Enhancing parasitism of the introduced ash tree pest, *Caloptilia fraxinella* by native parasitoid, *Apanteles polychrosidis* in Edmonton, Alberta: Not just a matter of more parasitoids being attracted to infested trees

by

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Abstract

Hymenopteran parasitoids are widely studied and employed as biological controls to reduce populations of pest insects. *Apanteles polychrosidis* Vierek (Hymenoptera: Braconidae) is a native parasitoid wasp that has shifted hosts, becoming the primary parasitoid of the ash leaf-cone roller, *Caloptilia fraxinella* Ely (Lepidoptera: Gracillariidae), an introduced nuisance pest of ash trees (*Fraxinus* spp.) in Edmonton, Alberta. This project explores two approaches to enhance *A. polychrosidis* as a biological control agent of *C. fraxinella*:

1) Baiting trees with synthetic semiochemical lures that release methyl salicylate (MS), and two green leaf volatiles (GLVs) (*Z*)-3 hexenyl acetate, and (*Z*)-3-hexanol to enhance wasp attraction and parasitism in infested ash trees. Mated female wasp attraction to these same compounds was tested also tested in an olfactometer. Male and female wasps were more attracted to ash trees treated with the combination of MS+GLV lures, at the lower release rate tested. Mated females did not demonstrate attraction to VOC compounds in the olfactometer.

2) Nutritional provisioning with 10% and 25% sucrose solution or natural nectar sources to enhance retention of wasps in ash trees and increase parasitism of *C. fraxinella.* The effect of sucrose nutrition on wasp longevity, female fecundity and parasitism, and offspring fitness were measured in the laboratory. Wasp longevity was greater in virgin male and female *A. polychrosidis* that were fed either 10 or 25% sucrose solution. Females lived significantly longer than males, when they were both fed 25% sucrose. Virgin female egg load was lower for wasps fed 25% sucrose, and egg load was negatively correlated with female longevity. Nectar feeding extended virgin female longevity similarly to those fed 25% sucrose. Neither sucrose solution, nor buckwheat flower provision in the field increased wasp retention. Wasp density was negatively correlated with *C. fraxinella* host density in field experiments.

Dedication

This thesis is dedicated to my mother, Marie Noël, and my father, Alexander McPike. You both have influenced and inspired me so much. Thank you for making me who I am. I love you both.

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List of Abbreviations

IPM- integrated pest management

GLV- green leaf volatiles

HIPV- herbivoreinduced plant volatiles

MS- methyl salicylate

VOC- volatile organic compound

Chapter 1: Introduction

1.1 Biological controls in Integrated Pest Management

The introduction of industrial scale use of chemical pesticides in the 1940s to agricultural practices quickly revolutionized crop production (Edwards 1993). Since then, widespread pesticide resistance has developed in pests exposed to intensive selection pressure through pesticide application (Georghiou and Saito 1983). This has decreased the effectiveness of pesticides, and increased the economic costs of pest control (Pimental and Burgess 2014). As well, evidence of environmental and human harm caused by the application of chemical control agents began to emerge in the 1960s (Debach and Rosen 1991). The desire to suppress pest species more efficiently, while minimizing negative environmental impact, lead to the development of Integrated Pest Management (IPM) (Koul and Cuperus 2007). This approach encourages practices that decrease the use of chemical pesticides such as: economic thresholds to determine when pesticide application is necessary, preventative practices to reduce the probability of pest outbreaks, employment of mechanical and cultural rather than chemical pest control methods, and biological controls (Minnick 1976).

Biological control involves the exploitation of natural enemies of a pest species to reduce the population of the target pest (Fleschner 1959). These natural enemies can include, but are not limited to, predatory and parasitic arthropods, and pathogenic micro-organisms (Debach and Rosen 1991). Classical biological control, beginning in the late 1800's, typically involves the introduction of natural enemies of an invasive pest to an area of pest invasion where the natural enemy does not naturally occur (Gurr *et al.* 2007). More recently, the recognition of the value of "ecological services", or natural processes that are perceived to benefit human society (Cairns 1995), has led to increased attempts to support native natural enemies, and the habitats and resources on which they rely. Conservation biological control is a branch of IPM which seeks to employ ecological knowledge to avoid practices that harm, and enhance the survival and success of native or established natural enemies (McCravy 2008). While the earliest motivations for, and applications of, IPM and biological controls were concentrated around agricultural systems, these practices have since been applied in other managed ecosystems, including urban forests (Olkowski *et al*. 1978).

1.2 Attracting natural enemies for biological control using plant volatile organic compounds

Plants emit a suite of volatile organic chemicals (VOCs) that can change in quality and quantity when the plant is under attack by herbivores. Herbivore-induced plant volatiles (HIPVs) can be constituent compounds, which are released in higher amounts due to physical damage to plant tissues, or can be actively produced *de novo* following enemy attack (Paré and Tumlinson 1995). Across 900 families of plants, ~2000 compounds have been identified as HIPVs in response to herbivore attack (War *et al*. 2011). Within the same plant species, HIPV production is variable and can differ by species of herbivore that attacks the plant (Rasmann and Turlings 2007, Pierre *et al.* 2011). Herbivore induced plant volatiles used to mediate interactions in tritrophic plant-pest- natural enemy interactions can potentially be exploited to modify the behaviour of natural enemies as a tactic in an IPM strategy (Dicke *et al*. 1990).

Herbivore-induced plant volatiles can act as direct or indirect defensive compounds (War *et al*. 2012). Compounds that act directly deter the herbivore, whereas HIPVs that act indirectly attract natural enemies of herbivores. Chemical cues produced by the herbivore, such as cues released from excrement or salivary excretions, can also be detected by natural enemies at close range (Godfray 1994). Herbivore induced plant volatiles generally do not indicate specific insect herbivores to foraging natural enemies but can be detected over long distances. Natural enemies, therefore, encounter a reliability-detectability problem (Vet *et al*. 1991, Vet and Dicke 1992) in host location. While many plants release common, ubiquitous compounds, both the quantity and quality of a given plants VOCs can be unique (McCormick *et al.* 2012). The degree to which a natural enemy can perceive differences in HIPVs seems linked to its level of host specialization. Natural enemies with a narrow host range are better able to detect variation in plant VOCs than generalist natural enemies (De Moraes *et al.* 1998, Röse *et al.* 1998, Ngumbi *et al.* 2010).

Among the arthropods studied to date, there is evidence for attraction of predatory mites, and five orders of carnivorous insects (Hemiptera, Neuroptera, Diptera, Coleoptera, and Hymenoptera) to VOCs emitted by herbivorized plants (Dicke and Baldwin 2010). One of the most studied groups is the hymenopteran parasitoids, which have been manipulated for biological control since the 1880s (Debach and Rosen 1991). Parasitoids are organisms that use one host individua during the lifecycle. Successful development of the parasitoid results in the death of the host (Doutt 1959). Numerous major agricultural pests from the orders Hemiptera, Lepidoptera, Coleoptera, and Diptera have at least one, sometimes multiple, wasp parasitoids, and over two thirds of successful biological control programs have employed parasitoid wasps (Debach and Rosen 1991).

Researchers use behavioural and electrophysiological assays to study the chemical ecology of tritrophic interactions involving parasitic wasps (Finidori-Logli *et al.* 1996, Smid *et al.* 2002, Gouinguene *et al*. 2005, Turlings *et al*. 2005, Williams *et al.* 2008, Mallinger *et al.* 2011). In behavioural bioassays, parasitoids are commonly attracted to chemicals emitted by the host plant of the wasp's herbivorous insect host (McCall *et al.* 1993, Finidori *et al*. 1996, Geervliet *et al*. 1997, Connor *et al*. 2007, de Lange *et al.* 2016). Often, a suite of compounds emitted from plants in specific combinations and ratios elicits the most wasp attraction (D'Alessandro *et al.* 2009, Tamiru *et al*. 2015). Some individual HIPVs can also attract parasitoid wasps in a dosedependant manner (Sasso *et al.* 2009, Yang *et al.* 2016).

Field investigation of parasitoid attraction to synthetic HIPVs outside of controlled laboratory settings is limited. Methyl salicylate, an ubiquitous HIPV produced by many plants in response to herbivory, is commercially available and used to enhance parasitoid attraction in several pest management systems. In grape vineyards, methyl salicylate lures attract wasps in the family Braconidae and also small (length <2mm) parasitic wasps (James and Price 2004). Dispensers of a synthetic blend of HIPVs produced by cabbage enhances parasitism of Diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) by *Cotesia vestalis* (Halliday) (Hymenoptera: Braconidae) (Uefune *et al*. 2012).

1.3 Carbohydrate food provision for natural enemies to enhance conservation biological control

Conservation biological control has been most intensively investigated for agricultural pest systems, where the planting of monocultures limits the efficacy of natural enemy foraging (Wolcott 1942, Marino and Landis 1996). Highly-disturbed monoculture agricultural settings are food-limited for beneficial insects (Jonsson *et al*. 2010, Tena *et al*. 2015). Promotion of natural enemy activity includes pesticide reduction and enhancement of habitat requirements through provision of water, food, shelter, and alternate hosts (DeBach and Rosen 1991). Much of this research has focused on providing carbohydrate resources to foraging adult parasitoids (Landis *et al.* 2000, Gardiner *et al*. 2008). Most parasitoid wasps feed on floral nectar, extra-floral nectaries, or other carbohydrate food sources such as hemipteran honeydew (Quicke 2015). Carbohydrate food consumed by parasitoids, post-eclosion, fuels somatic metabolism (Olson *et al*. 2000), flight (Amat *et al*. 2012), and can maintain developed eggs in females, preventing their resorption (Wang *et al.* 2014). Carbohydrate resources can significantly increase lifespan of parasitoids (Jacob and Evans 2000, Faria *et al.* 2007, Nafziger and Fadamiro 2010, Kant *et al.* 2016, Ashraf *et al*. 2017). Extended adult life is associated with increased opportunity for mate-finding, and host finding, both of which support increased parasitism of insect hosts. Carbohydrate provision can also increase egg production in female parasitoids (Riddick 2007, Wang *et al.* 2014, Zhu *et al.* 2015), as well as increase fertility (Cicero *et al.* 2012). More nutrients are required in adult wasps that develop eggs after adult eclosion, whereas wasps that eclose with a full egg complement can divert energy to flight and foraging activity (Jervis *et al.* 2008). Parasitoids acquire lipids required for oogenesis (Heming 2003) from the insect host, as larvae, or in some cases, by host feeding as adult wasps, as they are incapable of lipogenesis (Visser *et al*. 2010).

Energetic state affects parasitoid foraging behaviour (Sirot and Bernstein 1996, Varennes et al. 2015). Hungry female parasitoids are more attracted to floral nectar sources than to larval hosts and host plants, in olfactometer and wind tunnel choice experiments (Takasu and Lewis 1993, Seikmann et al. 2004, Lightle et al. 2010). Starvation can also reduce the propensity for oviposition in host patches (Tasaku and Hirose 1991). There is also evidence that nutrition level affects the neurophysiology of parasitoid wasps. The antennal response of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) to host plant volatiles is greater in satiated than in hungry wasps, and at a behavioural level, sucrose concentration in the diet is correlated with the response to host plant volatiles (Burrows *et al.* 2017).

In the “nectar provision hypothesis”, Heimpel and Jervis (2005) suggest that provision of flowers as a source of carbohydrate resources for parasitoid wasps should increase parasitism of pests in the proximity. This hypothesis is adapted from optimal foraging theory that proposes animals should spend more time in patches where foraging time is decreased (Pyke et al. 1977). For parasitoids, optimal patches will have food and larval hosts for oviposition in proximity (Jamont *et al.* 2010). In addition to providing food, some flowers have scents that attract parasitoids (Belz *et al*. 2013). Selection of flower species to attract and enhance parasitoid activity in a patch can be specific to the target parasitoid (Idris and Grafius 1995, Vattala *et al.* 2006, Bianchi and Wäckers 2008, Belz *et al.* 2013, Liu *et al*. 2017). The width and depth of flower corollae determine nectar accessibility (Vallata *et al.* 2006, Liu *et al.* 2017), and some floral scents such as yarrow, *Achillea millefolium* (Asteraceae) are repellent to certain parasitoids (Wäckers 2004). The effectiveness of nectar provision in managed ecosystems could be enhanced through attraction of wasps to synthetic HIPVs followed by provision of floral resources in an "attract and reward" system (Gordon 2013).

1.4 *Fraxinus/Caloptilia fraxinella*/*Apanteles polychrosidis* tritrophic system in Edmonton

The principles of conservation biocontrol can be applied to manage non-native herbivores if native natural enemies switch hosts to exploit an introduced host (Jones *et al.* 2015). The ash leaf-cone roller, *Caloptilia fraxinella* Ely (Lepidoptera: Gracillariidae), is native to eastern North America (Forbes 1923, Handfield 1997) but was first reported attacking horticultural ash (*Fraxinus*) trees in Edmonton, Alberta in 1999 (Pohl *et al.* 2004). Although ash is not native to the Prairies west of Manitoba (Nelson *et al.* 2014), its widespread use for horticultural plantings has allowed *C. fraxinella* to establish in many Western Prairie cities (Wist and Evenden 2013). In the urban forest, *C. fraxinella* is both an aesthetic and a nuisance pest. Eggs laid on newly flushed leaflets (Evenden *et al.* 2007) hatch into young larvae that mine leaflets to produce serpentine mines. Fourth instar larvae exit the leaflet and disperse on silken threads to new leaflets, which they roll into cone-shaped shelters for pupation (Evenden 2009) (Figure 1.1). In Edmonton, approximately 40% of boulevard trees are horticultural ash trees, the majority being green ash (*Fraxinus pennsylvanica* Marshall), followed by black ash (*F. nigra* Marshall) ("Trees-Count Distribution Edmonton - Open Data Portal" 2017). The infestation of ash by *C. fraxinella* in Edmonton varies by year with ~ 30% rolled leaflets in trees sampled in 2009 (Wist *et al*. 2015) to ~ 95% rolled leaflets in trees sampled in 2015 (McPike, unpublished data).

The Pest Management Laboratory for the City of Edmonton receives many requests from citizens for the pest to be controlled (Jenkins, personal communication). The City of Edmonton adheres to an IPM strategy, attempting to minimize pesticide use in the urban forest (City of Edmonton 2012). Therefore, a biological control strategy would best address this widespread urban pest.

Since its introduction to Edmonton, several native parasitoid wasp species have been recorded using *C. fraxinella* as a larval host (Pohl *et al*. 2004, Wist and Evenden 2013). *Apanteles polychrosidis* Viereck (Hymenoptera: Braconidae) (Figure 1.2) is the primary parasitoid of *C. fraxinella* in Edmonton, and has the potential to be an effective biological control agent (Wist and Evenden 2013). It is in the braconid subfamily, Microgastrinae (Quicke 2015). This braconid parasitoid is previously known from lepidopteran larval hosts in the Family Tortricidae including *Choristoneura rosaceana* (Harris), *Choristoneura fumiferana* Walsingham, *Pandemis limitata* Robinson, *Pandemis pyrucana* Kearfott*, Platynota idaeusalis* (Walker), and *Endopiza viteana* Clemens (Seaman *et al*. 1990; Biddinger *et al*. 1994; Li *et al*. 1999, Cossentine *et al.* 2004, Fernandez-Triana and Huber 2010, Pfannenstiel *et al* 2012). It is considered a generalist parasitoid (Wist *et al*. 2015). *Apanteles polychrosidis* has a wide Nearctic range (Fernández-Triana and Huber 2010). In the Okanagan region of British Columbia, *A. polychrosidis* is bivoltine, and overwinters in second or third instar larvae of *C. rosaceana* hosts, and has a second generation in the summer, in the same larval host (Cossentine *et al.* 2004). In Edmonton, the native larval hosts of *A. polychrosidis* have not been identified to species. Nine *A. polychrosidis* were reared from overwintered Tortricidae larvae, however, in late April and early May 2016 and 2017 (McPike unpublished data). The summer generation of *Apanteles polychrosidis* has shifted hosts to exploit *C. fraxinella* as its larval host (Wist and Evenden 2013). This is the first record of *A. polychrosidis* using a non-tortricid host.

Adult wasps of the first generation of *A. polychrosidis* emerge in late spring in Edmonton. Wasps mate quickly upon eclosion and females seek larval hosts for oviposition (Wist *et al.* 2013). Presumably, adult *A. polychrosidis* feed on nectar like most adult hymenopteran parasitoids (Quicke 2015). In the laboratory, mated females are capable of parasitism, on average, at four days post-eclosion (Cossentine *et al.* 2005). Based on field hostassociation studies, female *A. polychrosidis* target dispersing fourth instar *C. fraxinella* for oviposition (Wist and Evenden 2013).

Apanteles polychrosidis is a koinobiont parasitoid, as the parasitized host larva continues to grow and develop as the parasitoid develops within it (Quicke 2015). The parasitized 4th-instar *C. fraxinella* larva constructs the leaf roll but does not excavate the characteristic window in the leaf roll (Pohl *et al*. 2004). The mature *A. polychrosidis* larva emerges from the larva at this stage and pupates inside the leaf-roll in a rice-grain-shaped pupal case, suspended by silken threads at each end (Wist *et al*. 2013). Morphology of the pupal case can be used to distinguish between *A. polychrosidis* and *C. fraxinella* to quantify the level of parasitism in the field (Figure 1.3).

Previous work illustrates that the tritrophic relationship between *Fraxinus*/*C. fraxinella*/*A. polychrosidis* is mediated by semiochemicals (Wist *et al*. 2015) and could be manipulated to enhance biological control of *C. fraxinella* by *A. polychrosidis*. The antennae of male and female *A. polychrosidis* detect VOCs released from intact and herbivorized green ash leaflets (Wist *et al*. 2015). The compounds detected include: (1) (*Z*)-3-Hexenal, (2) (*Z*)-3-Hexenol, (3) (*Z)*-3Hexenyl-acetate, (4) ocimene, (5) linalool, (6) *E* 4,8-dimethyl-1,3,7-nonatriene, (7) methyl salicylate, (8) bourbonene, (9) *E*-β-farnesene, (10) α-farnesene, and (11) dendrolasin, and two unidentified compounds. Mated female wasps are differentially attracted to intact green ash leaflets and black ash leaflets with *C. fraxinella* larval mines. In the field, show decreased parasitism rate is decreased at higher host density in green ash, but not in black ash (Wist *et al.* 2015). These findings illustrate that foraging behaviour of *A. polychrosidis* is dictated by plantproduced semiochemicals.

Intact, mechanically damaged, and leaf-mined green ash leaflets produce different VOC profiles (Wist *et al*. 2015). The levels of green leaf volatiles (GLVs) ((*Z*)-3-Hexenal, (*Z*)-3Hexenol, (*Z)*-3-Hexenyl acetate), decrease or become too low to detect in the herbivore-mined leaflets, but increase eight hours after mechanical damage of leaflets (Wist *et al*. 2015). As a generalist parasitoid, *A. polychrosidis* may follow ubiquitous GLV cues to facilitate larval host location on multiple plant species (Ngumbi *et al*. 2010; McCormick 2012). Methyl salicylate (MS) is an HIPV, which is released at a higher rate from both mined, and mechanically damaged green ash leaflets (Wist *et al.* 2015). This compound attracts a number ofnatural enemies, including parasitoid wasps (James and Price 2004, Sasso *et al*. 2009, Degen *et al*. 2012). Because it is an HIPV, MS may play a role in indicating herbivore host presence and density to *A. polychrosidis*. Both MS, and the GLVs, *(Z*)-3-Hexenyl acetate and *(Z*)-3-Hexenol are commercially available lures, and show promise as compounds to attract *A. polychrosidis*.

Apanteles polychrosidis is presumed to be nectar feeding as an adult, and therefore the supplementation of carbohydrate resources near *C. fraxinella* patches should increase wasp retention, and provide nutrition for the wasp to sustain host foraging, longevity, and fecundity (Sirot and Bernstein 1996, Riddick 2007, Varennes *et al*. 2015, Wang *et al.* 2014).

1.5 Research objectives

Here, I test the hypothesis that parasitism levels of *C. fraxinella* by *Apanteles polychrosidis* can be enhanced in infested green ash trees using synthetic HIPVs in Edmonton, Alberta.
The research objectives are to:

1. Evaluate VOCs from green ash leaflets, MS and two GLVs), known to be detected by *A. polychrosidis,* as lures to attract wasps and increase parasitism of *C. fraxinella* in infested ash trees. (Chapter 2)
2. Test the attraction of mated female *A. polychrosidis* toMS and two GLVs in an olfactometer assay. (Chapter 2)
3. Evaluate the provision of two concentrations (10% and 25%) of sucrose solution to retain *A. polychrosidis* in green ash trees and increase parasitism of *C. fraxinella.* (Chapter 3)
4. Evaluate the effect of carbohydrate nutrition on wasp longevity, fecundity, parasitism rate, and offspring fitness. (Chapter 3)
5. Evaluate the effect of carbohydrate nutrition through the provision of buckwheat *Fagopyrum esculentum* Polygonaceae flowers on wasp longevity. (Chapter 3)
6. Evaluate the effect of buckwheat flowers in infested green ash trees, to support retention and foraging activity of *A. polychrosidis*. (Chapter 3)

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Chapter 1 Figures

Figure 1.1 **Green ash (*Fraxinus pennsylvanica*) leaflets rolled into "cones" by *Caloptilia fraxinella*, invasive ash pest in Western Canada.**

Figure 1.2 **Female *Apanteles polychrosidis* (~4mm length), pictured on buckwheat flower.**

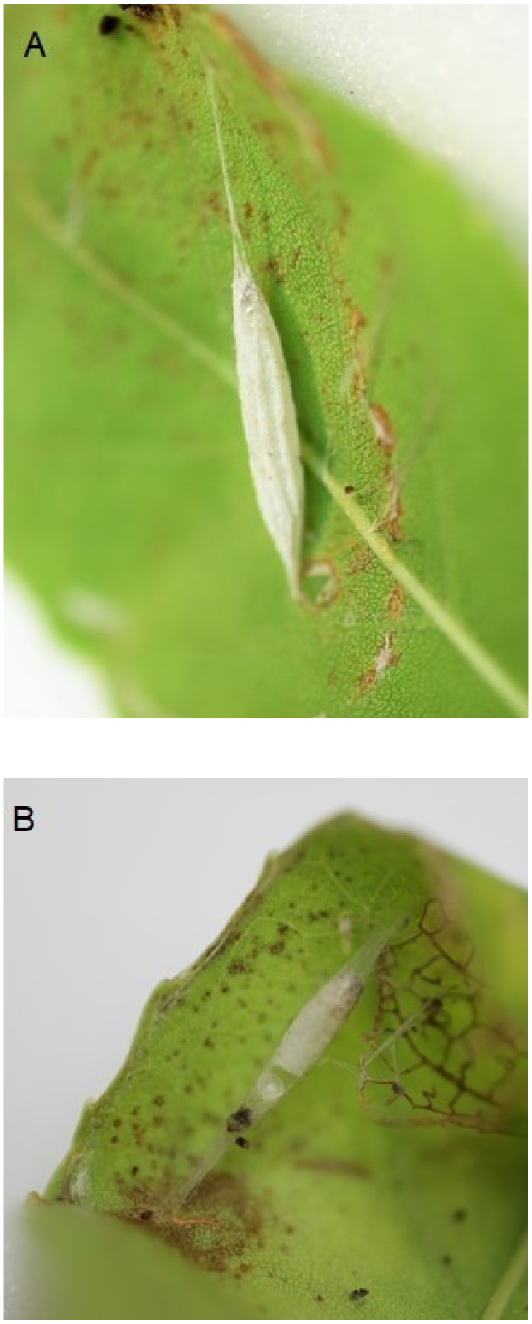

Figure 1.3 **A. Pupal case of *Caloptilia fraxinella* (length: 10 mm)*.* B. Pupal case of *Apanteles polychrosidis* (length: 3.5 mm).**

Chapter 2: Assessment of host plant volatile lures to attract *Apanteles polychrosidis* to ash trees infested with the ash leaf-cone roller, *Caloptilia fraxinella*.

2.1 Abstract

The introduced aesthetic pest *Caloptilia fraxinella* Ely (Lepidoptera: Gracillariidae)*,* or the ash leaf-cone roller, creates highly visible damage to the ubiquitous horticultural ash trees (Oleaceae, Genus *Fraxinus*) in Edmonton, Alberta, Canada. Because pesticide use is undesirable in an urban centre, and the City of Edmonton adheres to an Integrated Pest Management approach for the urban forest, a biological control for *C. fraxinella* is preferred. The native parasitoid wasp, *Apanteles polychrosidis* Vierek (Hymenoptera: Braconidae), has shifted hosts and is the primary parasitoid of *C. fraxinella* in Edmonton, and therefore has potential for biological control. Here, in an effort to increase parasitism of *C. fraxinella* by *A. polycrosidis*, synthetic lures releasing methyl salicylate and two green leaf volatiles (GLVs), (*Z)*-3-Hexenol, and *(Z)*-3-Hexenyl-acetate) at both low and high release rates are tested to enhance attraction and retention of *A. polycrosidis* in infested ash trees. Attraction of *A. polycrosidis* to baited trees was measured by capture on yellow sticky cards positioned in the tree canopy, and by parasitism rate. The same volatile organic compounds (VOCs) s were also tested in an olfactometer to evaluate the attraction of mated females. Both male and female *A. polycrosidis* were more frequently attracted to trees baited with the low dose of both MS+GLVs than to unbaited, infested ash trees. The increased attraction of wasps did not correlate with an increase in parasitism of *C. fraxinella*. The high release rate lures did not enhance attraction of *A. polycrosidis* to infested ash trees. The synthetic VOC treatments tested in the olfactometer assay did not attract mated female *A. polycrosidis*. Parasitism rate of *C. fraxinella* was impacted by VOC treatment at the high release rates, and was negatively correlated with host density in both field experiments.

2.2 Introduction

Plants produce a variety of volatile organic chemicals (VOCs). Many of these compounds are by-products of plant metabolism, and play many roles in plant fitness including herbivore defence, plant-plant communication, below ground defence, and attraction of herbivores and their natural enemies (Dudareva *et al*. 2006). Parasitoids, in particular, use VOCs released by the host plants of herbivores as long-range signals for insect-hostfinding. While chemicals produced by the insect host, such as frass or cuticular compounds are more reliable signals to parasitic Hymenoptera for host finding due to their specificity, they are often less detectable at long range (Vet and Dicke 1990). The ability of parasitoids to perceive differences in plant VOCs seems linked to the level of host specialization, with those having a narrow host range better able to detect variation than generalist parasitoids (De Moraes *et al.* 1998, Röse *et al.* 1998, Ngumbi *et al.* 2010).

Green leaf volatiles (GLVs) are ubiquitous, constitutive short-chain alcohol and aldehyde components of plants. They are released continuously in small amounts, and are almost instantly produced and emitted in much higher amounts when leaves are physically damaged. They have been implicated in the regulation of plant defence genes, and the priming of primary defences in a variety of plants (Engelberth 2004, Farag 2004), and also attract beneficial insects (Scala *et al.* 2013). The generalist parasitoid, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) prefers maize, *Zea mays* (Poaceae), varieties that release higher amounts of the GLVs (Hoballah *et al.* 2002). In addition, *C. marginiventris* prefers artificially damaged cotton plants, *Gossypium hirsutum* (Malvaceae), that release more GLVs than control plants (Rose *et al*. 1998). This species is also attracted in a dose dependent manner, to a blend of volatiles comprised of over 70% GLVs (Turlings *et al*. 1991). A specialist braconid parasitoid, *C. glomerata* is attracted to host plants (*Arabidopsis*) sprayed with the synthetic GLVs (*Z*)-3Hexenal, (*Z)*-3-Hexenol, and (*Z)*-3-Hexenyl acetate (Shiojiri *et al*. 2006).

Methyl salicylate (MS) is a herbivore-induced plant volatile (HIPV). Many plants produce and release volatile compounds, includingMS, when fed upon by insects. Methyl salicylate plays many roles in both direct and indirect plant defences (Garrido *et al.* 2009, Tang *et al.* 2015). As an indirect defence, MS attracts predatory and parasitic insects to plants infested with herbivore hosts. In sweet corn plots, braconid wasps are attracted to MS lures as well as synthetic copies of other HIPVs (Simpson *et al.* 2011). Methyl salicylate-baited blocks in a grape vineyard also attract braconid wasps (James and Price 2003). An aphid parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae), is attracted to MS, and response increases with dose (Sasso *et al*. 2009). Methyl salicylate lures are correlated with an increase in parasitism by *Cotesia marginiventris* of its host, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), at some concentrations (Degen *et al*. 2012).

There is evidence that a blend of volatile chemicals released from plants is important for parasitoid attraction. Tamiru *et al.* 2015 found that, despite 13 HIPVs eliciting a significant electroantennogram response in *Cotesia sesamiae*, only three of those compounds induced a behavioural response alone. *C. marginiventris* are attracted to both the natural and a synthetic blend of plant volatiles released from plants fed on by beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), and the response is dose-dependent (Turlings *et al*. 1991). *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) are successfully attracted in the field to HIPV dispensers emitting a synthetic blend of volatiles which mimic the compounds emitted from the herbivorized host plant, cabbage, *Brassica oleracea* (Brassicales: Brassicaceae) (Uefune *et al.* 2011).

Here, we test MS and GLVs as lures to attract a native parasitoid *Apanteles polychrosidis* Vierek (Hymenoptera: Braconidae), to increase parasitism of an invasive pest of ash trees, the ash leaf-cone roller, *Caloptilia fraxinellla* Ely (Lepidoptera: Gracillariidae) in Edmonton, Alberta, Canada. The ash leaf-cone roller was first recorded in Edmonton in 1999

(Pohl *et al.* 2004). Young larvae are leaf miners and mature larvae roll leaflets (Evenden 2009, Pohl *et al*. 2004) into aesthetically displeasing cones (Figure 1.1). Fourth instar larvae are considered nuisance pests when they hang from silk threads to disperse.

The primary parasitoid of *C. fraxinella* in Edmonton is *A. polychrosidis* (Wist and Evenden 2013). This wasp has a wide Nearctic range (Fernández-Triana and Huber 2010) and is native to Edmonton. Previous host records of *A. polychrosidis* include larvae from the moth family Tortricidae, including *Choristoneura rosaceana* (Harris), *Choristoneura fumiferana* Walsingham , *Pandemis limitata* Robinson, *Pandemis pyrucana* Kearfott*, Platynota idaeusalis* (Walker), and *Endopiza viteana* Clemens (all Lepidoptera:Tortricidae) (Seaman *et al*. 1990; Biddinger *et al*. 1994; Li *et al*. 1999, Cossentine *et al.* 2004, Fernandez-Triana and Huber 2010, Pfannenstiel *et al* 2012). In Edmonton, the native larval hosts of *A. polychrosidis* have not been identified to species. However, nine *A. polychrosidis* were reared from Tortricidae larvae, from leaf-rolls collected in late April and early May 2016 and 2017(McPike unpublished data). *Apanteles polychrosidis* has shifted hosts to exploit the introduced pest, *C. fraxinella*, as its larval host in summer (Wist and Evenden 2013). This is the first record of *A. polychrosidis* using a non-tortricid host.

Apanteles polychrosidis detect and orient to volatile organic compounds (VOCs) released from ash trees. The headspace volatiles from intact and damaged green ash seedlings contain 13 VOCs that elicit a response from both male and female *A. polycrosidis* antennae, in a gas chromatography- electroantennographic detection analysis (Wist *et al*. 2015). Those VOCs were identified as: (1) (*Z)*-3-Hexenal, (2) (*Z)*-3-Hexenol, (3) *(Z)*-3-Hexenyl-acetate, (4) ocimene, (5) linalool, (6) *E* 4,8-dimethyl-1,3,7-nonatriene, (7) methyl salicylate, (8) bourbonene, (9) *E*-βfarnesene, (10) α-farnesene, and (11) dendrolasin. Two other compounds detected by wasp antennae were not identified. Intact, mechanically damaged, and leaf-mined green ash leaflets produce different VOC profiles (Wist *et al*. 2015). Release of compounds classified as green leaf volatiles (GLVs) ((*Z*)-3-Hexenal, (*Z*)-3-Hexenol, (*Z*)-3-Hexenyl acetate), decreases after leafmining, but increases after mechanical damage of leaflets (Wist *et al*. 2015). Methyl salicylate (MS) release increases in both mined and mechanically damaged green ash leaflets (Wist *et al.* 2015). Wist *et al*. (2015) found *A. polycrosidis* were more attracted to black ash leaves mined by larval *C. fraxinella* than to unmined leaflets, indicating that the wasp is attracted to HIPVs. The GLVs (*Z)*-3-Hexenol, and (*Z)*-3-Hexenyl acetate, and the herbivore-induced plant volatile, MS, may have potential to attract *A. polycrosidis*, as they are attractive to other braconid parasitoids, and other parasitic Hymenoptera (Reddy and Guerrero 2000, James and Grasswitz 2014). Synthetic lures of ash VOCs may increase density pf *A. polychrosidis* at infested ash trees, and increase parasitism of *C. fraxinella*.

2.2.1 Objectives

I tested the hypothesis that synthetic VOC lures can attract *A. polychrosidis* to support parasitism of *C. fraxinella*. The following objectives were pursued:

1. Evaluate MS and two GLVs known to be detected by *A. polychrosidis,* as lures to attract wasps and increase parasitism of *C. fraxinella* in infested ash trees.
2. Test the attraction of mated female *A. polychrosidis* to MS and two GLVs in an olfactometer assay.

2.3 Methods

2.3.1 VOC lure field experiments

This study tested the effect of synthetic lures releasing MS or two GLVs alone or combined, on the attraction of, and parasitism by *A. polychrosidis*, in green ash trees infested with *C. fraxinella*. Experiments were conducted with lures at both low and high release rates (Table 2.1). The for the higher release rate lures, the rates were provided by Contech Enterprises Inc. (Delta, BC). Those of the lower release rate lures were measured and calculated in the laboratory (Appendix 2).

2.3.2 Experiment 1: Low release rate VOC lures

In the summer of 2016, ten sites were selected at least 600m apart in areas of Edmonton known to have *C. fraxinella* infestation (Figure 2.1). At each site, every second ash tree (14-35 m apart) was randomly assigned one of the following treatments: (1) A 250 µl eppendorf lure containing 200 µl of MS (99% purity, Thermo Scientific); (2) One 250 µl eppendorf lure of each of the GLVs ((*Z)*-3-Hexenol and (*Z)*-3-Hexenyl acetate) containing 200 mg of each compound (Contech Enterprises Inc., Delta, BC); (3) both MS and GLV lures together, or (4) Blank control (no lures). Each lure, or set of lures were secured with wire to the tree trunk, mid-canopy on a random side of the tree trunk with wire, mid canopy. To assess the activity of *A. polychrosidis* in each of the treated trees, three yellow sticky cards (18 cm x 9cm, 2cm x 2cm grid) (Alphascents Insect Monitoring Systems, West Linn, Oregon) were positioned in each tree at each site in the low-mid canopy. Sticky cards were changed weekly from 10 May through 21 July, 2016. After collection, the traps were wrapped in plastic and frozen until processed. Wasps were identified as *A. polychrosidis* based on wing characters, and femur colour (Wist and Evenden 2013), under a dissecting scope at 40X magnification. Wasps were separated by sex based on the presence of an ovipositor in females. Wasp density was calculated as the total number of the first-generation *A. polychrosidis* (10 May 2016-8 June 2016) captured in each treated tree divided by the number of traps collected from those trees across the first generation.

Parasitism of *C. fraxinella* on each treated tree at each site was assessed after the emergence of the second generation of *A. polychrosidis*, in August 2016. Approximately 100 leaf rolls were haphazardly collected from a 1.83 m ladder throughout the tree canopy (or as many as possible, if *C. fraxinella* infestation level was low). Pupal morphology was used to identify the number of *C. fraxinella* and *A. polychrosidis* (Figure 1.2) (after Wist *et al*. 2015) per each 100-roll sample. The overall density of *C. fraxinella* infestation was measured in August by visually estimating percentage of ash leaflets rolled, per treated tree (after principles of *Field manual for describing terrestrial ecosystems* 2010).

2.3.3 Experiment 2: High release rate VOC lures

In the summer of 2017, a similar experiment was conducted at the same field sites. Only the first generation of *A. polychrosidis* was targeted from 3 May to 21 June. The VOC lures were bubble packs (Contech Enterprises Inc., Delta BC) and released compounds at a higher release rate than lures in the 2016 experiment (Table 2.1). Wasps were captured and identified as in Experiment 1, but they were not separated by sex.

2.3.4 Statistical analyses: Experiments 1 and 2

All statistical analyses were conducted in R version 3.4.2 (R Core Team 2017). Data were evaluated visually for normality and heteroscedasticity.

In Experiment 1, the average count of wasps captured on yellow sticky traps positioned in trees over the first wasp generation was square-root transformed to approach normality. Average wasp count was the dependent variable in a linear mixed-effects model. Fixed explanatory variables were semiochemical treatment and the sex of *A. polychrosidis* (Table 2.2).

Site was included as a random variable. Non-significant interaction terms were removed from the model. Analyses were conducted using lme4 package version 1.1-11 (Bates *et al*. 2015). Post hoc multiple comparison Tukey tests were conducted with multcomp, version 1.4-8 (Hothorn *et al.* 2008), to determine differences between significant factors.

A second linear mixed model compared average wasp counts in trees treated with the high release rate lures, in Experiment 2 conducted in 2017. The average wasp count was square-root transformed. The fixed explanatory variable was semiochemical treatment, and site was included as a random variable. Analysis was conducted using lme4 package version 1.111 (Bates *et al*. 2015).

The parasitism rates of *C. fraxinella* in ash trees treated at low (2016) and high (2017) release rates, were compared with separate generalized linear mixed-effects models with a binomial error distribution in lme4 package version 1.1-11 (Bates *et al*. 2015). A vector (y), was constructed, binding the number of parasitized larvae, and the number of unparasitized larvae into a single object, to act as the response variable (Crawley 2015), with VOC treatment as the explanatory variable, host density as a covariate, and site specified as a random factor. The interaction between VOC treatment and host density was not significant for Experiment 1, and was removed from the model. For Experiment 2, the interaction between explanatory variables was significant, and multiple comparisons Tukey test was conducted using lsmeans package (version 2.27-2, Lenth 2016). Linear models for both Experiments 1 and 2 were used to show the relationship between parasitism level and host density.

A Welch's t-test was used to compare the overall mean number of first generation *A. polychrosidis* captured between years.

2.3.5 Experiment 3: Four-way olfactometer bioassay

The attraction of mated female *A. polychrosidis* to synthetic sources of MS and GLVs was tested in an olfactometer assay.

Insects

Apanteles polychrosidis were collected between 13-28 June, 2016 from sites across Edmonton. Leaf rolls without emergence "windows" were selected following the protocol of Wist *et al.* (2015). Leaf rolls were placed individually in 36 ml plastic cups positioned on trays. Ten15 trays were stacked in black plastic bags with moistened paper towels to maintain humidity. Leaf rolls were examined daily for wasp emergence and newly emerged males and females were paired within a single cup. Each pair of wasps was provided with a sucrose solution (25%, v/v) through a dental wick. Paired wasps were maintained at 21 °C, in a laboratory with fluorescent lights. Female wasps were assumed mated 24 hr after pairing, as they mate shortly after eclosion (Wist 2014). Wasps were maintained as pairs until females were used in trials at 9-11 days old, as *A. polychrosidis* is capable of parasitism up to 20 days post eclosion (Cossentine 2005).

Bioassay Methods

Methyl salicylate (Sigma-Aldrich, 99% purity) was diluted to 1mg/ml in hexane. Each of the two GLVs, were extracted from eppendorf tube lures (described above) and diluted to 1mg/ml in hexane. Five µl of each solution was applied with a micropipette to 1 x 1 cm filter paper on a straight pin, and allowed to evaporate for at least 10 seconds in a fumehood. The treatments were 5 µg MS, 5µg of each GLV (separate filter paper squares on 2 pins), 5 µg MS+5 µg each GLV (separate filter paper squares on 3 pins), or 5 µl of the solvent, hexane, as the clean air control. After solvent evaporation, filter papers were positioned in separate arms of the olfactometer and a female wasp was introduced to the bioassay within one minute.

The olfactometer was a circular six-way olfactometer (Analytical Research Systems Inc., Gainsville, FL.) with two airflow passages blocked to provide four odour sources. A circular hole in the center of the arena served as both the entrance point for the insect, as well as the attachment point for the air outflow (Figures 2.2, 2.3). Airflow into the arena from the four odour sources was 1L/min, and outflow at the center of the arena was 3L/min ("Standard Operating Procedure Four Chambered Olfactometer, IPM Lab" 2011), regulated with an airflow system (Model OLFM-6C-ADS+VAC, Analytical Research Systems Inc., Gainsville, FL.). Six 100-watt incandescent light bulbs diffused through white paper provided bright (600-650 lux), even light throughout the bioassay arena. Females were individually tested in 15 min trials conducted between 9 am and 4 pm. Odour sources were replaced for each wasp tested. A Logitech web camera was adhered to a ring clamp and positioned on a clamp stand at 50 cm above the platform, with the lens aiming downward at ~45º, to record the wasp movement in each assay. The stand with the camera was placed to have the whole olfactometer arena in the frame, but the camera out of view of the arena. Following each day of trials, the olfactometer was cleaned with 70% ethanol, glassware was rinsed three times with acetone, and three times with hexane, and dried in the oven at 60 °C overnight. Treatment positions were randomly rearranged for trials conducted on each of 3 days. Each wasp was only used once (n=21).

2.3.6 Statistical Analysis: Experiment 3

A grid dividing the arena into 6 triangular segments was superimposed to each recording with Photoshop (Adobe Creative Cloud) and the time spent in each sector was recorded. Female *A. polychrosidis* that did not move from their initial position were counted as nonresponders (n=3). The proportion of time each female spent in the sector of each treatment was calculated and those numbers were standardized over the observation period of 900 seconds (15 minutes). The number of seconds spent by each female wasp in the segment closest to each VOC treatment was cube-root transformed, and compared in a linear mixed model using lme4 package version 1.1-11 (Bates *et al*. 2015).

2.4 Results: VOC lure field experiments

2.4.1 Experiment 1:

Both semiochemical treatment and wasp sex significantly affected the average number of wasps captured on yellow sticky cards positioned in the variously treated ash trees in 2016 (sex: $\chi^2=34.84$, df=1, $p<0.0001$, treatment: $\chi^2=14.80$, df=3, $p=0.002$) (Table 2.2). The average number of females (0.58±0.08) captured per trap during the first generation was significantly higher than the number of males (0.22±0.06). Tukey pairwise testing showed a significantly higher number of wasps in trees baited with the low release rate MS+GLVs, compared to control trees (no lures) (Figure 2.4). The number of wasps attracted to trees baited with MS or GLV lures alone was intermediate.

Although more wasps were attracted to trees baited with the MS + GLV combined lures compared to unbaited trees in 2016, the parasitism rate of *C. fraxinella* by *A. polychrosidis* in these trees was not different than any of the other treatments. The Tukey pairwise comparison showed that parasitism in trees treated with GLV lures (30.9±7.78%) was marginally higher than that in trees treated with MS (21.83±6.12 %) lures ($p=0.059$). Trees treated with the low release rate MS had the lowest parasitism rate of the treated trees. Parasitism by *A. polychrosidis* was significantly negatively correlated with *C. fraxinella* density ($y= 33.027$, $x=-0.49$, $p=0.0028$, adjusted $R^2=0.20$) (Figure 2.5).

2.4.2 Experiment 2:

There was no significant difference in the average number of A. polychrosidis captured on yellow sticky cards positioned in the variously treated ash trees in 2017 ($\chi^2=1.82$, df=3, p=0.61).

Wasps capture was highly variable in trees treated with the high release rate lures. Wasps were equally attracted to trees treated with MS + GLV lures (1.80±1.09 per trap), MS lures (1.05±0.87 per trap), and GLV lures (1.50±0.40 per trap). Wasp capture on traps positioned in unbaited control trees seemed lower (0.8±0.33 per trap) than that in treated trees but was not significantly different.

There was a significant interaction between high release rate VOC treatment and host density ($\chi^2=11.607$, df=3, p=0.0089) that affected the parasitism rate of *C. fraxinella* by *A. polychrosidis* (Figure 2.6). Trees treated with the high release rate GLV lures alone had the lowest parasitism rates at higher host density. Parasitism rate was least affected by host density in trees with high release rate methyl salicylate lures alone, and MS decreased the effect of higher host density on parasitism rate in the trees with both lure types. The overall linear model showed parasitism rate to be significantly negatively correlated with host density ($y=36.7-0.54x$, $p<0.0001$, adjusted $R^2=0.29$) (Figure 2.7).

The mean average A. polychrosidis wasps captured over generation 1 in 2016 (1.0043± 0.092 per trap) was significantly higher than that in 2017 (0.14±0.034) (t=-9.14, df=196.83, p<0.0001).

2.4.3 Results: Experiment 3

There were no significant differences between the time spent by mated female wasps in the sections of the olfactometer supplied with the different odour treatments in the 15 min assay. There is a trend toward less time spent in the GLV-treated segment, but it was not significant (χ^2=3.24, df=3, p=0.36) (Figure 2.8).

2.5 Discussion

Male and female *A. polychrosidis* are attracted to low release rate VOC lures in a similar pattern but numerically more females are captured in traps positioned in treated ash trees. It is likely that more captured females reflects the female-biased sex ratio of *A. polychrosidis* (Wist and Evenden 2013) rather than an inherent difference in response to host finding cues between the sexes. As *A. polychrosidis* is protandrous, the female-biased sex ratio revealed in the current study could also be an artifact of emergence phenology within the season.

It is unclear why male *A. polychrosidis* are attracted to synthetic VOCs and potentially those produced by ash trees. The reliability-detectability hypothesis (Vet *et al*. 1991, Vet and Dicke 1992) provides a possible explanation. While pheromones are typically species specific, and therefore a “reliable” cue for mate finding, plant volatiles are ubiquitous and less costly as infochemicals. The biological cost of producing pheromones may have led to male *A. polychrosidis* eavesdropping on plant host cues as mate-finding kairomones (Kaplan 2017). Attraction of four braconid parasitoids (*C. glomerata*, *C. marginiventris, Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae), *Microplitis mediator* Haliday (Hymenoptera: Braconidae), to HIPVs in an olfactometer assay does not vary with wasp sex or mating status suggesting that plant cues can be used by both sexes (Xu et al 2014). The presence of cotton plants enhances attraction of male parasitoids, *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) to females, in an olfactometer bioassay (McAuslane *et al*. 1990). Close-range chemical mating cues are known for a number of parasitoids (Danci *et al*. 2016, Lo Pinto *et al*. 2013, Shu and Jones 1993), indicating the possibility that host plant VOCs function as long-range attractants for mate aggregation.

Despite the attraction of both sexes of *A. polychrosidis* to low release rate VOC lures in the field, I was unable to show attraction of mated female wasps to these same compounds using an olfactometer assay in the laboratory. Wasps in the olfactometer exhibited detection and orientation behaviours (Klomp 1981, Colazza 1999) to the tested odours including arrestment, antennal drumming and frequent turning. Retention in the area adjacent to the odour sources, however, did not vary with odour presented. Previous olfactometer studies on *A. polychrosidis* used a y-tube olfactometer positioned at a 45° angle to compare wasp response to natural semiochemical cues (Wist *et al.* 2015). In that study, wasps were also primed to respond by pre-exposure to a larval host before the bioassay (Wist *et al.* 2015). These methodological differences may partially account for reduced responsiveness of female wasps in the current study. It is also possible that VOCs tested in the olfactometer were not attractive alone to wasps . Lures releasing VOCs in the field are presented in the background of natural semiochemicals released by infested ash trees. A blend of plant compounds may be required to elicit oriented attraction in this species (D' Alessandro *et al*. 2009). Further, the release rate of the VOCs tested in the olfactometer may not elicit attraction. It appears that *A. polychrosidis* foraging behaviour is strongly influenced by VOC release rate as is illustrated here and by Wist *et al.* (2015).

Attraction of male *A. polychrosidis* to ash trees in the current study could feasibly be mediated by long-range female-produced sex pheromone signals. The chemical ecology of sex attraction in parasitoids is poorly studied, but female-produced long-range cues are known in some species (Ayasse *et al*. 2001). For *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of codling moth *Cydia pomonella* Linaeus (Lepidoptera:Tortricidae), male wasps are attracted to traps baited with female wasp pheromone lures (Suckling 2002). Males of *Macrocentrus grandii* Gooidanich (Hymenoptera: Braconidae) fly upwind to female pheromone in a wind tunnel, but are not attracted to any of the insect host plants tested (Jones 1996). Male *Itoplectis naranyae* Ashmead (Hymenoptera: Ichneumonidae), a generalist solitary parasitoid of a variety Lepidoptera and Coleoptera, respond to zero and one-day-old females both in laboratory and in field experiments (Itadani and Ueno 2014). The pattern observed in the current experiment could be the result of attraction of female *A. polychrosidis* to VOCs positioned in ash trees followed by male orientation to females via pheromones. Male antennae, however, are known to respond to ash VOCs (Wist *et al.* 2015), and this still requires an explanation under this hypothesis.

Increased attraction of male and female *A. polychrosidis* to trees treated with the combined low dose VOCs did not correlate to higher parasitism in treated ash trees. Increased aggregation of parasitoids at a host patch does not always lead to more parasitism of insect hosts in the patch (Morrison and Strong 1980). More encounters with conspecifics in a host patch can lead to more time spent making oviposition decisions (Visser *et al*.1999). Time may also be spent avoiding encounters with conspecifics. *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) avoid conspecific wasps with or without the presence of host kairomones (Castelo *et al*. 2003). Competition between conspecific females decreases longevity of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), a parasitoid of cabbage pest *Brevicoryne brassicae* (Linaeus) (Hemiptera: Aphididae) (Kant and Minor 2017). It is also possible that the attraction of more *A. polychrosidis* to trees treated with VOCs results in superparasitism one of larval hosts by more than one female parasitoid (Godfray 1994).

Detection and selection of unparasitized hosts increases foraging time and may decrease the efficiency of the female parasitoid under levels of high competition (Visser, van Alphen and Nell 1990).

In Edmonton, the effect of *C. fraxinella* density on parasitism by *C. fraxinella* varies with host plant and led Wist *et al.* (2015) to hypothesize that the HIPVs released by green and black ash mediate parasitism by *A. polychrosidis* in this system. Results of the current experiments support the findings of Wist *et al.* (2015) and illustrate that the parasitism rate decreases with an increase in host density in green ash trees. It is possible that parasitoids are swamped by high host numbers (Lessels 1985) and the overall rate of parasitism decreases with host density. It is also possible that high levels of HIPV released by plants attacked by many herbivores masks foraging cues (Schröder and Hilker 2008), and inhibits the close-range foraging behaviour of parasitoids. In the current study, parasitism was marginally lower in the trees treated with the low dose MS lures, compared to those with the GLV lures. Plant-produced MS may be an indicator of the health of the parasitoid's host. Koinobiont parasitoids rely on the nutrition of host larvae for development (Quicke 2015). Poor plant quality negatively affects herbivore performance in herbivore-plant interactions and this can also impact the third trophic level (Godfray 1994). Parasitoid larval growth and adult weight of two ichneumonid koinobiont parasitoids of *Plutella xylostella* (Linnaeus) (Lepidoptera: Noctuidae) larvae were reduced when the host larvae were reared on herbivorized cabbage (Bukovinszky *et al.*2012). The amounts released from the synthetic MS lures may signal low quality ash trees to *A. polychrosidis* that may support low quality larval hosts. Methyl salicylate lures attract predatory insects such as lacewings (Order Neuroptera), ladybird beetles (Coleoptera: Coccinellidae), and syrphid flies (Diptera: Syrphidae) (Mallinger *et al*. 2002, James and Price 2004). The quality of the prey item is less important to insect predators than parasitoids as they generally gain nutrition from more than one prey item. Reports of braconid wasp attraction to MS lures (James and Price 2004) may be idiobiont parasitoids, which are less dependent on well nourished larval hosts for success than konobiont parasitoids like *A. polychrosidis* (Sarfraz *et al*. 2009).

Interestingly, in Experiment 2, the presence of the high release rate GLV lures alone, led to decreased parasitism rate, in trees with high insect host density, compared to other treated trees. Defense chemical pathways are induced in plants exposed to a number of GLVs. Exogenous application of (*Z*)-3-Hexenol on corn plants activates the genes for, and increase the production of (*Z*)-3-Hexenyl acetate (100X increase) and MS (to levels similar to those found in herbivorized corn plants) (Farag *et al*. 2005). Tomato plants exposed to (*Z*) 3-Hexanol produce and release the HIPVs 2-carene, and β-phellandrene (Farag and Paré 2002). It may be that the higher doses of (*Z*)-3-Hexenol in Experiment 2 induced the production of multiple HIPVs, which affect *A. polychrosidis* foraging and parasitism behaviour, and this effect is amplified with higher herbivore attack on the tree.

Methyl salicylate is well known to induce pathogen defence in plants (Deng *et al.* 2004) and is an airborne signal that induces these reactions in plants in range of the signal (Shulaev *et al.* 1997). The high dose MS lures may be inducing the pathogen defence response in the treated trees. Pathogen/plant association has been linked to effects on parasitism in some systems. *Tischeria ekebladella* Bjerkander (Lepidoptera: Tischeridae), a leaf miner on oak, experiences higher parasitism on mildew-infected than uninfected oak leaves (Tack *et al.* 2012). The induction of plant pathogen pathways in ash trees exposed to high release MS lures may be affecting parasitism levels in this experiment.

Trap capture of *A. polychrosidis* and other specimens on yellow sticky cards was lower in 2017 than 2016. This is most likely due to less favourable environmental conditions in the spring of 2017. The coordination of the phenology of *A. polychrosidis* with *C. fraxinella* and *Fraxinus* is an important component of understanding this system that remains to be tested under field conditions.

It is clear that *A. polychrosidis* response to host plant is mediated by VOCs. The relationship between wasp attraction, and the induction of parasitism of the insect host need to be explored further.

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Figures

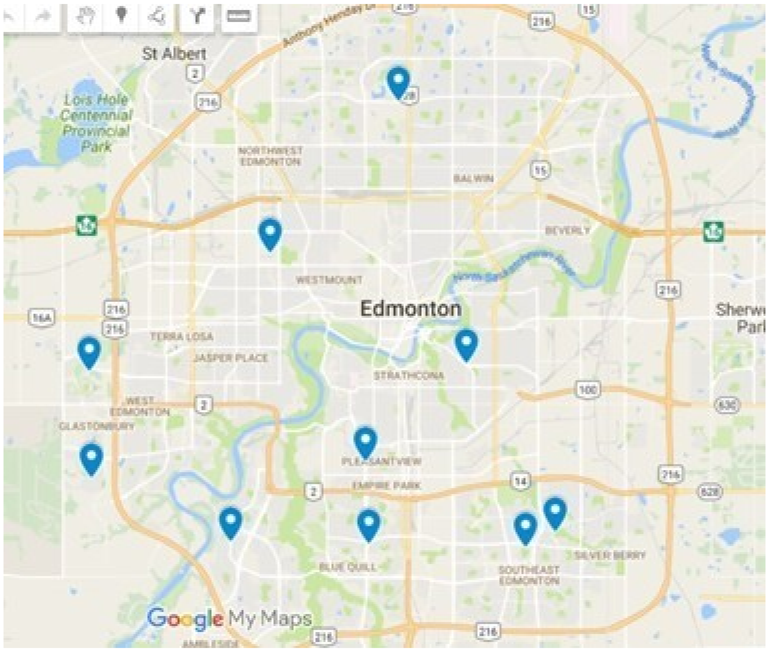

Figure 2.1 **Map of sites in Edmonton, Alberta, Canada (53.5444° N, 113.4909° W) for Experiments 1 and 2**. Experiments testedattraction of low and high release rate VOC lures to *Apanteles polychrosidis*, conducted in 2016 and 2017, respectively.(*Google Maps*, accessed Sept. 2017)

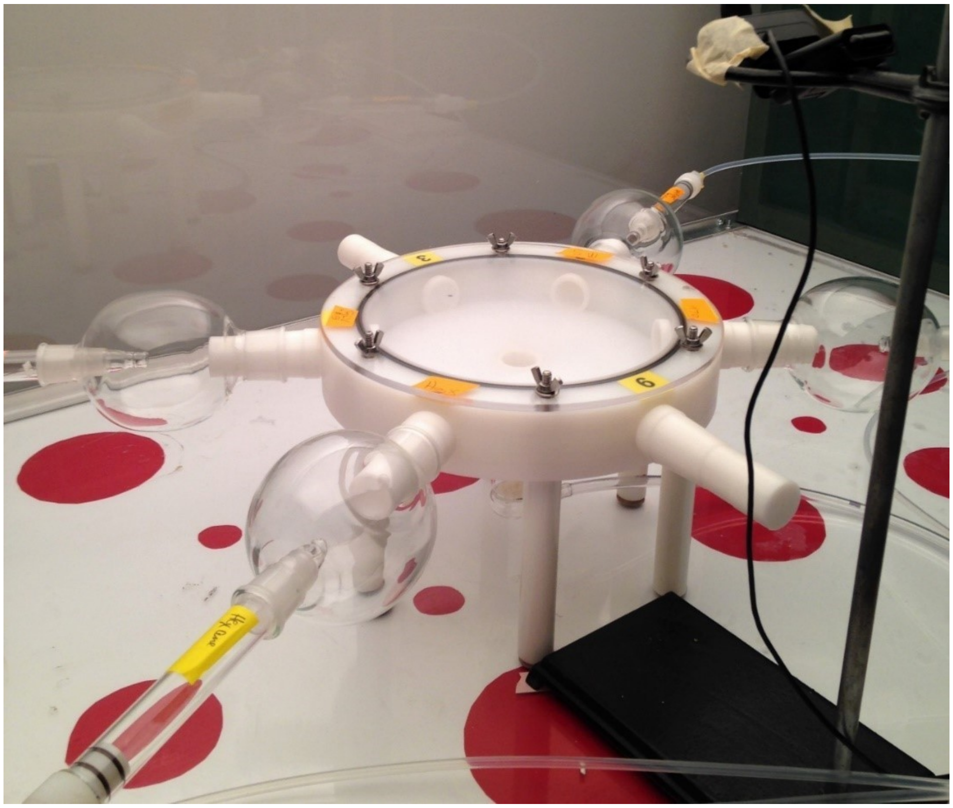

Figure 2.2 **Six-way olfactometer with two ports blocked.** Olfactometer was used to test response of mated female *Apanteles polychrosidis* to three odour sources and a blank control.

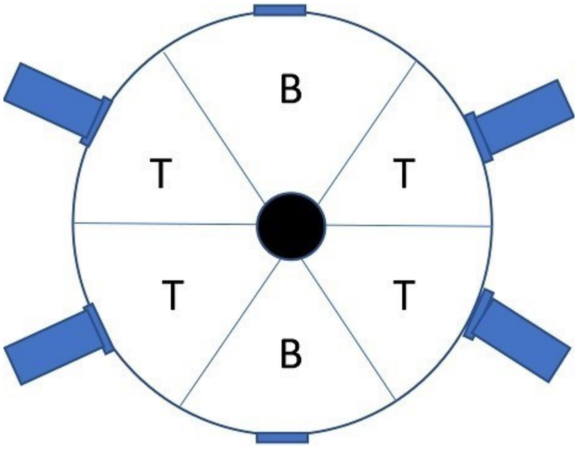

Figure 2.3 **Top view of six-way olfactometer with two ports blocked**. T=Treatment, B=blocked. The black circle in the centre represents the entrance where the insect is introduced into the arena and where the air outflow is attached, on the underside of the arena.

Figure 2.4. **Mean number of first generation *Apanteles polychrosidis* captured in trees baited with low release rate volatile organic compound (VOC) lures (n=10)**. One green ash tree at each site was treated with low release rate lures of (1) methyl salicylate (MS) (1.72 mg/day 20ºC): (2) green leaf volatiles (GLVs) ((*Z)*-3-Hexenol (0.005 mg/day, 20ºC) and (*Z)*-3Hexenyl acetate (0.32 mg/day, 20ºC); (3) both MS and GLVs (MS+GLVs); or (4) no lures (Control). A linear mixed model showed no interaction between sex of wasp and VOC treatment. Significantly more female wasps than males were captured. Bars marked with different letters within wasp sex are significantly different (compact letter display (cld) of Tukey pairwise comparisons).

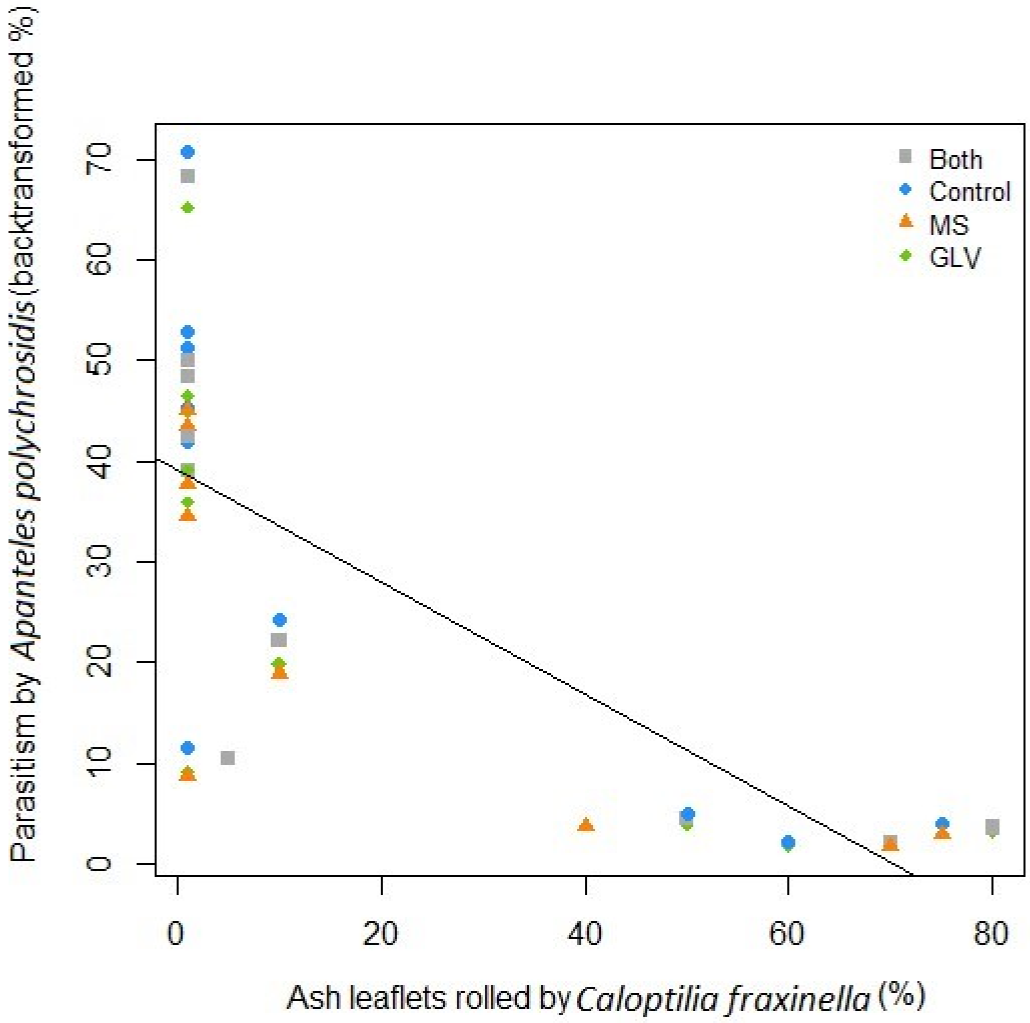

Figure 2.5 **Linear model of parasitism by *Apanteles polychrosidis*, as affected by host density**. Percent parasitism is the backtransformed value of the weighted vector, y= (parasitized, not parasitized). Green ash trees were treated with VOC treatments of low release rate lures of either methyl salicylate (MS) (1.72 mg/day 20ºC), green leaf volatiles (GLV) ((*Z)*-3Hexenol (0.005 mg/day, 20ºC) and (*Z)*-3-Hexenyl acetate (0.32 mg/day, 20ºC)), both MS and green leaf volatiles (Both), or no lures (Control) (y= 33.0-0.49x, p=0.0028, adjusted R²=0.20).

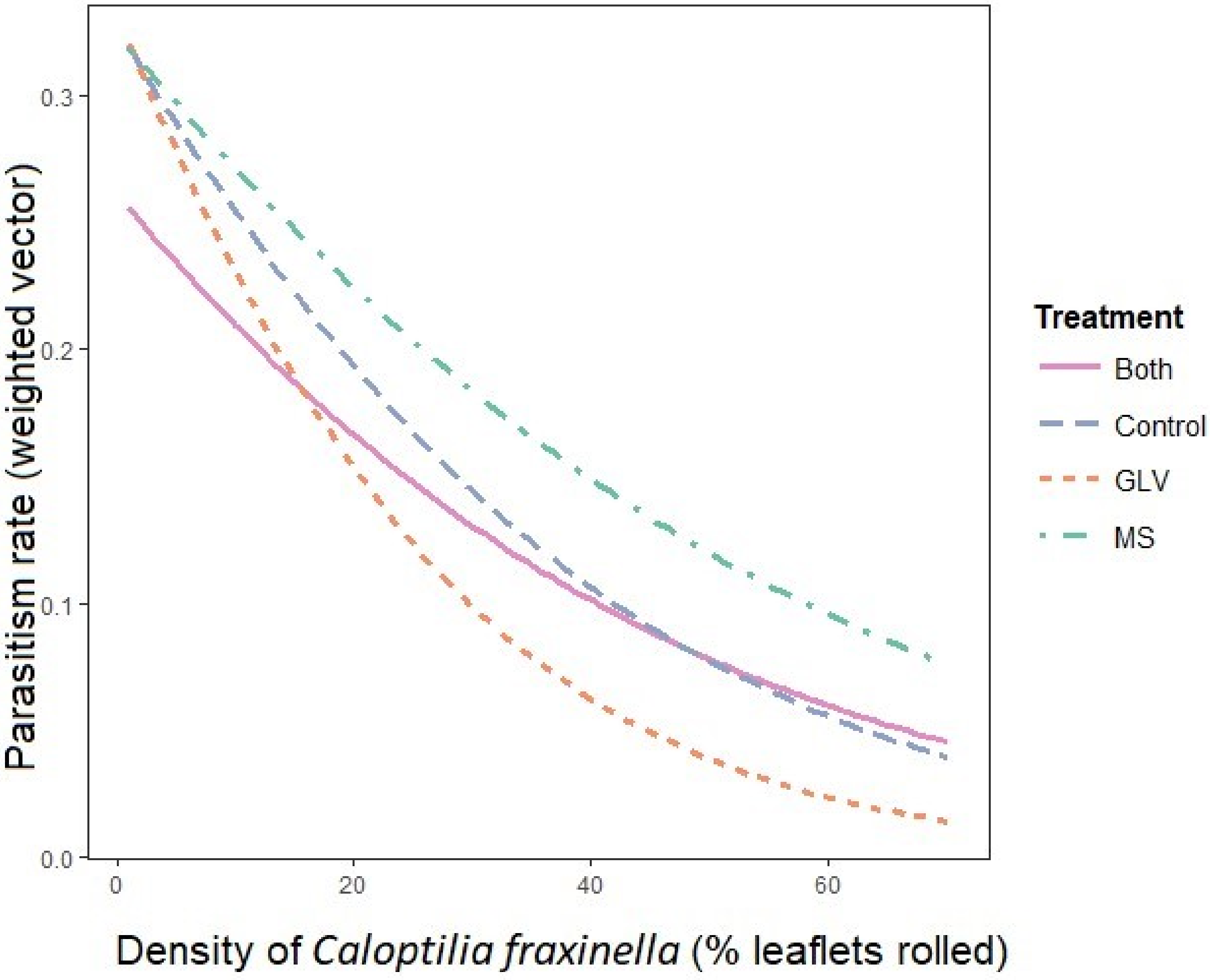

Figure 2.6 **Interaction plot of parasitism rate by *Apanteles polychrosidis*, as affected by *Caloptilia fraxinella* density, with volatile organic compound (VOC) treatment.** Percent parasitism is the value of the weighted vector, y= (parasitized, not parasitized). Green ash trees were treated with VOC treatments of high release rate lures of either methyl salicylate (MS) (5 mg/d, 20ºC), green leaf volatiles (GLV) ((*Z)*-3-Hexenol (3.7 mg/day, 20ºC) and (*Z)*-3-Hexenyl acetate (13 mg/day, 20ºC), both MS and green leaf volatiles, or no lures (Control).

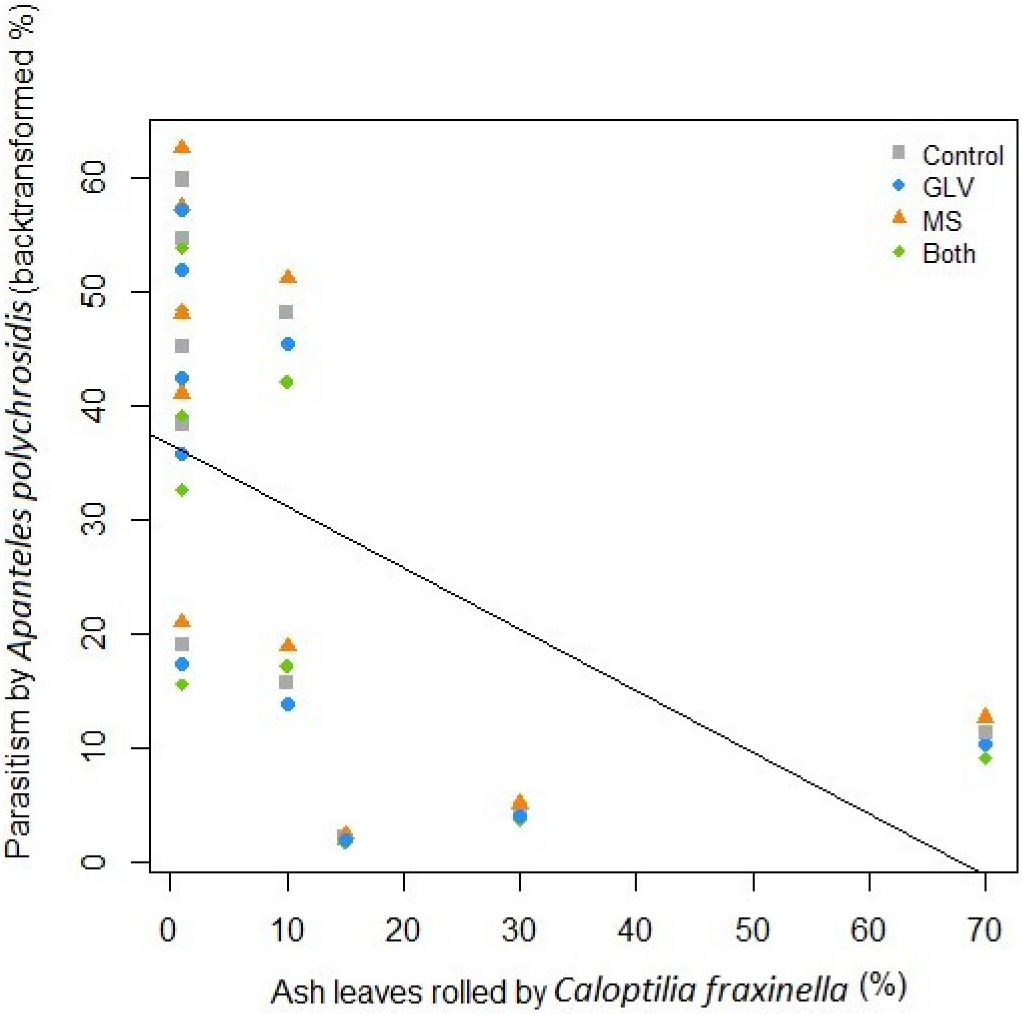

Figure 2.7. **Linear model of parasitism by *Apanteles polychrosidis*, as affected by *C. fraxinella* density, with volatile organic compound (VOC) treatment.** Percent parasitism is the backtransformed value of the weighted vector, y= (parasitized, not parasitized). Green ash trees were treated with VOC treatments of high release rate lures of either methyl salicylate (MS) (5 mg/d, 20ºC), green leaf volatiles (GLV) ((*Z*)-3-Hexenol (3.7 mg/day, 20ºC) and (*Z*)-3Hexenyl acetate (13 mg/day, 20ºC), both MS and green leaf volatiles, or no lures (Control) ($y=36.7-0.54x$, $p<0.0001$, adjusted $R^2=0.29$).

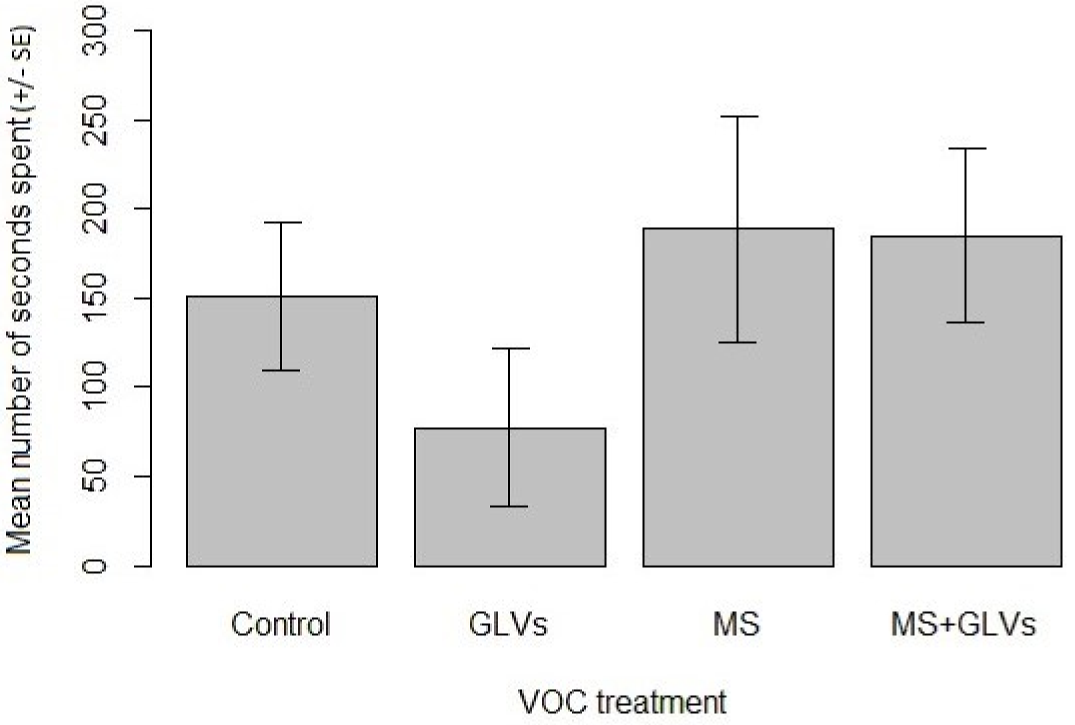

Figure 2.8. **Mean (± SE) number of seconds spent by mated female *Apanteles polychrosidis* (n=20) in proximity to various VOC treatments presented in an olfactometer**. A linear mixed model compared the mean time spent closest to each VOC treatment. Volatile organic compound treatments were: (1) 5 µg methyl salicylate (MS); (2)5µg of each GLV ((*Z)*-3-Hexenol and (*Z)*-3-Hexenyl acetate); (3) 5 µg MS+5 µg of each GLV; or (4) 5 µl of HPLC grade hexane(Control). Females were in the trial for 900 seconds.

Tables

Table 2.1 **Release rates of synthetic volatile organic compounds placed in *Caloptilia fraxinella* infested ash trees for Experiments 1 and 2.**

Compounds Tested	**Experiment 1: Low release rate (mg/day, 21ºC)**	**Experiment 2: High release rate (mg/day, 20ºC)**
methyl salicylate	2.2	5
(Z)-3-Hexenol	0.024	3.7
(Z)-3-Hexenyl acetate	0.43	13

Table 2.2. **Statistical Models for Experiments 1-3.**

Experiment 1	
Wasp density	lmer(sqrt(avg#wasps) ~ VOC treatment * sex + (1 | site))
Parasitism	glmer(y~VOC treatment*%rolled + (1 | site), family binomial), y=(parasitized , not parasitized)
Host density	lm(parasitism~%rolled)
Experiment 2	
Wasp density	lmer(sqrt(avg#wasps)~VOC treatment+(1|site))
Parasitism	glmer(y~VOC treatment*%rolled + (1 | site), family binomial), y=(parasitized , not parasitized)
Host density	lm(parasitism~%rolled)
Experiment1 & 2	
Total number wasps	t.test(average#wasps~year)
Experiment 3	
Seconds per treatment	lmer(#sec out of 900^(1/3)~VOC treatment + (1|ID))

Chapter 3: The provision of carbohydrate resources to attract and support *A. polychrosidis*, to increase parasitism of *C. fraxinella*

3.1 Abstract

The provision of nutritional resources for beneficial insects that support pest control, such as parasitoid wasps, is one strategy of conservation biological control. This could be an important element for the development of a biological control program to help control the ash leaf-cone roller, *Caloptilia fraxinella* Ely (Lepidoptera:Gracillariidae), an introduced pest of horticultural ash trees (*Fraxinus*) in a number of Prairie cities including Edmonton, Alberta, Canada. In the current study, I test the impact of carbohydrate food provision to support parasitism of *C. fraxinella* by its primary parasitoid, *Apanteles polychrosidis* Viereck (Hymenoptera: Braconidae), in Edmonton. Laboratory experiments compared longevity of wasps fed sucrose solutions at one of two concentrations (10 and 25%), or buckwheat flower, *Fagopyrum esculentum* (Polygonaceae). Parasitism rate and offspring fitness were compared for females fed on the different sucrose concentrations. Field experiments tested the effect of access to sucrose solution in feeders on retention of wasps and the parasitism rate of *C. fraxinella* by *A. polychrosidis*. Wasp attraction to buckwheat flowers positioned in pots in *C. fraxinella* infested ash trees was also assessed. Fed wasps lived longer than wasps with access to water only. The longevity of virgin female *A. polychrosidis* was similar when fed 25% sucrose or buckwheat flowers. Egg load did not change with sucrose provision. Adult longevity of fed virgin female *A. polychrosidis* was negatively correlated with egg load, suggesting that egg resorption occurs in this species. Female *A. polychrosidis* fed 25% sucrose produced offspring with a slightly female-biased sex ratio and higher fecundity than offspring from female *A. polychrosidis* fed the 10% sucrose solution. The provision of sugar feeders or buckwheat flowers

in infested green ash trees, did not enhance attraction of *A. polychrosidis*. There was an effect of sucrose treatment on parasitism of *C. fraxinella* through an interaction with host density.

3.2 Introduction

The “parasitoid nectar provision hypothesis” (Heimpel and Jervis 2005) contends that nutritional support of target parasitoids can enhance biological control. Carbohydrate resources can increase wasp longevity and fecundity (Benelli *et al.* 2017), and increase retention in treated patches (Jamont *et al.* 2014, Tena et al. 2015). When associated with floral volatile attractants, or visual cues, parasitoids may orient to sources of carbohydrate (Belz *et al.* 2013).

To maximize fitness, animals should spend more time in habitat patches where foraging time is optimized (Pyke *et al*. 1977). For adult parasitoids, optimal foraging occurs when nectar food sources are in close proximity to patches of insect hosts that provide oviposition sites. Artificial enhancement of carbohydrate sources can influence patch retention time in parasitoids. *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), a parasitoid of California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), is retained in patches of orchard habitat that are augmented with a carbohydrate solution (Tena *et al*. 2015). Patches of aphid infested *Brassica napus* (Brassicaceae) supplemented with plantings of *Vicia faba* (Fabaceae) retain higher numbers of the aphid parasitoid, *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). The extrafloral nectaries of V. *faba* provide nutrition for *D. rapae* and also attract wasps from outside the patch (Jamont *et al.* 2014). *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) parasitize more *Mamestra brassicae* Linnaeus (Lepidoptera:Noctuidae) when provided with both floral and extrafloral nectar, compared to extrafloral nectar alone (Geneau *et al.* 2013). This could be due to the nutritional benefits of additional nectar or attraction of parasitoids to floral volatiles.

Flowers release volatile compounds that attract parasitoid wasps (Belz *et al.* 2013), and can be manipulated to concentrate parasitoids where pest control is desired. Flower selection permits a more targeted approach to parasitoid attraction and retention than sugar feeding, as flower shape, colour, and volatile profile influence which wasps are attracted and can access the nectar (Wäckers 2004). Flower morphology affects nectar availability for parasitic wasps. The width and depth of the corolla determines nectar availability for *Microctonus hyperodae* Loan (Hymenoptera:Braconidae) (Vallata *et al.* 2006). The scent of yarrow, *Achillea millefolium* (Asteraceae) is repellant to *Cotesia glomerata, Pimpla turionellae* Linnaeus (Hymenoptera:Ichneumonidae)*,* and *Heterospilis prosopidis* Vierek (Hymenoptera: Braconidae) (Wäckers 2004). Buckwheat (*Fagopyrum esculentum* Polygonaceae) flowers are accessible and attractive to *M. hyperodae*, and access to buckwheat as compared to other flower species increases wasp longevity (Vallata *et al.* 2006).

In some systems, carbohydrate provision with flower nectar or sugar solutions enhances female parasitoid fecundity. The amount and type of carbohydrate resource required for egg development will differ depending on the reproductive strategy of the wasp. Synovigenic parasitoids continuously produce eggs after adult eclosion, whereas proovigenic wasps have a full egg complement at adult eclosion (Flanders 1950). There is a continuum of oovigenic strategies across the parasitic Hymenoptera that can be described with an index based on the proportion of developed eggs at eclosion (Jervis *et al*. 2001). Within the Braconidae studied to date, this index ranges from 0-1 (Jervis *et al*. 2001). Nutrients acquired post eclosion provide energy for egg building in synovigenic parasitoids, whereas nutrient allocation to flight and longevity may pervade in proovigenic parasitoids (Jervis et al 2008).

The fecundity of female *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a parasitoid of noctuid moths, increases when wasps have access to a honey solution (Riddick

2007). Sugar feeding by *Neochrysocharis formosa* (Hymenoptera: Eulophidae) prevents egg resorption and promotes new egg production (Wang *et al.* 2014). Access to nectar of the sesame flower, *Sesamum indicum* (Pedaliaceae), promotes lifetime egg production of the synovigenic parasitoid *Trichogramma chilonis* (Ishii) (Hymenoptera:Trichogrammatidae) (Zhu *et al.* 2017). Provision of buckwheat flowers in planted borders around host habitat increases fecundity and decreases egg maturation time in the parasitoid *Diadegma insulare* Cresson (Hymenoptera:Ichneumonidae) (Lee and Heimpel 2008).

Parasitoid efficacy is most commonly limited by adult female longevity rather than lifetime fecundity *per se* (Rosenheim 2011). The role of carbohydrate nutrition in extending the lifespan of adult parasitoids, therefore, may be as or more important than nutritional enhancement to egg production. In protrandrous parasitoid species, the length of adult life will affect the time that males can access females (Kant 2016). Parasitoid wasps access carbohydrates by feeding on flower nectar, extrafloral nectaries, and honeydew (Quicke 2015). Manipulation of carbohydrate sources in managed ecosystems through the provision of sugar solutions or floral resources has the potential to extend parasitoid lifespan (Jacob and Evans 2000, Irvin and Hoodle 2015, Ashraf *et al.* 2017 ,).

The types of carbohydrates available to parasitoids will vary in nature and may differ in their effect on parasitoid longevity. Consumption of glucose, fructose, and sucrose, the sugars most abundant in flower nectar, extends adult longevity of *Cotesia glomerata* and allows wasps to recover following starvation events (Wäckers *et al.l* 2001, Hausmann *et al.*2005). Provision with honey water lengthens the lifespan of an ichneumonid parasitoid, *Batheplectes curculionis*, of *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)(Jacob and Evans 2000). The longevity of braconid wasps *Apanteles ruficrus* (Haliday)and *Cotesia chilonis* Munakata that parasitize larvae of the rice pest, *Sesamia inferens* Walker (Lepidoptera: Noctuidae), is increased when wasps have access to sesame flowers, compared to those given water (Zhu *et al.* 2014). Provision of certain *Brassica* flowers to *D. insulare* increases wasp longevity to a similar extent as access to honey-water (Idris and Grafius 1995). The lifespan of *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) is equally increased by feeding on a sucrose solution or on honeydew (England and Evans 1997). In contrast, sucrose-feeding enhanced the longevity of the fruit fly parasitoid, *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Ichneumonidae), more than consumption of honey or golden syrup solutions (Zamek *et al*. 2012). The concentration of sugars can also impact parasitoid physiology. Food molecule transport in the insect gut can be disrupted by high sugar concentration (Chippendale 1978). Consumption of sucrose solutions with greater than 25% sucrose decreases both the longevity and fecundity of *Bracon hebetor* (Say) (Hymenoptera: Braconidae), a parasitoid of moth pests in the family Pyralidae (Lepidoptera) (Ashraf *et al*. 2017).

In addition to direct impacts on wasp fecundity and longevity, carbohydrate provisioning can also affect the neurophysiology of parasitoids. Starved, one-day-old *Cotesia rubecula* (Marshall) (Hymenoptera:Braconidae), parasitoid of *Pieris rapae* Lineaus (Lepidoptera: Pieridae), showed a reduced response to floral and host stimuli compared to satiated wasps and nectar-experienced wasps. Starved wasps also had increased flight latency, and slower response times to reach flowers for nectar or host-infested plants (Seikmann *et al*. 2004). The antennae of sucrose-fed *Microplitis croceipes* (Cresson) (Hymenoptera:Braconidae) have higher electrophysiological responses to cotton plant odours than starved wasps. In addition, parasitoid orientation to cotton plants infested with its host, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), increased with feeding in a dose dependent manner with the concentration of sucrose solution (Burrows *et al.* 2017).

The goal of the current study is to assess the effect of carbohydrate provisioning on the braconid parasitoid, *A. polychrosidis*, a native parasitoid currently exploiting an introduced pest of ash (*Fraxinus*), the ash leaf-cone roller, *Caloptilia fraxinella* (Lepidoptera: Gracillariidae), in Prairie communities of western Canada. Ash are desirable boulevard and park trees in urban centres on the Canadian Prairies as they are drought tolerant and cold hardy (Lane *et al*. 2016). In Edmonton, Alberta, Canada , almost 40% of the boulevard trees are ash trees, with the vast majority being green ash, *Fraxinus pennsylvanica* (Oleaceae) (City of Edmonton 2017).

The leaf-mining moth *Caloptilia fraxinella*, was first recorded on ash trees in Edmonton in 1999 (Pohl *et al.* 2004). The larvae emerge from eggs laid in spring, and are leaf-miners for the first three larval instars (Evenden 2009, Pohl *et al*. 2004). After dispersal on silken threads, the fourth larval instar rolls a leaflet into a cone shelter (Figure 1.1), inside which it completes development and pupates.

The primary parasitoid of *C. fraxinella* in Edmonton is *Apanteles polychrosidis* Vierek (Hymenoptera: Braconidae) (Wist and Evenden 2013). This wasp has a wide Nearctic range (Fernández-Triana and Huber 2010) and is native to the Edmonton area. It has been recorded from larval hosts in the moth family Tortricidae, including *Choristoneura rosaceana* (Harris), *Platynota idaeusalis* (Walker), and *Endopiza viteana* Clemens (all Lepidoptera:Tortricidae) (Seaman *et al*. 1990; Biddinger *et al*. 1994; Li *et al*. 1999, Cossentine *et al.* 2004). In British Columbia, *A. polychrosidis* is bivoltine and overwinters in first or second instar larvae of *C. rosaceana* with a second generation in the summer (Cossentine *et al.* 2004). In Edmonton, the native larval hosts of *A. polychrosidis* have not been identified. *Apanteles polychrosidis* has shifted hosts to exploit the introduced pest as its larval host in the summer generation (Wist and Evenden 2013) but the overwinter host is not known. Nine *A. polychrosidis* were reared from overwintered Tortricidae larvae, however, in late April and early May 2016 and 2017 (McPike, unpublished data). Adult *A polychrosidis,* like the majority of parasitoid wasp adults, are nectar feeders (Quicke 2015) but it is unknown if carbohydrate provision will enhance parasitism in this tri-trophic system.

3.2.1 Objectives

To test the hypothesis that carbohydrate provision can support *A. polychrosidis* to increase parasitism of *C. fraxinella*, this research pursues the following objectives:

1. Evaluate the provision of two concentrations (10% and 25%) of sucrose solution to retain *A. polychrosidis* in green ash trees and increase parasitism of *C. fraxinella*.
2. Evaluate the effect of carbohydrate nutrition on wasp longevity, fecundity, parasitism rate and offspring fitness.
3. Evaluate the effect of carbohydrate nutrition through the provision of buckwheat flowers on wasp longevity.
4. Evaluate the effect of buckwheat flowers in infested green ash trees, to support retention and foraging activity of *A. polychrosidis*.

3.3 Methods

Insects

For all laboratory feeding experiments *A. polychrosidis* were collected between 13-28 June 2015 and 2016 from green ash trees infested with *C. fraxinella* at sites across Edmonton, Alberta. Leaf-rolls containing wasp pupae were identified by the lack of a “window” in the leaflet tissue that is normally excavated by unparasitized *C. fraxinella* larvae before pupation (Wist et al. 2013). Leaf-rolls containing wasp pupae were placed individually in 36 ml plastic cups on cafeteria trays and stacked by a window (2015) or in plastic bags with moistened paper towels to prevent desiccation (2016), and maintained at room temperature (19-21°C) until wasp eclosion. Cups were checked daily for adult wasp emergence, and eclosed wasps were transferred to feeding treatments.

The larval host for Experiment 3 were colony-raised *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), reared from egg masses obtained from Pacific Agrifood Research Centre in Summerland, British Columbia. Larvae were hatched, and reared in 30 ml plastic cups with a pinto bean based diet (Shorey and Hale 1965) lining the bottom until use in trials. The colony was maintained at 24˚C, L16:D8 photoperiod, in a growth chamber.

3.3.1 Experiments 1- 3: Laboratory Sucrose-Feeding Experiments:

Laboratory feeding experiments tested the hypothesis that provision of sucrose solution impacts wasp longevity and egg load of virgin wasps (Experiment 1), and the longevity of mated wasps (Experiment 2). Newly eclosed wasps were transferred individually to new 36 ml plastic cups before (Experiment 1) or after (Experiment 2) mating (Wist 2014). Each wasp (n= 41-55) was provided with a feeding treatment through a dental wick (Figure 3.1). Feeding treatments for both experiments consisted of two sugar solutions: 10% (v/v) and 25% (v/v) sucrose in deionized water and a deionized water control. A no-water control treatment was also included in Experiment 1. Wasps were maintained at room temperature (19-21°C) and feeding treatments were changed every 5 days until death. In Experiment 1, dead females were frozen at -20°C until dissection to count eggs. Dissections were conducted in Ringer's solution to remove the ovaries. Ovaries were placed on a glass slide under a glass coverslip, which was tapped to liberate eggs. Eggs were counted under 60X magnification, using a dissecting microscope (Leica Microsystems Inc., Ontario, Canada) (Figure 3.2). As a covariate of wasp size (Visser 1994), the hind tibia length of each female wasp was measured using an ocular micrometer attached to a Leica MZ95 dissecting microscope at a 32X magnification (Figure 3.3).

To test the hypothesis that the level of carbohydrate nutrition affects parasitism rate and offspring fitness, a third laboratory feeding experiment was conducted using mated females fed either 25% (v/v) sucrose in deionized water solution (n=10) or 10% (v/v) sucrose in deionized water solution (n=9). As successful parasitism first occurs when *A. polychrosidis* females are 4 days old, on average (Cossentine *et al.* 2005), a water control treatment could not be included in this experiment because wasps would not survive long enough to parasitize hosts. After 9-15 days in 36 ml cups with access to the sucrose solution, individual female wasps were transferred to a 160 ml chamber. Females were provided with the same feeding treatment and had access to ten, first to third instar *C. rosaceana* larvae on a 1x1 cm cube of pinto bean diet (Shorey and Hale 1965) as an oviposition host (Cossentine *et al*. 2005). After 48 hrs, larvae were transferred, and the female received fresh larvae. This process was repeated until the female wasp died. Larvae that had been exposed to female wasps were maintained in pairs in 36 ml cups containing pinto bean diet in a growth chamber at 24 ˚C, L16:D8. Larvae were checked daily for signs of parasitism and wasp emergence. Emerged wasp offspring were removed from feeding containers within 24 hrs, and were frozen at -20 ˚C until dissection and measurement. Sex of emerged offspring was recorded, and the tibia length was measured using an ocular micrometer under a dissecting scope at 40X magnification. Female offspring were dissected as described in Experiment 1 to assess egg-load.

3.3.2 Experiments 1-3: Statistical Analyses

All statistical analyses were conducted in R (version 3.4.2) (R Core Team 2017). The effect of feeding treatment on the longevity of virgin (Experiment 1) and mated (Experiment 2) wasps was compared using separate generalized linear models with negative binomial error distributions, to account for overdispersion of the data, using MASS package (version 7.3-47, Venables and Ripley 2002) (Table 3.1). Explanatory variables included wasp sex and feeding treatment and the sex*treatment interaction term in each case. In Experiment 1, subsequent multiple comparisons were conducted using a pairwise Tukey's Test in the lsmeans package (version 2.27-2, Lenth 2016). In Experiment 2, the interaction term between sex and feeding treatment was not significant and was removed from the final model. In Experiment 1, the effect of nutrition treatment on egg load in virgin females was analysed with a simple linear model. The effect of wasp size and longevity on egg load was examined using a multiple regression model. Egg load was specified as the dependent variable, and body size (hind tibia length), and longevity were specified as independent variables.

In Experiment 3, a general linearized model (lme4 package, version 1.1-14, Bates *et al*. 2015) with a binomial error distribution was used to compare the rate of parasitism (a vector, y, binding the number of parasitized larvae, and the number of unparasitized larvae into a single object (Crawley 2015)) between females fed different concentrations of sucrose. The total number of offspring produced by females fed different levels of sucrose was compared with a generalized linear model with a Poisson error distribution (lme4 package, version 1.1-11 (Bates *et al*. 2015).

The proportion of male and female offspring produced by females fed different levels of sucrose solution was compared by constructing a vector (y) that bound the number of female offspring and the number of male offspring into a single object (Crawley 2015). The vector was used as the response variable in a generalized linear mixed model with a binomial distribution.

Maternal feeding treatment was specified as the explanatory variable and individual maternal wasp identity was considered a random factor (lme4 package, version 1.1-11, Bates *et al*. 2015). Tibia size of the offspring was compared with a linear model that specified maternal feeding treatment and wasp sex as the explanatory variables and maternal wasp identity as a random factor. The effect of maternal diet on the egg load of the female offspring was tested using a generalized linear mixed model with a Poisson error distribution (Table 3.1). Maternal feeding treatment and offspring tibia length were specified as the explanatory variables and individual female wasp identity was treated as a random factor. There was no significant interaction between the main effects, so the interaction term was removed from the final model.

3.3.3 Experiment 4: Laboratory Flower-Feeding Experiment

Experiment 4 tested the effect of access to buckwheat flower nectar compared to a sucrose solution on virgin female *A. polychrosidis* longevity. The previous longevity experiments demonstrated that the wasps live longer when fed the 25 % sucrose solution, so this concentration was selected for this experiment. The sucrose solution was prepared as explained previously, except that distilled water was used in place of deionized water as the solvent. Buckwheat flowers used as a food source were grown from seed (Apache Seeds, Edmonton, Alberta). Buckwheat seeds were sown in seedling trays (cells 4.0 X3.0 and 5.0 cm deep), in Sunshine Mix 2 soil (SunGro Horticulture Canada, Ltd, Seba Beach, Alberta) at a density of 3 seeds per cell. Trays were positioned in a growth room with full spectrum light (24 ˚C, L16:D8), and watered every two days. Plants were fertilized with 1g/L of 20-20-20 fertilizer (Plant-Prod All Purpose Fertilizer 20-20-20, Home Depot, Edmonton) on two occasions before the start of the feeding trial.

Newly emerged virgin female wasps were transferred individually to 36 ml plastic cups and supplied with one of four feeding treatments: 1) a cluster of 5-20 buckwheat blossoms on an intact plant (Figure 3.4); 2) a single buckwheat leaf on an intact plant; 3) 25% sucrose solution in distilled water; or 4) distilled water. Trays with the variously treated wasps were positioned within the same growth chamber in which plants were reared. For plant-based feeding treatments, wasps were transferred every second day to new blossoms or leaves. Other feeding treatments were changed every five days. An additional experiment was conducted to compare the effect of deionized and distilled water on wasp longevity (Appendix 1).

3.3.4 Experiment 4: Statistical Analysis

All statistical analyses were conducted in R (version 3.4.2) (R Core Team 2017). The longevity of wasps exposed to the various feeding treatments was compared using a generalized linear model with a negative binomial error distribution to account for overdispersion. Feeding treatment was specified as the explanatory variable (MASS package, version 7.3-47, Venables and Ripley 2002) (Table 3.1).

3.3.5 Experiment 5: Field Sucrose-Feeding Experiment

Experiment 5 tested the hypothesis that provision of sucrose solutions in the canopy of green ash trees infested with *C. fraxinella* could enhance foraging behaviour of *A. polychrosidis* and increase parasitism of *C. fraxinella*. The study was replicated at ten sites, established at least 600 m apart in Edmonton, Alberta (53.5444° N, 113.4909° W) (Figure 3.5) from June 3 to 22 July, 2015. Each site had a row of 5 green ash trees separated by 6-19 m. Four 36 ml feeders containing the treatments were placed in the mid-canopy of each tree, in the four cardinal directions. Treatments were randomly assigned to one tree at each site: 1) 25% sucrose solution; 2) 10% sucrose solution; 3) deionized water; 4) empty feeders; or 5) no feeders. Feeders consisted of a 10 cm diameter plastic Petri Plate with the 36 ml cup attached to the centre. Access to the sucrose solution in the cup was through a 3 cm dental wick attached to the cup lid. The entire feeding apparatus was spray-painted yellow (Tremclad Rust Paint, “Gloss Yellow”) and suspended within the canopy by wires (Figure 3.6). Feeders were replenished with new solutions each week for the duration of the experiment.

Apanteles polychrosidis activity within the tree canopy and parasitism rate of *C. fraxinella* were measured for treated trees at each site. Three yellow sticky traps (18cm x 9cm, Alphascents Insect Monitoring Systems, West Linn, Oregon), were placed in the low-mid canopy of treated trees at each site to measure wasp activity in each tree. Traps were collected each week and replaced with fresh traps. Collected traps were wrapped in plastic and frozen at -20˚ C until they could be processed. Wasps were identified as *A. polychrosidis* based on wing characters, and femur color (Fernadnez-Triana and Huber 2010), under a dissecting scope at 40X magnification. After the completion of the experiment in August, the parasitism rate of *C. fraxinella* on each treated tree was measured. At least one hundred leaf rolls were collected haphazardly from each tree as could be reached from a 1.83 m ladder. If there were fewer than 100 rolls on the tree, all leaf rolls were collected. Leaf-rolls were transported to the laboratory and assessed based on pupal morphology within the rolls as to whether they contained *C. fraxinella* or *A. polychrosidis* (Wist *et al.* 2015) (Figure 1.2). Density of *C. fraxinella* infestation was measured by visually estimating percentage of ash leaflets rolled per treated tree (after principles of *Field manual for describing terrestrial ecosystems* 2010).

3.3.6 Experiment 5: Statistical analyses

All statistical analyses were conducted in R (version 3.4.2) (R Core Team 2017). The mean number of wasps captured on sticky traps in trees with the various feeding treatments was compared with a generalized mixed effects model with a negative binomial error distribution to account for overdispersion. Site was specified as a random factor, using MASS package (version 7.3-47, Venables and Ripley 2002). Parasitism rate was compared by a generalized linear mixed model with a binomial error distribution. A vector (y), was constructed, binding the number of parasitized larvae, and the number of unparasitized larvae into a single object (Crawley 2015). The vector y was specified as the response variable and sucrose feeding treatment and host density as explanatory variables with site specified as a random factor. The generalized linear mixed model was conducted in lme4 package. A linear model compared parasitism rate with host density.

3.3.7 Experiment 6: Field Flower-Feeding Experiment

Experiment 6 tested the hypothesis *A. polychrosidis* would feed on buckwheat flowers positioned in *C. fraxinella* infested green ash trees. Sites were established at ten locations across Edmonton, separated by at least 600 m (Figure 3.7). At each site, two adjacent green ash trees were selected for treatment. Trees were separated by 7-14 m. Buckwheat plants used as feeding treatments were grown as described in Experiment 5 and transferred to 15 cm diameter plastic pots before transport to the field. Dark green plastic, 15-cm-diameter plant pots with attached saucers, were filled with a 3 cm layer of small stones and topped with 10 cm of Sunshine Mix 2 soil. Each pot contained 12 stems of blooming buckwheat or no buckwheat stems. Each tree at each site received one pot with flowering buckwheat and one pot with no buckwheat stems (soil only in pots) suspended by wire in the mid-canopy on opposite sides of the tree. Wasp attraction to flowers was measured by wasp capture on yellow sticky cards (18 cm x 9cm, 2cm x 2cm grid) (Alphascents Insect Monitoring Systems, West Linn, Oregon) positioned within each flower pot on a wooden skewer. Traps were changed weekly from 15

June-6 July 2016 (until there were no open blooms remaining). Traps were wrapped in plastic and frozen at -20 ˚C until they were processed. Trap capture was identified and enumerated as described for Experiment 5.

3.3.8 Experiment 6: Statistical Analyses

All statistical analyses were conducted in R (version 3.4.2) (R Core Team 2017). A generalized linear mixed model with a negative binomial error distribution tested for wasp association with buckwheat flowers with lme4 package version 1.1-11 (Bates *et al*. 2015). The mean capture of *A. polychrosidis* on sticky traps positioned in pots with or without buckwheat flowers was compared. Buckwheat feeding treatment was the explanatory variable, and the site, and tree within site were specified as nested, random factors.

3.4 Results

3.4.1 Laboratory Sucrose-Feeding Experiments

In Experiment 1, both sex and carbohydrate source affected virgin wasp longevity (sex: χ^2=11.49, df=1, p=0.0007, treatment: χ^2=1444.9, df=3, p<0.0001). There was a marginally significant interaction term between sex and carbohydrate source (sex*treatment: χ^2=7.35, df=3, p=0.061) that impacted wasp longevity. Virgin male and female wasps lived on average 13.38±0.88 and 16.04± 1.04 days, respectively when fed 10% sucrose solution. Wasps fed the 25 % sucrose solution lived 14.88±0.88 and 20.70±1.22 days for males and females, respectively (Figure 3.8). Both male and female virgin wasps lived significantly longer when fed either concentration of sucrose as compared to wasps given water, or those held in empty containers with no access to food or water. Male virgin wasps lived significantly longer when given water (2.46±0.12 days), compared to those in dry feeders (1.17±0.05) (z=-4.43, p=0.0002). Virgin males and females fed 10% sugar solution had similar longevity (z=2.121, p=0.401) as did male wasps fed either 10% or 25% sucrose (z=-1.267, p=0.911). Females fed 25% sucrose solution lived, on average, 4.66 days longer than females fed 10% sucrose solution (z=-2.881, p=0.077).

The sucrose feeding treatment had a significant effect on the egg load of virgin female *A. polychrosidis* (f=5.438, df=3, p=0.00159) (Figure 3.9). At dissection, wasps had between 5-52 eggs in their ovaries. Egg load was highest in females maintained on water (30.97±1.4 eggs) or fed the lower concentration of sucrose (10%) (30.04±1.68 eggs). Interestingly, the egg load of females fed the 25% sucrose solution was significantly lower than that in wasps provided with either 10% sucrose solution, or water alone (22.96±1.71). The egg load of females maintained in dry containers was intermediate (Figure 3.9). A significant regression equation for egg load was found (Egg load = -53.03 + 92.88 (Tibia) -0.25 (Age), R^2 = 0.1813, tibia slope p-value = 0.0001, age slope p-value = 0.003), with egg load increasing with female size, and decreasing with longevity (Figure 3.10).

In Experiment 2, mated wasp longevity was affected by wasp sex and feeding treatment (sex: χ^2=9.74, df=1, p=0.00180, treatment: χ^2=431.38, df=2, p<0.0001). Mated male and female wasps lived on average 12.83±1.04 and 17.6±0.97 days, respectively, when fed the 10% sucrose solution, and 17.76±0.34 and 20.63±2.17 days when fed the 25% sucrose solution. Male and female mated wasps fed the 25% sucrose solution lived significantly longer than those fed the 10% sucrose solution (z=-3.131, p=0.0048), or those given water (z=-19.064, p<0.0001) (Figure 3.11). When fed 10% sucrose solution, both sexes lived significantly longer than those fed water only (z=17.00, p<0.0001).

The parasitism rate did not differ between female wasps fed 10% or 25% sucrose solutions in Experiment 3 (χ^2=0.16, df= 1, p=0.69). The total number of larvae parasitized did not differ with maternal feeding treatment (χ^2=1.35, df=1, p=0.26), although females fed 25% lived marginally longer (25.25±2.99 days) than those fed 10% (18.5±2.26 d) (F=3.382, df=1, p= 0.085).

The offspring sex ratio did not differ with maternal feeding treatment (χ^2=1.42, df= 1, p=0.23). The fecundity of female offspring from mothers fed 10% and 25% sucrose solution was not significantly different (χ^2=1.40, df= 1, p= 0.24), but female offspring from mothers fed 25% sucrose contained, on average, nine more eggs than the offspring of those fed 10% sucrose. Overall offspring body size (males and females), as measured by tibia length, was marginally affected by maternal diet (χ^2=3.2732, df=1, p=0.0664), with offspring from females fed the higher sucrose concentration (25%) being slightly larger than those from mothers fed the 10% sucrose solution. Female offspring had longer tibiae than males (χ^2=45.77, df=1, p<0.0001).
Body size of female offspring, measured by tibia length, had a significant effect on egg load (χ^2=6.37, df=1, p=0.012).

3.4.2 Laboratory Flower-Feeding Experiment

In Experiment 4, virgin female longevity was affected by feeding treatment (Dev=265.42, df=3, p<0.0001). *Apanteles polychrosidis* fed either 25% sucrose solution or buckwheat flowers, lived significantly longer than females provided with distilled water, or a buckwheat leaf. Female wasps fed buckwheat had statistically similar longevity as females fed 25% sucrose solution (χ^2=93.79, df=4, p=0.221), although flower-fed females lived on average, four days longer than sugar-fed wasps (Figure 3.12).

3.4.3 Field Sucrose-Feeding Experiment

In Experiment 6, the number of wasps captured on yellow sticky cards in ash trees did not differ with feeding treatment provided to wasps (χ^2=4.28, df=4, p=0.37). The mixed model to describe parasitism showed a significant interaction between sugar feeding treatment and host density (χ^2=16.82, df=4, p=0.0021) (Figure 3.13). Parasitism rate in control trees with no feeders, and trees with feeders containing water, was most affected by host density, and displayed the lowest parasitism at high host density rates. The parasitism rate in trees with feeders containing 10% or 25%, or dry feeders, was much less affected by host density. The linear model showed that host density is negatively correlated with parasitism rate (y=60.24-0.46x, p= 0.00012, adjusted R^2=0.25) (Figure 3.14).

3.4.4 Field Flower-Feeding Experiment

There was no difference in the number of wasps captured on sticky cards positioned in pots of buckwheat with and without flowers that were positioned in *C. fraxinella*-infested green ash trees (χ^2=0.003, df=1, p=0.96). Traps positioned in pots with buckwheat flowers had 18.73±5 wasps, and control pots had 23.36±6.08, over the duration of the experiment.

3.5 Discussion

Sucrose feeding at both concentrations increased longevity of both virgin and mated male and female *A. polychrosidis* compared to starved wasps. This confirms that sucrose provides energy for *A. polychrosidis* that is available to wasps feeding at both the 10 and 25% sucrose concentrations. Female wasps live longer than males when fed on similar sucrose treatments. In general, female parasitoids live longer than males (Ashraf *et al.* 2017, Kant *et al.* 2016) and are typically larger than males (Hurlbutt 1987). In female *Itoplectis naranyae* Ashmead (Hymenoptera: Ichneumonidae), a parasitoid of lepidopteran pupae, large body size is correlated with increased longevity (Lui and Ueno 2012). Longevity and oviposition opportunities increase with female body size in a bean weevil parasitoid*, Lariophagus distinguendus* (Forster) (Hymenoptera: Pteromalidae) (van den Assem *et al*. 1989). Selection for larger females may, therefore, lead to increased longevity compared to males in *A. polychrosidis*.

Both virgin and mated females live longer when fed the 25% sucrose solution, compared to those fed on the 10% sucrose solution. This difference is significant in mated females and marginally significant for virgins. This indicates that female *A. polychrosidis* are able to acquire more resources from the higher sucrose concentration, allowing them to live longer. The present results also indicate that access to food sources post eclosion affects male wasp longevity. Mated males live longer when fed the higher concentration of sucrose but this effect of diet on longevity does not occur in virgin males. In male *Nasonia vitripennis* (Walker) (Hymenoptera:Pteromalidae), parasitoids of dipteran pupae, males which were allowed to mate repeatedly experienced decreased longevity, indicating an energetic cost of mating (BurtonChellew *et al*. 2007). Resource expenditure involved with copulation and insemination, therefore, may be compensated with carbohydrate replenishment in *A. polychrosidis*.

Apanteles polychrosidis appears to have a proovigenic egg production strategy, based on the present results. Females with access to water but no carbohydrate contain a similar number of eggs as those females fed the 10% sucrose solution. This indicates that egg development may halt before wasps are two days post eclosion. Females with access only to water live to ~2 days , compared to a life span of ~16 days for females fed the 10% sugar solution. As *Apanteles polychrosidis* is a konobiont parasitoid (Quicke 2015), egg development likely occurs primarily prior to adult eclosion (Jervis *et al*. 2001). As I did not dissect newly eclosed wasps, it is also possible that wasps develop some eggs during the first few days of the adult stage. This is the case for *Fopius arisanus* (Hymenoptera: Braconidae) that reach maximum egg load after 4 days of feeding as an adult (Wang and Messing 2003).

Eggs can be resorbed for somatic maintenance in female wasps as they age, and one egg resorbed supports about 10% of the energy required for daily somatic maintenance (Casas *et al.* 2005). Egg resorption is an example of a trade-off between reproductive potential and somatic maintenance (Richard and Casas 2009). My findings suggest that egg resorption occurs in *A. polychrosidis*. Long-lived virgin females have a reduced egg load compared to females that die early in the adult stage. Egg resorption occurs frequently in parasitoids with the synovigenic strategy in which the eggs are normally rich with yolk (Jervis *et al*. 2001). Sucrose provides energy that extends life and can fuel flight and locomotion (Casa *et al*. 2003, Amat *et al*. 2012), but it does not directly supply molecules for physical maintenance, such as amino acids and fats: molecules not produced by parasitoid wasps (Visser and Ellers 2008). Egg resorption occurs in some synovigenic parasitoids that feed on host hemolymph in addition to nectar when they are deprived access to oviposition hosts (Bodin *et al.* 2009, Chen 2014). Recycling of eggs by females may be necessary to replace some of the proteins and lipids usually acquired through hemolymph feeding in these species (Le Ralec 1995). *Apanteles polychrosidis*, however, does not host feed as an adult. More study is needed to assess the number of eggs in the ovaries of *A. polychrosidis* at adult eclosion as compared to various time points throughout the adult life stage to determine if and when egg resorption occurs in this species.

Water availability, not surprisingly, also influences wasp success. Virgin male wasps with access to water live significantly longer than those without water. The fecundity but not longevity of virgin female wasps was enhanced with access to water. Lipids are metabolized for water production in water-stressed beetles and flies (Marron *et al*. 2002). During desiccation, the wasp may resorb eggs for this purpose. Alternatively, the dehydration of eggs may make eggs invisible during enumeration.

The carbohydrate concentration of the diet influenced the sex ratio of the offspring of female *A. polychrosidis.* Females fed the high sucrose concentration produce more female offspring than females fed the lower sucrose concentration diet. The low numbers of total female offspring, however, may have decreased the power of the analysis. In general, "well-fed" female parasitoids produce more female offspring (Benelli *et al*. 2017). In the current study, there were more male offspring in total, which contradicts previous findings from field-collected wasps that illustrate a female-biased population (Wist and Evenden 2013). There are other factors that can influence sex ratio in parasitoid offspring. As haplodiploid organisms, hymenopteran females can refrain from fertilizing an egg, resulting in the production of male offspring (Godfray 1994). Insect host quality can determine parasitoid sex ratio, with male eggs laid in lower quality hosts (Ode and Hardy 2008). The larval hosts used in the present study (*Chorisoneura rosaceana*) were not the natal host species of the mothers, reared from *Caloptilia fraxinella*. Parasitoids prefer to lay eggs in the natal host (Jones *et al.* 2015). The novel host used in this bioassay may have been perceived as an inferior host to wasps reared from *C. fraxinella*. The diet of host insects influences host quality and can affect sex allocation. Broods of *Apanteles galleriae* (Wilkinson) (Hymenoptera: Braconidae), a parasitoid of the lesser wax moth, *Achria grisella* (Fabricus) (Lepidoptera: Pyralidae) became significantly male-biased when larval hosts were fed on an inferior diet (Uçkan and Ergin 2002). The *C. rosaceana* used as a larval host in this study were reared on a pinto-bean diet (Shorey and Hale 1965) that may render larvae less suitable for development of female pupae.

Little work has been done on maternal effects of nutrition on offspring fitness in parasitoids. The marginally larger offspring produced by females fed 25% sucrose could have a fitness advantage as larger female parasitoids have higher fecundity, and longevity (Lui and Ueno 2012). Female offspring whose mothers fed on the high sucrose diet produced slightly more eggs than daughters of females fed the low sucrose diet. The inability of adult parasitoids to synthesize lipids means that the nutritional support through carbohydrate feeding by mothers does not result in additional lipid production for egg development in offspring. Instead, carbohydrate consumption supports somatic maintenance in the mother and reduces the need for diversion of capital resources that sustain viable eggs (Wang *et al*. 2014). Larger males seem to have an advantage in conspecific fights. This is the case for two parasitoids in the superfamily Chalidoidea: a *Eurytoma sp*. (Hymenoptera: Eurytomidae) (Macedo *et al*. 2013), and *Melittobia acasta* (Hymenoptera: Eulophidae) (Innocent *et al*. 2007). Local mate competition is not as common in Ichneumonoidea (Quicke 2015), however, and it remains unclear if larger male *A. polychrosidis* have increased fitness.

While the rate of parasitism by females fed the two sucrose treatments did not differ under laboratory conditions, females fed 25% had more offspring. In the field, only host density is related to parasitism rate. Like Wist *et al.* (2015), the parasitism rate by *A. polychrosidis* is negatively correlated with C. *fraxinella* density on green ash but not on black ash.

In the present study, virgin female wasps had increased longevity when fed on buckwheat flowers compared to those fed the 25% sucrose solution. Nectar provided by the buckwheat flowers enhanced longevity of *A. polychrosidis*. *Cotesia glomerata* Linnaeus (Hymenoptera:Braconidae) has greater flight capacity after nectar feeding, as compared to sugar feeding (Wanner *et al.* 2006). The statistical non-significance of my result may be due to a lack of power in the analysis because of loss of flower-fed females during the trial. Females increase movement and flight propensity when fed buckwheat nectar (personal observation), and were prone to escape (n=21 in the buckwheat treatment completed the trial, n=7 escaped).

Provision of carbohydrates in the form of flowers or sucrose solution in the field setting did not increase wasp activity in *C. fraxinella*--infested green ash trees, as measured by counts on sticky cards in trees. The sugar feeders and sticky cards in our protocols were yellow, a colour known to be attractive to parasitoids (Vargas *et al*. 1991, Demirel and Cranshaw 2006, Perez *et al.* 2012). This may confound the results, as the cards act as a competitive attractant with the flowers and feeders. Recent studies use visual counts of parasitoid visits to quantify parasitoid attraction to patches with flower supplementation (Jamont *et al*. 2014, Tena *et al.* 2015). The urban setting is heterogenous, and the availability of carbohydrate resources near sites and within sites was variable. Most field studies that test the effect of carbohydrate provision on parasitoid activity occur in agricultural settings (Berndt *et al*. 2002, Bianchi and Wäckers 2008, Sivinski *et al*. 2011) where access to nectar may limit parasitoid activity and success. Access to carbohydrates within the ash tree canopy may not influence *A. polychrosidis* foraging activity if access to nearby nectar is available. Future studies should measure the dispersal capacity of *A. polychrosidis* and attempt to compare wasp density across the city to nectar resources. The dispersal capacity of *Hyposoter horticola* (Hymenoptera: Ichneumonidae) was assessed using DNA microsatellites to measure the distances between siblings (Couchoux *et al*. 2016). The flight capacity of *Microplitis mediator* (Halliday) (Hymenoptera:Braconidae) was measured on a computer-monitored flight mills (Yu *et al.* 2007). Knowing the dispersal capability of *A. polychrosisdis* would provide insight into the range of carbohydrate resources wasps can access proximal to infested host plants. Buckwheat flowers were chosen because of their previously demonstrated availability to closely related parasitoids (Vallata *et al.* 2006), ease of growing, and utility as a crop plant. This plant has been reported as an invasive plant in numerous states in the United States of America (Invasive Plant Atlas of the United States 2018), and therefore would need to be evaluated for its potential for invasion of the Edmonton area before encouraging its planting.

The interactive effect of the sucrose feeding treatments and host density on parasitism rate is difficult to interpret. It is unclear why the traps providing water would affect parasitism in a manner similar to control trees, with no feeders. It seems that sucrose provision at both 10, and 25% sucrose reduces the effect of host density on parasitism rate, but it remains to be explained why dry feeders would have a similar effect.

Addition of carbohydrate resources to *C. fraxinella*-infested trees did not enhance wasp activity. It is clear, however, that *A. polychrosidis* require carbohydrate sources to enhance longevity. Further, there is evidence that food quality influences wasp and possibly offspring sex ratio and fitness. Further knowledge of which food sources are exploited by *A. polychrosidis* in the field would help inform carbohydrate provision strategies to enhance their performance as a parasitoid of *C. fraxinella*.

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Figures

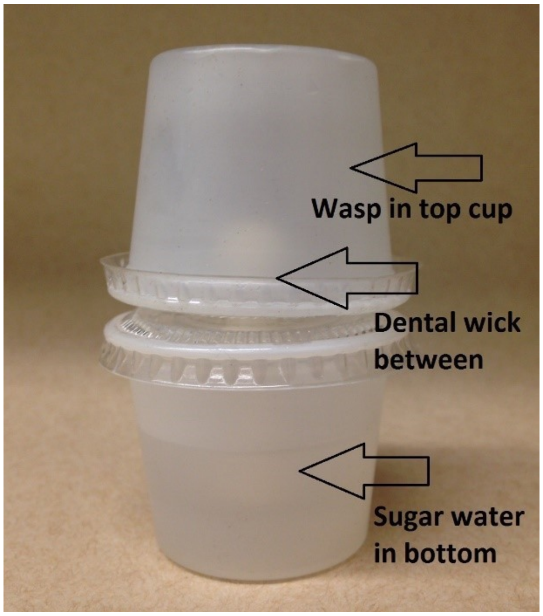


Figure 3.1 **Feeder for sucrose solution feeding experiments**. 36 ml plastic cups joined by a 3cm cotton wick.

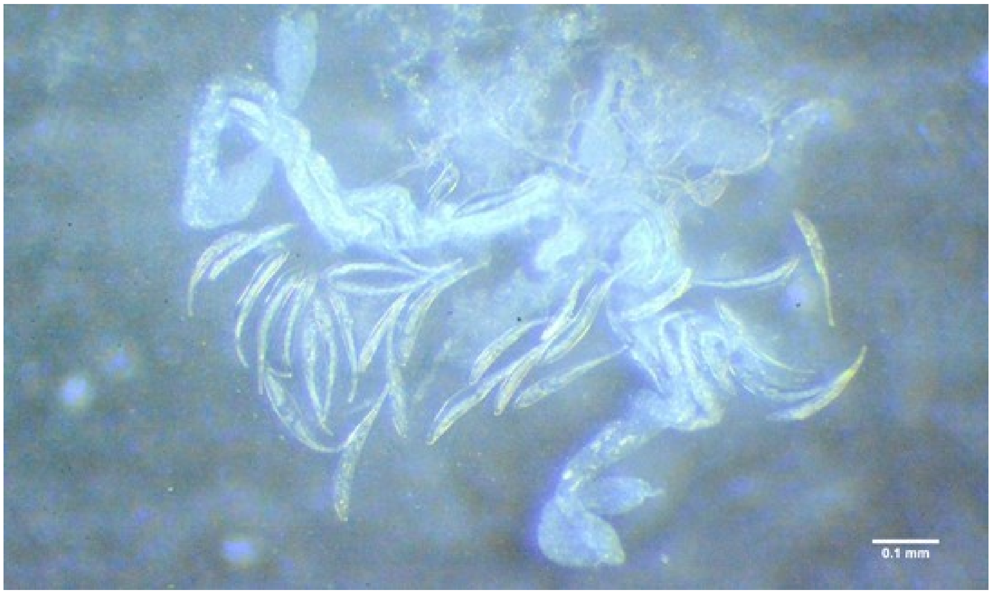


Figure 3.2 **Ovaries of *Apanteles polychrosidis* with eggs liberated (60x magnification)**.(photo credit: Rebecca Pain)

Figure 3.3 **Hind tibia of *Apanteles polychrosidis* measured using the ocular micrometer of a Leica MZ95 (Leica Microsystems Inc., Ontario, Canada) dissecting microscope at 32x magnification.** (photo credit: Rebecca Pain)

Figure 3.4 **Experimental set up for buckwheat plant treatment in Experiment 4.** Female *A. polychrosidis* were transferred upon emergence to 36 ml plastic cups clasped on to clusters of 5-20 buckwheat blossoms, or a buckwheat leaf control.

Figure 3.5 **Map of sites in Edmonton, Alberta, Canada (53.5444° N, 113.4909° W), for Experiment 5**. Conducted 3 June- 22 July 2015, sugar feeding treatments were placed in infested ash trees, in an attempt to increase *A. polychrosidis* retention. (*Google Maps*, accessed Sept. 2017)

Figure 3.6 **Feeders providing sucrose solutions used in Experiment 5**. Feeders consisted of a 10 cm diameter plastic Petri Plate with the 36 ml cup attached to the centre. Access to the sucrose solution in the cup was through a 3 cm dental wick attached to the cup lid. The entire feeding apparatus was painted yellow and suspended within the canopy of ash trees with wires.

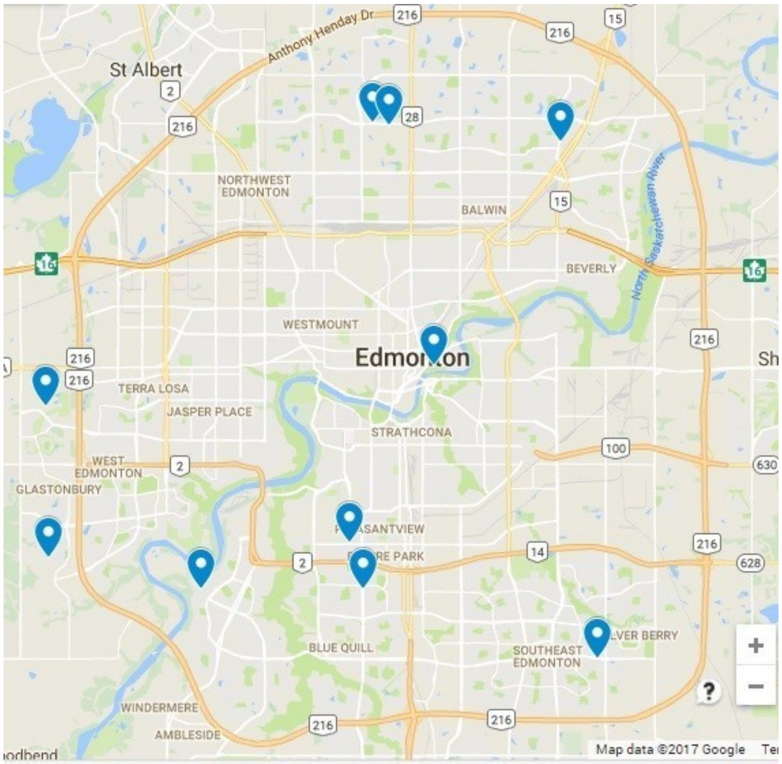


Figure 3.7 **Map of sites in Edmonton, Alberta, Canada (53.5444° N, 113.4909° W), for Experiment 6**. Conducted 15 June-6 July 2016. Pots containing buckwheat blossoms were placed in ash trees to *measure A. polychrosidis* attraction to flowers for feeding. (from *Google Maps*, accessed September 2017)

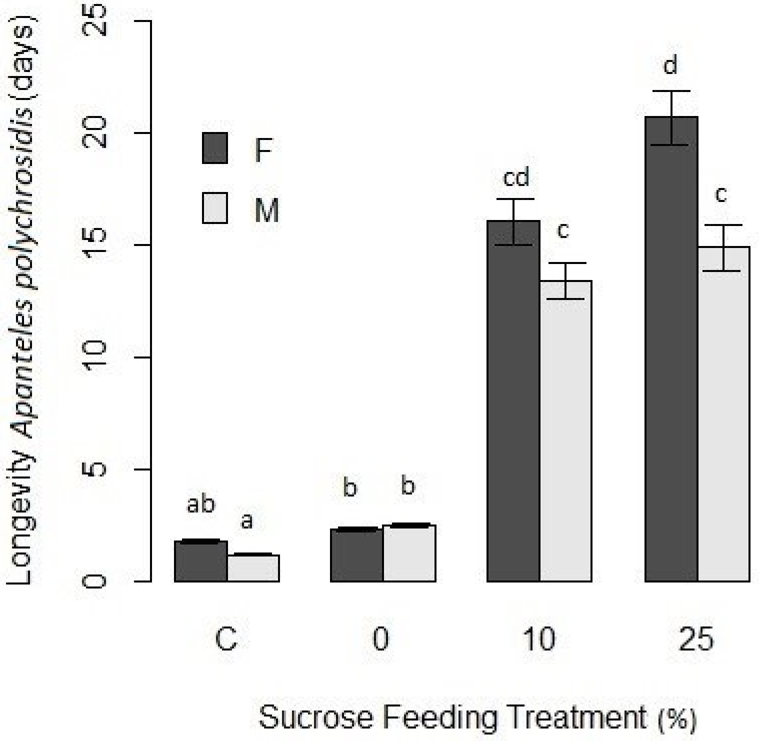


Figure 3.8 **Mean ± SE longevity of virgin *Apanteles polychrosidis* fed various sucrose feeding treatments.** Each wasp (n= 41-55 per treatment) was provided with a feeding treatment of either 10%or 25% (v/v) sucrose in deionized water, a deionized water control (0), or a dry feeder control (C). Bars marked with different letters are significantly different (compact letter display (cld) of multiple pairwise comparisons).

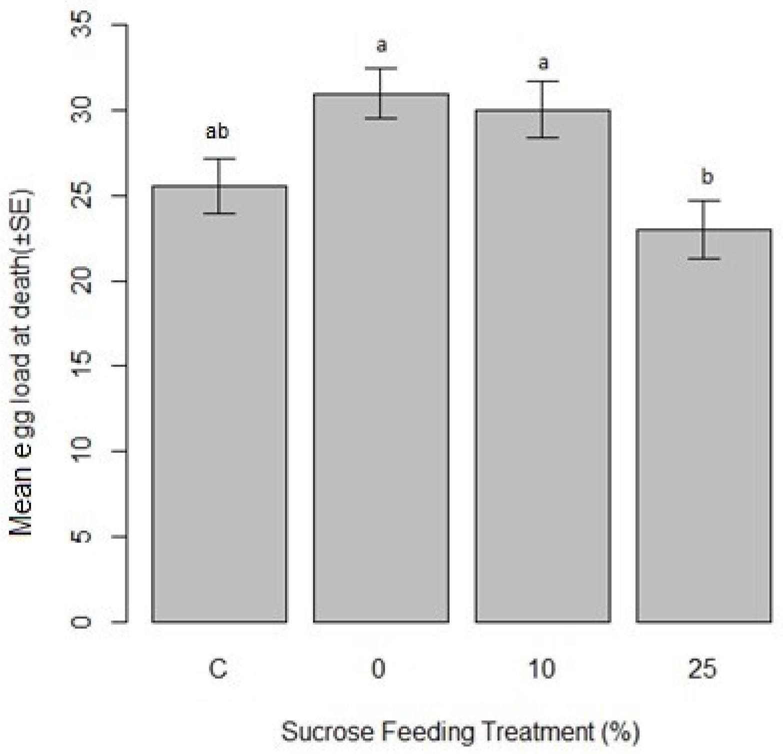


Figure 3.9. **Mean ± SE egg load at death of virgin female *Apanteles polychrosidis*.** Each wasp (n= 20-28) was provided with a feeding treatment of either 10 or 25%(v/v) sucrose in deionized water and a deionized water control (0), or a dry feeder control (C). Wasps were maintained at 21°C, and feeding treatments were changed every 5 days until death. Dead females were frozen at -20°C until dissection to count eggs. Bars marked with different letters are significantly different (compact letter display (cld) of multiple pairwise comparisons).

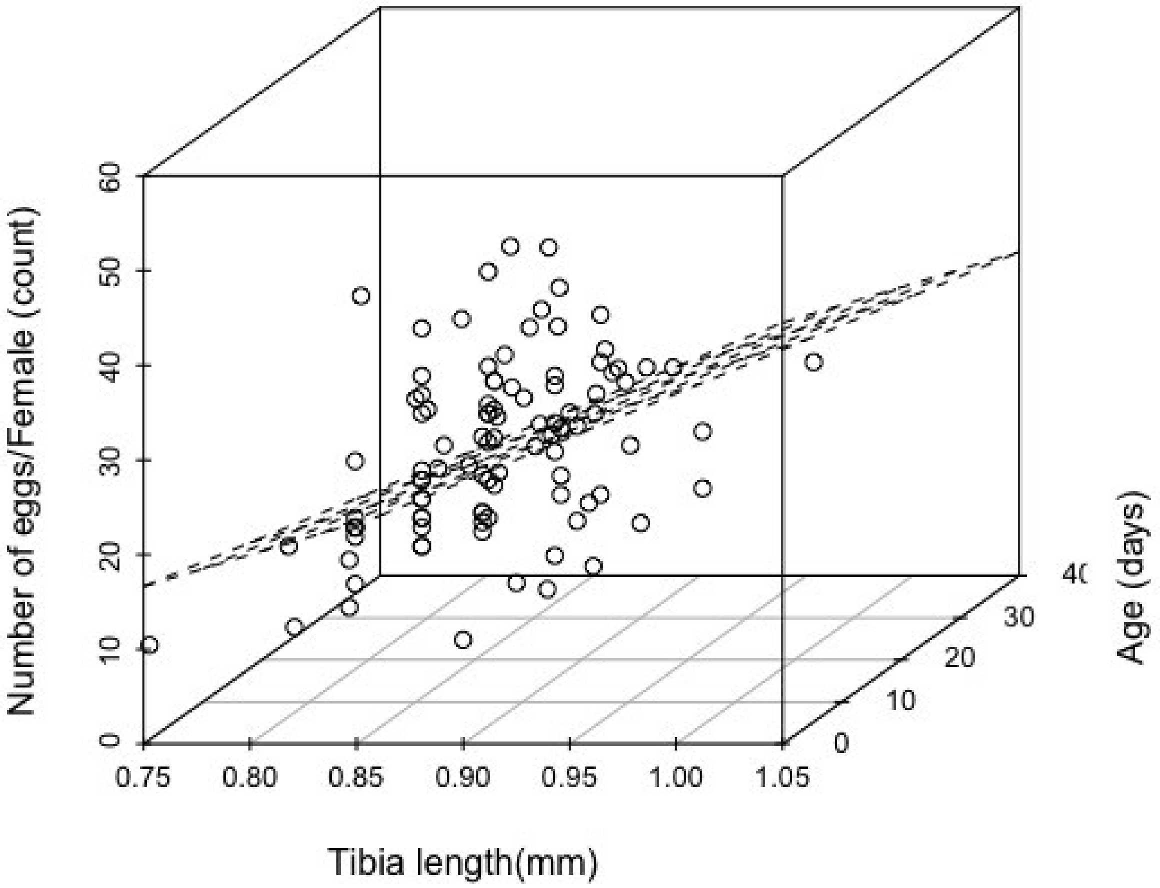


Figure 3.10**. Three-dimensional scatterplot with regression plane of the effect of tibia length and longevity on egg load.** Egg load = -53.03 + 92.88 (Tibia) -0.25 (Age), R^2 = 0.1813, tibia p= 0.0001, age p = 0.003.

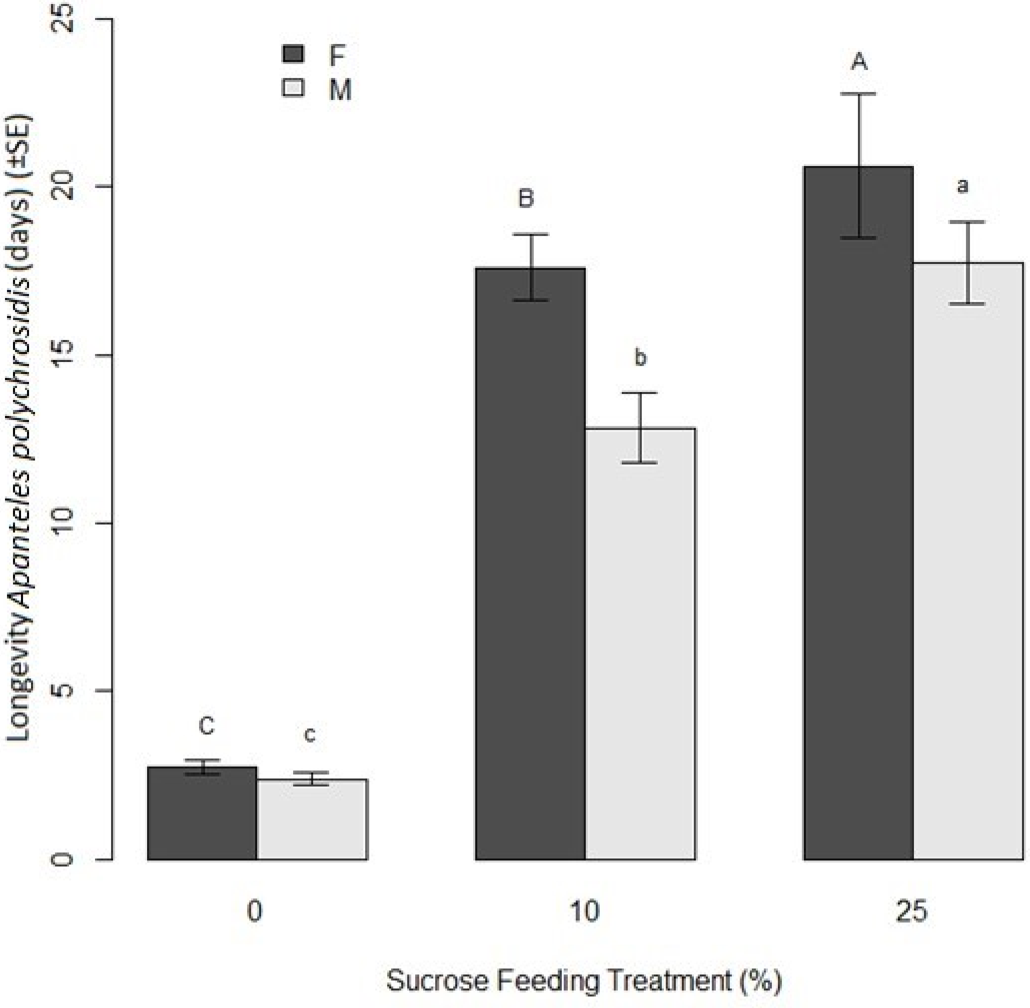


Figure 3.11 **Mean ± SE longevity (days) of mated *Apanteles polychrosidis***. Each wasp (n= 30-42 per treatment) was provided with a feeding treatment of either 10%(or 25%(v/v) sucrose in deionized water or a deionized water control (0). Bars marked with different letters are significantly different (compact letter display (cld) of multiple pairwise comparisons)

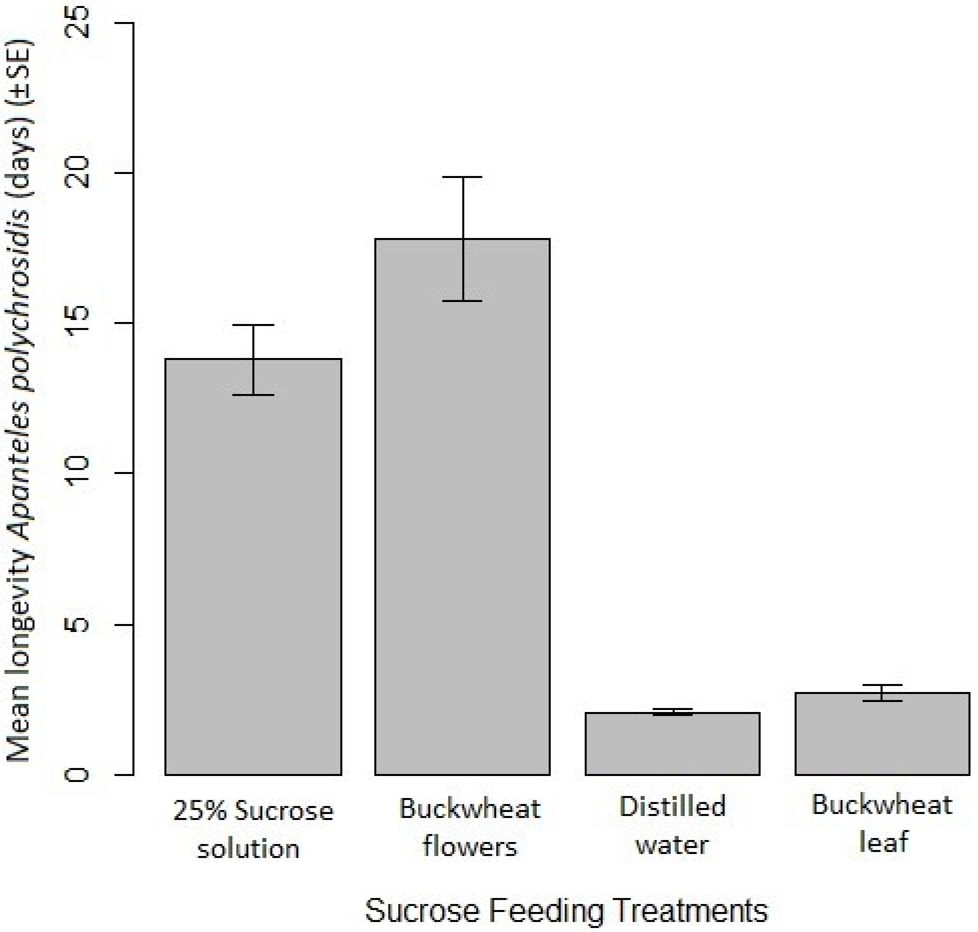


Figure 3.12 **Mean longevity (days) of virgin *Apanteles polychrosidis* with buckwheat nectar or sucrose feeding.** Each wasp (n= 41-55) was provided with a feeding treatment of either 25%(v/v) (25) sucrose in deionized water, a deionized water control (0), 5-20 buckwheat blossoms, or a buckwheat leaf control. Results were compared using a generalized linear model with a negative binomial error distribution. Differences between treatments, determined by compact letter display (cld) of multiple pairwise comparisons, shown with lower case letters.

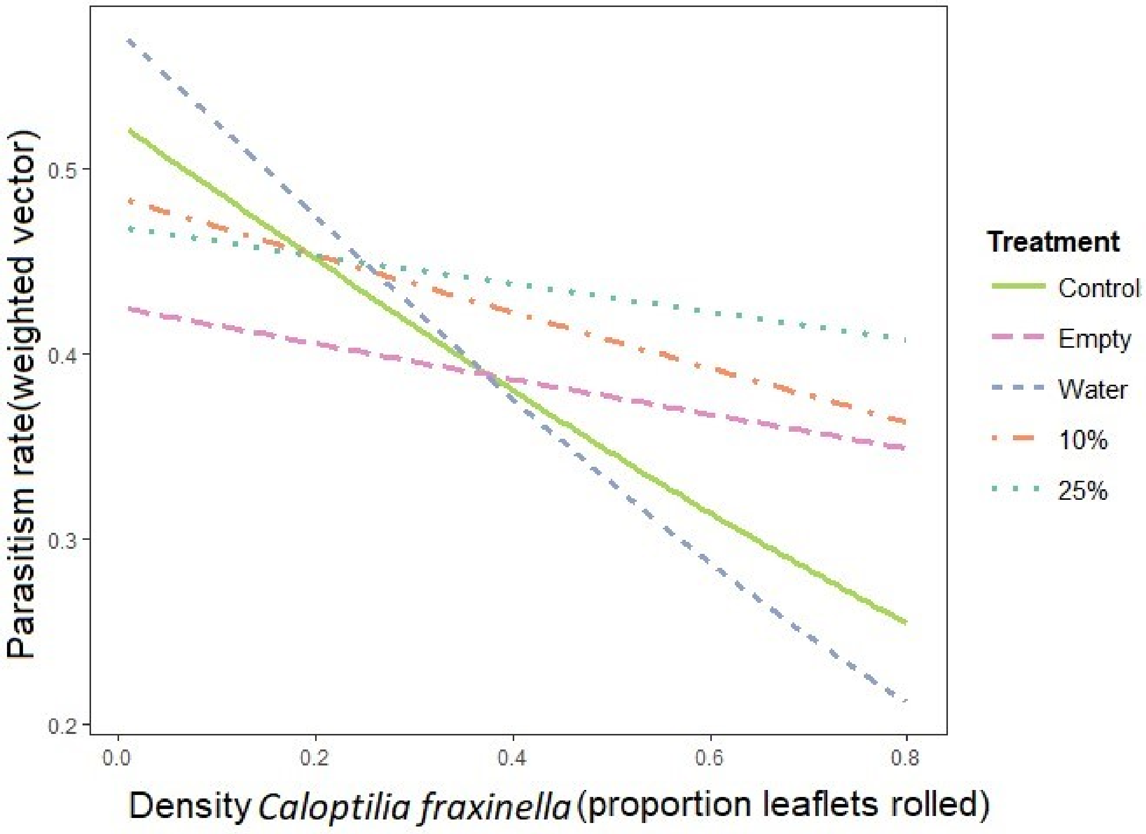
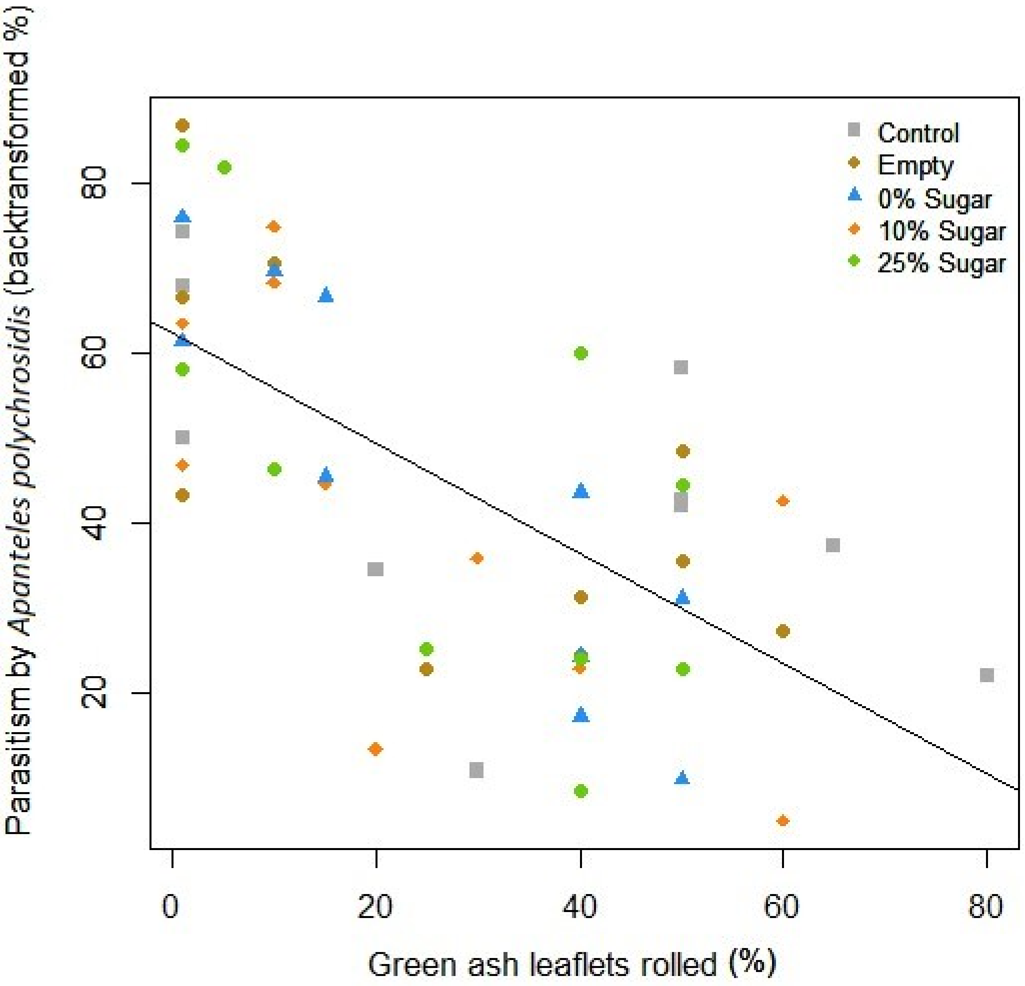


Figure 3.13. **Interaction plot of parasitism rate of *Caloptilia fraxinella* by *Apanteles polychrosidis* at different host densities, with sugar feeding treatments**. Parasitism rate is the value of the weighted vector, y= (parasitized, not parasitized). Trees were treated with either a feeder with 10 or 25%(v/v) sucrose in deionized water, a deionized water control (0%), empty feeders, or no feeder (Control).



3.15 **Linear model of parasitism of *Caloptilia fraxinella* by *Apanteles polychrosidis* compared to host density (% leaflets rolled per tree).** Percent parasitism is the backtransformed value of the weighted vector, y= (parasitized, not parasitized). Trees were treated with either a feeder with 10or 25%(v/v) sucrose in deionized water, a deionized water control (0%), empty feeders, or no feeder (Control) (y=60.24-0.46x, p=0.0001, adjusted R^2=0.25).

Tables

Table 3.1 **Statistical Models for Experiments 1-6**

Experiment 1	**Virgin wasps, sucrose feeding**
longevity, males and females	glm.nb(longevity~sex*feeding treatment)
egg load	lm(egg load~feeding treatment)
egg load, tibia length, dissected females	lm(egg load ~ tibia + longevity)
Experiment 2	**Mated wasps, sucrose feeding**
longevity, males and females	glm.nb(longevity~sex*feeding treatment)
Experiment 3	**Parasitism /maternal effects, sucrose feeding**
parasitism rate	glm(y~treatment, family = binomial) y=(not parasitized, parasitized)
parasitism total	glm.nb(totaloffspring~treatment)
longevity	lm(longevity~treatment)
offspring eggload	glmer(egg~treatment+tibia+(1|ID), family=poisson)
offspring sex ratio	glm(y~ maternal feeding treatment, family=binomial) y=(female offspring, male offspring)
Experiment 4	**Female wasps, buckwheat feeding**
longevity, buckwheat feeding	glm.nb(longevity~feeding treatment)
Experiment 5	**Field sucrose feeding**
wasp retention	glmer.nb(# wasps~treatment+(1|site))

parasitism	glmer(y~ treatment*%rolled+ (1 | site), binomial) y=(parasitized, not parasitized)
Experiment 6	**Field buckwheat feeding**
wasp attraction	glmer.nb(#wasps~treatment+(1|site/tree))

Chapter 4: Conclusion

4.1 Research conclusions

The ash trees (*Fraxinus*) of Edmonton, Alberta comprise ~40% of the boulevard trees, with the vast majority being green ash, *Fraxinus pennsylvanica* (Oleaceae) (City of Edmonton 2017). The introduced nuisance pest of horticultural ash trees, *Caloptilia fraxinella* (Lepidoptera: Gracillariidae), first recorded in Edmonton in 1999 (Pohl *et al*. 2004), inflicts aesthetic damage to ash trees and is a nuisance pest when larvae disperse. In Edmonton, the urban forest is managed with an Integrated Pest Management Strategy including biological control. The native parasitoid, *Apanteles polychrosidis* is a potential biological control agent for control of *C. fraxinella* in its expanded range. In Edmonton and other Prairie communities, *A. polychrosidis* has shifted hosts to become the primary parasitoid of *C. fraxinella* (Wist and Evenden 2013). The overall goal of my thesis was to investigate methods to enhance the performance of *A. polychrosidis* and increase suppression of *C. fraxinella*. Two strategies were investigated: 1) To attract the parasitoid with synthetic copies of volatile organic compounds (VOCs) released by green ash trees to increase the density of the wasp in infested trees; and 2) To provide carbohydrate food at infested green ash trees to increase parasitoid retention and support parasitism activity.

Previous studies have illustrated that *A. polychrosidis* use plant-produced VOCs to orient to infested ash trees (Wist *et al.* 2015). Female wasps are attracted to green ash (*Fraxinus pennsylvanica*) leaflets and black ash leaflets containing *C. fraxinella* larvae. Male and female wasps are responsive to plant chemicals released by both ash species as determined by electroantennogram studies. Of the 13 compounds that elicited a response from wasp antennae

(Wist *et al.* 2015), methyl salicylate and two green leaf volatiles ((*Z*)-3-Hexanol, and (*Z)*-3Hexenal acetate) were used as lures in experiments reported here. Evidence indicates that it is often a blend of volatiles, and the doses of compounds, rather than individual compounds that elicits natural enemy attraction (Turlings *et al*. 1991, Uefune *et al.* 2011, Tamiru *et al.* 2015). For my experiments, therefore, two release rates were tested, in the background of an infested green ash canopy, releasing natural VOCs.

The field experiments using VOC lures at the lower release rate showed that both male and female wasps were attracted to infested green ash trees that were baited with the combination of MS+GLV lures. This indicates that synthetic lures augmented orientation of wasps to infested trees, which could mediate both host and mate location strategies (Kaplan 2017). Some braconid parasitoids use long range sex pheromone signals to attract males (Jones 1996, Ayasse *et al*. 2001, Suckling 2002, Itadani and Ueno 2014). Further study is required to determine if female *A. polychrosidis* release a sex pheromone, but it is known that males detect plant VOCs (Wist *et al.* 2015). Recent work by Xu *et al*. (2017), shows that a number of braconid wasps use a combination of VOCs and pheromones for mate finding.

The rate of parasitism was not increased in the baited trees that had a higher density of wasps. Parasitism rate, however was affected by the low release rate VOC treatments, with parasitism in trees treated with GLV lures marginally higher than in trees treated with MS lures. A factor that appears to influence parasitism rate in this tritrophic system is insect host density. In green ash trees the rate of parasitism decreases as host density increases, (Wist *et al.* 2015). Results from field experiments conducted in 2016 and 2017confirm this relationship. The type of host density-dependence in *A. polychrosidis* appears to vary with plant host. In black ash (*Fraxinus nigra*), the parasitism rate is density-independent (Wist *et al*. 2015). The high release rate lure treatments had an interactive effect on parasitism rate in the present study, which may be explained by HIPV induction in treated trees. Further study on the influence of lure treatments, combined with the density of *C. fraxinella* on the VOC production of treated trees may help us to better understand this tri-trophic interaction.

Conservation biological control strategies include the preservation and enhancement of food sources for pest natural enemies (McCravy 2008). Adult parasitoids are typically carbohydrate feeders that consume floral nectar, extra-floral nectaries, or hemipteran honey dew (Quicke 2015). Both the composition (Zamek *et al*. 2012), and concentration (Ashraf *et al*. 2017, Burrows *et al.* 2017) of carbohydrate sources consumed can affect parasitoid performance. Carbohydrate feeding enhances wasp longevity and fecundity, and provides more energy to parsitoids (Seikmann *et al.* 2004, Lee and Heimpel 2008, Irvin and Hoodle 2015,Ashraf *et al.* 2017,). The experiments I conducted tested two concentrations of a sucrose solution, 10% and 25% v/v. The provision of sucrose solution extends the lifespan of adult *A. polychrosidis*. Both virgin and mated male and female parasitoids live longer when fed sucrose. The higher concentration of sugar consistently increased wasp longevity. Mated females fed 25% sucrose and offered *Choristoneura rosaceana* (Lepidoptera: Tortricidae) larvae as hosts parasitized marginally higher numbers of larvae than females fed 10%, because they lived longer. My results suggest that longer-lived virgin females when denied access to hosts, seem to resorb eggs for somatic maintenance. Parasitoids lack the ability to synthesize lipids (Visser and Ellers 2008). Once capital reserves acquired from larval feeding are expended, the material from eggs can be recycled (Casas *et al*. 2005). The benefit of longevity, therefore, may be undermined by the trade-off of using egg resources for somatic maintenance (Richard and Casas 2009). There did appear to be a slight maternal effect of feeding on offspring fitness. Females fed the higher concentration of sucrose showed a trend to produce more, larger, female offspring, with slightly higher egg counts.

This study determined that buckwheat flowers, *Fagopyrum esculentum* (Polygonaceae), are an adequate food source for adult *A. polychrosidis*. Flower plantings, in addition to providing nectar as food, have the added benefits of parasitoid attraction with floral volatiles (Belz *et al.* 2013), and provide parasitoid habitat (Landis *et al*. 2000). Virgin *A. polychrosidis* females with access to buckwheat flowers or a 25% sucrose solution had a similar longevity, and were observed to be very active when flower feeding. Buckwheat plants grew easily and flowered for an extensive time in the growth chamber, making it an attractive candidate for nectar provision near infested ash trees. In the field, however, my experiments failed to demonstrate wasp retention in ash trees containing sucrose feeders, or buckwheat flowers. The heterogeneous environment of the urban forest may not have the same sugar-limiting challenge as agroecosystems (Marino and Landis 1996).

The outcomes of my research provide further indications of how ash plant VOCs attract *A. polychrosidis*, and how host density impacts parasitism of *C. fraxinella* in green ash trees. From this work, it is clear that carbohydrate provision enhances the longevity of *A. polychrosidis*. Based on the research in this thesis, there are a number of research directions to further develop strategies to enhance *A. polychrosidis* as a parasitoid of *C. fraxinella*.

4.2 Future directions

The finding that male and female *A. polychrosidis* are attracted to infested ash trees baited with the MS+GLVs lures suggests that these plant volatiles are important for more than just host location. It is not known if male *A. polychrosidis* use a long-range sex pheromone. Yolfactometer choice experiments, or behavioural flight chamber studies could be used to investigate male attraction to female wasps (Fauvergue *et al.*1995, Jones 1996). If *A.*

polychrosidis does use sex pheromone communication, it could be developed to manipulate parasitoid behaviour in the field (Suckling *et al.* 2002). Male attraction to ash leaflets has not yet been tested. Olfactometer assays, such as those conducted to establish female attraction to green ash leaflets (Wist *et al*. 2015), could be conducted with male wasps. Knowing the role VOCs play in male attraction would provide insight into strategies for parasitoid manipulation.

A. polychrosidis is bivoltine, and makes use of other hosts in Edmonton (Wist and Evenden 2013). The alternate hosts need to be identified as the population dynamics of the host in which *A. polychrosidis* overwinters could determine the number of wasps available to parasitize *C. fraxinella* larvae in the spring. In the Okanagan region of British Columbia, *A. polychrosidis* uses second or third instar larvae of *C. rosaceana* as hosts for both generations (Cossentine *et al.* 2004). Observations made during this project indicate that several leafrollers (Tortricidae) that feed on a variety of tree species are overwintering hosts of *A. polychrosidis*. Further investigation to identify the overwintering hosts of *A. polychrosidis* and how wasps orient to these hosts is warranted.

Further experimentation to understand the nature of oovigeny, and the use of capital and adult acquired carbohydrate nutrients in *A. polychrosidis* would be beneficial. The present work shows that sucrose provision of adult wasps does not lead to egg development between the age of 2-20 days post eclosion. This would suggest that *A. poychrosidis* use a proovigenic strategy for egg development. The egg load of *A. polychrosidis* at emergence, however, was not studied here. Dissection and egg counting of newly-emerged females would provide data to establish the oovigeny index (Jervis *et al*. 2001) for *A. polychrosidis*. This knowledge would help establish the role carbohydrate provision might serve in egg development and foraging in this parasitoid.

Nearly all the research on conservation biological control is conducted in agroecosystems (DeBach and Rosen 1991). The urban forest environment is more heterogeneous than a monoculture landscape and may create a complex chemical environment (Wäschke *et al*. 2014). In addition, manipulation of the the olfactory environment in an urban forest maybe more difficult to achieve than in an agricultural setting. Background odour is an important consideration for understanding the effect of VOC lures in an environment (Cai *et al*. 2017). The measurement of headspace emissions from infested urban ash trees would aid in quantifying background odour in the urban forest.

To establish whether carbohydrate resources are a limiting factor for the performance of adult *A. polychrosidis* in urban ash trees, the dispersal ability of the wasp needs to be measured. Computer-linked flight mill assays could determine the flight capability of *A. polychrosidis*, as has been done in another braconid parasitoid (Yu *et al.* 2007). Once the dispersal range is known, the density of the parasitoid could be related to nectar and honeydew resource availability across Edmonton.

Further investigation of the host density effects on parasitism by *A. polychrosidis* is needed to better understand the potential for conservation biocontrol in this system. While it is clear that increased *C. fraxinella* density is correlated to decreased rate of parasitism by *A. polychrosidis*, the mechanism of this relationship is unknown. There are numerous tritrophic parasitoid systems that exhibit negative density-dependent relationships with the insect host (Stiling 1987). Proposed mechanisms which could be investigated for this system include egg limitation of parasitoids, inter- and intraspecific competition leading to increased handling time and decreased host-finding efficiency, and host phenology limiting larval exposure to *A. polychrosidis* (Wist *et al.* 2013).

The findings of this project provide further understanding of the role of semiochemical mediation of host location by *A. polychrosidis*. The data here also indicate the importance of carbohydrate resources for this parasitoid, which lays the foundation for future research into its reproductive strategies. The confirmation of the negative host density-dependent relationship that dictates parasitism on green ash is also an important finding. The results of this thesis should inspire future projects seeking to develop strategies to enhance the parasitism of *C. fraxinella* by *A. polychrosidis* in the horticultural ash trees of Edmonton.

4.3 Management Recommendations

This project sought to investigate two methods of increasing parasitism of *C. fraxinella* by *A. polychrosidis* in Edmonton's green ash trees: Volatile organic compound lures, and carbohydrate food provision. Having investigated these areas, the following are management recommendations for the City of Edmonton Pest Management Lab, based on experimental results, and informal observations from the duration of the project.

The rate of parasitism in an infested green ash tree is influenced by more than the mere presence of wasps. Increased number of *A. polychrosidis* on sticky traps in infested ash trees did not result in increased parasitism rate in those trees. The use of the low release rate MS and GLV lures combined to increase wasp attraction, is therefore not recommended as a strategy to increase parasitism.

The provision of sucrose solution in the laboratory clearly supported longevity of both male and female *A. polychrosidis*. Infested trees provisioned with sucrose solution feeders did not result in higher parasitism rates. Carbohydrate availability in the urban forest setting has not been quantified, and it is possible that *A. polychrosidis* is not sugar limited. Woolly ash aphid, *Prociphilus fraxinifolii* Riley (Hemiptera: Eriosomatidae), was observed in the green ash trees at a number of the experimental sites (personal observation). This insect was observed to produce honeydew that dripped out of curled leaves, when disturbed. As well, the urban forest is diverse, with many flowering trees and forbs. It has not been investigated which of these are accessible to *A. polychrosidis* as a nectar source in the field.

The laboratory experiments conducted here used buckwheat flowers to provide flower nectar to female *A. polychrosidis*. The longevity of the virgin female wasps was similar to that of wasps provided 25% sucrose solution. There have been additional benefits ascribed to plantings of flowers as a food source for a target parasitoid, as part of conservation biological control efforts including floral scent to attract parasitoids (Belz *et al.* 2013), and insect habitat provision (Blaauw and Isaacs 2015). Buckwheat has been planted as a nectar plant for parasitoids in a number of field studies (Irvin and Hoddle 2015, Lee and Heimpel 2005, Berndt *et al.* 2002). It is worth noting, however, that this plant is recorded as an introduced species, recorded in 28 States in the United States of America (Invasive Plant Atlas of the United States 2018). It is recommended, therefore, that any field planting of buckwheat be informed by a risk assessment of the plant's potential for invasion of the Edmonton area.

The support of alternate hosts for *A. polychrosidis* in Edmonton is a key factor for maintaining the wasp population, as it requires an overwintering host (Cossentine *et al.* 2005). Although not identified to species, a likely tortricid larval host on aspen, apple, and mayday has been identified (McPike, unpublished data). The apple and mayday trees from which the samples were collected were all within 50 meters of native stands of aspen. It is therefore recommended that the City of Edmonton retain present stands of aspen as reservoirs for the alternate host.

Pursuing the identification, to species, of the overwintering and alternate summer larval host of *A. polychrosidis* is recommended. It is suggested that if *A. polychrosidis* is reared from the overwintering host, that larval remains be collected, and preserved in 90% ethanol for DNA extraction and sequencing, to identify the parasitoid larval host to species. It is also recommended that leaf rolls be collected from aspen in June to determine if *A. polychrosidis* uses the same host in winter and summer, as an alternate host to *C. fraxinella*.

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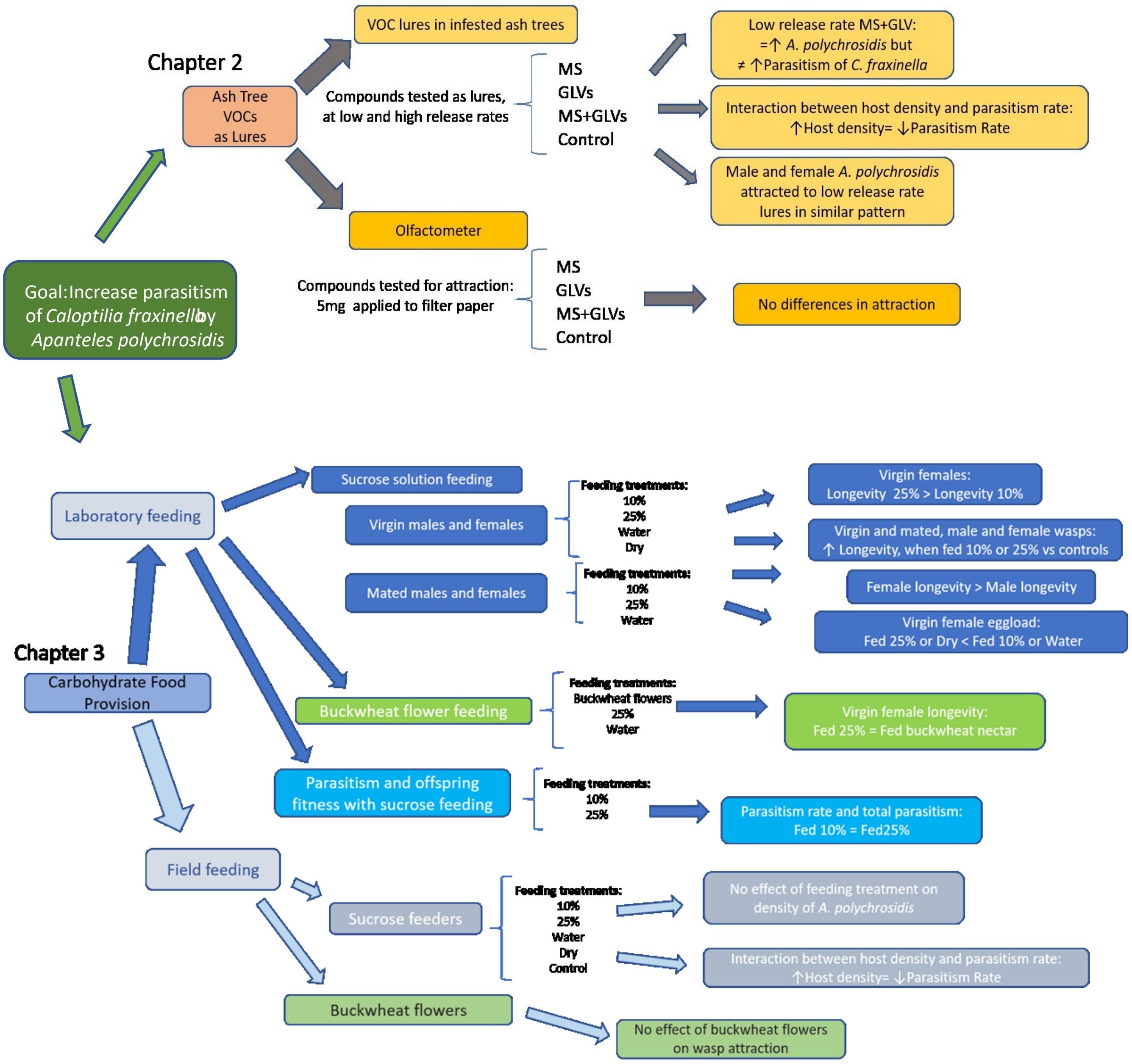
Figure 4.1 **Graphic Summary of thesis experiments and results.** Chapter 2 describes the use of synthetic volatile organic compounds (VOCs) produced by green ash trees (*Fraxinus pennsylvanica*) as lures to attract *Apanteles polychrosidis*. Chapter 3 focused on the provision of carbohydrate food resources to support *A. polychrosidis*. (MS=methyl salicylate, GLVs=green leaf volatiles, 10%=10% sucrose solution, 25%=25% sucrose solution)



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Appendix 1

***Apanteles polychrosidis* longevity with access to distilled water or deionized water**

Introduction

After having conducted multiple feeding experiments with *A. polychrosidis* using deionized water as the solvent, and control group, a paper referencing decreased longevity when zebra mussels, *Dreissena polymorpha* (Veneroidea:Driessenidae), were given deionized water (Ram 1993). There was concern that this effect could occur in other invertebrates, including my study organism, *A. polychrosidis.*

Research objective

To test the hypothesis that deionized water affects the longevity of *A. polychrosidis*, a longevity experiment was conducted in July 2017 comparing days-lived by wasps provided distilled water or deionized water.

Methods

A. polychrosidis were collected between 13-28 June 2017 from green ash trees infested with *C. fraxinella* at sites across Edmonton, Alberta. Leaf-rolls containing wasp pupae were placed individually in 36 ml plastic cups on cafeteria trays and stacked in plastic bags with moistened paper towels to prevent desiccation, and maintained at room temperature (19℃21℃) until wasp eclosion. Cups were checked daily for adult wasp emergence, and eclosed wasps were transferred to treatments.

Upon emergence, virgin females were placed in feeders with either distilled water or deionized water (Modulab BioScience water purification system). Date of emergence and death were recorded.

Analysis

A linear model was used to compare the longevity of the females in R version 3.4.2 (R Core Team 2017). The ANOVA showed no significant difference between the longevity of the treated groups (f= 0.61, df=1, p=0.44).

Conclusion

Longevity for the female A. polychrosidis was not affected by the use of deionized water as a feeding treatment. I conclude, therefore, that results from previous experiments conducted using deionized water are not compromised by confounding effects due to deionized water inclusion.

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Appendix 2

VOC Release Rate Measurement

Introduction:

Experiments to attract parasitoid *Apanteles polychrosidis* to synthetic volatile organic compounds (VOCs) known to be released by green ash trees (*Fraxinus pennsylvanica*, Oleaceae), to increase parasitism of *Caloptilia fraxinella*, were conducted in Edmonton, Alberta in the summer of 2016. The release rates of the lures were unknown. To calculate the release rates, the following experiment was conducted.

Methods:

The synthetic VOC lures consisted of the following:

1) 250 µl eppendorf tube with 200 µl of MS (99% purity, Thermo Scientific) which was pipetted into the tube in the lab, and was then sealed with a soldering iron.

2) 250 µl eppendorf lure containing 200 mg of (*Z)*-3-hexenol, manufactured by Contech Enterprises Inc. (Delta, BC)

3) 250 µl eppendorf lure containing 200 mg of (*Z)*-3-hexenyl acetate, manufactured by Contech Enterprises Inc. (Delta, BC)

Five of each of these lures was weighed to establish starting weights. The lures were maintained tube racks in a fume hood at 21˚C (measured by Hobo Pendant Temperature/Light Data Logger, Bourne, MA). Each lure was weighed daily, 5 days a week for 79 days.

To calculate the release rate, the average number of mg lost by 5 lures was divided by the number of days the lures were in the fume hood, and the temperature was established by the cumulative average temperature measured by the Hobo (Table 1).

Table 1. VOC lure release rate values

Lures	Average weight lost, 5 lures (mg)	Time in fume hood (days)	Release Rate, 21ºC (mg/day)
Methyl salicylate	173.84	79	2.2
(Z)-3-Hexenol	1.93	79	0.024
(Z)-3-Hexenyl acetate	33.83	79	0.43