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Pollination of Rubus arcticus L. in Alberta: microclimate effects on pollinator availability and the role of pollen limitation on fruit set

Burns, Cole

UNIVERSITY OF CALGARY

Pollination of *Rubus arcticus* L. in Alberta: microclimate effects on pollinator availability and the role of pollen limitation on fruit set

Cole Burns

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Abstract

Rubus arcticus Linnaeus is a widespread plant species that likely experiences changes in pollinator visitation and fruit production with microclimate. A pollen supplementation experiment with open controls, pure-selfed and outcross supplemented treatments was used to determine if *R. arcticus* fruit production is limited in the quantity of pollen deposited. This experiment was then complemented by a pollen tube analysis to determine the importance of pollen quality. To determine the effects of microclimate on *R. arcticus* pollinator availability and foraging behavior, pollinator surveys were conducted using time-lapse photography which was accompanied by micrometeorological monitoring with measurements from humidity/temperature loggers, an anemometer for wind speed, and hemispherical photography for solar irradiance. The results provide evidence that fruit production in *R. arcticus* can be extremely low in regions that are at the southern parts of its range, but this low fruit production does not necessarily reflect pollen limitation. The dominant pollinators of the study population were syrphid flies, which exhibited visitation frequencies that were influenced by temperature and humidity.

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List of Symbols, Abbreviations and Nomenclature

Symbol/Abbreviation/Term	Definition
%CanOpen	Percent Canopy Openness (%)
DESE	Presence of daily erroneous data logger reading due to excessive sun exposure (1 = yes, 0 = no)
DRHOV	Daily relative humidity of visits for given position (daily averaged RH (%) for a given observation day)
DST	Daylight savings time
DTOV	Daily temperature of visits for given position (daily averaged temperature (°C) for a given observation day)
DWSOV	Daily wind speed of visits for given position (daily averaged wind speed (m/s) for a given observation day
FDPC	Flower density per sampling grid cell (#)
FFOV	Flowers in field of view as observed by the time-lapse cameras
FOV	Field of view
FPS	Frames per second
GLA	Gap light analyzer
GLMM	General linear mixed-effects model
H	Global radiation incident on the ground
H_0	Extraterrestrial radiation incident on a horizontal surface outside
Handling Time	Total number of (time-lapse) frames an insect was observed on a focal flower (#)
H_b	Direct (beam) radiation energy
H_b/H	Beam fraction
HESE	Presence of hourly erroneous data logger reading due to excessive sun exposure (1 = yes, 0 = no)
HRHOV	Hourly relative humidity of visits for given position (hourly averaged RH (%))
HTOV	Hourly temperature of visits for given position (hourly averaged temperature(°C))
HWSOV	Hourly wind Speed of visits for given position (hourly averaged wind speed (m/s))
IESE	Presence of instantaneous erroneous data logger reading due to excessive sun exposure (1 = yes, 0 = no)
IRHOV	Instantaneous relative humidity of visits for given position (averaged RH (%) across all frames an insect was present)
ITOV	Instantaneous temperature of visits for given position (averaged temperature (°C) across all frames an insect was present)
Kt	Cloudiness index
MST	Mountain standard time
Outcross Supplemented	Treated with xenogamous pollen and left for open pollination
Pure-outcross	Strictly treated with xenogamous pollen only
Pure-self	Strictly treated with pollen from a stem's same flower
RH	Relative humidity (%)
R_p	Photosynthetically active radiation (PAR) or visible light in the spectrum of 400-700nm
R_p/R_s	Spectral Fraction
R_s	total shortwave radiation contributed by all wavelengths (0.25 μm to 25 μm)
RTU	Recognizable taxonomic unit
RTU Richness	Total number of RTUs that visited in a given observation day (#)
TransTot	Transmitted total solar radiation (mols/m^2/d)
UOC	Universal overcast sky model
Xenogamous Pollen	Pollen from a separate genetic individual plant

Chapter 1: General Introduction

Animal pollination is considered an important ecosystem service performed by insects, mammals, and birds that supports the existence of many plant species (Garibaldi et al. 2013). Currently, there are 295,383 known angiosperm (flowering plant) species (Christenhusz and Byng 2016), with approximately 87.5% of these being pollinated (at least in part) by animals (Ollerton et al. 2011). While often overlooked, this ecosystem service is a critical component of human existence. For instance, ~85% of the leading global food crops rely on animal pollination to some extent, which is mostly provided by insects (Klein et al. 2007). The economic benefits from pollination services (i.e. increased yields) amounts to 245 billion dollars ($CAD) and this value equates to 9.5% of the global costs of agriculture production (Gallai et al. 2009). However, there have been increasing global trends of pollinator decline (Vanbergen et al. 2013) and a main driver of this phenomenon is climate change (Potts et al. 2010a).

Many important groups of pollinators have experienced declines in their population sizes or experienced shifts in their distributions as a consequence of climate change. Among the most notable pollinator declines is the deterioration of managed/domesticated honey bees (*Apis mellifera*), in which the number of colonies has dropped by 16% from 1985-2005 (Potts et al. 2010b). Even more concerning, wild pollinators have also been suffering from the increasing effects of climate change. Not only are wild pollinators intrinsically important and contribute greatly to biodiversity, but they also can substantially increase yields and productivity when introduced to crop systems whereas crops with only honeybees introduced have much smaller yields (Garibaldi et al. 2013, 2014). The range size of bumblebees (*Bombus* spp.) has been steadily compressing since 2000 (Williams et al. 2007), with losses as large as ~300km in the

southern range limits of both European and North American bees (Kerr et al. 2015). On the other hand, butterfly ranges have shifted 14-240km northward within the past 100 years (Parmesan et al. 1999, Thomas et al. 2001, Hickling et al. 2006). Thus, climate change has had significant effects on pollinating insects at large regional scales, but climate can affect pollinators at much finer spatial scales as well.

More specifically, microclimate can dramatically impact the foraging behavior of most pollinators. Microclimate describes the differences in climate within small localized areas (i.e. forest edge vs. forest interior) (Geiger 1985), but these differences can greatly affect insects. As many pollinators are insects, they are thus ectothermic (i.e., their internal core body temperature is regulated via the external environment). Unlike endotherms/homeotherms, which can maintain stable body temperatures, the activity of ectotherms is heavily affected by the surrounding microclimate (Kearns and Inouye 1993). Ambient air temperature and solar radiation affect the minimum temperature thresholds in which the thoracic muscles can stimulate flight and are therefore thought to be the most important microclimate factors for pollinators. Consequently, many insects forage only within specific "microclimate" windows (Corbet et al. 1993), constrained by various temperature and irradiance regimes. Relative humidity has less of a direct effect on insects because of the impermeable materials within their exoskeletons (Oke 1987), yet has an effect on nectar concentration (Corbet et al. 1979), which may alter the feeding tendencies of prospective pollinators. Conversely, wind speed can directly affect the foraging behavior of insects as it not only influences their flight navigation and landing orientation (Chang et al. 2016), but can also negate the effects of temperature and irradiance via convective cooling (Church 1960, May and Casey 1983, Unwin and Corbet 1991). Therefore, climate even at a very small scale can have big implications for insect feeding and pollinating activities.

Whether these changes in the abundance, distribution, or activity of pollinators will have an effect on plant reproductive success will depend on many features of the plant population in question, but many studies indicate that these changes are resulting in amplified pollen limitation (Biesmeijer et al. 2006, Karbassioon 2017). Pollen limitation can simply be defined as a deficiency in pollen quality or quantity that limits the reproductive fitness of a plant (Ashman et al. 2004). To put it simply, some plants may not be receiving a sufficient number of conspecific pollen grains to initiate fertilization of ovules and the production of fruit/seeds or are receiving the wrong type of pollen; such as incompatible, heterospecific (pollen from other plant species) or inviable pollen. Pollen limitation may also be the result of pre-dispersal failure with pollen being lost to the environment or subject to herbivory from insects, and dispersal failure where there is a lack appropriate of pollinators (pollinator limitation) either through habitat fragmentation or poor environmental conditions (Wilcock and Neiland 2002). Decline in pollen quality is arguably the biggest contributor to pollen limitation. In one study, limitation on account of quantity only occurs in the lowest percentiles pollen delivery, as the fertilization efficiency of individual pollen grains is quite high (Aizen and Harder 2007). Should the majority of pollen delivered be incapable of fertilizing ovules or from an incompatible source, pollen quality will be more limiting to plant reproductive success.

A plant species whose pollination that may be affected by pollen limitation and microclimate is the Arctic/Dwarf Raspberry (*Rubus arcticus* L.: Rosaceae). *R. arcticus* is a long living fast growing perennial (Ryynänen 1973, Tammisola 1988), with a circumpolar distribution across the entire northern hemisphere of the globe (Maiz-Tome 2016). The species is found all across Canada in almost all the provinces and territories (Brouillet et al. 2010), growing in a wide variety of habitats, from wet forests, moist meadows, bogs, fens and other wetlands across

the boreal (Johnson et al. 1995); to thickets in the alpine, arctic, and tundra (Porsild 1951, Soper and Heimburger 1982, Robuck 1989, Scoggan 1989, Pojar and MacKinnon 1994, Hallsworth and Chinnappa 1997). *R. arcticus* has perfect bisexual flowers that are completely self-incompatible (Tammisola and Ryynänen 1970), but the species is also capable of asexual reproduction via rhizomes (Moss 1983) which produce dense aggregations of clonal patches (Ryynänen 1973, Tammisola 1988). The flowers of *R. arcticus* are entomophilous (insect pollinated) and are reported to be primarily pollinated by honeybees, bumblebees and to a lesser extent syrphid flies (Ryynänen 1973, Vool et al. 2003). When sufficiently pollinated, these flowers subsequently produce raspberry-like fruit with 15-30 drupelets (individual aggregate units) bound together by a centre receptacle (Ryynänen 1973). Most studies of the natural history and ecology of *R. arcticus* have taken place in Europe, possibly because the raspberries of *R. arcticus* are particularly valued in Northern Europe for their unique taste and aroma, in which they are cultivated commercially (Vool et al. 2009). While the Arctic Raspberry is known be wild harvested in Canada, there are very few studies of the determinants of fruit production of this species in North America.

Because *R. arcticus* is exclusively insect pollinated, it is very likely that microclimate has a pronounced effect on its pollinators. *R. arcticus* is found in a variety of different habitat types (i.e. arctic, alpine, tundra, wetlands and boreal forests) (Porsild 1951, Soper and Heimburger 1982, Robuck 1989, Scoggan 1989, Pojar and MacKinnon 1994, Johnson et al. 1995, Hallsworth and Chinnappa 1997) and this wide array of corresponding climatic conditions could influence the efficacy and availability of pollinators. On the other hand, *R. arcticus* is particularly shade and drought intolerant (Ryynänen 1973) often found growing near waterbodies and thus; temperature, irradiance, and humidity are all important variables for its growth and flowering

and may constrain the microclimate envelope. And as described above, these variables are also particularly important for pollinators, both directly and indirectly. Should microclimate significantly influence the pollination of *R. articus*, this may have cascading effects on pollen delivery and ultimately the productivity of fruit production (Vool et al. 2003).

The following thesis aimed to increase our knowledge of the determinants of fruit production in the Arctic Raspberry in Southern Alberta. In Chapter 2, I conducted the first examination of pollen limitation in this species. Fruit production/fruitset in *R. arcticus* may experience pollen limitation because the species is self-incompatible (Tammisola and Ryynänen 1970) with the dominant pollinators being bees (Ryynänen 1973). According to Larson and Barrett (2000), pollen limitation is more commonly found in self-incompatible species opposed to self-compatible ones. Further, because the species tends to grow in clumps of clones (Ryynänen 1973, Tammisola 1988) bees are likely to fly to nearest neighboring flowers (Zimmerman 1979), delivering selfed/incompatible (low quality) pollen. Consequently, pollen quality is more likely an issue to the species as opposed to deficiencies in pollen quantity. In Chapter 3, I investigated (1) the dominant pollinators of this species in the Rocky Mountains of Alberta and (2) how pollinator composition varies with microclimate. As summarized in Chapter 4 (Synthesis of Research), the highly clonal nature of this species is likely reducing the investment in fruit, even when substantial pollen is delivered. However, this species was observed to have extremely fragile stigmas that made the examination of pollen limitation difficult. While fruit production was low, the flowers still attracted a number of different pollinator guilds, some of which were observed to be sensitive to microclimate. The low fruit production and different pollinator composition observed in this study compared to others may be a reflection of the study sites being located near the southern range limit of this species.

Chapter 2: Pollen limitation in *Rubus arcticus*: the effects of pollen quality and quantity on fruit production

Introduction

Pollen limitation is one way in which we can measure how a lack of pollinators could negatively impact the reproductive capabilities of certain plant species. A common definition used to describe pollen limitation is a deficiency in pollen quality or quantity that limits the reproductive fitness of a plant (Ashman et al. 2004). More specifically, a plant may not have enough pollen deposited by pollinators individually or there may not be enough pollinators available collectively for sufficient delivery. Conversely, when pollen of a lesser quality is delivered, such as incompatible or selfed pollen, this can also result in pollen limitation. Wilcock and Neiland (2002) report other sources of pollen limitation such as pre-dispersal failure with pollen being lost to the environment or subject to herbivory, and dispersal failure where there is a lack appropriate of pollinators either through habitat fragmentation or poor environmental conditions.

The concept of pollen limitation originated from a model proposed by Haig and Westoby (1988) in which plants evolved traits (such as floral displays) that would optimize attractiveness to pollinators, increasing the amount pollen deposited as well as visits by pollinators. These traits would be selected for until investment in these costly traits reached equilibrium, such that female plant fitness would be limited equally by both the resources needed to produce ovules and the amount of pollen acquired. There is much debate as to how frequently pollen limitation occurs, with some studies indicating that pollen limitation is common in nature (Burd 1994, 2016) and others stating that it is highly variable (Rosenheim et al. 2014, 2016). Pollen limitation exhibits phylogenetic and geographical patterns (Vamosi et al 2006), and is positively correlated with

species richness, suggesting that areas of high biodiversity, such as the tropics, have more plant species prone to pollen limitation than other regions potentially due to higher levels of specialized plant-pollinator relationships. Few studies have examined how pollinator composition variability alters pollen limitation (Burd et al. 2009), but recent studies (e.g., Hegland and Totland 2008) suggest that pollen limitation does not give a simple metric of pollinator abundance (i.e., pollen limited species have often been observed to have high levels of visitation).

A standard experimental procedure of detecting pollen limitation in plants is pollen supplementation (Burd 1994). Pollen supplementation is performed by manually hand pollinating flowers with excess amounts of outcrossed pollen, with the assumption that any increase in fruit set or seed set compared to a naturally pollinated control group can be attributed to the artificial application of additional pollen; therefore implying that the plant is experiencing pollen limitation (Bierzychudek 1981). Ashman et al. (2004) suggest that, in comparison to naturally pollinated plants, supplemented plants will produce more seeds until they become pollen-saturated, reaching an asymptote with only resources limiting seed production beyond that point. There have been very few instances in which pollen supplementation resulted in a decrease in seeds and fruit, but this observation was attributed to experimental error from contamination or damaging flowers while supplementing them (Young and Young 1992a). Aizen and Harder (2007) caution the use of pollen supplementation experiments as they are incapable of further characterizing the cause of pollen limitation (i.e., determining whether pollen quality or quantity is the limiting factor).

The Arctic Raspberry (*Rubus arcticus* L.: Rosaceae) is a plant species that may experience pollen limitation based on a number of factors. Firstly, the polycarpellate flowers are

bisexual/perfect (Ryynänen 1973) yet they are also self-incompatible through a sporophytic-gametophytic system (Tammisola and Ryynänen 1970). Larson and Barrett (2000) found evidence that species that are self-incompatible are far more likely to be pollen limited than those that are autogamous and self-compatible. The potential negative fitness consequences of having flowers containing both female and male structures while being simultaneously self-sterile is further compounded by the configuration of these parts. Towards the centre of the flower, the anthers are heavily pressed together, bending down in middle, facing the stigmas (Figure 2.1). Saastamoinen (1930) originally proposed that the species was very likely to be self-compatible based on this floral arrangement, but this is not the case. Thus, the flowers have an inherent propensity of delivering low quality/selfed-pollen, and possibly hindering the production of viable fruit/seeds from high quality pollen through mechanisms like stigma or style clogging (Holland and Chamberlain 2007). Secondly, this species also reproduces asexually/vegetatively through rhizomes (Moss 1983), often forming dense patches of clones which can apparently reach up to 80m in diameter and grow as long as 160 years (Tammisola 1988). Furthermore, because bumblebees have been observed to be an important pollinator for the species (Ryynänen 1973) and have a tendency to alight to the nearest neighboring flower on foraging bouts (Zimmerman 1979), there is a high probability of selfed-pollen being excessively distributed amongst large patches of clonal/closely related *R. arcticus* individuals and fruit production will be thus inhibited by incompatible pollen. Since *R. arcticus* is an obligate outcrosser, fruit production appears to be most optimal in artificial/cultivated environments where separate genets can be planted in close proximity to each other, allowing for optimal outcrossing based on the foraging behavior of bees (Vool et al. 2003). Although bumble bees are a key pollinator of the species, *R. arcticus* is still thought to be pollinator limited as bumble bees have been observed to

actively select other flower species such a white clover even amongst large patches of *R. arcticus* (Kangasjarvi and Oksanen 1989). *R. arcticus* is pollinated to a lesser extent by syrphid flies, but they are lower quality pollinators compared to bees as they are not heavy enough to successfully deposit pollen past the densely crowded anthers (Vool et al. 2003). And with the majority of pollen being exhausted within the first few visits (Thomson 1986), *R. arcticus* flowers may lack adequate amounts of conspecific pollen as well have their stigmas crowded with selfed-pollen grains in addition to heterospecific pollen and thus will be unlikely to produce much fruit.

The reason why the effects of pollen limitation on fruit set are being more closely considered in this experiment rather than seed set is because of the importance of the fleshy fruit for this species. Many plant species have evolved dispersal mechanisms via vertebrates where the plants produce nutritious fleshy fruits that are ingested by animals (mostly birds and mammals), which then carry the seeds away from the parent plant, and eject them in distant areas where they can germinate and establish new populations (Herrera and Pellmyr 2002). Animal dispersal is likely to be important for *R. arcticus* considering that the fruits have been observed to be quickly consumed upon ripening by small mammals as well as birds (Ryynänen 1973, Young and Young 1992b). And since larger fruits generally have a higher preference from frugivores in contrast to smaller fruits (Jordano 1995), fresh fruit mass along with the number of drupelets per raspberry is likely to be very important for *R. arcticus* dispersal. Thus, understanding how pollen limitation influences these elements of fruit production is relevant. Besides, several pollen limitation studies focus on seed set as a metric of limitation; whether it is the collective number of seeds per treatment group, total number of seeds per fruit or seed mass (Agren 1989, Johnston 1991, Young and Young 1992a, Burd 1994, Ehlren et al. 2002). Many studies do incorporate fruit set, but this is mostly in the context of the total number of fruits

(Young and Young 1992a, Burd 1994, Abe 2001, Saez et al. 2014). Fewer experiments have incorporated fruit mass in regards to pollen supplementation, such as Sletvold et al. (2010). So examining how pollen limitation influences fruit mass can possibly add to a smaller body of literature.

Studying the role of pollen limitation on fruit production in this species is important for multiple reasons and among one of those is the significance of *R. arcticus* within human society. For instance, *R. arcticus* (especially R. *arcticus arcticus*) is a valuable cultivar in Northern Europe, particularly for the fine dining dessert and liqueur industry (Vool et al. 2009), being cultivated as early as 1762 by Linnaeus (Kostamo et al. 2015) and commercial cultivation commencing in the 1970s (Ryynänen 1972). Aside from having economic importance, *R. arcticus* is also culturally and historically significant as the berries supplement the traditional food crops of Canadian Aboriginal peoples such as the Inuit (Clark 2012) and Woodland Cree (Leighton 1985) while the leaves were used as an anti-diarrheal drug by the Shuswap (Palmer 1975). Although *R. arcticus* has a global IUCN red list status of "least concern" (Maize-Tome 2016), listed as "globally secure" by Natureserve (2017) and widespread throughout Canada (Brouillet et al. 2010), the species has been ranked as critically endangered since 2008 in Estonia (Vool et al. 2011). In Lithuania and Latvia, *R. arcticus* has since become extinct (Ingelög et al. 1993, Kukk and Kull 2005). Furthermore, the range of *R. arcticus* has been shrinking rapidly in Northern Europe due to the increased agriculture and forestry (Vool et al. 2011), a problem that could occur in Canada where such industries are also prevalent.

Based on the aforementioned traits, I thus sought to test the hypothesis that *R. arcticus* fruit set is pollen limited. Furthermore, I posit that pollen quality is more limiting to fruit production than quantity of pollen received. The flowers in nature likely receive an abundance of

low-quality selfed pollen or heterospecific pollen due to the foraging behavior of bumblebees and the life history trait of growing vegetatively in wide encompassing dense clusters of clonal individuals, but do not receive enough xenogamous pollen from individuals outside the same incompatibility class (Tammisola 1988). Consequently, I predict that flowers that are supplemented with an abundance of outcrossed (high quality) pollen will produce on average more drupelets (individual aggregate fruit units) per berry and also collectively heavier berries than naturally pollinated individuals would. Moreover, I predict that flowers that are supplemented only with selfed pollen will produce either very small quantities of fruit, with small stunted drupelets or no berries at all. Finally, I attempted to use pollen staining procedures to detect whether changes in pollen quality or quantity was associated with pollen limitation.

Methods

Study Site and Species

The study site was enclosed within a 625m^2 sampling grid (Figure A2), located in the north loop of the Jumpingpound Demonstration Forest (51.04794°N, -114.79494°W; NE-17-24-6-W5M; 1406m elevation), near Kananaskis, Alberta (Figure B1). This site was selected based on the location of a previously collected herbarium specimen (Wallis 1971), ease of access, and close proximity to the Barrier Lake Research Station. I refer to the subsite used for this component as the "Manipulated Treatment Site" to distinguish it from the subsite used for the project described in Chapter 3, which I refer to as the "Natural Undisturbed Site" (see Figure B2), similar to the experimental design of Reid (2011). Both sites were very similar in composition and stand type (Figure A1), but the "Manipulated Treatment Site" was more heterogeneous regarding shade cover and the soil moisture was greater with the east side transitioning into a treed fen. The presence of the fen and reduced shade cover was the reason

why this particular subsite was selected for this part of the research and not the "Natural Undisturbed Site" used in Chapter 3. Damp substrates are very beneficial for increased berry yields while the presence of the sphagnum moss within the site should increase the humidity and improve fertilization of flowers (Ryynänen 1973). Additionally, *R. arcticus* is generally quite shade intolerant so having less canopy cover would also promote successful fruit production.

Although I aimed to study *R. arcticus* at the species level, two subspecies were present within the "Manipulated Treatment Site". The three subspecies within *R. arcticus* are: *R. arcticus arcticus* Linnaeus., *R. arcticus stellatus* (J.E. Smith) B. Boivin and *R. arcticus acaulis* (Michaux) Focke. All subspecies grow in Canada, but *R. arcticus acaulis* is the most widely distributed (Brouillet et al. 2010). In Alberta, the dominant subspecies is *R. arcticus acaulis,* which commonly grows in wet forests, moist meadows, bogs, fens and other types of wetlands across the boreal and northern portions of the province (Johnson et al. 1995). Because the genetics of the different subspecies have not received adequate study, only *R. arcticus arcticus* specimens were used. Typically plant subspecies are parapatric, in which they are both geographically and reproductively isolated, but sometimes the more common subspecies range may expand to a less common/relict subspecies range becoming sympatric, in which increased gene flow and hybridization can occur (Ellstrand 1992). So the fact that these two subspecies were found together in such a small region is not completely unusual. As well, hybridization has only been reported to occur between *R. arcticus arcticus* and *R. arcticus stellatus* (Ryynänen 1973, Vool et al. 2009) and not the former sub-species pairing, so the confounding presence of hybrid is not as relevant. *R. arcticus arcticus* was mostly selected because it is the most widely distributed globally (Figure 2.2) and has received the most study, whereas some traits and characteristics for *R. arcticus acaulis* are often reconstructed by extrapolating information from *R. arcticus*

arcticus. For instance, Ladyman (2006) states that the incompatibility of *R. arcticus acaulis* is assumed from European studies of *R. arcticus arcticus* (Tammisola and Ryynänen 1970, Tammisola 1988). These two subspecies are very similar superficially (Figure 2.3), but the identification of the two sets of subspecies found within the "Manipulated Treatment Site" was confirmed at the University of Calgary Herbarium (B. Smith, *pers. comm.*, 2017). Voucher specimens for *R. arcticus arcticus* and *R. arcticus acaulis* were deposited into the University of Calgary's herbarium, with the following accession numbers respectively: UAC #94278 and UAC #94279.

Part A: Pollen Supplementation Experiment

To assess the effects of pollen limitation on *R. arcticus* fruit production, a pollen supplementation experiment was used to compare the difference in fruit set between naturally pollinated control flowers and flowers supplemented with outcrossed pollen (Bierzychudek 1981). Similar to Colling et al. (2004), an additional treatment group of bagged flowers supplemented with selfed pollen was included in the experiment to affirm the importance of pollen quality, even though the species is supposed to be completely self-incompatible. Because the rhizome networks are extremely difficult to distinguish from one another, this experiment was performed at the individual stem level assuming each ramet was independent concerning fruit development.

The pollen supplementation experiment commenced June 1, 2017 in which replicates and treatments were assigned to stems within the "Manipulated Treatment Site". Grid cells containing *R. arcticus arcticus* stems that possessed emerging floral buds were randomly selected from within the sampling grid and then assigned (with a random number generator) to one of the three treatments: control, pure-selfed, outcross supplemented. Each grid cell could

contain multiple stems from the same or separate treatments, depending on the random treatment assignment and the sequence in which stems produced floral buds. All control flowers were left undisturbed and open for natural pollination only. Pure-self treatments were performed by bagging unopened virginal flowers, supplementing them with their own pollen upon opening, and leaving them bagged indefinitely until fruit collection. Moreover, flowers were covered with bridal veil in which the mesh completely covered the whole stem and was held in place with rubber bands (Figure 2.4). After the flowers had fully opened and anthers had begun dehiscing, the pure-selfed flowers were supplemented with their own pollen by having the anthers gently pushed inward toward the stigmas with a fresh unused Q-tip. Outcrossed supplemented flowers received xenogamous pollen from at least two separate donor flowers (Fisogni et al. 2016) and hand pollination was performed by gently rubbing open dehiscing anthers of collected flowers directly on the receptive stigmas of supplemented flowers (Tammisola and Ryynänen 1970, Marshall and Ellstrand 1985, Sobrevila 1989). However, the distance at which the pollen donors were collected from was controlled for. Rhizomes and clonal networks of *R. arcticus arcticus* have been observed to spread several meters with rhizomes growing 0.25 m/year (Ryynänen 1973). Therefore, considering that *R. arcticus arcticus* can be a long living perennial with potentially fast spreading rhizomes and cross pollination may not be possible among clones as a patch may only contain 1-2 incompatibility genotypes (Tammisola 1988), the collected flowers for hand pollination were at least >10m away from the supplemented individuals. Additionally, pollen outcrossed from too far away can result in a phenomenon known as outbreeding depression, which is when mating individuals are too geographically/genetically isolated from one another which subsequently causes a decrease in fitness (Waser 1993). To control for outbreeding depression with spatial variation, the outcross supplemented group was divided into

two sub-treatments: one group had donors 10-20m away and the other had donors 20-30m away. However, these two sub-treatment groups were later collapsed to one single treatment of "outcross supplemented" due to lack of fruit produced between the two (see results section).

There were 30 replicates/stems for each of the three treatments and, because *R. arcticus arcticus* can produce up to three flowers per stem (Ryynänen 1973), all flowers per each stem received the respective treatment. Any replicate stem whose flowers had completely desiccated (Figure 2.5) prior to fruit production, was replaced by another replicate stem with a live flower and the treatment was repeated. To prevent herbivory from small rodents and birds (Young and Young 1992b), all control and outcross supplemented flowers were also covered with bridal veil bags following the senescence of flowers whereas pure-selfed flowers remained in their bags after being supplemented with self-pollen.

Fruit Harvesting and Collection

As recommended by Young and Young (1992b), all stems remained protected in their bags until the fruits were just ripe enough for collection (Figure 2.6). Bagging and early collection appeared to effectively circumvent herbivory, as no berries were lost. The berries were collected in late July to early August, with the last set of berries being collected on August 11, 2017. Outcross supplemented berries were harvested 6-8 weeks subsequent to the treatment of the first flower. All harvested berries were left with receptacles attached as they are connected to the ovaries and removing them will result in breaking the berries (Ryynänen 1973). The number of drupelets per berry was counted in the field and a portable food scale was used to measure the fresh mass of harvested berries, which included the receptacle.

Part B: Pollen Quantity Analysis

The initial goal of this experiment was to use pollen staining techniques to determine how pollen quality affects *R. arcticus* fruit production. The pollen staining techniques available (detailed in Appendix D) were not sufficient to visualize pollen tube length in *R. arcticus*. However, the pollen staining was sufficient to examine the effects of pollen supplementation and compare whether selfed and outcrossed supplemented flowers had in fact received more pollen than the control treatments, as well as inspect for other experimental errors such as stigma damage.

Within the "Manipulated Treatment Site", separate undisturbed and unopened flowers were divided into three groups: the open control group with flowers left for natural pollination, a bagged group with flowers being treated with selfed pollen, and a pure-outcrossed group in which flowers were emasculated and treated with xenogamous pollen from at least two donors 10-30m away (Chacoff et al. 2008); with each group consisting of at least 10 individual stems. Selfed and outcrossed flowers remained covered with bridal veil bags until collection, barring brief removal to apply treatments upon opening, whereas control flowers had bags removed immediately following opening. After receiving the respective treatment, all flowers were left undisturbed for at least 72 hours and then placed in microtubes filled ethanol-acetic acid (3:1 v/v) for 24 hours to fix floral tissues (Levin 1990, Kearns and Inouye 1993).

Forty-two flowers were collected in total for the pollen tube analysis experiment, but only six test flowers were used in the staining trials for said experiment, leaving the remaining amount to be used in this pollen quantity analysis experiment; 10 controls, 13 selfed and 13 outcrossed flowers.

Similar to Saez et al (2014), 10 pistils from each flower were randomly selected for staining and pollen grain counts, making a total of 360 pistils. These pistils were then stained in a few drops of 1% basic fuchsin (Snow 1982), rinsed in distilled water, and then mounted in 50% glycerin (Thomson et al. 1989) on glass slides with coverslips to be viewed under a conventional compound microscope. Both stigmas and all pollen grains (including heterospecific pollen) stained a pinkish to red hue, but *R. arcticus* grains appeared as bright pink (Figure 2.7 and Figure 2.8A). In addition to the brighter hue of pink, *R. arcticus* grains could also be identified by their tricolporate structure (Figure D1.A), which is a characteristic shared among members of the genus (Hebda and Chinnappa 1990). Since most of the stigmas resembled Figure 2.7.B+C whereas a small minority resembled Figure 2.7.D+E, *R. arcticus* grains could easily be counted under 400X magnification via simple visual inspection and fine focus adjustments rather than with assisted optics (Feinsinger et al. 1986) or particle counters (Wilson and Thomson 1991). All stigmas were examined for breakage and classified into a binary system where stigmas were either fully damaged with the lobe almost entirely removed (Figure 2.7A) or not fully damaged with the majority of the lobe left intact. Only the conspecific grains were counted on stigmas, as heterospecific grains (Figure 2.8) were essentially only found on control flowers and their abundance was negligible.

Data Analysis

For Part A , I used the statistical software R Version 3.4.3 (R Core Team 2017) to determine the difference between mean number of drupelet per berry and mean fresh berry mass was determined for control and outcross supplemented stems (pure selfed stems yielded no fruit) with a two tailed T test. The default *t.test* function was used assuming unequal variance for the analysis.

For Part B, I used a general linear mixed-effects model (GLMM) to assess if there were significant differences in pollen grain counts as well stigma breakage between the pollination treatment groups. Like Quesada et al. (1995), the formula for this model had the response variable as pollen grain count, treatment group as a fixed effect and the flower/plant as a random effect. Additionally, stigma breakage was also included as a fixed factor, similar to Saez et al. (2014), and was included as an interaction term with treatment type. Pollen grain count was square root transformed, assuming a Gaussian error distribution. Several researchers advocate that square root transformations are the most effective in normalizing count data (Sokal and Rohlf 1995, Zar 1999, Crawley 2003, Maindonald and Braun 2007). O'Hara and Kotze (2010) support that log-transformations should absolutely be avoided with count data and instead Poisson or negative binomial models should be used, yet they also found that square-root transformations were far more suitable and created substantially less bias than any log transformation. Each flower in each treatment was given a unique identifier (e.g. 1C for control flower #1, 1S for selfed flower #1, 1X for outcrossed #1, and etc.) so the model would recognize 'Flower' as a factor variable rather than an integer, analyzing each stigma from each flower separately. As with Suarez-Gonzalez and Good (2014), post-hoc Tukey pairwise comparisons were made to assess differences between treatments and was done using the 'glht' function in the 'multcomp' package (Hothorn et al. 2008). This model was constructed using the 'lme' function in the 'nlme' package (Pinheiro et al. 2017) in R Version 3.4.3 (R Core Team 2017).

Results

Part A: Pollen Supplementation Experiment

A total of 161 flowers were included among the 90 stems across the three treatments (Table 2.1). However, the pollen supplementation experiment only yielded 21 berries in total: 14

from the control replicates, zero from the pure-self replicates and seven from the outcross supplemented replicates. Despite some stems producing up to three flowers, all fruit produced was the result of a single flower per stem and the other remaining flowers completely desiccated. Between the control and outcross supplemented groups, there was little variation in the number of drupelets per berry (Figure 2.9) with the most common number of drupelets produced being two. Only two berries produced the maximum amount of seven drupelets and four berries yielded the minimum amount of one drupelet. There was no significant difference between the mean number of drupelets per berry for controls (2.64 drupelets) and outcross supplemented replicates (2.71 drupelets)(Two Tailed T-Test, $t = -0.0739$, $df = 11.315$, $p = 0.9424$). Regarding mean fresh berry mass, there was also little variation between the control and outcross supplemented groups (Figure 2.10). There was also no significant difference between the mean fresh berry mass for controls (0.179g) and outcross supplemented replicates, (0.171g) (Two Tailed T-Test, $t = 0.15133$, $df = 9.233$, $p = 0.883$).

Part B: Pollen Quantity Analysis

Control flowers appeared to have the highest quantities of conspecific pollen grains on stigmas, whereas flowers in the selfed and outcross had significantly less pollen present (Figure 2.11). Concerning stigma breakage, an opposite pattern manifested with the control and selfed treatments having fewer occurrences of stigma breakage. The amount of stigma breakage in the outcross group was significantly higher by more than double, and also exhibited a high degree of variation. For instance, some flowers in the outcross treatment would either have all 10 stigmas damaged, more than half of them damaged or none at all; whereas the highest number of broken stigmas in the control and outcross treatment was four per flower and the majority having only one or no broken stigmas at all. The GLMM analysis (Table 2.2) supports these observed

patterns, revealing that the outcross treatment and stigma breakage interaction with the outcross treatment have significant negative effects on pollen grain counts. The post-hoc Tukey pairwise comparisons (Table 2.3) can further confirm that there is a significant difference in pollen grain counts between the control and outcross treatment groups. However, there was no significant difference between the control and selfed groups or the selfed and outcrossed groups.

Discussion

The results of the pollen supplementation experiment indicate that fruit set of *R. arcticus* is not pollen limited. Because there was no significant difference between the control and outcrossed supplemented treatments for both mean number of drupelets per berry and mean fresh berry mass, these findings suggest that the species does not experience deficiencies in pollen delivery. This finding is further supported by the pollen quantity experiment which revealed that control stigmas often had an abundance of conspecific grains present, with an average of ~33grains/stigma (Figure. 1.11) and some stigmas containing as much as 100-200 pollen grains (Figure 2.7D+E). Therefore, taken together these results suggest that naturally pollinated flowers of *R. arcticus* experience sufficient pollen delivery. Because not a single replicate of the selfed treatment produced a single fruit, it suggests that pollen quality in naturally pollinated flowers was sufficient to produce the equivalent (albeit low) fruit production as that of outcrossed flowers. Hypothetically, if more fruits were produced in this experiment offering greater statistical power, my prediction that the outcross supplemented flowers would produce more drupelets per berry and heavier fruits may still occur; but not due to an excessive quantity of pollen deposited, but rather a greater proportion of high quality being delivered. Nevertheless, there are several other confounding factors that make it difficult to determine if this species is limited by the quantity or quality of pollen received.

Bearing in mind that 87% of the flowers used in this experiment had completely desiccated, it is possible that conditions were simply not favourable for fruit production. For instance, Kotilainen (1949) suggests that most wild *R. arcticus* flowers never reach fruiting due to excessive insolation during the peak of summer. This claim is in accordance with Saastamoinen (1930), who found lower fruit yields when conditions were outside optimal regions for *R. arcticus*; an average summer temperature of 12°C (across seven month growing season) and a maximum annual temperature of 24°C. Although located 23km away from the "Manipulated Treatment Site" (Figure B1), the Barrier Lake Research Station's climate data (F. Lodhawalla, *pers. comm*., 2017) is monitored daily for erroneous readings and can serve as an approximate reference for the summer conditions within the Jumpingpound region. The highest summer temperature reading occurred in July at 32.66°C whereas the average daily temperatures for June and July, the flowering season of *R. arcticus* in North America (Ladyman 2006), were 13.40°C and 16.38°C respectively (F. Lodhawalla, *pers. comm*., 2017). Consequently, it is reasonable to assume that the heat of July desiccated most of the flowers in the treatments, resulting in the small berry yield and thus lowering the power of the tests to detect if the species is pollen limited.

Aside from excessive heat and less ideal micrometeorological growing conditions, there are numerous other mechanisms that could have potentially caused the reduced berry yield in this pollen supplementation experiment. Although these are proposed in the circumstances of supplemented flowers producing lower seed set than controls, Young and Young (1992a) cite several instances of error in their meta-analysis that could also be applicable in this experiment. For example, Kwak and Jennersten (1986, 1991) suggest that hand pollination may actually only deposit a small quantity of pollen and subsequently reduce the number seeds produced. Despite

several anthers being used and applied repeatedly over all stigmas in the outcrossed flowers, this experimental error very likely occurred in this experiment. Hand pollination in the outcross treatment was significantly less effective in depositing pollen than that of natural pollination from insects in the control treatment, as reflected by the results of the pollen quantity analysis. This error could have been the result of hand pollination occurring outside the peak period of stigma receptivity (Young and Young 1992a). Such an outcome is very likely since the anthers in *R. arcticus arcticus* most readily produce viable pollen in the earlier stages of flowering when the petals are still folded and the flower is just about to open (Ryynänen 1973). Many of the donor flowers used had likely opened too much and thus the pollen is far less abundant in the anthers and is mostly deteriorated. Therefore, most of the few pollen grains deposited during the hand pollination were likely inviable, further compounding the lack of fruit production in the outcross supplemented treatment group. A protocol to correct for this matter would have been to hand pollinate for multiple consecutive days like Fisogni et al. (2016) who used two separate donors for two consecutive days. Hand pollination for this experiment was conducted with two donors immediately after one another, during one day only. Alternatively, the sampling regime of the pistils in the pollen quantity analysis could have skewed the results. Ryynänen (1973) found that pollen deposition was greatest on the middlemost stigmas. Therefore, it is possible that in some of the sample flowers, the majority of stigmas selected could have been outermost ones with less pollen present.

Conversely, the selfed and outcrossed flowers could have experienced stigma breakage due to excessive force from anthers being pressed against the stigmas during hand pollination (Young and Young 1992a). However, stigma breakage had a significant negative effect for pollen quantity within the outcross treatment only, so this suggestion is not as applicable for the

selfed treatment group, and the Q-tip method appeared to be less damaging in applying selfed pollen compared to pressing whole anthers down on stigmas. Secondly, because the outcross-supplemented treatment group was not purely outcrossed/bagged and rather also left for open pollination like the control group (Chacoff et al. 2008), it is possible pollinators could have been attracted to the unusually high amounts of pollen on the stigmas, resulting in indirect removal of the supplemented pollen (Young and Young 1992) or damage from pollen thieves (Mcdade and Kinsman 1980, Grant and Grant 1981).

But since these flowers were repurposed from the former pollen tube analysis and the outcrossed flowers were emasculated + bagged to contrast the effects of pollen quality on pollen tube growth, they do not entirely represent what may have been occurring for the outcrossed flowers in the main pollen supplementation experiment. More specifically, the outcrossed flowers in the pollen tube analysis were purely-outcrossed receiving only xenogamous pollen whereas the outcrossed flowers in the pollen supplementation experiment were outcrossed supplemented receiving xenogamous pollen and any additional pollen delivered by insect visitors. Therefore, outcross supplemented flowers could have not only received both selfed and outcrossed conspecific pollen, but also heterospecific pollen. Nevertheless, the purely-outcrossed flowers can still serve as references for the effectiveness of the hand pollinating and any stigma damage that may have occurred in the pollen supplementation experiment.

Wesselingh (2007) recommends that experimenters check flowers for tiny accidental pollinators, such as thrips, that can pass through even the smallest of mesh sizes. Additionally, Baker and Cruden (1991) support that including treatment groups with bagging/caging in addition to insecticide application is necessary to control for unintended pollination from such small insects. These suggestions have relevance because thrips were found during dissection of

flowers used in the pollen quantity analysis experiment (Figure 2.12), with up to three individuals per flower. The thrips could have interfered with the treatments by delivering excessive heterospecific pollen to the bagged pure-selfed and pure-outcrossed groups, depositing unwanted xenogamous pollen on the pure-selfed flowers, or moving incompatible pollen from clones within the same incompatibility class (Tammisola 1988) to the pure-outcrossed treatments. Regarding heterospecific pollen delivery, pollen grains from at least four heterospecific species were found on some of the control stigmas (Figure 2.8), but their abundance was negligible compared to that of the conspecific pollen. Furthermore, the pure-selfed and pure outcrossed stigmas very rarely had any heterospecific pollen grains present and if so, there at most would be two grains from a separate single species. Ryynänen (1973) and Tammisola (1988) posit that thrips do not actively pollinate *R. arcticus* flowers, but are rather substantial pests that can supposedly reduce berry yields significantly in nature. The thrips achieve this by living in the flowers, drinking fluids of the various essential floral organs and subsequently kill the whole flower. So it is more likely that the thrips presence within flowers was detrimental in terms of directly reducing the berry yield via parasitism rather than altering the pollination treatments by depositing unwanted types of pollen.

The cause of the small berry yield may not have been the result of less ideal weather, experimental error, thrips infestations or even pollen limitation, but rather predominant selection by the species for asexual reproduction (Knight et al 2005). A total of 2305 vegetative *R. arcticus* stems were found within the $625m^2$ sampling grid of the "Natural Undisturbed Site" in Chapter 3. Additionally, the maximum daily floral density for the entire subsite was 128 flowers, and only 11 berries (each from separate individual stems) were later produced from these. Therefore, the highest ratio of flowering stems to vegetative stems at this subsite was 0.056

whereas the ratio of fruiting stems to vegetative stems was 0.004. Based on this finding, it would appear that this population of *R. arcticus* at this location does not invest nearly as much into sexual reproduction with flowers and fruit as it does for asexual reproduction with rhizomes and cloning. This assumption is supported by numerous others who also found that *R. arcticus* in North America was locally abundant with vegetative stems numbering in the thousands, whereas flowering and fruiting individuals were substantially less frequent or incredibly rare (Spackman et al. 1997, Fertig 2000, Ladyman 2006). European studies also corroborate that the species exhibits sporadic fruiting in the wild (Saastomoinen 1930, Ryynänen 1973). Ladyman (2006) theorizes that *R. arcticus* populations experiencing a lack of fruiting/flowering individuals may be due to the fact that they are triploid. However, Tammisola (1988) found the majority of *R. arcticus* populations in Finland to be diploid, finding only one triploid population. Information on the distribution of cytotypes and ploidy for *R. arcticus* in North America is extremely restricted, with only one finding of a *R. arcticus acaulis* population from Manitoba, Canada being diploid (Löve 1987).

Increased resource allocation to asexual reproduction and cloning can evolve under conditions of environmental stochasticity (Menges 1991) by maintaining population stability during periods of reduced pollinator availability (Kingsolver 1986). To account for resource allocation in pollen limitation studies, Knight et al. (2006) advises that pollen limitation experiments should be performed across multiple years (unlike this experiment which was for only one growing season) for polycarpic species like *R. arcticus*. If a plant receives an excess of pollen in a given year, it may invest heavily in reproduction via seed for that year, but in future years allocate more energy towards storage or growth (Primack and Hall 1990, Calvo 1993, Ehrlen and Eriksson 1995, Primack and Stacy 1998). This is supported by Rosenheim et al.

(2014, 2016) suggestion that pollen limitation is very uncommon in parent optimizing species (plant species that produce far more embryos and seeds than the number of seedlings that actually will reach maturation) and lack of consideration for resource allocation results in exaggeration of pollen limitation. Rosenheim et al. (2014) found that *R. chamaemorus* had pre-pollination costs much higher than the post-pollination costs, suggesting a parental optimism strategy, contradictory of Agren (1989) findings that the species is pollen limited. Furthermore, the expected increase with pollen supplementation in *R. chamaemorus* was much less compared to the other 79 species tested in the meta-analysis. Thus, Rosenheim et al. (2014) would probably argue that *R. arcticus*, like its relative, would have a parental optimism strategy, as it produces far more seeds than necessary to reach maturation and likely would not be pollen limited with pre-pollination costs being less than post-pollination costs. Additionally, Rosenheim et al. (2016) would likely support that *R. arcticus* may not invest heavily into fruit production as the pre-pollination costs are very low and the species does not require substantial resources to produce ovules and flowers. On the other hand, post-pollination costs are likely to be very high for *R. arcticus* as the germination is very low in nature ($\leq$ 40%, Ryynänen 1973) and seedlings are highly susceptible to competition Saastomoinen (1930). Therefore, pollen supplementation may shift the reproduction future-present trade-off in this species even more towards the future as pre-pollination cost may already be quite low. However, to confirm this quandary, future studies such as this one must be conducted across multiple growing seasons rather than just one.

Conversely, Bliss (1971) suggests that sexual reproduction becomes less important during periods of environmental stress and selection of asexual over sexual reproduction is common in harsh regions such as the arctic, tundra and alpine. For instance, Marchand and Roach (1980) found that despite being capable of producing viable seeds, the rapid colonization

of *Potentilla tridentata* (a very distant relative of *R. arcticus* within Rosaceae) in the alpine can be attributed to vegetative reproduction with extensive rhizome networks whereas seed germination occurred only within the highest temperature ranges. The *R. arcticus* population at the study site was located within the montane subregion of Alberta. Thus, it is plausible that this population infrequently exhibits sexual reproduction, doing so only when conditions are the most favorable. On the other hand, being a cold adapted species which have been found to have their southernmost ranges in mid-latitude mountain ranges (Abeli 2008), this population of *R. arcticus acticus* is very likely to be within its southernmost range in North America (USDA 2018) and could be so genetically depauperate, that it rarely employs sexual reproduction.

Conclusion

Although the statistical analyses of the pollen supplementation experiment suggests that the fruit set of *R. arcticus* is not pollen limited, the low fruit yield and weak statistical power limit the confidence with which we can interpret this finding. Further, the findings of the pollen quantity analysis lend evidence to the idea that the species may not necessarily be quantity limited as an abundance of conspecific grains were found on naturally pollinated flowers. Moreover, there are several other confounding factors that make it difficult to conclude if this species fruit production is pollen limited. Such factors may include: poor growing conditions and less ideal weather, mechanical damage and insufficient delivery during hand pollination, thrips parasitism, resource allocation and predominant selection for asexual reproduction. Consequently, I would recommend that field experiments should be accompanied by greenhouse/lab trials in which: growing conditions can be more directly controlled to ensure successful fruit production, herbivory and parasitism can be prevented, cytotypes and ploidy can be kept constant, genets can be grown separately to examine pollen limitation at the whole plant

level and the effects of interconnected clonal networks on pollen limitation can be tested. Extra care should also be taken during hand pollination as *R. arcticus* stigmas are especially frail and prone to breaking from excessive force. The phenology of the donor flowers should also receive special attention, only using flowers that have folded petals and not yet fully open to ensure abundant quantities of viable pollen may be administered. Lastly, pollen limitation experiments involving *R. arcticus* should be performed across multiple years to account for resource allocation as well as seasonal changes in growth periods.

Tables

Table 2.1 The respective quantities of flowers and fruit produced in each treatment of the pollen supplementation experiment.

	Control	Selfed	Outcrossed
Flowers	50	58	53
Fruit	14	0	7

Table 2.2 The summary output of the GLMM used for the pollen quantity analysis experiment. * Denotes significance within a confidence interval of 95% , *** denotes significance within a confidence interval of 99.9% and **** denotes significance within a + 99.99% confidence interval

Fixed Effects	**Estimate**	**Standard Error**	**t-value**	**p-value**
TreatmentOutcross	-2.135171	0.9865069	-2.16438	0.0378*
TreatmentSelf	-0.699209	0.9750028	-0.71714	0.4783
StigmaBreakage	-2.388181	0.5450538	-4.38155	< 0.0001****
TreatmentOutcross:StigmaBreakage	2.74404	0.7254854	3.78235	0.0002***
TreatmentSelf:StigmaBreakage	-0.109838	0.6999138	-0.15693	0.8754

Table 2.3 The summary output from the post-hoc multiple comparison test on the treatment factor within the GLMM model used for the pollen quantity analysis experiment.* Denotes significance within a confidence interval of 95%.

Comparison	Estimate	Standard Error	z-value	p-value
Outcross - Control	-2.1352	0.9865	-2.164	0.0304*
Self - Control	-0.6992	0.975	-0.717	0.4733
Self - Outcross	1.436	0.9218	1.558	0.1193

Figures

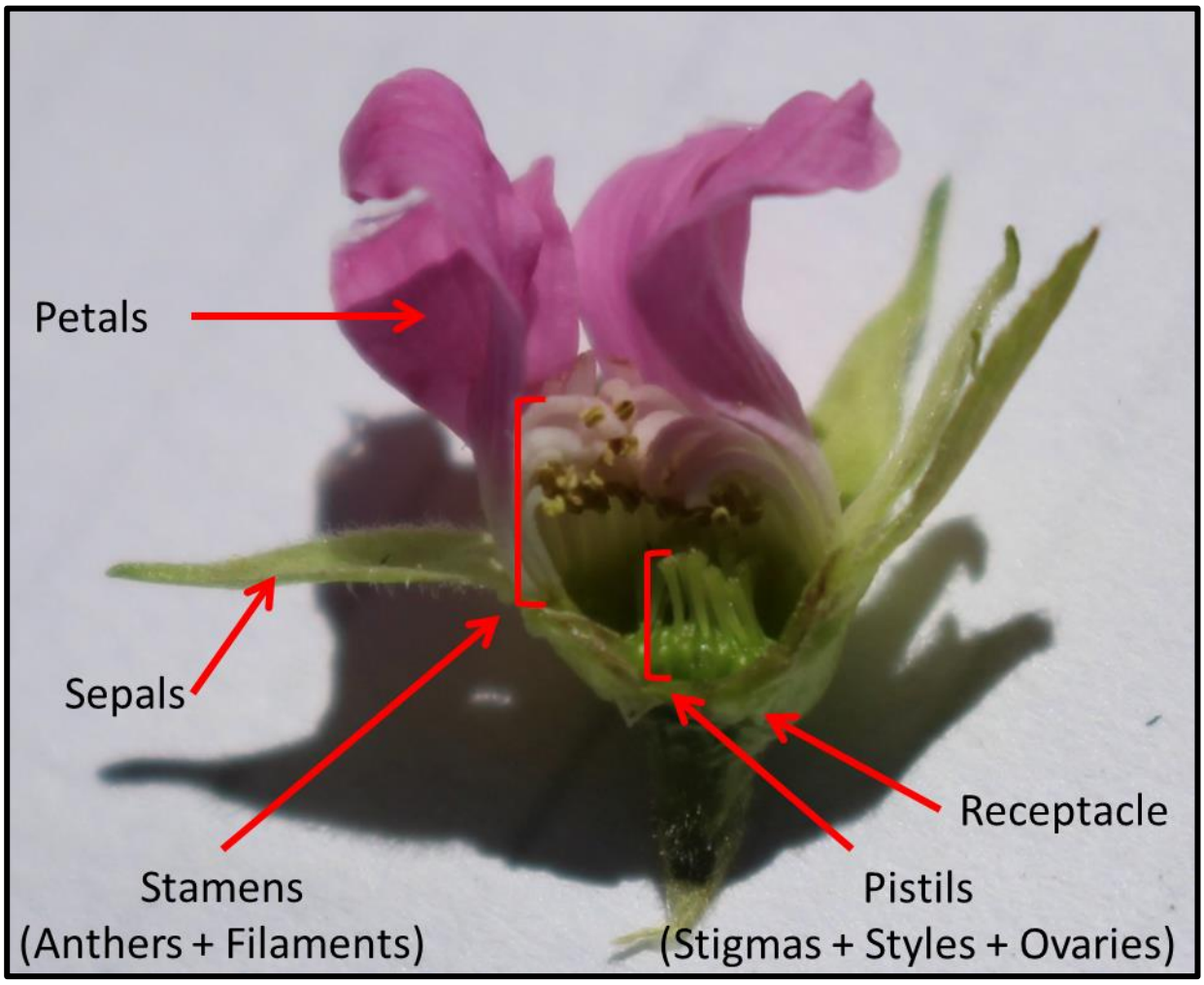


Figure 2.1 A *Rubus arcticus* flower that was sliced in half longitudinally, the red arrows and labels denote the respective floral structures.

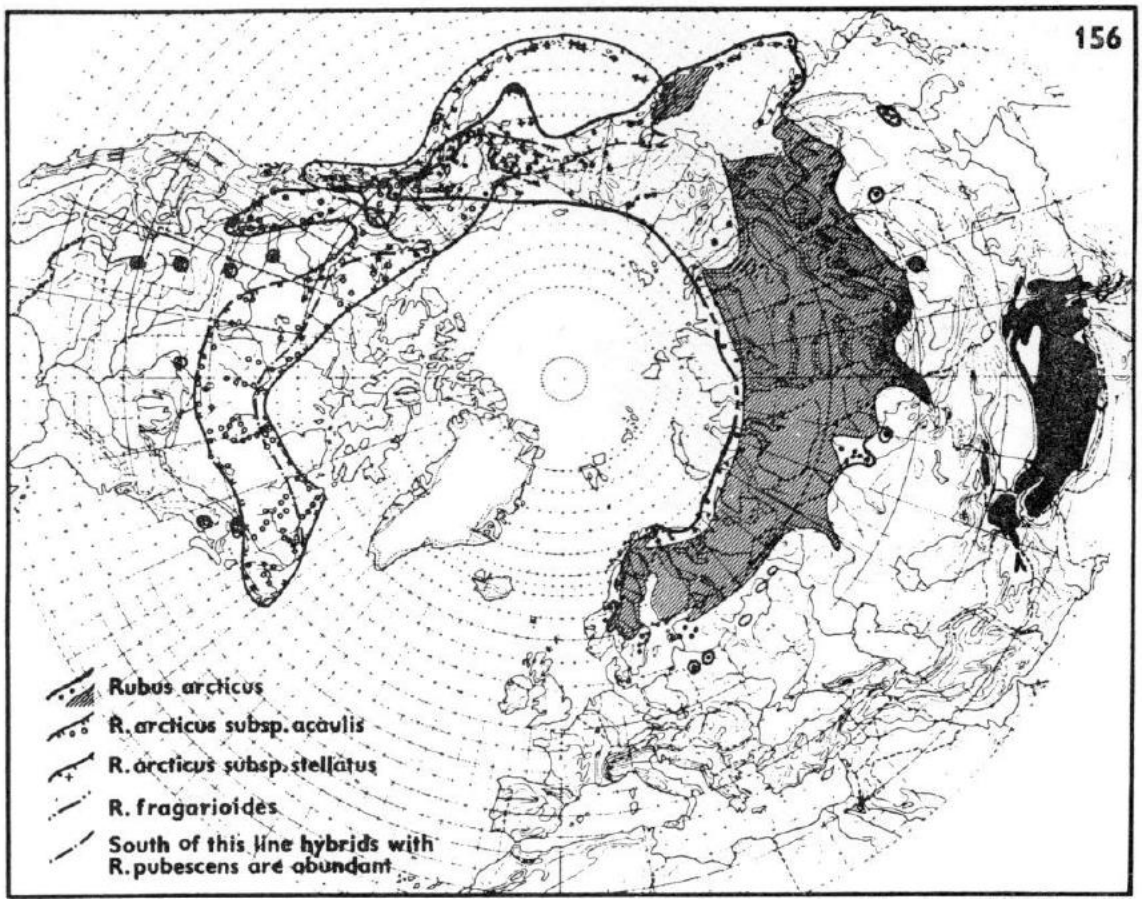


Figure 2.2 A map from Hulten (1971) displaying the global distribution of *Rubus arcticus* and its subspecies.

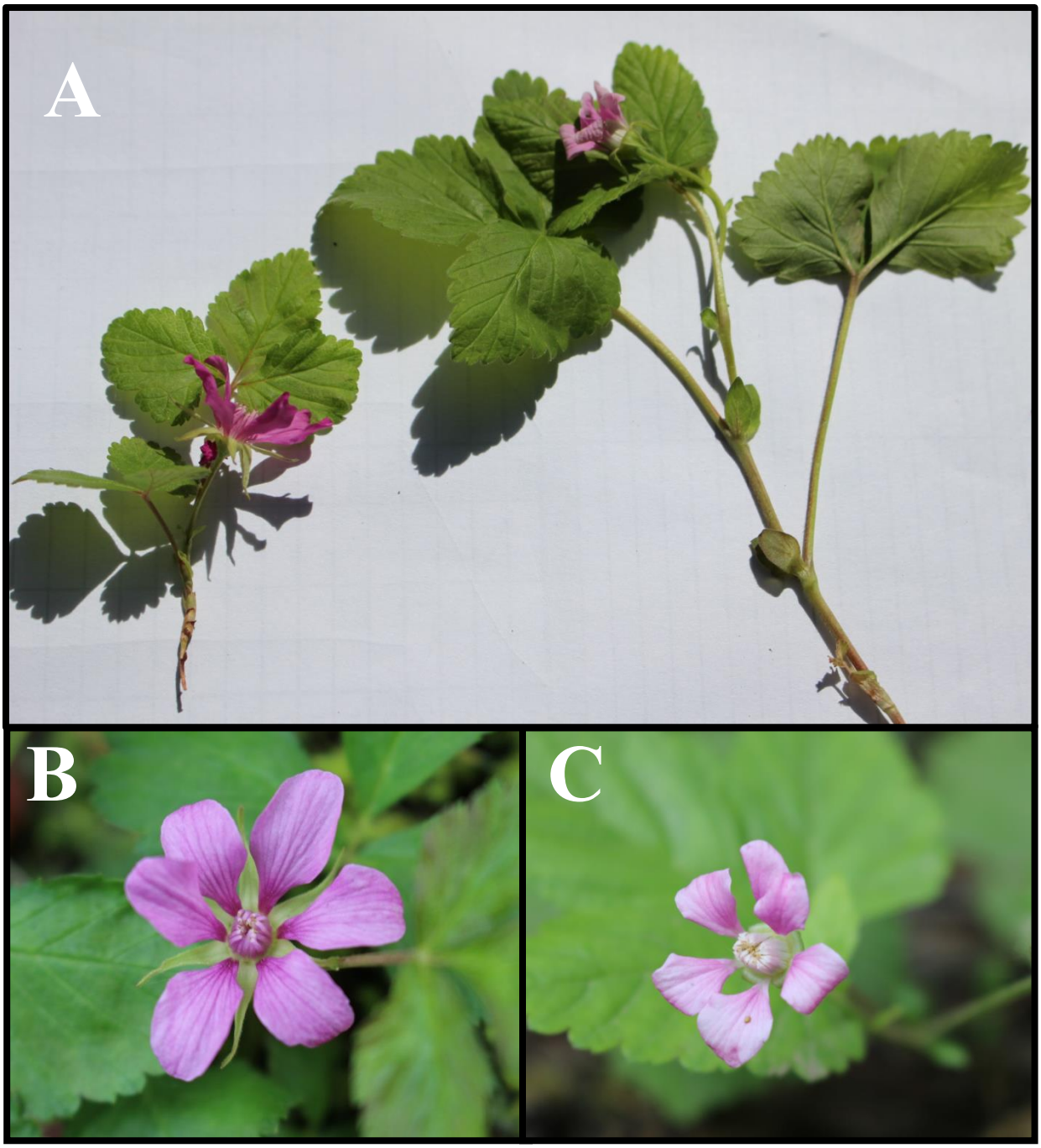


Figure 2.3 ‘A’ – right & ‘C’ = *Rubus arcticus arcticus*, ‘A’- left & ‘B’ = *Rubus arcticus acaulis*. *R. arcticus acaulis* is distinguished by its longer glabrous sepals, distinctly clawed narrow petals and bearing a single flower which is usually below leaves whereas *R. arcticus arcticus* has pubescent glandular (often with yellow glands) sepals, broader petals and bearing up to three flowers well above the leaves (Alice et al. 2015).

Figure 2.4 A replicate of the pure-selfed treatment covered with bridal veil to prevent natural pollination and visitation from insects. The mesh bag was held in place with a rubber band for easy removal. Control and outcross supplemented flowers were also covered with the same bags at the onset of fruit production and when flowers had fully senesced.

Figure 2.5 An image displaying a completely desiccated *Rubus arcticus arcticus* flower.

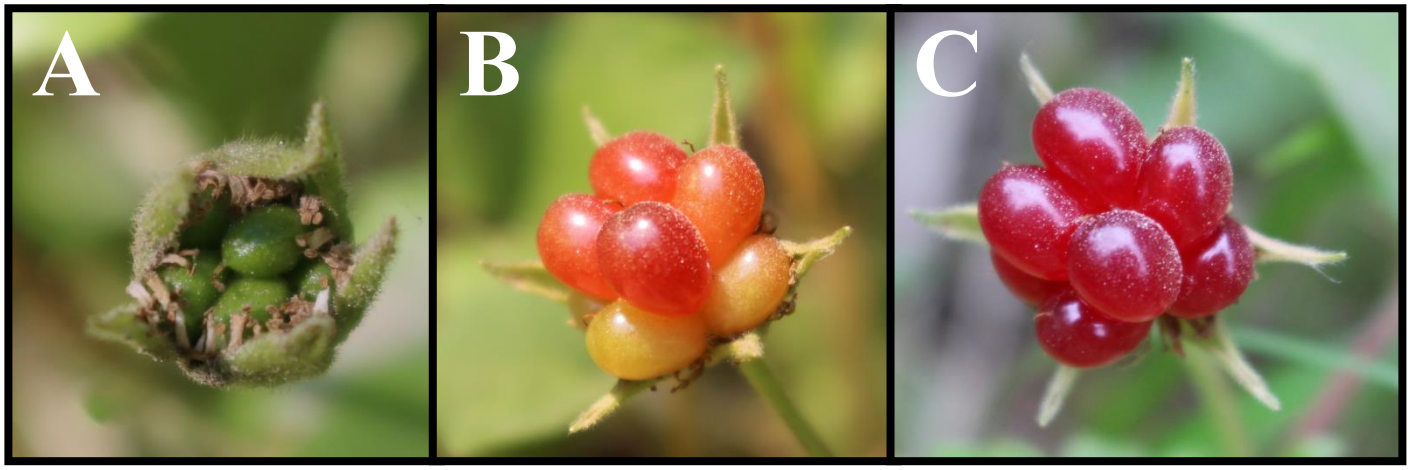


Figure 2.6 The fruit development and ripening stages of a *Rubus arcticus arcticus* berry. 'A' was taken at three weeks post ovule fertilization/three weeks prior to harvesting. 'B' was taken at < six weeks post ovule fertilization/three days prior to harvesting. 'C' was taken six weeks post ovule fertilization/day of harvesting. The time span of these images was from June 27th – July 20th, 2017.

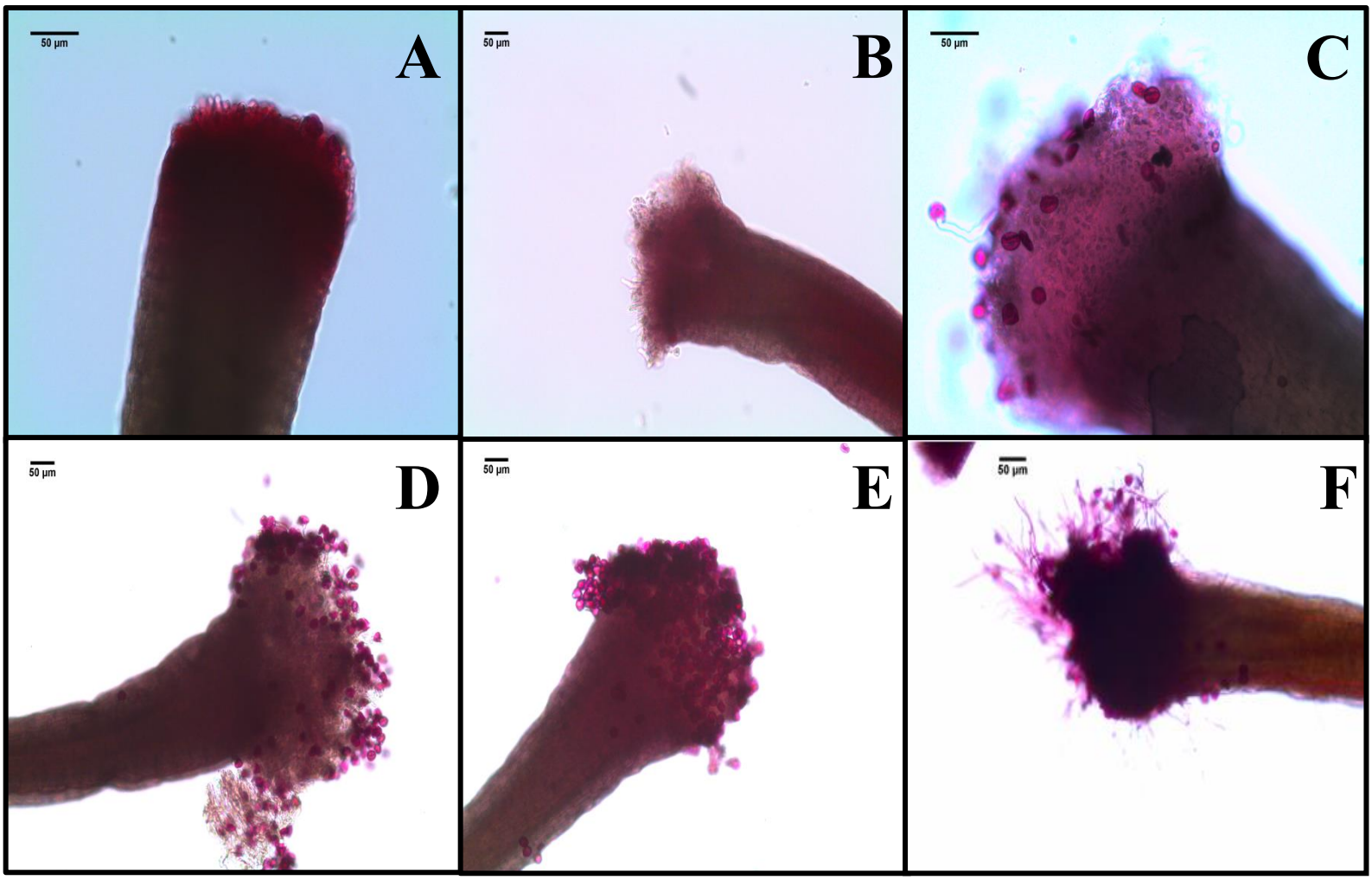


Figure 2.7 A set of micrographs displaying stained *Rubus arcticus* stigmas. All stigmas were stained in a few drops of 1% basic fuchsin for a few seconds, rinsed with water and mounted on glass slides with coverslips in 50% glycerin to be view under a compound microscope with white light. 'A' features a broken stigma in the outcross group at 200X magnification in which only the most basal part is still left intact. 'B' features a stigma in the control group at 100X magnification that is completely bare of any pollen. 'C' features a stigma in the control group at 200X magnification that is lightly/moderately loaded with pollen. 'D' features a stigma in the control group at 100X magnification that is heavily loaded with pollen and germinating tubes. 'E' features a stigma in the control group at 100X magnification that is saturated with pollen. 'F' features a stigma supplemented with selfed-pollen at 100X magnification and is inundated with germinated pollen tubes.

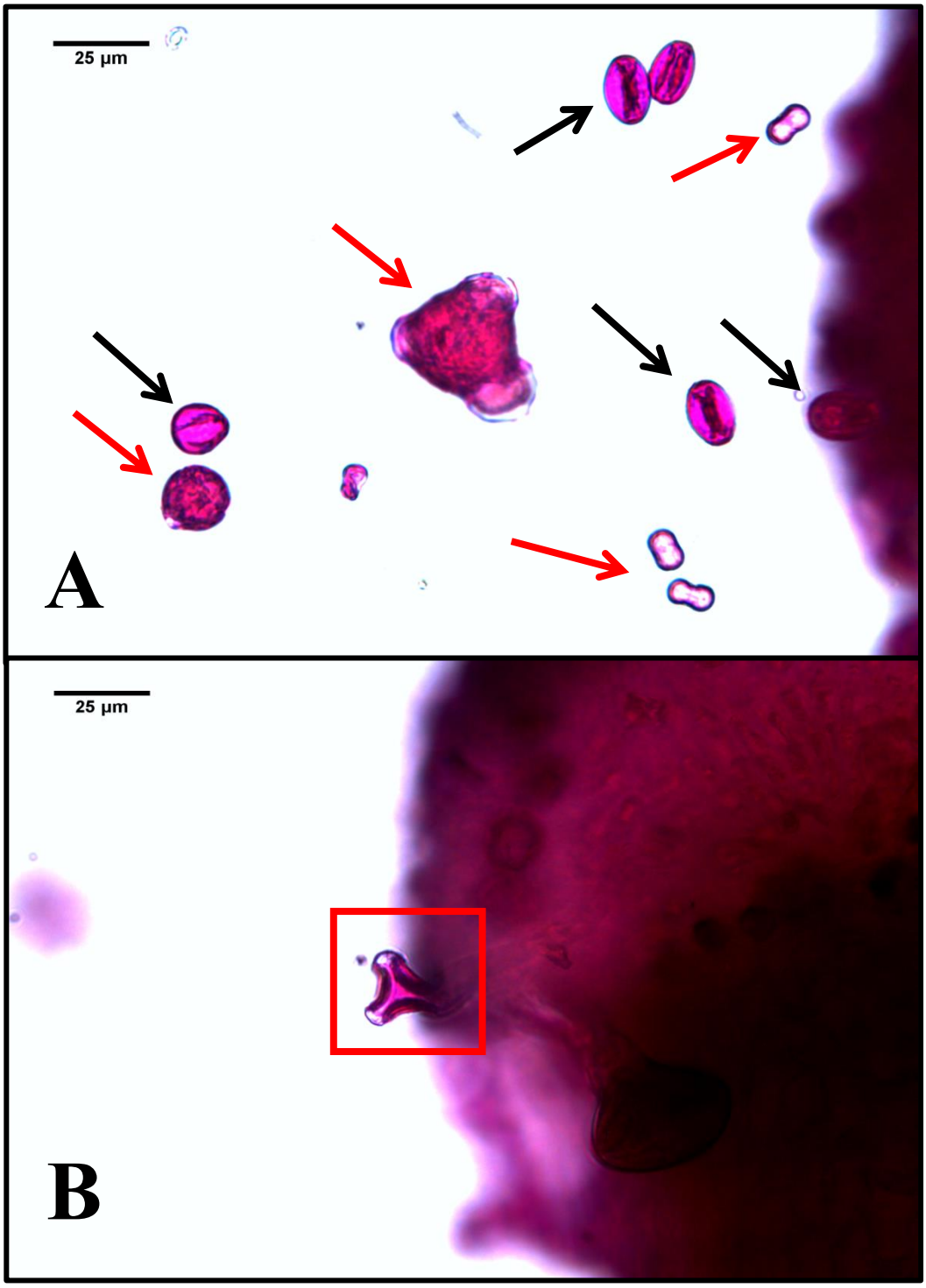


Figure 2.8 Stained heterospecific and conspecific grains on *Rubus arcticus* stigmas. Stigmas and pollen were stained in a few drops of 1% basic fuchsin for a few seconds, rinsed with water and mounted on glass slides with coverslips in 50% glycerin to be view under a compound microscope with white light under 400X magnification 'A' features conspecific grains denoted by black arrows and heterospecific pollen with red arrows. 'B' features another species heterospecific pollen grain highlighted by the red square.

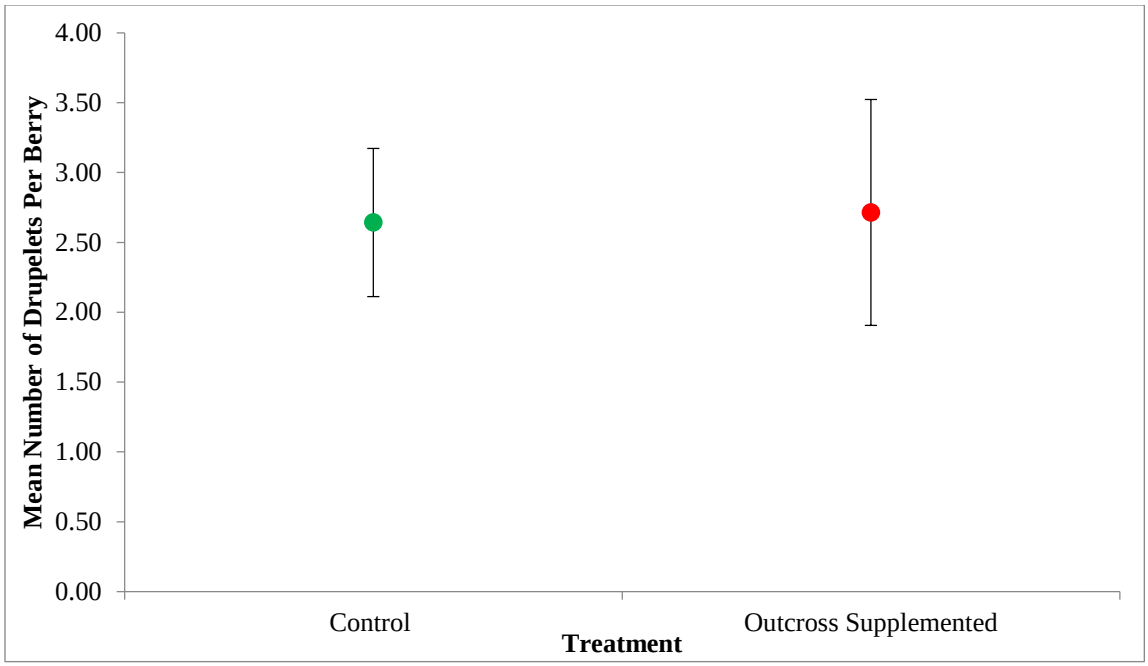


Figure 2.9. The mean number of drupelets per berry for the control (N=14) and outcross supplemented (N=7) treatment groups in the pollination supplementation experiment. Error bars represent standard error.

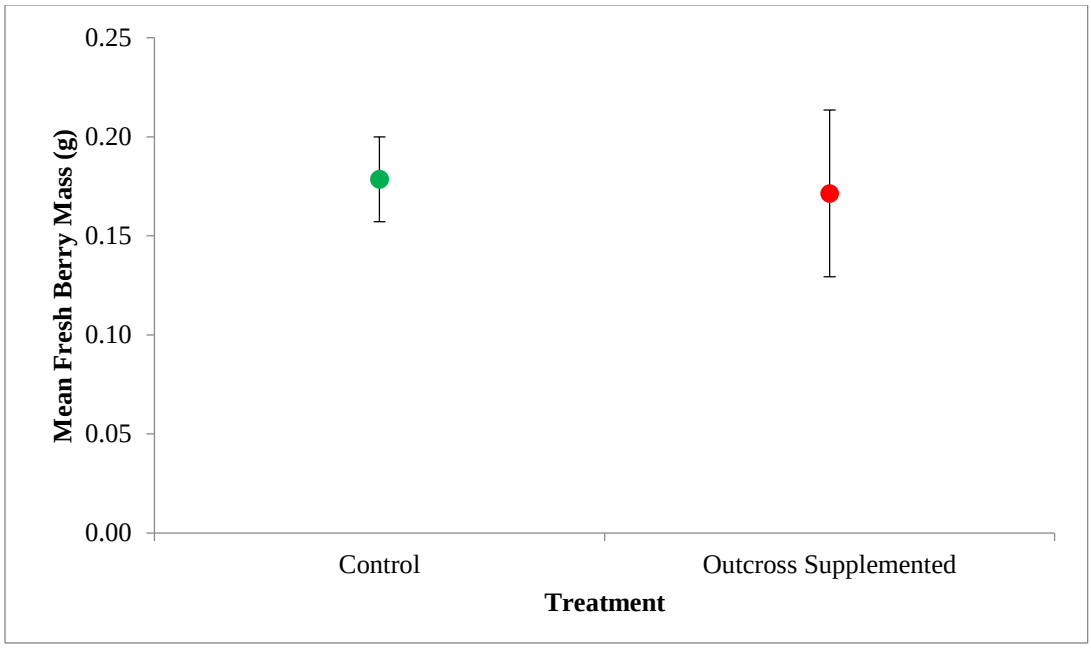


Figure 2.10 The mean fresh berry bass (g) for the control (N=14) and outcross supplemented (N=7) treatment groups for the pollination supplementation experiment. The mean berry mass includes the collective mass for all the drupelets as well as the receptacle when freshly harvested in the field. Error bars represent standard error.

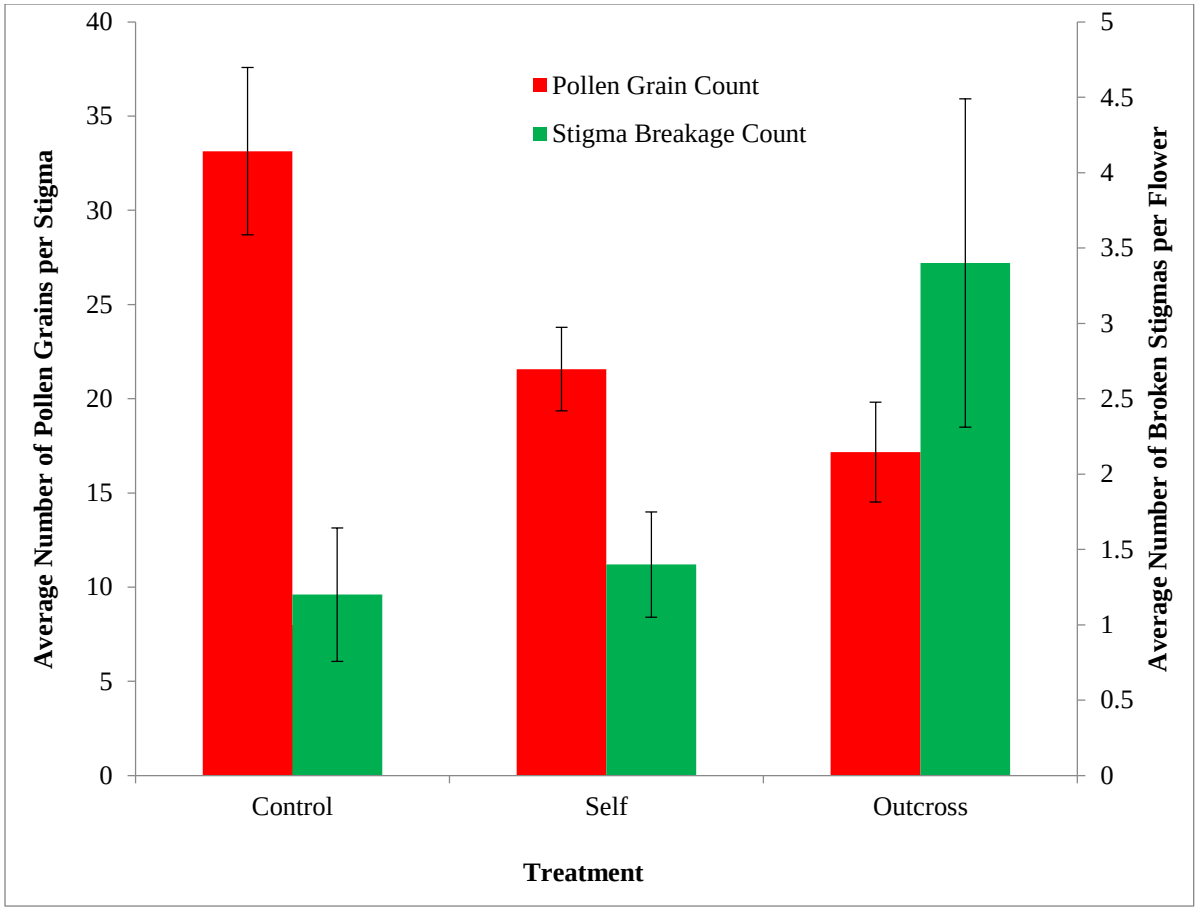


Figure 2.11 The average amounts of pollen grains found on stigmas and frequency of stigma breakage across each treatment in the pollen quantity analysis. Ten pistils were randomly selected from each flower of each treatment (N = 10, 13, 13). Error bars represent standard error.

Figure 2.12 A thrips (Order Thysanoptera) that was collected while dissecting a *Rubus arcticus arcticus* flower. The image was captured under 40X magnification using a dissecting scope.

Chapter 3: The impact of microclimate on pollinator availability and foraging behavior for *Rubus arcticus*

Introduction

With climate change, ecosystems will be subjected to the highest global temperatures seen over the past 740,000 years and record CO_2 emissions seen over the past 650,000 years by 2100, which will inevitably threaten the services they offer (Fischlin et al. 2007). Among those services is pollination, as climate change is reported to be one of the biggest contributing factors of increasing global trends of pollinator declines (Vanbergen et al. 2014). In addition to global declines in domesticated/managed pollinators such as honey bees (van Engelsdorp et al. 2008, Potts et al. 2010a), climate change has affected the distribution of wild pollinators like butterflies, whose ranges have shifted northward by 14-240km within the past 100 years (Parmesan et al. 1999, Thomas et al. 2001, Hickling et al. 2006). Moreover, bumblebee diversity has been steadily declining in Europe (Goulson et al. 2008) and their range sizes have been steadily compressing in light of recent climate change (Williams et al. 2007, Kerr et al. 2015). Some studies have been able to find that these pollinator declines have corresponded with paralleled plant range declines (Biesmeijer et al. 2006, Potts et al. 2010b). Consequently, climate change has numerous cascading effects in regards to pollinators and their recipient plant species. But in order to fully understand how climate affects pollinators, one must first consider the relationships of pollinators with climate at micro-scales.

Microclimate can be defined as the climate within small localized spaces on the earth (i.e. an opening in the forest, hilltop and etc.) which can correspond in very slight differences between surrounding areas (Geiger 1985), but these differences can be substantial enough for small organisms such as pollinating insects. Some pollinators such as birds, bats and a few

insects are capable of regulating their core body temperature endothermically or are homeothermic (Kearns and Inouye 1993). However, the majority of pollinating insects are ectothermic and behavior is heavily constrained by the external environment. Solar radiation and ambient air temperature are especially important microclimate variables for insects as they influence their core body temperatures necessary for flight. For instance, some insects rely heavily on sunflecks (patches of direct sunlight transmitted through the forest canopy) to forage in very shady environments (Beattie 1971). Furthermore, Corbet et al. (1993) found that for social bees (i.e. honeybees and bumblebees), foraging activity is positively correlated with increased solar radiation and air temperature. Additionally, the minimum temperature threshold for foraging flight for each species of social bees is unique and their activity is constrained within a specific "microclimate window". The effects of solar radiation and air temperature extend to other taxa of insects such as lepidopterans. For instance, Bergman et al. (1996) observed butterflies visiting flowers most frequently around solar noon when daily temperatures and solar radiation had peaked whereas hawkmoths (Sphingididae) will not pollinate *Mirabilis jalapa* L. (Nyctaginaceae) when temperatures are beneath 13°C at dusk (del Rio and Burquez 1986).

In addition to solar radiation and air temperature, relative humidity and wind speed can also influence the foraging activity of pollinators. Anthophorinid bee abundance has been observed to be positively correlated with relative humidity whereas abundance of megachilid and halictid bees along with syrphid flies has been found to be negatively correlated with increasing humidity (Sgolastra et al. 2016). However, Kearns and Inouye (1993) suggest that relative humidity does not necessarily affect pollinators directly, but rather indirectly by affecting pollen and water reserves in the plant. Because insects have impermeable materials (such wax in the

cuticle) on their exteriors, relative humidity is less important in their energy balance (Oke 1987). Nevertheless, relative humidity is still important for pollen production in the anthers as well as nectar concentration (Corbet et al. 1979). For instance, Ruban and Kurlovic (2000) found that humidity can alter the sugar concentration in nectar by 2.8-5.3 times. Thus, in environments where the relative humidity is really low, nectar production is strongly correlated with this microclimate variable (Corbet and Delfosse 1984). On the other hand, wind speed can directly influence insect foraging, and if the wind speed is high enough, it can completely prevent visitation from all insects (Kearns and Inouye 1993). For instance, wind speeds > 8 m/s have been observed to completely restrict bumblebee foraging (Bergman 1996) whereas speeds of 4-9 m/s has been found to limit honey bee activity (Lundie 1925). Wind applies drag to insects' wings and bodies during flight which can reduce flight speed and fine body control as well as restrict the angles at which they can approach a flower/inflorescence (i.e. flying upwind versus cross wind or downwind) (Chang et al 2016). And not only can wind speed can affect the successful navigation of flying insects, but also their convective cooling (Church 1960, May and Casey 1983, Unwin and Corbet 1991).

Microclimate likely has an influence on the pollination of *R. arcticus*. Because the species is self-incompatible and strictly entomophilous, fruit and seed production requires insect pollination (Vool et al. 2003). Having a circumpolar distribution across the northern hemisphere (Maiz-Tome 2016, Figure 2.2) and occurring in habitats like low elevation bogs/wetlands to moist woodlands in the sub-alpine/alpine (Porsild 1951, Robuck 1989, Pojar and MacKinnon 1994, Hallsworth and Chinnappa 1997), arctic (Soper and Heimburger 1982), and tundra (Scoggan 1989); the variety of climatic conditions that *R. arcticus* likely presents the species with variability in pollination. Furthermore, *R. arcticus* is drought and shade intolerant, often

flourishing near waterbodies like stream sides (Ryynänen 1973) and therefore often present only within homogenous microclimates (Cole et al. 2015). This may be especially important for potential pollinating insects as different taxa actively seek or avoid shade depending on their thermal flight requirements (Herrera 1997). For example, some taxa of syrphid flies have been observed to exclusively visit shade covered plants. This chapter aims to examine how certain pollinators of *R. arcticus* will decline with changing microclimate availability

Consequently, the objective for this component of the project was to test my hypothesis that microclimate significantly affects the availability and behavior *R. arcticus* pollinators. I predicted that solar radiation and air temperature will have strong positive effects on pollinator visitation and diversity, as these regulate the lower limits in which insects can achieve flight (Kearns and Inouye 1993). Humidity will have a weaker positive effect on visitation (if any at all) as it only directly affects nectar availability and nectar amounts have been observed to have no effect on visitation frequency, but instead strong positive effects on pollinator handling times (Manetas and Petropoulou 2000). So I predict that humidity will have a strong positive effect on handling times, as there should be more nectar with increased humidity, prompting the visiting insects to probe the flower for longer periods. Solar radiation and temperature should also have positive effects on handling times. Firstly, nectar concentration in *R. arcticus* has been observed to increase with increasing air temperature (Karp et al 2004). Secondly, solar irradiance has been observed to be positively correlated with floral temperature and bees have been observed to spend longer durations within flowers with increasing air temperature (Herrera 1995a). Wind speed should have a negative effect on pollinator visitation and diversity, because it simultaneously decreases insect flight stability (Combes and Dudley 2009, Ravi et al. 2013) and their landing capability (Chang et al. 2016). Conversely, wind speed will have a positive effect

on handling time since it has been observed that at higher wind speeds, pollinators have delayed take off times and subsequently spend longer time on flowers (Brown and McNeil 2009).

Methods

Study Site and Species

The study location for this portion of the project was also conducted within the Jumpingpound Demonstration Forest North Loop (51.04719 ° N, -114.79501 ° W; NE-17-24-6-W5M; 1406m elevation) (Figure B1). More specifically, this experiment was performed at the "Natural Undisturbed Site" (Figure B2) within a separate 625m^2 sampling grid (Figure A2), located < 50m from the subsite for Chapter 2. This subsite was in a very similar forest stand type as the "Manipulated Treatment Site" (Figure A1), but had more canopy cover on the south side of the sampling grid due to homogenous spruce tree abundance and had a more diverse assemblage of species in the understory/forest floor (Table C1). Additionally, this site had less *Salix* spp. and *Betula glandulosa* in the lower canopy, allowing for easier navigation through the grid cells, limiting the amount of trampling and ensuring simpler set-up/take-down of monitoring equipment each day. For the same reasons as in Chapter 2, only *R. arcticus arcticus* flowers were used for this experiment as opposed to *R. arcticus acaulis*. Regardless, there appeared to be no *R. arcticus acaulis* shoots growing within this subsite alongside any of the *R. arcticus arcticus* individuals anyway.

Pollinator Surveying

Surveys for this experiment spanned from June 7 to July 10, 2017; occurring five days a week (Monday – Friday), with a total of 23 observation days. In Canada, *R. arcticus* flowers from June to July (Moss 1983, Johnson 1995, Ladyman 2006), so the initial proposed

observation period was going to extend across the entire two months, with as many observation days as logistically possible. However, flowering within the population at this subsite ended sooner than anticipated, therefore the observation period could not have encompassed the full flowering season as originally intended. Surveys did not occur during days of inclement weather as this posed a safety risk. Additionally, surveys did not occur on rainy days because precipitation is a seasonal climatic variable outside of scope of this experiment and was not a subject of concern for this research. Only two observation days had interrupted surveying periods due to rain.

Pollinator surveying made use of time-lapse photography. Direct *in situ* pollinator surveys (i.e. transects and sweep netting) have the severe drawback of observer interference, in which some visiting insects may be deterred by the presence of the experimenter and subsequently will not display normal foraging behavior (Edwards et al. 2015). Moreover, time-lapse photography can be very effective as many cameras are portable and affordable, have an extensive battery life and are capable of capturing near complete records of flower visits while simultaneously reducing sampling bias. Other researchers have made use of indirect surveying protocols with videography instead. For instance, Gilpin et al. (2017) used GoPro cameras on account of their popularity and higher resolution whereas Lortie et al (2012) and Reid (2011) used the very affordable 5th generation Apple iPod Nanos whose cameras also boast very clear videos. However, in these studies, battery life and memory was a concern in which storage cards/batteries had to be manually replaced during recording periods or supplemented with external battery packs. Steen (2009; 2017) also notes that the videos with these devices are recorded in real time so their effectiveness for reducing observer interference is diminished by the large amount of time required to score through the footage, whereas motion capture

photography is far superior regarding this matter. However, motion detection camera systems may not only be quite costly and lack portability, but they record very small scales and can only capture visitation on a single inflorescence (Gilpin et al. 2017) while also mistakenly capturing useless footage when the cameras are triggered by non-insect movement like wind (Edwards et al. 2015).

Five Brinno TLC200 HD time lapse cameras (Brinno, Taipei City, Taiwan) were used for the pollinator surveys in this experiment. Although the TLC200 HDR Pro model was primarily used in Edwards et al. (2015) possessing better features such as a shorter focal length and manual focusing, the regular TLC200 HD model was still found to be effective for capturing insect visitation. Additionally, the TLC200 HD was far less expensive and the videos were a third/half the file size of videos recorded by TLC200 HDR Pro for the same period of time. The TLC200 HD cameras only require four AA batteries per unit and have incredibly long battery life. For instance, all five cameras did not require their batteries to be changed once over the 23 observation days and were still well over half capacity by the end of the last observation day. Each camera was mounted in Brinno ATH110 weather resistant housing cases (Brinno, Taipei City, Taiwan) to protect the lenses from moisture as well as dust from the nearby road < 25m from the subsite. Anti-desiccant silica packs were also placed inside each housing case to limit any potential fogging or condensation within. The five cameras were also placed on flexible octopus tripods with a max height of 25cm, which allowed for cameras to be placed at an optimal angle as *R. arcticus* stems which can grow up to 15cm high (Johnson et al. 1995).

Excluding the first day of surveying when there were only three flowers open in the entire sampling grid (so only three cameras could be used) and the second day of surveying when one of the cameras was not turned on properly, all five cameras were deployed and functioning

for each observation day. To capture the maximal pollinator activity, the time lapse cameras recorded from 09:00 to 17:00 (MST) for a total of eight hours each observation day, excluding the aforementioned rain days as well as a third day in which there was a mandatory safety meeting in the morning at the Barrier Lake Research Station which delayed the recording start time to 10:30. There were also three instances in which a camera was accidentally bumped, wind blew other vegetation in the way obscuring the focal flower or blew the flower out of the field of view (FOV), making the actual surveying time less than eight hours. Although TLC200 HD cameras possess LCD screens that provide real time previews of the cameras' perspectives, the display cannot be turned on when recording is in progress. Therefore, the cameras had to be positioned perfectly prior to recording and if the focal flowers moved out of the FOV or became obscured, there was no possible way to tell without stopping and restarting the recording process. All cameras were set to record on the default "Better" image quality option as the "Best" option produced marginally better image quality. Furthermore, cameras were set to record at 10 frames-per-second (FPS) and with an image capture rate of every three seconds, which Edwards et al. (2015) found was an optimal capture rate that would yield near complete records of floral visitation. Additionally, the time-stamp option was turned on and every morning prior to recording, all cameras had their date/times synced with Mountain Standard Time (UTC – 7:00). The default exposure on the cameras had proven to be too high and would result in over-exposed images, so the exposure was lowered to the third lowest setting.

A random sampling protocol was used for the positioning of these five cameras. Any of the sampling grid cells (Figure B2) containing open *R. arcticus arcticus* flowers were randomly selected each day using a random number generator, such that each of the five cameras were in separate grid cells. Flowers were then randomly selected inside each grid cell. However, during

the beginning and end of the flowering season for the subsite, only 2-3 of the 25 grid cells would contain open flowers and thus, there would be more than one camera per grid cell for some observation days. Because *R. arcticus* also reproduces asexually via rhizomes and forms distinct patches of clones (Ladyman 2006, Figure 3.1), cameras were positioned to fit as many open flowers within the FOV as possible (ranging from 1-6 flowers in the FOV), allowing for better visitation records. The cameras were also slightly angled down on inflorescences creating a top view from above and allowing for better identification of floral visitors (Figure 3.2). The minimum focus distance specified by the manufactures for the TLC200 HD model is 75cm. However, as demonstrated by Figure 3.2A and Figure 3.3, these cameras still provide reasonable image quality and clarity of insect visitors if placed well before that minimum focus distance.

From the 23 observation days, 866.39 useable observation hours (time period the cameras were left recording for) was collected. Although this time-lapse footage was substantially compressed by recording with a 10 FPS framerate and three second capture rate, a standard full eight hour observation period would still result in a ~2GB video that was 16 minutes or longer. Therefore, 112 videos would equate to ≤ 29.87 hrs of actual footage. Similar to Edwards et al. (2015) and Lortie et al (2012), QuickTime Version 7.7.9 was used to score through the videos on a frame-by-frame basis. Each time an insect had landed on a focal flower or probed it, it was considered to be a pollinator. Furthermore, the time of first contact was recorded because the footage was time-stamped with the date/exact time. Since the capture rate of the cameras was every three seconds and an insect could have landed anywhere within that time period, the total number of frames that the insect spent in contact with a flower was also recorded as a measure of handling/foraging time. The number of flowers within each camera's FOV was also documented. And lastly each insect was classified into recognizable taxonomic units (RTUs) or

morphotypes/morphospecies based on body size, color, taxonomy and obvious morphological features because the resolution of the TLC200 HD cameras was not high enough to permit identification to species. RTU classification allows for rapid identification of large quantities of specimens while simultaneously offering a fair estimate of species richness (Oliver and Beattie 1993).

Microclimate Monitoring: Temperature and Relative Humidity

Each camera was also paired with a data logger measuring ambient temperature and relative humidity (RH). Omega OM-92 temperature/RH data loggers (Spectris Canada, Larval, Quebec, Canada) were used for this experiment, which have an accuracy of ± 0.03 °C from 5-60°C for temperature and for humidity; ±3% over 20 to 80%, ±5% below 20% or above 80%. This particular series of data loggers was selected not only for their capability to measure both temperature and RH, but also for their affordability, ability to record at very small time intervals with a memory of 65,520 measurement readings and included software, which allowed for easy data extraction into Microsoft CSV files. The internal clock of each data logger was synced each day with Mountain Standard Time (MST) prior to recording measurements (like the time-lapse cameras) and was set to record at one second intervals so the precise temperature and RH could be applied to the corresponding time-stamp(s) of a visiting insect. However, these data loggers may have been a poor choice due to their sensitivity to excessive sun exposure and black plastic casing. If left in the open sun, these data loggers would give massive spikes in temperature and RH that were most likely inaccurate. Similar to Karbassioon (2017) who attached weather loggers to wooden stakes and covered them with aluminum foil, the data loggers for this experiment were placed on wooden sticks 5-15cm off the ground (depending on the height of the focal flower) and were sheltered by a white (colour selected for high albedo value) plastic bowl

in attempt to reduce surface heat transfer from the soil as well as excessive solar radiation exposure skewing measurements (Figure 3.4A). The data loggers were placed at least 15cm away flowers to allow accurate representation of local temperature/RH and to limit the interference of visiting insects (Figure 3.4B)

Microclimate Monitoring: Wind Speed

To have a similar set up like the temperature/RH loggers recording every second would be more complex and expensive regarding measurement of wind speed. Such a set up would require five separate cup anemometers and additional data loggers to record the measurements at the same temporal resolution. Thus, a more straightforward approach similar to Fijen and Kleijn (2017) protocol was used to measure wind speed. More specifically, a HoldPeak 866B digital anemometer (Zhuhai Jida Huapu Instrument Co. Ltd, Zhuhai, China) was used to measure wind speed at each of the five focal flower/camera positions every 30 minutes, starting at 9:00 and ending at 17:00 MST. As recommended by Kearns and Inouye (1993), the anemometer was held at the same level as flowers and facing the direction of oncoming wind to provide the most accurate data. Additionally, the HoldPeak anemometer has a built in "average" function which will display the average wind speed for the entire duration in which it was recording. Therefore, the average wind speed was collected after 10 seconds of measuring at each of position.

Microclimate Monitoring: Solar Radiation

Numerous pollinator-microclimate monitoring studies use pyranometers to directly and continuously measure solar radiation (Corbet et al. 1993, Herrera 1995a & b, Bergman et al. 1996, Herrera 1997). However, these instruments are costly, often requiring complex calibrations (Brooks 2007). A more inexpensive alternative could have been to use a light meter instead, but they have the major disadvantage of measuring light visible to humans and not necessarily to

insects or utilized in photosynthesis by plants (Kearns and Inouye 1993). Instead, hemispherical photography was used in this experiment to simulate the irradiance received by the focal flowers. Hemispherical photography has been utilized by numerous forestry studies for several years as an indirect approach to approximate understory light regimes (Hardy et al. 2004, Gonzalez-Tagle et al. 2011, Murray et al. 2012, Zorer et al. 2013, Jia et al. 2015, Tichy et al. 2016, Chandler 2017, Dion et al 2017). Circular/hemispherical images are taken with a fisheye lens with 180°FOV facing the canopy on a vertical axis from the reference point (Figure 3.5) and these images can be subsequently digitized to assess light penetration (Jennings et al. 1999).

Using a Canon EOS Rebel SL1 digital SLR camera with a Canon 15-58mm lens (Canon Canada, Brampton, Ontario, Canada) mounted with an Opteka HD² 0.20X professional super AF fisheye lens adapter (47th Street Photo Ltd., New York City, NY, USA), reference hemispherical photographs (with a field of view of at least 180° as specified by the manufacturer) were captured at each focal flower/time-lapse camera position for each observation day. This fisheye lens adapter was selected for its affordability as well as its use in previous research projects involving hemispherical photography (Hayduk 2012, Landert 2016). At each of the five positions, the camera was positioned with the lens directing facing the canopy vertically (Figure 3.6A) and with the top of the camera facing true north, using a compass with the corrected declination for the region as a reference (14° 20.10' East, Natural Resources Canada 2017). As recommended by Zorer et al. (2013), the camera was positioned as close to the focal *R. arcticus* flowers as much as possible. Conversely, Tagle et al. (2011) suggests taking hemispherical photos 1.3m above the ground to control for interference from understorey vegetation, but this is not ideal for a low, prostrate growing species like *R. arcticus*. Because the flexible octopus tripods could not bear the full weight of a DSLR camera with the attached fisheye lens, a stable

Xit XT50TRS 50-Inch Pro Series Tripod (Xit Group, Brooklyn, NY, USA) was used instead. This tripod can be collapsed to a minimum height of 15.5 inches and this length was always used when taking the reference hemispherical images to provide the most relevant solar radiation data (Figure 3.6B). The camera lens and tripod set-up was levelled horizontally using a bubble level (Beckschäfer et al. 2013, Glatthorn and Beckschäfer 2014). The slope, aspect, elevation and geographical position was recorded daily for each of the five focal flower positions, as these were additional input parameters required for the software package to simulate solar radiation exposure. Slope was calculated using a Suunto PM-5/360 PC Clinometer (Suunto, Vanta, Finland), aspect was collected using a Suunto MC-2/360/D/CM/IN/NH compass (Suunto, Vanta, Finland), and the exact field coordinates and elevation of focal flower positions was recorded using a Garmin eTrex® 30 GPS navigator (Garmin Ltd., Olathe, KS, USA).

These hemispherical images were either taken in the early morning, late afternoon or during cloudy periods as recommended by Weiss et al (2004) to limit the amount of direct sunlight entering the camera (Rich 1989) and the pinhole effect (Figure 3.7, Rich 1990). However, this alone is not enough to control for overexposure from the vegetation, in which the contrast between the sky and canopy may not be sufficient for computer programs to isolate the threshold value (Rich 1990). As recommended Beckschäfer et al. (2013), the histogram-exposure protocol was used to limit the amount of over-exposure from the canopy vegetation. More specifically this was performed by using the “P” (programmable exposure) setting on the Canon EOS Rebel SL1 and utilizing the histogram display with the ISO value set to 400.The exposure was adjusted until the “highlight alert” warning had disappeared along with the grey value spikes on the rightmost side of the x –axis on the histograms (Figure 3.8) and then images were subsequently captured.

Solar radiation exposure in all the reference hemispherical photographs for each focal flower position was assessed using Gap Light Analyzer (GLA, Frazer et al. 1999). Using a reference hemispherical photograph, GLA computes the total amount of solar radiation received by simulating the sun's path through the sky regions (Figure 3.9A) and then estimates the amount of light transmitted by creating a binary image threshold in which the sky pixels are white whereas the canopy pixels are black (Figure 3.9B). In addition to the reference hemispherical photographs, GLA also requires numerous user input parameters to compute the solar radiation transmitted through the canopy and characterize understorey light regimes (see Appendix E). Following the simulations from each reference hemispherical photo for each focal flower position, "% Canopy Openness" and "Trans Total" (Total transmitted radiation which is the sum of direct and diffuse radiation) were recorded from the output summary.

Microclimate Monitoring: Canopy Openness and Floral Density

Canopy openness is not necessarily a micrometeorological variable, but it can directly alter the microclimate for pollinators and receptive flowers. Canopy gaps not only alter the understory moisture and temperature regimes (Herrera 1995b), but they can improve the visibility of flowers below for pollinators due to the greater light penetration (Walters and Stiles 1996). For this reason, percent canopy openness was recorded from the GLA simulation outputs and included as an additional variable within the experiment. Moreover, the greater light penetration from these gaps can stimulate changes in plant resource allocation or growth (McConnaughay and Coleman 1999) and change floral density (Stiles 1979, Horvitz & Schemske 1995, Bruna 2003), which has been observed to influence the foraging behavior of pollinators (Cartar and Real 1997, Ishii et al. 2008). For instance, floral density has been observed to be positively correlated with pollinator visitation in which denser patches have

higher inter-individual pollination (Totland and Matthews 1997) whereas thinner patches may receive greater pollination per plant, but less inter-individual pollination (Ghazoul 2005). Although floral density may also not be a microclimatic variable, it could be another useful factor to include as it affects pollinator behavior and pertains to microclimate. Therefore, for each observation day, the number of open flowers in each of the sampling grid cells was recorded. Also, flower counts were performed at the end of the monitoring period (17:00) to account for any flowers that may have opened while pollinator surveys and microclimate monitoring was in progress. Both percent canopy openness and floral density are particularly relevant to *R. arcticus* as it tends to aggregate in dense clonal patches and thrives in open canopies being a shade intolerant species (Ryynänen 1973, Ladyman 2006)

Data Analysis

Several GLMMs were constructed using R Version 3.4.3 (R Core Team 2017) to determine if microclimate does have an influence on the activity and availability of *R. arcticus* pollinators. More specifically, separate GLMMs were made for each of the following response variables: pollinator visitation, foraging/handling time and diversity.

Some useful indices of pollinator diversity may be species richness or Shannon's diversity index (Hubalek 2000), but unfortunately the time-lapse cameras had too poor of image quality to permit identification to species level. Thus, the total number of RTUs or RTU richness was used a means to characterize the diversity of *R. arcticus* pollinators, similar to Reid (2011) and Lortie et al. (2012). RTU richness was assessed on a daily scale since the range of different RTUs on an hourly scale was too small of a response to model. Therefore, the response variable of the pollinator diversity model was the daily RTU richness as measured by each time-lapse camera. Fixed factors for the model included: an interaction term between flowers in the field of

view for each camera (FFOV) and flower density per sampling grid cell (FDPC), an interaction between daily average temperature of visits (DTOV) and daily average relative humidity of visits (DRHOV), daily average wind speed of visits (DWSOV), presence of daily erroneous data logger readings due to excessive sun exposure (DESE), an interaction between percent canopy openness (%CanOpen) and transmitted total radiation per day (TransTot). The only random effect for this model was the date of the observation day to account for seasonality and temporal oscillation (Karbassioon 2017). DESE was included in the model to account for the aforementioned sun exposure issue of the data loggers and was a binary factor in which "0" corresponded with no inaccurate readings throughout the day and "1" noted there were some inaccurate readings present. The Barrier Lake Research Station's climate data (F. Lodhawalla, *pers. comm.*, September 10, 2017) served as a reference for characterizing this factor. If a data logger had any irregular temperature and RH readings during a given observation day in contrast to the station's climate data, it was assigned a 1 for DESE for that day. Finally, this model was created using the 'lme' function in the 'nlme' package (Pinheiro et al. 2017) with a Gaussian error distribution.

Another model was constructed with pollinator handling/foraging time as the response variable. Handling time was simply characterized as the number of frames in which an insect was observed interacting with a flower. Since the capture rate of the cameras was every three seconds and an insect could have entered the FOV anywhere within that period (Figure 3.10), frame count is a more quantifiable index of time. Because handling time was characterized on such a fine scale, the temporal resolution differed between the fixed variables. For instance, because the data loggers recorded temperature and RH every second during the monitoring period, these measurements could correspond to a pollinator's exact foraging period within the

same time scale. On the other hand, wind speed was measured every 30 minutes and GLA could only compute transmitted solar radiation for an entire day at the minimum. Therefore, these two variables could not be interpolated down to same scale as temperature and RH, operating as very restricted linear predictors of handling time. This model also had pollinators grouped by functional group/clade rather than RTU. There were several instances in which many of the RTUs rarely visited the flowers and would act as influential outliers skewing the data. Furthermore, a hemipteran was observed on a flower for over an hour (or the equivalent in frame count) and this was the only visitation event for the clade. Similarly, there were only two instances of a cerambycid beetle visiting flowers in which one visit was very long whereas the other was very short. Thus, these clades were also removed from the model because they acted as influential data points skewing the outliers and it is more likely these insects were basking rather than serving as active pollinators. As well, RTUs in this case would serve as a categorical variable unlike in the diversity model in which RTUs was a numerical variable for richness. And because there were several different RTUs, this many levels within one variable combined with the numerous other variables in the equation would overcomplicate the model and result in convergence errors. The fixed factors for this model were: an interaction between FFOV and FDPC, an interaction between instantaneous temperature of visit (average temperature across all frames an insect was present, ITOV) and instantaneous relative humidity of visit (average RH across all frames an insect was present, IRHOV), hourly average wind speed of visit (HWSOV), presence of instantaneous erroneous data logger readings due to excessive sun exposure (IESE), and an interaction between %CanOpen and TransTot. Date and the hour [of the day] of each visit were included as random factors to control for temporal variation (Karbassioon 2017) as seasonal and daily rhythms were beyond the scope of this research. This model was constructed

with the ‘glmmTMB’ package (Magnusson et al. 2017), using a truncated Poisson error distribution. All the previously discussed models in this project had Gaussian error distributions, but this was only because the data fit better with that error family. A truncated error distribution is necessary for count data in which there are no zeros present (Zuur et al. 2009), which is applicable to this model because handling time could never be zero. Further, Poisson and/or negative binomial linear models are predominantly used in ecology for analyzing count data (Zuur et al. 2009, O’Hara and Kotze 2010). A post-hoc effects test (Type III Anova) was performed on this model as it contained both microclimate variables and pollinator group type as fixed effects and using parameter estimates (as with the other models) would create dummy variable slopes (R. Cartar, *pers. comm.,* 2018). This effects test was then followed by another post-hoc test (Tukey HSD) to determine significant differences in handling times between each pollinator group, using the ‘lsmeans’ and ‘cld’ functions from the ‘lsmeans’ (Lenth 2016) and ‘multcomp’ (Hothorn et al. 2008) packages respectively.

The final sets of models were made with hourly visitation counts as the response variable. Using whole number counts as opposed to a rate is considered better for modelling visitation as using rates can introduce errors and misrepresent the data (Reitan and Nielsen 2016). A separate visitation GLMM was made for each clade/functional group. However, only the dominant clades (bumblebees, mosquitoes, syrphid flies and other brachycerans) had their hourly visitation count modelled whereas butterflies were excluded because they were very rare/infrequent pollinators. Each model had the exact same equation, with date and the hour of the day as random factors to account for differences in temporal structure (Karbassioon 2017) across the daily monitoring period and observation days. The fixed factors for each visitation model were: an interaction between FFOV and FDPC, an interaction between hourly average temperature of visits (HTOV)

and hourly average relative humidity of visits (HRHOV), hourly wind speed of visits (HWSOV), presence of hourly erroneous data logger readings due to excessive sun exposure (HESE), and an interaction between %CanOpen and TransTot. Each model was created using the 'glmmTMB' package (Magnusson et al. 2017), with a Poisson error distribution. Furthermore, each model equation had the "ziformula" parameter set to "~1", which allows the model to run assuming zero inflation (Brooks et al. 2017). Accounting for zero inflation in this particular model was necessary as there were several hours in which the visitation count was zero for a given clade, an assumption also made by Reitan and Nielsen (2016).

All the models mentioned above contain interactions terms in the equations. These interactions were included because I had anticipated that the effect of one dependant variables may change at a given value of another dependant variable. Therefore, I wanted to account for inter-dependent effects between the microclimate variables and how they might ultimately influence the response variables. For demonstrative purposes, consider the relationship between canopy openness, solar radiation and humidity on butterfly diversity. According to Weerakoon et al. (2015), most butterflies prefer sunnier conditions, yet tend not to forage when humidity is either very high or very low. The aforementioned authors also found that the diversity of butterflies was greatest under moderately open canopies. So the effect of humidity on butterfly diversity changes with differing levels of irradiance and canopy openness. However, three-way interactions would only overcomplicate the model equations listed above and result in convergence errors, so only two-way interactions were used between the most likely inter-dependant variable pairs. The 'plot_model' function from the 'sjPlot' package (Lüdecke 2018) was used to visualize these interactions and to subsequently interpret their relationships.

To determine if the number of recording hours was adequate to sufficiently estimate diversity, species accumulation curves were constructed. Species accumulation curves are created by graphing the number of species or taxonomic units as a function of sampling effort, either by individuals processed or some other metric (Colwell et al. 2004). Presence of a horizontal asymptote on the logarithmic curves is indicative of ample sampling effort. Many studies also use rarefaction functions and curves to classify species discovery between sites/regions which is calculated via extrapolation/interpolation when there are concurrent differences in sampling effort. However, since this study was performed at a single site, using rarefaction is not applicable and species accumulation curves were more appropriate. The 'specaccum' function in the 'vegan' package (Oksanen et al. 2018) in R (R Core Team 2017) was used to create species accumulation curves with RTU richness as a function of both the total number of recording hours by the time-lapse cameras and the number of individual insect visitation events processed, similar to Edwards et al. (2015) and Lortie et al. (2012). To confirm if the curves had reached a horizontal asymptote or not, the 'specpool' function in the 'vegan' package (Oksanen et al. 2018) was also used to determine the Chao1 value (R. Cartar, *pers. comm.*, 2018), which is an estimate of the actual minimum/lower bound limit of taxonomic units in reality (Gotelli and Colwell 2011,). These additional data analyses were necessary since Williams et al. (2001) suggests that essentially all pollinator surveying studies fail to fully capture pollinator diversity as they sufficient sampling effort.

Results

The field season spanned 23 observation days resulting in 866.39 usable observation hours and 112 different time-lapse camera/focal flower position iterations within the 625m^2 sampling area. The maximum flower density across the entire sampling grid was 128 flowers and

the maximum number of flowers in an individual grid cell was 44. From the pollinator surveys, the time-lapse cameras captured a total of 1497 visitation events in which syrphid flies were the most frequent visitors measured as both hourly visitation rate (0.967 visits/hour) and total number of visits (844), comprising ~56% of all visits (Table 3.1). Other brachyceran flies were the next frequent pollinator group at ~26%, followed by bumblebees at ~10% and mosquitoes at ~7% of all visits. Handling time appears to have a negative pattern with increasing visitation. For instance, butterflies, hemipterans and cerambycid beetles had the lowest visitation frequencies, but had the longest average foraging times, with a frame count equivalent to nearly 10 minutes. Mosquitoes had the next largest average handling time and the most frequent visitors, syrphid and brachyceran flies with very similar average handling times, had much smaller foraging periods by comparison. Bumblebees had the lowest handling time, spending on average less than two frames at the focal flowers.

Pollinator diversity and RTU richness also had some noticeable trends. Some time-lapse cameras would have footage where no insects had visited the focal flower(s) and thus would have an RTU richness of zero, but the highest RTU count per camera per observation day was 10; despite a total of 30 RTUs visiting the flowers across the entire season (Table 3.2). The GLMM for pollinator diversity revealed that RTU Richness was only influenced by FFOV, FDPC and %CanOpen individual as well as the interaction between FFOV and FDPC (Table 3.3). FFOV, FDPC and %CanOpen all had significant strong positive effects on RTU Richness, whereas all the other microclimatic variables had no significant effects whatsoever.

In regards to handling time of the *R. arcticus* pollinators, there were numerous discernable relationships. All the microclimate variables along with FDPC and %CanOpen had significant positive effects on handling time (Table 3.4) whereas FFOV had a negative effect. All

the interactions had significant (negative) effects with %CanOpen:TransTot having the highest magnitude and ITOV:IRHOV have smallest effect. The only fixed factor that had no significant effects was IESE. Clade/functional group of the pollinators had exponentially higher (positive) effects on handling time in comparison to the direct/indirect microclimate variables. The post-hoc pairwise comparison test (Table 3.5) confirmed this result, in that the handling time of each clade was unique.

There were numerous patterns between the hourly visitation rates and each of the pollinator groups. FFOV, HTOV, HRHOV and the interaction between HTOV and HRHOV all had significant effects on the visitation rates of syrphid flies (Table 3.6). HTOV and HROV both had negative effects whereas FFOV was positive and the interaction had a strong positive effect. A very similar set of results also occurred for the other brachyceran flies in which FFOV had significant positive effect whereas temperature and humidity had negative effects (Table 3.7) as well as a negative effect from the interaction between FFOV and FDPC. The interaction between temperature and humidity was also significant, but not as large of a positive effect as seen with the syrphid flies. Bumblebee visitation rates were significantly impacted by FFOV, FDPC (which both had strong positive effects) as well as the (negative) interaction between FFOV and FDPC (Table 3.8). Lastly, HRHOV had a significant negative effect for hourly visitation of mosquitoes and there was a positive effect for the interaction between HRHOV and HTOV (Table 3.9).

Despite the date and time of day being modelled as random factors in all the GLMMs, there still appears to be some distinguishable temporal trends. Firstly, syrphid fly visitation was most frequent when the average hourly temperature was highest during the day and when the hourly average RH was lowest in a unimodal distribution (Figure 3.11). Other brachyceran flies

have a bimodal distribution with visitation peaking in the late morning when RH had started to steadily decrease as well as at 14:00 when temperature was rapidly increasing. As for the other pollinators, there are no discernable patterns in their visitation frequencies throughout the day. Syrphid flies had the highest visitation frequency at the start of the flowering season (early June) and their maximal visitation coincided with the peak period in flowering (Figure 3.12). However, later in the flowering season (late June/early July), the frequency of syrphid fly visits dropped substantially. On the other hand, the visitation of the other pollinators remained either relatively variable throughout the season or did not change with increasing/decreasing floral density.

Both species accumulation curves display logarithmic trends (Figure 3.13). In the smaller ranges of observation hours and individual visits processed, the discovery rate of RTUs increases rapidly, but substantially decreases in the much larger ranges. There appears to be no asymptote for both curves whereas the Chao1 estimates by total recording hours was 36.11 ± 6.072 RTUs and total number of insects processed was 47.99 ± 23.61 RTUs.

In summary, there appears to be some support for the hypothesis that microclimate does influence the diversity, foraging time and visitation frequency of *R. arcticus* pollinators. Firstly, direct and indirect microclimate variables had a significant effect on pollinator handling times, but only canopy openness and floral density affected RTU richness. Further, the hourly visitation of the most frequent visitors, syrphid flies, was negatively affected by temperature and humidity, yet the temperature:humidity interaction had relatively high positive effect. Brachyceran flies exhibited similar patterns as the syrphid flies whereas for mosquitoes, only humidity had an effect (negative) and there was a positive temperature:humidity interaction. Bumblebee visitation was impacted only by flower density. Also, humidity, temperature, solar radiation and wind speed had significant positive effects on pollinator handling times. Nonetheless, the strength of

effects from these factors was small in comparison to the effect of clade/functional group/pollinator type. Conversely, none of the direct microclimate variables had significant effects on overall diversity. Humidity and temperature had a negative rather than positive effect for two pollinator groups; the opposite of my prediction. As well, wind speed had no significant effect on visitation frequency for any of the pollinator groups, rendering my third prediction incorrect.

Discussion

The positive effect of canopy openness on pollinator diversity is consistent with previous research. Several studies have observed that forests with larger canopy openings foster pollinator biodiversity (Fye 1972, Rudolph and Ely 2000, Klein et al. 2003, Campbell et al. 2007, Romey et al 2007, Grundel et al. 2010, Taki et al, 2010, Hanula et al. 2015). One would expect a similar effect for solar radiation given the relationship of canopy openness with forest light regimes, but the results of the RTU richness analysis suggest otherwise. The lack of a significant effect of transmitted radiation on pollinator diversity as well as the interaction between %CanOpen and TransTot is perplexing. Datillo and Dyer (2014) observed that while sunlight availability had no positive impact on ant diversity in the Brazilian Amazon rain forest, the diversity of ant-to-plant interactions did increase with canopy openness by reducing the niche overlap and species evenness. These ecological processes may also be occurring for this *R. arcticus* population seeing as there was a very uneven distribution among the pollinators. For instance, syrphid flies made up more than half the visitation events. Regardless, there was no significant effect of %CanOpen and TransTot on the visitation of syrphid flies or any of the other pollinator groups.

The impact of FDPV and FFOV on pollinator diversity is also congruent with other studies. Increased flower density and patch size has been commonly observed to increase the

visitation and floral constancy of pollinators (Kunin 1997, Dauber et al. 2010), but increased blossom density has also been observed to increase pollinator diversity as well (Hegland and Boeke 2006). In this aforementioned study, blossom density was even a stronger linear predictor of pollinator diversity than plant species richness. As the authors suggest, the reason that flower density is more influential than floral diversity could be due to the fact that most pollinators are generalists (Waser et al.1996). Consequently, having more flower species in a given area may not be as valuable to the pollinators as the quantity of open flowers because many of these insects do not actively forage on particular species, but rather any flower present. For this study, syrphid and other brachyceran flies not only were the most frequent visiting clade, but they also contributed the most variety of RTUs (Table 3.2). Brachycerans can be very specialized, like the Bombyliidae that feed on long tubular flowers, but many members of this suborder (including Syrphidae) are generalists with variable mouthparts feeding on a wide array of flower species (Kevan and Baker 1983). Although the pollinator composition was more so affected by indirect microclimate variables like canopy openness and floral density, I interpret this finding with caution because the species accumulation curves did not show obvious asymptotes whereas the Chao1 estimates had large standard error values. Therefore, there is much uncertainty if this experiment had enough sampling effort to fully capture pollinator diversity and subsequently characterize taxonomic richness with microclimate.

Edwards et al. (2015) found that one of their time-lapse cameras at one of their sites had an increased number of insect visits with increased number of inflorescences within the FOV. Increased sampling effort (i.e. number of insects processed) corresponds in an increased discovery of rate of species or taxonomic units as seen in species accumulation curves (Colwell et al.2004). Therefore, the greater number of flowers there are within the time-lapse cameras'

FOVs, the more likely it will be for the cameras to record more pollinator species visiting. This same theory is also applicable for the results where FFOV had a positive effect on the visitation frequency of syrphid flies, brachycerans and bumblebees. Krell (2004) argues that parataxonomic identification like the use of RTUs can actually inflate species richness estimates and in most cases cannot serve as a reliable means of classification. However, RTUs can still be trustworthy for non-comparative descriptions of species richness at single site scales as well in circumstances in which very strong or obvious patterns manifest (Krell 2004), both of which apply in this experiment. Conversely, identification to species may have been more ideal when characterizing visitation frequencies as some insects such as syrphid flies display species-specific relationships with microclimate variables (Gilbert 1985).

As predicted, all the microclimate factors had positive effects on handling time. Canopy openness also had a positive effect which is to be expected as it is closely tied to transmitted solar radiation. Interestingly, wind speed had a stronger positive effect in comparison to ITOV, IRHOV, TransTot and %CanOpen which were relatively weaker. I had predicted that temperature, solar radiation and especially humidity would have strong positive effects. A potential reason for this outcome could be the convective cooling properties of wind on insects (Church 1960, May and Casey 1983, Unwin and Corbet 1991) which could negate the effects of temperature/radiation. Then again, the stronger effect of wind could be attributed to the fact pollinators tend to spend more time on flowers during faster wind periods with delayed takes offs (Brown and McNeil 2009). The aforementioned researchers observed that pollinators of *Rubus chamaemorus* would quickly take off when observers approached flowers when the wind was < 4 m/s, but would remain on flowers longer at greater speeds at the sight of observers.

Another explanation for the weaker effects of temperature, humidity, radiation and canopy openness on handling time could be the fact that there are numerous conflicting interactions between each of these variables concerning nectar production/foraging. Firstly, some insect taxa have been observed to select more dilute nectar in very dry conditions as opposed to more concentrated nectar produced during more humid periods, prioritizing water over energy intake (Ohguchi and Aoki 1983, Willmer 1986, Conteras et al. 2013). Furthermore, nectar concentration and viscosity appear to positively correlated, which results in increased handling times as the insects have slower intake rates due to the thicker sugary nectar (Josens and Farina 2001). However, increased air temperature and sunshine also increases the temperature of the nectar, making it less viscous (Kovac and Stabentheiner 2011, Nicolson et al. 2013), which has been found to be more ideal for bees, in spite of the concurrent decrease in nectar concentration. The effects of temperature on nectar and handling time appears complex and potentially species-specific (Afik and Shafir 2007; Herrera 1995a; Willmer et al. 1994, Karp et al. 1994) and a future study that integrates floral temperature, nectar volume, concentration and/or viscosity in addition to the microclimate variables, to isolate the complicated effects of nectar on pollinator availability and behavior is warranted.

The relative differences in handling times between each taxonomic group of pollinators listed by Herrera (1989) are comparable to those in this experiment, which further reinforces that intrinsic differences within taxa is more influential than the direct effects of microclimate on foraging duration. In this study, syrphid fly handling times were 10X greater than that of bumblebees, butterfly handling times were 105X greater than that of bumblebees, and butterfly handing times were 11X greater than that of syrphid flies.

Mosquitoes also have unique foraging behavior in comparison to other taxa of pollinators. Although mosquitoes are commonly perceived as troublesome blood feeding parasites, they are known to provide pollination services (Fang 2010). For instance, mosquitoes are essential pollinators for *Platanthera obtusata* (Orchidaceae) (Stoutamire 1968, Thien 1969, Gorham 1976), a common orchid found in essentially all regions of Canada (Brouillet et al. 2010). Furthermore, males are complete obligate nectar feeders and although females require blood meals for egg production, nectar is still actively sought after as the sugars and lipids found within are absolutely necessary for flight and maintenance (Foster 1995). However, there appears to be less literature concerning mosquito handling times and the effects of microclimate on the foraging behavior of pollinating mosquitoes. Sandholm and Price (1962) observed numerous species of mosquitoes collecting nectar from the flowers of several plant species, including *Dasiphora fruticosa* (Rosaceae) which is a relative of *R. arcticus*. Based on their propensity to remain on flowers well after their actual nectar meal (Sandholm and Price 1962), it is more plausible that the mosquitoes were just lingering on the *R. arcticus* flowers. Thus, it is also very unlikely that microclimate had a pronounced effect on their handling times in comparison to other pollinator groups.

Similar to the nectar production and foraging, this study generated conflicting results regarding the effects of microclimate on syrphid fly visitation frequency. Humidity was found to have a negative effect on the visitation counts of syrphid flies. This finding is consistent with Sgolastra et al. (2016) who had the same finding as well as Gilbert (1985) who observed that as RH increased, syrphid fly nectar and pollen collection decreased. Temperature in this study was found to also have a significant negative effect on syrphid visitation, while the previous literature has syrphid visitation to respond positively (Zimina 1957) and negatively (Bankowska 1964;

Nielsen 1966; Maier and Waldbaer 1979) to temperature and irradiance. The effects may be species-specific as Gilbert (1985) observed that some species of syrphid flies were more likely to be observed in the shade, but collectively the syrphid flies foraged more regularly for nectar in the sun. It is important to note that the highest average syrphid visitation counts occurred at early afternoon when the average hourly temperature was also highest at ~ 20°C. The other brachyceran flies had nearly the same set of results as the syrphids concerning visitation frequency.

There were also no effects of temperature, humidity, solar radiation, wind speed and canopy openness on bumblebee visitation. Nevertheless, this set of results is quite consistent with previous research. Despite Corbet et al. (1993) suggestion that bumblebees have a specific "microclimate window" in which they can achieve flight and actively forage for food, they are considered hardy pollinators because they have been observed to forage in a wide range of microclimates that would deter most other insects. For instance, Tuell and Isaacs (2010) found that bumblebees were able to forage far more regularly in poor weather conditions (i.e. low temperature and solar radiation, high wind speed and humidity) in both years of the two year study opposed to honeybees that were only able to forage frequently during good weather. This set of results has been found repeatedly in several previous studies (Free 1960, Boyle and Philogene 1985, Boyle 1987). Therefore, unless present in extreme regions like the arctic or alpine, bumblebees are far less constrained by microclimate in comparison to other pollinators. Bumblebee visitation was more strongly affected by floral density, which is also consistent with the literature. Since bees appear to have a predisposition to alight to nearest neighboring flowers (Zimmerman 1979), flower density would have a pronounced effect on bumblebee visitation, as demonstrated by multiple previous studies (Klinkhamer and de Jong 1990, Cartar and Real 1997,

Goulson 2003, Ishii 2008). Shorter distances between flowers in denser patches corresponds with smaller inter floral travel time, less energy being expended for flight, and overall increased efficiency.

The interactions in all the models produced interesting yet contradictory results. My initial theory of the interaction between the number of flowers within a camera's FOV and the flowers in the given sampling grid cell can serve as an example. To elaborate, I speculated that when there were less focal flowers in the camera's FOV (i.e. only one flower) while the encompassing sampling grid cell had high floral density; pollinator diversity/handling time/visitation frequency would be less as the insects would be drawn away by the high number of neighboring conspecific blooming flowers. Conversely, when there was perhaps a larger patch flowers (i.e. six flowers) in the camera's FOV, the effect of floral density with the sampling grid cell would be negated as the pollinators would likely visit all the flowers within that said patch before moving on to other ones within the grid cell; indicating an overall positive effect in this interaction. However, the complete opposite pattern manifests as indicated by Figure 3.14A Handling time increases with increasing surrounding floral density within the cell when there are fewer focal flowers in the FOV, but handling time decreases rapidly with increasing floral diversity within the cell when there are several flowers within the FOV. One may argue that the effect size of this interaction is small compared to pollinator group type, but this negative interaction is substantial in the diversity and handling time models as well the syrphid, brachyceran and bumblebee visitation models. Hegland and Boeke (2006) found that bloom density was a much stronger predictor of fly visitation compared to floral species richness, yet the effect of bloom density did not have as strong of a positive effect as anticipated. These authors then suggest that this discrepancy could be due to the fact flies have far less optimal

foraging patterns in contrast to highly efficient pollinators like bumblebees. Flies likely also have lower energy requirements and the need to visit as many flowers within a patch as fast as possible to maximize energy gain is less relevant, so flower density is likely to be less important to them and they can afford to have longer residence time on flowers (Hegland and Boeke 2006). This rationale is quite relevant considering that flies made up the majority of visits as well as the diversity of RTUs (Table 3.1 + 3.2). However, this still does not explain why there was a significant negative interaction between FFOV and FDPC for bumblebee visitation as bumblebees have a high tendency to visit nearest neighboring flowers (Zimmerman 1979). When there are several flowers within the camera's FFOV, a bumblebee should be more likely to visit this patch rather than a single *R. arcticus* flower as closer dispersed flowers patches have been demonstrated to be selected for by bumblebees (Klinkhamer and de Jong 1990, Cartar and Real 1997, Goulson 2003, Ishii 2008); yet this does not seem to occur.

There were also conflicting interactions between temperature and humidity in some of the models. As indicated by Figure 3.14B floral handling times were longer when humidity was high while temperature was low and vice-versa, indicating an overall negative interaction. The former scenario can be easily interpreted as when relative humidity is highest and temperature is within the lowest ranges, sucrose concentrations would also be high and the nectar would be more viscous which would ultimately slow down the insects intake rates (Josens and Farina 2001). But when temperature is high and humidity low, the nectar would be more dilute and watery which would correspond in faster intake rates and smaller handling times (Kovac and Stabentheiner 2011, Nicolson et al. 2013), which creates an opposing set of results. However, the effect of this interaction is quite small in comparison to the effect pollinator group type, which again is likely attributed to the fact that intrinsic behavioral differences across insect taxa may outweigh the

effects of microclimate on handling time. On the other hand, there was a positive interaction between temperature and humidity for both syrphids and other brachyceran flies visitation models, which implies that visitation frequencies would be highest when both temperature and humidity were high. This outcome is especially puzzling considering that when examining temperature and humidity individually, both have negative effects on visitation frequency for these pollinator groups.

Finally, the interaction between canopy openness and transmitted radiation on handling time can be somewhat be rationalized. According to Figure 3.14C handling time was the least when both percent canopy openness and transmitted radiation were high. Some taxa of syrphid flies actively avoid the sun and prefer the shade such as *Volucella inanis and V. zonaria* (Herrera 1997) or *Melanostoma scalare* (Gilbert 1985). One could then understand how both high canopy openness and transmitted radiation would correspond to decreased handling time, as larger canopy gaps can allow for more intense direct sun exposure which may be less desirable to more shade preferring insect taxa. Conversely, on very cold cloudy days with less solar radiation, larger canopy gaps may be important to less hardy insects as they can bask on flowers soaking up as much sunlight as possible, enabling them to reach stable body temperatures in spite of the poorer weather. However, the effect of this interaction on handling time again was small in comparison to the effect of pollinator group type. Nevertheless, this was the only instance of a significant interaction between canopy openness and transmitted radiation, with absences of interaction between these variables in the diversity and visitation models. Therefore, it's difficult to ascertain how important this interaction actually is for *R. arcticus* pollinators.

Another factor that should have been considered is heterospecific flower presence, density and dispersion. Although bumblebee visitation has been found to be weakly explained by

floral species richness in comparison to total bloom density similar to syrphids and other flies (Hegland and Boeke 2006), several observations were made in this study in which bumblebees would actively select other nearby flowering species even amongst large patches of *R. arcticus* flowers. For instance, from the beginning observation period (early June) to the middle (mid/late June), bumblebees would constantly fly over patches of clear unobscured *R. arcticus* flowers and instead more frequently visit *Mertensia paniculata* (Boraginaceae) flowers (Figure 3.15A). Later in the flowering season and observation period (early/mid-July) when *M. paniculata* stopped flowering, the bumblebees would select *Astragalus americanus* (Fabaceae) flowers (Figure 3.15B) instead. A possible explanation for this occurrence could be that bumblebees tend to prefer flowers with deep corollas because only longue tongued insects can reach the nectaries and thus will provide more nectar than open cup/saucer shaped flowers that are visited by a wider variety of insects (Oleson et al. 2007). However, it is more likely that the bumblebees were selecting these two species as they have very large inflorescences with flowers clustered closely together, whereas *R. arcticus* flowers are typically solitary (Moss 1983) and the nearest neighboring flower could be on a completely separate stem several centimetres away. The inclusion of these factors could explain the low visitation rate compared to other more regular and floral constant visitors like syrphid flies. Consequently, future studies concerning microclimate of *R. arcticus* pollinators should also consider the effects of separate flower species and dispersion rather than just *R. arcticus* bloom density.

Aside from missing some additional variables, one must also consider other possible short-comings of the statistical analyses used in this study. Despite being a common tool for examining pollinator-microclimate interactions (Corbet et al. 1993, Herrera 1995a+b, Brown and McNeil 2009, Reid 2011, Fijen and Kleijn 2017, Karbassioon 2017), GLMMs and linear models

in general have their drawbacks. Firstly, one must consider that pollinators are more likely to have non-linear relationships with microclimate. To elaborate, there is likely an optimal range for some microclimate variables and then outside of this range, pollinator abundance steadily decreases. Butterflies can reinforce this notion as they tend to rest during periods of either very high or very humidity, but actively forage at moderate levels (Weerakoon et al. 2015). The only microclimate variable in this experiment that may have been linearly directional was wind, corresponding in a negative relationship as very high wind speeds can prevent visitation from virtually all insects (Kearns and Inouye 1993). Regardless, the inclusion of wind speed in the models was of little use anyway considering the study site experienced slight variability in wind speed with the highest measurement being 1.85 m/s (likely negated by the dense *Picea glauca* trees in the site), resulting in a lack of an effect similar to Heard and Hendrikz (1993). Models that incorporate non-linear relationships may be more ideal, such as Sanchez-Lafuente et al. (2005) who used a quadratic term for temperature responses or Boscolo et al. (2017) who used General Additive Models (GAMs) for bee visitation patterns. Many other studies use ordination analyses to examine how pollinator assemblages change with micro-environmental variables and floral traits or diversity (Morales and Aizen 2005, Tasen et al. 2010, Bates et al. 2011, Williams 2011). Alternatively, as pollinators can exhibit species-specific relationships with microclimate (Gilbert 1985), perhaps one could instead use ordination analyses to examine how pollinator traits (i.e. body size, mouthparts, feeding habits, tongue length and etc.), rather than pollinator taxa, vary with microclimate. However, Jamil et al. (2013) argues that GLMM approaches are far superior to ordination, as GLMMs account for pseudo-replication and heteroscedascity.

Finally, future pollinator-microclimate experiment may want to consider the effects of temporal oscillation, unlike this study. In this study, the peak flowering of *R. arcticus* coincided

with its most frequent visitor, members of Syrphidae. Iler et al. (2013) found that though most alpine floral species maintain phenological synchrony with syrphids over long periods even within a wide set of changing abiotic conditions (i.e. snowmelt, degree days and precipitation). On the other hand, the authors found that mid or late-blooming species in the Colorado Rocky Mountains may have fewer plant-pollinator interaction periods with the onset of global warming and inter-annual variation due to faster advancement of snowmelt, whereas early-blooming species would have increased overlapping days. This set of results is supported by Totland (1993) who suggests that with alpine floral species, selection for early blooming is greatest. Tiusanen et al. (2016) despite making the same prediction that peak early blooming would be more beneficial for the arctic growing *Dryas* spp., found that the diversity of pollinators including muscids (which are very important pollinators for the genus) was greater later in the season. However, there appears to be no phenological mismatch for this population of *R. arcticus* flowers for this current flowering season, since the peak period of flowering coincides with the peak period of visits, and syrphid fly visitation also occurs earlier in the season (June). Vool et al. (2003) found that syrphid fly visitation did not match with the peak period of *R. arcticus* blooming, but the flowering season in Estonian populations differs from North American ones, and *R. arcticus* is a generalist and could receive ample pollination through other visitors. Nevertheless, performing pollinator-microclimate surveys across multiple years is worth considering in regards to possible phenological mismatches with increasing climate change.

Conclusion

In conclusion, microclimate does have some influence on the pollinators of *R. arcticus*. None of the direct microclimate variables had significant effects on the diversity, assemblage, or RTU richness of the pollinators with only canopy openness and floral density having significant

positive effects. On the other hand, all the microclimate variables (both direct and indirect) weakly explained the variation in pollinator foraging duration whereas the clade/functional group type had strong effects. This outcome is most likely due to the fact that there are intrinsic differences among taxa of insects and pollinators will exhibit particular foraging tendencies regardless of microclimate. The most frequent visiting pollinators were syrphids and brachyceran flies in which humidity and temperature had the strong (negative) effects on their hourly visitation, yet unexpectedly there was also a strong positive effect from the interaction between temperature and humidity.

Future studies should include factors associated with nectar foraging/production as they have many conflicting and counterintuitive relationships with the different microclimate variables. This suggestion is further reinforced by the conflicting interactions that each microclimate variable have with one another and how they subsequently influences pollinator availability. These future studies may also want to consider using models or statistical analyses that account for non-linear relationships that may exist between microclimate variables and pollinator taxa, or even possibly examine how pollinator traits vary with microclimate to avoid any species-specific relationships. Furthermore, the effects of heterospecific plant species presence, density and dispersion should also be included as several observations were made in which bumblebees would constantly select other neighboring species opposed to even large patches of *R. arcticus* flowers. Including these factors could potentially account for the low visitation of the bumblebees and may explain why in this case they were not the dominant pollinators as the literature would suggest. And finally, pollinator-microclimate experiments should be performed across multiple years to account for any possible temporal oscillation that

could result in phenological mismatches between peak periods in flowering and pollinator visitation.

Tables

Table 3.1 The total number of visits across the 23 observation day period, percentage of total visits, average hourly visitation rate (# of visits/hour), and handling time (# of frames observed on flower in field of view) for each respective clade/functional group of observed insect pollinators/visitors.

Clade/Functional Group	Bumble Bees	Brachyceran Flies	Cerambycid Beetles	Hemipterans	Butterflies	Mosquitoes	Syprhid Flies	All Insects
Total # of Visits	143	394	2	1	8	105	844	1497
% of Total Visits	9.55	26.32	0.13	0.07	0.53	7.01	56.38	N/A
Average Hourly Visitation Rate	0.164	0.451	0.002	0.001	0.009	0.120	0.967	1.715
Average Handling Time	1.65	15.91	192	1735	172.63	63.15	15.72	N/A

Table 3.2 A list of abbreviations, description and taxonomic information for the 30 RTUs identified within the time-lapse camera footage for the pollinator surveys.

RTU	Description	Lowest Identifiable Taxon
Bomb	Bumblebee	*Bombus* (Genus)
Syrph1	Medium syrphid fly with yellow spots and black stripes	Syrphidae (Family)
Syrph2	Medium syrphid fly with black and orange/yellow spots	Syrphidae (Family)
Syrph3	Small round yellow and black striped syrphid fly	Syrphidae (Family)
Syrph4	Metallic syrphid with round body	Syrphidae (Family)
Syrph5	Medium syrphid with long thin body	Syrphidae (Family)
Syrph6	Large round syrphid with pale yellow bracket abdominal shaped stripes	Syrphidae (Family)
Syrph7	Medium syrphid fly resembling a wasp	Syrphidae (Family)
Syrph8	Medium tube bodied yellow and black spotted syrphid	Syrphidae (Family)
Syprh9	Small tube bodied syrphid fly with white dot on side of abdomen	Syrphidae (Family)
Syprh10	Medium syrphid fly with longitudinal abdominal black stripe	Syrphidae (Family)
Syrph11	Large syrphid fly that closely resembles a bumble bee	Syrphidae (Family)
Syrph12	Large syrphid fly with very thin light yellow stripes across abdomen	Syrphidae (Family)
Syrph13	Medium Syrphid fly with elongated linear abdomen and bulbous tip	Syrphidae (Family)
Syrph14	Medium syrphid fly, also bumble bee mimic, but smaller with more black than yellow	Syrphidae (Family)
Syrph15	Medium syrphid fly that has two yellow dots separated by black stripe,	Syrphidae (Family)
Syrph16	Medium Syrphid fly with top bracket shaped abdominal stripes (rest are solid)	Syrphidae (Family)
Mosq	Mosquito	Culicidae (Family)
Lep1	Small butterfly	Lepidoptera (Order)
Lep2	Medium butterfly with speckled wings and two white stripes	Lepidoptera (Order)
Lep3	Medium butterfly black wings and one large white stripe	Lepidoptera (Order)
Hemi	Hemipteran	Hemiptera (Order)
Dipt1	Medium grey/dull brown fly	Brachycera (Sub-Order)
Dipt2	Small linear black fly	Brachycera (Sub-Order)
Dipt3	Medium black metallic fly	Brachycera (Sub-Order)
Dipt4	Medium dark non-metallic fly	Brachycera (Sub-Order)
Dipt5	Tiny black fly	Brachycera (Sub-Order)
Dipt6	Very large fly	Brachycera (Sub-Order)
Dipt7	Orange fly with red eyes	Brachycera (Sub-Order)
Ceramb	Cerambycid beetle	Cerambycidae (Family)

Table 3.3 The output summary of the GLMM used to model daily pollinator diversity/RTU Richness. * Denotes significance within a confidence interval of 95% and ** denotes significance within a confidence level of 99%.

Fixed Effects	**Estimate**	**Standard Error**	**t-value**	**p-value**
FFOV	0.840036	0.248005	3.387173	0.0011**
FDPC	0.124678	0.05146	2.422803	0.0177*
DTOV	-0.49649	0.376203	-1.31974	0.1908
DRHOV	-0.21319	0.125003	-1.70551	0.0921
DWSOV	0.328912	0.367903	0.894018	0.3741
DESE	-0.26359	0.953607	-0.27642	0.783
%CanOpen	0.354285	0.137209	2.582079	0.0117*
TransTot	0.204816	0.188737	1.085194	0.2812
FFOV:FDPC	-0.055	0.0199	-2.76395	0.0071**
DTOV:DHROV	0.011117	0.006784	1.638814	0.1053
%CanOpen:TransTot	-0.02582	0.015261	-1.69194	0.0946

Table 3.4 The output summary for the effects test on the GLMM with pollinator handling time as the response variable. * Denotes significance within a 95% confidence interval, *** denotes significance within a 99.9% confidence interval whereas **** denotes significance within a +99.99% confidence interval.

Effect	**Direction of Effect**	**Chisquare (χ^2)**	**df**	**p-value ($> \chi^2$)**
FFOV	-	50.111	2	$< 0.0001^{****}$
FDPC	+	34.47	2	$< 0.0001^{****}$
FFOV:FDPC	-	17.416	1	$< 0.0001^{****}$
ITOV	+	11.642	2	0.002965^{**}
IRHOV	+	31.378	2	$< 0.0001^{****}$
ITOV:IRHOV	-	6.2154	1	0.01266^{*}
IESE	-	0.5644	1	0.4525
HWSOV	+	91.222	1	$< 0.0001^{****}$
%CanOpen	+	83.093	2	$< 0.0001^{****}$
TransTot	+	74.491	2	$< 0.0001^{****}$
%CanOpen:TransTot	-	45.416	1	$< 0.0001^{****}$
Pollinator Group/Clade	+	11790	4	$< 0.0001^{****}$

Table 3.5 The output summary of the post-hoc Tukey test used to determine significant differences in handling times between the different pollinator groups. Unique letters indicate significant differences and groups sharing the same letters are not significantly different.

Pollinator Group	lsmean	Standard Error	Lower Confidence Limit	Upper Confidence Limit	Significance
Bumblebees	-0.0715	0.160995	-0.485053	0.3420629	a
Brachycerans	2.55137	0.129394	2.218987	2.8837537	b
Syrphids	2.685556	0.129307	2.353397	3.0177147	c
Mosquitos	3.804178	0.129733	3.470924	4.1374321	d
Butterflies	5.225315	0.132292	4.885487	5.5651428	e

Table 3.6 The output summary of the GLMM used to model the hourly visitation counts of syrphid flies. * Denotes significance within a 95% confidence interval whereas *** is within a 99.9% interval, and **** is significance within +99.99%.

Fixed Effects	**Estimate**	**Standard Error**	**z-value**	**p-value**
FFOV	0.199279	0.070298	2.835	0.00459**
FDPC	-0.00409	0.013058	-0.313	0.75395
HTOV	-0.07631	0.037926	-2.012	0.0442**
HRHOV	-0.09833	0.018726	-5.251	< 0.0001****
HWSOV	-0.05881	0.152367	-0.386	0.69954
HESE	0.1591	0.125037	1.272	0.20322
%CanOpen	-0.00251	0.046431	-0.054	0.95696
TransTot	-0.11596	0.067625	-1.715	0.0864
FFOV:FDPC	0.001797	0.0043	0.418	0.67596
HTOV:HRHOV	0.004825	0.000856	5.637	< 0.0001****
%CanOpen:TransTot	0.005016	0.006245	0.803	0.42186

Table 3.7 The output summary of the GLMM used to model hourly visitation counts of other brachyceran flies. * Denotes significance within a 95% confidence interval, ** denotes confidence within a 99% confidence interval, *** denotes significance within a 99.9% confidence interval, **** denotes significance within a +99.99% confidence interval.

Fixed Effects	Estimate	Standard Error	z-value	p-value
FFOV	0.375398	0.090842	4.132	< 0.0001****
FDPC	0.033143	0.021877	1.515	0.129777
HTOV	-0.26594	0.075556	-3.52	0.000432***
HRHOV	-0.08458	0.025748	-3.285	0.001021**
HWSOV	-0.23496	0.177972	-1.32	0.186771
HESE	-0.13796	0.24191	-0.57	0.568483
%CanOpen	0.098184	0.055408	1.772	0.076388
TransTot	0.080685	0.076483	1.055	0.291451
FFOV:FDPC	-0.01688	0.007742	-2.18	0.029292*
HTOV:HRHOV	0.005501	0.001435	3.834	0.000126**
%CanOpen:TransTot	-0.00783	0.006149	-1.273	0.202977

Table 3.8 The output summary of the GLMM used to model hourly visitation counts for bumblebees. *** Denotes significance within a confidence interval of 99.9% whereas **** denotes significance within a +99.99% confidence interval.

Fixed Effects	Estimate	Standard Error	z-value	p-value
FFOV	0.694802	0.148525	4.678	< 0.0001****
FDPC	0.11768	0.033174	3.547	0.000389***
HTOV	-0.03319	0.071326	-0.465	0.641706
HRHOV	-0.0386	0.029927	-1.29	0.197093
HWSOV	-0.00554	0.275995	-0.02	0.983999
HESE	0.47179	0.320932	1.47	0.141545
%CanOpen	0.163549	0.097397	1.679	0.093115
TransTot	0.149241	0.119804	1.246	0.21287
FFOV:FDPC	-0.04445	0.012932	-3.437	0.000587***
HTOV:HRHOV	0.0021	0.0015	1.4	0.161401
%CanOpen:TransTot	-0.01702	0.010344	-1.645	0.100003

Table 3.9 The output summary of the GLMM used to model hourly visitation counts of mosquitoes. * Denotes significance within a 95% confidence interval.

Fixed Effects	**Estimate**	**Standard Error**	**z-value**	**p-value**
FFOV	-0.2189	0.193609	-1.131	0.2582
FDPC	0.018054	0.040171	0.449	0.6531
HTOV	-0.17572	0.113965	-1.542	0.1231
HRHOV	-0.09369	0.040955	-2.288	0.0222*
HWSOV	0.001111	0.339533	0.003	0.9974
HESE	-0.28699	0.485709	-0.591	0.5546
%CanOpen	0.152777	0.117004	1.306	0.1916
TransTot	0.098852	0.145511	0.679	0.4969
FFOV:FDPC	0.00233	0.013985	0.167	0.8677
HTOV:HRHOV	0.005225	0.002202	2.372	0.0177*
%CanOpen:TransTot	-0.00796	0.011585	-0.688	0.4918

Figures

Figure 3.1 A clonal patch of *Rubus arcticus arcticus* stems, with four open flowers present.

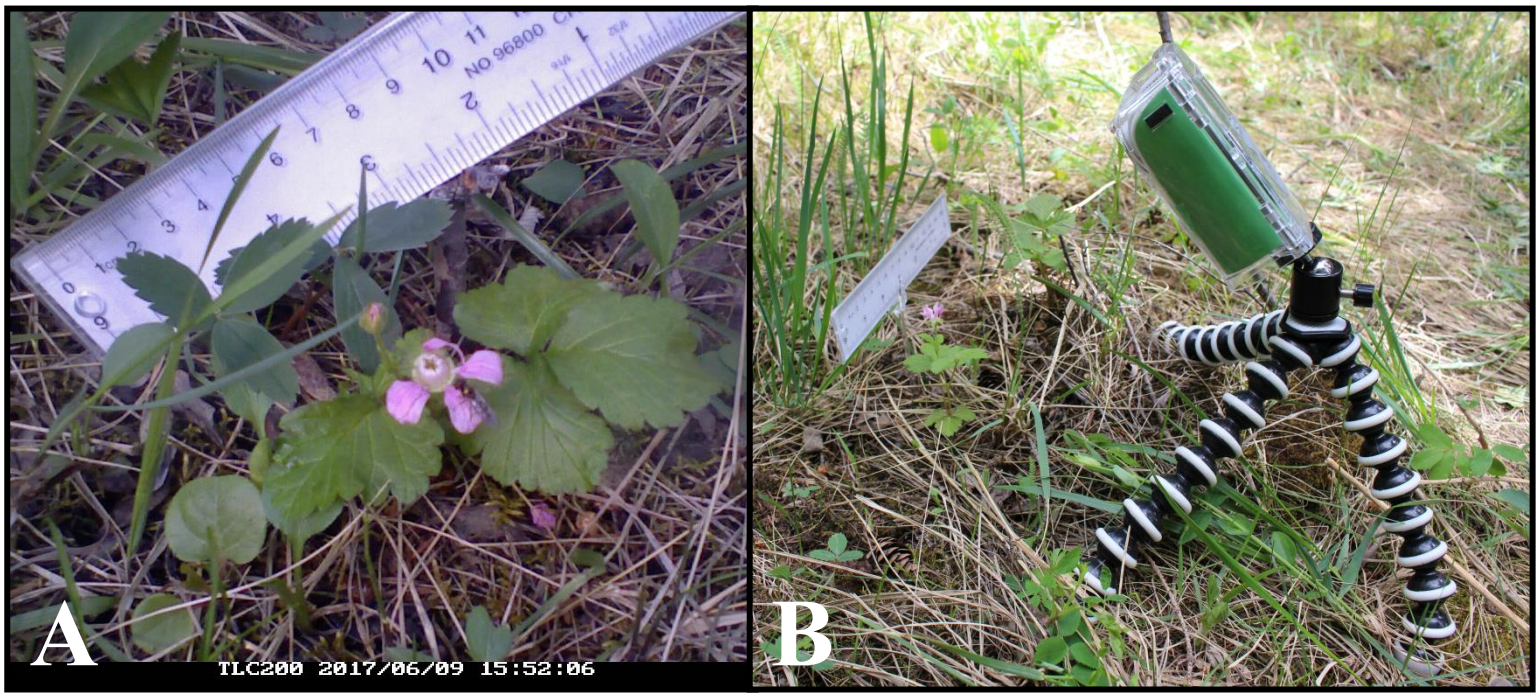


Figure 3.2 The time-lapse camera set up used for the pollinator surveying. ‘A’ is example footage from the Brinno TLC200 HD camera, with exposure set to third lowest setting and on “Better” image quality. ‘B’ displays the placement distance and angle of the time-lapse camera from the focal flower.

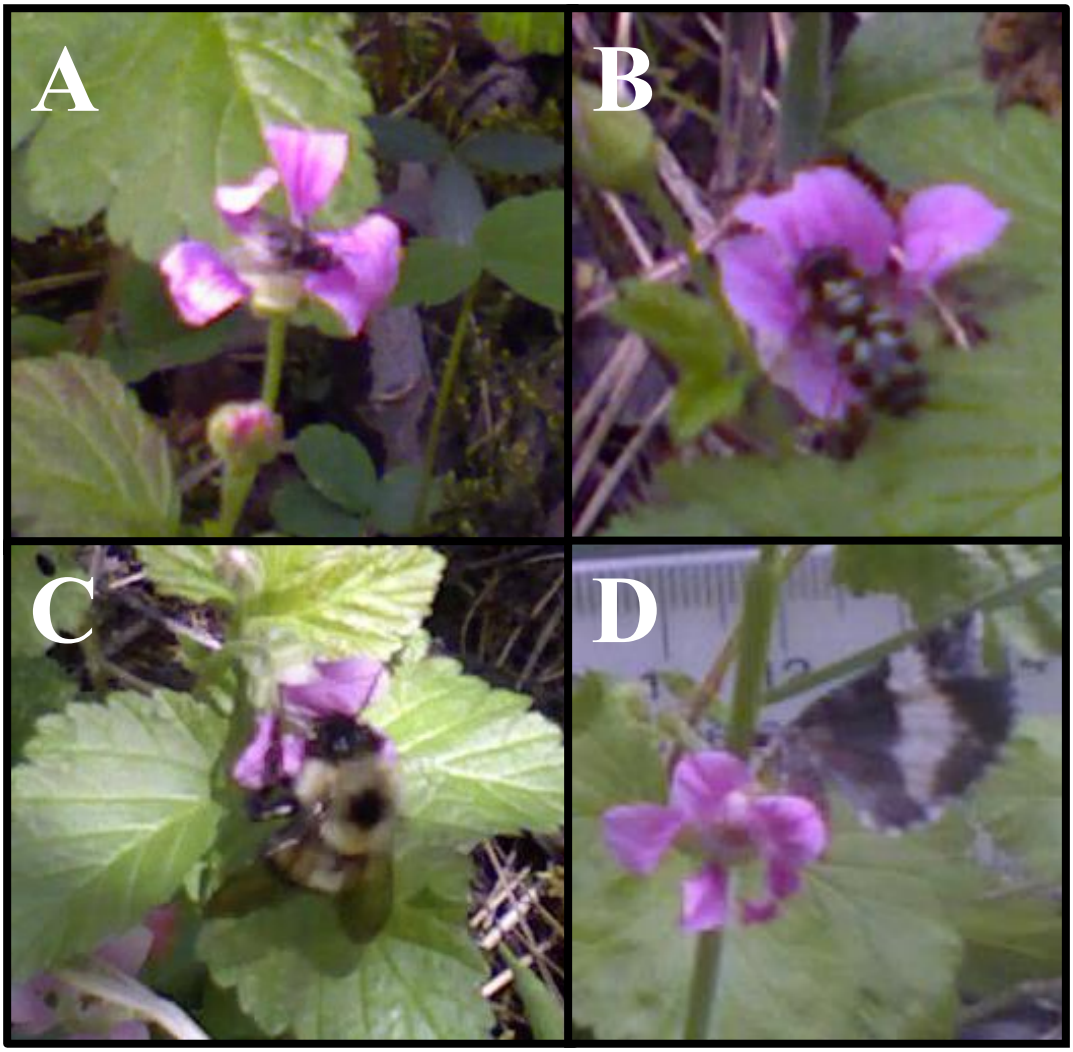


Figure 3.3 Footage collected by Brinno TLC200 HD time-lapse cameras. ‘A’ features a Brachyceran fly (most likely a Muscid), ‘B’ features a syrphid fly, ‘C’ features a bumblebee and ‘D’ features a butterfly.

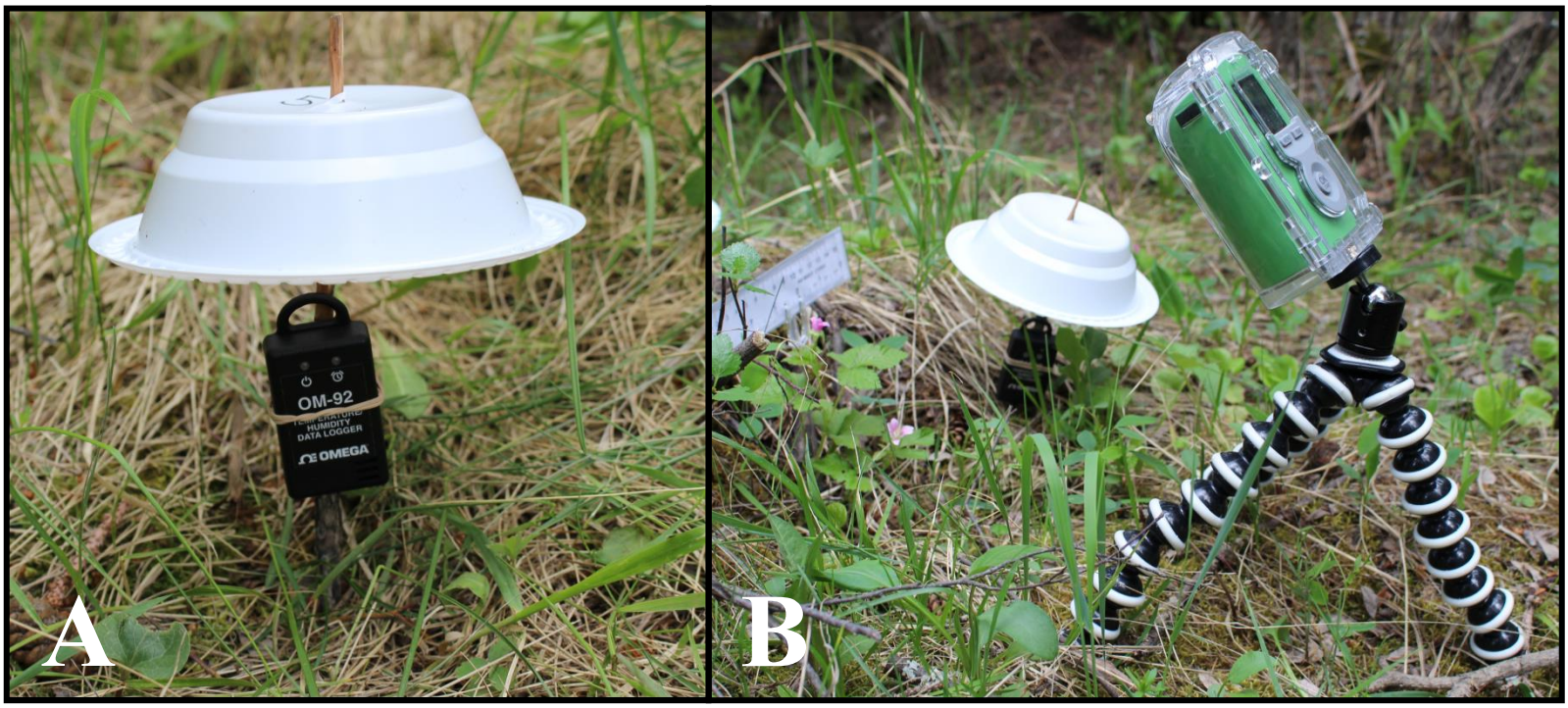


Figure 3.4 The temperature/humidity data logger set up. ‘A’ demonstrates the use of the wooden sticks to raise the data loggers to same level as *Rubus arcticus arcticus* flowers as well as the white plastic bowl shield it from excessive sun exposure. ‘B’ demonstrates the placement of the data logger set-up in relation to the focal flower.

Figure 3.5 An example of a hemispherical photograph that was used for simulating the amount of solar radiation received by the focal *Rubus arcticus arcticus* flowers in the pollinator surveying and microclimate monitoring experiment..

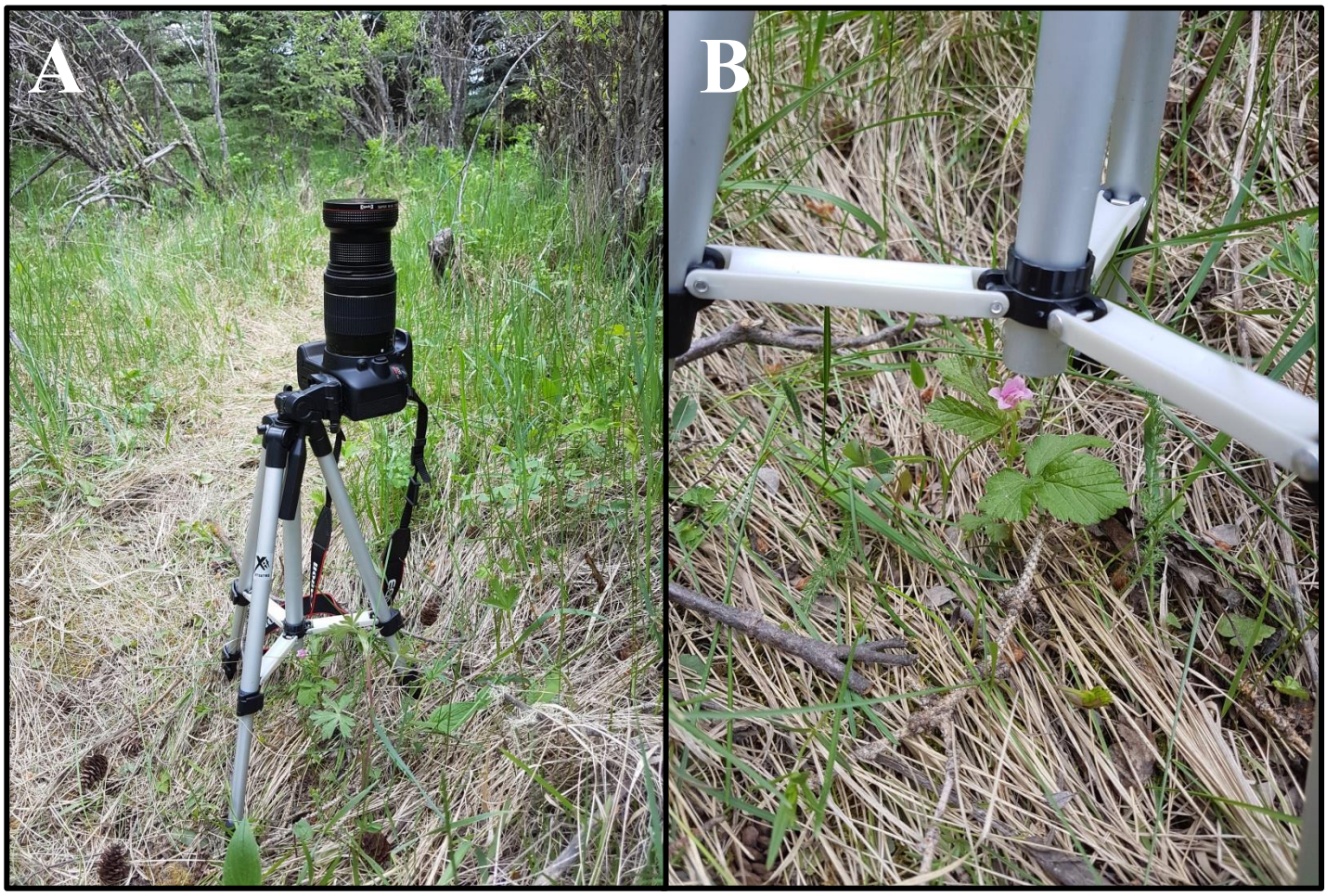


Figure 3.6 The camera setup for capturing the reference hemispherical photographs to simulate solar radiation exposure. ‘A’ features the Canon EOS Rebel SL1 DSLR camera mounted with an Opteka HD^2 0.20x Fisheye Lens vertically facing the canopy, with the top of the camera facing true north using a compass with the corrected regional declination and horizontally leveled using a bubble level. ‘B’ displays the minimum distance between the camera and the focal flower.

Figure 3.7 A hemispherical image in which direct sun light had penetrated though the canopy into the fisheye lens. The red circle highlights the "pin-hole effect" (Rich 1990) where the sunlight that is poking through the trees creates a gap in which the light and over exposure results in a loss of the tree/vegetation pixels.

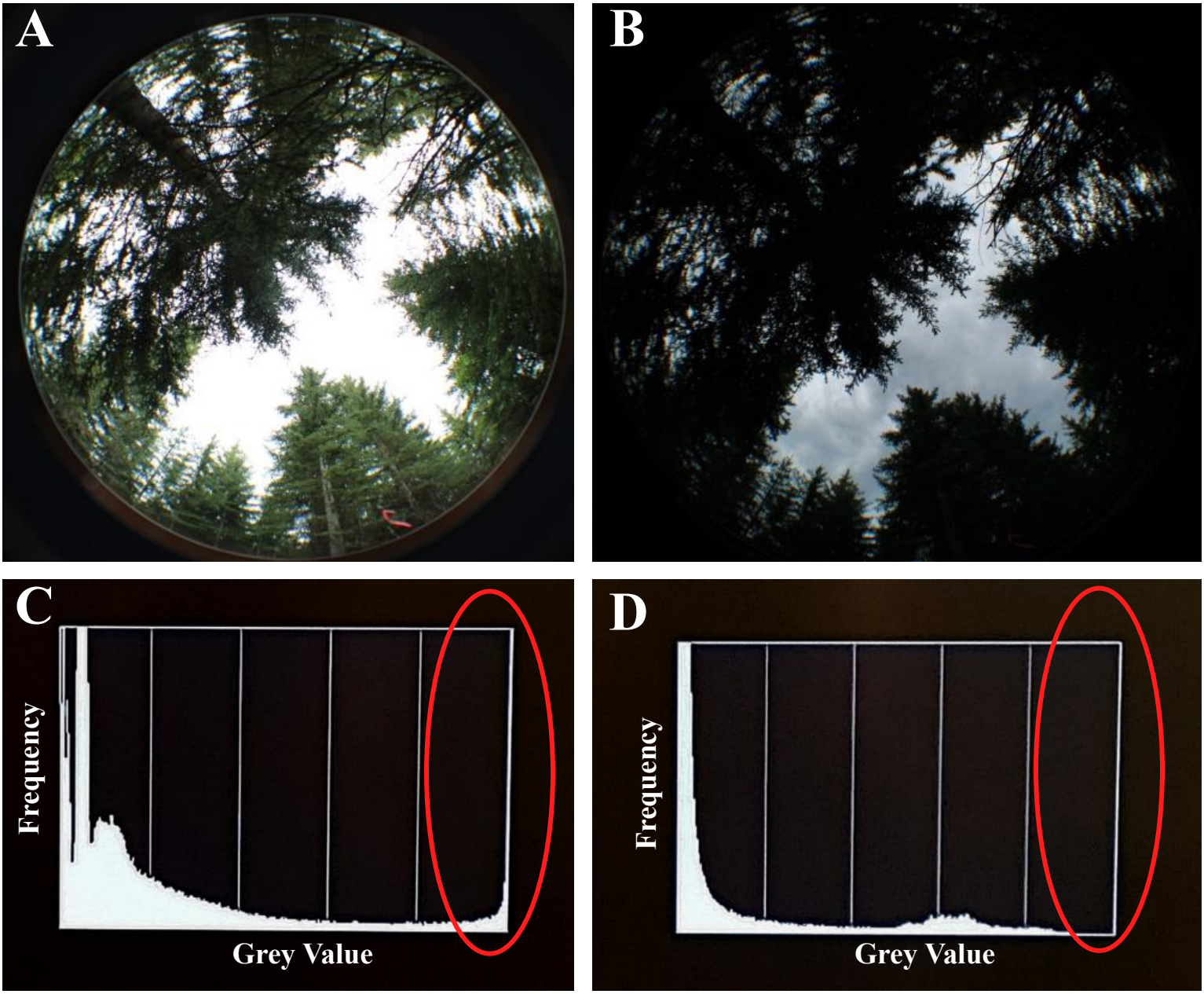


Figure 3.8 The histogram exposure protocol (Beckschäfer et al. 2013) in taking digital hemispherical photographs. 'A' is a hemispherical photograph captured with the "auto" (automatic exposure) setting whereas 'B' was captured with the "P" (programmable exposure) setting with ISO set to 400. 'C' is the grey value histogram for the overexposed photo (A) in which there is a sharp spike on the right most side of the x-axis highlighted by the red circle. 'D' is the grey value histogram for the underexposed photo (B) and completely lacks a spike or any dramatic peaks on the right side of the x-axis highlighted by the red circle. 'C' and 'D' were both captured from the image playback mode on the Canon EOS Rebel SL1 with the histogram function turned on.

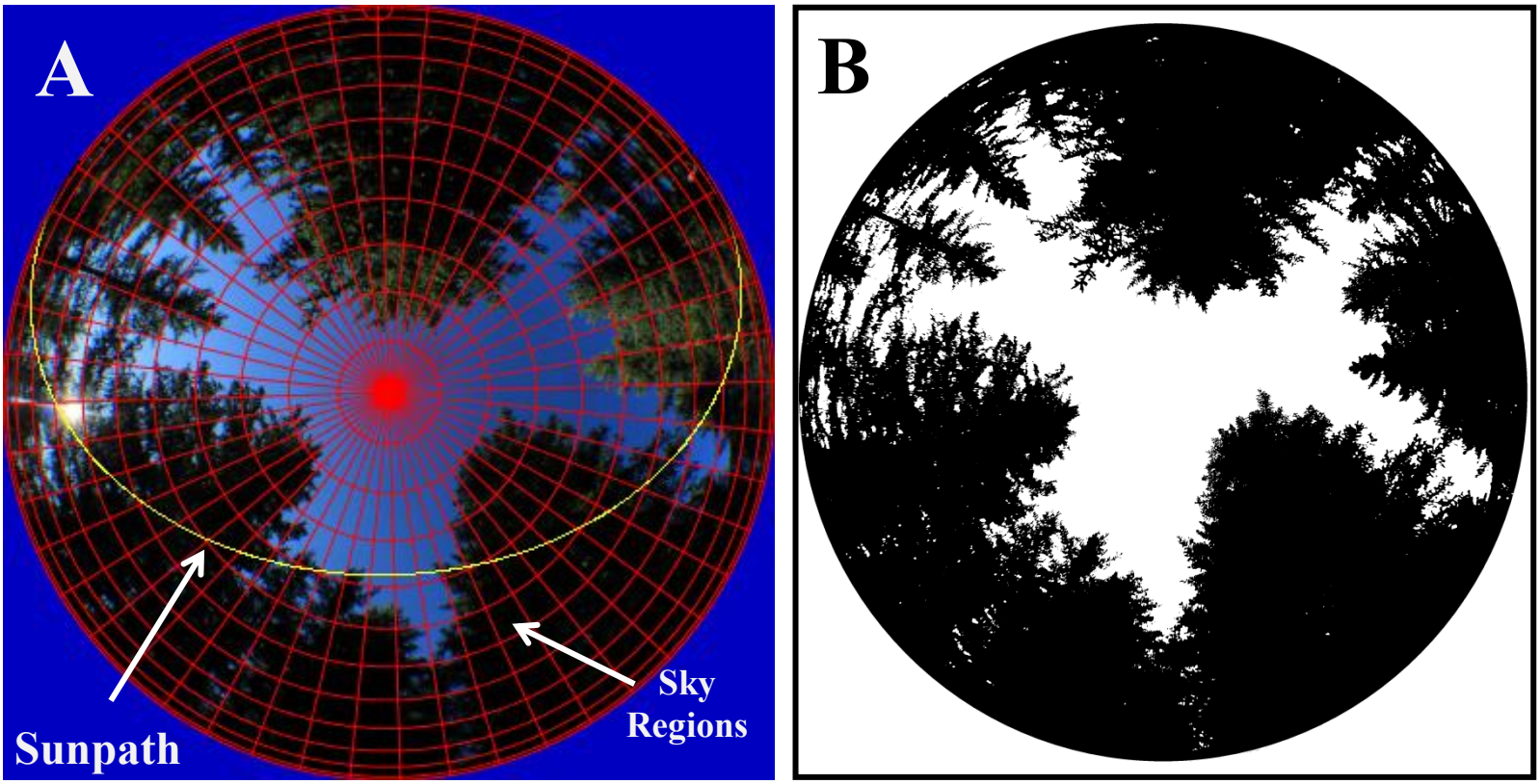
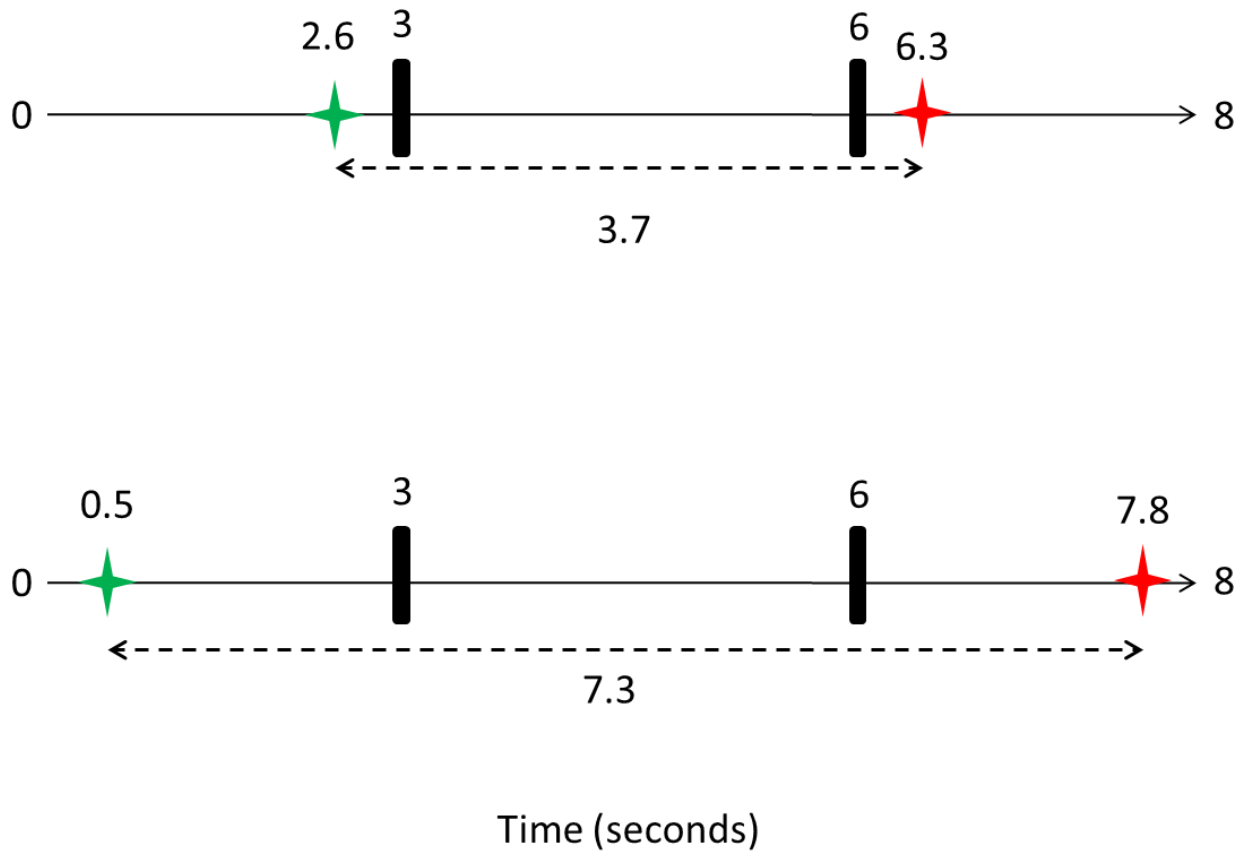


Figure 3.9 The GLA simulation process to assess solar radiation exposure. 'A' shows a hemispherical image with the "overlay mask" function (non-usable areas in blue) with the sun path and sky region graticules on top the image. 'B' shows the threshold binary image in which the sky pixels are white and canopy/vegetation pixels



$$\{Handlingtime(seconds) \approx ((Handlingtime(\#\ of\ frames)\ \times 3) \pm < 3)\}$$

Figure 3.10 A diagram demonstrating how actual pollinator handling time in seconds differs from handling time as the number of camera frames an insect was observed on the flower. The green stars represent when the pollinator first lands on the flower whereas the red star represents its departure. The solid black line represents real time, the black rectangles are the images that the time-lapse cameras capture every three seconds and the dashed line is the actual handling time of a pollinator. The top time line illustrates that even though an insect may have been present for two frame captures, its actual handling time was less than the frame count equivalent of six seconds. The bottom shows the opposite in that the actual handling time of a pollinator can be greater than the frame count equivalent of six seconds. The relationship between handling time recorded in frames versus seconds is also displayed in the equation at the bottom of the diagram.

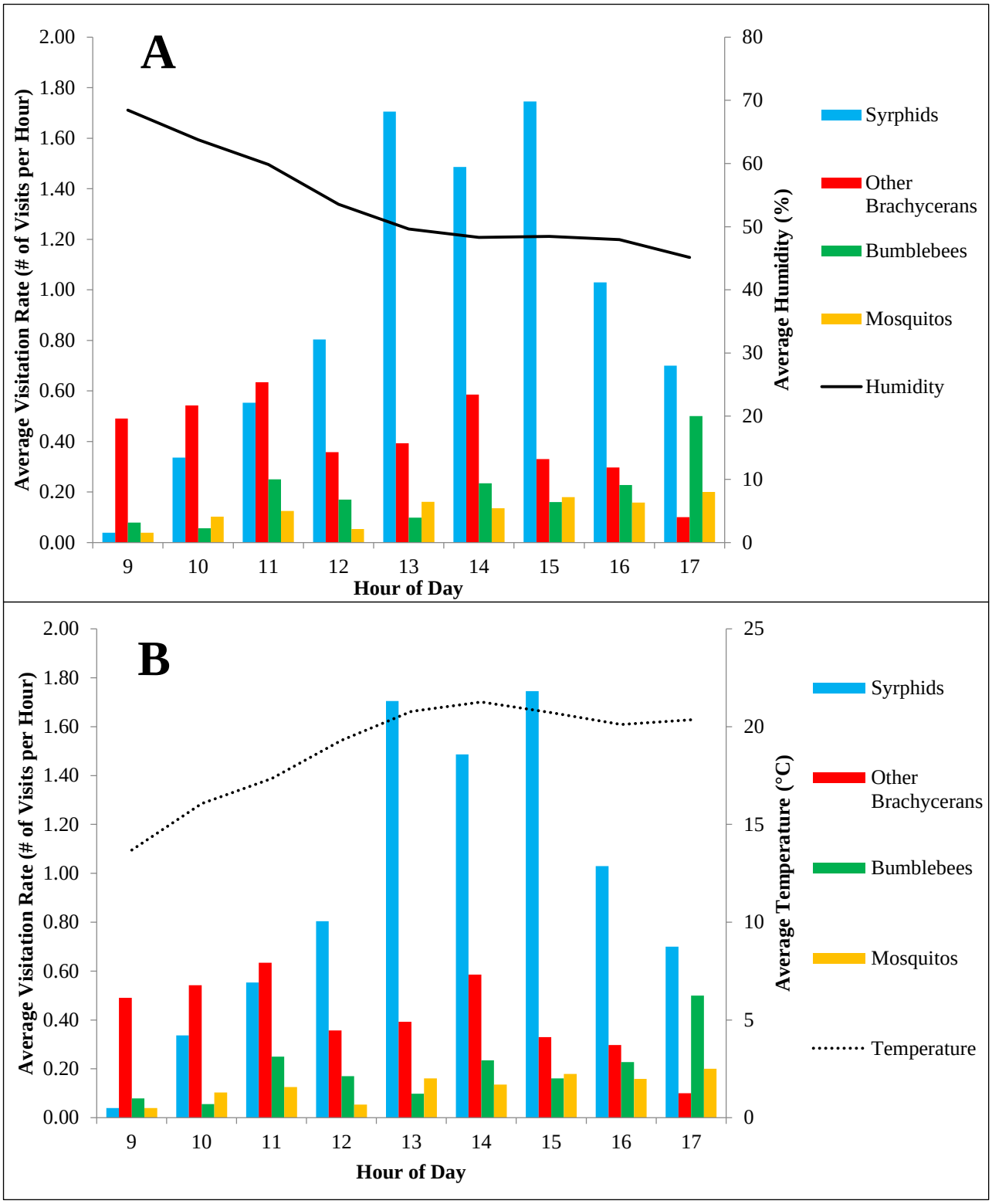


Figure 3.11 The visitation rates for each pollinator clade and hygrothermic factors as a function of the time of day. 'A' is visitation in relation to average humidity whereas 'B' is visitation in relation to average temperature.

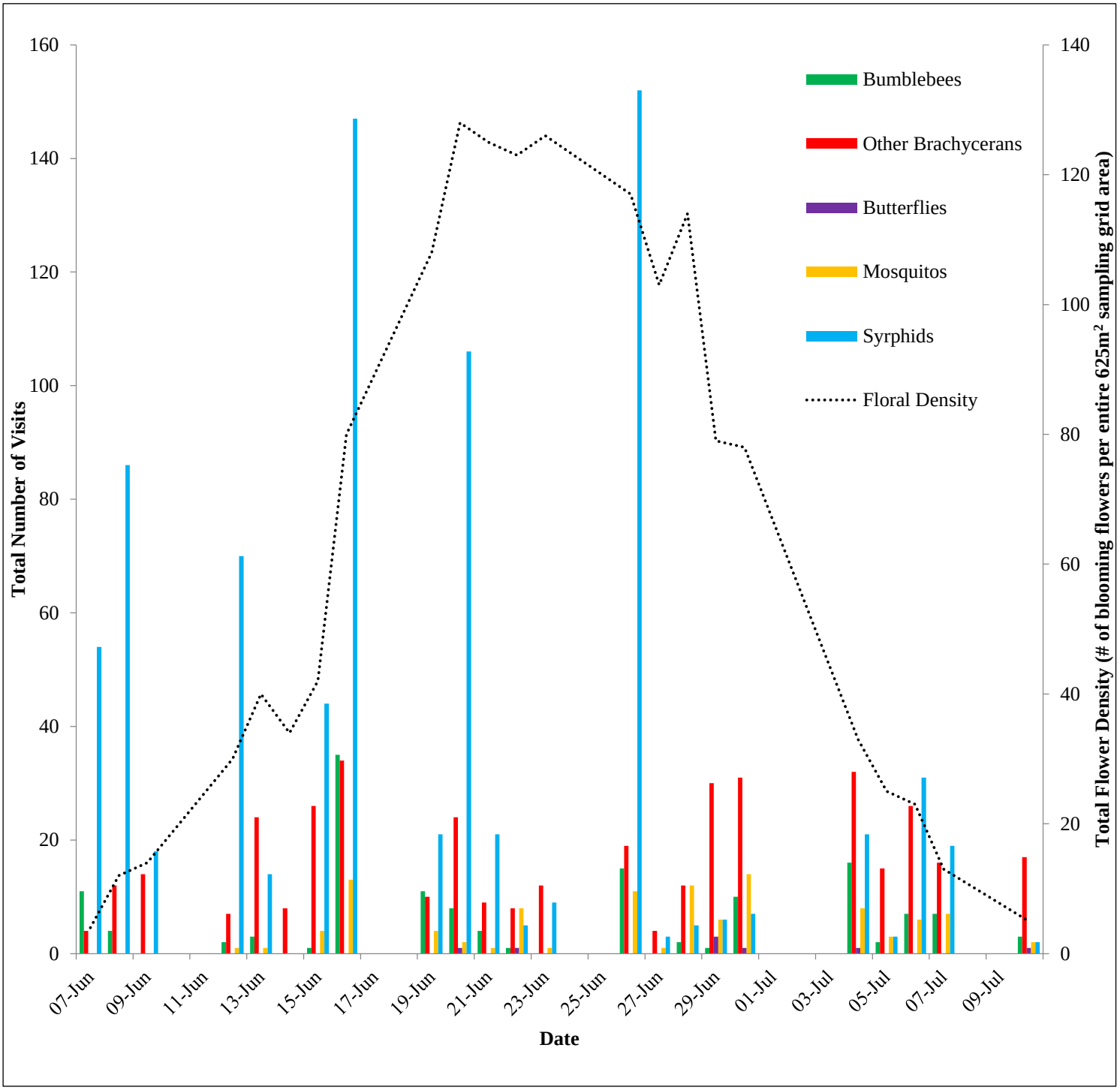


Figure 3.12 The changes in pollinator visits and flower density within the sampling grid for the "natural undisturbed site" across the entire observation period.

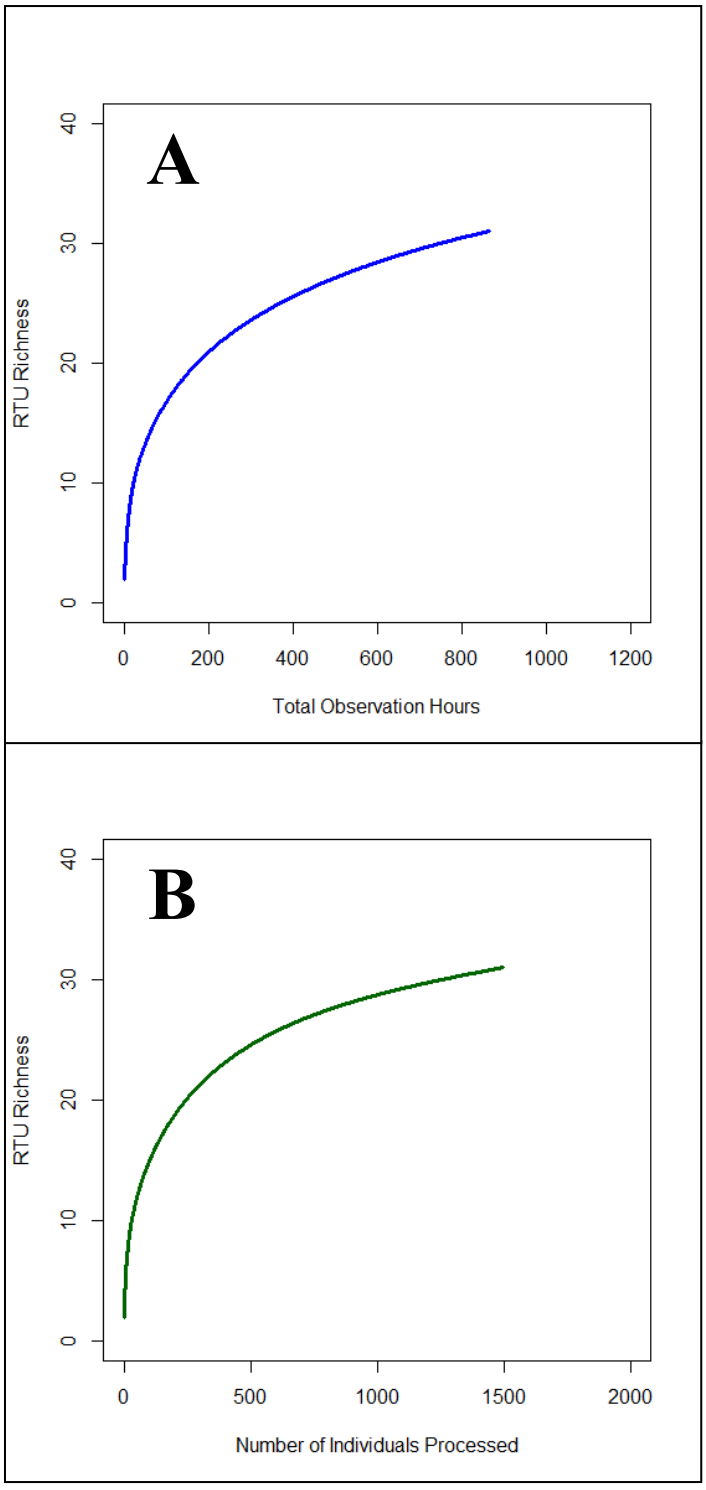


Figure 3.13 Species accumulation curves with daily Recognizable Taxonomic Unit (RTU) richness as a function of sampling effort. 'A' has the sampling effort as total observation hours recorded by the time-lapse cameras whereas 'B' is effort by the number of insects processed or observed visiting focal *R. arcticus* flowers.

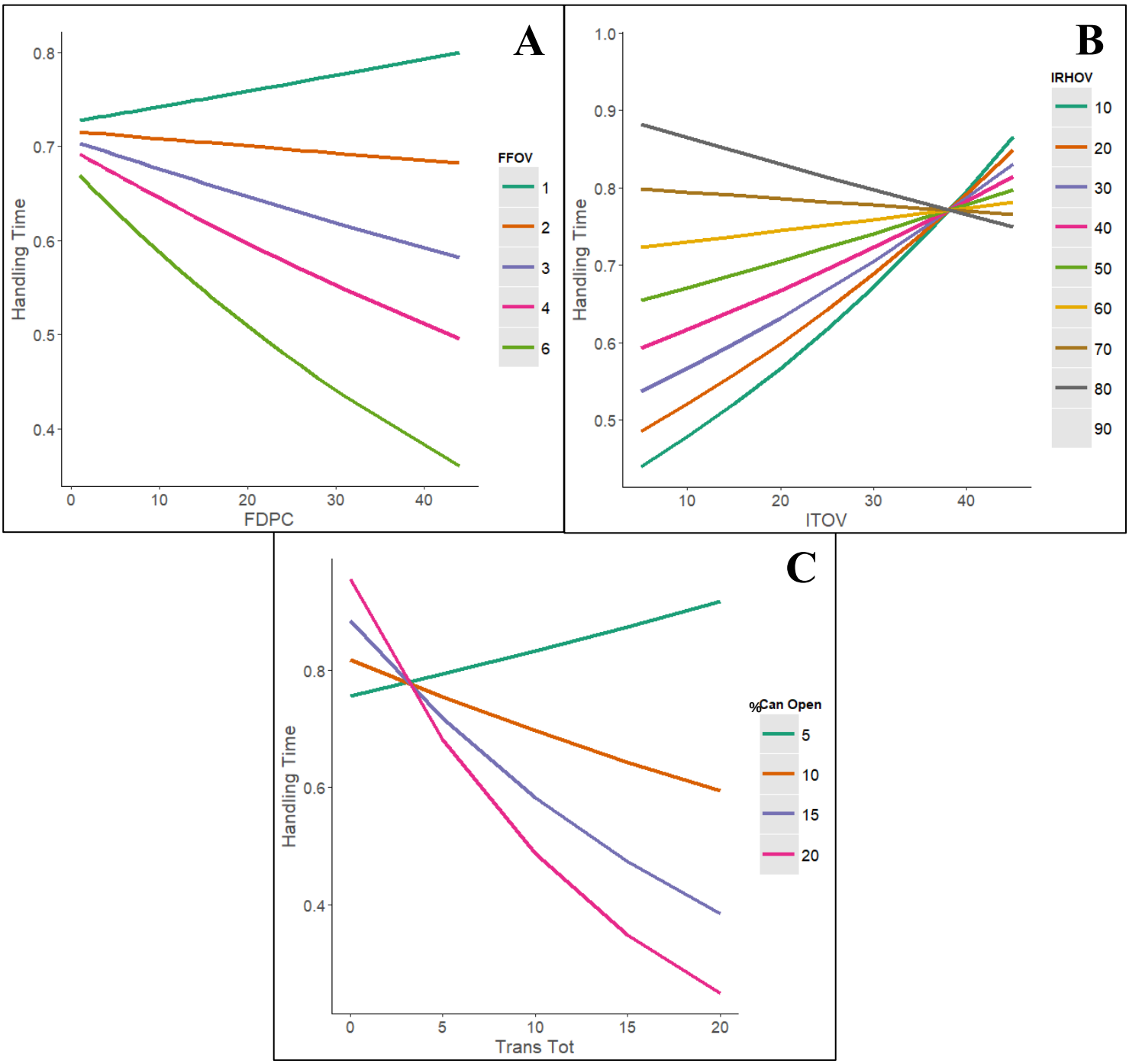


Figure 3.14 Graph plots illustrating interaction effects on handling time (# of frames an insect was observed on a focal flower) as a function of: 'A' FDPC:FFOV, 'B' ITOV:IRHOV, 'C' TransTot:%CanOpen.

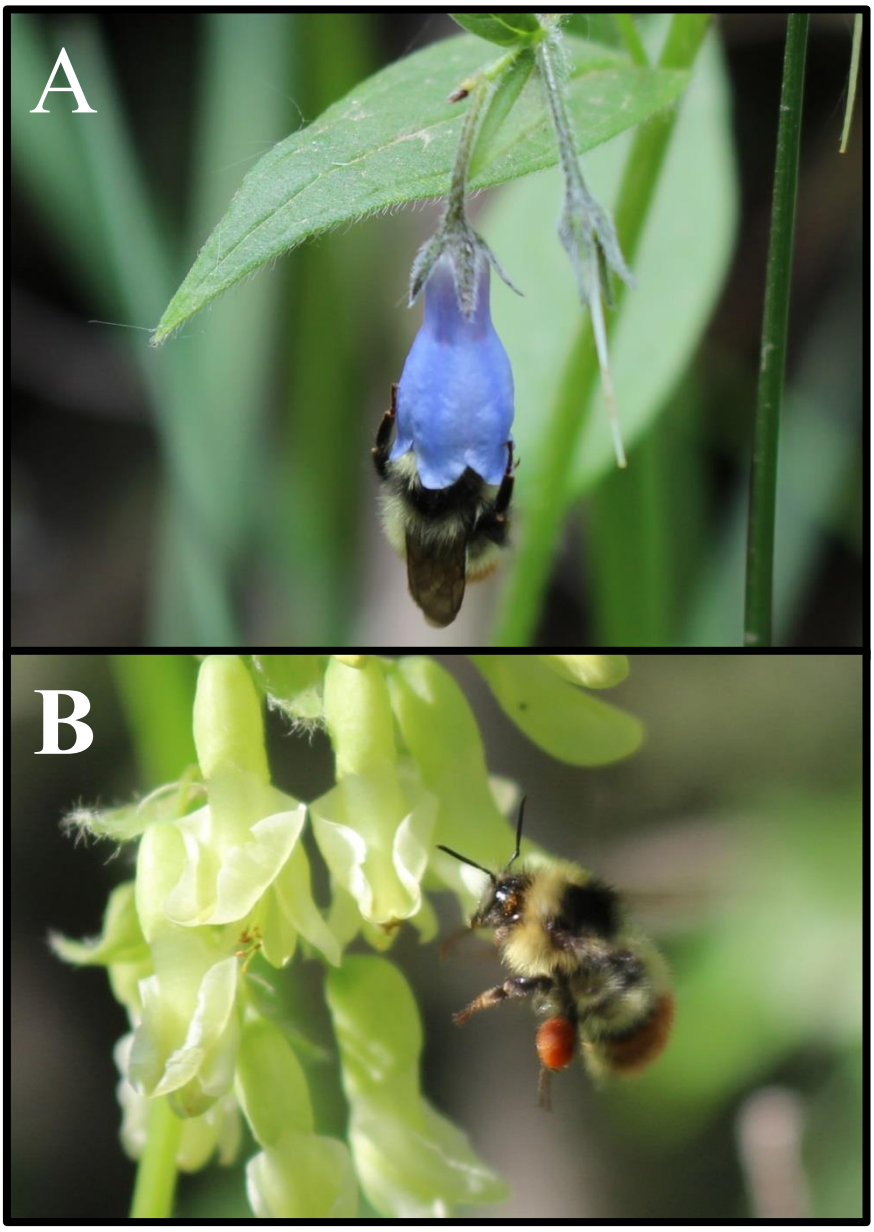


Figure 3.15 Bumblebees (*Bombus* spp.) visiting separate flower species, nearby *Rubus arcticus* focal flowers. ‘A’ features a *Mertensia paniculata* flower (Boraginaceae) whereas ‘B’ features an *Astragalus americanus* (Fabaceae) inflorescence.

Chapter 4: Synthesis of Research

Microclimate does have some effect on the availability of *R. arcticus* pollinators, at least concerning visitation frequencies for the dominant pollinators. Syrphid flies were the most frequent pollinators essentially throughout entire daily surveying periods as well as most of the flowering season. This occurrence of syrphid flies being the dominant pollinator of *R. arcticus* flowers for this population is a novel finding as previous studies found either honeybees or bumblebees comprising the vast majority of floral visits (Ryynänen 1973, Tammisola 1988, Kangasjarvi and Oksanen 1989, Vool et al. 2003). This finding further solidifies Larson et al. (2001) suggestion on the importance of syrphid flies as alpine/montane pollinators. Moreover, Lefebvre et al. (2018) finding of flies becoming the predominant pollinators at elevations of ~1500m could also explain why the syrphid flies were more abundant than bumblebees, as the elevation of the study site was 1406m. Conversely, one must also consider that this experiment had pollinator survey spanning from 9:00 – 17:00 and pollinators have been found to be quite active after this time period, as demonstrated in Edwards et al. (2015). Furthermore, bumblebees are actually homoeothermic and can maintain stable body temperatures via shivering (Heinrich 1979, 1993). This ability even allows bumblebees to fly in extremely cold temperatures, such as ≤ 0°C (Bruggeman 1958, Heinrich, 1993), while still maintaining thoracic temperatures > 35°C (Heinrich 1975, 1993). So the bumblebees could have visited more frequently in the evenings than the syrphid flies and thus, the pollinator surveys could have created an exaggerated juxtaposition between syrphids and bumbles in regards to visitation frequency.

Some may suggest that although bumblebees were far less frequent visitors in this study, they compensated for their irregularity by being more efficient and higher quality pollinators. For

instance, Vool et al. (2003) supports that syrphid flies are actually lower quality pollinators of *R. arcticus* because they do not weigh enough to successfully navigate past the densely packed anthers, whereas honeybees and bumblebees can. Therefore, one may argue that the greater abundance of syrphid flies acting as pollinators for this population could possibly correspond in pollen limitation of fruit set. Additionally, the handling times of the bumblebees may seem exponentially smaller than syrphid flies, but they are extremely efficient pollinators as they have been observed to have much larger pollen loads and deposition rates than flies (Kearns and Inouye 1994). Nevertheless, the effect of handling time on pollen deposition is heavily debated as many have found either that longer residing insects have increased pollen transfer (Thomson 1986, Galen and Stanton 1989, Conner et al. 1995, Hurlbert et al. 1996, Ivey et al. 2003), shorter staying insects deposit more pollen (Gomez and Zamora 1999), or handling time had no effect on pollen transfer (Pettersson 1991, Mitchell and Waser 1992, Cresswell 1999). Additionally, with many syrphid flies being generalists (Haslett 1989), some may suggest that this foraging strategy detracts from their effectiveness. Nonetheless, even generalist syrphid species have been found to be effective pollinators of plants (McGuire and Armbruster 1991). Furthermore, multiple studies have found no existing trade-off between handling time and visitation frequency (Herrera 1989, Jones et al. 1998, Utelli and Roy 2000, Ivey et al. 2003). So regardless of their lesser pollen loads and inefficient pollen transfer, syrphid flies may compensate by being more constant visitors than bumblebees (Kearns and Inouye 1994), as was the case in this study.

Despite being inefficient pollinators, the high frequency of syrphid visitation in this study may be associated with the higher level of pollen delivery observed. As suggested by Ladyman (2006) based on Kangasjarvi and Oksanen (1989) observations, it is very likely that *R. arcticus* is pollinator limited in other parts of its range. Even in cultivated and plantation settings, *R.*

arcticus appears to have low visitation events. For instance, Vool et al. (2003) recorded < 50 visitation events when surveying the Vasula and Kambja plantations, Estonia; in which pollinators were surveyed for 30 minute periods in the morning, noon and afternoon in extremely dense *R. arcticus* rows (~80 flowers/m^2) for five days spanning from May 12 – June 16. The low visitation by bees could be due to competition with other more locally abundant species such as *Mertensia paniculata* and *Astragalus americanus* in which bumblebees were observed to often select over *R. arcticus* flowers. Evans et al. (2017) supports that locally rare plant species are more prone to pollinator limitation and ultimately pollen limitation in florally rich habitats. On the contrary, this population only had 26 other associated flowering plant species (Table C1).

The levels of visitation to *R. arcticus* observed in the region (Chapter 3) appear to be sufficient to deliver ample pollen because I find no evidence to suggest pollen limitation (Chapter 2). There were no statistical significant differences in drupelet yield and fresh berry mass between the naturally pollinated controls and the pollen supplemented outcrossed flowers, which would suggest the species is not pollen limited, although power to detect difference was small. Furthermore, the pollen quantity analysis revealed that stigma breakage was significant in the outcross flowers (Table 2.2) which corresponded with decreased pollen counts that were significantly lower than the control flowers (Table 2.3). Regardless, the number of pollen grains on control flowers was quite substantial, with an average of ~33 grains/stigma. In contrast to *R. idaeus*, a close relative that is not pollen limited, has been observed to have an average of 30 grains/stigma in naturally pollinated flowers, which is more than enough for ample fertilization of ovules (Saez et al. 2014). Because the pollen tube analysis failed to produce any definitive quantitative results, elucidating the effects of pollen quality on *R. arcticus* fruit production is challenging. None of the flowers purely supplemented with selfed pollen produced any fruit or

drupelets whatsoever whereas the control and outcrossed flowers managed to at least produce some berries, so it is reasonable to assume that pollen quality is therefore sufficient to ensure some fruit production albeit very little.

Even if *R. arcticus* is pollinator limited or pollen limited in terms of receiving less outcross pollen, this outcome may not necessarily be as important for the species survival and continuity. In the "Natural Undisturbed Site", of the 2305 *R. arcticus* stems counted within the sampling gird, less than 10% of these produced flowers and less than 1% produced actual fruit. Outside of cultivated settings, others have observed that *R. arcticus* flowers very irregularly in the wild and rarely produces fruit (Spackman et al. 1997, Fertig 2000, Ladyman 2006), which gives the implication that *R. arcticus* predominantly reproduces clonally via rhizomes. Asexual reproduction is the norm for many other alpine and arctic species like *R. arcticus*, as it enables them to still reproduce during harsher periods of environmental stress which may be outside the ranges of sexual reproduction to occur (Bliss 1971). Alternatively, *R. arcticus* is a cold adapted species (Ryynänen 1973) and cold adapted species have been commonly observed to have their southernmost ranges in mid-latitude mountain chains which act as refugia (Abeli et al. 2018). This study location is very likely to be within the southernmost range of *R. arcticus arcticus* in North America based on USDA (2018) distribution maps, so it is quite possible that this population is so genetically isolated, that under no circumstances will it ever be capable of effective sexual reproduction. As well, despite having very high viability (95%, Wada and Reed 2011), *R. arcticus* seeds have a very low germination rate (Ryynänen 1973) and its rapid colonization abilities after disturbance can be attributed almost entirely by vegetative propagules rather than seedlings, that rapidly die off as other plant species encroach (Saastamoinen 1930). As well, *R. arcticus* clonal patches can reach up to 80m in diameter, growing 25cm laterally/year

and have been found to be as old as 160 years old (Tammisola 1988). Knight et al. (2005) suggest that sexual reproduction may not be as important to species that predominantly utilize asexual reproduction because they can divert their resources to other aspects of their life history and still maintain a moderate degree of fitness. Consequently, sexual reproduction may be unnecessary for the species as it is quite capable of survival and colonization via asexual reproduction.

However, with the increasing effects of climate change on plant-pollinator interactions and range shifts, *R. arcticus* may be at risk in the future. Asexual reproduction and cloning is advantageous with short term environmental perturbation as it allows species to rapidly colonize and sustain populations during inhospitable periods (Callaghan et al. 1992). But with abrupt and permanent changes such as the effects of climate change, clonal plant species may be more vulnerable as they may lack sufficient seed recruitment and genetic variation that permits adaptation to the changing environmental conditions. Outside of cultivated settings, *R. arcticus* has relatively low germination rates, seedlings have reduced competitive ability, and populations tend to rely heavily on cloning via rhizome growth (Saastamoinen 1930, Ryynänen 1973, Tammisola 1988). Furthermore, alpine and environments have been documented to have increased summer temperatures and noticeable altitudinal/elevational shifts of plant species towards summits and past treelines (Walther et al. 2002, Parmesan 2006). Therefore, *R. arcticus* populations such as the ones examined in this experiment could potentially suffer as syrphid fly visitation frequencies appear to be negatively affected by temperature. With less pollinators visiting the flowers, *R. arcticus* populations could then be pollen limited and fail to produce viable fruit and seeds with genetic variability that will allow the species adapt with climatic warming. However, this is very likely to be a grandiose assumption as one must consider that

this experiment examined pollinators in regards to microclimate rather than climate. These two environmental processes function across very different spatial scales. Climate occurs on a global to regional scale (> 10,000km – 200km), whereas microclimate can be within a range of < 10m (Pearson and Dawson 2003). So microclimate can drastically change within a site due to factors such as canopy closure/openness, structure and species (i.e. foliage type, deciduous vs. coniferous) proximity to water (i.e. riparian areas) or forest edges (Chen et al. 1999). Therefore, it's uncertain as to how microclimates may vary with climate change and how this ultimately affects pollinator availability. Regardless, as other plant species shift in elevation/altitude with global warming, *R. arcticus* may be outcompeted as bumblebees could have even further decreased visitation with increased heterospecific floral diversity.

Literature Cited

Abe, T. 2001. Flowering phenology, display size, and fruit set in an understory dioecious shrub, *Aucuba japonica* (Cornaceae). American Journal of Botany 88: 455-461.

Abeli, T., J.C.Vamosi and S. Orsenigo. 2018. The importance of marginal population hotspots of cold-adapted species for research on climate change and conservation. Journal of Biogeography 00:1-9.

Afik, O. and S. Shafir. 2007. Effect of ambient temperature on crop loading in the honey bee, *Apis mellifera* (Hymenoptera: Apidae). Entomologia Generalis 29:135-148.

Agren, J. 1989. Seed Size and Number in *Rubus chamaemorus*: Between-Habitat Variation, and Effects of Defoliation and Supplemental Pollination. Journal of Ecology 77: 1080-1092.

Aizen, M.A. and L.D. Harder. 2007. Expanding the limits of pollen-limitation concept effects of pollen quantity and quality. Ecology 88: 271-282.

Alice, L.A., D.H. Goldman, J.A. Maclin and G. Moore. 2015. *Rubus*. In: Flora of North America Editorial Committee (eds.). 1993+. Flora of North America North of Mexico. 20+ vols. New York and Oxford. Vol. 9.

Ashman, T.L, T.M. Knight, J.A. Steets, P. Amaskare, M. Burd, D.R. Campbell, M.R. Dudash, M.O. Johnston, S.J. Mazer, R.J. Mitchell, M.T. Morgan and W.G. Wilson. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85: 2408-2421.

Baker, J.D. and R. W. Cruden. 1991. Thrips-mediated self-pollination of two facultatively xenogamous wetland species. American journal of Botany 78:959-963.

Bankowska, R.1964. Studies on the Palaearctic species of the genus *Sphaerophoria* St. Farg. et Serv. Annotates Zoologici 22: 285-353.

Bates, A.J., S.P. Sadler, A.J. Fairbrass, S.J. Falk, J.D. Hale and T.J. Matthews. 2011. Changing Bee and Hoverfly Pollinator Assemblages along an Urban-Rural Gradient. PLoS ONE 6: e23459.

Beattie, A.J. 1971. Itinerant pollinators in a forest. Madroño 21: 120-124.

Biesmeijer, J.C., S.P. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters, A.P. Schaffers, S.G. Potts, R. Kleukers, C.D. Thomas and J. Settele. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313: 351-354.

Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. American Naturalist 117:838-840.

Boscolo, D., P.M. Tokumoto, P.A. Ferreira, J.W. Ribeiro and J.S. dos Santos. 2017. Positive responses of flower visiting bees to landscape heterogeneity depend on functional connectivity levels. Perspectives in Ecology and Conservation 15: 18-24.

Boyle-Makowski, R.M.D. 1987. The importance of native pollinators in cultivated orchards: their abundance and activities in relation to weather conditions. Proceedings of the Entomological Society of Ontario 118: 125-141.

Boyle-Makowski, R.M.D. and B.J.R. Philogene. 1985. Pollinator activity and abiotic factors in an apple orchard. The Canadian Entomologist 117: 1509-1521.

Brouillet L., F. Coursol, S.J. Meades, M. Favreau, M. Anions, P. Bélisle, and P. Desmet. 2010. *Rubus arcticus* in VASCAN. The Database of Vascular Plants of Canada.

Brouillet L., F. Coursol, S.J. Meades, M. Favreau, M. Anions, P. Bélisle, and P. Desmet. 2010. *Platanthera obtusata* in VASCAN. The Database of Vascular Plants of Canada.

Brooks, D. 2007. Measuring Sunlight at Earth's Surface: Build Your Own Pyranometer [Online]. Institute for Earth Science Research and Education. Available from: http://www.instesre.org/construction/pyranometer/pyranometer.htm

Brooks, M.E., K. Kristensen, K.J. van Benthem, A. Magnusson, C.W. Berg, A. Nielsen, H.J. Skaug, M. Maechler and B.M. Bolker. 2017. Modeling Zero-Inflated Count Data with glmmTMB. bioRxiv preprint bioRxiv:132753.

Brown, A.O. and J.N. McNeil. 2009. Pollination Ecology of the High Latitude, Dioecious Cloudberry (*Rubus chamaemorus*:Rosaceae). American Journal of Botany 96: 1096-1107.

Bruggeman, P.E. 1958. Insect and environment of the high Arctic. Proceedings 10th International Congress of Entomology 1: 695-702.

Bruna, E.M. 2003. Are plant populations in fragmented habitats recruitment limited? Tests with an Amazonian herb. Ecology 84: 932-947.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60:83-139.

Burd, M. 2016. Pollen limitation is common – should it be? (A Comment on Rosenheim et al., "Parental Optimism versus Parental Pessimism in Plants: How Common Should We Expect Pollen Limitation to Be?"). The American Naturalist 187: 388-396.

Burd, M., T.L. Ashman, D.R. Campbell, M.R. Dudash, M.O. Johnston, T.M. Knight, S.J. Mazer, R.J. Mitchell, J.A. Steets and J.C. Vamosi. 2009. Ovule number per flower in a world of unpredictable pollination. American Journal of Botany 96: 1159-1167.

Callaghan, T.V., B.A. Carlsson, I.S. Jónsdóttir, B.M. Svensson and S. Jonasson. 1992. Clonal Plants and Environmental Change: Introduction to the Proceedings and Summary. Oikos 63: 341-347.

Calvo, R. N. 1993. Evolutionary demography of orchids: intensity and frequency of pollination and the cost of fruiting. Ecology 74:1033-1042.

Campbell, J.W., J.L. Hanula and T.A. Waldrop. 2007. Effects of prescribed fire and fire surrogates on floral visiting insects of the blue ridge province in North Carolina. Biological Conservation 134: 393-404.

Cartar, R.V. and L.A Real.1997. Habitat structure and animal movement: the behavior of bumble bees in uniform and random spatial resource distributions. Oecologia 112: 430-434.

Chacoff, N.P., D. Garcia and J.R. Obeso. 2008. Effects of pollen quality and quantity on pollen limitation in *Crataegus monogyna* (Rosaceae) in NW Spain. Flora 203: 499-507.

Chandler, J.L. 2017. A slow opportunist: physiological and growth responses of an obligate understory plant to patch cut harvesting. Oecologia 183:677-687.

Chang, J.J., J.D. Crall and S.A Combes. 2016. Wind alters landing dynamics in bumblebees. Journal of Experimental Biology 219: 2819-2822.

Chen, J., S.C. Saunders, T.R. Crow, R.J. Naiman, K.D. Brosofske, G.D. Mroz, B.L. Brookshire and J.F. Franklin. 1999. Microclimate in Forest Ecosystem and Landscape Ecology: Variations in local climate can be used to monitor and compare the effects of different management regimes. BioScience 49: 288-297.

Christenhusz, M. and J.W. Byng. 2016. The number of known plant species in the world and its annual increase. Phytotaxa 216: 201-217.

Church, N. S. 1960. Heat loss and the body temperatures of flying insects. II. Heat conduction within the body and its loss by radiation and convection. Journal of Experimental Biology 37: 186-211.

Clark C. 2012. Inuit ethnobotany and ethnoecology in Nunavik and Nunastiavut, northeastern Canada [Thesis]. University of Montreal: Montreal, Quebec, Canada.

Cole, L.J., S. Brocklehurst, D. Robertson, W. Harrison and D.I. McCracken. 2015. Riparian buffer strips: their role in the conservation of insect pollinators in intensive grassland systems. Agriculture, Ecosystems and Environment 211: 207-220.

Colling, G., C. Reckinger and D. Matthies. 2004. Effects of Pollen Quantity and Quality on Reproduction and Offspring Vigor in the Rare Plant *Scorzonera humilis* (Asteraceae). American Journal of Botany 91: 1774-1782.

Colwell, R.K., C.X. Mao and J. Chang. 2004. Interpolating, extrapolating and comparing incidence-based species accumulation curves. Ecology 85: 2717-2727.

Combes, S. A. and R. Dudley. 2009. Turbulence-driven instabilities limit insect flight performance. Proceedings of the National Academy of Science USA 106: 9105-9108.

Conner, J.K., R. Davis and S. Rush. 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. Oecologia 104: 234-245.

Conteras, H.L., J.Goyret, M. von Arx, C.T. Pierce, J.L. Bronstein, R.A. Raguso and G. Davidowitz. 2013. The effect of ambient humidity on the foraging behavior of the hawkmoth *Manduca sexta*. Journal of Comparitive Physiology A 199: 1053-1063.

Corbet, S. A., D. M. Unwin, and O.E. Prys-Jones. 1979. Humidity, nectar and insect visits to flowers, with special reference to *Crataegus, Tilia* and *Echium*. Ecological Entomology 4: 9-22.

Corbet, S.A., and E.S. Delfosse. 1984. Honeybees and the nectar of *Echium plantagineum* L. in southeastern Australia. Australian Journal of Ecology 9:125-139.

Corbet, S.A., M. Fussel, R. Ake, A. Fraser, C. Gunson, A. Savage and K. Smith. 1993. Temperature and the pollinating activity of social bees. Ecological Entomology 18: 17-30.

Crawley, M.J. 2003. Statistical Computing. An Introduction to Data Analysis using S-Plus. John Wiley&Sons Ltd: London, UK.

Cresswell, J.E. 1999. The influence of nectar and pollen availability on pollen transfer by individual flowers of oil-seed rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidarius*). Journal of Ecology 87: 670-677.

Datillo, W. and L. Dyer. 2014. Canopy Openness Enhances Diversity of Ant–Plant Interactions in the Brazilian Amazon Rain Forest. Biotropica 46: 712-719.

Dauber, J., J.C. Biesmeijer, D. Gabriel, W.E. Kunin, E. Lamborn, B. Meyer, A. Nielsen, S.G. Potts, S.P.M. Roberts,V. Sober, J. Settele, I. Steffan-Dewenter, J.C. Stout, T. Teder, T. Tscheulin, D. Vivarelli and T. Petanidou. 2010. Effects of patch size and density on flower visitation and seed set of wild plants: a pan-European approach. Journal of Ecology 98: 188-196.

Dion, P.P., J. Bussières and L. Lapointe. 2017. Late canopy closure delays senescence and promotes growth of the spring ephemeral wild leek (*Allium tricoccum*). Botany 95: 457-467.

del Rio, C. and A. Burquez. 1986. Nectar production and temperature dependent pollination in *Mirabilis jalapa* L. Biotropica 18:28-31.

Edwards, J., G.P. Smith and M.H.F. McEntee. 2015. Long-term timelapse video provides near complete records of floral visitation. Journal of Pollination Biology 16: 91-100.

Ehlren, J., S. Kack and J. Agren. Pollen Limitation, Seed Predation and Scape Length in *Primula farinose*. Oikos 97: 45-51.

Ellstrand, N.C. 1992. Gene Flow by Pollen: Implications for Plant Conservation Genetics. Oikos 63: 77-86.

Evans, T.M., S. Cavers, R. Ennos, A.J. Vanbergen and M.S. Heard. 2017. Florally rich habitats reduce insect pollination and the reproductive success of isolated plants. Ecology and Evolution 7: 6507-6518.

Fang, J. 2010. Ecology: A world without mosquitoes. Nature 466: 432-434.

Feinsinger, P., K.G. Murray, S. Kinsman and W.H. Busby. 1986. Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. Ecology 67:449-464.

Fertig, W. 2000. Ecological assessment and monitoring program for northern blackberry (*Rubus acaulis*) in the Bighorn National Forest, Wyoming. Wyoming Natural Diversity Database: Laramie, Wyoming, USA.

Fijen, T.P.M. and D. Kleijn. 2017. How to efficiently obtain accurate estimates of flower visitation rates by pollinators. Basic and Applied Ecology 19: 11-18.

Fischlin, A., G.F. Midgley, J.T. Price, R. Leemans, B. Gopal, C. Turley, M.D.A. Rounsevell, O.P. Dube, J. Tarazona and A.A. Velichko. 2007. Ecosystems, their properties, goods, and services. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson (eds.). Cambridge University Press: Cambridge, UK.

Fisogni, A., M. Rossi, L. Bortolotti, G. Bogo, N. de Manicor, M. Quaranta and M. Galloni. 2016. Seasonal and annual variations in the pollination efficiency of a pollinator community of *Dictamnus albus L.* Plant Biology 18: 445-454.

Foster, W.A. 1995. Mosquito nectar feeding and reproductive energetics. Annual Review of Entomology 40: 443-474.

Frazer, G.W., C.D Canham and K.P. Lertzman. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true - colour fisheye photographs, users manual and program documentation. Copyright © 1999: Simon Fraser University: Burnaby, British Columbia, Canada and the Institute of Ecosystem Studies: Millbrook, New York, USA.

Free, J. B. 1960. The pollination of fruit trees. Bee World 41:141-169.

Fye, R.E., 1972. The effects of forest disturbances on populations of wasps and bees in northwestern Ontario (Hymenoptera: Aculeata). The Canadian Entomologist. 104. 1623-1633.

Galen, C. and M.L. Stanton. 1989. Bumble bee pollination and floral morphology: factors influencing pollen dispersal in the alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). American Journal of Botany 76: 419-426.

Gallai, N., J.M. Salles, J. Satelle and B.E. Vaissière. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68: 810-821.

Garibaldi, L.A., I. Steffan-Dewenter, R. Winfree, M.A. Aizen, R. Bommarco, S.A. Cunningham, C. Kremen, L.G. Carvalheiro, L.D. Harder, O. Afik and I. Bartomeus. 2013. Wild pollinators enhance fruit set of crops regardless of honeybee abundance. Science 339:1608-1611.

Garibaldi, L.A., L.G. Carvalheiro, S.D. Leonhardt, M.A. Aizen, B.R. Blaauw, R. Isaacs, et al. 2014. From research to action: Enhancing crop yield through wild pollinators. Frontiers in Ecology and the Environment 12: 439-447.

Geiger,R. 1965. The Climate near the Ground. Harvard University Press: Cambridge, Massachusetts, USA.

Ghazoul, J. 2005. Pollen and seed dispersal among dispersed plants. Biological Review 80: 413-443.

Gilbert, F.S. 1985. Diurnal activity patterns in hoverflies (Diptera, Syrphidae). Ecological Entomology 10: 385-392.

Gilpin, A.M., A.J. Denham and D.J. Ayre. 2017. The use of digital video recorders in pollination biology. Ecological Entomology 42: 383-388.

Gomez, J.M. and R. Zamora. 1999. Generalization vs. specialization the pollination system of *Hormathophylla spinosa* (Cruciferae). Ecology 80: 796-805.

Gonzalez-Tagle, M.A., J. Jimenez Perez and W. Himmelsbach. 2011. Impact of firewood extraction on leaf area index and canopy openness in mixed pine-oak forests in northeast Mexico. Forstarchiv 82:20-25.

Gorham, J R. 1976. Orchid pollination by *Aedes mosquitoes* in Alaska. The American Midland Nationalist 95:208-210.

Gotelli, N.J. and R.K. Colwell. 2011. Chapter 4: Estimating Species Richness. In: Magurran, A.E. and B.J. McGill (eds.). Biological Diversity – Frontiers in Measurement and Assessment. Oxford University Press: Oxford, UK.

Goulson, D.2003. Bumblebees: Behaviour and Ecology. Oxford University Press: Oxford, UK.

Goulson, D., G.C. Lye and B. Darvill.2008. Decline and conservation of bumblebees. Annual Review of Entomology 53: 191-208.

Grant, B. R. and P. R. Grant. 1981. Exploitation of *Opuntia* cactus by birds on the Galapagos. Oecologia (Berlin) 49: 179-187.

Grundel, R., R.P. Jean, K.J. Frohnapple, G.A. Glowacki, P.E. Scott and N.B. Pavlovic. 2010. Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. Ecological Applications 20: 1678-1692.

Haig, D. and M. Westoby. 1988. On limits to seed production. American Naturalist 131:757-759.

Hallsworth, B. and C.C. Chinnappa. 1997. Plants of Kananaskis Country in the Rocky Mountains of Alberta. University of Alberta Press: Edmonton, Alberta, Canada.

Haslett, J.R.1989. Interpreting patterns of resource utilization: randomness and selectivity in pollen feeding by adult hoverflies. Oecologia 78: 433-442.

Hardy, J.P., R. Melloh, G. Koenig, D. Marks, A. Winstral, J.W. Pomeroy and T. Link. 2004. Solar radiation transmission through conifer canopies. Agricultural and Forest Meteorology 126: 257-270.

Hebda, R.J. and C.C. Chinnappa. 1990. Studies on pollen morphology of Rosaceae in Canada. Review of Paleobotany and Palynology 64: 103-108.

Hayduk, E.A. 2012. Using LiDAR Data to Estimate Effective Leaf Area Index, Determine Biometrics and Visualize Canopy Structure in a Central Oregon Forest with Complex Terrain [Thesis]. Evergreen State College: Olympia, Washington, USA.

Hanula, J.L., S. Horn and J.J. O'Brien. 2015. Have changing forests conditions contributed to pollinator decline in the southeastern United States? Forest Ecology and Management 348: 142-15.

Heard, T.A. and J.K. Hendrikz. 1993. Factors Influencing Flight Activity of Colonies of the Stingless Bee *Trigona carbonaria* (Hymenoptera : Apidae). Australian Journal of Zoology 41: 343-353.

Hegland, S.J. and Ø. Totland. 2005. Relationships between species' floral traits and pollinator visitation in a temperate grassland. Oecologia 145: 586-594.

Hegland, S.J. and L. Boeke. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community Ecological Entomology 31: 532-538.

Hegland, S.J. and Ø. Totland. 2008. Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? Oikos 117: 883-891.

Heinrich, B.1975. Thermoregulation in bumblebees. II. Energetics of warm-up and free flight. Journal of Comparative Physiology A 96: 155-166.

Heinrich, B. 1979. Bumblebee Economics. Harvard University Press: Cambridge, MA, USA and London, UK.

Heinrich, B.1993. The Hot-Blooded Insects. Strategies and Mechanisms of Thermoregulation. Springer-Verlag: Berlin and Heidelberg, Germany.

Herrera, C.M. 1989. Pollinator Abundance, Morphology, and Flower Visitation Rate: Analysis of the "Quantity" Component in a Plant-Pollinator System. Oecologia 80: 241-248.

Herrera, C.M. 1995a. Floral Biology, Microclimate, and Pollination by Ectothermic Bees in an Early-Blooming Herb. Ecology 76: 218-228.

Herrera, C. 1995b. Microclimate and individual variation in pollinators: flowering plants are more than their flowers. Ecology 76: 1516-1524.

Herrera, C.M. 1997. Thermal Biology and Foraging Responses of Insect Pollinators to the Forest Floor Irradiance Mosaic. Oikos 78: 601-611.

Herrera, C.M. and O. Pellmyr. 2002. Plant-Animal Interactions: An Evolutionary Approach. Blackwell Science: Oxford and Malden, Massachusetts, USA.

Hickling, R., D.B. Roy, J.K. Hill, R. Fox and C.D. Thomas. 2006.The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology 12: 450-455.

Horvitz, C.C. and D.W. Schemske. 1988. A test of the pollinator limitation hypothesis for a neotropical herb. Ecology 69: 200-206.

Høye, T.T., E. Post, N.M. Schmidt, K. Trøjelsgaard and M.C. Forchhamme. 2013. Shorter flowering seasons and declining abundance of flower visitors in a warmer Arctic. Nature Climate Change 3: 759-763.

Holland, J.N. and S.A. Chamberlain. 2007. Ecological and Evolutionary Mechanisms for Low Seed: Ovule Ratios: Need for a Pluralistic Approach? Ecology 88: 706-715.

Hothorn, T., F. Bretz and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50: 346-363.

Hubalek, Z. 2000. Measures of species diversity in ecology: an evaluation. Folia Zoologica 49:241-260.

Hulten, E. 1971. The circumpolar plants. II. Dicotyledons. Kungl. Svenska Vetenskaps Akademiens Handlingar. 4. Ser. 13, 1: 164-165, 373-375.

Hurlbert, A.H., S.A. Hosoi, E.J. Temeles and P.W. Ewald. 1996. Mobility of *Impatiens capensis* flowers: effect on pollen deposition and hummingbird foraging. Oecologia 105: 243-246.

Iqbal, M. 1983. An introduction to solar radiation. Academic Press: Orlando, Florida, USA.

Ingelög. T., R. Anderson, and M. Tjernberg. 1993. Red Data Book of the Baltic Region. Part 1. Lists of threatened vascular plants and vertebrates. Swedish Threatened Species Unit: Upsala, Sweden.

Iler, A.M., D.W. Inouye, T.T. Hoye, A.J. Miller-Rushing, L.A. Burkle and E.B. Johnston. Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. Global Change Biology 19: 2348-2359.

Ishii, S.H., Y. Hirabayashi and G. Kudo. 2008. Combined effects of inflorescence architecture, display size, plant density, and empty flowers on bumble bee behavior: experimental study with artificial inflorescences. Oecologica156: 341-350.

Ivey, C.T., P. Martinez and R. Wyatt. 2003 Variation in Pollinator Effectiveness in Swamp Milkweed, *Asclepias incarnata*(Apocynaceae). American Journal of Botany 90: 214- 225.

Jamil, T., W.A. Ozinga, M. Kleyer and C.J.F. ter Braak. 2013. Selecting traits that explain species-environment relationships: a Generalized Linear Mixed Model approach. Journal of Vegetation Science 24: 988-1000.

Jauh, G.Y. and E.M. Lord. 1995. Movement of the tube cell in the lily style in the absence of the pollen grain and the spent pollen tube. Sexual Plant Reproduction 8: 168-172.

Jennings, S.B., N.D. Brown and D. Sheil. 1999. Assessing forest canopies and understorey illumination: canopy closure, canopy cover and other measures. Forestry 72: 59-74.

Jia, X., R. Huang, Y. Zhou and Z. Su. 2015. Patterns of Understory Plant Diversity in Response to Transmitted Solar Radiation in a Subtropical Forest. International Symposium on Material, Energy and Environment Engineering.

Johnson, D., L. Kershaw, A. MacKinnon and J. Pojar.1995. Plants of the western boreal forest and aspen parkland. Lone Pine Publishing and the Canadian Forest Service: Edmonton, Alberta, Canada.

Johnston, M.O. 1991. Pollen Limitation of Female Reproduction in *Lobelia Cardinalis* and *L. Siphilitica*. Ecology 72: 1500-1503.

Jones, K.N., J.S. Reithel and R.E. Irwin. 1998. A trade-off between the frequency and duration of bumblebee visits to flowers. Oecologia 117: 161-168.

Jordano, P. 1995. Angiosperm Fleshy Fruits and Seed Dispersers: A Comparative Analysis of Adaptation and Constraints in Plant-Animal Interactions. The American Naturalist 145: 163-191.

Josens, R.B. and W.M. Farina. 2001. Nectar feeding by the hovering hawk moth *Macroglossum stellatarum*: intake rate as a function of viscosity and concentration of sucrose solutions. Journal of Comparative Physiology A 187: 661-665.

Kangasjarvi, J. and J. Oksanen. 1989. Pollinator behaviour in cultivated and wild arctic bramble (*Rubus arcticus* L.). Journal of Agricultural Science in Finland 61: 33-38.

Karbassioon, A. 2017. Pollinator Activity along an Environmental Gradient [Thesis]. University of Oslo: Oslo, Norway.

Karp, K., M. Mand, M. Starast and T. Paal. 2004. Nectar production of *Rubus arcticus.* Agronomy Research 2: 57-61.

Kearns, C.A. and D.W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado: Niwot, Colorado, USA.

Kearns, C.A. and D.W. Inouye. 1994. Fly pollination of *Linum lewisii* (Linaceae). American Journal of Botany 81: 1091-1095.

Keep, E. 1968. Incompatibility in *Rubus* with special reference to *Rubus ideaus* L. Canadian Journal of Genetics and Cytology 10: 253-262.

Kerr, J.T., A. Pindar, P. Galpern, L. Packer, S.G. Potts, S.M. Roberts, P. Rasmont, O. Schweiger, S.R. Colla, L.L. Richardson, D.L. Wagner, L.F. Gall, D.S. Sikes and A. Pantoja. 2015. Climate change impacts on bumblebees converge across continents. Science 349: 177-180.

Kevan, P.G. and H.G. Baker. 1983. Insects as flower visitors and pollinators. Annual Review of Entomology 28: 407-453.

Kho, Y.O. and J. Baër. 1968. Observing pollen tubes by means of fluorescence. Euphytica 17:298-302.

Kingsolver, R.W. 1986. Vegetative reproduction as a stabilizing feature of the population dynamics of *Yucca glauca*. Oecologia 69:380-387.

Klein, A.M., B.E. Vaissière, J.H. Cane, I. Stephan-Dewenster, S.A. Cunningham, C. Kremen and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B 274: 303-313.

Klinkhamer, P.G.L and T.J. de Jong. 1990. Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). Oikos 57:399-405.

Knight, T.M., J.A. Steets, J.C. Vamosi, S.J. Mazer, M. Burd, D.R. Campbell, M.R. Dudash, M.O. Johnston, R.J. Mitchell and T.L. Ashman. 2005. Pollen Limitation of Plant Reproduction: Pattern and Process. The Annual Review of Ecology, Evolution and Systematics 36: 467-497.

Knight, T.M., J.A. Steets and T.L. Ashman. 2006. A Quantitative Synthesis of Pollen Supplementation Experiments Highlights the Contribution of Resource Reallocation to Estimates of Pollen Limitation. American Journal of Botany 93: 271-277.

Kostamo, K. A. Toljamo, P. Palonen, J.P.T. Valkonen, S.O. Karenlampi and H. Kokko. 2015. Control of downy mildew (*Peronospora sparsa*) in arctic bramble (*Rubus arcticus ssp. arcticus*). Annals of Applied Biology 167: 90-101.

Kotilainen, M. J. 1949. Luonnonkasvien hallavaurioista.Suom. Tiedeakatemian esitelmät ja pöytäkirjat 1948: 137-153.

Kovac, H. and A. Stabentheiner. 2011. Thermoregulation of foraging honeybees on flowering plants: seasonal variability and influence of radiative heat gain. Ecological Entomology 36: 686-699.

Krell, F.T. 2004. Parataxonomy vs. taxonomy in biodiversity studies - Pitfalls and applicability of 'morphospecies' sorting. Biodiversity and Conservation: 795-812.

Kukk, T. and T. Kull. 2005. Easti taimede levikuatlas [Atlas of the Estonian Flora]. EMÜ põllumajandusja keskkonnainstituut: Tartu, Estonia.

Kunin, W.E. 1997. Population Size and Density Effects in Pollination: Pollinator Foraging and Plant Reproductive Success in Experimental Arrays of *Brassica kaber*. Journal of Ecology 85: 225-234.

Kwak, M. M., and O. Jennersten. 1986. The significance of pollination time and frequency and of purity of pollen loads for seed set in *Rhinanthus angustifolius* (Scrophulariaceae) and *Viscaria vulgaris* (Caryophyllaceae). Oecologia (Berlin) 70:502-507.

Kwak, M. M., and O. Jennersten. 1991. Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris*: heterospecific pollen and pollen limitation. Oecologia (Berlin) 86:99-104.

Ladyman, J.A.R. 2006. *Rubus arcticus* L. ssp. *acaulis* (Michaux) Focke (dwarf raspberry): A technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available from: https://www.fs.usda.gov/detail/r2/landmanagement/?cid=stelprdb5206999

Landert, K.A. 2016. Comparing Photographic and GIS-based Applications for Estimating Canopy Cover in Southern Appalachian Bogs. Proceedings of The National Conference On Undergraduate Research (NCUR). University of North Carolina: Asheville, North Carolina, USA.

Larson, B.M.H. and S.C.H. Barrett. 2000. A comparison of pollen limitation in flowering plants. Biological Journal of the Linnaean Society 69:503-520.

Larson, B.M.H., P.G. Kevan and D.W. Inouye. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. Canadian Entomologist 133: 439-465.

Lawrence, M.G. 2005. The Relationship between Relative Humidity and the Dewpoint Temperature in Moist Air: A Simple Conversion and Applications. Bulletin of the American Meteorological Society 86: 225-233.

Lee, Y.J., R.I. Alfaro and G.A. Van Sickle. 1983. Tree-crown defoliation measurement from digitized photographs. Canadian Journal of Forest Research 13: 956-961.

Lefebvre, V., C. Villemant, C. Fontaine and C. Daugeron. 2018. Altitudinal, temporal and trophic partitioning of flower-visitors in Alpine communities. Scientific Reports 8: 4706.

Leighton A.1985. Wild Plant Use by the Woods Cree (Nihithawak) of East-Central Saskatchewan. National Mercury Series: Ottawa, Ontario, Canada.

Lenth, R.V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69: 1-33.

Levin, D.A. 1990. Sizes of natural macrogametophyte populations in pistils of *Phlox drummondii*. American Journal of Botany 77: 356-353.

Lortie, C.J., A.E. Budden and A.M. Reid. 2012. From birds to bees: Applying video observation techniques to invertebrate pollinators. Journal of Pollination Ecology 6: 125-128.

Löve, Á. 1987. Chromosome number reports XCVI. Taxon 36:659-661.

Lüdecke, D. 2018. sjPlot: Data Visualization for Statistics in Social Science. R package version 2.4.1. Available from: https://CRAN.R-project.org/package=sjPlot

Lui, B.Y.H., and R.C. Jordan. 1960. The interrelationship and characteristic distribution of direct, diffuse and total solar radiation. Solar Energy 4: 1-19.

Lundie, A. E.1925. The flight activities of the honeybee. United States Department of Agriculture Bulletin 1328.

Magnusson, A., H.J. Skaug, A. Nielsen, C.W. Berg, K. Kristensen, M. Maechler, K.J. van Bentham, B.M. Bolker and M.E. Brooks. 2017. glmmTMB: Generalized Linear Mixed Models using Template Model Builder. R package version 0.1.3. Available from: https://github.com/glmmTMB

Maier, C.T. and G.P. Waldbauer.1979. Diurnal activity patterns of flower flies (Diptera: Syrphidae) in an Illinois sand area. Annals of the Entomological Society of America 372: 231-245.

Maiz-Tome, L. 2016. *Rubus arcticus*. The IUCN Red List of Threatened Species 2016: e.T64323866A67730712

Maindonald, J. and J. Braun. 2007. Data Analysis and Graphics Using R – An Example-Based Approach [2nd Edition]. Cambridge University Press: Cambridge, UK.

Manetas, Y. and Y. Petropoulou. 2000. Nectar amount, pollinator visit duration and pollination success in the Mediterranean shrub *Cistus creticus*. Annals of Botany 86: 815-820.

Marchand, P.J. and D.A. Roach. 1980. Reproductive Strategies of Pioneering Alpine Species: Seed Production, Dispersal, and Germination. Arctic and Alpine Research 12: 137-146.

Marshall, D. L. and N. C. Ellstrand. 1985. Proximal causes of multiple paternity in wild radish, *Raphanus sativus*. American Naturalist 126:596-605.

Martin, F., 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology 34: 125-128.

May, M. L. and T.M. Casey.1983. Thermoregulation and heat exchange in euglossine bees. Physiological Zoology 56: 541-551.

McConnaughay, K.D.M. and J.S. Coleman. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80: 2581-2593.

McDade, L. and S. Kinsman. 1980. The impact of floral parasitism in two neotropical hummingbird pollinated plant species. Evolution 34:944-958.

McGuire, A.D. and W.S. Armbruster.1991. An experimental test for reproductive interactions between two sequentially blooming *Saxifraga* species (Saxifragaceae). American Journal of Botany 78: 214-219.

Menges, E.S. 1991. The application of minimum viable population theory to plants.In: D.A. Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press: New York City, New York, USA.

Mitchell, R.J. and N.M. Waser. 1992. Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. Ecology 73: 633-638.

Morales, C.L. and M.A. Aizen. 2006. Invasive mutualisms and the structure of plant–pollinator interactions in the temperate forests of north-west Patagonia, Argentina. Journal of Ecology 94: 171-180.

Moss, E.H. 1983. Flora of Alberta [2nd Edition]. Packer, J.G. (ed.). University of Toronto Press: Toronto, Ontario, Canada.

Mulcahy, G. B., and D. L. Mulcahy. 1982. The two phases of growth of *Petunia hybrida* (Hort. Vilm-Andz.) pollen tubes through compatible styles. Journal of Palynology 18:61-64.

Murray, B.D., S.A. Holmes, C.R. Webster and J.C. Witt. 2012. Post-Disturbance Plant Community Dynamics following a Rare Natural-Origin Fire in a *Tsuga canadensis* Forest. PLoS One 7: e43867.

Natural Resources Canada. 2017. Magnetic Declination Calculator. Government of Canada. Available from: http://www.geomag.nrcan.gc.ca/calc/mdcal-en.php

Nicolson, S.W., L. de Veer, A. Kohler and C.W.W. Pirk. 2013. Honeybees prefer warmer nectar and less viscous nectar, regardless of sugar concentration. Proceedings of the Royal Society B 280: 20131597.

Nielsen, T.K. 1966. Species of the genus *Helophilus* (Dipt., Syrphidae) found on Jaeren, Rogaland. Norsk Entomologisk Tidsskrift 13: 427-439.

Nobis, M. and U. Hunziker. 2005. Automatic thresholding for hemispherical canopy-photographs based on edge detection. Agricultural and Forest Meteorology128: 243-250.

O'Hara, R. and J. Kotze. 2010. Do not log-transform count data. Methods in Ecology and Evolution 1: 118-112.

Ohguchi, O. and K. Aoki. 1983. Effects of colony need for water on optimal food choice in honey-bees. Behavior Ecology and Sociobiology 12:77-84.

Oke, T.R. 1987. Boundary Layer Climates [2nd Edition]. Routledge: New York City, New York, USA.

Oksanen, J., F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R. B. O'Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs and H. Wagner. 2018. vegan: Community Ecology Package. R package version 2.4-6. Available from: https://CRAN.R-project.org/package=vegan

Oleson, J.M., Y.L. Dupont, B.K. Ehlers and D.M. Hansen. 2007. The openness of a flower and its number of flower-visitor species. Taxon 56: 729-736.

Oliver, I., and A. J. Beattie. 1993. A possible method for the rapid assessment of biodiversity. Conservation Biology 7:562-568.

Ollerton, J., R. Winfree and T. Tarrant. 2011. How many flowering plants are pollinated by animals? Oikos 120: 321-326.

Palmer G.1975. Shuswap Indian Ethnobotany. Syesis 8: 29-51.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annual Review of Ecology, Evolution, and Systematics 37: 637-669.

Parmesan, C., N. Ryrholm, C. Stefanescu, J.K. Hill, C.D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, W.J. Tennent, J.A. Thomas and M. Warren. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399: 579-583.

Pearson, R.G. and T.P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography 12: 361-371.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2017. Linear and Non Linear Effects models. R package version 3.1-131. Available from: https://CRAN.R-project.org/package=nlme

Pojar, J. and A. MacKinnon. 1994. Plants of the Pacific Northwest Coast. British Columbia Ministry of Forests. Lone Pine Publishing: Edmonton, Alberta, Canada.

Porsild, A.E. 1951. Botany of southeastern Yukon adjacent to the Canol Road. Natural Museums of Canada Bulletin 121:1-400.

Potts, S.G., J. Beismeijer, C. Kremem, P. Neuman, O. Schweiger and W.E. Kunin. 2010a. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25: 345-353.

Potts, S.G., S.P.M. Roberts, R. Dean, G. Marris, M.A. Brown, R. Jones, P. Neumann and J. Settele. 2010b. Declines of managed honey bees and beekeepers in Europe. Journal of Apicultural Research 49: 15-22.

Primack, R.B., and P. Hall. 1990. Costs of reproduction in the lady's slipper orchid: a 4-year experimental study. American Naturalist 136: 638-655.

Primack, R.B. and E. Stacy. 1998. Cost of reproduction in the pink lady's slipper orchid, (*Cypripedium acaule*, Orchidaceae): an eleven- year experimental study of three populations. American Journal of Botany 85: 1672-1679.

Quesada, M., K. Bollman and A. Stephenson. 1995. Leaf Damage Decreases Pollen Production and Hinders Pollen Performance in *Cucurbita texana*. Ecology 76: 437-433.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria. Available from: https://www.R-project.org/

Ravi, S., J.D. Crall, A. Fisher and S.A. Combes. 2013. Rolling with the flow: bumblebees flying in unsteady wakes. Journal of Experimental Biology 216: 4299-4309.

Reid, A.M. 2011. Cushion plants in the alpine of British Columbia, Canada: systematic review, trophic facilitation, and pollen limitation [Thesis]. York University: Toronto, Ontario, Canada.

Reitan, T. and A. Nielsen. 2016. Do Not Divide Count Data with Count Data; A Story from Pollination Ecology with Implications Beyond. PLoS ONE 11: e0149129.

Rich, P.M.1989. A manual for analysis of hemispherical canopy photography. Technical Report. Alamos National Lab: New Mexico, USA.

Rich, P.M. 1990. Characterising plant canopies with hemispherical photographs. Remote Sensing Reviews 5: 13-29.

Robuck, W.O. 1989. Common Alpine Plants of Southeast Alaska. USDA Forest Service, Pacific Northwest: Portland, Oregon, USA.

Romey, W.L., J.S. Ascher, D.A. Powell and M. Yanek. 2007. Impacts of logging on midsummer diversity of native bees (Apoidea) in a northern hardwood forest. Journal of the Kansas Entomological Society 80: 327-338.

Rosenheim, J.A., N.M. Williams and S.J. Schreiber. 2014. Parental Optimism versus Parental Pessimism in Plants: How Common Should We Expect Pollen Limitation to Be? The American Naturalist 184: 75-90.

Rosenheim, J.A., N.M. Williams, S.J. Schreiber and J.M. Rapp. 2016. Modest Pollen Limitation of Lifetime Seed Production Is in Good Agreement with Modest Uncertainty in Whole-Plant Pollen Receipt (A Reply to Burd). The American Naturalist 187: 397-404.

Rotheray, G.E. and F. Gilbert. 2011. The Natural History of Hoverflies. Forrest Text: Ceredigion, UK.

Ruban, N. and T. Kurlovich. 2000. Productivity of nectar of *Oxycoccus macrocarpus.* In: Problems of Rational Utilization and Reproduction of Berry Plants in Boreal Forests on the eve of the XXI Century. Proceedings of the Conference 11-15: 210-213.

Rudolph, D.C. and C.A. Ely. 2000. The influence of fire on lepidopteran abundance and community structure in forested habitats of Eastern Texas. Texas Journal of Science: 52:127-138.

Ryynänen A.1972. Arctic bramble (*Rubus arcticus* L.) a new cultivated plant. Annales Agriculturae Fenniae 11: 1-76.

Ryynänen, A. 1973. *Rubus arcticus* and its cultivation. Annales Agriculturae Fenniae 12: 1-76.

Saastamoinen, S. 1930. Mesimarja (*Rubus arcticus* L.) Suomessa. Annales Botanici Societatis Zoologicae-Botanicae Fennicae Vanamo 13: 355-414.

Saez, A., C.L. Morales, L.Y. Ramos and M.A. Aizen. 2014: Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. Journal of Applied Ecology 51:1603-1612.

Sanchez-Lafuente, A.M., J. Guitian, M, Medrano, C.M. Herrera, P.J. Rey and X. Cerda. 2005. Plant Traits, Environmental Factors, and Pollinator Visitation in Winter-flowering *Helleborus foetidus* (Ranunculaceae). Annals of Botany 96: 845-852.

Sandholm, H.A. and R.D. Price. 1962 Field observations on the nectar feeding habits of some Minnesota mosquitoes. Mosquito News 22: 346-349.

Scoggan, H.J. 1978. The Flora of Canada. National Museum of Natural Sciences: Ottawa, Ontario, Canada.

Sgolastra, F., A. Fisogni, M. Quaranta, G. Bogo, L. Bortolotti and M. Galloni. 2016. Temporal activity patterns in a flower visitor community of *Dictamnus albus* in relation to some biotic and abiotic factors. Bulletin of Insectology 69: 291-300.

Sletvold, N., J.M. Grindeland and J. Agren. Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. New Phytologist 188: 385-392.

Snow, A.A. 1982. Pollination Intensity and Potential Seed Set in *Passiflora vitifolia.* Oecologia 55: 231-237.

Sobrevila, C. 1988. Effects of distance between pollen donor and pollen recipient on fitness components in *Espeletia schultzii*. American Journal of Botany 75:701-724.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry [3rd Edition]. Freeman and Company: New York, New York, USA.

Soper, J.H. and M.L. Heimburger. 1982. Shrubs of Ontario. National Museum of Natural Sciences: Ottawa, Ontario, Canada.

Spackman, S., B. Jennings, J. Coles, C. Dawson, M. Minton, A. Kratz, and C. Spurrier. 1997. Colorado Rare Plant Field Guide. Prepared for the Bureau of Land Management, the USDA Forest Service, and the U.S. Fish and Wildlife Service by the Colorado Natural Heritage Program: Fort Collins, Colorado, USA.

Spitters, C.J.T., Toussaint, A.J.M., and Goudriaan, J. 1986. Separating the diffuse and direct component of global radiation and its implications for modeling canopy photosynthesis. Part I. Components of incoming radiation. Agricultural and Forest Meteorology 38: 217-229.

Steen, R. 2009. A portable digital video surveillance system to monitor prey deliveries at raptor nests. Journal of Raptor Research 43: 69-74.

Steen, R. 2017. Diel activity, frequency and visit duration of pollinators in focal plants: in situ automatic camera monitoring and data processing. Methods in Ecology and Evolution 8: 203-213.

Stiles, G. F. 1979. Notes on the natural history of *Heliconia* (Musaceae) in Costa Rica. Brenesia 15: 151-180.

Stoutamire, W.P. 1968. Mosquito pollination of *Habenaria obtusata* (Orchidaceae). Michigan Botanist 7: 203-212.

Suarez-Gonzalez, A. and S.V. Good. 2014. Pollen limitation and reduced reproductive success are associated with local genetic effects in *Prunus virginiana*, a widely distributed self-incompatible shrub. Annals of Botany 113: 595-605.

Taki, H., T. Inoue, H. Tanaka, H. Makihara, M. Sueyoshi, M. Isono and K. Okabe. 2010. Responses of community structure, diversity, and abundance of understory plants and insect assemblages to thinning in plantations. Forest Ecology and Management 259: 607-613.

Tammisola, J. 1988. Incompatibility classes and fruit set in natural populations of arctic bramble (*Rubus arcticus* L.*)* in Finland. Journal of Agricultural Science in Finland 60: 327-446.

Tammisola, J. and A. Ryynänen. 1970. Incompatibility in *Rubus arcticus* L. Hereditas 66: 269-278.

Tasen, W., P. Duengkae, D. Marod, K. Ogata and P. Pianhanuruk. 2010. Effects of Some Environmental Factors to Species of Major Insect Pollinators in Flowering Season of Teak (*Tectona grandis* L.f.). The Thailand Natural History Museum Journal 4: 3-14.

Thien, L.B.1969. Mosquito pollination of *Habenaria obtusata* (Orchidaceae). American Journal of Botany 56: 232-237.

Thomas, C.D., E.J. Bodsworth, R.J.Wilson, et al. 2001. Ecological and evolutionary processes at expanding range margins. Nature 411: 577-581.

Thompson, C. 2016. Techniques for Viewing Pollen Tubes in Angiosperm Flowers. Honors Research Projects. Paper 390.

Thomson, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. Journal of Ecology 74: 329-341.

Thomson, J.D., M.A. McKenna and M.B. Cruzan. 1989. Temporal patterns of nectar and pollen production in *Aralia hispida*: implications for reproductive success. Ecology 70:1061-1068.

Tichy, L. 2016. Field test of canopy cover estimation by hemispherical photographs taken with a smartphone. Journal of Vegetation Science 27:427-435.

Tiusanen, M., P.D.N. Hebert, N.M. Schmidt and T. Roslin. 2016 One fly to rule them all—muscid flies are the key pollinators in the Arctic. Proceedings of the Royal Society B: Biological Sciences 283: 20161271.

Totland, Ø. 1993. Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. Canadian Journal of Botany 71: 1072-1079.

Totland, Ø. and I. Matthews. 1997. Determinants of pollinator activity and flower preference in the early spring blooming *Crocus vernus*. Acta Oecologica 19: 155-165.

Tuell, J.K. and R. Isaacs.2010. Weather during bloom affects pollination and yield of high bush blueberry. Journal of Economic Entomology 103: 557-562.

Unwin, D. M. and S.A. Corbet.1991: Insects, Plants and Microclimate. Richmond Publishing: Slough, UK.

USDA, NRCS. 2018. The PLANTS Database. National Plant Data Team: Greensboro, North Carolina, USA. Available from: http://plants.usda.gov

Utelli, A.B. and B.A. Roy. 2000. Pollinator abundance and behavior on *Aconitum lycoctonum* (Ranunculaceae): an analysis of the quantity and quality components of pollination. Oikos 89: 461-470.

Vamosi, J.C., T.M. Knight, J.A. Steets, S.J. Mazer, M. Burd and T.L. Ashman. 2006. Pollination decays in biodiversity hotspots. Proceedings of the National Academy of Science 103: 956-961.

Vanbergen, A.J. and The Insect Pollinators Initiative. 2013. Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment 11: 251-259.

van Engelsdorp, D., J. Hayes Jr, R.M. Underwood and J. Pettis. 2008. A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008. PLoS ONE 3: e4071.

Vool E., M. Noormets, K. Karp, R. Mand and M. Starast.2003. Pollinators and their foraging behavior in arctic bramble (*Rubus arcticus* L.) plantations in Estonia. Integrative Approaches in the Baltic Sea Region 15: 481-489.

Vool, E., K. Karp, M. Noormets, U. Moor and M. Starast. 2009. The productivity and fruit quality of the arctic bramble (*Rubus arcticus ssp. arcticus*) and hybrid arctic bramble (*Rubus arcticus ssp. arcticus*×*Rubus arcticus ssp. stellatus*). Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 59: 217-224.

Vool, E., K. Karp, M. Starast, T. Paal and A. Luik. 2011. Growth Habit of Arctic Bramble (*Rubus arcticus*) within Kansoo Conservation Area in Estonia. Baltic Forestry 17: 170-178.

Walters, B.B. and E.W. Stiles. 1996. Effect of canopy gaps and flower patch size on pollinator visitation of *Impatiens capensis*. Bulletin of the Torrey Botanical Club 123: 184-188.

Walther, G.R., E. Post, P. Convey, A. Menzel, C. Parmesan, T.J.C. Beebee, J.M. Fromentin, O. Hoegh-Guldberg and F. Bairlein. 2002. Ecological responses to climate change. Nature 416: 389-395.

Waser, N.M. 1993. Population Structure, Optimal Outbreeding, and Assortative Mating in Angiosperms. In: Thornhill, N.W. (ed). 1993. The natural history of inbreeding and outbreeding. University of Chicago Press: Chicago, Illinois, USA.

Waser, N.M., L. Chittka, M.V. Price, N.M. Williams and J. Ollerton. 1996. Generalization in pollination systems and why it matters. Ecology 77: 1043-1060.

Wallis, C. 1971. *Rubus acaulis* Michx. University of Calgary Herbarium [Accession No. 29679].

Weerakoon, B.M.B, A.M.R.S. Bandara and K.B. Ranawana. 2015. Impact of Canopy Cover on Butterfly Abundance and Diversity in Intermediate Zone Forest of Sri Lanka. Journal of Tropical Forestry and Environment 5: 41-46.

Weiss, M., F. Baret, G.J. Smith, I. Jonckheere and P. Coppin. 2004. Review of methods for in situ leaf area index (LAI) determination. Agricultural and Forest Meteorology 121: 37-53.

Wesselingh, R.A. 2007. Pollen limitation meets resource allocation: towards a comprehensive methodology. New Phytologist 174: 26-37.

Wilcock, C. and R. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. TRENDS in Plant Science 7: 270-277.

Williams, N.M., R.L. Minckley and F.A. Silveira. 2001. Variation in native bee faunas and its implications for detecting community changes. Conservation Ecology 5: 7 [Online].

Williams, P.H., M.B. Araujo and P. Rasmont. 2007. Can vulnerability among British bumblebee (*Bombus*) species be explained by niche position and breadth? Biological Conservation 138: 493-505. Routledge: New York City, New York, USA.

Williams, N.M. 2011. Restoration of Nontarget Species: Bee Communities and Pollination Function in Riparian Forests. Restoration Ecology 19: 450-459.

Willmer, P.G. 1986. Foraging patterns and water balance: problems of optimization for a xerophilic bee, *Chalicodoma sicula*. Journal of Animal Ecology 55: 941-962.

Willmer, P.G., A.M. Bataw and J.P. Hughes. 1994. The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. Ecological Entomology 19: 271-284.

Wilson, P. and J.D. Thomson. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. Ecology 72:1503-1507.

Young, H. J., and T. P. Young. 1992a. Alternative outcomes of natural and experimental high pollen loads. Ecology 73:639-647.

Young, J.A. and C.G. Young. 1992b. Seeds of woody plants in North America. Dioscorides Press: Portland, Oregon, USA.

Zar, J.H. 1999. Biostatistical Analysis [4th Edition]. Prentice Hall: Englewood Cliffs, New Jersey, USA.

Zimina, L.V.1957.New data on the ecology and faunistics of hoverflies (Diptera. Syrphidae) in the Moscow region.Byulletin Moskva O-Valspyt Prirod, Otdrlii Biologii 64: 51-62.

Zimmerman, M. 1979. Optimal foraging: a case of random movement. Oecologia 43: 261-264.

Zorer, R., T. Moffat, A. Strever and J.J. Hunter. 2013. Hourly simulation of grape bunch light microclimate using hemispherical photography. Ciência e Técnica Vitivinícola 28:1031 - 1034.

Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev and G.M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York City, New York, USA.

Appendix A: Study Site and Sampling Grid

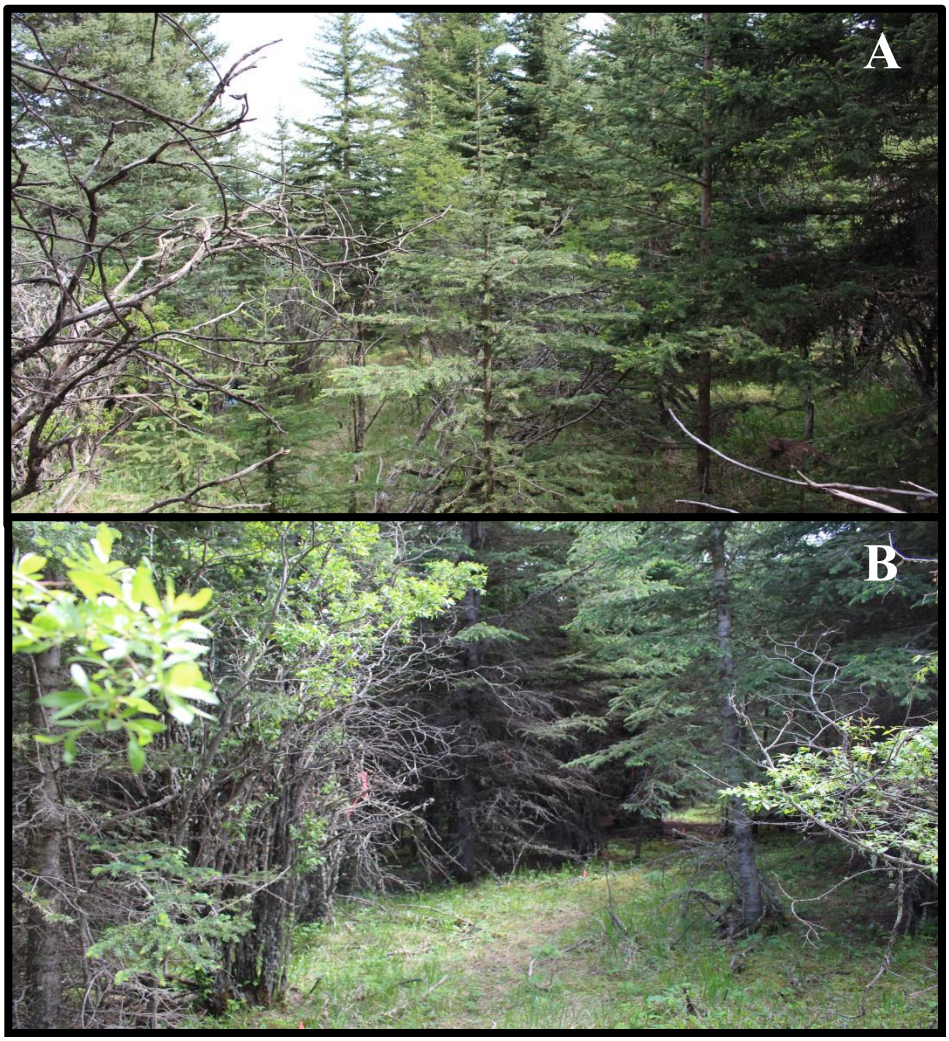


Figure A1 'A' displays the "manipulated treatment site" where the pollen supplementation experiment had occurred whereas 'B' displays the "natural undisturbed site" where the pollinator/microclimate monitoring was conducted.

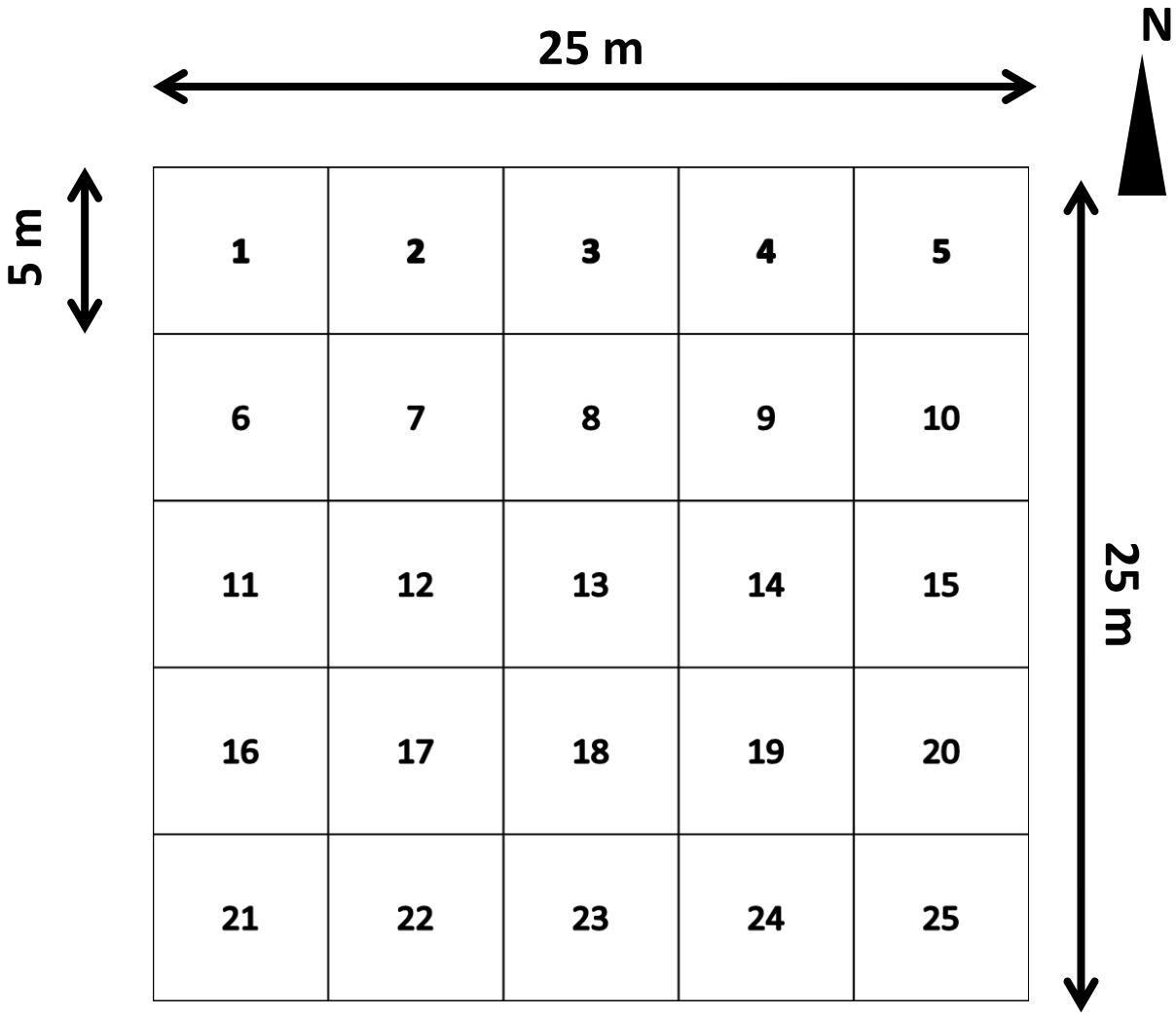


Figure A2 The dimensions, arrangement and orientation of the sampling grids used for the "Manipulated Treatment Site" for the pollen supplementation experiment and the "Natural Undisturbed Site" for the pollinator/microclimate monitoring. The numbers inside each $25m^2$ square of the $625m^2$ sampling grid denote the given quadrate/grid cell location. The sampling grids for both sites were orientated north using a compass with the appropriate declination for the region.

Appendix B: Site Maps

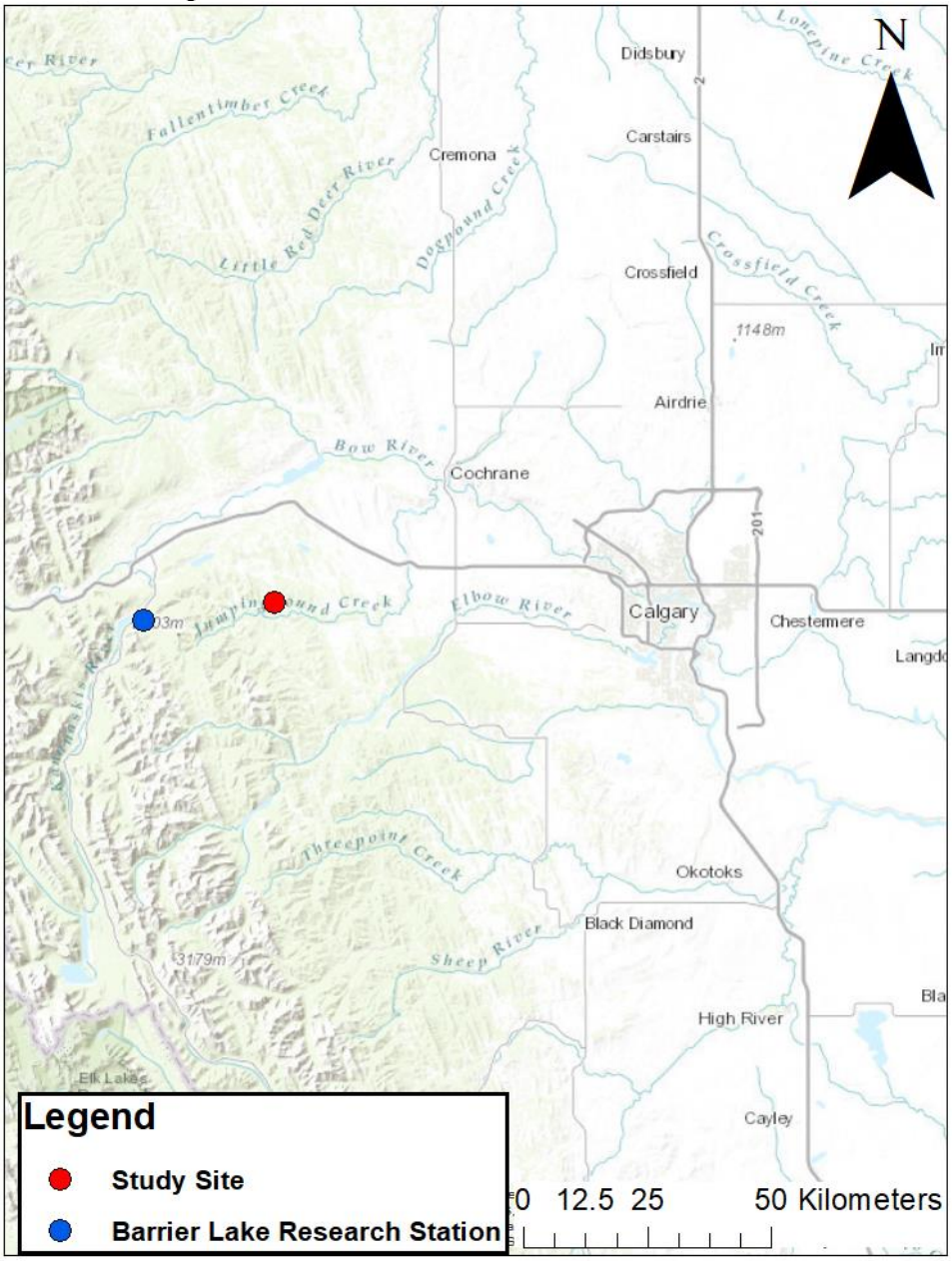


Figure B1 A map displaying the location of the study site for the entire project (red), in the south-western portion of Alberta, Canada. The study site was approximately 23km east of University of Calgary's Barrier Lake Research Station (blue) where specimens and data was temporarily stored during the field season from June – August 2017. This map was constructed with ArcGIS Version 10.4.0.5524 (Esri 2015) using the built in topography base map.

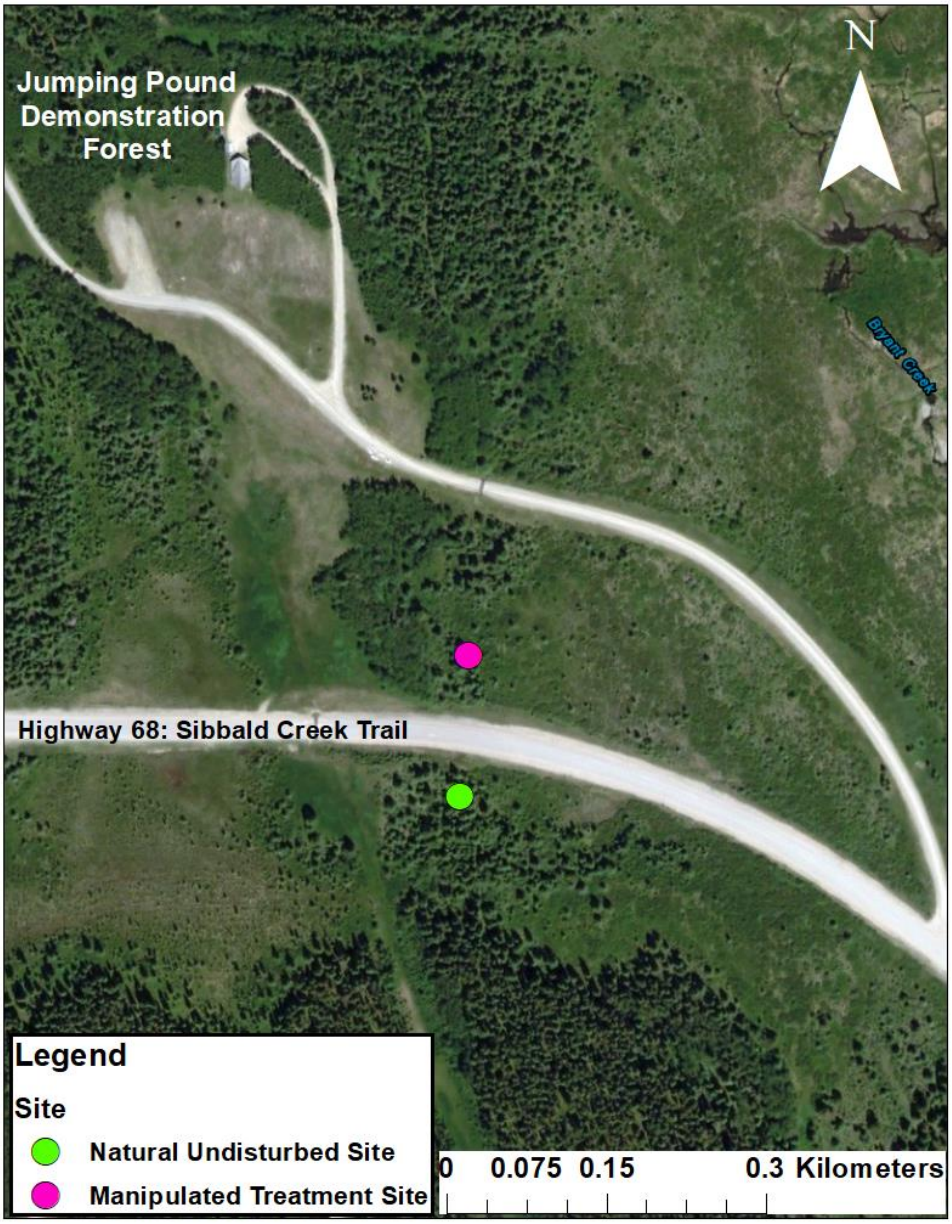


Figure B2 A map displaying the location of the study site separately into the two subsites. The "Manipulated Treatment Site" (pink) was where the pollen supplementation experiment was conducted whereas the "Natural Undisturbed Site" (green) was where the pollinator/microclimate monitoring had occurred. Both subsites were divided by the Sibbald Creek Trail/ Highway 68, ≤ 25m from the road. This map was constructed with ArcGIS Version 10.4.0.5524 (Esri 2015) using the built in satellite imagery base map.

Appendix C: Associated Species

Table C1 The associated flowering plant species for *Rubus arcticus* specimens by study subsite.

"Natural Undisturbed Site"	**"Manipulated Treatment Site"**
Geum rivale	*Mertensia paniculata*
Fragaria virginiana	*Equisetum arvense*
Taraxacum officinale	*Galium sp.*
Rosa acicularis	*Trifolium pretense*
Equisetum arvense	*Pyrola asarifolia*
Potentilla gracilis	*Petasites frigidus var. palmatus*
Geranium richardsonii	*Thalictrum venulosum*
Delphinium glaucum	*Dodecathon sp.*
Mertensia paniculata	*Geum rivale*
Astragalus americanus	*Stellaria longipes*
Vicia americana	*Delphinium glaucum*
Pyrola asarifolia	
Moneses uniflora	
Galium sp.	
Platanthera obtusata	
Linnaea borealis	
Chamaenerion angustifolium	
Ribes oxyacanthoides	
Smilacina stellata	
Ozmorhiza sp.	
Vaccinium vitis-idaea	
Lathyrus ochroleucus	
Petasites frigidus var. palmatus	
Achillea millefolium	
Thalictricum venulosum	
Ranunculis acris	

Appendix D: Pollen Tube Analysis

Introduction

Pollen supplementation experiments cannot distinguish whether pollen quality or quantity is responsible for pollen limitation (Aizen and Harder 2007), and so a separate experiment was conducted to evaluate the role of pollen quality in *R. arcticus* fruit production. Chacoff et al. (2008) was able to assess the effects of quality on pollen grain germination and pollen tube growth in *Crataegus monogyna* (Rosaceae) using stain technology. Further, Keep (1968) found that in self-incompatible *Rubus* spp., essentially all pollen tubes originating from selfed pollen grew only a third or quarter the way down the length of the styles. I hypothesized that low-quality selfed pollen will most likely result in stunted pollen tubes on account of the species' gametophytic pre-fertilization self-incompatibility mechanism (Tammisola and Ryynänen 1970), whereas tubes from high quality outcrossed pollen will have an increased probability of either successfully reaching the ovary or progressing nearly down the full lengths of styles. From this hypothesis, I predicted that: outcross flowers would have high counts of full length pollen tubes reaching style bases per pistils; selfed flowers would have a higher abundance of stunted pollen tubes per pistil; and control flowers would have a mix of stunted and full length tubes, but the vast majority will be stunted in pistils indicating that the species is naturally pollen limited quality-wise.

Methods

This sub-experiment was comprised of three separate treatment groups similar to the pollen supplementation experiment for Chapter 2 and was conducted within the "Manipulated Treatment Site" on separate undisturbed and unopened/virginal flowers, with slight modifications. There was an open control group with flowers left for natural pollination, a

bagged group with flowers being treated with selfed pollen, and a pure-outcrossed group in which flowers were emasculated and treated with xenogamous pollen from at least two donors 10-30m away (Chacoff et al. 2008); with each group consisting of at least 10 individual stems. Similar to Saez et al. (2014) study with *R. idaeus,* 10 stems/flowers per treatment would be sufficient as *R. arcticus* is also polycarpellate and numerous pistils can function as sub-replicates within each treatment. Selfed and outcrossed flowers remained covered with bridal veil bags until harvesting, barring brief removal to apply treatments upon opening, whereas control flowers had bags removed immediately following opening. Emasculation and bagging was administered in outcrossed flowers to isolate the effects of self-pollination and allow for greater contrast of pollen tube length between pollen types (high quality outcrossed vs. low quality selfed). After receiving the respective treatment, all flowers were left undisturbed for at least 72 hours, since this has been observed to be ample time for *Rubus* spp. pollen grains to successfully germinate and have pollen tubes reach the base of style, penetrating into the ovary (Keep 1968). Following the 72 hour period, flowers were harvested and placed in microtubes filled ethanol-acetic acid (3:1 v/v) for 24 hours to fix floral tissues (Levin 1990, Kearns and Inouye 1993). After 24 hours, the ethanol-acetic acid was replaced with 70% ethanol in which flowers were stored in until dissection. All flowers were later dissected in which pistils were extracted with ovaries left intact and transferred back to 70% ethanol filled microtubes. Many pollination experiments fix pistils in Farmer's Solution/formalin acetic-acid-alcohol (Kearns and Inouye 1993), but Thompson (2016) found ethanol-acetic acid (3:1 v/v) solution was nearly as adequate in preserving tissues and preventing decomposition.

Two separate staining protocols were used to visualise *R. arcticus* pollen tubes. The first staining procedure was slightly modified from Levin (1990) in which pistils were stained in

0.01% basic fuchsin: 0.01% fast green (4:1) for at least 24 hours, then later de-stained and softened with 85% lactic acid for at least 12 hours. Pollen grains and tubes appeared as a light blue/green whereas the stigma/stylar tissue stained a bright pinkish red (Figure D1.B). The original protocol specified that pistils should be stained in 1% basic fuchsin:1% fast green with tubes appearing maroon against a nearly white background, but using that concentration resulted in overstaining of *R. arcticus* pistils, making the pollen tubes completely indistinguishable from all other tissues. Furthermore, the original protocol was used for *Phlox drummondii*, which has flowers that have a vastly different structure/morphology as well as a different chemical composition. All test pistils were mounted in 85% lactic acid on glass slides and squashed with a cover slip, to be viewed under a conventional compound microscope with white light.

The second staining protocol involved a slightly modified version from Martin (1959) which involves the use of aniline-blue epifluorescence microscopy. Test pistils were rinsed in water and then left in 1N NaOH (Kho and Baër 1968) for 1 hour at 60°C (Mulcahy and Mulcahy 1982) to soften the tissues for staining. Subsequently, the pistils were rinsed with water and left in decolourized aniline blue stain for 30 minutes, while being stored in a culture plate wrapped in aluminum foil to prevent light from interfering with the staining process (A. Kumar, *pers. comm.*, January 5, 2018). These pistils were then mounted on a glass slide with 70% glycerin, gently squashed with a coverslip, viewed under a compound microscope fitted with a UV filter/laser in which pollen tubes appeared bright blue against a black background (Figure D1.D)

Results

Both staining protocols were successful in that they allowed for pollen grains and tubes to be easily visualized on stigmatic surfaces, yet they both failed to allow for proper measurement of pollen tube lengths. In the basic fuchsin-fast green stain, the pollen tubes may have been

visible on the stigma, but as soon as they progressed into the style, they became indistinguishable from the rest of the tissue. The aniline blue protocol was used in attempt to correct for this issue and allow for a greater contrast between the style and pollen tubes, but it appears that the tissue in styles also contained material that fluoresces under UV light. This was the result for all test pistils and trials for both sets of staining protocols.

Discussion/Conclusion

Perhaps squashing alone was insufficient and these staining procedures would have been more successful if pistils were sectioned with a microtome to allow for better visualization of pollen tube movement through the stylar tissue (Jauh and Lord 1995). However, such equipment was unavailable at the time of these staining trials and beyond the budget of this research project. Thus, based on these findings alone, it is difficult to discern whether the pollen tubes are stunted or full length and subsequently assess if pollen tube growth can function as an indicator of pollen quality in *R. arcticus*. Consequently, no quantitative results can be gathered from this experiment, but some qualitative observations can be made to better understand the pollination process of the species at the microscopic level.

Figures

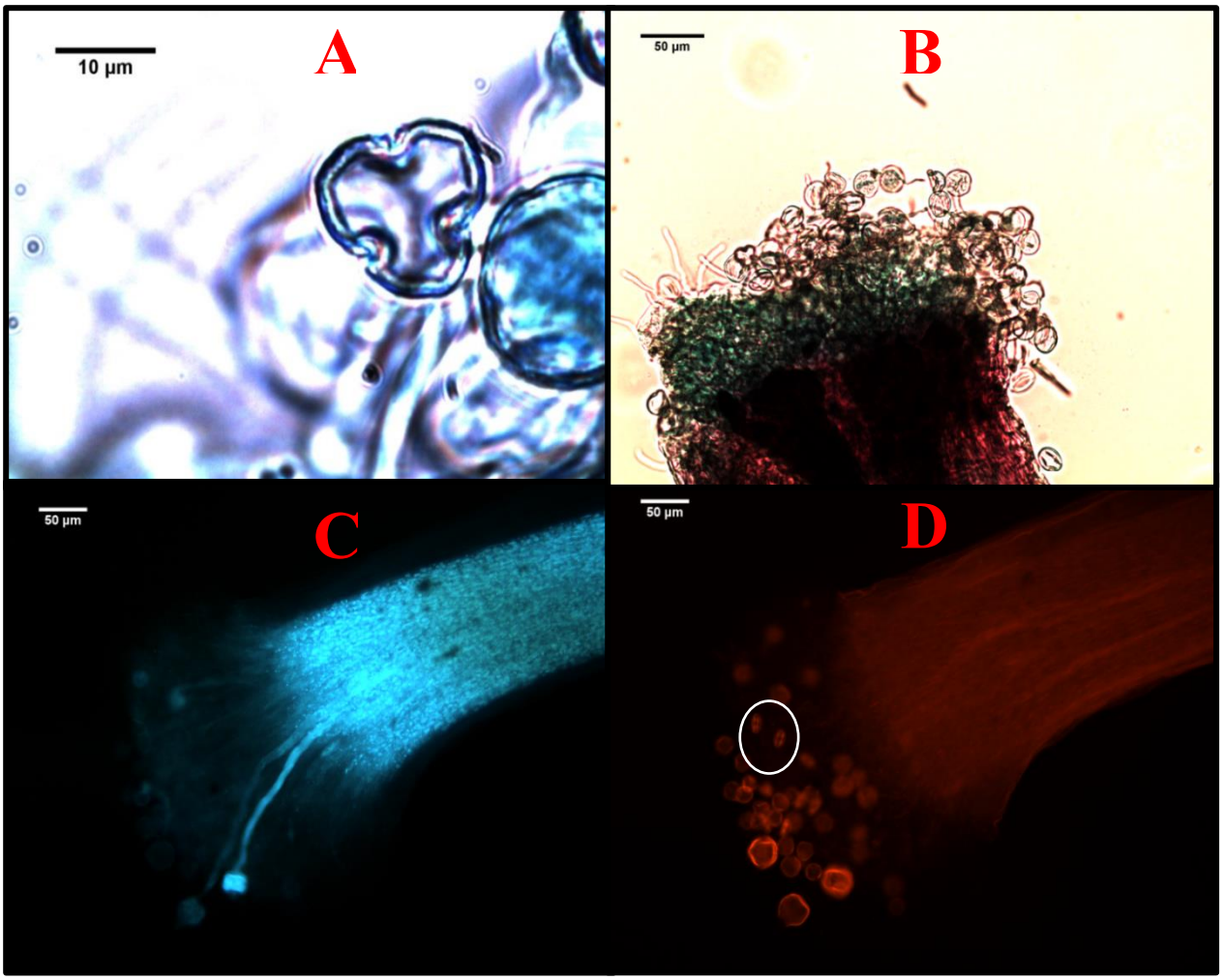


Figure D1 *Rubus arcticus* stigma/styles, pollen grains and tubes. 'A' features an image of a *R. arcticus* pollen grain (in polar view) with the tricolporate ultrastructure that is characteristic of the genus (Hebda and Chinnappa 1990) and was captured under 1000X magnification in white light. 'B' features an image of a *R. arcticus* stigma (stained pink) with several pollen grains on the surface along with germinating pollen tubes (both stained green) and was captured under 200X magnification in white light. 'C' displays an obvious germinated pollen grain with a pollen tube progressing into the stylar tissue, with some other tubes slightly out of focus. 'D' features the presence of numerous *R. arcticus* pollen grains on the stigma surface, along with some heterospecific pollen with different grain ultrastructures, highlighted by the white circle. 'A-B' pistils were stained using a modified protocol from Levin (1990). 'C-D' were stained using a slightly deviated version of the standard aniline blue fluorescence protocol (Martin 1959) and were captured under 200X magnification using a compound microscope fitted with a UV filter and laser; different channels were used to separately highlight the pollen tubes and grains respectively for each photo.

Appendix E: GLA Configuration Settings and Methods

Description

The first step in using GLA is registering the hemispherical image. This process involves manually overlaying the image with a reference circle which defines the actual portions of the image from the non-required black background (Figure E1). Each image had a unique registration as some photos varied in size due to some very slight zoom differences, but the initial cursor point was always placed at the top centre of each hemispherical image. After registering a hemispherical image, GLA requires additional "Configuration Settings" which can be divided into four different categories: image, site, resolution and radiation. For the "Image" tab, all reference hemispherical images had the "registration" set with the initial cursor point at north along with the top of the image being "Geographic North" and the "Projection Distortion" set to "Orthographic". In the "Site" tab; the latitude, longitude, elevation, aspect and slope input was set to the corresponding values for each respective focal flower position. Within the "Resolution" tab, solar time step was set to 1 minute and the growing season start date and end date were both set to the corresponding observation day's date, which allows GLA to compute the total radiation received for just that day only (Frazer et al. 1999). The finest temporal resolution GLA can compute to is one day, but Zorer et al. (2013) managed to create hourly simulations within the program. However, they did this with the assistance of a pyranometer coupled with their hemispherical reference photographs. The "Number of Azimuth Regions" was set to 48 and "Number of Zenith Regions" set to 12 as done in Zorer et al (2013). In the "Radiation" tab and for the "Sky Region Brightness" settings, the clear sky coefficient was kept at 0.65 since in North America, this value falls somewhere between 0.6 – 0.7 (Frazer et al. 1999).

And lastly, the default Universal Overcast Sky Model (UOC), which assumes all sky regions to be equally bright, was left unchanged.

The "Radiation" tab also has a series of "Model Parameters". Solar constant was always kept at the default 1367 W/m^2 as recommended by Frazer et al (1999) and units were set to mols/m^2/d. However, cloudiness index (Kt), spectral fraction (R_p/R_s) and beam fraction (H_b/H) are all regional specific parameters and could not be remain constant and had to be calculated separately for each observation day. Kt is a measure of cloudiness for a given site and the following equation (Lui and Jordan 1960, Iqbal 1983) was used to compute it:

$$Kt = H/H_0$$

H = Global radiation incident on the ground
H_0 = Extraterrestrial radiation incident on a horizontal surface outside

H can be data from regional solar radiation measurements, thus solar radiation data from the climate data set from the Barrier Lake Research Station (F. Lodhawalla, *pers. comm.,* 2017) was used as reference for this variable. H_0 was computed by using GLA's "Compute Extraterrestrial Radiation" tool in the "Utilities Menu". Both variables have to be for the same time period and same units (Frazer et al. 1999). Since the data from the Barrier Lake Research Station was in W/m^2, H_0 was also computed in W/m^2. Lastly, to have the H in the same temporal resolution as H_0, the solar radiation was averaged across all 24 hourly measurements from the Barrier Lake Research Station's pyranometer to produce a daily average for a given observation day.

Spectral fraction is simply the ratio of photosynthetically active radiation (PAR) or visible light in the spectrum of 400-700nm (R_p) to the total shortwave radiation (R_s) contributed by all wavelengths (0.25 µm to 25 µm). This ratio can be directly collected by having a pyranometer paired with quantum sensor to measure PAR, side by side (Frazer et al. 1999).

However, no regional PAR data was available for use and instead, the following equation was used to predict the ratio:

$$\frac{R_p}{R_s} = 1 - \exp(-.499Kt^{-0.219})$$

Beam fraction is a ratio of direct (beam) radiation energy (H_b) to total radiation global radiation incident on the ground. The portions of radiation that is scattered by the earth's atmosphere is diffuse radiation whereas the portion that reaches the earth's surface uniformly is direct radiation. Similar to spectral fraction, an equation was used to predict beam fraction. Numerous functions have been created which allow Kt to seperate H_b from H (Iqbal 1983, Spitters et al. 1986, Reindl et al. 1990), which ultimately resulted in the conception of the Atmospheric Environment Service algorithm (Frazer et al. 1999). This algorithm is as follows:

$$\frac{H_b}{H} = [1 - \exp(-3.044Kt^{2.436}]$$

After all the configuration settings had been specified for each registered reference hemispherical image, the blue colour channel was selected within the "Choose A Colour Pane" tool. As recommended by several authors (Lee et al 1983; Frazer et al. 1999, 2001; Nobis and Hunziker 2005, Zorer et al. 2013), this colour best separates the canopy from the sky (Figure E2). The working images were then transformed into binary images (Figure 3.8B) using the "Threshold" tool. The threshold value was manually adjusted in every reference image to include as much of the actual canopy vegetation as possible to accurately compute the solar radiation. In the few cases when the sun was visible through the canopy creating the "pinhole effect" (Rich 1990), GLA's "Draw" tool was used to manually fill in these gaps as accurately as possible. Following the thresholding of the reference image, the "Calculate" function was run with "Canopy" Structure and Transmitted Gap Light" selected. GLA then will provide an output

summary log with numerous results, but only “% Canopy Openness” and “Trans Total” (Total transmitted radiation which is the sum of direct and diffuse radiation) were recorded.

Figures

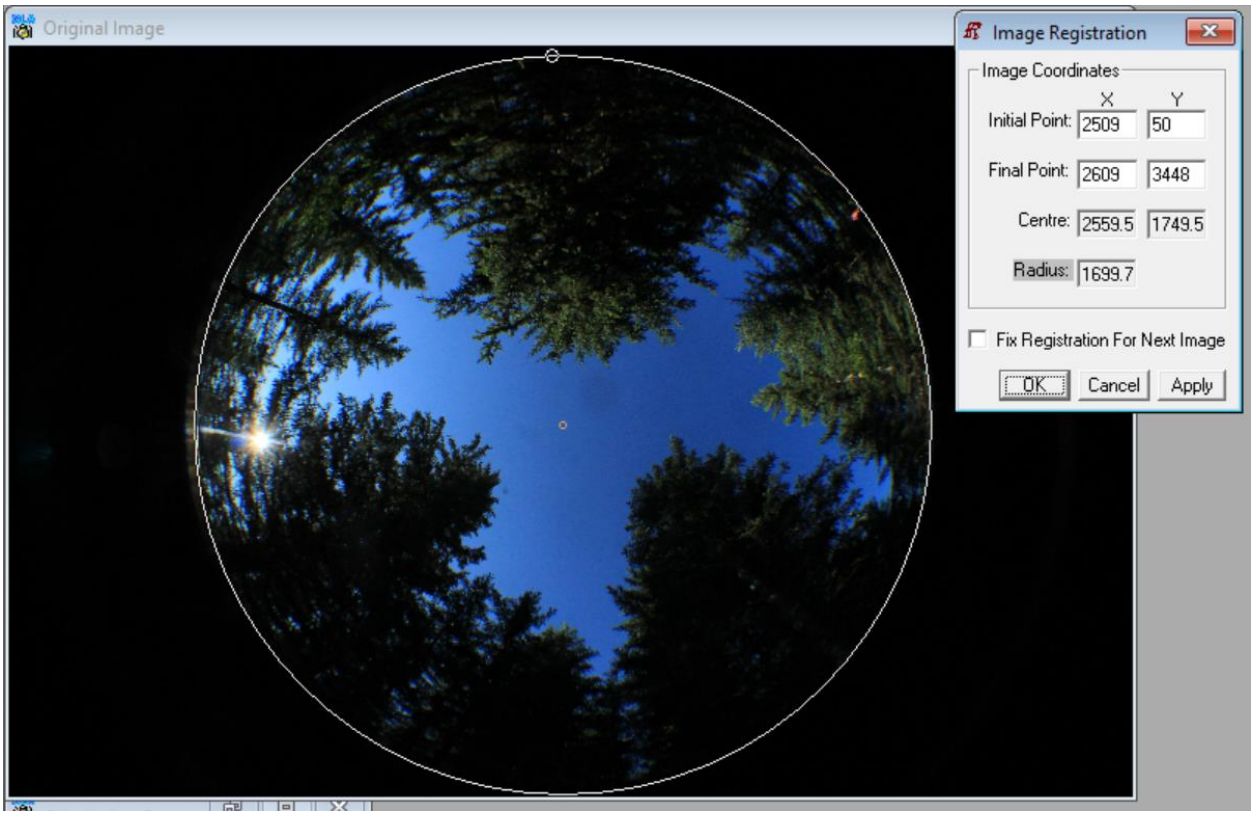


Figure E1 The image registration process in the Gap Light Analyzer Program.

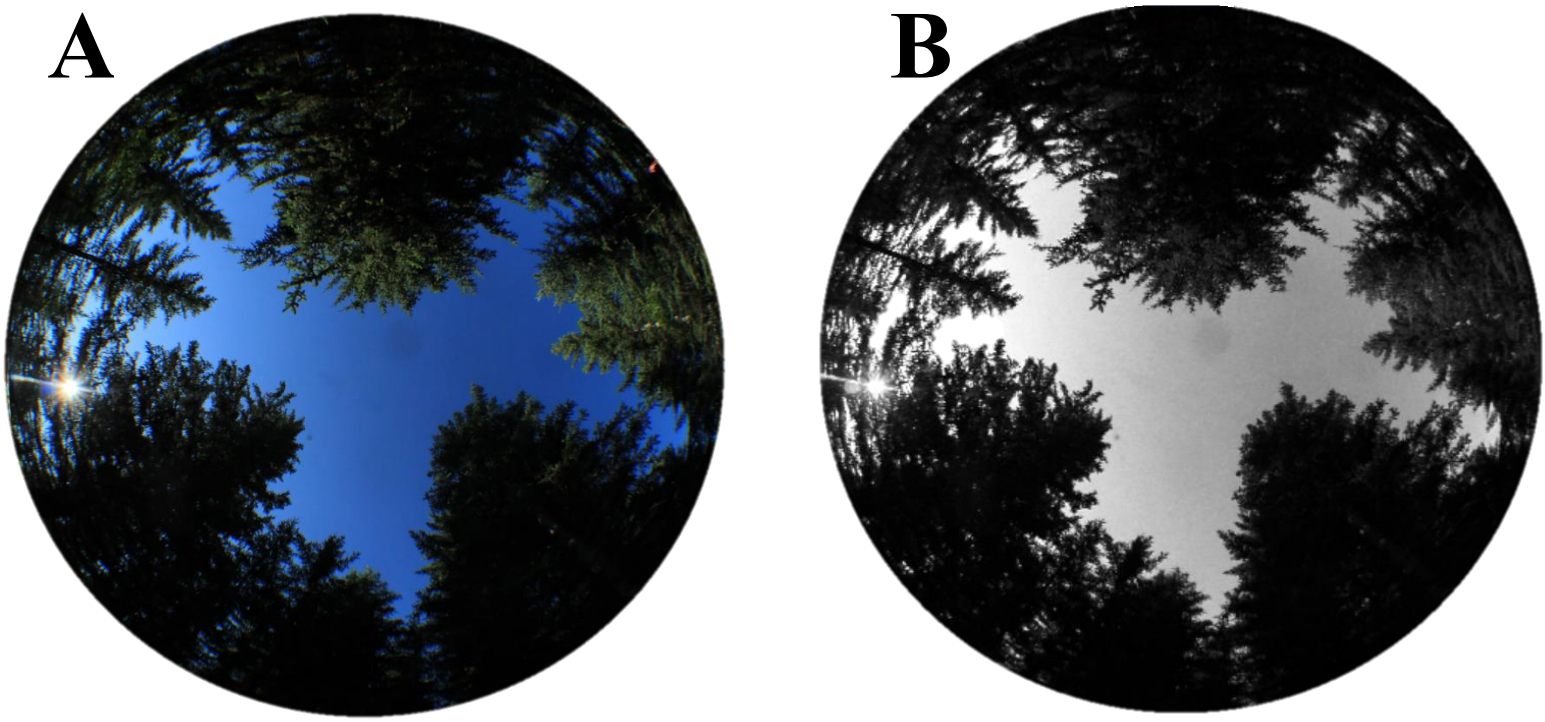


Figure E2 The "Choose Colour Pane" tool in the Gap Light Analyzer Program. 'A' is the unaltered references image whereas 'B' is the working image in which the blue channel has been applied to allow greater contrast between the canopy and sky.