

TIMING OF FLOWERING AFFECTS POLLINATION AND FRUIT SET IN *VIBURNUM*
EDULE IN BOREAL FORESTS OF ALASKA

By

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Abstract

Spring flowering in Alaskan boreal forests is happening earlier on average; how this relates to the pollination of plants and their pollinator community is unknown. Highbush cranberry (*Viburnum edule*) is one of the first herbaceous understory plants to flower every year, and in years when it flowers early there are fewer other species in bloom compared to years when it flowers at a more average time. Highbush cranberry is also important as a subsistence food and many boreal animals consume these fruits as a regular part of their diet. The potential for change may lead to differences in the response of pollinators and plants under early season conditions which could alter resources for pollinators and impact fruit production. This research looks at the impact of flowering timing on pollen deposition on *Viburnum edule*, and the composition of the pollinator community visiting the available flowers. Using an experiment with flowers placed in boreal forest sites either at an early time or at a peak flowering time across two years, we found that early flowering highbush cranberry received fewer pollen grains than peak flowering highbush cranberry and were visited less. *V. edule* was primarily visited by syrphid flies, native bees, and muscoid flies. We also observed a lower total number of visitors, and a lower proportion of visitors that were bees during an early flowering time than at peak flowering time. Floral visitors were more abundant during the advanced flowering year than during an average flowering year. We do not currently think that pollen limitation is causing a reduction in fruit set of early flowering *V. edule* because at all flowering times observed, we found over 50% of flowers to have been presumed visited while less than half of flowers in an inflorescence form fruits on average. More information on boreal pollinator triggers for diapause break and floral visitation is necessary to make more reliable predictions of the future impacts of phenology shifts in flowering plants and insect pollinators.

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1.1 Introduction

Interactions between plants and pollinators with historically similar flowering and emergence timing are potentially threatened by climate change (e.g., Høye and Forchhammer, 2008; Fabina *et al.*, 2010; Inouye *et al.*, 2012; Iler *et al.*, 2013). When plants and their pollinators respond to climate change differently there is the potential for a lack of synchrony between the phenology of consumers and their resources, a phenomenon known as phenological or trophic mismatch (e.g. Root *et al.*, 2003; Hegland *et al.*, 2009; Yang and Rudolf, 2010; McKinney *et al.*, 2012; Doiron *et al.*, 2015). Plants are generally responding to warmer temperatures in spring by flowering earlier, but the size of the shift differs by species (e.g., CaraDonna *et al.*, 2014; Parmesan & Yohe, 2003; Wolkovich *et al.*, 2012) and the phenology of insect emergence and activity may respond differently (Maglianesi *et al.*, 2020; Schmidt *et al.*, 2016).

In boreal ecosystems climate change is happening substantially faster than at lower latitudes, with spring onset occurring up to two weeks earlier than the historic average, and a temperature increase of 1.4°C over the last century (Wendler & Shulski, 2009). Plants in this region have a short growing and flowering season with highly synchronous flowering within individuals and populations (e.g., Molau, 1993; Mulder & Spellman, 2019a; Song *et al.*, 2022). The combination of rapid warming and short flowering periods make high latitude ecosystems particularly vulnerable to plant-pollinator mismatches, especially if shifts in flowering times and pollinator emergence are in response to different environmental cues. Plant-pollinator studies have shown a wide range of results for potential mismatches due to phenological shifts, from plants emerging before the needed pollinators (Gezon *et al.*, 2016) to pollinators emerging first (e.g., Gaku *et al.*, 2004; Sargent & Ackerly, 2008), to concurrent shifts in phenology (Forrest, 2015; Kudo & Ida, 2013).

In many early flowering boreal plant species, growth is largely triggered by ground thaw, which in turn is determined by an interaction between the early season air temperatures and total snow depth from the winter (e.g., Aerts *et al.*, 2006; Bliss, 1971; Diggle & Mulder, 2019; Fitter & Fitter, 2002; Iler *et al.*, 2013; Inouye *et al.*, 2012; Legault & Cusa, 2015). Some boreal species appear to initiate flowering later in the summer based on different environmental cues than early flowering species, or changing day length cues (Carlson pers. obs.), and species differ in the magnitude of the shifts of their flowering time annually (Mulder & Spellman, 2019b). Plants that typically flower early in the season are most responsive to early ground thaw, but are also most likely to suffer from a lack of pollinators because a smaller pool of potential pollinators is available (e.g., the size of bumble bee colonies grow through the summer, and the likelihood of multiple species of adults insect pollinators, even if short-lived, being active should increase from early spring to summer), the weather is more likely to limit pollinator activity in the spring, and pollinators may respond to environmental cues differently than the plants they forage from.

Flower morphology may also influence the vulnerability to phenological mismatch. Generalist flowers attract a wide variety of pollinators, and are therefore less likely to have a mismatch (Renner & Zohner, 2018), although there is still the potential for flowering and insect emergence to respond differently to environmental triggers. Much of the current research on potential mismatches in plant pollinator interactions is focused on bee-pollinated plants (Forrest, 2015; Ignasi *et al.*, 2011), and there are few studies that focus on plants pollinated by other insects. Boreal plants that flower early and attract general pollinators may be at risk of missing their pollinators, or seeing reduced pollination when flowering early in the spring (Molau, 1993).

Many boreal pollinators are generalists and visit multiple species for rewards such as pollen and nectar (Armbruster & Guinn, 1989; Kevan *et al.*, 1993). In Alaska there are many

species of bumble bees, solitary bees, flower-visiting flies, flower-visiting beetles, and butterflies recorded in the UAM Arctos Database (Sikes & Rykken, 2020). Interior Alaska pollinators likely overwinter under tree bark, leaf litter, or underground where they are able to break diapause early to ensure a longer development period for their offspring, although there is high variability in the phenology of solitary bees, and flies (e.g., Armbruster & Guinn, 1989; Danks, 2004; Howarth & Edmunds, 2000). Their overwintering locations are typically in sunny areas that have limited precipitation, although a limited amount is known about what truly drives pollinator overwintering location (Kevan *et al.*, 1993). In particular, flies, both muscoid and syrphid, have been found to be critical to the pollination of arctic and subarctic flowers in various habitats, including many early spring flowering plants (e.g., Brown & McNeil, 2009; Elberling & Olesen, 1999; Fulkerson *et al.*, 2012; Kevan, 1972; Kevan *et al.*, 1993; Totland, 1994; Tiusanen *et al.*, 2016).

Viburnum edule (Michx.) Raf. (Adoxaceae, formerly Caprifoliaceae), also referred to as highbush cranberry or squashberry, is an abundant early flowering generalist boreal forest plant species that shows especially large interannual shifts in flowering time between early and late springs relative to other boreal understory plants (Mulder & Spellman, 2019b). A study of 39 understory species that included a very advanced and a very delayed spring showed up to three weeks of variation in flowering times depending on the timing of spring for *V. edule* (Mulder & Spellman, 2019b). In a year with a highly advanced spring there is the potential for *V. edule* to flower before its typical co-flowering species (*Rosa acicularis*, *Mertensia paniculata*, *Cornus canadensis*) because these species have not shown as large of shifts in flowering time. These shifts in the timing of flowering could cause a change in the quantity of pollen deposited. *V. edule* has minimal nectar (Kornhauser pers. obs.) and is an exposed flower, but it does provide

visitors with pollen rewards which may be enough to attract a variety of visitors including bees, muscoid flies, and syrphid flies. Whether *V. edule* is self-compatible and capable of auto pollination has not been studied to our knowledge.

We aim to understand how variation in date of flowering impacts plant-pollinator relationships for an early-flowering, generalist plant that shows large shifts in response to the timing of spring. Before we could figure out if a trophic mismatch was occurring, we wanted to know if *V. edule* can set fruits in the absence of pollinators. We completed a pollinator exclusion experiment to determine the necessity of pollination service for fruit set production in *V. edule*. Through experimental addition of flowers we manipulated the timing of flowering in two successive years, one with an advanced spring and one with an average spring. We wanted to know if the flowering time and the year impacted the amount of pollen, the proportion of stigmas visited, or the number of visitors. We also aimed to document who the pollinators are for this species in interior Alaskan boreal forest communities.

1.2 Methods

1.2.1 Study Species

V. edule flowers are bisexual, with five short and spreading stamens and a short style with a single-rounded stigmatic surface. It is not known when anthers dehisce and when stigmas are receptive, but with a very short flowering time of less than 1 week for all flowers on an individual it is likely that there is overlap between the two. The five connate flower petals are white or cream, to pink, 4 to 7 mm wide, flat, and spreading. The flowers occur in a compound corymbs that are found in opposite pairs, forming a semi-sphere of on average 25 flowers that originate at the leaf axils (Figure 1). The fruits are drupes 5–8 mm in diameter and are bright red

with 1 seed. *V. edule* flowers very synchronously in each inflorescence (rarely > 48 hrs variation in anthesis among flowers), as well as among plants in an area. In Fairbanks, Alaska, *V. edule* plants flower in a two week period (Mulder & Spellman, 2019b).

1.2.2 Study Area and Timing

Primary sites were located in the University of Alaska Fairbanks (UAF) forest in Fairbanks, Alaska, USA (Table 1). The two primary sites at UAF, sites A and B, were forest patches about 300 m apart. We used one additional site (C) during the first study year that was located about 2 km from the primary sites. We selected sites that had some co-flowering species that were expected to be visited by similar pollinators, but where *V. edule* was the dominant species present. Co-flowering plants include *Rosa acicularis* (prickly rose), *Mertensia paniculata* (bluebell), *Geocaulon lividum* (pumpkin berry), and *Calypso bulbosa* (fairy slipper orchid). Two of the three sites were primarily birch (*Betula neoalaskana*) forests with some white spruce (*Picea glauca*) trees present (A & C), while one site (B) was a mix of white spruce and birch. Canopy cover data were collected at each plot by using a concave crown densiometer (Forestry Suppliers, Canada) at a height of 1.5 meters in each cardinal direction and averaging the resulting values (Table 1).

Table 1: Site Information. The decimal degree locations, elevation, and mean canopy cover for each site.

Site Name	Location	Elevation (m)	Site Area (m ²)	Mean Canopy Cover (Percent Closed)
A	64.859°N, 147.856°W	180	600	76.53 ± 0.05
B	64.857°N, 147.858°W	180	500	72.53 ± 0.12
C	64.896°N, 147.814°W	210	400	73.8 ± 0.11

Flowering in year 1 (henceforth referred to as advanced year) was slightly earlier than average while flowering in year 2 (henceforth referred to as average year) occurred at an average time relative to the phenology data collected by Mulder and Spellman (2019b). Both years fell within the timeframe of flowering measured by Mulder and Spellman (2019a, b). The precipitation was measured using the sum of rainfall at the Fairbanks airport for the three days of each experimental period.

1.2.3 Fruit Set

To see if *V. edule* could self-fertilize, we conducted a pollinator exclusion experiment during the early spring of 2020. We chose 16 plants nearby sites A, B and C (5 at site A, 5 at site B, 6 at site C). The focal plants were within a small radius of 20 meters at each of the sites. Small mesh bags were placed over one inflorescence of an opposite inflorescence pair while the other inflorescence was left open. The inflorescences were labeled and bagged while still in bud (Figure 1B), then left from early May until mid-August at which point the unripe fruits were counted to determine whether pollinator access affected fruit production.

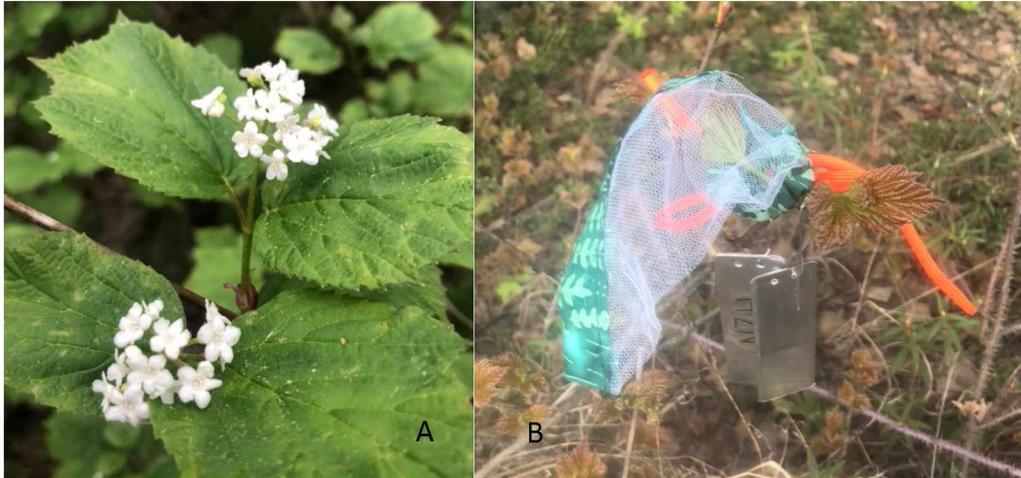


Figure 1: *V. edule* flowers (A) and netting experiment (B). Photo A shows a pair of *V. edule* inflorescences. Photo B shows a net bag over one inflorescence and another exposed inflorescence, both with metal tags and both still in bud.

1.2.4 Phenology and Pollination Success

We selected the start of the experimental period based on the first flowering *V. edule* plants we could find in the Fairbanks area. We designated the “early flowering week” as the first week that flowers were opening in the Fairbanks area, while “peak flowering” was the week that the majority of *V. edule* plants were flowering (Table 2). We found the earliest flowering plants in the area by looking in sunny areas where flower buds were present earlier than other areas. We were not able to add a third week because all plants had completed flowering. Although the date of snow melt was later in 2020 than in 2021, May 7th and April 30th respectively, temperatures during the early growth to flowering period were much higher in 2020 than in 2021 (50 vs. 45 degree days found using the total number of degrees > 0 °C during the 3 day experimental period).

Table 2: Environmental data by experimental week. This table provides the sum of degree days for the 3 experimental days as well as degree days from January 1 of each year through the end of the experimental week.

Year/ treatment	Flower timing treatment	Julian Date	Average Temp	Max Temp	Min Temp	Precip- itation Sum (mm)	Degree days for each week (3 days)	Degree days through week (Jan1- end of week)
Advanced year 2020	Early flowering	149- 151	16.7 ± 0.65	26.1	6.1	0	50.5	420
Advanced year 2020	Peak flowering	155- 157	13.6 ± 0.17	22.8	7.2	19	41.5	508
Average year 2021	Early flowering	152- 154	17.83 ± 0.23	22.8	7.2	12	45.6	478
Average Year 2021	Peak flowering	158- 160	14.1 ± 0.15	23.9	7.8	38	45	572

There was little co-flowering of other species in the early flowering period while there was some co-flowering during the peak flowering period. During experimental week 1 (early flowering) of both years *Calypso bulbosa* was the only nearby plant in flower and was only found near site B. It is known for having a deceptive pollination syndrome and for relying on naive queen bumble bees (Alexandersson & Ågren, 1996). During experimental week 2 (peak flowering) of 2020 and of 2021 many flowering plants began to bloom including *Rosa acicularis*, *Mertensia paniculata*, *Geocaulon lividum*, and two *Taraxacum* species. Other than *R. acicularis* at site C in year 1 (advanced year), these flowering plants were not found directly

occurring in the sites, but were present nearby along trails and roads within a few hundred meters of the sites.

1.2.5 Experimental Procedure

Branches with *V. edule* inflorescences were collected on a sunny south-facing slope near site C (see Table 1) because there were a large number of *V. edule* patches containing shrubs that flowered earlier than many other locations. Each branch had 2-6 inflorescences. Each inflorescence had a mean of 40 ± 27 SD flower buds (range: 5-130), and most of the buds bloomed within a day of clipping. Any buds that had opened were removed prior to being placed in plots. The branches of inflorescences were kept in flower tubes (small plastic vials of water) throughout the experiment and were refilled with water daily.

At each of the three sites, we placed five plots in a circle, approximately 50 meters apart. Each plot consisted of a rectangular tray that held 6 cone-shaped containers with a diameter of approximately 4 cm and a height of approximately 12 cm. Each cone held one *V. edule* branch with at least 2 inflorescences, each with 15 to 40 flowers. The inflorescences were held in the containers at 50 cm above the ground. There were no other *V. edule* plants flowering in any site at any time treatment, either through removal or due to the understory composition. There were *V. edule* plants flowering nearby each site, but fewer at the early time period for all sites in both years. The plots also had a GoPro camera (GoPro Hero 7 Black, GoPro Inc.) mounted on a fence post at a height of 1 meter off the ground and a Hobo temperature logger (Onset, HOBO Pendant Temperature/Light 64K Data Logger) about 1 meter off the ground. The GoPro Hero 7 cameras were outfitted with external battery packs and 128 GB microSD cards and were used to monitor the insect visitors at the plot for at least 4 hours a day for 3 consecutive days in each

experimental week. Videos were taken during the middle of the day (10:00-14:00), but not taken during periods of rain. This 4-hour time block was selected because it was the most active period of day for floral visitors (K. Kornhauser pers. obs.). Evening observation further confirmed the period of recording (K. Kornhauser pers. obs.). We recorded videos at a resolution of 1080 p at 30 frames per second. A Hobo temperature logger was used to gather information about the plot specific temperature. In 2021, the second experimental year, we only used sites A and B and three plots from each site due to the practical difficulties of collecting data from site C and additional plots.

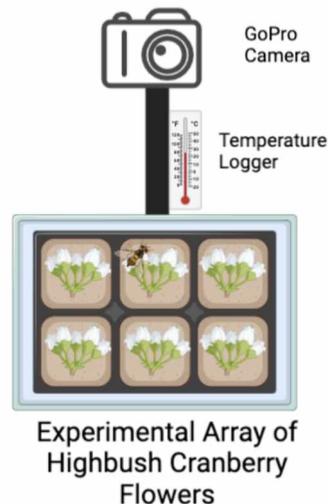


Figure 2: Experimental plots for phenology experiment. A GoPro camera, temperature logger, and an array of clipped *V. edule* flowers were used to observe the effects of early flowering on the pollination of *V. edule*.

1.2.6 Flower Visitors

We reviewed 2 hours of video from each day to identify the flower visitors (muscoïd flies, syrphid flies, solitary bees, bumble bees, beetles, aphids) and which inflorescence and plot they had visited. Flies were classified into the Muscoïdea or Syrphidae families but not classified further. Bees included *Bombus*, and species of Halictidae, and Andrenidae. Bees were typically

not distinguishable to genus from video recordings. Beetles (Coleoptera) and aphids (Superfamily Aphidoidea) were also found visiting *V. edule* flowers. Visits were classified as “flyby” (insect was in frame but did not land on any part of *V. edule*), “landed on flowers” (insect was observed interacting with flowers of *V. edule*), and “landed on leaves” (insect landed on a *V. edule* plant but did not engage in any potential pollinating behavior) depending on what the flower visitors did throughout their time in the video.

1.2.7 Pollination

At the end of the three-day experimental period, the stigmas were removed from the flowers and placed into a petri dish with a base of agar for transport and storage. We mounted up to 15 stigmas from each inflorescence onto microscope slides with a basic fuchsin gel stain within 36 hours of collection (Kearns, 1993). Pollen grains were counted under a compound light microscope at 40× magnification. Heterospecific pollen grains were found on < 0.01% of stigmas and were noted, but not identified or included in the total pollen count.

1.2.8 Analyses

To determine whether the timing of flowering (early vs. peak) or the year (advanced vs. average) affected pollination, we evaluated differences between experimental periods in pollen deposition and pollinator visitation. We performed statistical analyses using R (Version 1.3.1093) considering plots (each plot of 6 branches, $n = 15$ in year 1, $n = 6$ in year 2) as our experimental units. Inflorescences within a plot were not considered independent of one another due to their proximity and the likelihood that a visitor attracted to one inflorescence would be attracted to others nearby. We used two measures of pollen deposition: the mean pollen grains

per inflorescence and the proportion of stigmas that had *V. edule* pollen per plot; both measures use the 15 stigmas collected rather than every flower from each inflorescence. The proportion of “stigmas with pollen” was defined using a threshold of 5 pollen grains on a stigma to indicate a flower had sufficient pollen deposition for fertilization and were likely to represent flowers visited by insects. We selected a threshold of 5 pollen grains because we are focused on pollinator visitation, not the occasional pollen grain that gets to a stigma by wind or during the stigma removal procedure. We ran the same model using a threshold of 1 pollen grain and 10 pollen grains to evaluate how our definition affected the results. We ran a linear model with mean pollen grains per plot as a response variable as well as a model with the proportion of stigmas visited as the response variable. In both models the explanatory variables were the year (advanced and average), the relative flowering time (early and peak), the interaction between relative flowering time and year, and the random variables were the number of flowers and the site. Although we only have 2 years of data, we used year as an explanatory variable due to the shift of timing between years. Interannual variation in flowering time was not replicated due to the scope of the study, and is not a fully robust test.

To determine if timing, both time of flowering and year, had an effect on the number of visitors observed, we ran a linear regression with the total number of visitors per plot as the response variable. The number of visitors per plot was found using 2 hours of video data of pollinator visitation. We considered year (advanced vs. average), relative flowering time (early and peak), the interaction between year and relative flowering time as the explanatory variables. We ran these models twice, one with all sites for both years, and once without site C for both years because site C was not included in year 2 and we wanted to ensure that we were comparing the same set of sites. For the linear models we used the package ‘lme4’. Additionally, we ran chi-

square tests of independence to test whether the pollinator community composition was dependent on the year or the week. We also ran chi square tests to compare the community of visitors at each time period.

1.3 Results

1.3.1 Fruit Set

V. edule flowers rarely produced fruits in the absence of pollinators, indicating that they rely on floral visitors for pollination. The flowers that remained exposed to visitors were 7.5 times more likely to produce an immature fruit than the flowers that had visitors excluded (2 vs. 0.125 unripe fruits per inflorescence) ($F_{(1, 15)} = 30.0$, partial $R^2 = 0.47$, $p < 0.0001$). In general, the plants included in the pollinator exclusion experiment did not produce many fruits even when exposed to visitors (mean: 2 fruits per 20 flowers when exposed to visitors). Additionally, most exposed flowers did not produce fruits: 11% of flowers exposed to visitors produced a fruit, while 0.9% of pollinator-excluded flowers produced a fruit.

1.3.2 Flower Visitors

V. edule flowers are most commonly visited by syrphid flies, while muscoid flies and solitary bees were the next most likely to visit respectively (Figure 3B). There were very few bumble bees, beetles and aphids and as such they were not included in figures. The proportion of floral visitors made up by syrphid flies, muscoid flies, and bees changed between early and peak flowering times in the advanced year regardless of whether site C was included (Figure 3B) (all sites: $\chi^2 = 16.1$, $p = 0.0032$; only sites A and B: $\chi^2 = 10.8$, $p = 0.004$) with a higher proportion of muscoid flies and a lower proportion of syrphid flies during the early flowering time. In the

average year (sites A and B only), the shift was much smaller ($\chi^2 = 6.2$, $p = 0.05$) and bees were overrepresented in the early versus the late flowering period. In comparing the two years there is a similar shift regardless of whether site C was included (All sites: $\chi^2 = 51.4$, $p = < 0.0001$; only sites A and B: $\chi^2 = 47.8$, $p = < 0.001$) with a higher proportion of bees in year 1, and a higher proportion of syrphid flies in year 2 with all sites considered.

The number of visitors to each plot was affected by the flowering time (early vs. peak) and the year of flowering (Figure 5C, Table 3). Visitors were twice as likely to visit in an average year (year 2) than they were in an advanced year (year 1), and were twice as likely to visit during peak flowering than during early flowering. Plants at peak flowering in both years had approximately double the number of visitors than during the early flowering period (Figure 3A). The relative abundance of syrphid flies increased from early flowering to peak flowering (Figure 3B).

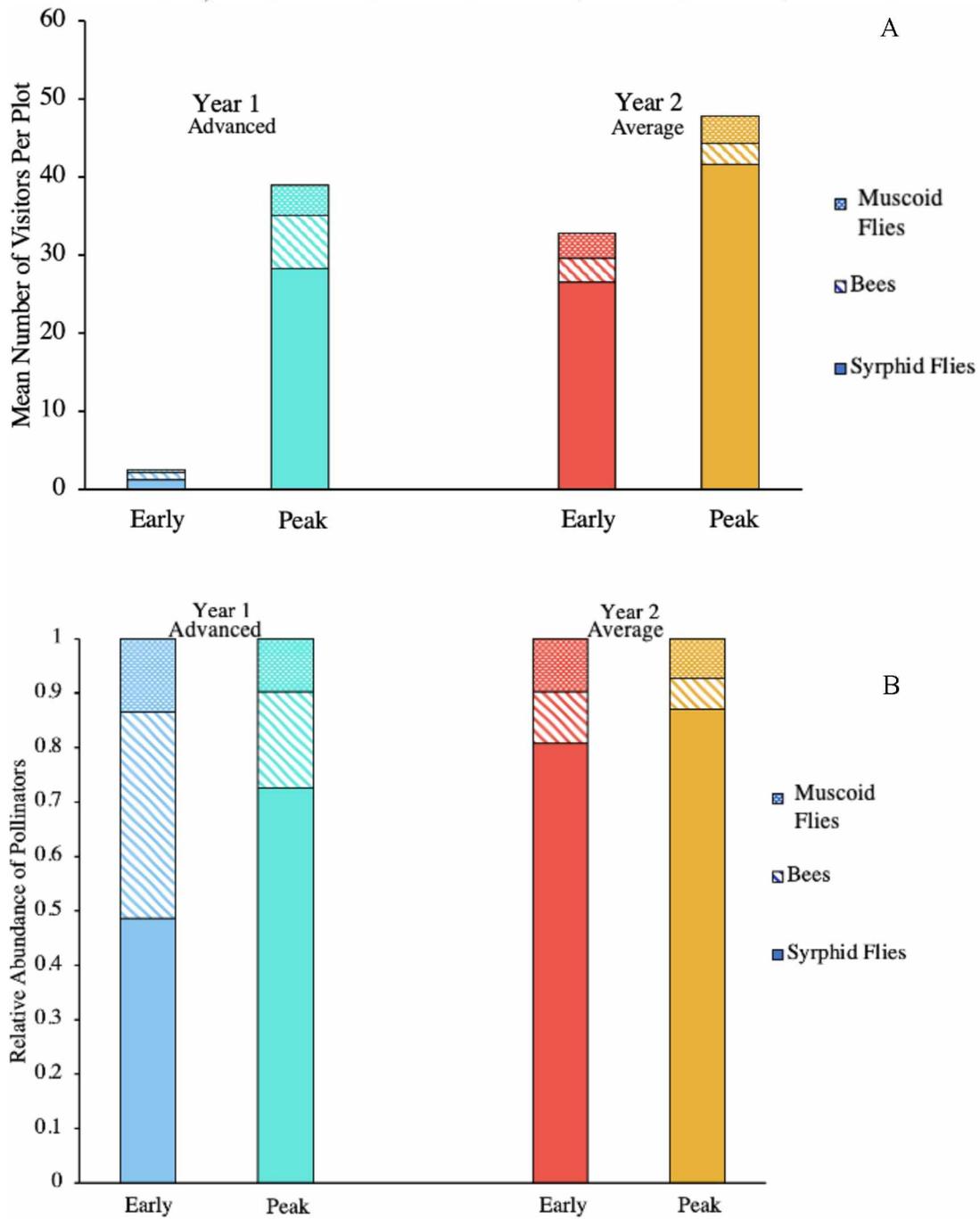


Figure 3: Bar graphs showing the absolute number of visitors and the relative abundance of visitors present at different time periods. A) Average number of visitors per plot and when they visited. B) Relative abundance of visitors.

The minimum percent of stigmas visited for a given number of pollinators, with five or more pollen grains per stigma, increased with the number of visitors (Figure 4). As the number of visitors increased, the variability in the number of stigmas pollinated decreased.

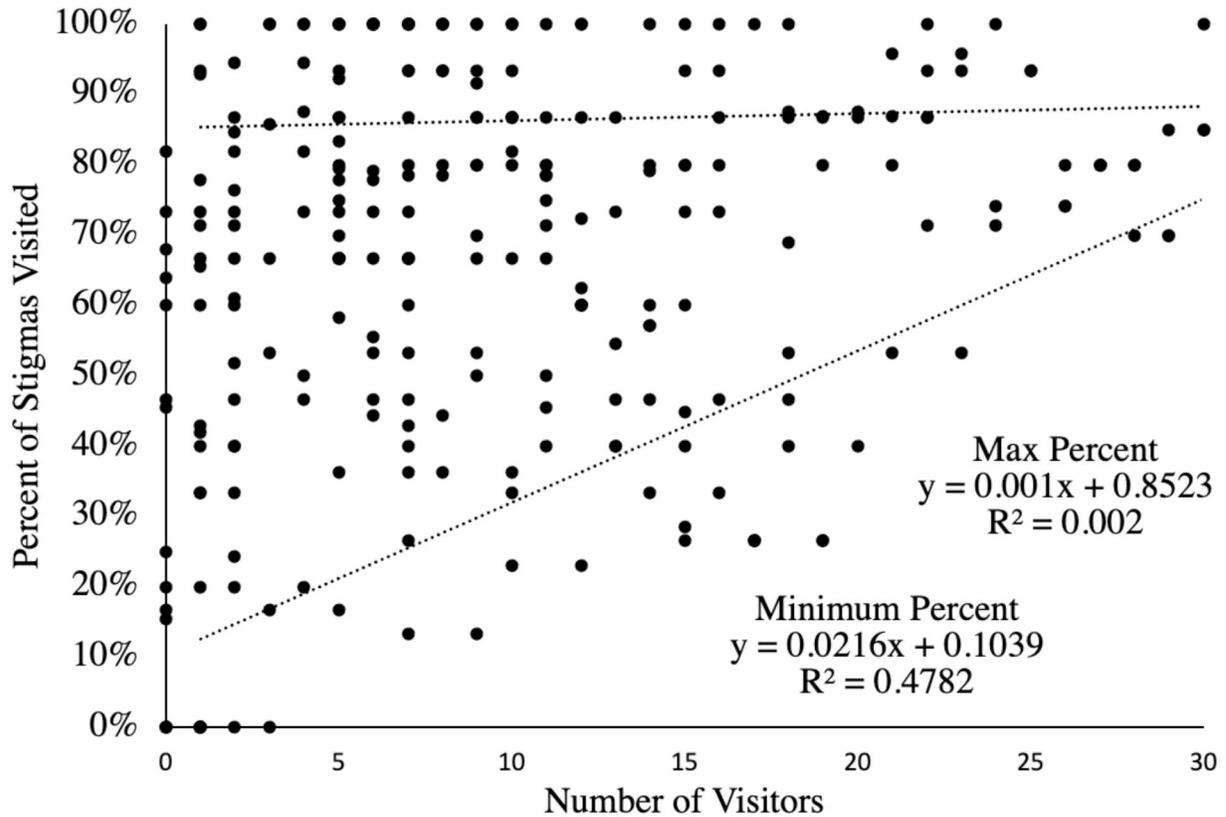


Figure 4: Percent of stigmas visited vs. the number of visitors. This plot shows a triangular relationship between the number of visitors and the percent of stigmas pollinated with maximum and minimum regression lines.

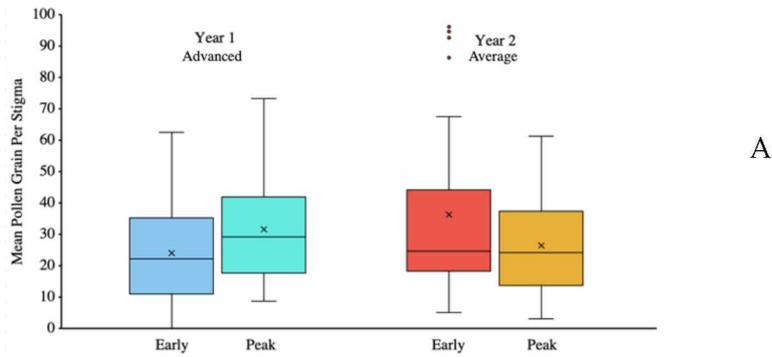
1.3.3 Pollination

V. edule plants flowering at peak time had 66% more pollen grains than plants that flowered early (Figure 5A). For mean pollen grains per plot, the interaction between early or peak flowering and an advanced or average spring year had a significant effect on the mean pollen grains deposited per stigma, so when flowering occurred early in an advanced year it had

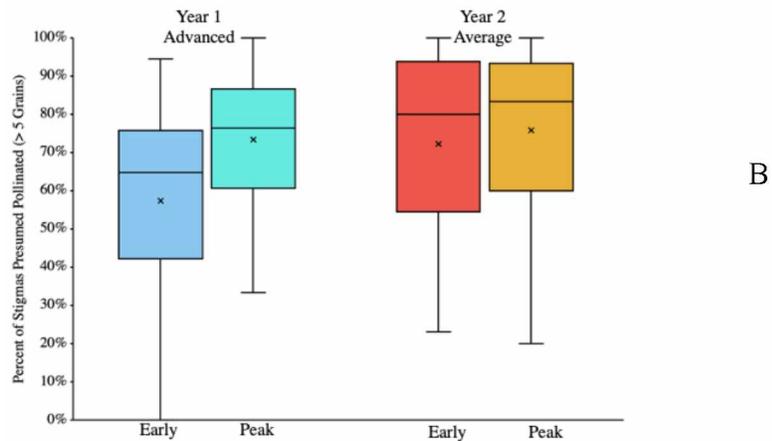
less pollen than if it flowered during any other time and year (All sites: $F = 8.79$, $p = 0.006$, only sites A and B: $F = 5.88$, $p = 0.024$, Table 3).

The proportion of stigmas with five or more pollen grains per stigma at each plot was 1.5 times greater during the peak flowering time than during the early flowering time (All sites: $F = 7.97$, $p = 0.008$, only sites A and B: $F = 7.73$, $p = 0.012$, Table 3, Figure 5B). Thresholds of 1 and 10 pollen grains were tested and we found qualitatively similar results. In all observed experimental periods the mean of the percent of stigmas with five or more pollen grains per stigma was greater than 50%.

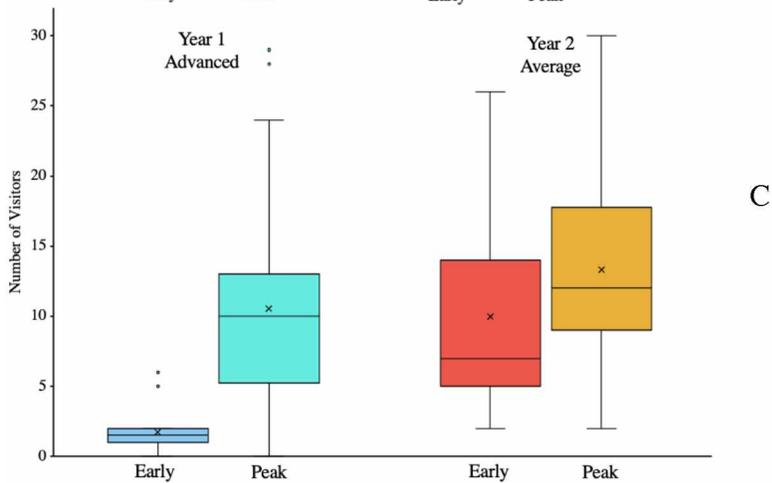
The number of visitors was significantly affected by the year of flowering, the week of flowering (early vs. peak), and the interaction between the two (Figure 5C, Table 3). When plants flowered early in an advanced year they were visited by fewer insects than at any other time period. As the number of flower visitors increased, the proportion of stigmas with higher numbers of pollen grains also increased. When there were few visitors, variation in pollen loads on stigmas were high (Figure 5). Analyses with and without site C are included in the tables but, for simplicity, not in the figures.



A



B



C

Figure 5: Box and Whisker Plots Comparing the Flowering Time with Three Response Variables. Plot A shows the mean pollen grains per stigma for each of the 2 years of monitoring for early and peak flowering. Plot B shows the percent of stigmas presumed pollinated for each of the 2 years monitored for early and peak flowering. Plot C shows the number of visitors each of the 2 years for early and peak flowering. In all three figures, year 1 (advanced) is shown with the left two boxes and year 2 (average) is shown in the right two boxes. Outliers are shown as points above/below each box.

Table 3: ANOVA values for three response variables. *F*-values, degrees of freedom, and *p*-values are reported for the year (advanced or average), the relative timing of flowering (early or peak), and the interaction between the two.

Response Variables	All Sites (A, B and C)								
	Year			Relative Timing of Flowering			Interaction		
	<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Mean Pollen Grains	0.61	1	0.44	0.96	1	0.33	8.79	1	0.006
% Stigmas Adequately Pollinated	2.06	1	0.16	7.97	1	0.008	2.81	1	0.103
Number of Visitors	21.4	1	<0.001	17.8	1	<0.001	1.19	1	0.233
Sites A and B only									
Mean Pollen Grains	0.19	1	0.66	0.19	1	0.66	5.88	1	0.024
% Stigmas Adequately Pollinated	1.64	1	0.21	7.73	1	0.012	1.23	1	0.28
Number of Visitors	22.0	1	<0.001	27.1	1	<0.001	3.51	1	0.07

1.4 Discussion

V. edule produces very few fruits in the absence of visitors and is largely dependent on floral visitors for fruit and seed-set. The small number of fruits produced in the pollinator exclusion bags suggest that this species is self-compatible, but that herkogamy or dichogamy limit auto-pollination. However, other species of *Viburnum* are known to be self-incompatible

(Nebot & Mateu, 1990) and low levels of contamination with outcrossed pollen, or low levels of apomixis is also possible in this study. Fruit set in the open pollination treatment was significantly greater than when visitors were excluded but still low, suggesting pollinator service limitation or resource limitation. On average, 11% of flowers in an inflorescence exposed to pollinators produce a fruit, with a maximum of just 5 fruits per 40 flowers in an inflorescence. Both resource and pollinator limitation has been shown to be severe in other high-latitude systems (Carlson *et al.*, 2008; Fulkerson *et al.*, 2012), and despite lower pollinator abundance and diversity many northern species are partially or wholly dependent on pollinators (Kevan 1972, Fulkerson *et al.* 2012). These lines of evidence suggest resource limitation is a strong factor in limiting fruit production. However, as spring timing and flowering are trending earlier, there is a potential for pollen limitation. If pollen availability is reduced even further as flowering advances, pollen limitation could become an issue. Previous research focused on trophic mismatches of plants and pollinators have had similar results, finding that pollen limitation is not currently impacting fruit set due to a trophic mismatch (Gillespie *et al.*, 2016; Scaven & Rafferty, 2013). Most stigmas in this study had more than the 5 pollen grains that is expected to be adequate to fertilize the single ovule, but if this species is self-incompatible it may be limited by the number of outcrossed pollen grains. Even during the period with the lowest visitation rate (early flowering in the advanced year) we saw more than 55% of flowers were adequately pollinated (>5 pollen grains on stigma) which is far greater than the proportion of flowers converted to fruits. Given the tight inflorescence and behaviors of pollinators typically visiting multiple flowers within an inflorescence, one would expect high levels of geitonogamous pollen movement.

Syrphidae, Muscoidea, Andrenidae, and Halictidae were the most common floral visitors to *V. edule* at our sites. Syrphid flies were the overwhelming majority of observed visitors on *V. edule*, followed by muscoid flies and solitary bees; very few bumble bees were observed visiting *V. edule*. Other studies have also observed the importance of flies as pollinators in high latitude flowering plants, especially in spring (e.g., Brown & McNeil, 2009; Elberling & Olesen, 1999; Fulkerson *et al.*, 2012; Kevan, 1972; Tiusanen *et al.*, 2016). *V. edule* provides very little, if any, nectar and it is not surprising that guilds of pollinators that primarily forage nectar on flowers, such as butterflies, were not observed. Pollen as the sole primary reward for pollinators has the negative consequence on the plants of potentially more pollen grains being consumed and fewer pollen grains being moved (Nicholls & Hempel de Ibarra, 2017), and may be partially linked to higher levels of pollen limitation.

While syrphid flies were the most common visitors at all times, they made up a smaller proportion of visitors in the early treatment (Figure 3B). During an early flowering period in the advanced year, muscoid flies made up a larger proportion than they did in any other flowering period. In both years we observed an increase in the proportion of syrphid flies with peak flowering. It is not clear if this change is associated with syrphid fly emergence, or if syrphids are moving into these areas when resources are more abundant. Syrphid fly emergence in the Rocky Mountains is based on the timing of snow melt, which is also a factor in flowering phenology (Iler *et al.*, 2013). Given how important syrphid flies appear to be as pollinators, a better understanding of what drives their emergence is needed. Muscoid flies also make up a large proportion of visitors to *V. edule*. Muscoid flies have been found to be a main driver of pollination in the high Arctic, and are also important pollinators in the boreal forests of the subarctic (Kevan *et al.*, 1993; Tiusanen *et al.*, 2016).

To better understand the various potential trophic mismatches in early spring boreal forest ecosystems, it is critical to gain a more thorough understanding of the syrphid and muscoid flies, specifically the triggers for their break of diapause and plant associations. Further research on the pollinator communities and their responses to earlier spring time, as well as a better understanding of the plant-pollinator network in interior Alaskan boreal forests, would help expand our understanding of the impacts of a changing climate on the plant-pollinator interactions within this ecosystem. Another important step would be to complete hand-pollination experiments on *V. edule* along with other boreal plants that have a shifting flowering time. These hand-pollination experiments would provide information on the degree of pollen limitation; if the flowers are self-incompatible, high numbers of pollen on stigmas could cause pollen limitation via incompatible pollen. This information would help to better understand the impacts of phenologic shifts of plants and of insect pollinators.

1.5 Conclusions

V. edule plants that flower early had fewer pollen grains deposited on them, had a more highly variable proportion of their flowers presumed visited, and had fewer visitors than if they flower at a peak time. Prior to this study, we did not know what insects visited *V. edule* or if it even relied on pollination from insects, but now we know that syrphid flies and muscoid flies appear to be crucial to the pollination of this species. It is also important to note that we do not currently think that pollen limitation is an issue, but we cannot be certain because we did not have a way to assess fruit production due to our study design. We do not know if the pollen we counted is self-pollen or pollen from other plants. If highbush cranberry is self-infertile, and it is getting a lot of self-pollen, it would still not be able to produce fruits.

If spring timing continues to advance, pollen limitation may become an issue, especially if the trends we observed continue and if *V. edule* is self-incompatible. If spring comes earlier and *Viburnum* flowers earlier, and we see the same trends we currently see, then we may observe a decrease in the pollination and visitation of *Viburnum* and may at some point reach pollen limitation. If this does occur it is possible that there would be fewer fruits and there would be fewer fruits for the animals and people that rely on this plant for food. It is possible that *V. edule* will not continue to flower earlier due to other climate triggers and photoperiod restrictions, and in this case the trends we observed may not continue.

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Appendix: Visitor Photos



Photo 1: Syrphid fly visiting *V. edule*.



Photo 2: Syrphid fly visiting *V. edule*.



Photo 3: Syrphid fly visiting *V. edule*.



Photo 4: Muscoïd fly visiting *V. edule*.

Visitor video link: <https://www.youtube.com/watch?v=vgwQfFFkqHc>