor of growth, color and vigor (Comba et al. 1999). As a result, different cultivars of annuals may vary considerably in their nectar production and quality.

In papers published on variation in pollinator visitation rates among cultivars, focus has largely been placed on ornamental perennial plants instead of annual flowers (Garbuzov and Ratnieks 2014B, Mach and Potter 2018, Rollings and Goulson 2019, Erickson et al. 2021). For example, Garbuzov and Rateniks (2014B) measured pollinator visitation to 13 different cultivars of the perennial *Lavandula* spp. two separate years, along with other perennials, and found large variation in pollinator visitation among cultivars. Similarly, Rollings and Goulson (2019) showed pollinator visitation among 111 plant cultivars, 16 annuals of them annuals, with most cultivars showing variation in visits. Other studies also focus on sampling plants for visitation of certain pollinator groups such as bumble bees (Sikora et al. 2016), honey bees (Sponsler et al. 2020) and butterflies (Shackleton and Ratnieks 2016), but lack specific cultivars for plants sampled.

A few previous studies have specifically addressed variation in pollinator visitation among cultivars of annual flowers (Yeargan and Colvin 2009, Erickson et al. 2020). Yeargan and Colvin (2009) focused on butterfly visitation to four cultivars of *Zinnia* spp. and found one cultivar, Lilliput, was visited more often that the other three cultivars. Erickson et al. (2020) evaluated pollinator visitation to five different cultivars each of the annuals *Zinnia* spp., *Lantana* spp., *Lobularia* spp., *Salvia* spp. and *Tagetes* spp. (marigold), and found variation among pollinator visitation and taxa to specific cultivars. Erickson et al. (2020) has demonstrated that

variation in pollinator visitation among cultivars of annual flowers is expected with their work has identifying cultivars that can be recommended. However, the five annuals evaluated are a small proportion of the number of cultivars being sold, which means no information is available for a majority of the annual flowers being sold. For example, marigold, one of the top ten most sold annuals (USDA-NASS 2019), had five cultivars evaluated for pollinator visitation, but has 181 varieties listed on the Ball Seed (Chicago, IL) website for purchase. With so many cultivars on the market for consumer purchase, more studies on variation in pollinator visitation are needed to help inform consumers and growers on what cultivars receive the most pollinator visits.

To provide better information on cultivar preferences, we looked at pollinator visitation rates to cultivars of three annual flowers, *Tagetes* spp. (marigold), *Portulaca* spp. and *Bidens* spp., which have been advertised by growers like Ball Seed as being attractive to pollinators. Of these three, only marigold is among the top twenty most popular annuals in terms of sales (USDA-NASS 2019). In total, six cultivars of *Tagetes* spp. (marigold), eight cultivars of *Portulaca* spp. and ten cultivars of *Bidens* spp., were chosen to be evaluated for two years through collecting pollinators during sample periods to obtain pollinator visitation rates for each cultivar. Since nectar could be an important floral trait that promotes pollinator visitation, in the second year we also looked at nectar production and nectar sugar concentrations in the most and least pollinator visited cultivars of *Portulaca* spp. and *Bidens* spp. and *Bidens* spp. based on pollinator visitation data from the first year. We hypothesized (1) that pollinator visitation would vary greatly among the cultivars of each plant, and (2) that cultivars with a higher pollinator visitation rate would produce more nectar or have higher sugar concentrations.

MATERIALS AND METHODS

Plant Preparation

The cultivars of the annuals chosen for the experiment consisted of six cultivars of *Tagetes* spp. (marigold), eight cultivars of *Portulaca* spp. and ten cultivars of *Bidens* spp. All cultivars annual species and cultivars are listed in Table 1. In 2020 and 2021 seeds, plugs or cuttings were purchased from commercial greenhouse growers for planting at the Michigan State University Plant and Soil Sciences Greenhouses. The plants were grown starting in mid-February to have them at a height of at least 0.4 m and in full flower before June 1st, when they were planted outside. Seedlings started in plug trays were transferred into 18.9 L plastic pots after six weeks, with five plugs put into each pot. No pesticides were used in the growing process. Predatory mites were used for biological control of thrips and spider mites, and parasitoids were released for aphid control. One week before the annual flowers were planted in field plots, Ozmacote® slow-release fertilizer (Marysville, OH) was applied to pots at the labeled rate to provide fertility throughout the summer season.

All plants were planted in the ground at the MSU Pollinator Performance Center, East Lansing, Michigan, on June 1st each year. A 32×10 m area was cleared of any vegetation and rototilled for planting. The surrounding area consisted of alfalfa and a mix of weeds. The plot area was divided into three smaller areas: one for *Bidens* spp. (10×6 m), one for *Portulaca* spp. (8×6 m), and one for marigold (6×6 m). A cleared border of 3 m separated the three plot areas. All annuals were planted in a randomized complete box design for each type. Plants were placed into six rows within each plant block, so that each row contained one of each cultivar in a randomized order. The layout of the plot in 2020 was repeated in the year 2021. The *Bidens* spp.

plot consisted of 60 plants (10 cultivars in each row); the Portulaca spp. plot consisted of 48 plants (8 cultivars in each row); and the marigold plot consisted of 36 plants (6 cultivars in each row) (Fig. 2). Plants were placed a half meter apart in rows to allow adequate spacing for plant growth. While planting, potting soil was added and mixed with the natural ground soil to help the plants with growth and rooting. The plants were watered with 10 cm of irrigation from a sprinkler each week if there was no natural rainfall for that week. The entire plot was maintained with weekly weeding so that a 1 m perimeter around the plot remained cleared. Three hives of *Bombus impatiens* (Cresson) were ordered from Biobest Inc. (Leamington, Ontario) each year and placed about 20 m away from the field plots. Two *Apis mellifera* (Linnaeus) hives were placed 15 m from the plots both years. For the year of 2021, five extra colonies of *Apis mellifera* were placed around 100 m away from the plots, and were maintained according to standard beekeeping management. It was not known how many total honey bee colonies may have been within the flight range of the plots, except for the colonies within 150 m of the plot.

Pollinator Collection

In the months of June to August of 2020 and 2021, pollinators were collected from all annuals. Collections were made twice per week unless weather conditions were unsuitable, for a total of 24 collection periods in 2020 and 20 collection periods in 2021. Pollinators were only collected on days when the temperature was between 15° and 33° C, or wind speed was below 25 kph. Pollinators were also not collected during rain or when rain had occurred earlier in the morning. For each collection period, every plant was observed for one minute. Any bee or syrphid that was previously on the flowers or landed on the flowers of the annual during the

one-minute period was collected. All specimens were collected with an 18-volt insect vacuum (Bioquip Inc., Rancho Domingo, CA). The pollinators were then euthanized with ethyl acetate, pinned, labeled, and stored in Cornell University drawers (Bioquip Inc., Rancho Domingo, CA).

All pollinators collected were identified at least to the genus level, with some being taken to the species level. *Bombus impatiens*, *Apis mellifera*, and syrphid flies were identified by comparison to voucher specimens collected one or two years earlier in a related research project at the Michigan State University Horticulture Teaching and Research Center, a site that is located 1.5 km away, and also surrounded by agricultural fields. Syrphid flies were also identified to species using the Field Guide to the Flower Flies of Northeastern North America (Skevington et al. 2019). All identifications, other than for wild bees, were confirmed by Gary Parsons, Emeritus Curator of the Michigan State University Arthropod Research Collection. All wild bee identifications were made by Jason Gibbs, Curator of the J. B. Wallis and R. E. Roughley Museum of Entomology, with the exception of *Xylocopa virginica* (Linnaeus). Voucher specimens for all species collected for this research are deposited in the collection.

Nectar Collection

In 2021 nectar was collected from the highest and lowest visited cultivar of *Bidens* spp. and *Portulaca* spp., based on the mean pollinator visitation per cultivar the previous year. For both cultivars of *Bidens* spp. (Pretty in Pink and Bee Happy Red Imperial) and *Portulaca* spp. (Pazzaz Tangerine and Happy Hour Rosita), four standard pollinator exclusion bags were placed in different directions on four individual annuals of each cultivar. This gave a total of 16 bags for each cultivar that all contained at least three flower heads. Bags were placed on mature flowers

for 24 hours before sampling to allow time for the flowers to produce nectar. Nectar sampling took place throughout two days in mid-August at one-hour intervals from 9:00 to 16:00. Weather conditions were sunny or partly cloudy with no rain. Nectar was not collected from the highest and lowest visited cultivars of marigold because the amount of nectar produced was too small to measure.

Nectar from both *Portulaca* spp. cultivars was collected from bagged flowers with 1.0 μ l capillary tubes. A total of 20 nectar samples from Pazzaz Tangerine and 20 samples from Happy Hour Rosita were collected. We initially attempted to get nectar samples from bagged flowers of both cultivars of *Bidens* spp. with 1.0 μ l capillary tubes. Since *Bidens* spp. flowers are composite flowers that contain multiple florets within one floral head, nectar samples were taken from five florets per floral head. The five nectar samples were then summed together and divided by five to give the mean nectar volume per floral head. A total of 20 nectar samples were taken from Pretty in Pink using the capillary method. We were unable to collect nectar using the capillary method from the lowest visited cultivar, Bee Happy Red Imperial, due to the small amount of nectar collected in all replicate samples. The volume of nectar for each sample collected by the capillary method was determined by using a digital caliper (AdoricLife, Orlando, FL) to measure how much of the 1.0 μ l capillary tube was filled. The length of the filled capillary tube was then converted into microliters. Sugar concentrations for each sample were determined by using a Reichert Inc. (Depew, NY) Brix50 refractometer.

Due to the lack of sufficient nectar from sampling Bee Happy Red Imperial with capillary tubes, both Pretty in Pink and Bee Happy Red Imperial were sampled using a centrifugation method. For each cultivar, mature floral heads were removed from the bagged flowers and placed individually into 500 µl PCR tubes after all petals were removed. The PCR tubes were

then placed into an Eppendorf (Hamburg, Germany) 5415D centrifuge that was run for two minutes at 3000G to extract the nectar. The nectar was collected from the bottom of the PCR tube with a 1.0 µl capillary tube. Pretty in Pink had a total of five samples taken and Bee Happy Red Imperial had a total of 14 samples taken. The volume of nectar for each sample was determined using the same method above. Sugar concentrations were also determined for each sample as previously described.

Data Analysis

All analyses were conducted in R Version 4.1.2 (R Core Team 2021). Pollinators collected from research plots were split into four pollinator groups: *Apis mellifera*, *Bombus impatiens*, Wild Bees, and Syrphids. Although mean visitation rates for each group of pollinators to each cultivar are shown in bar graphs, the statistical comparison of mean visitation rates among cultivars of marigold, *Portulaca* spp. and *Bidens* spp. were made after combining the four groups of pollinators into an overall pollinator visitation rate. One-minute sampling periods to each of the six replicate cultivars were summed for all collection periods each year to determine a mean visitation rate for each annual. The results are represented as overall pollinator visits per minute for each cultivar and each year. A Poisson regression approach was used to assess the differences in pollinator visitation rates among the cultivars of each annual. This analysis was done for each year separately, with pollinator visitation counts set as the response variable and the time of sampling as the offset. Marigold, *Portulaca* spp. and *Bidens* spp. were analyzed separately to compare visitation rates among the cultivars of each. Nectar metrics are displayed as box plots and analyzed using a unpaired Wilcoxon test to compare the differences in nectar

volume and nectar sugar concentration of the two chosen *Portulaca* spp. and *Bidens* spp. cultivars.

RESULTS

In 2020 and 2021 a total of 2,558 pollinators were collected from all annuals in our field plots (Table 2). In general, there was a wide variation in pollinator visitation among cultivars of *Tagetes* spp. (marigold), *Portulaca* spp. and *Bidens* spp., as well as a difference in the observed visitation rates in 2020 compared with 2021 (Figs. 3, 4 and 5). Nectar collected from flowers of the most and least pollinator visited cultivars of 2020 also shows a large variation in nectar volume and sugar concentration among cultivars (Figs. 6, 7, 8 and 9). In the following three sections, complete results are presented separately for marigold, *Portulaca* spp. and *Bidens* spp. that demonstrate variation among cultivars in pollinator visitation rate as well as nectar production and sugar concentrations.

Marigold

Within each year, Taishan Orange in 2020 and 2021 (0.22 ± 0.08 , 0.23 ± 0.08 , respectively) and Single Disco Marietta in 2020 (0.37 ± 0.1), had greater mean pollinator visitation rates than the remaining four cultivars that all had visitation rates less than 0.10 visits per minute (Fig. 3). Mean pollinator visits per minute for both years did not vary among the remaining cultivars; Crested Bonanza Yellow, Antigua Yellow, Antigua Primrose, and Antigua Orange (Fig. 3). Only one cultivar, Single Disco Marietta, varied among years, with a smaller mean pollinator visitation rate in 2021 (0.18 ± 0.07) than in 2020 (0.37 ± 0.1) (Fig. 3).

Portulaca

Within each year, Pazzaz Tangerine and Colorblast Lemon Twist had greater mean pollinator visitation rates in 2020 (1.42 ± 0.22 , 1.02 ± 0.16 , respectively) and 2021 (1.24 ± 0.2 ,

1.09 \pm 0.19, respectively) than all other cultivars, with the exception of Pazzaz Red Flare which had similar, but slightly lower visitation rates in 2020 (0.88 \pm 0.15) and in 2021 (0.77 \pm 0.16) (Fig. 4). Pazzaz Red Flare had greater visitations rates in both years than all remaining cultivars with the exception of Happy Hour Deep Red in 2021 (0.67 \pm 0.14) (Fig. 4). There were no differences among the five least visited cultivars in 2020 or 2021 (Fig. 4).

Happy Hour Deep Red and Happy Hour Rosita had higher mean pollinator visitation rates in 2021 (0.67 ± 0.14 , 0.48 ± 0.12 , respectively) than in 2020 (0.21 ± 0.07 , 0.19 ± 0.07 , respectively) (Fig. 4). All other cultivars (Pazzaz Tangerine, Colorblast Lemon Twist, Pazzaz Red Flare, Happy Hour Banana, Happy Hour Fuchsia, and Happy Hour Coconut) did not vary from year to year for overall pollinator visits per minute (Fig. 4).

Bidens

Within each year, Pretty in Pink had the greatest mean visitation rate in 2020 (1.17 \pm 0.17) and in 2021 (2.47 \pm 0.28) compared with all other cultivars (Fig. 5). In both years Bee Happy Orange had greater mean pollinator visits per minute in 2020 (0.47 \pm 0.11) and 2021 (0.63 \pm 0.14) than Blazing Embers, BeeDance Painted Red, BeeDance Red Stripe, and Bee Happy Red Imperial (Fig. 5). In 2021 Bee Happy Orange also had more pollinator visits than Sunbeam (0.19 \pm 0.08), BeeDance Yellow (0.12 \pm 0.06), and Yellow Splash (0.06 \pm 0.04) (Fig. 5). Mean pollinator visits per minute for both years did not vary among the cultivars of Blazing Embers, BeeDance Red Stripe, and Bee Happy Red Imperial (Fig. 5).

Pretty in Pink had a higher mean pollinator visitation rate in 2021 (2.47 ± 0.28) than in 2020 (1.17 ± 0.17) (Fig. 5). Yellow Splash also varied among years and had a lower mean pollinator visitation rate in 2021 (0.06 ± 0.04) than in 2020 (0.27 ± 0.08) (Fig. 5). All other

cultivars (Bee Happy Orange, Sunbeam, Bee Bold, BeeDance Yellow, Blazing Embers, BeeDance Painted Red, BeeDance Red Stripe, and Bee Happy Red Imperial) did not vary in mean pollinator visitation rates from year to year (Fig. 5).

Nectar

The volume of nectar collected from the cultivar of *Portulaca* spp. with the greatest mean pollinator visits per minute in 2020, Pazzaz Tangerine, was greater than the volume of nectar collected the least visited cultivar, Happy Hour Rosita (W = 421, p < 0.0001) (Fig. 6). Similarly, the sugar concentration of nectar from Pazzaz Tangerine flowers was greater than that from Happy Hour Rosita flowers (W = 372, p < 0.001) (Fig. 7).

The volume of nectar collected from the cultivar of *Bidens* spp. with the greatest mean pollinator visits per minute in 2020, Pretty in Pink, was greater than the volume of nectar collected from the least visited cultivar, Bee Happy Red Imperial, for both the capillary method (W = 258, p < 0.0001) and centrifuge method (W = 53, p < 0.01) (Fig. 8). Similarly, the nectar sugar concentration from the flowers of Pretty in Pink was greater than that from Bee Happy Red Imperial flowers using the capillary method (W = 266, p < 0.0001) and the centrifuge method (W = 56, p < 0.001) (Fig. 9).

DISCUSSION

Our results confirm that the pollinator visitation rates to marigold, *Portulaca* spp. and *Bidens* spp., which have been advertised as being attractive to pollinators, varies greatly among cultivars of each (Figs. 3, 4 and 5); so that as a group they cannot be accurately described as 'attractive to pollinators' or recommended as 'pollinator-friendly' without cultivar-specific data. It is known that nectar quality and availability tend to drive the visitation rate of bees and syrphids to flowers. Nectar quality and availability among the different cultivars of annuals could affect how often pollinators visit their flowers. Our comparison of nectar production and sugar concentration of one highly visited cultivar with a lesser visited cultivar of both *Portulaca* spp. and *Bidens* spp., supports this (Figs. 6, 7, 8 and 9). In the future, evaluating nectar production and sugar concentrations may be one way that plant breeders could collect data to support labeling of cultivars as 'pollinator-friendly'.

In 2021, honey bee visitation rates to annuals in our research plots were greater than in 2020, and in contrast the visitation rates of wild bees was greater in 2020 than in 2021. Syrphids visited flowers at a similar rate in 2020 and 2021. The focus of our research, comparison of pollinator visitation among cultivars of marigold, *Portulaca* spp. and *Bidens* spp., was based on the combined visits of honey bees, bumble bees, wild bees and syrphids within the year of 2020 and 2021, separately. Since our results are based on combined pollinator visitations and sampling times took place during similar weather parameters each year, greater visitation to the majority of cultivars by honey bees in 2021 and by wild bees in 2020 should not impact within-year cultivar comparisons.

Pollinator visitation rates changed significantly from 2020 to 2021 for one cultivar of marigold, Single Disco Marietta; two cultivars of *Portulaca* spp., Happy Hour Deep Red and

Happy Hour Rosita; and three cultivars of *Bidens* spp., Pretty in Pink, Bee Dance Yellow, and Yellow Splash. It is unlikely that these changes were due to any differences in general pollinator activity in the area. The decreased visitation rate by pollinators in 2021, compared with 2020, to Single Disco Marietta marigold is opposite of what would be expected from the general increased honey bee activity seen in 2021. It is also unlikely that an increase in honey bee activity explains any of the differences in combined pollinator visits to a particular cultivar from 2020 to 2021, with the possible exception of Pretty in Pink *Bidens* spp., which had a major increase in honey bee visits in 2021.

The most likely explanation for the change in pollinator visitation for a cultivar from 2020 to 2021 is that the flowers did not produce the same amount or quality of nectar from year to year. Since nectar samples were collected multiple times throughout the day to account for variation in nectar secretion at different times of day, overall nectar volumes and sugar concentrations should accurately represent the differences seen in cultivars. Apparently, a cultivar of an annual may look nearly identical from year to year, but vary in characteristics important for attracting pollinators. We did not collect nectar from cultivars in both years, so we cannot conclude that the large differences in visitation rate to a particular cultivar was due to a change in nectar production. However, our data suggests that this is likely, and future research should explore this possibility. Also, in the future, breeding programs for annual flowers that will be labeled as 'pollinator-friendly' should measure nectar production or pollinator visitation each year.

Complimentary with other studies, our research indicates that pollinator visitation varies greatly among cultivars. However, comparing results with previous research is complicated because different approaches have been used to determine rates of pollinator visitation. We used

one minute collection intervals, where all insect visitors to flowers on one plant were collected during the one minute sample period. All specimens were later pinned and identified. Many previous studies use a 'snapshot' method of observation, where each plant is observed for 10 to 30 sec by trained observers that visually identify flower visitors (Garbuzov and Ratnieks 2014B, Mach and Potter 2018, Rollings and Goulson 2019). These studies all classify pollinators into groups like our study, however visual identification may sometimes not be as accurate, particularly for identifying wild bees and syrphids to the genus and species level. Some researchers used longer observations time for each plant, such as the 10-minute observations seen in both Erickson et al. (2020, 2021) studies. Longer observation periods are helpful for collecting data on less frequently observed pollinator taxa, such as butterflies. In the future, standardization of methods used for determining pollinator visitation to cultivars of ornamental flowers would be helpful when attempting to compile information for making recommendations.

Apis mellifera visitation rates to cultivars of *Tagetes* spp. (marigold), *Portulaca* spp. and *Bidens* spp. varied from year to year with more honey bees visiting in 2021 than 2020. This could possibly be attributed to the five extra colonies placed out in 2021, or unknown hives in the area as honey bees have been recorded to travel up to 9.5 km away to forage (Beekman and Ratnieks 2000). Another possible influence for increased honey bee visitation could be changes in the availability of floral resources outside of the research area. Honey bees are generalist pollinators that visit many flowering plant species (Goulson 2003) and can communicate the distance and quality of floral resources to other members of the hive (Von Frisch 1967). The large variation of honey bee visitation seen throughout sampled cultivars (Figs. 3, 4 and 5) highlights that honey bee visitation could be largely variable in similar future studies due to their generalist foraging behavior.

Wild bees represent a large portion of local pollinator communities and are valuable pollinators in agricultural settings (Garibaldi et al. 2014). Woody ornamentals and native shrubs have been evaluated in replicated trials to determine which species are most suitable for pollinators in Michigan (Rowe et al. 2018), and to boost populations of pollinators for the purpose of improved pollination of fruit crops (Isaacs et al. 2009). The area surrounding our research plots is agricultural, with the nearest woodlot being 0.5 km away. Even so, we collected 26 spp. of wild bees (Table 2) showing wild bees could still benefit from ornamental annuals in agricultural areas. Wild bees are also considered important pollinators and valued wildlife in urbanized areas (Wilson and Jamieson 2019). Urban or suburban areas often have an even greater diversity of wild bees compared with agricultural settings (Prendergast et al. 2022). Although annual flowers in urbanized areas may not be as important of a source for nectar and pollen as perennial flowers, more annuals are purchased and planted each year. Knowing what cultivars are highly visited by wild bees is important as more people have become interested in planting flowers that support pollinators.

Visitation rates of bumble bees were low compared with the other groups of pollinators, with most of the bumble bees being collected from the top two cultivars of *Portulaca* spp. (Fig. 4). The only species of bumble bee collected throughout all pollinator collections was *Bombus impatiens*. Due to the low numbers of bumble bees collected we assume most if not all came from the *Bombus impatiens* colonies placed out near our field plots. Bumble bee population density and diversity have been found to be suppressed by intensively managed land (Larkin and Stanley 2021), which could be why we did not see a large presence of other bumble bee species as the surrounding area is mostly managed agricultural land.

Syrphid flies were collected from every cultivar of marigold, *Portulaca* spp. and *Bidens* spp. during both years of pollinator collections (Figs. 3, 4 and 5). Attention to syrphid flies as a functional pollinator group, as well as a form of biological control, has increased in recent years (Klecka et al. 2018, Doyle et al. 2020, Dunn et al. 2020). Although the public may not be aware of the importance of syrphids as pollinators and predators, they can play a valuable role in gardens and agricultural areas, and should be included when evaluating overall pollinator attractiveness. The first and most important practice for gardeners that want to increase pollinators in their garden is to avoid the use of insecticides. Because the larvae of syrphids are predators of aphids and psyllids, which are common garden pests, they are important for natural control of garden pests in the absence of insecticides (White et al 1995, Irvin et al. 2021). Providing floral resources for syrphids could be beneficial perk in garden pest control efforts.

A lack of floral resources in intensely managed areas is an important factor causing pollinator decline. Annual ornamental plants can benefit pollinators in urban areas, as well as providing forage in the early and late season. Cultivars of annuals such as *Zinnia* spp., *Lobularia* spp., *Tagetes* spp. (marigold), *Lantana* spp. and *Pentas* spp., have previously been shown to vary significantly in visitation by pollinators, as well as the pollinator taxa visiting. Our results show similar results of different pollinator visitation rates among the selected cultivars. One possible reason for this difference in visitation could be nectar availability and quality of varying cultivars, as our results show different nectar production and sugar concentrations among cultivars. The public is interested in learning what they can do to help pollinators, and many are planting 'pollinator-friendly' plants. Marigold, *Portulaca* spp. and *Bidens* spp. are three types of annual flowers that could be used to support pollinators while adding the color and beauty to gardens consumers look for. This research informs entomologists, horticulturalists, growers and

educators about which cultivars of marigold, *Portulaca* spp. and *Bidens* spp. can be recommended to attract pollinators, and further emphasizes the need to evaluate all 'pollinator friendly' flowers at the cultivar level.

APPENDIX

| Annual Flower | Species | Cultivar |
|----------------|--------------------------------|------------------------|
| Tagetes spp. | Tagetes erecta L. | Taishan Orange |
| | Tagetes erecta L. | Antigua Orange |
| | Tagetes erecta L. | Antigua Primrose |
| (marigold) | Tagetes erecta L. | Antigua Yellow |
| | Tagetes patula L. | Crested Bonanza Yellow |
| | Tagetes patula L. | Single Disco Marietta |
| | Portulaca oleracea L. | Colorblast Lemon Twist |
| | Portulaca oleracea L. | Pazzaz Red Flare |
| | Portulaca oleracea L. | Pazzaz Tangerine |
| | Portulaca grandiflora Hook. | Happy Hour Banana |
| Portulaca spp. | Portulaca grandiflora Hook. | Happy Hour Coconut |
| | Portulaca grandiflora Hook. | Happy Hour Deep Red |
| | Portulaca grandiflora Hook. | Happy Hour Fuchsia |
| | Portulaca grandiflora Hook. | Happy Hour Rosita |
| | Bidens ferulifolia (Jacq.) DC. | BeeDance Painted Red |
| | Bidens ferulifolia (Jacq.) DC. | BeeDance Red Stripe |
| | Bidens ferulifolia (Jacq.) DC. | BeeDance Yellow |
| | Bidens ferulifolia (Jacq.) DC. | Bee Happy Orange |
| D: 1 | Bidens ferulifolia (Jacq.) DC. | Bee Happy Red Imperial |
| Bidens spp. | Bidens ferulifolia (Jacq.) DC. | Bee Bold |
| | Bidens ferulifolia (Jacq.) DC. | Blazing Embers |
| | Bidens ferulifolia (Jacq.) DC. | Yellow Splash |
| | Bidens ferulifolia (Jacq.) DC. | Pretty in Pink |
| | Bidens aurea (Aiton) Sherff | Sunbeam |

Table 1. List of all annual plant cultivars and species.

Table 2. List of all pollinators collected with identification to genus or species and number collected.

| Pollinator Group | Family | Genus | Species | Collected (n) |
|------------------------|-----------------|---------------|---------------|---------------|
| Apis mellifera | Apidae | Apis | mellifera | 551 |
| Bombus impatiens | Apidae | Bombus | impatiens | 49 |
| | Andrenidae | Calliopsis | andreniformis | 7 |
| | | Melissodes | bimaculatus | 4 |
| | Apidae | Melissodes | sp. | 3 |
| | | Xylocopa | virginica | 3 |
| | | Agapostemon | sericeus | 6 |
| | | Agapostemon | texanus | 1 |
| | | Agapostemon | virescens | 19 |
| | | Augochlorella | aurata | 12 |
| | | Halictus | confusus | 130 |
| | | Halictus | ligatus | 247 |
| | | Halictus | rubicundus | 12 |
| | | Lasioglossum | admirandum | 2 |
| Wild Bees ¹ | | Lasioglossum | coriaceum | 2 |
| | Halictidae | Lasioglossum | ellisiae | 6 |
| | Trailetrade | Lasioglossum | ephialtum | 18 |
| | | Lasioglossum | hitchensi | 2 |
| | | Lasioglossum | imitatum | 1 |
| | | Lasioglossum | leucocomus | 3 |
| | | Lasioglossum | leucozonium | 3 |
| | | Lasioglossum | paradmirandum | 6 |
| | | Lasioglossum | pectorale | 1 |
| | | Lasioglossum | pilosum | 169 |
| | | Lasioglossum | weemsi | 4 |
| | | Lasioglossum | sp. | 257 |
| | | Sphecodes | mandibularis | 1 |
| | Megachilidae | Anthidium | oblongatum | 1 |
| | rphid Syrphidae | Allograpta | obliqua | 9 |
| | | Eristalis | flavipes | 2 |
| | | Eristalis | arbustroum | 7 |
| | | Eristalis | obscura | 7 |
| 0 1.1 | | Eristalis | tenax | 178 |
| Syrphid | | Helophilus | fasciatus | 23 |
| | | Helophilus | latifrons | 22 |
| | | Sphaerophoria | scripta | 22 |
| | | Syritta | pipiens | 14 |
| | | Syrphus | ribesii | 15 |
| | | Toxomerus | sp. | 735 |

Table 2. (cont'd)

| Syrphid | Syrphidae | Tropidia | quadrata | 4 |
|---------|-----------|----------|----------|---|
|---------|-----------|----------|----------|---|

¹Wild Bees refers the category of pollinator that includes all bees with the exception of honey bees (*Apis mellifera*) and the common eastern bumble bee (*Bombus impatiens*).



Figure 1. Pictures of field plots (from left to right: Tagetes spp. [marigold], Portulaca spp., and Bidens spp.).

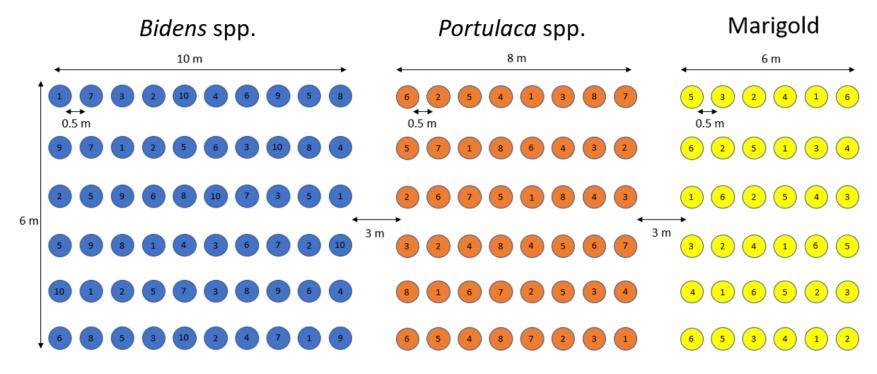


Figure 2. Plot diagram for 2020 and 2021 showing the randomized placement of cultivars in each row and block with the number inside each circle representing a particular cultivar.

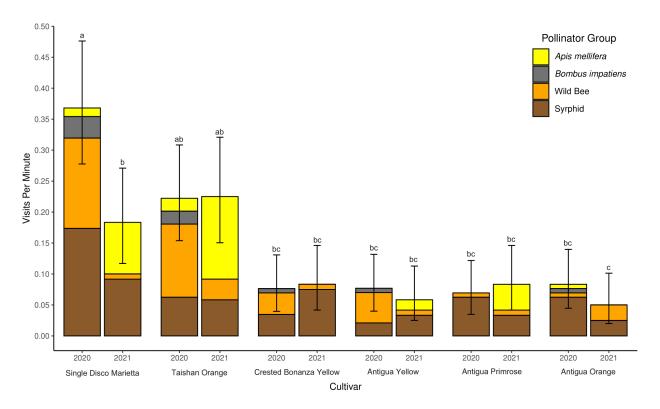


Figure 3. Mean pollinator visitation rate to six cultivars of *Tagetes* spp. (marigold) in 2020 and 2021. Error bars are 95% confidence intervals.

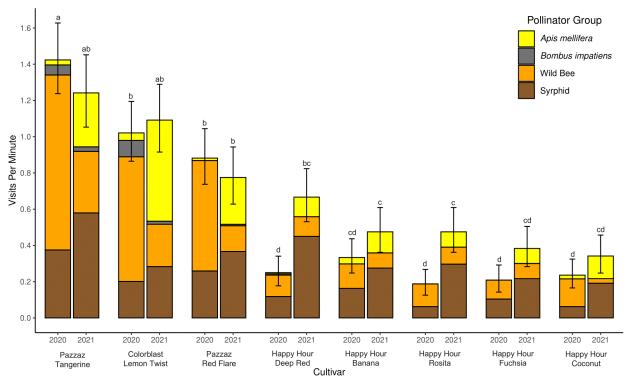


Figure 4. Mean pollinator visitation rate to eight cultivars of *Portulaca* spp. in 2020 and 2021. Error bars are 95% confidence intervals.

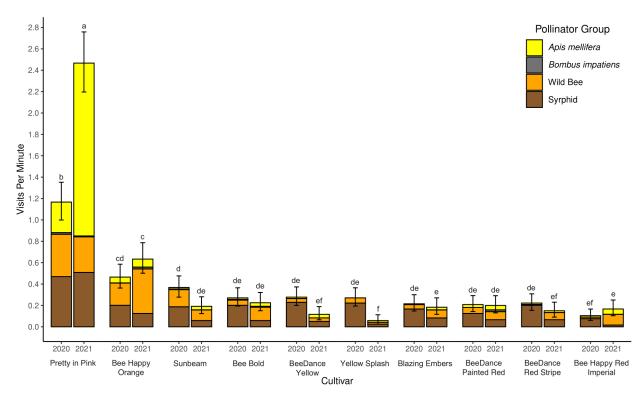


Figure 5. Mean pollinator visitation rate to ten cultivars of *Bidens* spp. in 2020 and 2021. Error bars are 95% confidence intervals.

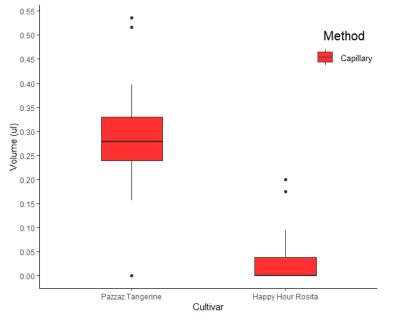


Figure 6. Box plot showing median and range of nectar volume produced by two different *Portulaca* spp. cultivars (W = 421, p < 0.0001).

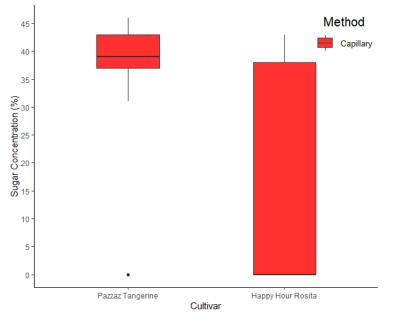


Figure 7. Boxplot showing median and range of nectar sugar concentrations of two different *Portulaca* spp. cultivars (W = 372, p < 0.001).

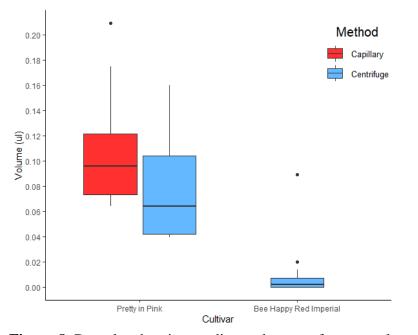


Figure 8. Box plot showing median and range of nectar volume produced by two different *Bidens* spp. cultivars with the capillary (W = 258, p < 0.0001) and centrifuge (W = 53, p < 0.01) method.

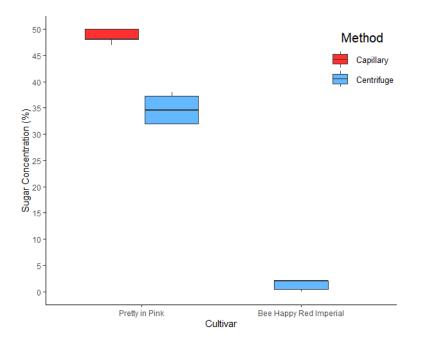


Figure 9. Box plot of showing median and range of nectar sugar concentrations of two different *Bidens* spp. cultivars with the capillary (W = 266, p < 0.0001) and centrifuge (W = 56, p < 0.001) method.

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