

**In Communication with Semaa: Exploring Floral Nectar  
Secretion in *Nicotiana Rustica***

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## Abstract

In some Indigenous creation stories, Semaá (*Nicotiana rustica*) was one of the first plants on Turtle Island, given to humanity to facilitate discussions between the spiritual and physical realms. The original communicator, this paper explores one of the ways Semaá is in communication with the natural world. Through measuring the volume, sugar concentration, and sugar available in floral nectar secreted by Semaá over their flowering period, better insight was gained into the condition's pollinators, florivores, and other animals may experience when visiting these plants. Semaá flowers secrete larger volumes of nectar throughout the night compared to during the day, while nectar concentrations stayed consistent throughout. Consequently, more nectar sugars are available to nighttime floral visitors. This finding suggests Semaá may produce nectar at relatively constant concentrations, regulating instead how much is secreted in response to the time of day.

## Foreword

The three components that constitute my Master in Environmental Studies degree include A) Pollinator Ecology and Plant-Pollinator Interactions, B) Wildlife Management and Conservation, and C) Environmental Education. This major research primarily explores the first facet, providing myself with hands-on, in-field experience working with and learning from Sema, to explore the rhythms of the plant's nectar secretion in the context of their potential pollinator interactions. It takes an exploratory ecological approach influenced by Indigenous ways of knowing, and is a largely mensurative experiment, as the plants were not overly manipulated during the study – the knowledge was collected in natural conditions, with all the intrinsic variability of the outdoors. The pollinator portion of component A and the other components of this degree are well-covered through a combination of university and self-directed courses, as well as experiential learning and teaching assistantships; this research seeks to fill in some of my own knowledge gaps surrounding plants and their role within plant-pollinator interactions.

## Dedication

To Dr. Sheila Colla, for welcoming me into your lab, giving me research to do, and constantly sending opportunities my way; I could not have asked for a more knowledgeable or supportive advisor.

To Lisa Myers, for your encouragement, interest, and wonderful advice.

To my lab mates, Dr. Amanda Liczner, Briann Dorin, Hadil Elsayed, Sarah MacKell, Shelby Gibson, and Taylor Kerekes, for letting me participate in and learn from your work, for always being sounding boards to my research ups and downs.

To my classmates and friends, Elizabeth Cho McMillan and Zoe Parco. Without your COVID camaraderie and our billion study sessions, I don't think I ever would have finished this paper. To Mitchell McMillan, our tireless bartender.

To Jenna Polson, Joanna Wong, Megan Perrins, Sarah Leong, and Shakiba Sedighi. I'm grateful to have such wonderful friends in you all, thank you for always reaching out when I withdraw, for being my entertainment and reprieve outside of school.

And finally, to my endlessly supportive family, who readily encourage every choice I make, even when none of us quite know where I'm going. All my confidence comes from knowing you are in my corner and will always be there to pick me back up.

I can't thank you all enough.

## Acknowledgement and Positionality

In this paper I will refer to the plants who made this work possible primarily by their Anishinaabemowin name *Semaa*, rather than their scientific name *Nicotiana rustica*. This is purposeful, to recognize their spirit and combat the reductionism innately built into Western knowledge and the scientific method. In many Indigenous creation stories, plants are one of the oldest beings and consequently our earliest teachers, and this naming choice is meant to reflect their knowledge and convey respect. It is also an effort to remove the scientist-subject dynamic and replace it with one of co-creation.

I am a colonial-settler of European descent, but so far removed from any ancestral or cultural ties that I would only feel comfortable identifying as Canadian. Being a Canadian means recognizing that I live on Indigenous Land that is currently occupied by the Canadian state, off which I inherently profit from due to my privilege as a white person. While my upbringing and education has been heavily steeped in Western knowledge, this research seeks to both build from and challenge this foundation by incorporating Indigenous ways of knowing, to honour my *Semaa* teachers who were fundamental to this work.

This research owes itself to the Land that made it possible. The *Semaa* seeds originate from Tkaronto soil, the territory of the Anishinabek Nation, the Haudenosaunee Confederacy, the Huron-Wendat, and the Mississaugas of the Credit First Nation. Grown to seedlings out west, the Land here has longstanding relationship with the Kainai, Piikani, and Siksika of the Blackfoot Confederacy; the Stoney Nakoda Bearspaw, Chiniki, and Wesley First Nations; and the TsuuT'ina. The *Semaa* grew to maturity further north on Plains Cree, Dene, Blackfoot, Nakota Sioux, and Saulteaux Land. This is also to acknowledge the numerous First Nations, Métis, and Inuit peoples not named here who have called this Land home since time immemorial. This acknowledgement is a recognition of the false and broken promises of the treaties, specifically the 6, 7, and 13 territories where this research took place, and which have long excluded and disrupted the Indigenous ways of life on this Land. In acknowledging the Land and its Indigenous peoples, it is a commitment to restoring the true spirit and intent of those sacred agreements, because “as long as the grass grows, the river flows, and the sun shines... we are to be in those good relations” (Live Green Toronto & Crawley, 2021, 18:15).

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## Introduction

As the most diverse group of plants on the earth, angiosperms and their proliferation reflect the sheer efficacy of the more than hundred-million-year-old relationships between themselves and pollinators. In its simplest explanation, flowering plants use animals to deliver their reproductive units to other compatible partners, paying their messengers with materials that may offer nutrition, provide defensive benefits, or even facilitate mating. However, this definition conceals an evolutionary game of tug of war, whereby both parties seek to minimize costs and maximize benefits; their long-lasting partnership characterized by constant adaptation, response, and readaptation. It has borne many exciting studies, and with more than 350,000 flowering plant species to investigate, there are innumerable ways to learn about and from angiosperms. This exploration allies with *Semaa* (*Nicotiana rustica*; Goodspeed, 1954), a species of wild tobacco, to delve into the dynamics of their nectar secretion and reveal potential pollinator associations.

*Semaa* (*Asemaa/Asamaa*), also known as *Oien'kwa'ón:we* in Kanien'kéha, is a plant significant to many Indigenous peoples across Turtle Island (Milne, 2020). The plant originated in central South America, but has since been introduced and naturalized as far north as Canada (Sadik, 2014). The archeological and ethnographic evidence supports widespread use of wild tobacco throughout the Americas, the cultivation and movement facilitated by Indigenous peoples (Winters, 2000). The presence of this plant in Canada dates back as early as 700 AD, with evidence of the Huron people growing it along the northern shores of Lake Ontario (Sadik, 2014). Historically, wild tobacco has filled a variety of roles, featuring prominently in “religious and secular ceremonies, rites of passage, economic and political alliances, [and] social events” as well as being used for relaxation (Sadik, 2014, pp. 6). Today, this traditional use continues; *Semaa* is considered to be one of four sacred medicines, along with sage, cedar, and sweetgrass, and central to contemporary Indigenous ceremonies, offerings, prayers, gifts, and medicines (Maron, 2018; Sadik, 2014).

*Semaa* are annual flowering plants with large, alternating, ovate leaves and trumpet-like, greenish-yellow flowers that blossom off a single, central stem as a panicle inflorescence (Figure 1). Key pollinator groups for *Semaa* are largely unknown, with bees (specifically the Apidae family) and moths suspected to play an important role based on the plant's pollination syndrome (Raguso et al., 2003; Tiedge & Lohaus, 2017). Pollination syndromes describe a group of physical



**Figure 1:** *Scientific illustration of Semaia*. Whole plant shown in centre, excluding root system; 0.5 – 1.5 m tall. A. Floral calyx and corolla, 20 – 32 mm long; open for pollination, B. Corolla tube, nectaries found at the narrower base C. Top-down corolla view, 5 stamens and stigma absent. D. Seed pod. Retrieved from “Tobacco Use by Native North Americans” by J.C. Winter, 2000, University of Oklahoma Press.

characteristics that complement and encourage the mutualistic interaction between plants and pollinators, considering floral color, odor, shape, quantity and quality of floral rewards, among other morphological traits to predict potential types of compatible pollinators (Dellinger, 2020). The assertion follows that a given plant's pollination syndrome has developed and evolved over time in response to the influence of dominant pollinators. A review by Rosas-Guerrero et al. (2014) of plants and their floral traits supported the presence of pollination syndromes and also demonstrated that the predictability of plant-pollinator interactions is greater in plants that are from tropical regions or in those that are known to be pollinator dependent. However, the overall applicability and accuracy of pollination syndromes has been challenged in recent years as an oversimplification of these interspecies interactions (Ollerton et al., 2009). Floral traits are not singly shaped by mutualistic pollinator interactions, but also antagonistic “herbivores, florivores, seed feeders, [and] nectar robbers” (Kessler et al., 2015, pg. 3). Pollination syndromes also may fail to capture the flexibility of plants and their respective pollinator networks, which are known to change across seasons and over years in response to their environment (Olesen et al., 2008; Olesen et al., 2011). Experimental evidence to elucidate plants' pollinator assemblages are necessary to better characterize their current ecological networks.

Research has revealed correlations between the volume, concentration, and composition of floral nectar, all aspects included in a pollination syndrome, and different groups of pollinators, although the mechanisms behind these relationships have been more difficult to illuminate (Pyke et al., 2020). Primarily composed of carbohydrates, nectar is an aqueous solution that functions as the primary source of energy and nutrients for pollinators. Sucrose, fructose, and glucose sugars dominate the liquid, but “amino acids, organic acids, lipids, proteins, inorganic ions” among other compounds are also present and play important roles (Tiedge & Lohaus, 2017, pg. 2). Floral visitor's primary source of nitrogen comes from the amino acids found in nectar, which also can act as phagostimulants (Tiedge & Lohaus, 2017). Sema contains a high concentration of amino acids, particularly aspartic acid, in comparison to other members of the *Nicotiana* genus (Tiedge & Lohaus, 2017). The dominant sugar in Sema appears inconclusive; two studies observed Sema's nectar to be fructose-rich, while another found a higher proportion of sucrose present (Silva et al., 2018; Silva et al., 2020; Tiedge & Lohaus, 2017). Flowers that secrete nectar with high fructose concentrations are associated with “short-tongued bees, [new world] bats, perching birds, and flies”, in comparison to sucrose-rich nectars which are correlated with “hummingbirds,

[old world bats], long-tongued bees, moths and butterflies” (Perret et al., 2001, pg. 267). Along with nectar composition, pollinators have preferences for different nectar concentrations. Flowers with high sugar concentrations (35% w/v or 35 mg/ul) tend to be bee-pollinated, while lower concentrations (20-25% w/v or 20-25 mg/ul) attract hummingbirds and Lepidoptera (Pyke & Waser, 1981). This difference can be explained by their mouthparts; hummingbirds, butterflies, and moths rely on active suction to drink nectar, where high volumes and low viscosity are preferential (Kim et al., 2011). Bees in contrast collect nectar by dipping their proboscis into floral nectaries, where a viscous, highly-concentrated solution would be favoured (Kim et al., 2011).

Nectar secretion is highly variable within plant populations and across time, in response to both the environmental conditions and the visitors, mutualist or antagonist, plants experience (Gilbert et al., 1991). The variation is further increased when considering flowers have the ability to stop and start nectar production, and in some cases, reabsorb the secretion completely (Nicolson, Nepi, and Pacini, 2007). This level of control allows plants to attract, manipulate, and sometimes even deter interactions (Pyke et al., 2020). Floral nectar influences the actions of pollinators, who will make their next foraging decision based on what they encounter, which ultimately affects the success of pollen transfer and eventual plant reproduction (Pyke et al., 2020). As pollinators can detect differences in volume, concentration and composition while sampling nectar, and neighbouring flowers on a given plant are known to be positively correlated across these nectar attributes, a probing pollinator can expect to find a similar quality of nectar in a nearby flower (Pyke et al., 2020). However, these attributes are not significantly consistent across days; a pollinator returning a day later could not expect to find the conditions they were originally met with (Pyke et al., 2020). These nectar dynamics were elucidated recently and first examined in a bird-pollinated plant, *Blandifordia grandiflora*; the authors recommending further research is necessary to confirm these trends (Pyke et al., 2020).

While pollinators cannot determine the quality of nectar without sampling the flower, plants can advertise their floral resources through the use of scent. Fragrance emission is known to follow circadian rhythms, where odor production can be both modified or halted in response to external factors like pollination (Theis, 2006). Floral odor plays a significant role in attracting pollinators as well as when deterring predators, in which *Semaa* is no exception (Kessler et al., 2015). *Semaa*'s fragrance is suspected to have been “shaped by pollinator- and herbivore-mediated

selection, biosynthetic pathway dynamics and shared evolutionary history” with the genus *Nicotiana* (Raguso *et al.*, 2003, pg. 265). The fragrance produced by *N. rustica* is both species-specific and temporal – distinct from other members of the *Nicotiana* genus and distinct depending on the time of day the plant is encountered (Raguso *et al.*, 2003). While the main compounds found in both the day and night odor cocktails include  $\alpha$ -cedrene (cedar), benzaldehyde (almond), benzyl alcohol (aromatic, floral), epoxyphorone (camphor), and nicotine (tobacco), the concentrations of each volatile differ markedly, creating the distinct scent profiles (Raguso *et al.*, 2003). It is also of note that the scent emitted by *Semaa* is more than two times greater at night than it is during the day (Raguso *et al.*, 2003). The nighttime floral headspace is dominated by benzaldehyde, a pollinator and florivore attractant (Raguso *et al.*, 2003; Theis, 2006). In contrast, daytime *Semaa* flowers release 5-fold more nicotine (Raguso *et al.*, 2003). Nicotine is a toxic alkaloid present in high concentrations in all tissues of *Semaa*, functioning as a chemical defense to discourage predation – consequently, it is also known to deter pollinators including hawkmoths, hummingbirds, and bees (Adler *et al.*, 2012). In *N. attenuata*, a *Semaa* relative, the presence of nicotine was found to decrease both the length of pollinator visits and the volume of nectar removed, but increase the number of pollinator visits (Kessler *et al.*, 2007).

These highlights of *Semaa*’s odor profile give a simplistic view of the 22-compound blend of volatiles emitted by the plant’s floral tissues, which likely interact in complex (and largely unknown) ways. How the fragrance bouquets are perceived by floral visitors is even less well-understood, but some trends have been observed in other plant species. Phenylacetaldehyde, a sweet, honey-like aromatic found in the blend is known to be strongly attractive to both pollinators and florivores; in a field study with Canada thistle (*Cirsium arvense*), honey bees (*Apis mellifera*) and ants (*Formicidae*) were significantly attracted to the pure compound (Theis, 2006). The same study found beetles from the mordellid family to be associated with the floral aromatic benzyl alcohol (Theis, 2006). Ketoisophorone and the previously mentioned epoxyphorone elicit strong antennal responses in lepidoptera species (Andersson & Dobson, 2003; Guédot *et al.*, 2008).

All of these findings hint at, but do not fully capture the pollinator castes *Semaa* may be catering their nectar to attract. This research is part of a larger project conducted by York University PhD student Shelby Gibson, who found in previous field studies while investigating the pollination requirements of *Semaa*, plants pollinated at night produced larger seed pods and

more seeds per pod (unpublished data). This suggests that the pollination the Semaas plants received during the nighttime was more effective in facilitating production of the plant's reproductive units, and has led to the hypothesis that this species of wild tobacco may cater the production of their floral resources to the preferences of nighttime pollinators. The objective of my experiment is to complement this previous work by determining if the nectar secreted by *N. rustica* varies in volume ( $\mu\text{l}$ ), sugar weight (mg), or sugar concentration ( $\text{mg}/\mu\text{l}$ ) depending on the time of day.

## Hypotheses

H<sub>0</sub>: *Nicotiana rustica*'s nectar attributes, including nectar volume ( $\mu\text{l}$ ), weight of sugar present within the nectar (mg), or the nectar concentration ( $\text{mg}/\mu\text{l}$ ), will not vary depending on the time of day it is secreted.

H<sub>a</sub>: One or more of *Nicotiana rustica*'s nectar attributes will vary depending on the time of day it is secreted.

## Methods

*Research Ethics*: Following Brittany Luby's precedent with Manomin, the Land and Semaas are recognized here as "spirited being(s) worth ethical consideration", non-human teachers whose ways of knowing deserve respect (2021, pg. 8). This is further necessitated by my positionality as a western researcher – if I care about my Indigenous peers, I must also care about the Land and Semaas, as the "welfare [of Indigenous peoples is] intimately tied to the wellbeing of other-than-human beings" (Luby et al., 2021, pg. 9). Luby's work with Manomin explores how consent works when communication is non-verbal; while Semaas and myself do not necessarily speak the same language, communication was still possible. An early instance includes when the seedlings were stretching high in their trays, their thin, leggy stems reaching upwards, the Semaas were telling me that they needed more sunlight, otherwise they would refuse to produce more leaves. This is how consent was interpreted throughout this research; at any time throughout the experiment that the Semaas communicated their needs and I failed to adequately respond, they could and would withdraw consent.

This interpretation is consistent with Reo's (2019) definition of consent, which insists consent not be some singular event, but an ongoing process that requires continued thoughtfulness, maintenance, and care. My relationship with Semaa was continuously built up over the growing season, in which we communicated with physical and visual cues; ultimately 23 participants consented to the nectar sampling, with the remaining seven individual's flowering only after experimental period was complete. It is important to note that the consent process did not stop after the field season was completed; Luby et al. (2021, pg. 6) stresses the importance of waiting until the plants are fully mature and have "lived as full a life as possible", and thereby consent to being harvested. Seeds and leaves were only taken at the very end of Semaa's growing season, before they returned to the earth with the first frost. My accountability to Semaa and the Land is something I must continue to explore after our research, when I have determined how to properly share the knowledge gained from our relationship.

*Growing Conditions:* Organic Non-GMO Tobacco Hopi from [Urban Harvest](#) (Toronto, Ontario) was started indoors from seed in six 22" x 11" x 2" [plastic plant trays](#) at the beginning of April, 2020. Four packets of seeds (175 seeds/package) were sown on top of damp [seed starting potting mix](#) and the trays loosely covered with bubble wrap to create a greenhouse effect. Seeds began to germinate about a week after initially sown, and were thereafter watered on a regular basis to keep soil moist. At the end of April, the seed trays were moved into a [walk-in greenhouse](#). The largest seedlings with true leaves began to be transplanted into individual 4" [plastic nursery pots](#) at the start of May; 95 seedlings ultimately were transferred into their own pots. The plants continued to grow indoors throughout May and were hardened off outdoors in June. The 15' x 15' garden plot located in Lamont, Alberta (53° 45' 35.7876" N 112° 46' 38.712" W) was prepared at the beginning of July, with fifteen 30 L bags of topsoil added to create five north to south garden rows. The 30 largest plants were transplanted into the garden plot, with six plants/row, spaced two feet apart in each direction. The garden plot was manually weeded and watered as needed throughout July and August.

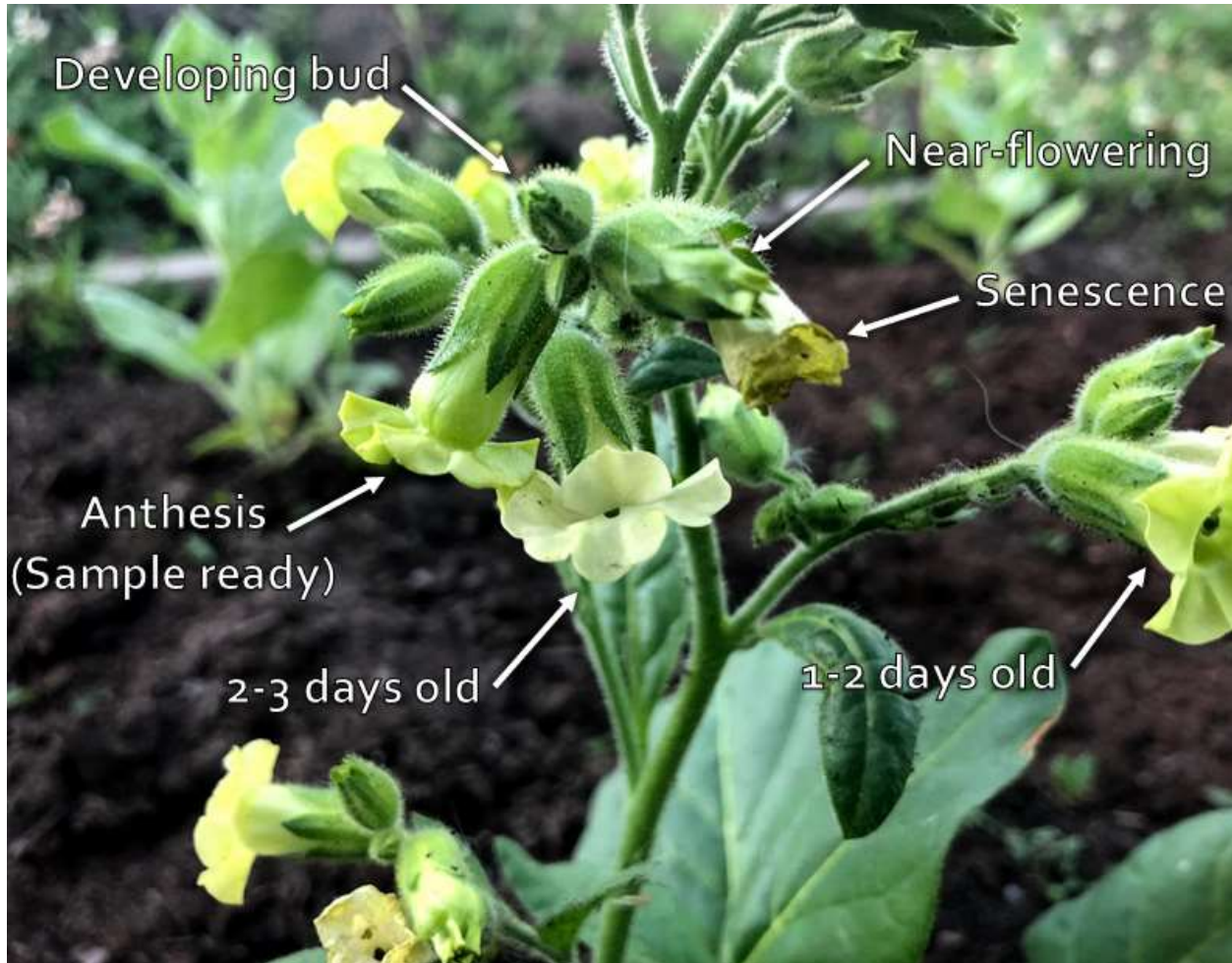
*Field Experiment:* The first plant flowered on July 27<sup>th</sup>. Nectar sampling began August 5<sup>th</sup>, 2020 and took place every day, weathering permitting, until August 20<sup>th</sup>. Each day, buds on participating plants were bagged with [4" x 6" sheer drawstring pouches](#) to exclude pollinators from the sample flowers. The selected buds were ones that appeared to be near-flowering, so that when eventually

sampled they would be in anthesis (Figure 2). Each flower selected was sampled twice in a twelve-hour period, between 8:00 – 10:00 and 20:00 – 22:00. When the initial sample was taken (8:00 – 10:00 or 20:00 – 22:00) alternated each sampling round, so that the final samples of the entire data set would reflect both the nectar produced over the day and overnight. Only one flower/plant/round was sampled to reduce confusion. The initial sample period began by removing the exclusion bags and marking flowers in anthesis on each plant. Nectar samples were then collected from the newly opened flowers with [75 mm Drummond™ Capillary Tubes](#). The length of the nectar along the tube was measured with a ruler and recorded to be converted to a volume later. After this measurement the nectar was expelled from the microcapillary tube onto the sample well of a [Fisherbrand™ Handheld Digital Brix/RI Refractometer](#), which had been previously zeroed and cleaned with distilled water. In a shaded area with the cover closed, the refractometer read off and produced the average of 15 Brix° measurements of each nectar sample (automatically correcting for temperature), and the number recorded. The sample well was cleaned, the flower re-bagged, and the process repeated for the next marked flower. These same flowers were then resampled 12 hours later using the same process, to determine the volume and Brix° concentration of the nectar produced between sample periods.

*Data Transformation:* The nectar volume in microlitres ( $\mu\text{l}$ ) was found using the formula  $V = \pi r^2 l$ , where  $V$  is the volume ( $\mu\text{l}$ ),  $r$  is the inner radius of the microcapillary tubes (0.2 mm), and  $l$  is the volume originally measured as the length (mm) of nectar in the microcapillary tubes. The Brix° measurements were converted to nectar concentrations (mg/100  $\mu\text{l}$ ) using the formula  $y = 0.226 + 0.937x + 0.00585x^2$  (as seen in Pyke *et al.*, 2020) where  $x$  is the Brix° measurements collected in the field and  $y$  is the mg of sugar/100  $\mu\text{l}$  nectar, better known as the nectar's sugar concentration. The weight of sugar (mg) present in each sample can be calculated simply by multiplying the sample's nectar volume ( $\mu\text{l}$ ) by its corresponding nectar concentration (mg/ $\mu\text{l}$ ).

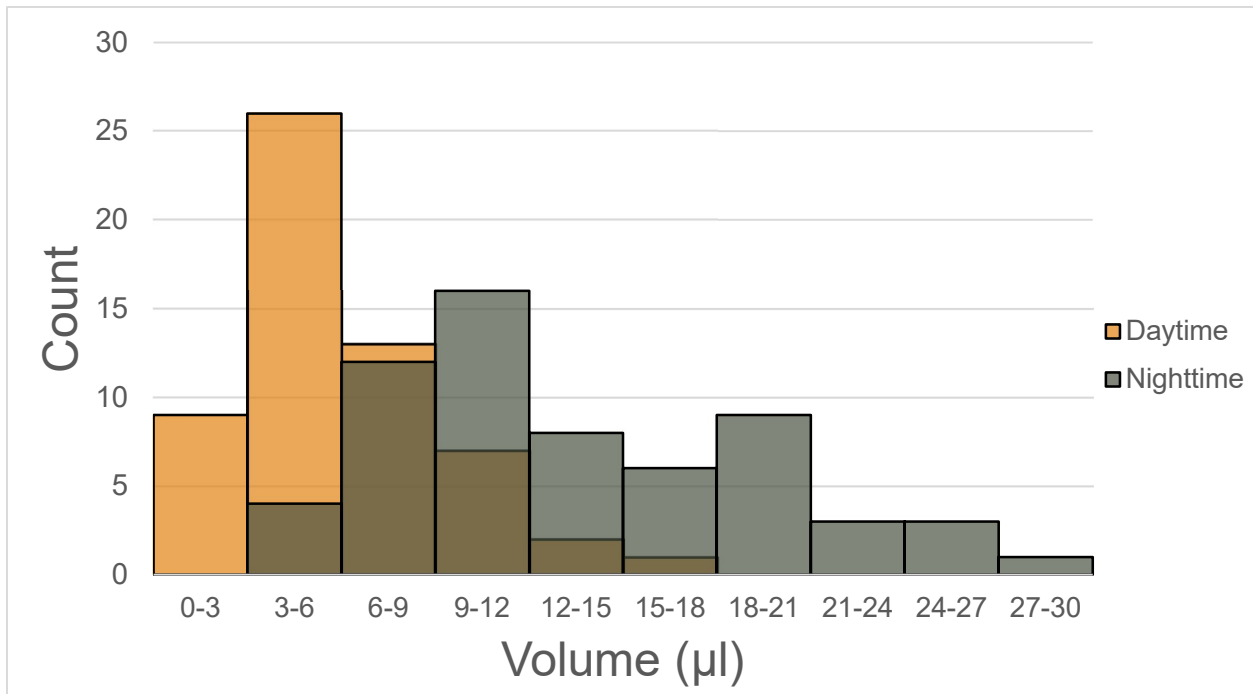
*Data Analysis:* In total, 294 samples were taken, representing 147 flowers from 23 participating plants. The minimum dataset was selected for analysis, containing only samples where both the initial and final sample could be taken. Any samples inhibited by thunderstorms, rain water, rough handling, or attempted dilutions to get a refractometer reading were removed - flowers that could be sampled but did not produce nectar were included. This left 240 samples, reflecting 120 flowers (Daytime, n=62; Nighttime, n=58) for analysis. A two-tailed student's t-test assuming equal

variances was used to determine if there was a significant difference in the volume ( $\mu\text{l}$ ), sugar available (mg), or sugar concentration of the nectar ( $\text{mg}/\mu\text{l}$ ) between the daytime and nighttime treatments.

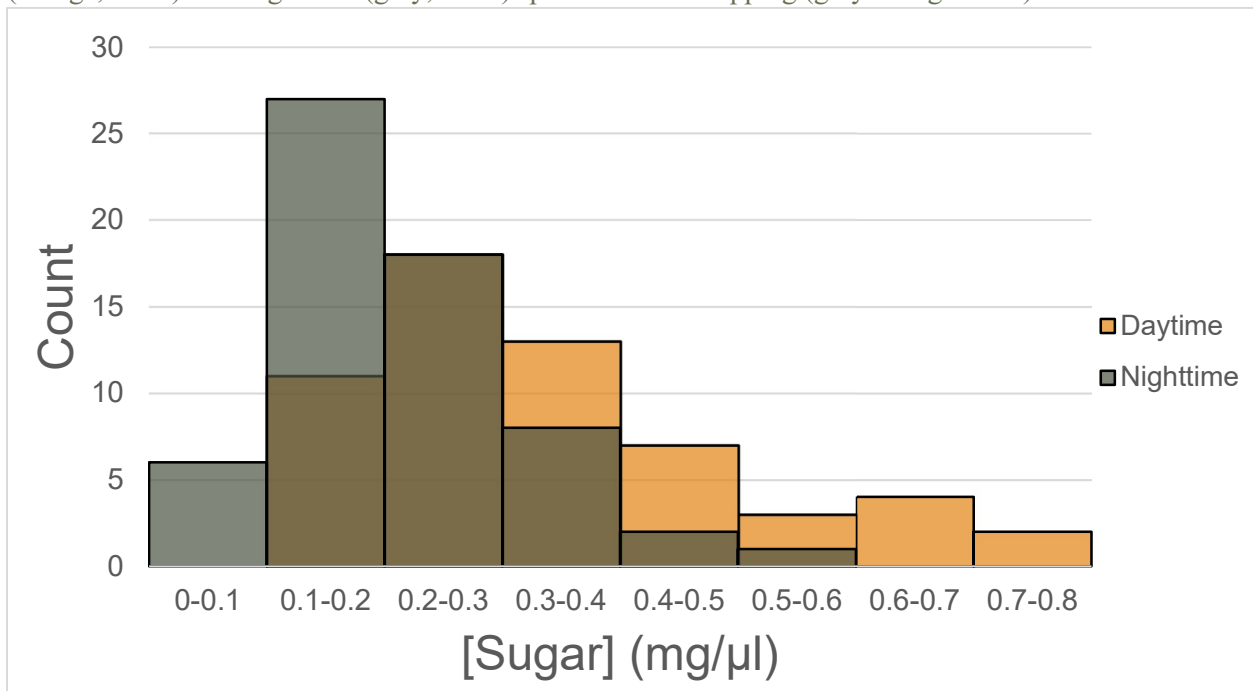


**Figure 2: Life Stages of *Sema* Flowers.** In this species of wild tobacco, blooms are continuous and unsynchronized. From anthesis to senescence, each flower lasted around 4 days with the stages distinguished by color and form. At anthesis, the flowers are lime green and firm; this is the time when sampling would begin and nectar was first collected for analysis. In the following days the color warms to a bright yellow and the flower softens as the petals open further. In the final days of each flower's life cycle, they turn a pale, translucent yellow before deteriorating.

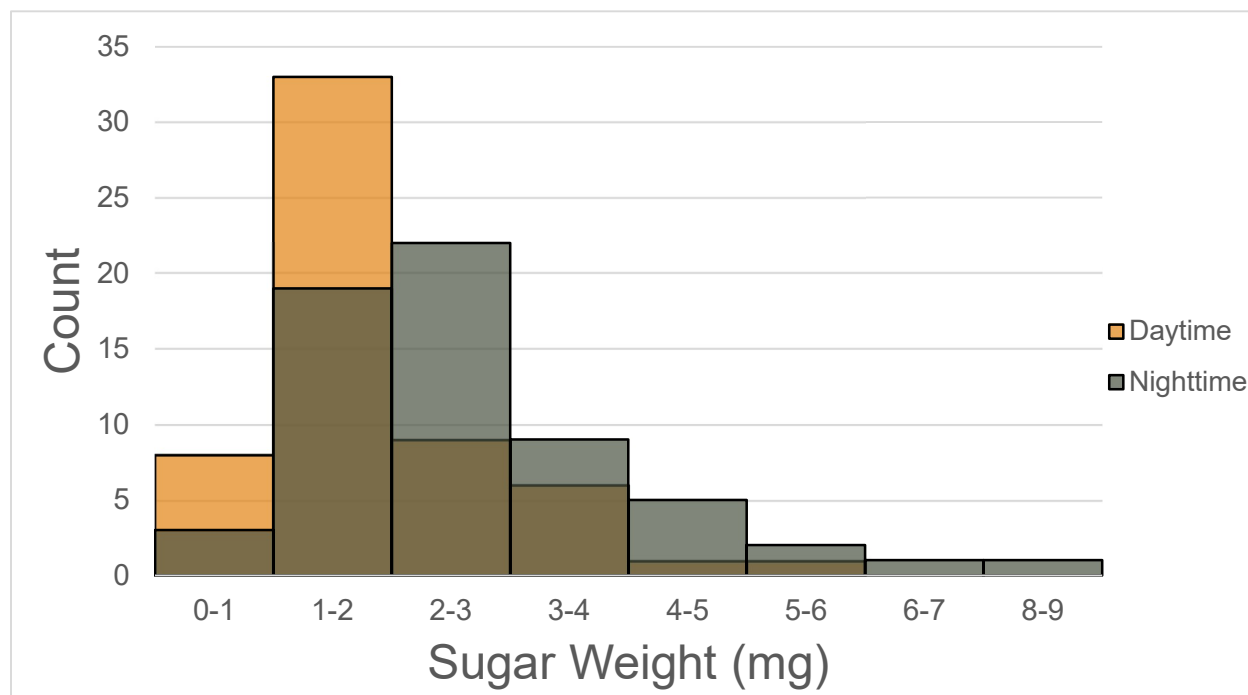
## Results



**Figure 3: *Spread of Anthesis Nectar Volumes.*** Distribution of nectar volumes ( $\mu\text{l}$ ) measured during the initial sampling period, loosely reflective of the volume available at the onset of anthesis ( $n=120$ ). Daytime (orange,  $n=62$ ) and Nighttime (grey,  $n=58$ ) spreads are overlapping (grey-orange color) and color-coded.



**Figure 4: *Spread of Anthesis Sugar Concentrations.*** Distribution of nectar concentrations ( $\text{mg}/\mu\text{l}$ ) measured during the initial sampling period, loosely reflective of the concentration of sugars (glucose, fructose, and sucrose) in the nectar at the onset of anthesis ( $n=120$ ). Daytime (orange,  $n=62$ ) and Nighttime (grey,  $n=58$ ) spreads are overlapping (grey-orange color) and color-coded.



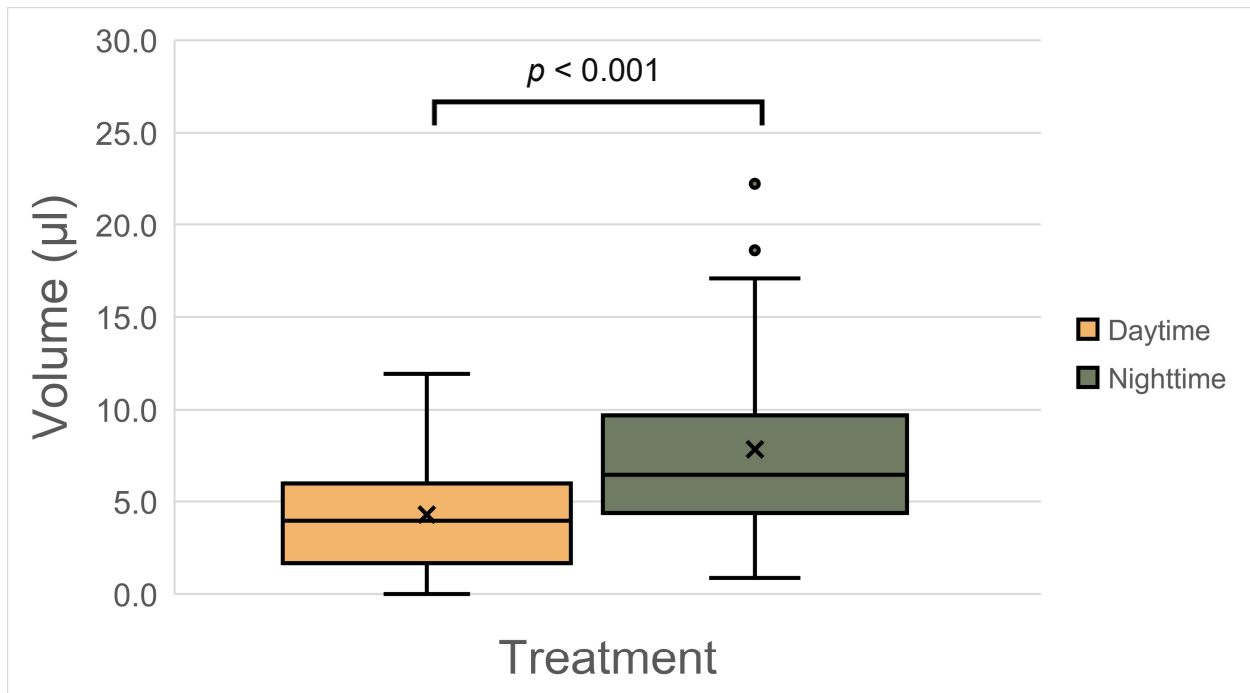
**Figure 5: Spread of Anthesis Sugar Availability.** Distribution of sugar available (weight in mg) in the nectar collected during the initial sampling period, loosely reflective of the sugar available at the onset of anthesis (n=120). Daytime (orange, n=62) and Nighttime (grey, n=58) spreads are overlapping (grey-orange color) and color-coded.

No statistical analysis was undertaken with the data from the initial samples as the timing of this first nectar collection varied; while sampling always took place between either 8:00 to 10:00 or 20:00 to 22:00, the sample flowers themselves may have just opened, or been open for a couple hours. All that could be ensured was that they were closed the sampling period before, and newly opened during the current period. Determining the exact timing and duration of anthesis was out of this experiment's scope. Instead, general patterns were explored with histograms to characterize this data.

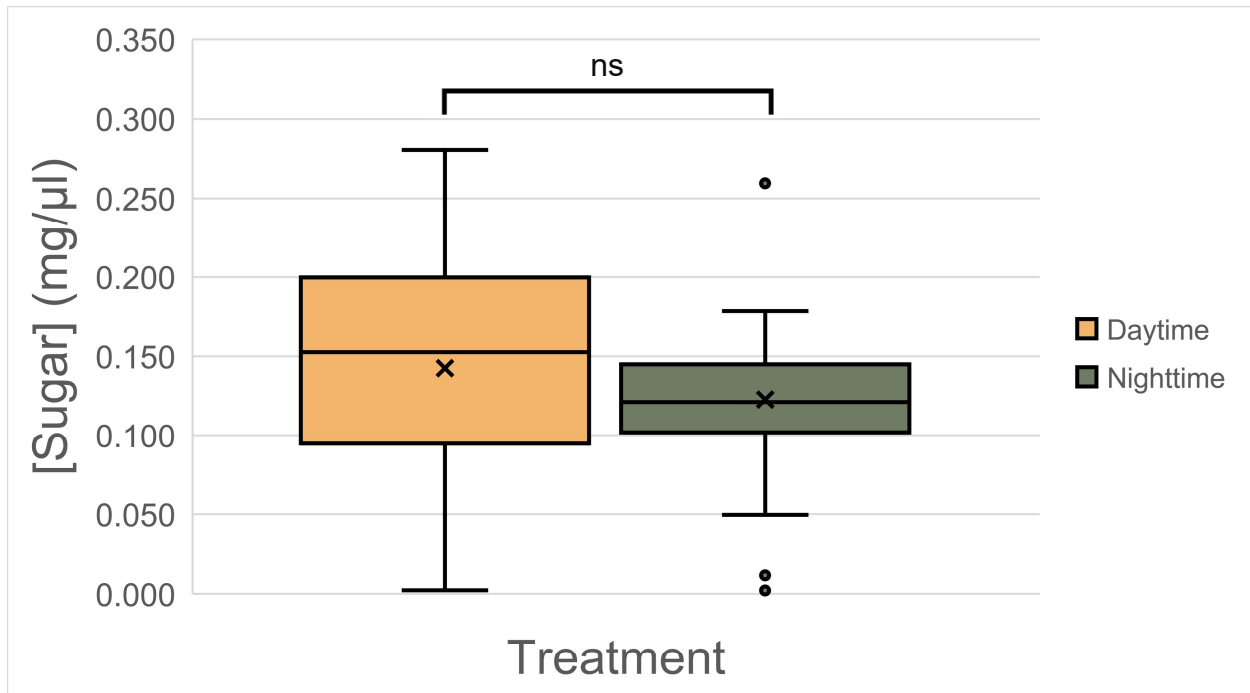
During anthesis, the mean volume of nectar collected was 9.8  $\mu\text{l}$ , with volumes ranging from 0.9 – 29.5  $\mu\text{l}$ , although half the samples were between 5.3 – 13.5  $\mu\text{l}$  (Figure 3). The highest volumes were encountered during the initial nighttime collection hours (Figure 3). The average sugar concentration of these initial samples was 0.270 mg/ $\mu\text{l}$ , with a range of 0.045 – 0.743 mg/ $\mu\text{l}$  (Figure 4). Half of the sugar concentrations measured during this anthesis period can be found between 0.166 – 0.326 mg/ $\mu\text{l}$ . The highest sugar concentrations measured were during the initial daytime collection hours (Figure 4). The sugar weight (mg), or mass of sugar available to potential pollinators, was calculated using these previous two measurements. On average, 2.3 mg of sugar

was available during anthesis, but this weight could range as low as 0.3 to as high as 8.2 mg (Figure 5). Half of the weights calculated were between 1.3 and 2.9 mg. The largest sugar weight calculated was encountered during the initial nighttime collection period (Figure 5).

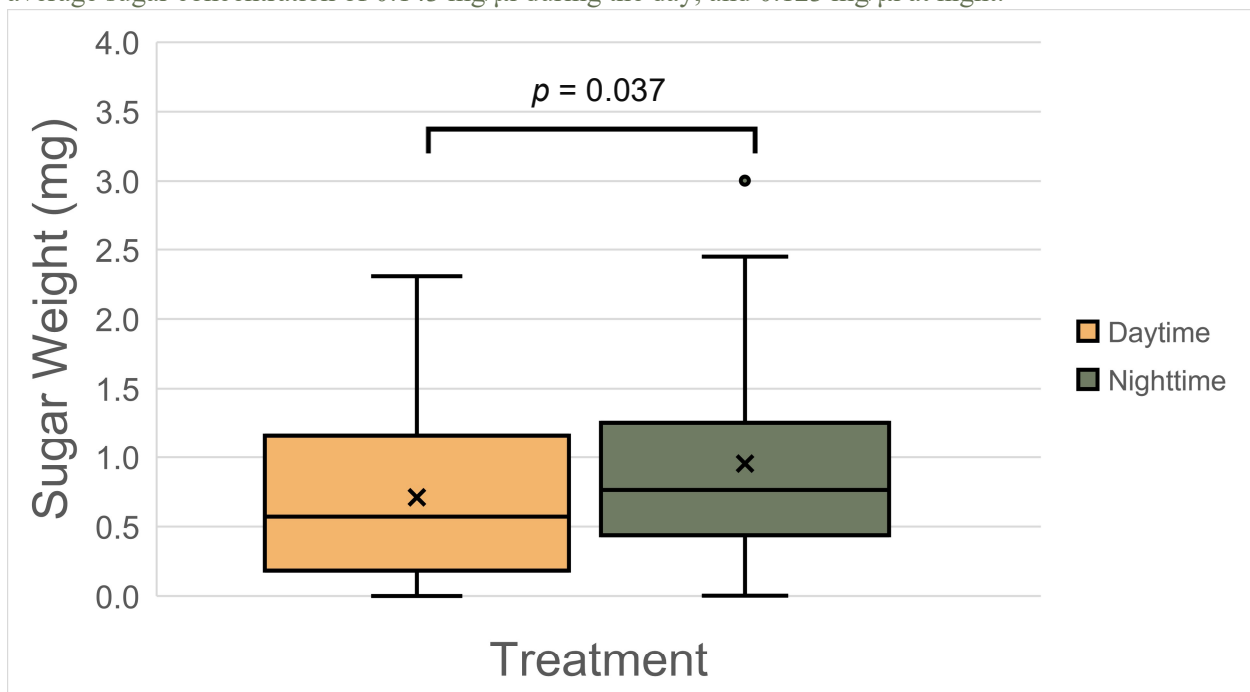
The final samples reflect 12 hours of nectar replenishment over either the day or night hours. Four flowers, all within the daytime treatment, did not produce nectar in the 12 hours after the initial sample period. After the two collection periods, all sampled flowers produced little to no further nectar. Both the nectar volume and the sugar available (weight in mg) across treatments was found to be significantly different, with a higher volume of nectar ( $t$ -test,  $t = -4.78$ ,  $p < 0.001$ ) and more sugar available ( $t$ -test,  $t = -2.12$ ,  $p = 0.037$ ) over the course of the night (Figure 6 and 8). The average volume replenished over each treatment was 4.23  $\mu\text{l}$  during the day and 7.81  $\mu\text{l}$  at night (Figure 6). The distribution of daytime collections ranged from 0.0 – 11.9  $\mu\text{l}$ , while the night treatment had a spread of 0.9 – 17.1  $\mu\text{l}$ , with two outliers at 18.6 and 22.2  $\mu\text{l}$  (Figure 6). Half of the daytime samples were between 1.7 and 6.0  $\mu\text{l}$ , in comparison to the night where half of the data is between 4.4 and 9.7  $\mu\text{l}$  (Figure 6). The average sugar available was calculated to be 0.71 mg during the day and 0.95 mg at night (Figure 8). The distribution of daytime collections ranged



**Figure 6: Daytime and Nighttime Nectar Volumes.** The nectar volume ( $\mu\text{l}$ ) secreted over daytime (8:00 – 20:00) and nighttime (20:00-8:00) treatments was found to be significantly different ( $t$ -test,  $t = -4.78$ ,  $p < 0.001$ ). The means are indicated with an “x”, with the Sema flowers secreting nectar with an average volume of 4.23  $\mu\text{l}$  during the day, and 7.81  $\mu\text{l}$  at night.



**Figure 7: Daytime and Nighttime Sugar Concentrations.** The sugar concentrations (mg/ $\mu$ l) measured over daytime (8:00 – 20:00) and nighttime (20:00-8:00) treatments were found to be non-significant ( $t$ -test,  $t = 1.68$ ,  $p = 0.096$ ). The means are indicated with an “x”, with the Semaa flowers secreting nectar with an average sugar concentration of 0.143 mg/ $\mu$ l during the day, and 0.123 mg/ $\mu$ l at night.



**Figure 8: Daytime and Nighttime Sugar Availability.** The sugar available (mg) was calculated from the original concentration and volume measurements. The daytime (8:00 – 20:00) and nighttime (20:00-8:00) treatments were found to be significantly different ( $t$ -test,  $t = -2.12$ ,  $p = 0.037$ ). The means are indicated with an “x”, with the Semaa flowers having an average of 0.71 mg available during the day, and 0.95 mg available at night.

from 0.0 – 2.3 mg, while the night treatment had a spread of 0.0 – 2.5 mg, with a single outlier at 3.0 mg (Figure 8). The sugar concentrations across treatments were non-significant ( $t$ -test,  $t = 1.68$ ,  $p = 0.096$ ). The Sema flowers secreted nectar within a range from 0.00 – 0.28 mg/ $\mu$ l during the day, with an average sugar concentration of 0.143 mg/ $\mu$ l (Figure 7). The range of the nighttime sugar concentrations was smaller than that of the daytime treatment, from 0.05 – 0.18 mg/ $\mu$ l, with a slightly lower average concentration of 0.123 mg/ $\mu$ l as well (Figure 7). The nighttime treatment had three outliers, one of which seemingly had a nectar concentration of 0 mg/ $\mu$ l (despite a sufficient volume to test with). The other low outlier has a concentration of 0.012 mg/ $\mu$ l, while the high outlier was measured at 0.260 mg/ $\mu$ l (Figure 7).

It was found that regardless of the treatment, the nectar parameters (volume, weight, concentration) for the first collection were significantly different than that of the follow-up sample period; in every instance the  $p$  value was  $< 0.001$ . The nectar parameters of these sampled flowers averaged around two times higher at anthesis compared to the follow-up samples that represented 12 hours of nectar replenishment.

## Discussion

The nectar volume and the amount of sugar available in the Sema flowers sampled was found to be higher during the nighttime treatment for both the anthesis samples and the samples reflecting 12 hours of nectar replenishment, while the nectar's sugar concentration was slightly, but non-significantly higher during the day. Nectar volume and concentration was found to be greatest at anthesis, declining as the flowers age, consistent with the existing literature (Silva et al., 2018). These findings confirm the alternate hypothesis; some nectar attributes will vary significantly depending on the time of day the flower is visited. Nighttime visitors of Sema can expect to find larger volumes of nectar present and consequently more sugar available to consume. As there was no significant difference in the sugar concentration of nectar across treatments, this suggests that the flowers produce nectar at a consistent concentration, controlling instead how much they secrete in response to the time of day. While evaporation from higher daytime temperatures could explain the reduced volumes observed during the daytime treatment, this reasoning would be better substantiated if nectar concentrations were also observed to significantly increase (as the water evaporates, the ratio of sugar molecules to water molecules increases).

This research, taken in concert with the existing literature, adds to the ever-growing picture of Semaas network of associations. While high nectar concentrations were observed at anthesis (up to 0.74 mg/ul), the average nectar concentrations across the sample population reflected concentrations more consistent with the preferences of hummingbirds, moths, and butterflies (under 0.25 mg/ul). The higher nighttime volumes of nectar recorded may be related to or causal of the larger amounts of scent released at night observed in previous studies (Raguso et al., 2003). Hypothetically, as Semaas flowers do not close throughout the duration of their lifetime, the plant may accept the visitation of pollinators undaunted by the higher nicotine concentrations the plant releases during the day to deter florivores, but then attempt to actively attract potential nighttime visitors with more nectar and more nectar sugars, advertising these wares through greater amounts of scent and distinct and attractive odour profiles. This postulation could be confirmed or rejected entirely by a study dedicated to observing Semaas floral visitors across time. Anecdotally, *Bombus centralis*, a long-tongued species of bumble bee, was observed many times actively drinking nectar from Semaas flowers throughout the experiment. Other floral visitors included syrphid flies, a lygus bug, and what appears to be thrips (but not confirmed) hiding near the nectaries (Figure 9).

Some caveats must be considered when evaluating and comparing these results to the existing literature on Semaas. This field study took place well out of both the native and naturalized ranges of *N. rustica* due to pandemic-related constraints, and did not last the duration of the flowering season. The flowering of the participating plants was delayed by about two weeks compared to Semaas plants located in Ontario, Canada, a location of which Semaas has been naturalized. As the sample flowers were bagged, the nectar standing crop was not measured – a measurement that would reflect all the intrinsic variation experienced by flowers, which in turn could influence nectar secretion. Future research could benefit from including both bagged and unbagged treatments, as well as multiple field sites within native/naturalized ranges to confirm the biological relevance of these findings. Empirical growth plots and multi-level modelling are other statistical analyses that may be useful in illuminating any other trends in this dataset.

In Indigenous research methodologies where participants consider Semaas to be sacred, the offering of these plants is meant to represent the respect and gratitude necessary to meaningfully engage in research (Wilson & Restoule, 2010). The acceptance of such an offering represents the

consent necessary to begin the research, to do the research in a good way (Wilson & Restoule, 2010). Semaas mediate communication, a role in which they are well-versed, having facilitated discussions on Turtle Island since its inception. It is important to note that these discussions are not limited to those between humans; Semaas are sacred because of their ability to connect the physical and spiritual worlds (Wilson & Restoule, 2010). In this research and the research upon which it is built, we see Semaas in constant, distinctly non-human modes of communication with all that exists around them. I am grateful to have been privy to one such mode, and to share it in the form of this paper.



**Figure 9:** *Semaas' Insect Visitors.* A) *Bombus centralis* B) Lygus bug C) Syrphid fly D) Unidentified, commonly seen, nectary-dwelling insect, potentially thrips.

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