FIELD AND LABORATORY STUDIES ON THE EFFECTS OF HOST FRUIT VOLATILES ON *RHAGOLETIS MENDAX* (DIPTERA: TEPHRITIDAE) ADULTS

by

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So say we all.

PREFACE

This thesis contains four chapters.

Chapter 1

This chapter includes a general introduction and literature review for the thesis.

Chapter 2

This chapter is a manuscript in preparation for submission to *The Canadian Entomologist*.

Kwasniewska, A.B., Buddle, C.M. and C. Vincent. Effects of host fruit volatiles on field captures of blueberry maggot, Rhagoletis mendax*, adults flies (Diptera: Tephritidae) on green sphere traps*.

Chapter 3

This chapter is a manuscript in preparation for submission to *Journal of Insect Behaviour*.

Kwasniewska, A.B., Vincent, C., Buddle C.M. and S. Gaul. Effects of host fruit volatiles on the olfactory responses of Rhagoletis mendax *(Diptera: Tephritidae) flies.*

Chapter 4

This chapter is a general conclusion to the thesis.

CONTRIBUTION OF AUTHORS

A.B. Kwasniewska, C.M. Buddle and C. Vincent designed the research presented in Chapters 2 and 3. A.B. Kwasniewska collected the field and wind tunnel data, analyzed it and presented the results. The project was co-supervised by C.M. Buddle and C. Vincent. They gave advice on the experimental design, and helped with interpretation of results, as well as edited all chapters in this thesis. S. Gaul provided the chemical extracts from host fruit.

ABSTRACT

I examined olfactory responses of the blueberry maggot fly *Rhagoletis mendax* Curran towards organic volatile compounds extracted from host fruit, in both field and laboratory experiments. First, I investigated the effects of eight volatile treatments on the capture rate of adult flies, in Saint-Chrysostome, QC. A second study focused on testing the attractiveness of the same volatile treatments in wind tunnel bioassays. I also evaluated the effect of experience, sex and population on the olfactory response of these flies.

Ammonium acetate was one of the more effective lures in field experiments during the early season. The treatment blend of terpenes and alcohols had the highest fly capture rate in early and late season but it did not differ significantly from ammonium acetate. Ammonium acetate elicited the strongest response in wind tunnel bioassays, followed by a blend of aldehydes and alcohols. Nova Scotia flies had an overall higher response to treatments than the Quebec population and male flies exhibited a higher flight frequency. Experienced flies were also less stimulated by the treatments than naive flies.

This research has provided an initial step in identification of attractive host fruit volatiles to *R. mendax* adults, supporting the hypothesis that volatile host blends of specific compounds are attractive to the insect. It is also suggested that future experiments use either male or female, naive flies from the same geographical location.

RÉSUMÉ

J'ai examiné les réponses olfactives de la mouche de bleuet, *Rhagoletis mendax* Curran, face à des composés organiques volatils extraits de bleuets, lors d'expériences de terrain et de laboratoire. Premièrement, j'ai étudié les effets de huit traitements de produits chimiques volatiles distincts sur les niveaux de capture de mouches adultes, à Saint-Chrysostome, QC. Une deuxième étude a concernait le potentiel d'attraction de ces mêmes traitements lors d'essais biologiques en tunnel de vol. J'ai aussi évalué l'effet de l'expérience, du sexe et de la population sur la réponse olfactive de ces mouches.

L'acétate d'ammonium a été l'appât le plus efficace lors des expériences de terrain en début de saison. Le mélange de terpènes et d'alcools a procuré le niveau de capture de mouche le plus élevé en début et en fin de saison, mais n'a pas manifesté de différences significatives face à l'acétate d'ammonium. L'acétate d'ammonium a suscité la plus forte réponse dans les essais biologiques en tunnel de vol, suivie par un mélange d'aldéhydes et d'alcools. Globalement, les mouches de Nouvelle-Écosse répondaient davantage aux traitements que celles du Québec, tandis que les mouches mâles exhibaient un plus grand nombre de vols. Les mouches ayant acquis de l'expérience étaient moins stimulées par les traitements que les mouches naïves.

Cette recherche a franchie une étape préliminaire à l'identification de substances volatiles de fruits hôtes pouvant attirer des adultes *R. mendax*. Ces résultats appuient l'hypothèse selon laquelle les mélanges d'appâts volatiles doivent être produits de composés spécifiques afin d'être attirant pour l'insecte. Il est également suggéré pour des expériences futures d'utiliser des mouches inexpérimentées, soit mâles ou femelles, provenant du même emplacement géographique.

To my parents, for their unwavering support and understanding throughout my education.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

Fruit flies (Diptera: Tephritidae) are considered to be among the most economically important pest groups worldwide (Aluja and Norrbom, 2001). When present in valuable fruit crops, these insects fall under strict management involving regulation and control. Studies dealing with behavioural and ecological aspects of tephritids are therefore very important, since they are the key to understanding and managing such insects.

In Canada, fruit crops of importance include apples, apricots, blueberries, cherries, cranberries, grapes, peaches, pears, plums, strawberries and raspberries (Statistics Canada, 2008). As one of the major fruit crops, blueberry fields cover approximately 62,000 hectares of land and produce 77,500 metric tons of fruit per year (Statistics Canada, 2008). The estimated farm gate value for both highbush and lowbush blueberries is $189,000,000 (Statistics Canada, 2008). These statistics include both cultivated and wild blueberries.

Blueberries are extremely susceptible to various insect species, a critical one being the blueberry maggot fly, *Rhagoletis mendax* Curran, a native fly of North America (Payne and Berlocher, 1995). Susceptibility to infestation varies according to cultivar. Early maturing blueberry cultivars, such as Earliblue and Bluetta, contained significantly fewer *R. mendax* maggots when compared to Bluehaven, Coville, Darrow, Elizabeth and Lateblue (Liburd et al., 1998a). Once infested, blueberries become soft, mushy and unmarketable. When poorly managed, maggots can surface in processed berries, such as jams and preserves. To prevent this, as well as to prevent further spread of *R. mendax* to uninfested areas, strict regulations impose zero tolerance for any *R. mendax* larvae found in Canadian commercial berries (CFIA, 2009).

1.1 Biology of *Rhagoletis mendax*

Rhagoletis mendax is a small, black fly, approximately 5 mm in length with an 8 mm wing span. Its wings have a distinctive black banded pattern which closely resembles that of *R. pomonella* Walsh, its sibling species. *R. mendax* can be identified by the smaller ovipositor in females, a darker shading of the frontal femora, as well as an overall smaller size (Lathrop and Nickels, 1932)

Hosts

In the eastern United States, *R. mendax* is most commonly found infesting deerberries, *Vaccinium stamineum* L., which are considered its principle host (Payne and Berlocher, 1995). Deerberry used to be considered a range limiting plant for *R. mendax*, but many infestations are now occurring in areas completely devoid of *V. stamineum*.

Along with infesting high bush blueberries, *V. corymbosum* L., and lowbush blueberries, *V. angustifolium* L. (Barry et al., 2004), the blueberry maggot fly also shows some preference for black huckleberries, *Gaylussacia baccata* W., occurring in wild berry populations in Canada (Smith et al., 2001).

The pattern of host plants used by *R. mendax* remains confusing. Payne and Berlocher (1995) found many discrepancies in the types of infested hosts. In many areas, the fly failed to infest all of the available host fruit, including *V. corymbosum* and *V. angustifolium*. Although puzzling, this may be explained by the relatively inflexible diapause characteristics of *R. mendax*, restricting it from colonizing all the hosts when they are at a ripe stage.

Rhagoletis mendax larvae are also found in other wild berries, although at low levels of infestation. Larvae can be found in blue huckleberries, *Gaylussacia frondosa* L. (Payne and Berlocher, 1995), as well as wintergreen, *Gaultheria procumbens* L., which can frequently be found growing around the edges of infested blueberry fields (Smith et al., 2001).

Life cycle

The life cycle of blueberry maggot flies is closely synchronized with the seasonal ripening of host fruit. Blueberry maggot flies live for approximately 19-24 days in nature, and an average of 20 days in captivity. Eggs are oviposited beneath the skin of berries, where they experience an incubation period that can vary between four to eight days in the field, and may be influenced by prevailing temperatures (Lathrop and Nickels, 1932).

Maggots start to appear in fruit around the end of July. They remain inside the fruit, feeding on the pulp for approximately three to four weeks. *Rhagoletis mendax* go through three instars during their larval period and maggot populations reach their peak abundance around mid-August, coinciding with most blueberry harvest times (Lathrop and Nickels, 1932). At the end of the third larval instar, maggots drop to the ground, either inside the berry or outside, where they pupate and overwinter (Prokopy and Papaj, 2001).

The pupal stage is spent within the first few inches of soil and takes place during the winter. Most flies emerge the following season, but about 5-20% may remain in this stage for two to four years. Emergence usually occurs in July (Lathrop and Nickels, 1932). Some pupae develop into adults much later in the season, emerging as late as August or September (Teixeira and Polavarapu, 2002). Pupae may also experience heat stress which interferes with further maturation. When exposed to sudden high temperatures, the pupae arrest their development at approximately 10 days prior to emergence (Teixeira and Polavarapu, 2005). This delays the emergence of adults or can cause defects during metamorphosis.

Adults emerge as soon as berries begin to ripen. They aggregate loosely on host leaves for the next two weeks to feed (Smith and Prokopy, 1981). Flies mature sexually in approximately 7-10 days (Smith and Prokopy, 1981). Sexual maturity in females is recognized by the presence of fully developed ovaries (Prokopy and Papaj, 2001). Most successful matings occur on leaves, where copulation is usually initiated by the frontal approach from males (Smith and Prokopy, 1982). As mating activities move from leaves to fruit, the males generally preceed the female (protandry) (Smith and Prokopy, 1981), but copulation occurs most frequently when the females are the first to arrive, since the presence of males on a fruit seems to deter them from landing (Smith and Prokopy, 1982). Still, more sexual encounters occur on fruit, since most are forced while the female is attempting oviposition. It is unknown if sperm competition exists within this species. Each mated female lays from 25 to 100 eggs within 19 to 25 days.

Distribution

The current range of *R. mendax* encompasses all of the eastern United States, as well as parts of Canada. In Canada, *R. mendax* has been found in the eastern provinces of Nova Scotia, New Brunswick and Prince Edward Island for almost a century (Lathrop, 1932). More recently, its range has expanded into the southern parts of Quebec and Ontario (CFIA, 2009). For the moment, only small, isolated populations are present in quarantined cultivated fields, but more unknown populations may be occurring in the wild.

Diet

Like other frugivorous flies, *R. mendax* adults need a sufficient intake of carbohydrates and water for survival. They also require a satisfactory amount of protein or amino acids for the development of eggs (Prokopy and Papaj, 2001). Natural sources of food for these flies can include foliar leachate, insect honeydew, flower nectar, juices from wounded fruit and bird droppings (Hendrichs and Prokopy, 1994). Blueberry maggot flies have also been observed to feed on wounded fruit juices, as well as bird feces (Smith and Prokopy, 1981).

Learning

Since flies spend time on the host before mating, further activities such as courtship and ovipositional site location will occur on the same host because of previous experience (Prokopy et al., 1989). In theory, acquisition of experience could occur during habitat location, locating a plant within the habitat, finding a prospective egg laying and/or reproductive site within a plant, examining the prospective site, and ovipositing or attracting a female (Prokopy et al*.,* 1994). Averill et al. (1996) have demonstrated that previous experience with a certain host can greatly influence the response towards that host. For example, *R. mendax* and *R. pomonella* adults are much more likely to locate a particular host fruit after being exposed to it prior to the trials.

Learning in *Rhagoletis* spp. can occur during short or long term exposure to hosts. As Prokopy et al. (1993) have demonstrated with *R. pomonella* and *R. mendax*, long term prior experience with host fruit of at least three days can induce significant changes on the strength of response to different hosts. Short term exposure has also been shown to have an effect on the responses of apple maggot flies (Averill et al., 1996). After allowing a single ovipositional attempt on blueberries prior to testing, female *R. mendax* were much more successful at finding their host.

Natural enemies

Diachasma alloeum Muesebeck, a wasp that originally parasitized *R. pomonella*, has a potential to act as a natural biological control agent in maggot infestations. These natural predators have been observed attacking *R. mendax*, and have been found parasitizing larvae in many blueberries (Stelinski et al., 2004). It was found that *D. alloeum* is capable of distinguishing between non-infested and *R. mendax*-infested fruit, prior to alighting on fruit. Stelinski et al. (2006) showed that current monitoring and control methods, such as kaolin and imidacloprid treatment for *R. mendax,* are severely affecting the behaviour of their parasitic wasps, preventing *D. alloeum* from correctly identifying maggot infested fruit and alighting on them.

1.2. Host location and volatiles

Visual host cues

Most *Rhagoletis* flies use similar methods to detect hosts. *Rhagoletis pomonella* has been studied the most extensively in this genus, therefore posing as a good model species. As observed with *R. pomonella*, these fruit flies are using shape and colour intensity contrasts to detect hosts visually (Owens and Prokopy, 1986).

Olfactory host cues and olfactory receptor neurons

Olfactory cues are volatile blends of chemicals naturally emitted from host plants. These cues serve a very important role since fruit flies rely heavily on them when visual cues start diminishing (Berlocher and Feder, 2009). Flies can detect chemicals through olfactory receptor neurons (ORNs) located in their antennae (Olsson et al., 2006a,b). The antennae of different flies' exhibit variation in ORN types and numbers, although *R. pomonella* flies reared from apple, dogwood and hawthorn, as well as *R. mendax* flies reared from blueberries have the same types of ORNs present (Olsson et al., 2006a).

Stimulation occurs when a particular volatile compound comes in contact with a certain type of ORN, specifically tuned to that chemical (Olsson et al., 2006a). Although many *Rhagoletis* flies have the same ORNs, behavioural responses towards preferential host volatiles can differ significantly (Linn et al., 2005). Following this discovery, Olsson et al. (2006b) found that specific host blends for different *Rhagoletis* species are identified by varied sensitivities of ORN antennal regions, and not the lack of response. In this study, *R. pomonella* flies exhibited different sensitivities in the same ORN regions, when reared from different hosts. This variation in ORN numbers can increase the range of response to a particular volatile, allowing the fly to distinguish between minute proportion differences in blends.

In other studies involving *R. pomonella*, an attractive blend of specific volatiles emitted from host fruit was found (Fein et al., 1982, Zhang et al., 1999, Nojima et al., 2003a,b). Fein et al. (1982) demonstrated that the apple maggot was attracted to a blend of seven different compounds: hexyl acetate, (E)-2-hexen-1-yl acetate, butyl 2-methylbutanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate and hexyl butanoate at a ratio of 35:2:8:12:5:28:10. Zhang et al. (1999) found that a blend of five compounds proved to be even more attractive to the fly than the previous. This blend included butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate and pentyl hexanoate at a ratio of 10:4:37:44:5. These studies also demonstrated that *R. pomonella* is attracted to a blend that consists of uneven ratios of compounds. This illustrates the specificity of chemicals need to attract the insect. Recent studies show that *Rhagoletis* flies not only preferentially locate their own host volatile blends in the field (Linn et al., 2003), but they also avoid the blends emitted by plants of no interest (Linn et al., 2005), even if the same types of chemicals are present. Liburd (2004) has also demonstrated that *R. mendax* can be attracted to some synthetic chemicals; butyl butanoate and cis-3-hexen-1-ol, which similarly attract the apple maggot. Averill et al. (1996) showed that *R. mendax* is significantly more attracted to its berry host and not to hosts of other *Rhagoletis* species. Pelz-Stelinski et al. (2005) confirmed this again by capturing more flies on traps adjacent to host fruit.

Other volatiles

Attractive volatiles may also be emitted from other sources than the host itself. These may include bacteria living on the surfaces of leaves or fruit. It has been reported that *Enterobacter (Pantoea) agglomerans* is attractive to *R. mendax* as well as *R. pomonella* flies (MacCollom et al., 1992, 1994, 2009, Lauzon et al., 1998, 2000). Flies are attracted to only certain strains of Enterobacteriaceae, which include bacteria that fix nitrogen from the atmosphere (Murphy et al., 1994, Lauzon et al., 1998). Tephritids locate the right bacteria on the leaves or fruit of host plants, and ingest them while foraging. *Enterobacter agglomerans* can thus be isolated from the alimentary tracts of *R. pomonella* and *R. mendax* flies. This suggests that the bacteria have a symbiotic relationship with these flies since they provide them with nitrogen to metabolize directly inside the gut (Drew et al., 1983, Lauzon et al., 2000). Females also need sufficient amounts of nitrogen in order to fully mature their ovaries (Prokopy and Papaj, 2001). Since *E. agglomerans* is attractive to *R. mendax*, MacCollom et al. (2009) found that adding it to Pherocon-AM traps significantly increases the number of fly captures.

1.3. Population studies

Within the *Rhagoletis* genus, the *R. pomonella* sibling complex is frequently used as a model demonstrating how reproductive isolation can facilitate sympatric speciation in the absence of geographic isolation (Bush, 1969). The species of *R. mendax* arose by a host shift experienced by *R. pomonella* flies. Therefore, many studies on *R. mendax* populations rely on comparisons to *R. pomonella*. Most population studies outline the differences found between the two species populations.

Berlocher (1995) found that the population structure of *R. mendax* differs greatly from *R. pomonella*, even though the two are so closely related. Blueberry maggot flies have a very uniform genetic structure throughout their populations, which may be influenced by the specialization on only two host genera.

Genetic differences were found between *R. mendax* flies inhabiting the same location (Teixeira and Polavarapu, 2003). In New Jersey, adult emergence occurs in July and it is followed by a secondary emergence much later in the season, in August or September. Genetic differences exist between the early and the late emerging populations (Teixeira and Polavarapu, 2002, 2003). It was also found that the genetic structure of *R. mendax* populations from blueberry cultivars, is much less diverse than the wild populations. Since infestations in cultivated blueberries are likely established by a small sample of individuals, loss of genetic diversity may indicate that a founder effect is responsible for the genetic differences between the wild and the cultivar populations (Teixeira and Polavarapu, 2003).

1.4. Monitoring and management

Pherocom-AM traps

Monitoring techniques today involve the use of yellow panel Pherocon-AM traps. These traps are baited with ammonium acetate, which mimics a food source for *R. mendax* and therefore is attractive (Wood et al., 1983, Neilson et al., 1984). The capture rate of flies is affected by several factors. Traps folded at a 45° angle and placed with the apex of the 'V' pointing downward are significantly more effective at capturing blueberry maggot adults (Geddes et al., 1989, Liburd et al., 1998b). Also, placement of traps 5 to 10 cm above the bush canopy is essential for maximum fly captures (Gaul et al., 1995, Liburd et al., 2000).

Although effective to a certain extent, these traps come with a few disadvantages. First, they have been found attractive only early in the fly's life cycle, with fly captures decreasing drastically in the late season (Liburd, 2004). The decline of attractiveness occurs later in the life cycle, when blueberry maggot flies are not as dependent on sources of ammonium needed to mature. Second, Pherocon-AM traps are also not as effective when monitoring for *R. mendax* at low infestation levels (Neilson et al., 1984). Finally, Pherocon-AM traps attract many other insects (Neilson et al., 1984), which may be beneficial in the regulating populations of *R. mendax,* or pollination, essential for blueberry production. Valuable pollinators such as bumble bees, *Bombus impatiens* Cr., honey bees, *Apis mellifera* L. (Javorek et al., 2002, Dedej and Delaplane, 2003, Desjardins and de Oliviera, 2006), as well as some beneficial predatory insects, are present in blueberry fields.

Sphere traps

Sphere traps have good potential for controlling and monitoring blueberry maggot fly populations. A decrease in fly populations has been demonstrated in many studies where spheres are used (Liburd et al., 1999, Stelinski and Liburd, 2001, Liburd et al., 2003, Barry and Polavarapu, 2005). Sphere traps have been researched extensively. Hamill et al. (2003) compared wooden, plastic and biodegradable spheres and investigated on how they work when coated with imidacloprid. They found no differences between the three sphere types, although flies did spent more time on biodegradable spheres. Plastic spheres were deemed the best, since they are the easiest to use with insecticidal coatings, as well as they are much cheaper than traps made of other materials.

Various studies dealing with *R. mendax* have demonstrated that green or red spheres can capture significantly more flies than Pherocon-AM traps, when baited with the same lure (Liburd et al., 1998b, Teixeira and Polavarapu, 2001). Teixeira and Polavarapu (2001) have also found that there can be differences in reproductive maturity of females between captures on green plastic spheres and Pherocon-AM traps. Green sphere traps seemed to catch more immature females than Pherocon-AM traps. Also, when traps are placed just above or within 15 cm of the bush canopy, fly captures significantly increase (Liburd et al., 2000, Teixeira and Polavarapu, 2001).

Colours of traps can have an impact on the response of blueberry maggot flies. Flies are significantly more attracted to green or red spheres than blue (Liburd et al., 1998b, Teixeira and Polavarapu, 2001). The size of traps also has an influence on the number of fly captures. For example, Liburd et al. (2000) showed that traps 9.0 cm in diameter catch more flies than 3.6 or 15.6 cm traps, since these concentrate the ammonium acetate coating to an attractive level, without limiting the surface area to catch flies.

Coated with insecticides, green spheres have shown a potential to significantly decrease infestation levels in surrounding berries when baited with ammonium acetate (Liburd et al., 2003). Still, ammonium acetate baited spheres do not reduce infestations to desirable levels, when compared to insecticidal spray treatments. Insecticide coated traps combined with bait that is sought out as food by the flies, can act together as a good attract-and-kill system. Barry and Polavarapu (2004) found that baits with ammonium acetate have the highest potential to be ingested along with the insecticide, since flies spend the most time feeding on it. They also tested this bait with different insecticidal coatings and deduced that fipronil and spinosad are the most effecting at killing *R. mendax* on contact (Barry and Polavarapu, 2005). Deployment strategies for imidacloprid-treated spheres have also been evaluated by Stelinski and Liburd (2001). Although none of the deployment strategies differed significantly in degree of fruit injury caused by blueberry flies, fields with spheres harboured significantly fewer larvae than untreated control fields.

IPM and pesticides

Integrated pest management (IPM) is a system developed as a monitoring and control strategy for farmers who may have to deal with crop pests. IPM was developed to enable farmers to make ecologically and biologically rational decisions to use biological control agents in their crop management, as much as technologically and economically possible (Kogan and Hilton, 2009). The use of synthetic insecticides should, as stated in the original IPM theory, occur as a last option (Kogan and Hilton, 2009). A monitoring program for the closely related species of *R. pomonella* (the apple maggot) has been developed by Prokopy (2003), where a specific deployment of spheres was used in apple orchards to control infestations. This small scale demonstration showed that an IPM program is capable of reducing apple maggot infestation levels to 1%, similar to levels in chemically treated fields.

Current Canadian Food Inspection Agency (CFIA) protocol requires deployment of Pherocon AM traps in fields to determine if an infestation exists. Due to the strict regulations imposed during blueberry maggot presence, an appropriate schedule is determined for insecticidal spray application (Gaul et al., 1995, Gaul et al., 2002, Peltz et al., 2005). Spraying may take place once or twice in the season, and number of sprays may increase depending on insecticide type used. Some insecticidal sprays used today include Imidan, Sevin and Guthion.

Collins and Drummond (2004) also studied field edges to determine if these can serve as sources of flies to further infest cultivated berries. They discovered that the edges of blueberry fields hold the highest densities of blueberry maggots. Since their results showed a decrease in the overall maggot infestation, as well as decreased the amount of insecticide application needed, they implemented a perimeter spraying method in their IPM program.

An option to spray with organic pesticides also exists. Organic insecticides, such as Spinosad (Entrust) and pyrethrum (PyGanic), compared favourably in terms of effectiveness with Imidan (Barry et al., 2005). These were effectively controlled *R. mendax* populations and can be incorporated into blueberry maggot management programs under organic standards.

Other control methods involve the use of Surround WP. The active ingredient in this commercial powder is kaolin; a white, non-porous, fine grained aluminosilicate mineral ($Al_4Si_4O_{10}(OH)_8$). Once sprayed as a suspension mixed with water, this substance coats the berry and acts as a physical barrier, preventing the insect from recognizing the fruit as its host (Lemoyne et al., 2007). Furthermore, its fine granules stick onto the legs of the insect, hindering movements such as walking and ovipositing. In choice bioassays, Surround WP treated fruit had a significantly lower number of visits by *R. mendax* (Lemoyne et al., 2007). The duration of walking bouts on fruit was significantly shorter as well. Kaolin coated fruit also deterred all females from ovipositing.

Gamma irradiation

Ionizing radiation is a technique currently used with some tephritids to prevent the spread of infestations, especially in guava and mango fruit in the southwestern United States (Hallman, 1999). Fruit is irradiated after collection, while being passed on conveyer belts. Irradiation is not considered harmful to the fruit, and berries with a radiation dose of 750 Gy still retain their high qualities such as taste, firmness, color, shelf life, and consumer choice (Miller et al., 1994).

This method was tested with *R. mendax* pupae by Sharp and Polavarapu (1999). It was found that gamma radiation doses of over 80 Gy prevented adult emergence from blueberries infested with the maggot. Even lower doses of 10 Gy, caused deformities as early as the third instar (Hallman and Thomas, 1999). Therefore, these techniques can be useful in preventing the spread of blueberry maggot infestations.

1.5. General objectives and research questions

Pest management is very important to healthy crop growth and production of good quality food. With consumers exerting constant pressure to use more environmental and sustainable methods, organically grown crops have become the focus of many farmers.

The overall goal of my research is to identify an attractive volatile extract from the primary hosts of *R. mendax* flies that can be used in trapping techniques for monitoring and/or control of this species. Traps with more effective lures will be better able to determine the population densities better so that insecticidal applications may be accurately scheduled with less spraying. Traps with strong, attractive lures can also act as an alternative tool to our current control methods. I also wanted to test whether experience with host fruit prior to testing affects the blueberry maggot's behavioural response to the treatment, and finally, if there are any differences in responses between the two sexes.

In this thesis, Chapter 2 evaluates the effectiveness of green sphere traps baited with different host fruit volatile blends in a field context. This work was done under field conditions at Réserve Écologique du Pin-Rigide and surrounding forest area near Saint-Chrysostome, Quebec. This area contains many wild hosts, including black huckleberries (*G. baccata*), lowbush blueberries (*V. angustifolium*) and highbush blueberries (*V. corymbosum*). Chapter 3 presents results from a laboratory study done in the facilities at Agriculture and Agri-Food Canada research station in Saint-Jean-sur-Richelieu, Quebec. There I performed wind tunnel bioassays to evaluate the olfactory responses of *R. mendax* males and females. The same host fruit volatile blends are tested in this experiment as in the field. In the wind tunnel, it was also evaluated whether experience has an impact on the response of the fly.

In Chapter 2 my objective is to evaluate the attractiveness of host fruit volatile treatments with green sphere traps for adult* Rhagoletis mendax *flies under field conditions

Green sphere traps were deployed in blueberry and huckleberry bushes. They were baited with 9 different treatments; 7 chemical volatiles, ammonium acetate (standard) and a blank. The host fruit volatile compounds included mixtures of terpenes-aldehydes-alcohols, terpenes-aldehydes, terpenes-alcohols, aldehydes-alcohols, terpenes, aldehydes and alcohols. Traps were checked twice weekly and adult flies were counted and removed. Sampling occurred from 3 July to 29 August 2008. I predict that one of the volatile blends will capture more flies, and that it will be better and longer lasting than the standard of ammonium acetate.

In Chapter 3 my objective is to assess which host fruit volatile generates a change in the olfactory responses of* Rhagoletis mendax *adult flies in wind tunnel bioassays

In this experiment I addressed a series of more specific research questions:

(i) Which host fruit volatile blends stimulate the most activity in *R. mendax* flies during wind tunnel bioassays?

The wind tunnel consisted of three sections (i.e. downwind, central, and upwind). Each section contained a small artificial plant resembling the host. Sexually mature blueberry maggot flies were released individually inside the wind tunnel at the downwind section. They were observed for 20 minutes each. The following behaviours were recorded; total time active, frequency of flight, movement through tunnel and behaviour in each section of the tunnel. I predict that one of the volatile blends will create a stronger attractive response in the flies, and that this mixture will be more attractive than the standard of ammonium acetate during all the season.

(ii) Do flies with host experience exhibit a change in response to volatile blends?

After being tested in the naive state, the flies were placed inside cages with market purchased organic blueberries. Exposure with host fruit was of long duration; selected flies were allowed to remain on blueberries for at least three days prior to testing (Prokopy et al., 1993, Prokopy et al., 1994). I predict that experienced flies will have a higher response to the volatile treatments since they will be familiar with the host and searching for its odour.

(iii) Do males and females differ in their responses towards different volatile blends?

A sample of females and males were tested inside the wind tunnel with each blend of volatiles. I predict that there will not be any differences in the behavioural responses of the two sexes towards the volatiles.

(iv) Do flies originating from different geographical locations exhibit a different olfactory response to treatment blends?

Flies reared from two different geographical populations (Nova Scotia and Quebec) were observed in the wind tunnel under the influence of the same treatments. I predict that responses will differ according to geographic location because genetic differences have been found between flies from different regions.

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CONNECTING STATEMENT

In chapter 1, I reviewed the literature concerning the biology, host location and management of *Rhagoletis mendax* adults. Few studies have attempted to identify a specific host fruit volatile blend that is attractive to these flies. Chapter 2 examines the effect of different blends of host fruit volatiles extracted from ripe fruit, on the capture rate of blueberry maggot flies on green spheres in field studies.

CHAPTER 2: EFFECTS OF HOST FRUIT VOLATILES ON FIELD CAPTURES OF BLUEBERRY MAGGOT, *RHAGOLETIS MENDAX*, ADULT FLIES (DIPTERA: TEPHRITIDAE) USING GREEN SPHERE TRAPS.

2.1. ABSTRACT

Rhagoletis mendax Curran is a persistent agricultural pest in the Maritime Provinces, as well as in Quebec and Ontario. The current monitoring strategy involves the use of ammonium acetate baited Pherocon-AM traps to determine population densities and fly emergence. This method needs to be refined. The objectives of this study are to identify a volatile blend that will effectively attract *R. mendax* adults. I tested seven volatile treatments, as well as ammonium acetate (standard), as lures on green sticky spheres, at Saint-Chrysostome, QC. The treatment blend of terpenes and alcohols was the most attractive, followed closely by ammonium acetate during the early and late phases of the blueberry season. This mixture was not significantly different from the standard. Ammonium acetate also captured more flies in the early season. Since host volatile treatments did not surpass the standard, results suggest that even ratios of chemical blends do not have a strong enough effect for monitoring this pest, and should be further manipulated or combined with other odour sources. For now, a combination of host fruit volatiles and the standard can be used as lures to maximize the monitoring potential for the entire season.

2.2. INTRODUCTION

The blueberry maggot fly, *Rhagoletis mendax* Curran, is a key pest of highbush blueberries, *Vaccinium corymbosum* L., and lowbush blueberries, *V. angustifolium* L. (Barry et al., 2004). It also shows some preference for huckleberries (*Gaylussacia baccata* W.) occurring in wild berry populations (Smith et al., 2001). *Rhagoletis mendax* is currently distributed throughout the eastern provinces of Canada (Nova Scotia, New Brunswick and Prince Edward Island), as well as in the southern portions of Quebec and Ontario (CFIA, 2009). Adult flies lay eggs inside ripe berries, where a larva hatches and feeds on the pulp of the fruit making it soft, mushy and unmarketable (Prokopy and Papaj, 2001). If not monitored closely, maggots can surface in processed berries, such as jams and preserves. Therefore, regulations impose zero tolerance for any *R. mendax* larvae found in Canadian commercial berries (CFIA, 2009). Fruit can continue to be sold only if growers comply with CFIA programs and regulations. Integrated pest management for the fly involves first monitoring for the pest with Pherocon-AM traps, followed by rigorous insecticidal spraying with Spinosad, Sevin or Imidan, if an infestation is confirmed (Peltz et al., 2005).

Standard monitoring practices for blueberry maggot flies rely on the use of yellow panel Pherocon-AM traps. These traps are baited with ammonium acetate, which has been shown to attract *R. mendax* adults since it acts as a lure mimicking a food source (Wood et al., 1983, Neilson et al., 1984). Although relatively effective, these traps have been found more attractive for sexually immature adults (Liburd, 2004). They are also ineffective when monitoring for *R. mendax* at low infestation levels (Neilson et al., 1984). Another disadvantage to Pherocon-AM traps is that many beneficial insects are attracted to the same bait as well as the colour, and this may reduce population numbers of natural enemies and other insects that act as biological control agents regulating the populations of *R. mendax* flies (Neilson et al., 1984).

Depending on the placement, size, colour and material, using sphere traps as a method for capturing fruit flies in infested fields is promising concept (Liburd et al., 1998, 2000, Teixeira and Polavarapu, 2001). Green or red spheres placed 15 cm within the blush canopy, capture significantly more *R. mendax* than Pherocon-AM traps when baited with the same lure (Liburd et al., 1998, Teixeira and Polavarapu, 2001). When coated with insecticides, green spheres have shown a potential to significantly decrease infestation levels in surrounding berries when baited with ammonium acetate (Liburd et al., 2003). Nevertheless, ammonium acetate baited spheres are not able to reduce infestations to levels low enough to compete with insecticidal spray applications in fields. It is therefore important to find more attractive lures to enable higher capture rates of flies.

The fly's emergence is synchronized with seasonal maturation of host fruit in the field, which they locate by the use of visual and olfactory cues that are mimicked by the traps (Visser, 1986). A good example is the monitoring and control strategy for *R. pomonella* Walsh, the apple maggot, a sibling species to *R. mendax*. It involves the use of red sphere traps, baited with a volatile chemical blend that has been identified from host fruit to be attractive (Fein et al., 1982, Zhang et al., 1999). Both species also seem to be using shape and colour intensity contrasts to detect the traps visually (Owens and Prokopy, 1986, Teixeira and Polavarapu, 2001).

The objective of this study is to determine which volatile mixture, extracted from ripe host fruit, will have the highest capture rate of *R. mendax* flies in wild populations of berries throughout the entire season. I also evaluated the host fruit volatile treatments against the current monitoring standard of ammonium acetate. I predict that one of the treatment blends will be more attractive and therefore capture higher numbers of flies. I also predict that this mixture will attract adult *R. mendax* throughout the entire season.

2.3. MATERIALS AND METHODS

Volatile lures. Concentrated volatile solutions were obtained from (Agriculture and Agri-Food Canada, Kentville, Nova Scotia). They were extracted from lowbush blueberry and huckleberry hosts. The chemicals were grouped into the broad classes of terpenes, aldehydes and alcohols, since these compounds are volatile and are associated with head space volatiles of berries. These were then mixed in all possible combinations at even ratios (Table 2.1). Using even ratios is a good strategy for initial studies dealing with host volatile identification, since the results can indicate which chemicals are of interest to *R. mendax* flies for monitoring purposes.

Concentrated volatiles were diluted in hexane (4mL concentrate per 100mL of hexane) to ensure even coverage of rubber septa. The septa were immersed in each treatment solution and placed in a fume hood to allow the hexane to evaporate, leaving only the desired volatile coating. Treated septa were placed separately in several plastic bags and kept in the freezer to preserve the chemicals. Ammonium acetate (1.5g), in crystallized form, was sprinkled onto the traps designated for that treatment, and mixed into the TangleTrap® glue.

Field site and Sampling Methods. Experiments were conducted at Réserve Écologique du Pin-Rigide and surrounding forest area near Saint-Chrysostome, Quebec (Figure 2.1). This area was selected because of its historically high levels of *R. mendax* infestation. Host plants growing in this area include *V. corymbosum*, *V. angustifolium* and *G. baccata*.

Trap locations were chosen only in dry areas where sphagnum moss growth was minimal. Each trap was surrounded by host plants to a radius of at least 5 m; distances between traps were kept at no less than 10 m to prevent overlap in trapping area. Chosen trap sites consisted of a mix of *V. angustifolium* and *G. baccata* host plants.

Commercially available green plastic spheres, purchased from Distributions Solida (Saint-Ferréol-Les-Neiges, Quebec), were used as prescribed by Liburd et al. (1998). Only green spheres were used in this study as a precaution in order to avoid any attractive effects of colour since I was testing for volatile attraction only. The spheres were 9 cm in diameter, the most effective size for capturing *R. mendax* (Liburd et al., 2000). Each sphere was coated in TangleTrap® at the beginning of the study, and recoated 5 weeks later. The traps were placed above the canopy of the host plant by the use of steel poles, and the lures were hung just above the sphere.

The experiment consisted of 9 treatments; 7 chemical volatiles, ammonium acetate (standard) and a blank (Table 2.1). The traps were placed in a completely randomized block design. Each treatment was replicated eight times, resulting in a total of 72 traps.

Traps were monitored from 3 July to 29 August 2008. The traps were checked twice weekly and adult flies were counted and removed. Rubber septa were renewed and ammonium acetate traps were recoated weekly.

Statistical Analyses. Field data were grouped according to two different periods of time (early and late season). Early season was designated to the month of July, and late season to the month of August.

Fly capture data were tested using a two-factor mixed Analysis of Variance (ANOVA), with SAS (SAS Version 9.1, SAS Institute, 2001). This model consisted of two variables: treatment and block. Treatments were considered as a fixed effect and corresponded to the application of terpenes-aldehydes-alcohols, terpenes-aldehydes, terpenes-alcohols, aldehydes-alcohols, terpenes, aldehydes, alcohols, ammonium acetate, as well as a blank. Blocks were considered random effects; there were eight blocks. The response variable was the number of *R. mendax* adults captured on spheres during the early and late seasons. These variables were entered as temporal repeated measures.

Raw data were transformed to meet assumptions of normality required for ANOVA. Data for both early and late season fly captures were square root transformed $\left(X' = \sqrt{X + 0.5}\right)$. For all tests, Type I error was established at $\alpha = 0.05$. Multiple treatment comparisons of means for number of fly captures were adjusted with Tukey's post hoc test.

2.4. RESULTS

A total of 754 *Rhagoletis mendax* adult flies was captured. During the fly's peak season, when the density of ripe host fruit was the highest (18 July to 11 August), the rate of fly captures was the greatest (Figure 2.2). During this time, 83% of flies were captured. In the early and late seasons, there was a total of 388 and 366 flies captured respectively.

During the early season (July 2008), the standard of ammonium acetate (treatment 8) had the highest number of fly captures ($F_{8,15}=3.67$, $p=0.0017$). This treatment, as well as the blend of terpenes and alcohols (treatment 3), captured significantly more flies than the control treatment and the blend of aldehydes and alcohols (treatment 4) (Figure 2.3).

During the late season (August 2008), blended terpenes and alcohols (treatment 3) captured the highest mean number of flies. This blend captured significantly more flies than the control treatment, the blend of aldehydes and alcohols (treatment 4), the blend of terpenes (treatment 5) and blended aldehydes (treatment 6) (Figure 2.3, $F_{8,15}=10.04$, $p<0.0001$). No significant differences were detected between the two periods.

There was an effect of block for early ($F_{7,15}=10.43$, $p<0.0001$) and late season ($F_{7,15}=5.28$, $p<0.0001$). On the basis of general observation, there seemed to be a population density increase from south-west to north-east (Figure 2.1), indicating spatial heterogeneity of fly populations at the field site.

2.5. DISCUSSION

Rhagoletis mendax adults were attracted to a blend of terpenes and alcohols at even ratios. This blend captured the highest number of flies and was significantly different from control treatments in both the early and late season. During the early season, traps coated with ammonium acetate also collected significantly more flies compared to blank traps. Although the blend of terpenes and alcohols had a high capture rate, it did not differ significantly from the standard of ammonium acetate. Since the standard is not efficient enough to decrease infestation levels to a desirable tolerance (Wood et al., 1983, Neilson et al., 1984), this attractive blend of terpenes and alcohols would likely have a similar effect on the degree of infestation and may not be able to decrease larval densities in berries to levels accepted by regulations.

In the early season, ammonium acetate was the treatment with the highest rate of capture, but in the late season, mean capture rate for ammonium acetate decreased to the extent that it did not differ significantly from the control treatment (Figure 2.3). This is similar to the results presented by Liburd (2004), who showed that ammonium acetate has a much higher capture rate of *R. mendax* adults earlier in the season. This may occur because, during the early season, most flies are still immature and are foraging for food instead of ovipositional or mating sites (Smith and Prokopy, 1981). During that period, female flies need to find suitable sources of nitrogen to achieve sexual maturity (Prokopy and Papaj, 2001). The ovaries of *R. mendax* females will not develop completely until adequate amounts of nitrogen are ingested (Prokopy and Papaj, 2001). Nitrogen can usually be found on foliage, in either bird droppings or by ingestion of nitrogen producing bacterial strains (Drew et al., 1983).

The blend of terpenes and alcohols was the most effective host volatile mixture in fly captures during both seasonal phases. Comparatively, the capture rate of this blend remained high throughout the season. Mean number of captures was also slightly higher in the late season for this treatment. Although this blend had a high mean capture rate, it was not significantly different from ammonium acetate in either seasonal phase. Still, it continued to capture flies at the same rate. This blend, therefore, has a potential to monitor *R. mendax* populations more efficiently for the entire berry growing season.

These results demonstrate how different mixtures of the host chemical compound groups elicit a change in the rate of *R. mendax* captures on traps. This strengthens the assumption that flies are attracted to specific mixtures produced by their hosts (Bruce et al., 2005). The insect's response changes when the compounds are tested separately. In nature, flies must constantly discern between different blends of volatiles emitted from all the plants around them. From an ecological perspective, flies must be able to identify the specific blend of its host while surrounded by a multitude of volatile mixtures emanating from all plants in the area, both host and non-host. This is achieved if blends are comprised of specific chemicals and ratios attractive to the fly (Visser, 1986, Bruce et al., 2005). Attractiveness of specific blends has been shown in many studies involving *R. pomonella*. Fein et al. (1982) demonstrated that the apple maggot was attracted to a blend of seven different compounds: hexyl acetate, (E)-2-hexen-1-yl acetate, butyl 2-methylbutanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate and hexyl butanoate at a ratio of 35:2:8:12:5:28:10. Zhang et al. (1999) found that a blend of five compounds proved to be even more attractive to the fly than the 7-compound blend. This blend included butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate and pentyl hexanoate at a ratio of 10:4:37:44:5. These studies demonstrated that *R. pomonella* is attracted to a blend that consists of uneven ratio of compounds. One of the next steps for research dealing with *R. mendax* is to investigate the ratios of terpenes and alcohols emitted from host fruit.

I also detected a block effect in my study, and this may have been caused by gradients in bush growing medium, which sometimes varied from sand to damp *Sphagnum* spp. moss. Other environmental factors might have influenced capture rate, including potential effects of surrounding vegetation, amount of direct sunlight, weather changes and other environmental conditions, which can be expected in a natural forest setting. The block effect creates implications that spatial heterogeneity can have while managing this pest. Traps should be evenly distributed over fields to avoid over or under estimation of actual population numbers. Future study would be required to assess why fly populations appear aggregated in space.

Future avenues of research with *R. mendax* could include a more complete assessment of the range of attractive volatiles that could be emitted from sources other than the host fruit. Bacteria living on leaf or fruit surfaces, and green leaf volatiles that include some similar chemical groups such as aldehydes, alcohols and acetates (Hansson et al., 1999) present some possibilities for further research. *Enterobacter (Pantoea) agglomerans* is attractive to *R. mendax* as well as *R. pomonella* flies (MacCollom et al., 2009, Lauzon et al., 2000). The bacteria fix nitrogen from the atmosphere (Murphy et al., 1994), and provide it for the flies' diet. MacCollom et al. (2009) showed that adding *P. agglomerans* to Pherocon-AM traps had a significant increase in the number of fly captures. This suggests that combining different lures, such as host fruit volatiles with attractive bacterial cultures or ammonium acetate, may increase the effectiveness of lures. Bacterial odours may be involved in host location (MacCollom et al., 2009), therefore when mixed with host fruit volatiles, flies may be attracted further towards the trap after locating the source. Green leaf volatiles may also have a similar effect in *R. mendax* attraction, as their volatile composition usually includes terpenes, alcohols and acetates (Natale et al., 2003).

My research has demonstrated that specific blends of host fruit volatiles can act as lures for monitoring *R. mendax* infestations, especially when used in conjunction with 9 cm, plastic, green sphere traps. The results of this study can be used by farmers to effectively monitor the flies throughout the season, but the blended terpenes and alcohols may not be powerful enough to be used in traps with the intent to exert population control of flies. Although this blend did not achieve that, its chemical composition may be a step to creating a more attractive mixture. For the most effective monitoring strategy for *R. mendax*, it is recommended to bait traps with both ammonium acetate and the host fruit blend of terpenes and alcohols for maximum capture rate.

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Table 2.1. Blends of various classes of volatile compounds that were extracted from ripe host fruit and were found in both black huckleberries (*Gaylussacia baccata*) and lowbush blueberries (*Vaccinium angustifolium*). Courtesy of S. Gaul (AAFC/Kentville, NS).

Treatment	Compound groups used
1	Terpenes + aldehydes + alcohols
2	Terpenes + aldehydes
3	Terpenes + alcohols
4	Aldehydes + alcohols
5	Terpenes
6	Aldehydes
7	Alcohols
8	Ammonium Acetate (standard)
0	Blank (control)

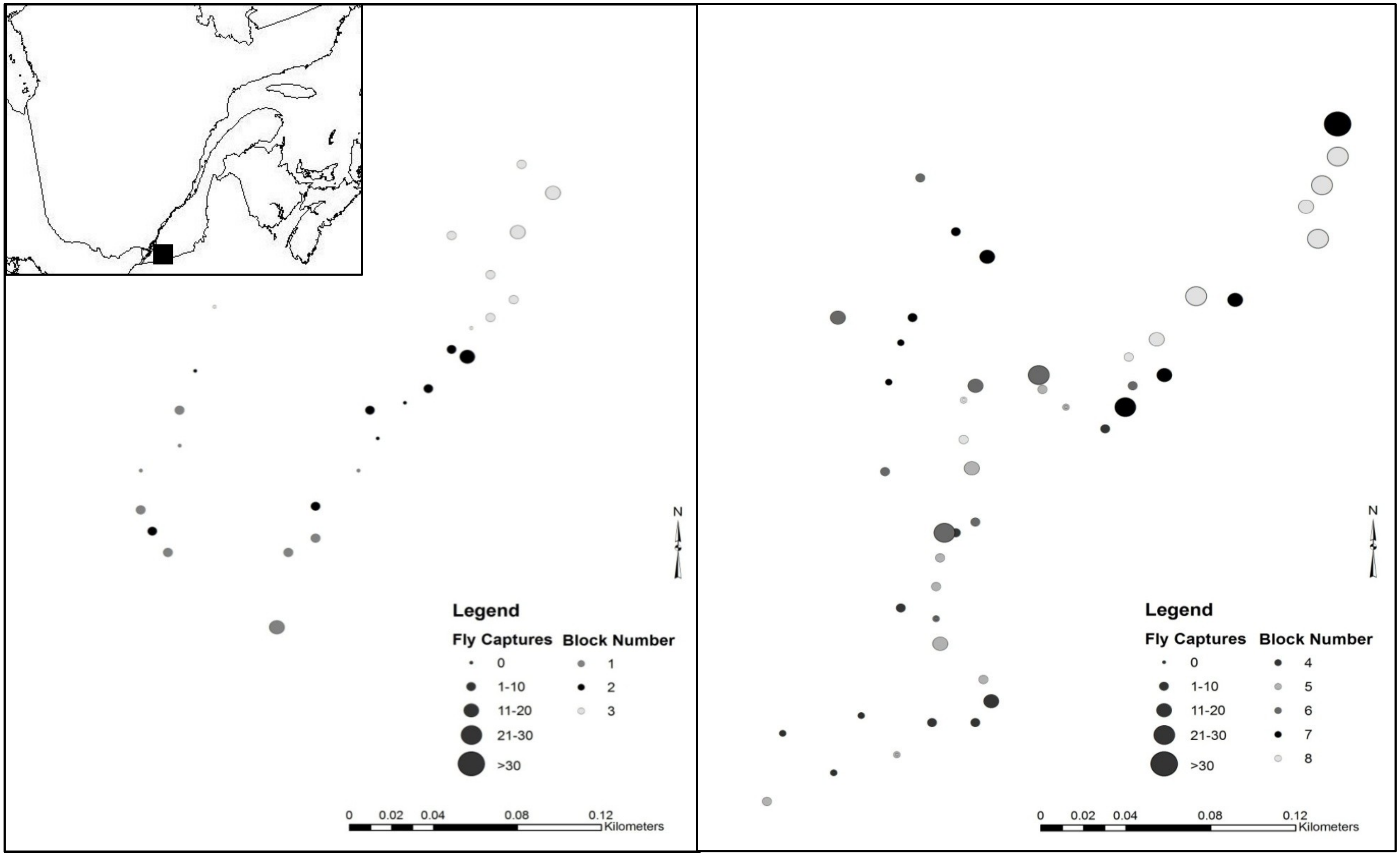

Figure 2.1. Location of green sphere traps in and around the Réserve Écologique du Pin-Rigide, Saint-Chrysostome, Quebec, July-August, 2008. Symbols of different size represent the number of flies captured. Changes in greyscale indicate different blocks. Inset graph shows the field location in the Province of Quebec, Canada.

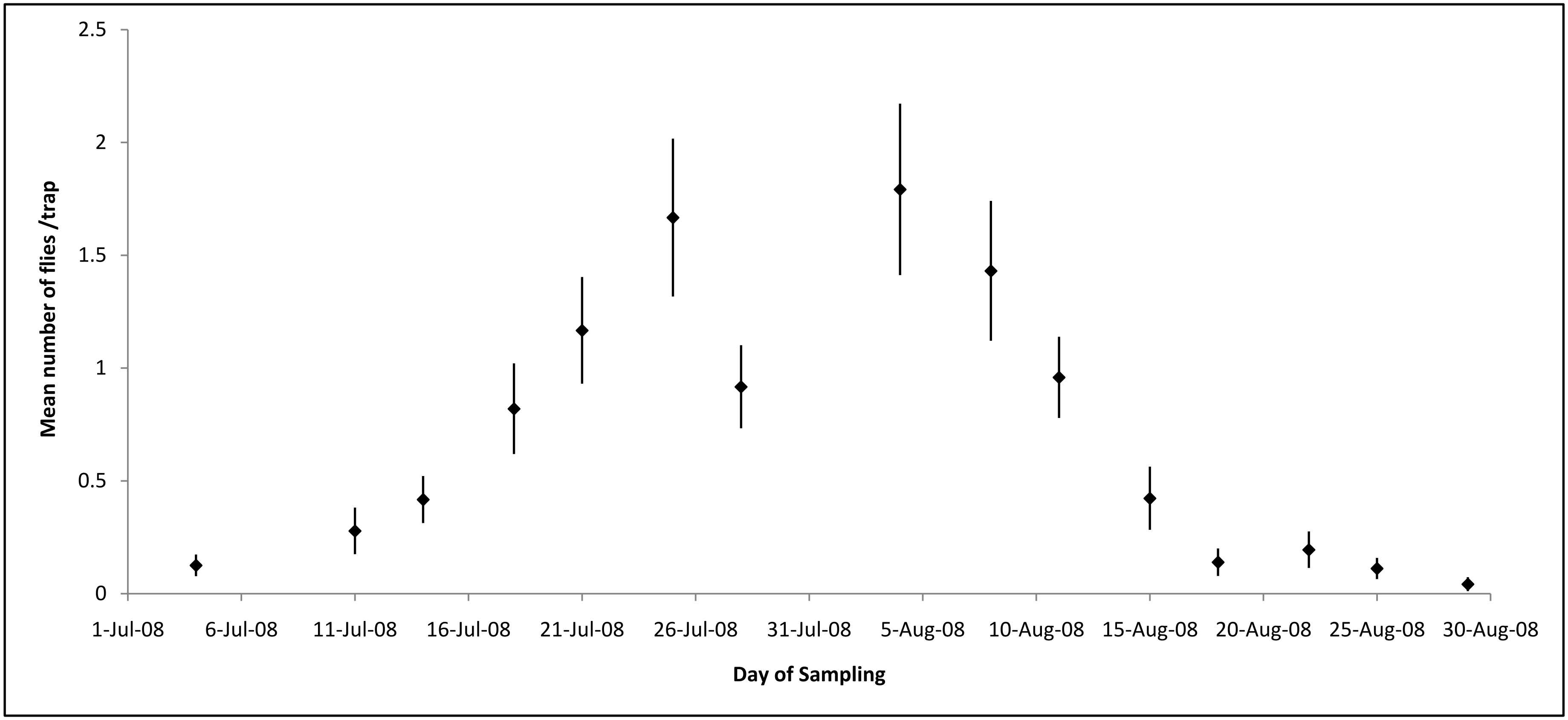

Figure 2.2. Mean (± SE) of fly captures for each day of sampling for all treatments. Collections took place at Reserve Ecologique Pin-Rigide, Saint-Chrysostome, Quebec. A total of 72 traps were checked twice weekly from 4 July 2008 until 29 August 2008.

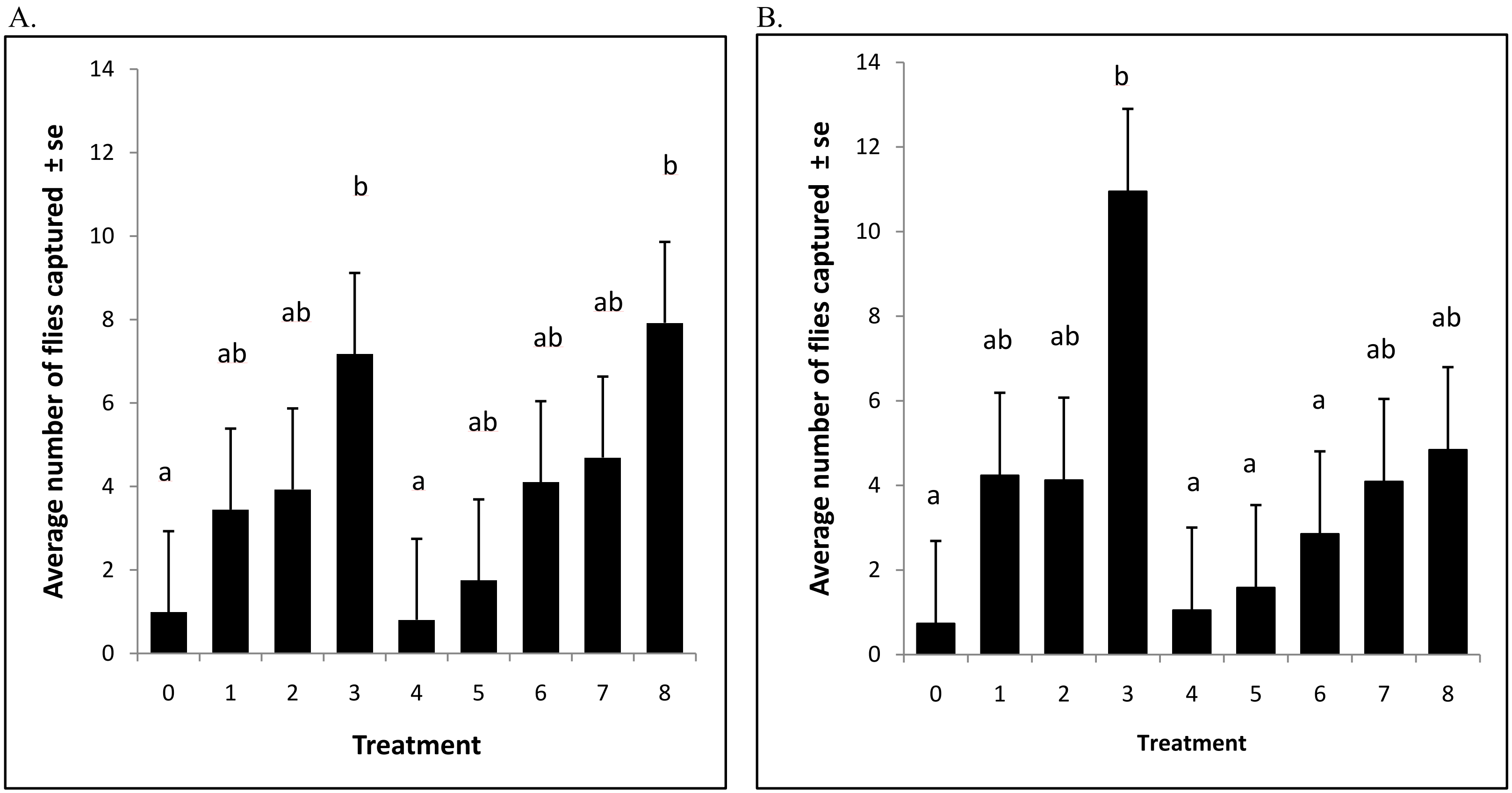

Figure 2.3. Mean (+ SE) of *R. mendax* fly captures during the early[a] (A) and late[b] (B) phases of the season. Different letters indicated a significant difference between treatments. See Table 1 for definition of treatments.
[a] Early season occurs during the month of July
[b] Late season occurs during the month of August.

CONNECTING STATEMENT

Chapter 2 has shown that a blend of terpenes and alcohols can increase the capture rate of *R. mendax* adults on green spheres in the field. Chapter 3 focuses on the olfactory response of blueberry maggot flies towards the same volatile blends in wind tunnel bioassays. It also examines if these responses are influenced by sex, experience and population.

CHAPTER 3: EFFECTS OF HOST FRUIT VOLATILES ON THE OLFACTORY RESPONSES OF *RHAGOLETIS MENDAX* (DIPTERA: TEPHRITIDAE) FLIES

3.1. ABSTRACT

Rhagoletis mendax Curran is a key pest of wild and cultivated blueberries in north-eastern North America. Current monitoring strategies involve the use of ammonium acetate baited Pherocon-AM traps, and need to be refined for more accurate pest detection and monitoring. The objective of this study is to identify a volatile blend, extracted from ripe host fruit that will create the strongest olfactory response in *R. mendax* adults. The effects of long term (three days) experience with ripe blueberries, as well as sex were evaluated with behavioural responses of active time and frequency of flight. A total of eight volatile blends were tested, all with even ratios; seven extracted from host fruit, and one standard being ammonium acetate. The treatment blend of aldehydes and alcohols, as well as ammonium acetate stimulated the highest response in time spent active in the wind tunnel. Males were more prone to flight than females and an interaction between experience and sex showed that naive females spend more time active than experienced flies. Since host volatile treatments did not surpass the standard, results suggest that even ratios of chemical blends do not have a strong enough effect and should be further manipulated or combined with other odour sources to elicit a stronger behavioural response in adult blueberry maggot flies.

3.2. INTRODUCTION

The blueberry maggot fly, *Rhagoletis mendax* Curran, is an important pest of cultivated highbush blueberries, *Vaccinium corymbosum* L., and lowbush blueberries, *V. angustifolium* L. (Barry et al., 2004). The fly also shows some preference for huckleberries, *Gaylussacia baccata* W., occurring in wild berry populations (Smith et al., 2001).

Rhagoletis mendax has been present in the eastern provinces of Nova Scotia, New Brunswick and Prince Edward Island for almost a century (Lathrop, 1932). More recently, its range has expanded into the southern portions of Quebec and Ontario (CFIA, 2009). The larvae hatch from eggs laid by adults in berries and, after feeding on the pulp, drop to the ground where they pupate and overwinter (Prokopy and Papaj, 2001). Blueberries become unmarketable after such infestations, thus, there is zero tolerance for any *R. mendax* larvae found in Canadian commercial berries (CFIA, 2009). Integrated pest management for this pest involve monitoring for fly captures, followed by rigorous insecticidal spray applications if an infestation exists (Peltz et al., 2005).

Standard monitoring techniques rely on Pherocon-AM® traps. These yellow cardboard traps are baited with ammonium acetate, which mimics a food source for *R. mendax* and therefore is attractive (Wood et al., 1983, Neilson et al., 1984). Although relatively effective, these traps could be improved. First, they are attractive mostly during the early stages of the fly's life cycle, with fly captures decreasing drastically in the late season (Liburd, 2004). Second, they are ineffective when monitoring for *R. mendax* at low infestation levels (Neilson et al., 1984). Finally, Pherocon-AM traps attract many beneficial insects, which may act as biological control agents that can regulate the populations of *R. mendax* flies (Neilson et al., 1984).

The life cycle of *R. mendax* is closely synchronized with seasonal maturation of host fruit and adults emerge as berries begin to ripen. The hosts are located through visual and olfactory cues. As observed with *R. pomonella* Walsh (the apple maggot), *R. mendax* adults are using shape and colour intensity contrasts to detect the traps visually (Owens and Prokopy, 1986, Teixeira and Polavarapu, 2001). Olfactory cues come in forms of volatile blends emitted from host plants, and serve a very important role since fruit flies seem to rely heavily on these when visual cues start diminishing (Berlocher and Feder, 2009). In other studies involving *R. pomonella*, a specific blend of volatiles emitted from host fruit was found to be very attractive and can be used for trapping adult flies (Fein, 1982, Zhang et al., 1999, Nojima et al., 2003a,b). This blend consists of butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate and pentyl hexanoate (Zhang et al., 1999). Liburd (2004) has demonstrated that *R. mendax* can be attracted to some synthetic chemicals; butyl butanoate and cis-3-hexen-1-ol, and other recent studies show that *Rhagoletis* flies not only preferentially locate their own host volatile blends in the field (Linn et al., 2003), but they also avoid the blends of emitted by non-host plants (Linn et al., 2005).

Insects of different taxa, such as *Ceratitis capitata* W. (Diptera: Tephritidae) and *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), have demonstrated that experience can play an important role in identifying and accepting host plants and have shown a change in response during upwind flight bioassays (Visser and Thiery, 1986, Papaj et al., 1989). Also, since *R. mendax* flies spend time on the host before mating, further activities such as courtship and ovipositional site location will occur on that host because of previous experience (Prokopy et al., 1989). In theory, learning could occur during habitat location, locating a plant within the habitat, finding a prospective egg laying and/or reproductive site within a plant, examining the prospective site, and ovipositing or attracting a female (Prokopy et al., 1994).

Learning in *Rhagoletis* spp. can be of short or long term duration. As Prokopy et al. (1993) have demonstrated with *R. pomonella* and *R. mendax*, long term prior experience with host fruit of at least three days can have a significant change on the strength of response to different hosts. Short term exposure has also been shown to have an effect on the responses of apple maggot flies (Averill et al., 1996).

Differences in behaviour concerning males and females may also provide further insight into olfactory responses of blueberry maggot flies. For instance, as mating activities move from leaves to fruit, the males tend to appear there first, followed by females (Smith and Prokopy, 1981). Females forage from fruit to fruit in search of ovipositional sites, without much regard for the males, who still attempt forced copulation (Prokopy and Papaj, 2001).

The objectives of this study are to: (1) identify a volatile blend of compounds extracted from ripe host fruit, that will elicit an olfactory response by *R. mendax* adult flies; (2) test if *R. mendax* flies have a stronger response to host fruit volatiles in wind tunnel bioassays after long-term experience with host fruit; (3) determine if the olfactory response differs between male and female adults; (4) evaluate if flies from two geographical populations differ in their responses.

3.3. MATERIALS AND METHODS

Insects. *Rhagoletis mendax* larvae were collected from two different geographical locations and two different hosts in early August 2007. The Nova Scotia population was collected from Parrsboro and Sheffield, and came from a lowbush blueberry (*V. angustifolium*) cultivar. Larvae collected from L'Acadie and Saint-Chrysostome in Quebec came from wild occurring huckleberries (*G. baccata*), as well as some highbush blueberries (*V. corymbosum*).

The berries were placed indoors in boxes and over sand for approximately 30 days at Agriculture and Agri-Food Canada at Saint-Jean-sur-Richelieu (QC, Canada). This ensured that all the larvae dropped into the sand to pupate. The boxes were then placed outdoors until mid-December, which allowed the overwintering pupae to experience a seasonal decrease in temperatures. The sand was subsequently sifted and pupae were removed and placed in a Petri dish with a thin layer of moist sand. These Petri dishes were then placed in a cold chamber at 4°C for at least 6 months. Pupae were removed from storage as needed and placed in an incubator. Flies were reared in cages (36 x 36 x 36 cm) with screen walls at 25 ± 1°C and 70 ± 10% RH, and a photoperiod of 16:8 (L:D) h.

Flies emerged between 30 to 50 days after start of incubation, depending on the population. They were fed *ad lib* on a diet consisting of an amino acid and vitamin mixture (Vitamin diet fortification mixture, from MP Biomedicals Inc., Irvine, CA, USA) as prescribed for the apple maggot (Neilson, 1965). Amino acid mixture was created from a blend of casein hydrolysate acid (MP Biomedicals Inc., 93 g), tryptophan (Aldrich Chemical Company, Milwaukee, WI, USA, 2 g), arginine (MP Biomedicals Inc., 2 g), histidine (MP Biomedicals Inc., 2 g), and cystine (MP Biomedicals Inc., 1 g). This blend was mixed with minerals (salt mixture No. 2 USP (MP Biomedicals Inc.)) in a 4:1 ratio. Sucrose and distilled water were also provided separately in 5 cm Petri dishes.

Flies were randomly selected from an age cohort of 7-14 days, since this ensured that reproductive organs are fully developed and that sexual maturation has been reached (Lemoyne et al., 2007). Both males and females were kept together in rearing cages for the entire duration of the experiments.

Volatile lures. Concentrated volatile solutions were obtained from Agriculture and Agri-Food Canada (Kentville, Nova Scotia). They were extracted from ripe host fruit, ie. *Gaylussacia* spp. and *Vaccinium* spp. The chemicals were grouped into broad classes of terpenes, aldehydes and alcohols, since these compounds are volatile and are associated with head space volatiles of the berries. These were then blended in all possible combinations at even ratios (Table 3.1).

Treatments. Bioassays were conducted in a series of 9 treatments; ie. 7 chemical volatiles blends, ammonium acetate (standard) and a blank (Table 3.1). A drop of the treatment solution (0.5 mL) was dripped onto a strip of filter paper. Ammonium acetate (1.5g), in crystal form, was placed in a 2 cm Petri dish when presented as a lure.

Experience. Due to low emergence and shorter life of laboratory reared flies from the Quebec population, effect of experience with ripe host fruit prior to testing was performed with Nova Scotia flies only. After being tested once as naive, the Nova Scotia flies were placed back in rearing cages and exposed to market-purchased organic blueberries to induce learned behaviour, so that they can acquire experience of preferential host under controlled conditions. The flies were allowed to remain on blueberries for three days prior to testing (Prokopy et al., 1993, Prokopy et al., 1994). Although it has been shown that flies do not need long term exposure to fruit to acquire enough experience (Averill et al., 1996), long duration was used because of the experimental set up, which was limited by day time hours for testing trials and life span of flies.

Wind tunnel. Bioassays were conducted in a Plexiglas wind tunnel (0.3 x 0.3 x 1.0 m). Air was pulled into the tunnel through a charcoal filter, by an exhaust fan connected at the downwind end of the tunnel (Figure 3.1). The charcoal filter was of same dimensions as width and height of the wind tunnel. Air velocity was measured with an anemometer (HH-600 series, Model HHF615M, Omega Engineering Inc., Stamford, CT, USA) where air travelled through the tunnel at 10-15cm/sec, and was vented from the enclosed experimental room by ducts. A lure was placed near the filter in the upwind section. At the bottom of the tunnel black stripes of varying width were drawn perpendicular to the length of the tunnel. This enabled the flies to judge their movement during flight. Plastic plant models were placed inside each of the tunnel sections (upwind-lure, central, downwind-release), analogous to the design by Aluja et al. (1993). The plants were simulated by the use of plastic terrarium vegetation, and selected for a similar leaf shape as that of host plants. The use of artificial vegetation was to ensure that the bioassay tested for the effect of fruit volatiles only, without any potential mixing of volatiles emitted from live vegetation.

Experimental design. All treatments were presented to female and male flies. A total of 16 naive flies (eight females and eight males) were tested for each treatment. In the Nova Scotia population, the flies were retested after having experience of three days with ripe host fruit. The Quebec population of flies was only tested as naive due to a lower sample size of flies available. This generated a sample size of 144 adult *R. mendax* flies for the Quebec population, 288 for Nova Scotia population, and a total sample size of 432.

Prior to the bioassays, flies were placed in the tunnel for 10 minutes to acclimate. Prior to testing a volatile blend two control flies were observed individually. Only one blend was tested per day, as a precaution to avoid the treatments from mixing with each other.

An individual fly was released into the wind-tunnel and allowed 20 minutes to reach the lure and its behaviour was recorded with the Observer 5.0 (Noldus Information Technology, Wageningen, The Netherlands). Behaviour was classified and recorded by the use of two categories; active time and flight frequency, since these indicate the level of stimulation towards volatile treatments. Active time in the tunnel was calculated by subtracting the total time spent in tunnel from inactive moments (Total time – time spent grooming – time spent sitting). Frequency of flight was recorded every time lift off occurred from the surface. Both of these behaviours were also tallied per tunnel section; i.e. downwind, central, and upwind.

Once tested, flies were removed from the tunnel and placed back in a rearing cage, separate from the remaining population. Since all volatile compounds used were water-soluble, the wind tunnel and plant models were thoroughly washed with water after each day of trials, and allowed to air dry overnight.

Data analyses. Response variables used for this analysis were as follows; time spent active in the tunnel and frequency of flight. A three-factor analysis of variance (ANOVA) was performed on data.

Due to the incomplete structure of the data (two levels of experience tested on Nova Scotia flies, one level of experience only for Quebec flies), two different subsets of data were created and each was tested with different models. The first data set consisted of the Nova Scotia fly population only. For these data, the model included the following fixed factors: sex (male and female), experience (naive or experienced) and treatment (terpenes-aldehydes-alcohols, terpenes-aldehydes, terpenes-alcohols, aldehydes-alcohols, terpenes, aldehydes, alcohols, ammonium acetate, blank). The second data set included only naive flies from both populations. The model for these tested the effects of population (Nova Scotia, Quebec), sex (male and female) and treatment (same as above). Here, the population factor was considered random, while the other factors remained fixed.

As none of the data met the assumptions of normality required for parametric statistics, they were transformed as follows: Nova Scotia data for active time were cube root transformed $\left(X' = \sqrt[3]{X}\right)$; data for active time for population difference analysis were log transformed $(X' = \log(X + 2))$; data for Nova Scotia flight frequency were cube root transformed $\left(X' = \sqrt[3]{X}\right)$; and the population difference data for flight frequency were log transformed $(X' = \log(X + 0.5))$.

PROC GLM and PROC MIXED (SAS Version 9.1, SAS Institute, 2001) were used to analyse the Nova Scotia fly data set and naive Quebec and Nova Scotia data set, respectively. An α level of 0.05 was used for all analyses. Multiple treatment comparisons were adjusted with Tukey’s post hoc test.

The second set of analyses addressed the effect of tunnel section (downwind, central, upwind) on the same two response variables (time spent active and frequency of flight). Rather than comparing different tunnel sections, this analysis has been done to estimate the interaction between the tunnel sections and treatments. This interaction measured the association between the treatments and the three tunnel sections. For example, a significant interaction between treatment and tunnel section in the time spent active would indicate that the relative active time spent in each of the three sections would vary significantly between treatments. Because the active time in one section was not independent to the active time spent in another tunnel section, the data were placed in contingency tables and analysed with chi-square tests (JMP Version 6.0, SAS Institute, 2006).

3.4. RESULTS

Rhagoletis mendax flies were most active when presented with ammonium acetate (treatment 8) as a lure in the tunnel. They spent significantly more time moving during this treatment than the control treatment (Table 3.2, Figure 3.2). Flies were significantly more active when presented with a blend of aldehydes and alcohols (treatment 4) than the control treatment. Although these treatments were significantly higher than the blank, they did not differ significantly from each other (Table 3.2, Figure 3.2).

Results of the data set including only naive flies from both Quebec and Nova Scotia had a significant effect of population in both behaviours (Table 3.2). Flies originating from Nova Scotia were significantly more active than Quebec flies. They also had a significantly higher frequency of flight. There were no significant effects of population interacting with other factors (Table 3.2).

Results of the data involving only the Nova Scotia population of *R. mendax* displayed a significant effect of sex in flight frequency, as well as, a significant interaction effect between experience and sex in activity time (Table 3.2). The effect of sex demonstrated that male *R. mendax* flies had a significantly higher frequency of flight than females (Table 3.2, Figure 3.3). Also, the interaction between experience level and sex was significant among the naive and experienced females. Here, naive females spent significantly more time active than experienced females (Table 3.2).

Contingency table analysis demonstrated that, for both data sets and behaviours, the model had an overall significant effect (Table 3.3), indicating that behaviours in each tunnel section varied significantly between treatments. Interpreting contingency tables is somewhat subjective, but results do suggest several trends. The results did not show many differences in the deviations from expected values between the two populations and the tables were similar to each other. A significantly higher deviation from the expected value occurred for the ammonium acetate treatment and the blend of aldehydes and alcohols. The flies spent more time active in the central and upwind tunnel sections when compared to other treatments in those same sections. The same results were present for frequency of flight. The treatment blend of terpenes, aldehydes and alcohols also had a significant association with the upwind section of the wind tunnel (Table 3.3).

3.5. DISCUSSION

Our laboratory results demonstrate that *R. mendax* adult flies were attracted to a blend of aldehydes and alcohols at even ratios (Table 3.2, Figure 3.2). Flies responded to this blend by increased activity inside the wind tunnel. When actively searching for the source emitting volatiles, the length of time spent walking and flying was significantly longer than the control treatment (Figure 3.2). Although this blend was the most attractive from the seven host volatile blends it was not significantly higher than the standard of ammonium acetate, which was also significantly different from the control treatment. Activity time for ammonium acetate was also slightly higher than the time for the aldehydes-alcohols blend. However, ammonium acetate is not efficient enough to decrease infestation levels to a desirable tolerance in the field (Wood et al., 1983, Neilson et al., 1984). Therefore, this attractive blend of aldehydes and alcohols most likely would have a similar effect on the degree of berry infestation in the field and would not be able to decrease larvae to acceptable levels.

This study strengthens the idea that specific mixtures from hosts are attractive to flies (Bruce et al., 2005). When tested separately, the same compounds had a different effect, as seen in the activity times of individual aldehyde and alcohol blends (Figure 3.2). In the wild, flies must constantly discern between different blends of volatiles emitted from all the plants around them. From an ecological perspective, the air surrounding the fly is a mixture of chemicals which come from host plants, as well as non-host plants. In such context, the fly must be able to identify the specific blend of its host. This can be achieved if the blend is comprised of specific chemicals and ratios attractive to the fly (Bruce et al., 2005, Visser, 1986). The attractiveness of specific blends has been shown in many studies, notably with *R. pomonella*. Fein et al. (1982) demonstrated that the apple maggot was attracted to a blend of seven different compounds: hexyl acetate, (E)-2-hexen-1-yl acetate, butyl 2-methylbutanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate and hexyl butanoate at a ratio of 35:2:8:12:5:28:10. Zhang et al. (1999) found that a blend of five compounds proved to be even more attractive to the fly than the blend created by Fein et al. (1982). This blend included butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate and pentyl hexanoate at a ratio of 10:4:37:44:5. These studies also demonstrated that *R. pomonella* is attracted to a blend that consists of uneven ratios of compounds. The next research step for *R. mendax* and host volatiles is, therefore, to determine what ratios the blend of aldehydes and alcohols are emitted from the host fruit.

Population differences were found between Nova Scotia and Quebec flies (Table 3.2). Teixeira and Polavarapu (2003) also demonstrated that genetic population differences exist between *R. mendax* flies inhabiting the same geographical location. Although it has been shown that most *Rhagoletis* species possess the same olfactory receptor neurons (ORNs) in their antennae (Olsson et al., 2006a), behavioural responses towards preferential host volatiles still differ significantly (Linn et al., 2005). Following this discovery, Olsson et al. (2006b) found that *R. pomonella* flies reared from different hosts, exhibit different sensitivities in the same ORNs when exposed to specific host blends. This may suggest why there is a population difference between the two locations of Nova Scotia and Quebec. *R. mendax* flies reared from different hosts may be exhibiting different sensitivities in their ORNs, and therefore a slightly different response to presented volatiles.

The population difference may also indicate a start of a host shift within this species. Such phenomenon has been documented for the *Rhagoletis* species complex. *Rhagoletis pomonella*, a sibling species to *R. mendax*, has demonstrated that it's capable to switch host preferences. In a time span of approximately 150 generations, this species has managed to diverge from using hawthorn (*Crataegus* spp.) as its host to apples (*Malus pumila*) (Olsson et al., 2006a). Blueberry maggot flies may be experiencing a potential host shift between blueberries and huckleberries. This poses a problem when considering management options for this pest, since wild populations of huckleberries occur frequently within Quebec and Ontario forests and may act as a suitable host for further range increases. Huckleberries occur only in wild berry populations, and thus are left unmanaged and not monitored. Therefore, these berries have a potential to act as sources for *R. mendax* fly infestations that later occur in farmers' fields. The population effect also suggests that management of this pest will have to be context-specific, and adjusted to the geographical location, since these results suggest plastic life history characteristics.

It is interesting that there was a significant difference between males and females in the frequency of flight. This variable demonstrated a level of excitement and stimulation experienced by the fly, and males were much more prone to flight than females (Figure 3.3). Also, the effect of sex did not interact with any treatment (Table 3.2). This indicates that the males may be naturally more prone to flight and may be more active than females. Since there was no interaction with treatment, the overall higher flight frequency may have been caused by a stimulation of another type. For example, the presence of sexually mature females in rearing cages prior to testing, may have excited the males causing their flight to be more sporadic. Males may also be biologically more active than females since they need to establish territories and initiate copulation. This variation in responses between sexes suggests that further studies should be performed either males or females to avoid a bias.

The effect of experience was only detected in the interaction with sex. In this case, a significant difference was present for the time they were active. Here, naive females were more active than experienced females. Experienced females may have spent less time active because they were exposed to fruit volatiles earlier, and were able to oviposit for the three days prior to testing. This may have decreased need for location of ovipositional sites. Towards the end of the season, where most flies are fully mature, mated and familiar with ovipositional sites, propensity to visit fruit decreases, but does not change for residency on leaves (Smith and Prokopy, 1981). This result also indicates that experience is important when flies need to discern between two different host species, but it does not influence behaviour when presented with the same host.

There are many research directions to pursue with *R. mendax* and its olfactory responses. Attractive volatiles may be emitted from sources other than host fruit. These may include bacteria living on the surfaces of leaves or fruit, and green leaf volatiles that include some similar chemical groups such as terpenes and alcohols (Hansson et al., 1999). *Rhagoletis mendax* and *R. pomonella* are attracted to the bacteria *Enterobacter (Pantoea) agglomerans* (Lauzon et al., 2000, MacCollom et al., 2009). These bacteria fix nitrogen from the atmosphere (Murphy et al., 1994), and provide it for the flies' diet. MacCollom et al. (2009) also demonstrated that adding *P. agglomerans* to Pherocon-AM traps had a significant increase in the number of fly captures. This suggests that combining different lures, such as host fruit volatiles with attractive bacterial cultures or ammonium acetate, may increase fly capture rate. Bacterial odours may act as olfactory cues (MacCollom et al., 2009), and once the fly approaches the trap, host plant volatiles could further attract it. Green leaf volatiles can also work in a similar manner. Their volatile composition usually includes aldehydes, alcohols and acetates (Natale et al., 2003) and may have an impact on the way *R. mendax* flies perceive their host.

This study demonstrated that specific blends of host fruit volatiles, especially those of aldehydes and alcohols, can induce a change in behaviours of *R. mendax* flies, indicating stimulation and attraction towards the odour. The results also indicate that behavioural responses towards different volatile treatments are not influenced by sex. Behaviour still may be influenced by the experience of the fly, decreasing the attraction, therefore bioassays may benefit from tests with naive insects only when tested with the same host. The treatment results of this study should be tested further in the field, manipulating proportions of chemicals in this blend may create a stronger effect against ammonium acetate. Blended aldehydes and alcohols are not powerful enough to be used in traps for infestation level control. However, its chemical composition may be the key to creating a more attractive mixture.

3.6. LITERATURE CITED

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Table 3.1. Mixtures of different classes of volatile chemicals that were extracted from the headspace of ripe huckleberries (*Gaylussacia baccata*) and lowbush blueberries (*Vaccinium angustifolium*). Courtesy of S. Gaul (AAFC/Kentville, NS).

Treatment	Compound groups used
1	Terpenes + aldehydes + alcohols
2	Terpenes + aldehydes
3	Terpenes + alcohols
4	Aldehydes + alcohols
5	Terpenes
6	Aldehydes
7	Alcohols
8	Ammonium Acetate (standard)
0	Blank (control)

Table 3.2. ANOVA results (F and p-values) in wind tunnel bioassays for *Rhagoletis mendax*. Behaviours were recorded for 20 minutes, for 9 different treatment blends of volatiles extracted from ripe host fruit. Active time in the tunnel was calculated by subtracting the total time spent in tunnel from inactive moments (Relative time = total time – time spent grooming – time spent sitting). Frequency of flight was recorded every time lift off from the surface occurred. Nova Scotia and Quebec population results included data for naive flies only (A). Nova Scotia population data includes previous experience with host fruit (B).

	Effect	df	Active Time		Flight Frequency	
			F	p	F	p
A.	*Nova Scotia and Quebec population*					
	Treatment	8, 27	4.06	0.0320[a]	2.47	0.0802
	Population	1, 27	9.18	0.0027[a]	5.94	0.0155[a]
	Sex	1, 27	2.96	0.0866	1.55	0.2139
	Population*Sex	1, 27	0.41	0.5229	0.02	0.8824
	Population*Treatment	8, 27	0.98	0.4489	0.87	0.5424
	Sex*Treatment	8, 27	1.78	0.0810	1.85	0.0691
B.	*Nova Scotia population*					
	Treatment	8, 35	0.98	0.4559	1.94	0.0548
	Experience	1, 35	4.62	0.0326	3.60	0.0590
	Sex	1, 35	0.19	0.6634	11.32	0.0009[a]
	Experience*Sex	1, 35	7.85	0.0055[a]	3.50	0.0626
	Experience*Treatment	8, 35	1.85	0.0690	1.01	0.4286
	Sex*Treatment	8, 35	1.38	0.2072	0.83	0.5789
	Experience*Sex*Treatment	8, 35	1.39	0.2023	0.84	0.5676

[a] indicates a significant effect

Table 3.3. Results for contingency table analysis between the three sections of the wind tunnel and each of the nine treatment blends of volatiles extracted from ripe host fruit. + indicate that the behaviour has a significantly higher deviation from the expected value occurring in the tunnel section for that treatment (Chi squared value > 3.84). - indicate that the behaviour has a significantly lower deviation from the expected value occurring in the tunnel section for that treatment (Chi squared value > 3.84). Blank cells indicate no significant effect was present.

	Time Spent Moving			Frequency of Landing		
Treatment	Downwind	Central	Upwind	Downwind	Central	Upwind
A. *Nova Scotia and Quebec population*						
Blank	+	-	-	+	-	-
Terpene, aldehyde, alcohol		-	+			+
Terpene, alcohol	+	-	-	+	-	-
Terpene, aldehyde		+	-		+	-
Aldehyde, alcohol	-	+	+	-	+	+
Terpene	+	-	-	+	-	-
Aldehyde	+	-			-	+
Alcohol	-	+	-		+	-
Ammonium acetate (standard)	-	+	+	-	+	+
B. *Nova Scotia population*						
Blank	+	-	-	+		-
Terpene, aldehyde, alcohol		-	+		-	+
Terpene, alcohol	+	-	-	+	-	-
Terpene, aldehyde		+	-		+	-
Aldehyde, alcohol	-	+	+	-	+	+
Terpene	+	-	-	+	-	-
Aldehyde	+	-			-	
Alcohol		+	-		+	-
Ammonium acetate (standard)	-	+	+	-	+	+

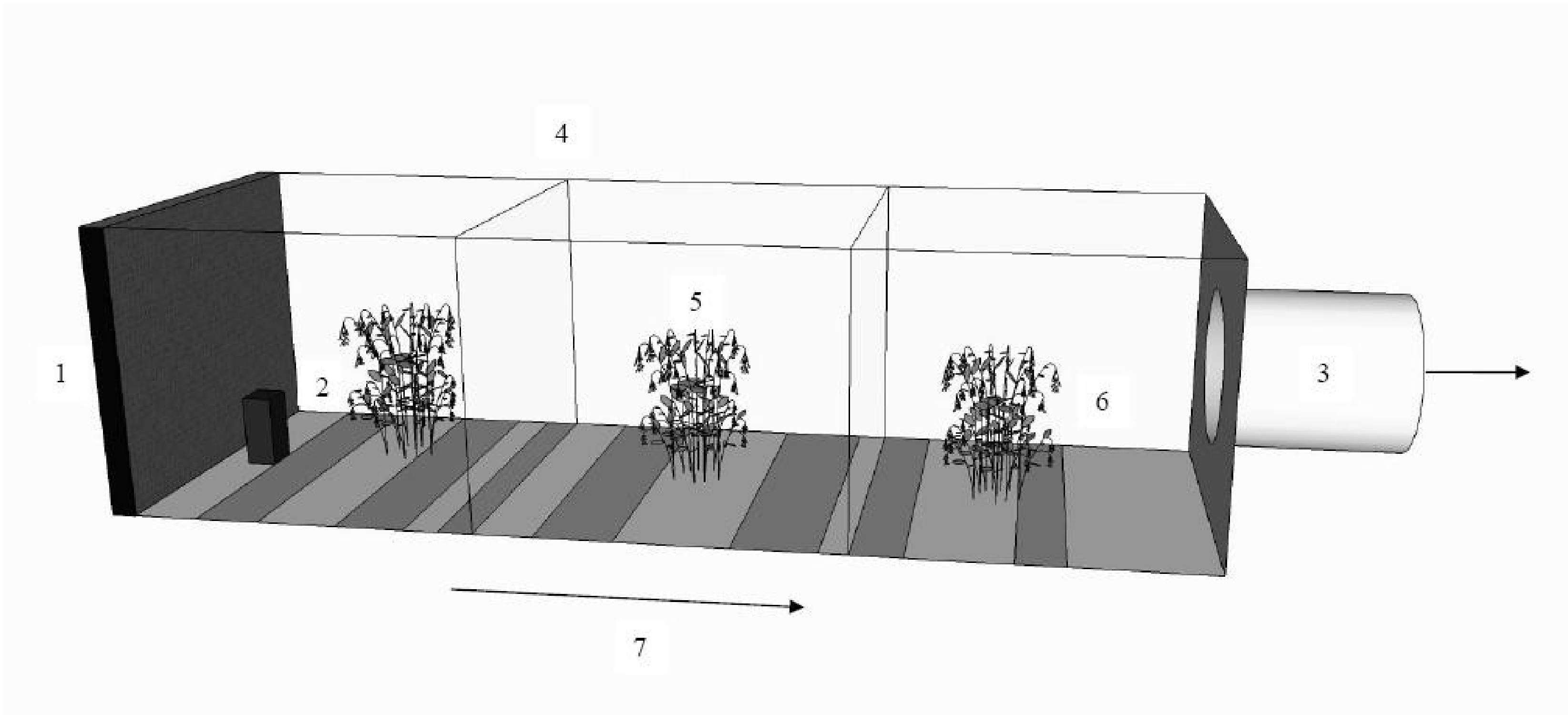

Figure 3.1. Wind tunnel (100 x 30 x 30 cm: L,W, H). 1) charcoal filter; 2) location of lure; 3) location of fan generating air current, as well as exhaust tube; 4) sub-division between tunnel sections; 5) artificial vegetation; 6) release area; 7) direction of air flow.

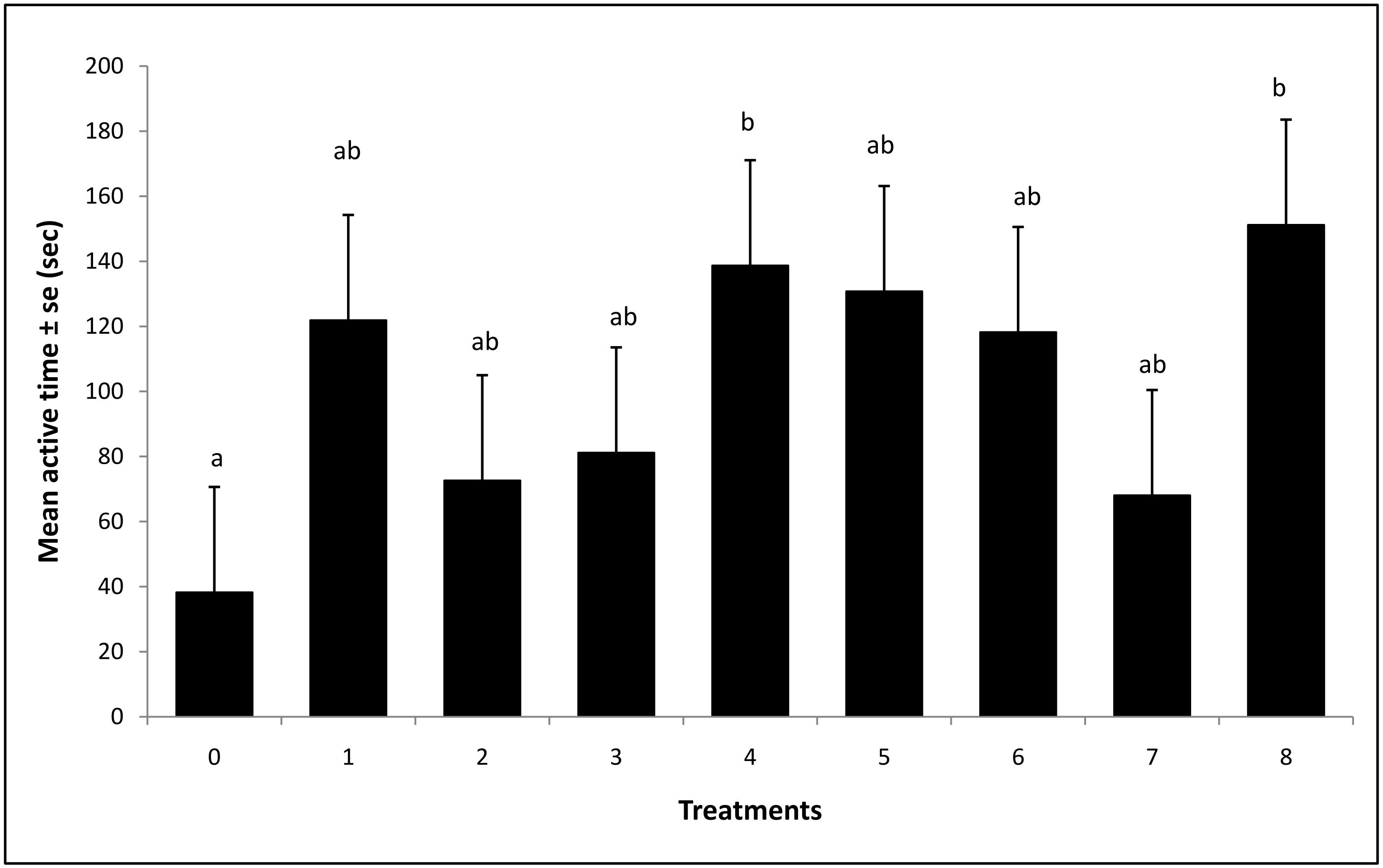

Figure 3.2. Mean (+ SE) for active time for *R. mendax* adults in response to different treatments within the wind tunnel. See Table 3.1 for definition of treatments. Different letters indicated a significant difference between treatment (Tukey's post hoc test).

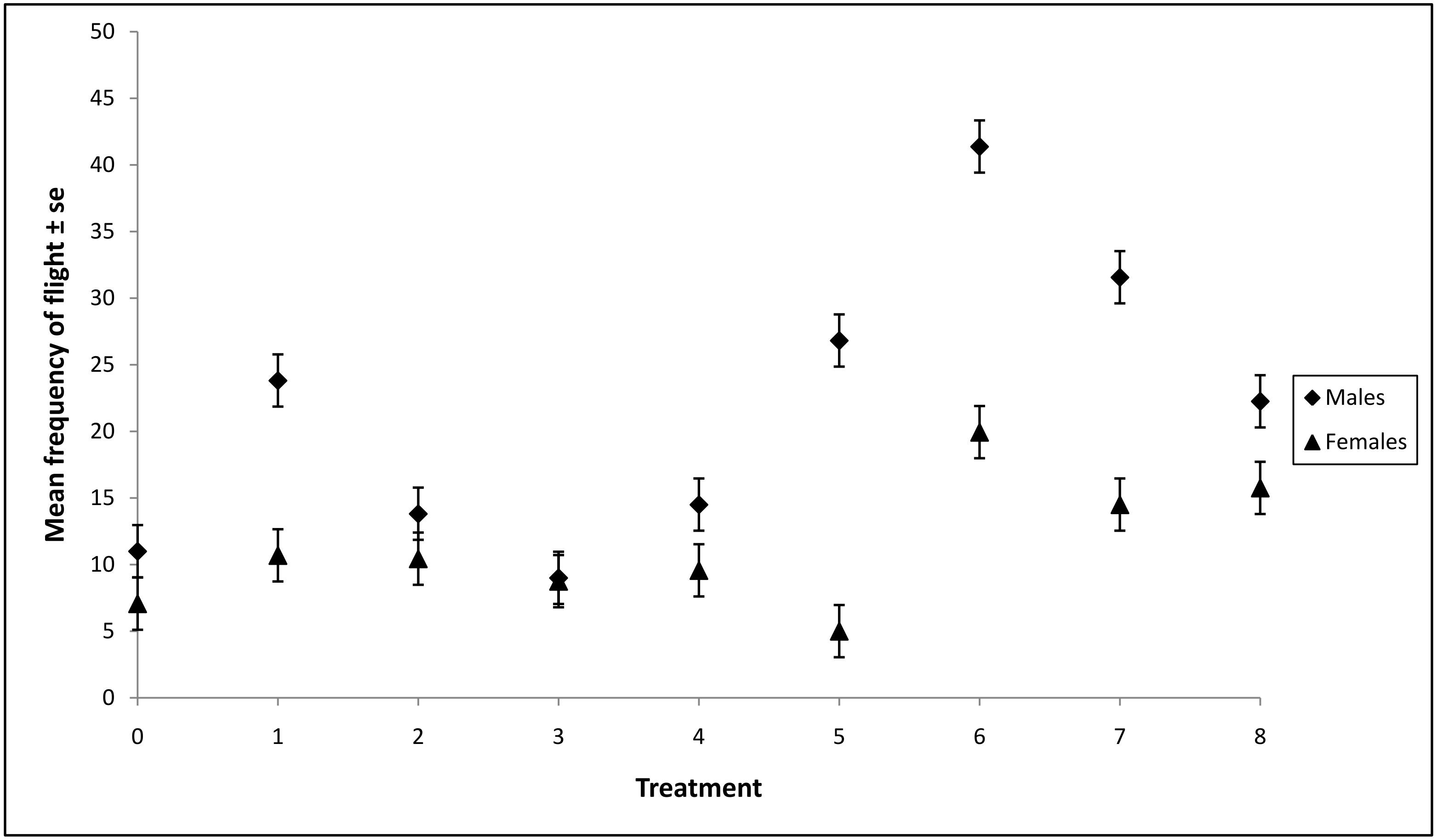

Figure 3.3. Mean (± SE) for frequency of flight for *R. mendax* male and female adults in the wind tunnel for each of the 9 treatments.

CHAPTER 4: GENERAL CONCLUSIONS

Through my research investigating the effects of host volatiles on blueberry maggot fly (*Rhagoletis mendax* Curran) capture rates and olfactory behaviour, new information has been obtained regarding the specific composition of the volatile blend attractive to this species. I examined seven possible blends at even ratios, as well as the standard of ammonium acetate that may help in the identification of a more specific chemical mixture of compounds emitted from host fruit. My research contributes to the efforts of developing efficient and reliable monitoring and management tools of blueberry maggot flies in culvitated blueberry, by identifying a more attractive lure to use as trap bait. This work also has potential to improve integrated pest management programs, and decrease the use of insecticidal applications.

Chapter 2 evaluated the effectiveness of host fruit volatile blends, consisting of different combinations of terpenes, aldehydes and alcohols, as well as ammonium acetate, on the capture rate of *R. mendax* adults on green sphere traps in the field. Increased capture rates have been documented when trapping *R. mendax* adults with green spheres instead of Pherocon-AM traps with same lures (Liburd et al., 1998, Teixeira and Polavarapu, 2001). Currently, ammonium acetate is the monitoring standard, since it is relatively attractive but only early in the season (Liburd, 2004). My work confirms this since ammonium acetate had a significantly higher capture rate only in July. The host fruit volatile blend composed of terpenes and alcohols had the highest capture rate throughout the entire berry season, however, it did not differ from ammonium acetate. This finding suggests that this blend is an improvement over the standard when monitoring *R. mendax* populations, but may not be sufficient to control maggot infestations to a desired level.

The experiment in Chapter 3 was designed to evaluate the attractive behavioural response of blueberry maggot flies towards the same treatment blends of host fruit volatiles and ammonium acetate. The response differed between sexes, populations and experience and flies demonstrated the highest attractive response towards ammonium acetate and a blend of aldehydes and alcohols. This result strengthens the idea that only specific blends at preferred ratios are attractive to flies (Bruce et al., 2005, Visser, 1986). The factors of sex, population and experience did not significantly interact with treatments, but had a significant effect when compared individually. These results suggest that volatile identification studies should be performed with either male or female flies from same geographical origins to avoid biases. Differences in responses to volatiles can exist for same fly species when reared from different hosts (Olsson et al., 2006a,b). My results show that previous host experience does not elicit a stronger response in bioassay treatments involving the same host, but Averill et al. (1996) demonstrated that it can when testing attractiveness to other potential hosts.

The findings of my experiments identified two different attractive volatile blends under different conditions. This further suggests that environmental settings may influence the perception of volatile blends in flies, and controlled laboratory tests may not elicit the same response as field test. In nature, trap baits are in competition with natural host volatiles, thus flies may react differently to lures when mixed with natural host odours. Due to this variation, it is always beneficial for laboratory bioassays to be accompanied by field work.

4.1. LITERATURE CITED

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