Nectar Monitoring of *Salvia* species in the UC Berkeley Bee Garden

Introduction

In mid 2003, a bee garden was established in a small plot at the University of California, Berkeley Oxford Tract in order to attract local native California bee species. Bees were sampled in 2004 and their host flowers monitored (Wojcik et al 2008). The results of the 2004 study showed that in a short time, a high diversity of native bees can be attracted to a garden containing diverse floral resources. Since then, the UC Berkeley bee garden has changed in terms of floral composition as new floral resources are added to test their attractiveness to native bees (Frankie, personal communication). New flowers planted since 2004 include *Salvia* species from the Lamiaceae family, and these have been successful in attracting bees (personal observation). Proper monitoring of the frequency and diversity of bees visiting these new floral resources would be invaluable in assessing the attractiveness of these new plants to bees. Information about the attractiveness of plants to native bees would be useful for urban gardeners who are interested in attracting native pollinators to their gardens.

Aside from the observation that these new *Salvia* species attract bees, there remains the question of how these plants attract bees. A pollinator will move among plants to obtain floral rewards such as pollen and nectar. In 1976, Frankie et al. discovered that there is considerable interspecific and intraspecific variation in nectar flow patterns of flowering trees in the Costa Rican dry forest. The study also concluded that these variations could be largely responsible for the movement of pollinators among mass-flowering trees. Pleasants (1981) also found that nectar production rates affected floral preferences of bumblebees. To the best of my knowledge, no such study has been conducted on *Salvia* species.

The study conducted in Spring 2012 aimed to determine the attractiveness of the four *Salvia* species to native bees; *Salvia brandegei*, *Salvia melissadora* ‘Grape Scented Sage’, *Salvia munziii,* and *Salvia mellifera*. Specifically, I looked at the interspecific and intra-stalk variation in the volume and quality of nectar over time in these *Salvia* species, and if these variations affect bee movement over time. I tested the following hypotheses: (1) the native *Salvia* species (*Salvia brandegei*, *Salvia munziii*, and *Salvia mellifera*) would be more successful in attracting native bees as compared to the exotic *Salvia melissadora* ‘Grape Scented Sage’; (2) the trends in nectar volume and quality over time are different among different *Salvia* species and within stalks of the same species; (3) the number of bees attracted to a plant is positively correlated to the volume of nectar produced by the plant. To test these hypotheses, I conducted several bee frequency counts in the UC Berkeley bee garden and measured the amount of nectar produced by the selected stalks of the different *Salvia* species over time.

The study conducted in Summer 2012 focused on variation in nectar flow on a smaller scale. Variation in nectar flow among different flowers of the same plant and within the same flower over time were recorded. As different flowers were in season over the summer, the following *Salvia* species were assessed: *Salvia melissadora* ‘Grape Scented Sage’, *Salvia* ‘Indigo Spires’, *Salvia chamaedryoides*, and *Salvia uliginosa*. I tested the hypotheses that the amount of nectar produced differed amongst individual flowers and over time. To test these hypotheses, I measured the amount of nectar produced by selected flowers of the different *Salvia* species over time.

Methods

Study Site

The study was conducted on the Oxford Tract which is located on the University of California, Berkeley campus, at 1751 Walnut Street, Berkeley CA, 94720. The site is surrounded by mostly urban residential buildings, and a plot of agricultural land for field trials of various crops. The bee garden in the Oxford Tract was established in 2004 and mostly native plants were planted in order to attract native bees. The garden provides a continuum of diverse floral resources throughout the months of April to October, and several native and nonnative bees visit the garden throughout the flowering season (Wojcik et al. 2008). Sampling days were timed such that the weather would be warm (at least 68F) to control for the potential effects of temperature.

Study Species

Salvia was selected for the study species as there were several species already established in the garden and were known to attract various medium to large bees (Wojcik et al 2008). *Salvia* was also chosen because nectar from the flowers were readily accessible through capillary tubes (personal observation). All *Salvia* plants were in full flower during the course of the study.

Four different type of *Salvia* were chosen for the Spring 2012 study: *Salvia brandegei, Salvia munzii*, *Salvia mellifera*, and *Salvia melissadora* 'Grape Scented Sage'. Of these 4 species, the first 3 are native to California, while the last is native to mountains in Southern Mexico.

In Summer 2012, most of the spring *Salvia* species declined with the exception of *Salvia melissadora* 'Grape Scented Sage'. Instead, three new summer *Salvia* species which were popular horticultural elements in both farm hedgerows and urban gardens were selected. These include *Salvia* 'Indigo Spires', *Salvia chamaedryoides*, and *Salvia uliginosa*. *Salvia* 'Indigo Spires' is a hybrid cross between the non-natives *Salvia longispicata* and *Salvia farinacea*. *Salvia chamaedryoides* is native to Mexico, while *Salvia uliginosa* is native to Brazil, Argentina, and Uruguay.

Pollinators

Medium to large bees which frequented the *Salvia* species were represented from two different families, with *Xylocopa, Apis, Bombus,* and *Habropoda* from the family Apidae and *Anthidium* from the family Megachilidae. These bees are assumed to be pollinators of the *Salvia*.

Preparation for Nectar Monitoring

The methods for this section were originally styled after a similar research project conducted by Frankie and Haber 1983 on flowering trees in Costa Rica, where flowers on all trees were bagged prior to the study and most flowers were one-day flowers which opened before sunrise. This allowed sampling from a pool of new flowers, thus controlling for the age of the flower. For both studies, all flowers except buds on selected stalks were removed, bagged and secured with wooden clothespins at 6pm, after most bee activity had ceased. This was done to exclude bees from obtaining nectar from the sample flowers. On the next day, the bags were removed during nectar measurements. In the Spring 2012 study, plastic bags with small holes punched in with an awl were used, but mesh bags were used instead during the Summer 2012 study.

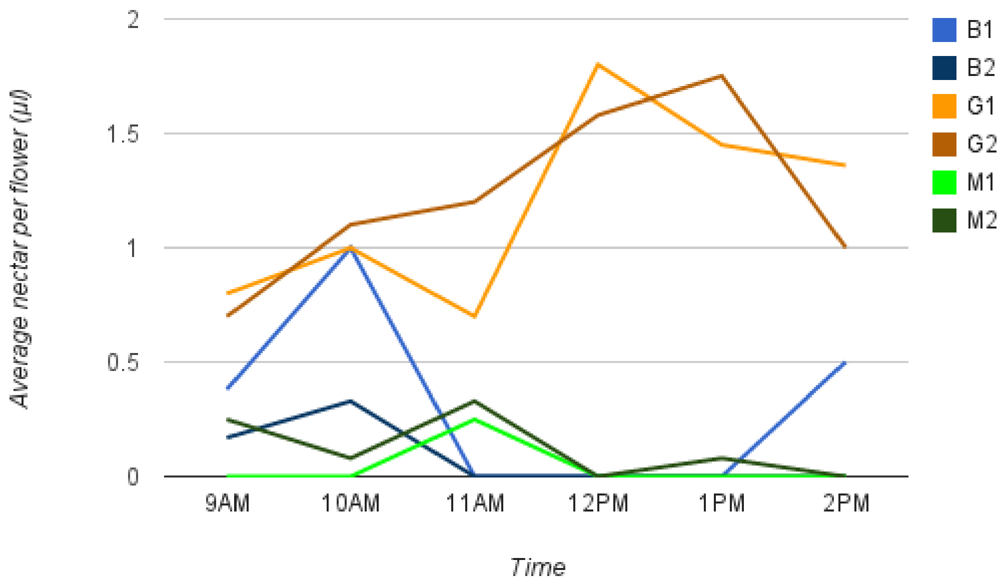
Nectar Monitoring

Nectar was sampled by careful probing through the center of the flower using precalibrated Drummond microcapillary tubes. Nectar was removed hourly from all open flowers from the stalk from 9am - 2pm. Sampling was initially conducted until 5pm but it was observed that most flowers stopped producing nectar after 2pm, and several flowers would be too damaged after repeated hourly sampling. Flower sample size either increased or decreased through time on a given stalk since flowers either opened or fell off periodically during the sampling period. For the Spring 2012 study, a mean amount of nectar per flower was calculated hourly for each stalk and each plant. For the Summer 2012 study, open flowers were labelled based on their position on the plant (row number followed by clock position assuming each row were split into twelve sections like a clock), and the amount of nectar per flower recorded.

Initially, there were concerns that by removing the nectar from the flower every hour, the flower may not be able to produce any more nectar. The results of average nectar per flower from the pilot study show that this is not the case (Graph 1), and individual flowers do not stop producing nectar after the nectar has been removed (personal observation).

Bee Monitoring

Bee counts were conducted on a certain portion of the plant every hour. Observation of bees were conducted a metre away from each plant and the portion of the plant observed was limited to roughly 1.5mx1.5m where the majority of the selected stalks for nectar monitoring were. The number of bees observed landing on flowers in that portion were recorded. Each frequency count lasted 3 minutes.



Graph 1: Average nectar production per flower (µl) over time. B1&2: *Salvia brandegei*, G1&2: *Salivia melissadora* 'Grape Scented Sage', M1&2: *Salivia munzii*

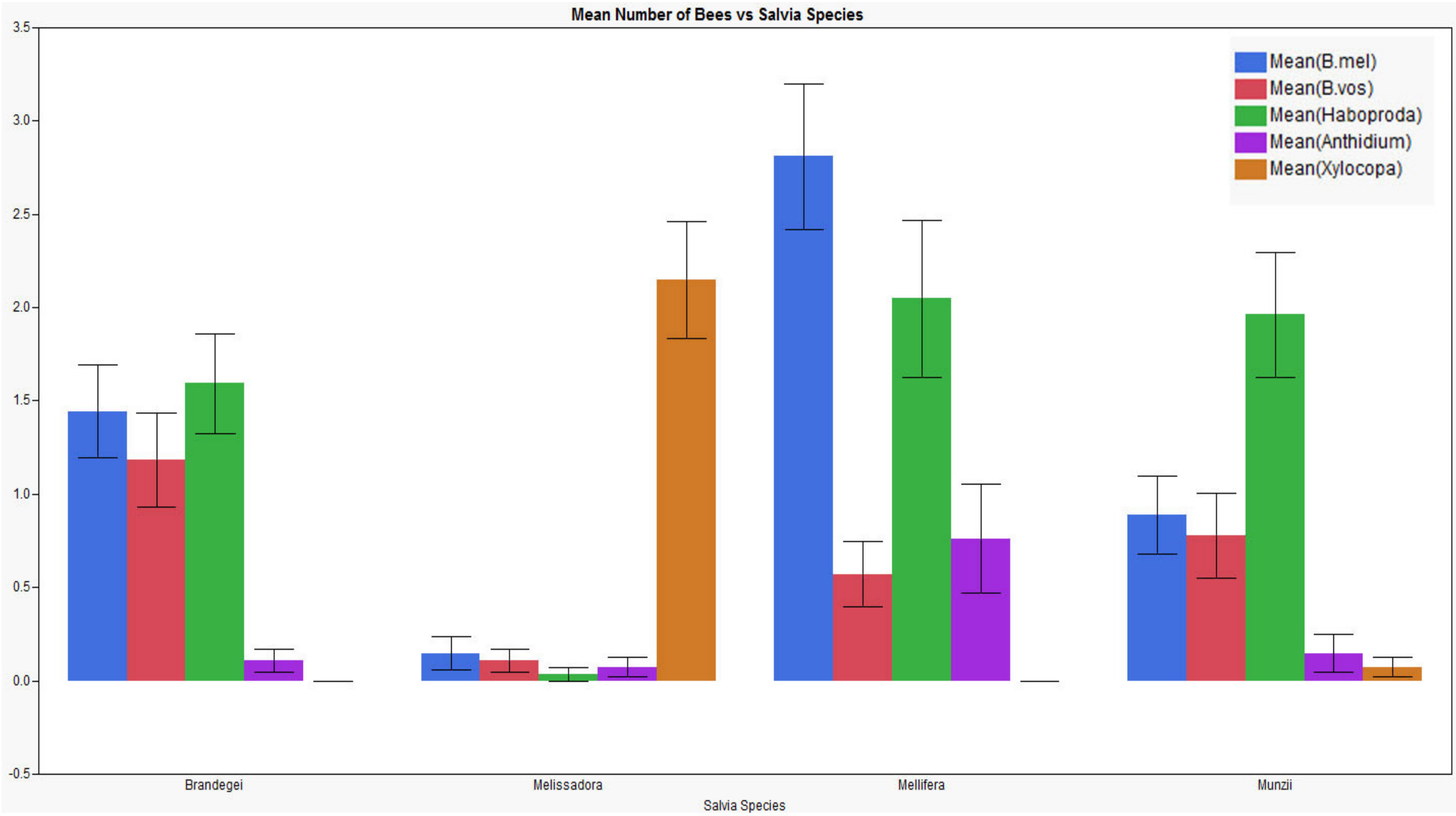
Results (Spring 2012 Study)

The bee diversity results show that the exotic *Salvia melissadora* attracts *Xylocopa* bees at a significantly higher rate than the other native *Salvia* (one-way ANOVA, df = 3,98, $p<0.0001$) but attracts less bees of other species (Graph 2).

Number of bees observed during peak nectar flow is not significantly higher than the number of bees observed during low nectar flow for each *Salvia* species (Table 1).

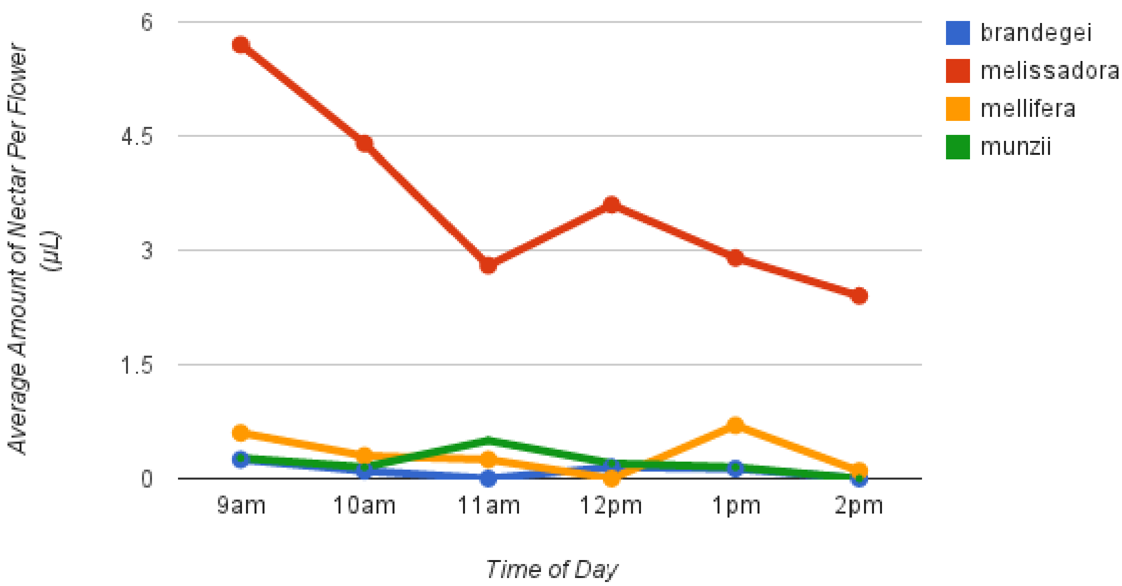
Plant	P-value	R^2 value
Salvia brandegei	0.1142	0.0246
Salvia melissadora	0.4423	0.0058
Salvia munzii	0.0180*	0.0559
Salvia mellifera	0.0856	0.0343

Table 1: Logistic regression of average volume of nectar versus the mean number of bees observed for the four different Salvia species. * indicates significance at the $P<0.05$ level. Although the P-value for Salvia munzii indicates a significant trend of mean number of bees increasing as nectar volume increases, the low R^2 value indicates that this trend is too weak to be considered meaningful.



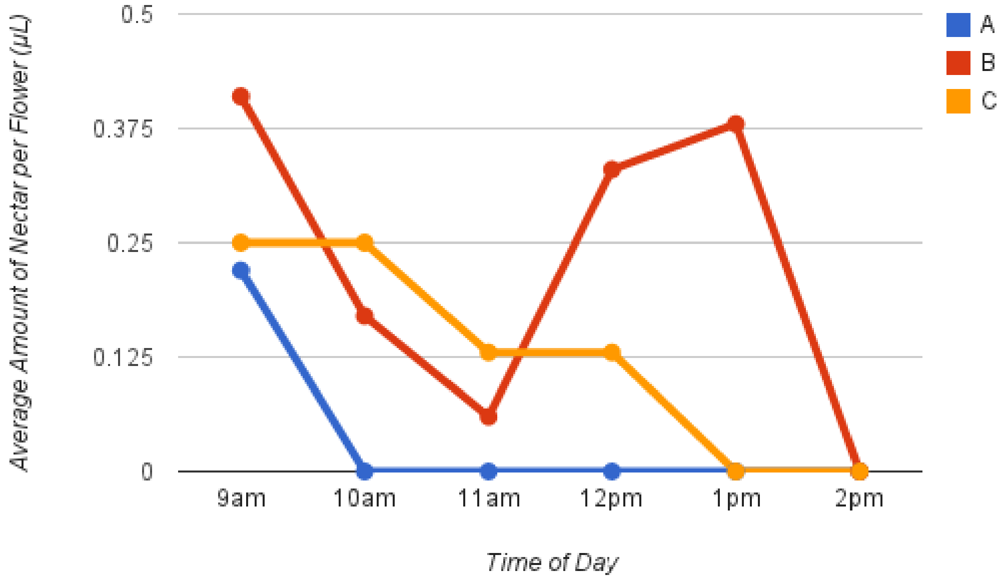
Graph 2: Mean Number of Bees vs *Salvia* Species, Error bars represent 1SE above and below the mean. Tukey's test reveals that mean number of *Xylocopa* is significantly higher in Salvia melissadora as compared to other *Salvia* species ($P < 0.05$)

Different *Salvia* species had different nectar flow patterns over time (Graph 3). In general, *Salvia melissadora* produced the highest average nectar per flower (one-way ANOVA, df = 3,98, $P<0.0001$) and the nectar was evenly spread over the flowers. This was in contrast to the other *Salvia*s, which had little consistency in nectar volume from flower to flower and some flowers had significantly higher volumes of nectar than others during the same period of time (personal observation).

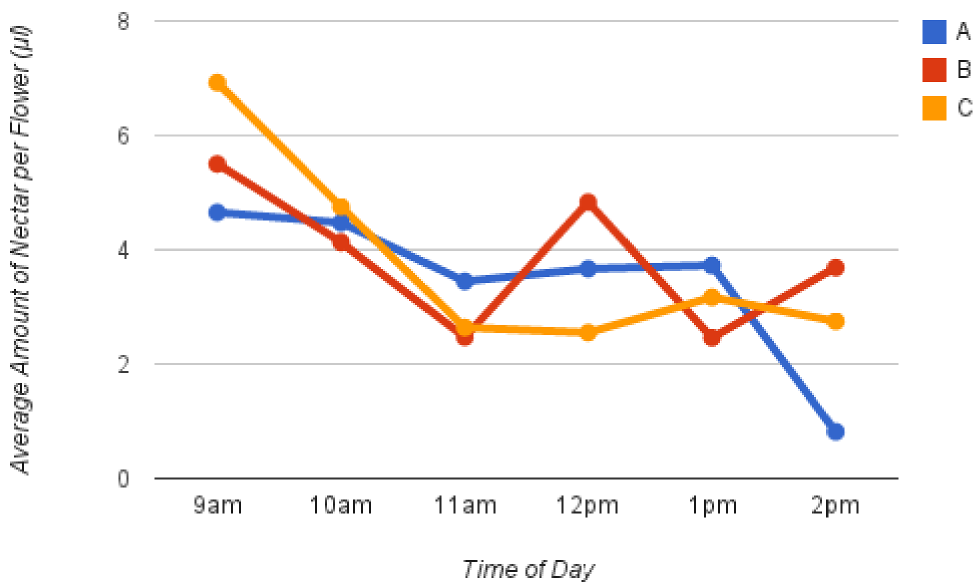


Graph 3: Average nectar production per flower (μl) over time for the four *Salvia* species.

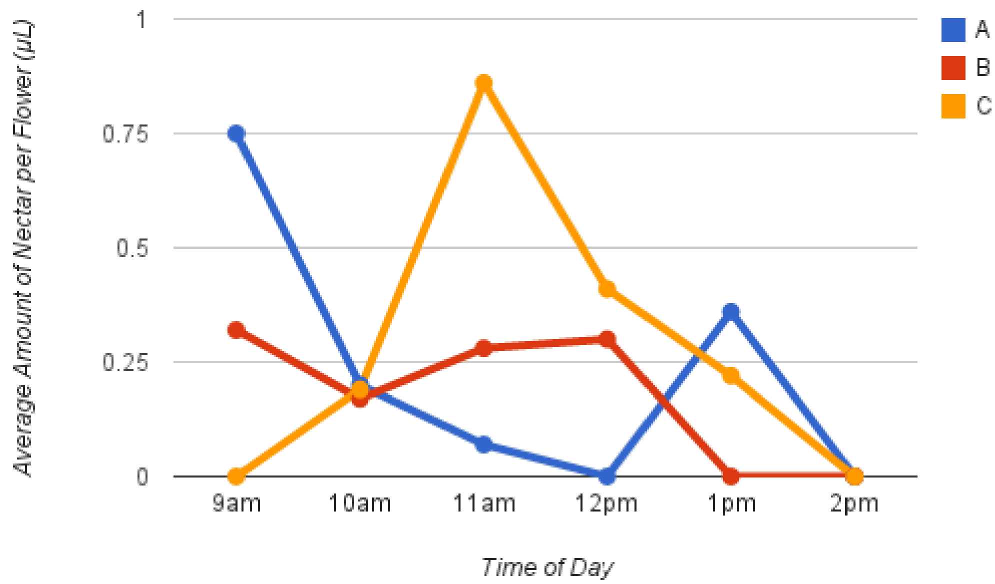
Even different stalks from the same *Salvia* species had different nectar flow patterns over time, with some stalks producing two peaks of average nectar volume per flower while other stalks produced a steady decline in nectar flow. (Graph 4a-d). For *Salvia brandegei*, different stalks exhibited different nectar flow patterns. Stalk A had an immediate decrease in nectar after the first hour. Stalk B showed the same decrease but had an extra peak of nectar at 1pm, while Stalk C showed a decline in nectar production, but in a stepwise pattern (Graph 4a). Stalks of *Salvia melissadora* also generally showed a decrease in nectar production over time, though only Stalk B had a second significant peak in nectar flow (Graph 4b). Stalks of *Salvia munzii* displayed drastically different patterns. Stalk C started with no nectar but had extremely high flow at 11am. Stalk A showed typical nectar production decline with an extra peak at 1pm, while Stalk B maintained a steady nectar flow which declined after 12pm (Graph 4c). Stalks of *Salvia mellifera* showed the most consistency in nectar flow, with all stalks having an extra peak at 1pm, though each peak were at significantly different magnitudes (Graph 4d).



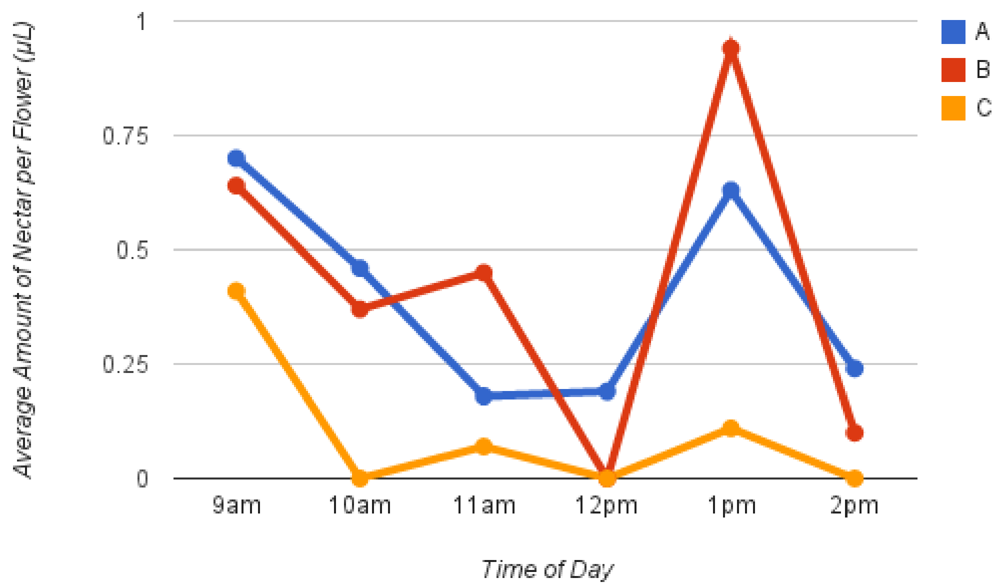
Graph 4a: Average nectar production per flower (µl) over time for stalks of *Salvia brandegei*.



Graph 4b: Average nectar production per flower (µl) over time for stalks of *Salvia melissadora*.



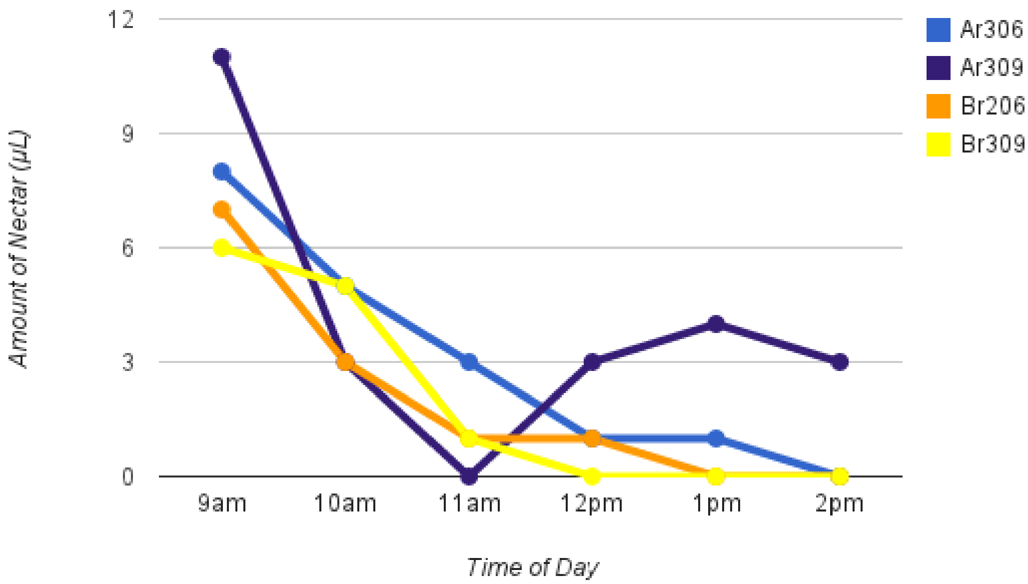
Graph 4c: Average nectar production per flower (µl) over time for stalks of *Salvia munzii*.



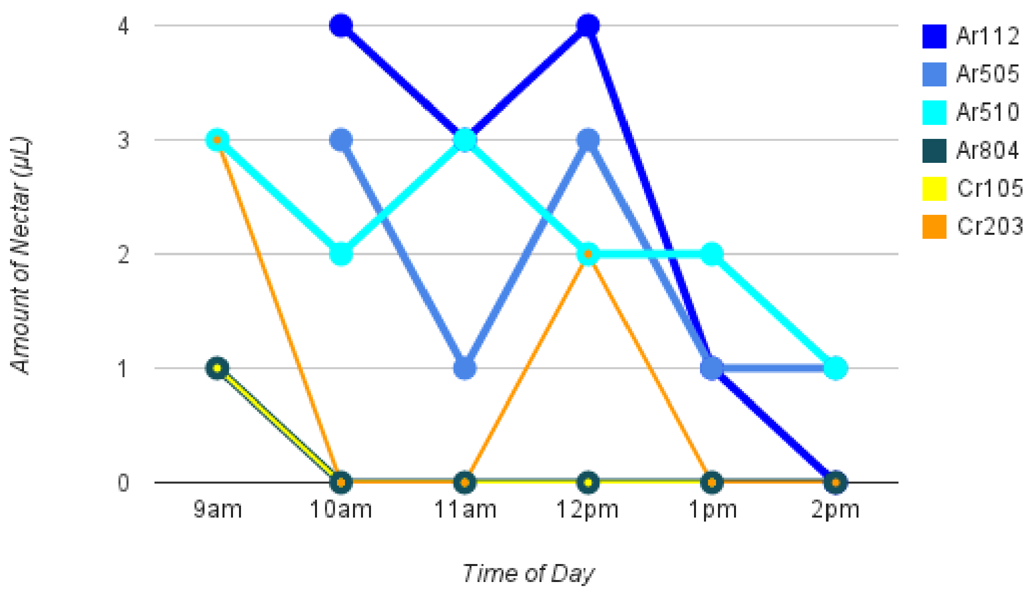
Graph 4d: Average nectar production per flower (µl) over time for stalks of *Salvia mellifera*.

Results (Summer 2012 Study)
A total of 130 flowers were marked and recorded for this study, excluding flowers which were discarded due to the presence of ants. When a flower contained ants, there was no nectar produced (personal observation). To prevent clutter, only selected nectar flow patterns for each *Salvia* species with representatives from different stalks recorded on the same day were presented. The results of the Summer 2012 study show that not all flowers of the same stalk or species had the same nectar flow pattern. However, there were a couple of set trends per *Salvia* species which the nectar flow patterns of each flower tended to follow. The first would be a large peak of nectar flow earlier in the morning with another smaller peak later on. The second would be a decline in nectar flow.

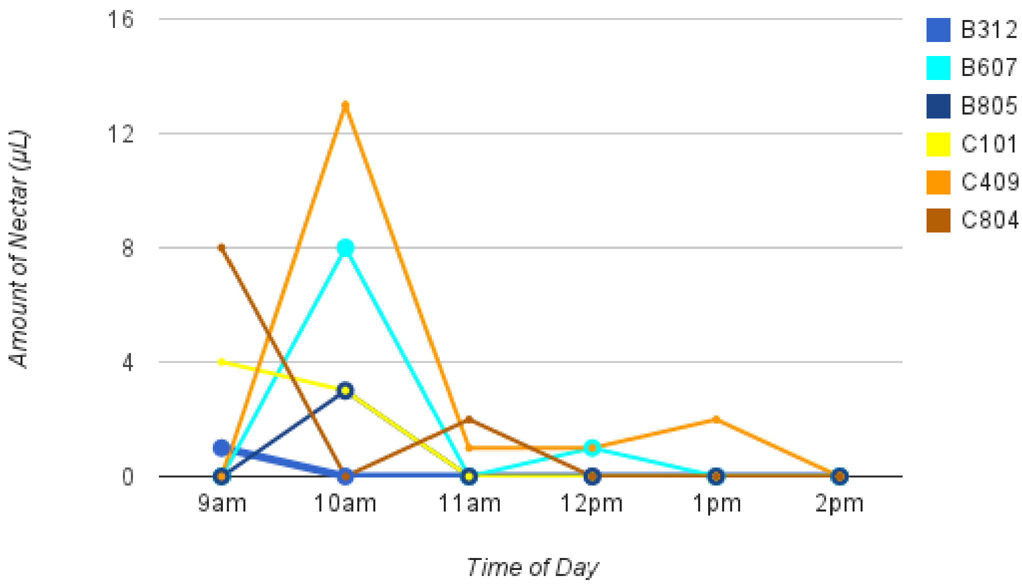
For example, in flowers of *Salvia melissadora*, most flowers displayed typical declines in nectar flow; however, a few flowers would display an additional peak in nectar flow around 1pm, such as flower Ar309 in Graph 5a. The flowers of *Salvia uliginosa* also had two general patterns of nectar flow, with flowers producing peak nectar flow at both 9am and 11am or 12pm, or an immediate decline in nectar flow (Graph 5b). Interestingly, flowers Ar112 and Ar505 opened one hour later than Ar510, but produced very similar nectar flow patterns to Ar510, although the patterns were shifted to the right by their hour lag in opening (Graph 5b). It seems that the majority of flowers of *Salvia* 'Indigo Spires' had extreme peaks of nectar flow at 10am, though of different magnitudes, with some flowers showing and additional slight peak of nectar flow at 12pm or 1pm (Graph 5c). Flower C804 however seemed to display the same nectar flow pattern as mentioned, though with the trend shifted to the left. Some flowers such as B312 and C101 showed very little nectar flow at 9am which declined to nothing by 12pm (Graph 5c). All flowers of *Salvia chamedryoides* showed a general decline in nectar flow, although some flowers such as D202 and D205 produced a slight peak of nectar flow at 11am (Graph 5d).



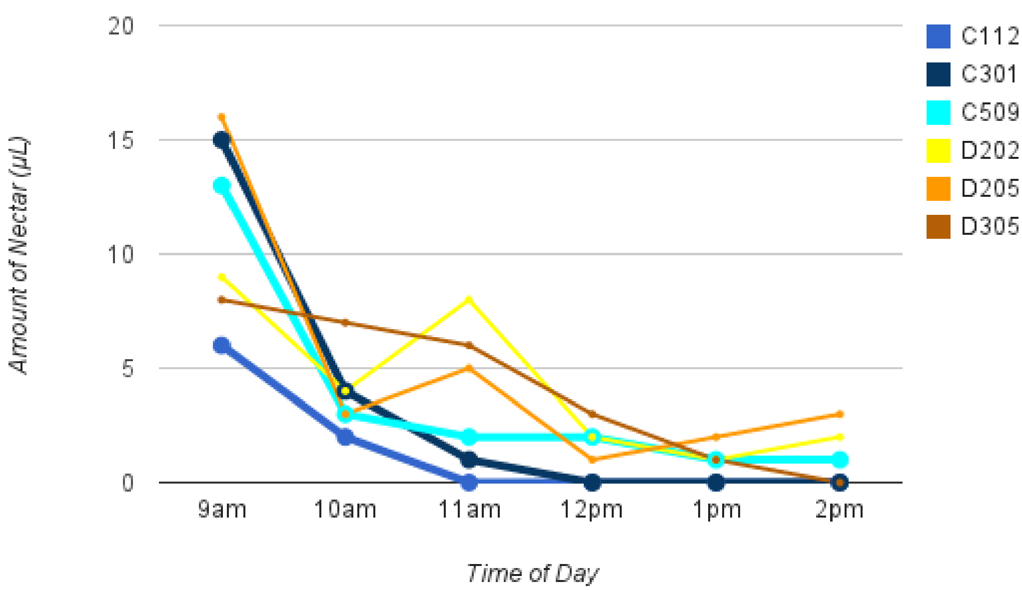
Graph 5: Amount of nectar produced by individual flowers of *Salvia melissadora* over time. Blue lines represent flowers from stalk A, while yellow lines represent flowers from stalk B of the same plant.



Graph 6: Amount of nectar produced by individual flowers of *Salvia uliginosa* over time. Blue lines represent flowers from stalk A, while yellow lines represent flowers from stalk B of the same plant.



Graph 6: Amount of nectar produced by individual flowers of *Salvia* 'Indigo Spires' over time. Blue lines represent flowers from stalk B, while yellow lines represent flowers from stalk C of the same plant.



Graph 6: Amount of nectar produced by individual flowers of *Salvia chamaedryoides* over time. Blue lines represent flowers from stalk C, while yellow lines represent flowers from stalk D of the same plant.

Discussion

The results of bee monitoring in the Spring study showed that *Xylocopa* bees show a strong preference for the exotic *Salvia melissadora*, while other native bees exhibit preferences for other native *Salvia*. This could be due to a variety of reasons, such as the unfamiliarity of local bees to an exotic species, as their native ranges do not overlap. Another reason could be the size of the flowers of *Salvia melissadora*, which are larger than the flowers of the other Salvia species. The larger size of the flowers of *Salvia melissadora* may mean that smaller bees are unable to reach far enough to obtain the nectar, as *Xylocopa* are the largest bees in the garden. A comparison between proboscis lengths of bees and nectary depth of flowers from different Salvia species could be useful in determining if this were true.

Interestingly, bees did not seem to follow nectar flow, as the number of bees observed during peak nectar flow is not significantly higher than the number of bees observed during low nectar flow. This may be due to the small sample size of stalks measured, as each stalk produced different rates of nectar flow, a smaller sample may not be sufficient to capture an accurate estimate of the average nectar flow of the plant at a certain period of time. Furthermore, bees were counted when they landed on a flower, but not how long they stayed on the flower, which is often indicative of the floral rewards present. In that case, visitation lengths should be considered in bee counts, and the use of video equipment during bee monitoring would be better in determining visitation lengths of each bee visitor. Bees may not also be following nectar flow as some may require certain temperatures to be able to optimize foraging activity (Stone and Jenkins 2008; Kwon and Saeed 2003), hence changes in the weather could be a more important factor in explaining the movement of bees. In addition, the contents of nectar could be important in determining the attractiveness of the plant to bees. Sugar concentration, the presence of certain amino acids and other chemicals are crucial aspects of the energy provided by nectar (Nicolson 2011), though not much is known of the different combinations which are optimal to specific species of bee. A combination of measurements of nectar contents with nectar quantity could be an effective predictor of bee movement. Unfortunately, since the amount of nectar collected in the pilot study is often too low for an accurate reading on the refractometer, I was unable to obtain any meaningful measurements of sugar concentration in nectar.

The different patterns of nectar flow among different *Salvia* species in the Spring study were not unexpected. Intra-plant variation of nectar flow was diverse, with different stalks of the same plant exhibiting different times of peak nectar flow and different trends of nectar volume over time. This indicates that a plant's strategy would involve variation in nectar production within the plant such that there are always nectar resources available to attract bees without the energy expenditure of constantly producing nectar in all flowers. In order to further our understanding of the level of variation in nectar production in *Salvias*, more research was conducted during Summer 2012 to investigate variation at the individual flower level. The results show that flowers of *Salvia melissadora, Salvia uliginosa, Salvia* 'Indigo Spires' and *Salvia chamaedryoides* fall under two general categories of nectar flow, some with two peaks of nectar flow while others with a general decline in nectar flow. It is possible that by varying the number of flowers producing each type of nectar flow pattern, the plant also varies the amount nectar produced per stalk over time. There could be a myriad of other factors that affect nectar variation which could be applicable to the bee garden. Some of these factors include: plant sex (Klinkhamer and de Jong 1990), nectary depth (Brink and deWet 1980), and relative humidity

(Bertsch 1983). These factors could also be investigated in conjunction with nectar production to determine if they could explain the observed variations in nectar flow in the unique Californian climate.

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