Phasgonophora sulcata Westwood (Hymenoptera: Chalcididae): A Potential Augmentative Biological Control Agent For The Invasive Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) In Canada

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Abstract

The emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), is an invasive pest of ash (Fraxinus spp.) in North America. Since its accidental introduction in the early 1990’s, A. planipennis has killed millions of ash trees in 22 states and 2 provinces. The widespread establishment of this pest has made eradication impossible. Therefore, long-term management protocols that minimize future populations to non-damaging levels are required. One possible management strategy is biological control using parasitic wasps. In Canada, an endoparasitic chalcid wasp, Phasgonophora sulcata Westwood, has demonstrated potential as possible augmentative biological control agent of A. planipennis. Unfortunately, very little information exists concerning the interactions of this wasp and its hosts. The goal of my work was to determine the information necessary for both evaluating its effectiveness as an
augmentative biological control agent and to provide information that would be useful for parasitoid production and release. I conducted four studies that addressed this problem, specifically (1) determination of the species’ basic life history characteristics; (2) analysis of the wasp’s courtship behaviours and elucidation of adult-produced pheromones; (3) identification of semiochemicals used in host searching; and (4) observations on the within-tree and temporal distributions of *P. sulcata* and other parasitoids in *A. planipennis*-infested sites in Southwestern Ontario. My results indicate that *P. sulcata* possesses several characteristics associated with effective biological control agents, including synchronization with the preferred host stage, low super-/multiparasitism, and high egg loads at emergence. However, its solitary and delayed development reduces the feasibility of mass rearing. Natural populations may however be effective in areas of high host densities or where augmentation is possible.
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Chapter 1: Literature review

1.1 *Agrilus planipennis* in North America

*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), emerald ash borer, is an invasive wood-boring pest of ash (*Fraxinus* spp.) in North America. It was first discovered in the Detroit/Windsor region during the summer of 2002 (Haack et al. 2002), however dendrochronological evidence indicates that it was in that area for at least 10 years prior its discovery (Poland and McCullough 2006). The native range of *A. planipennis* includes northeastern China (Chinese Academy of Science 1986), Korea (Ko 1969), Japan (Wei et al. 2004) and the Russian Far East (Haack et al. 2002). Females lay their eggs in bark crevices and under bark scales on trees. Larvae emerge through the ventral surface of the eggs, whereupon they burrow through the bark down to the cambial layer. Larvae feed in the cambium between the bark and wood until 4\(^{th}\) instar (Haack et al. 2002). In the early to late fall, 4\(^{th}\) instar larvae excavate pupal chambers, either in the bark or about 1.5 cm into the sapwood (Haack et al. 2002), and overwinter as prepupae (Poland and McCullough 2006). These prepupae begin pupation the following spring and emerge as adults usually in late spring; however, adult emergence can vary with latitude. A 1-year life cycle is most common, but a 2-year life cycle may occur in more northerly latitudes and in new infestations (Poland and McCullough 2006).

*Agrilus planipennis* is a significant alien invasive species because of its ability to attack a wide range of ash trees. *Agrilus planipennis* has been recorded from five eastern North American *Fraxinus* spp., including green ash (*F. pennsylvanica* Marsh.), white ash (*F. americana* L.), black ash (*F. nigra* Marsh.), blue ash (*F. quadrangulata* Michx.) (Poland and McCullough
also feeds on non-native species such as *F. chinensis* subsp. *chinensis* Roxb., *F. chinensis* subsp. *rhynchophylla* (Hance) E. Murray, *F. mandshurica* Rupr. (Yu 1992), *F. rekytina* Roxb. (Wei et al. 2004) and *F. excelsior* L. (Pureswaran and Poland 2009). Its wide host range, coupled with the widespread planting of *F. pennsylvanica* and *F. americana* in urban settings, will allow it to spread throughout the natural and planted ranges of ash in Canada and the United States (MacFarlane and Meyer 2005). Such spread will be economically devastating as the compensatory value of ash timber in the United States is about $282 billion dollars (Poland and McCullough 2006). Ash is used to make a variety of goods, including baseball bats, furniture, paper products, and crating (Poland and McCullough 2006), and subsequent job losses associated with reductions in ash-dependent industries could have important economic impacts in both Canada and the United States. In Canada, costs associated with removal, treatment, and replacement of ash trees has been is likely to cost up to $1.5 billion (McKenney et al. 2012). Economic effects caused reduced property and aesthetic values, increased storm water runoff, variation in temperature regulation, and reductions in associated wildlife will also be magnified in urban areas where *A. planipennis* is present (Sydnor et al. 2007).

Apart from the aforementioned economic consequences, ecological impacts will be also occur. Several ash species are important constituents of North American hardwood forests. For example, green ash is a significant constituent of the overstory in forests with moist soils (Poland and McCullough 2006), while black ash is common in riparian regions. In Central Ontario, low-lying swampy areas or “swales” are often occupied by only pure stands of black ash (Strickland et al. 1987). These trees provide habitat for insects, birds, and mammals that may not occur in other environments. The elimination of ash from these such habitats could lead to the extinction
of these rare organisms found only in these habitats. Many species, including birds, insects, and mammals rely upon ash as an important food source (Heyd 2005), consuming ash seeds (Poland and McCullough 2006) and bark of young trees. A reduction in the primary food for these species would have many direct and indirect negative effects on species associated with these habitats. The loss of a dominant species such as ash will have important effects on many ecological processes, such as altering the amounts of course woody debris available, diversity of canopy and floral fauna, and nutrient cycling (Gandhi and Herms 2010). An analogous situation was observed following the arrival of zebra mussels in Ontario lakes, which resulted in unhindered consumption of zooplankton within affected water bodies. Plankton is the base of food webs in freshwater ecosystems and its loss has led to major negative effects on primary, secondary, and tertiary consumers. Because ash is a numerically important primary resource in both common and specialized forest habitats, its elimination from Ontario’s ecosystems would be similarly devastating. It is thus clear that the management and containment of *A. planipennis* must be achieved to prevent these potentially irreversible ecological and economical impacts.

Biological control using insect parasitoids was identified as a potential long-term management objective during the early planning for research on North American *A. planipennis* populations (Liu et al. 2003). The search for classical biological control agents of *A. planipennis* in China began soon after its discovery in North America (Haack et al. 2002). In 2003, surveys of natural enemies were conducted across the native range of *A. planipennis* in China (Liu et al. 2003). These surveys identified three hymenopteran parasitoids that showed potential for use against *A. planipennis* in North America. These included two larval parasitoids; *Tetrastichus planipennisi* Yang (Eulophidae), and *Spathius agrili* Yang (Braconidae); and an egg parasitoid *Oobius agrili* Zhang and Huang (Encyrtidae). Following host range testing, which indicated that
non-target effects would be minimal, small field releases of all three of these parasitoids were undertaken in Michigan in 2007 (Bauer et al. 2010). These were followed by subsequent releases in Ohio and Indiana, as well as again in Michigan in the next year. In 2008, *S. agrilus* and *O. agrili* were recovered from dissected ash trees near the 2007 release site, indicating that both of these species were able to successfully attack *A. planipennis* and overwinter (Bauer et al. 2010). Subsequent samplings of trees at regular distances from the original release point of *S. agrilus* in September 2008 yielded no additional parasitoids. In sites in Michigan where *T. planipennisi* has been released however, parasitism rates in both release and control sites were shown to increase in consecutive years indicating likely establishment (Duan et al. 2013a). At present, all three parasitoids are being reared in the United States, and have been released at sites in Illinois, Indiana, Kentucky, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, and Wisconsin (USDA-APHIS/ARS/FS 2012). Releases of *T. planipennisi* were undertaken in southern Ontario by the Canadian Forest Service in 2013; however, evidence of establishment will likely not be known until 2014 (NRCAN 2013).

Augmentative biological control of *A. planipennis* using North American parasitoids has also been proposed for long-term *A. planipennis* management (Lyons 2010). Surveys conducted in infested sites across Canada and the United States have identified several native parasitoid species that attack *A. planipennis*. These include several braconid wasps in the genus *Atanycolus* (Hymenoptera: Braconidae), such as *A. cappaerti* (Marsh and Strazanac) and *A. hicoriae* Shenefelt (Cappaert and McCullough 2009), *Balcha indica* (Many & Kaul) (Hymenoptera: Eulophidae) (Duan et al. 2009), and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) (Lyons 2010). Although early surveys indicated that parasitism rates of *A. planipennis* by North American parasitoids was low (Liu et al. 2003), more recent surveys in
southwestern Ontario suggested that *P. sulcata* may parasitize up to 40% of *A. planipennis* larvae at some sites (Lyons 2010).

The report of such high levels of parasitization warranted continued research into the potential use of *P. sulcata* as an augmentative biological control agent for *A. planipennis*. Information concerning its interactions with its new host however is sorely lacking. Some knowledge concerning the relationship between *P. sulcata* and some North American *Agrilus* spp. exists (Haack et al. 1981; Haack and Benjamin 1982); however, it cannot be applied in the evaluation of this parasitoid with its novel host without further study. In addition, information relating to host location abilities and potential rearing protocols is also lacking. In order for a potentially effective augmentative biological control program to be designed, a complete and comprehensive basis of knowledge of the host-parasitoid system must be developed. This body of knowledge will be important not only to the evaluation of this parasitoid’s potential effectiveness, but will provide scientists with the information required to develop a release protocol that will have the greatest chance for success. The goal of my thesis is to provide much needed information that will satisfy both of these requirements.

This introductory chapter contains five sections. In section 1.2, I provide a brief overview of biological control using wasp parasitoids, including its definition, and its three most common types. I also outline several important aspects of wasp parasitoid biology that are associated with the effectiveness of a biological control agent, and discuss how environmental- and host-related factors can influence these life history traits. In section 1.3, I outline the typical courtship and mating sequences in parasitic Hymenoptera, and their importance in a successful biological control program. In section 1.4, I introduce the importance of chemical ecology in host finding in
parasitic Hymenoptera. I also identify several semiochemicals that may be used by parasitoids, and how they may be utilized in a biological control program. In section 1.5, I provide background to the absolute importance of spatial and temporal synchronization between the target host and the enemy for maximizing both host mortality and management. I conclude with a brief overview of each thesis chapter, and how each relates to my overall research goal.

1.2 Biological control using parasitic wasps

1.2.1 Biological control: Definition and types

Biological control is defined as the actions of a predator, parasitoid, or pathogen that maintains another pest organism’s population to a level lower than its economic threshold (DeBach and Rosen 1991). Both the target organisms and biological control agents can include insects and other invertebrates, as well as pathogens, and vertebrates (Van Driesche and Bellows 1996). Based on the number of biological programs enacted against them, insect pests are the most common group targeted in biological control programs (Laing and Hamai 1976). The organisms most often used as biological control agents are insect parasitoids (Greathead 1986). Insect biological control is most often used to suppress forest or agricultural pests, or to restore areas that have been affected by a non-native pest. During the latter half of the 20th century, these problems were usually addressed through the application of broad-spectrum pesticides. Although highly effective during their initial use, constant application can lead to several important drawbacks, including contamination of the environment, damage to human health, the extirpation of natural enemies and other non-target species, and the evolution of pest resistance (Van Driesche and Bellows 1996). Additionally, the stigma of pesticide applications as not a ‘green’ method of pest control has also affected the willingness of some people and
organizations to use them (Zadoks and Waibel 2000). Consequently, there is a strong demand for other pest management strategies like biological control to replace pesticide applications as the primary method for managing pest populations.

Biological control encompasses three specific types: classical, conservation, and augmentation. Classical biological control is the purposeful introduction of exotic enemies into a new region to manage a pest (Van Driesche and Bellows 1996). Usually the target pest is non-native, and the enemies are from the original range of the target pest, however exotic enemies may occasionally be introduced into a new area as a means of controlling an indigenous pest (Van Driesche and Bellows 1996). The process of determining which species to import begins with observations of the natural enemies of the target pest in its natural range. Candidate species are first reared from the pest and identified. They are then evaluated in relation to the potential host and non-target species, reared, and then released into their new range. The first recorded instance of successful classical biological control of an insect pest occurred in California in 1887 where a parasitic fly Cryptochetum iceryae Williston (Diptera: Cryptochetidae) and the predacious beetle Rodolia iceryae Jensen (Coleoptera: Coccinellidae) from Australia were released as part of a management program for the cottony cushion scale (Icerya purchasi Maskell, Hemiptera: Monophlebidae) (Caltagirone and Doutt 1989; Van Driesche and Bellows 1996). Within two years, the imported enemies had successfully controlled the pest throughout the affected region. Since this first instance, the importation of classical biological control agents has become an increasingly popular method of control for hundreds of different pest species in dozens of countries (DeBach and Rosen 1991).
Conservation biological control is the manipulation of a site’s physical characteristics in ways that benefit the locally present natural enemy populations (DeBach and Rosen 1991). In many situations, such as if the natural enemy is effective in some regions, but not others, conservation practices can be used to enhance its effectiveness (DeBach and Rosen 1991; Van Driesche and Bellows 1996). Common practices for insect enemies include the planting of attractive flowers, enhancement of semiochemicals that are used by parasitoids in host location, and the provision of specific foods that can positively influence traits related to efficacy such as fecundity, longevity (Van Driesche and Bellows 1996; Dahlsten and Mills 1999). Although conservation biological control can be carried out in a wide variety of settings, physical manipulations of the environment may be best suited to forest environments. These areas in particular often possess the large size, lack of perturbation, and complexity that can allow for the successful conduction of this strategy (Dahlsten and Mills 1999). This is not to say that other settings, such as agricultural or urban should not be subjected to conservation biological control, but rather the positive effects may be more pronounced in forests.

Augmentation biological control is the increase of natural enemy populations through releases. Augmentation may be required to counteract impediments that are preventing effective management. These may include asynchrony between the adult parasitoid and the preferred host stage, low parasitoid fecundity, or adverse abiotic and biotic factors (Van Driesche and Bellows 1996). Depending on the physical attributes of the enemy, augmentation can be achieved through either inundative or inoculative releases. Inundation is the mass release of natural enemies that are expected to provide immediate control of the pest. These parasitoids or predators may or may not possess the ability to establish themselves in the environment, but, regardless, would possess the necessary host searching and attack abilities to exert immediate control of the pest (Van
Driesche and Bellows 1996). Conversely, inoculation consists of the release of small numbers of reproductively competent parasitoids at various times in the season with the goal of establishing an enemy population in the site. With inoculation, the burden of managing the pest population lies on future generations of natural enemies rather than that of the released individuals (Van Driesche and Bellows 1996). The progeny of the released enemies may provide pest control at the site for years to come. Often the augmentation programs are coupled with conservation biological control to ensure the establishment of the natural enemy and management of the pest.

Selection of a suitable natural enemy is the most important aspect of implementing a biological control program. Several authors have listed characteristics that are consistently present in successful biological control agents (Huffaker and Messenger 1976; Coppel and Mertins 1977; van Lenteren and Woets 1988; DeBach and Rosen 1991; Smith 1996). Important attributes include female-biased sex ratios, high fecundity and longevity. One of the most important attributes of a successful parasitoid, the dominant class of natural enemies used in biological control of insects, however, is high host specificity (Huffaker and Messenger 1976; Coppel and Mertins 1977; DeBach and Rosen 1991). Specialists are more effective than generalists because they do not waste valuable resources such as eggs and searching time attacking non-target hosts. A high degree of host specificity also minimizes the risk of exotic parasitoids attacking non-target hosts after release (Wilson and Huffaker 1976; Coppel and Mertins 1977). High host specificity is usually a common trait of classical rather than augmentative biological control agents due to the long evolutionary relationship between the host and the enemy. Augmentative biological control agents of non-native pests most often do not have such an evolutionary relationship with the pest, and consequently augmentative specialists are therefore less frequent. However, an important drawback of a classical biological control
agent is its occasional inability to replicate its effectiveness in the new range due to adverse environmental or biotic factors. When choosing a parasitoid for use in a biological control program, it is therefore necessary to conduct a variety of studies on the effects of both the host and the environmental conditions on natural enemy efficacy.

1.2.2 Evaluating efficacy: Life history traits of the agent

In order for an enemy to be used as part of a biological control program, a comprehensive understanding of its life history parameters as they relate to both the host and the release environment must be established (DeBach and Rosen 1991). Specifically, traits such as parasitoid longevity, fecundity, emergence times, and sex ratios, which may influence parasitoid effectiveness, must be evaluated as they relate to the specific environment and host with which the parasitoid will be associated (Coppel and Mertins 1977). These traits must also be known for the development of a rearing protocol for insect production. Traits such as these, however, do exhibit plasticity and may vary depending on several factors, including the size of the host in which the parasitoid is reared, environmental conditions of the release site, and the conditions present at parasitoid emergence. An inability to evaluate these traits as they relate to the specific conditions of the control program may result in the production of insects that are ineffective due to environmental conditions at the release site, or in an inability to estimate parasitoid effectiveness due to differing reproductive rates between laboratory and field populations. Such investigations are therefore critical in formulation of a biological control program.

1.2.2.1 Influence of host size on parasitoid effectiveness

It is well established that the host in which the parasitoid developed can have an effect on several traits expressed in the adult (Salt 1940). Fecundity, longevity, and progeny sex ratios for
example may be affected by the size of the host in which the parasitoid develops. In some cases, significant differences between parameters within the same species can occur. For example, Arakawa et al. (2004) reared *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) from three different pentatomid host species of different sizes and observed that longevity and fecundity of adult parasitoids were influenced heavily by the host from which they were reared. At 7 days old, the fecundity of adult female parasitoids reared from *Nezara viridula* (L.), *Plautia cossota stali* Scott, and *Halyomorpha halys* Uhler were 70.1 ± 1.0, 75.8 ± 1.7, and 90.7 ± 1.2 eggs per female respectively, while female longevity was 11.0 ± 0.5, 25.1 ± 1.3, and 28.5 ± 1.6 days respectively. In both experiments, the authors observed positive correlations between host size and both fecundity and longevity. The authors concluded that increased nutrient availability in larger hosts allowed developing parasitoids to sequester more resources that would allow for increased adult longevity and fecundity.

Progeny sex ratios can also be influenced by host size. As females are required to parasitize hosts, the possession of a female-biased sex ratio is critical to population establishment and pest management. As immature females require more nutrients than males in order to produce the reproductive system and its eggs, adult females will often place female eggs in only the best, and often the largest, hosts (Charnov et al. 1981). In areas where host quality is poor, adult females will lay more male eggs in an effort to save female eggs for better hosts. Unless better hosts are available, the female eggs may not be laid, and the population will have an equal, or occasionally male-biased sex ratio. As progeny sex ratios are vital to not only population establishment, but also the effectiveness of the parasitoid species, it is necessary to evaluate the host species-specific progeny sex ratio during parasitoid evaluation.
Due to the variability caused by the rearing host, investigations into the size-dependent traits for any potential biological control agent should be undertaken during evaluation. This is required if the potential effectiveness of released parasitoids is to be approximated based on the most abundant field host. If parasitoid effectiveness is evaluated based on a host different from the target species, then estimates of host mortality will be inaccurate. Future releases will then be incorrectly calculated and the biological control program may not succeed. For optimal release numbers to be determined, the longevity, fecundity, and sex ratio of the parasitoid in relation to the dominant host that will be encountered by the released parasitoid species should be calculated. This is a problem at the moment in the literature available on *P. sulcata*. As indicated previously, some literature exists on the relationships between *P. sulcata* and the North American *Agrilus bilineatus* (Weber) (Haack et al. 1981) that may be useful in the formulation of an augmentative biological control program for *A. planipennis*. However, these results cannot be relied upon for determining the effectiveness of *P. sulcata* against *A. planipennis* as the rearing host can have an effect on some of the aforementioned life history traits. It is therefore necessary to determine all required life history traits as they relate specifically to the target host.

1.2.2.2 Influence of environmental conditions on parasitoid life history

An important attribute of a successful biological control agent is the ability to survive under to the environmental conditions of the release site (Huffaker and Messenger 1976; Coppel and Mertins 1977; van Lenteren and Woets 1988; DeBach and Rosen 1991; Smith 1996). Temperature, humidity, and photoperiod could all affect parasitoid attributes that are linked to efficacy. In an analysis of several biological control programs, Collier and van Steenwyk (2004) noted that most field release programs which failed were due to parasitoids being unable to overcome adverse environmental conditions at the release site. These effects negatively affected
attributes such as longevity and fecundity, causing a failure in parasitoid establishment. The effect of the environment is a particularly important to the success of classical biological controls as the parasitoid itself is also non-native. It is important, however, that environmental effects on life history attributes be tested for all possible biological control agents, especially where the selection of specific parasitoid strains is possible. An example of this is in the augmentative use of *Trichogramma* spp. where strains within a species are judged on their effectiveness in a variety of environmental conditions and used accordingly (Smith 1996). From the judging of strains, the insects that show the best potential for survival and success in the release environment can be selected and used.

Like all insects, parasitoids are poikilothermic and thus possess many life traits that are directly influenced by the environmental conditions, especially temperature. One such trait is longevity (Tingle and Copland 1989; Lysyk 2000; Ferreira de Almeida et al. 2002; Hansen and Jensen 2002). In some cases, differences in the longevity of parasitoids reared at different temperatures can be large. In a study of adult *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae) McDougall and Mills (1997) observed that mean longevity for adults reared at 10°C and 35°C were approximately 53 and 3 days respectively. Uçkan and Ergin (2003) also observed a similarly large discrepancy in adult longevities for *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). Adults kept at 20°C lived 41.0 ± 2.5 days, while those kept at 30°C lived 20.7 ± 2.5 days. Temperature has also been shown to influence fecundity (Force and Messenger 1964; James and Warren 1991; Borchier and Smith 1996; Urbaneja et al. 2001; Eliopoulos and Stathas 2005), along with humidity (Ouedraogo et al. 1996; Rousse et al. 2009), and photoperiod (Sagarra et al. 2000). For example, Zandi-Sohani and Shishehbor (2011) observed that the mean number of eggs oviposited per day by adult *Encarsia acaudaleyroides*
Hayat (Hymenoptera: Aphelinidae) kept at 25°C was more than twice the value for females kept at 32°C (54.6 ± 5.6 versus 20.1 ± 1.6). Emana (2007) also observed that relative humidity (RH) significantly influenced the mean number of progeny produced per *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) females kept at 30°C, with insects kept at 60 – 70 % RH producing 21.3 ± 1.4 eggs, while those kept at 70 – 80 % R.H. produced only 54.2 ± 3.8 eggs. Other traits such as emergence time (Payne 1933; Ferreira de Almeida et al. 2002; Zamani et al. 2007; Mainali and Lim 2012) and progeny sex ratio (King 1987; Duale 2005) have also been observed to vary with environmental conditions. As with host species, it is essential to observe a potential biological control agent’s life history parameters in laboratory conditions that mimic those of the release site. By doing this, the effectiveness of the parasitoid within the specific site can be accurately estimated. The testing of parasitoids at different laboratory conditions can also be effective in optimizing insect production. For these reasons, rigorous observations of parasitoids in different environmental conditions are vital during the selection of potential biological control agents.

### 1.2.2.3 Influence of proovigenesis and synovigenesis on life history traits

Longevity, fecundity, and emergence times are also influenced by the condition of the parasitoid at the time of adult emergence. Parasitoids may emerge as either synovigenic or proovigenic adults (Quicke 1997). Proovigenic wasps emerge with most of the mature eggs developed, while synovigenic species emerge with few or no mature eggs. In order to parasitize hosts, synovigenic species must obtain nutrients to support mature egg development during their adult lives. During this preoviposition period, synovigenic adults are unable to parasitize hosts. The production of mature eggs in proovigenic species, however, begins during immature development. During development, parasitoids must divide the available nutrients between the
needs of the reproductive system, including egg development, and the energetic needs of other functions such as locomotion. Increased reproductive effort early in life has been shown to come at a cost to other body functions, with longevity often being lower in proovigenic species compared to synovigenic species. This has been observed in not only proovigenic parasitic Hymenoptera (Jervis et al. 2001) but also in Lepidoptera and Trichoptera (Jervis et al. 2005). Because little energy is spent on reproductive systems during development, synovigenic species are typically longer lived than proovigenic species.

The requirement for additional resources in proovigenic species is linked with whether or not the host is paralyzed during oviposition. During parasitization, idiobiont parasitoids paralyze their host during oviposition, whereas koinobionts do not. Therefore, hosts containing koinobionts continue to develop and sequester nutrients from the host, while those containing idiobionts cannot provide additional nutrients outside of what the host contained at parasitization. As a result of a nutrient deficiency, most idiobionts cannot emerge with a complete mature egg complement and are thus synovigenic. In koinobiont parasitoids, the additional nutrients sequestered by the active host allow for not only pupation and eclosion, but the generation of the reproductive system. Therefore, most koinobionts are able to produce a mature egg complement prior to emergence, and are therefore proovigenic (Jervis et al. 2001).

Delayed immature development is common with proovigenic parasitoids (Quicke 1997). For koinobionts to sequester the maximum amount of nutrients from the host, it is in the interest of the parasitoid to delay their own development. For example, female *Chalcis canadensis* Cresson (Hymenoptera: Chalcididae) parasitize the eggs of *Stratiomyia* spp. (Diptera: Stratiomyide), but do not initiate prolonged feeding until the host has reached the latter stages of
its larval development. Only at this point will the parasitoid quickly complete its development and emerge from the host pupa (Cowan 1979). By allowing the host to continue to develop, C. canadensis larvae are able to sequester more nutrients from its host than would be available in the host egg at the time of parasitization. A significant drawback of delayed development, however, is that an extended developmental time leaves the parasitoid vulnerable to hyperparasitism, predation on the host, or multi-parasitism by ectoparasitoids. In environments or niches where such sources of mortality are high, there is a selection pressure for rapid development and shorter emergence times. Consequently, idiobionts are more common in sites where such pressures exist (Jervis et al. 2001).

Whether or not a parasitoid is proovigenic or synovigenic is important to understanding its potential effectiveness as a biological control agent. Firstly, synovigenic parasitoids should only be released only when they have completed their pre-oviposition period. Otherwise, parasitoids may disperse from the release site or be consumed by predators before they are able to cause appreciable amounts of mortality to pest populations. As the development of a mature egg complement has already taken place, proovigenic parasitoids may be released immediately after emergence and can be expected to cause host mortality immediately (Ellers and Jervis 2004). However, the shortened lifespan observed in proovigenic species may require several releases over the span of a season if the preferred host stage is present for long periods. This may not be necessary in longer-lived synovigenic species, as released individuals may be able to survive for a large proportion of the season. An important trait of synovigenic species is their ability to both reabsorb and generate eggs throughout their lifetime (Jervis et al. 2001). These abilities are essential if host density is patchy. If host density is low, synovigenic species may be able to reabsorb nutrients from their eggs until they reach an area of more consistent host
densities (Ellers and Jervis 2004). Proovigenic species usually do not possess oobssorption abilities, and are thus only efficient in areas where hosts are both easily accessible and in high densities. If proovigenic species are released in patchy sites, then many parasitoids may expire before they can locate a suitable host patch (Ellers and Jervis 2004). Consequently, the host density and availability in a release site must be taken into account when choosing the parasitoid species for release. In cases where no available parasitoid species is suitable for the site conditions, then other management protocols such as mechanical or chemical treatments should be investigated.

1.2.3 Influence of super-/multiparasitism on agent effectiveness

Avoidance of super-/multiparasitism is an important attribute of effective biological control agents (DeBach and Rosen 1991). Superparasitism is the parasitization of a host by two or more parasitoids of the same species, while multiparasitism is parasitization of the same host by two or more parasitoids of different species (Quicke 1997). Both events can be detrimental to solitary parasitoids because these species often require all of the available nutrients from a host in order to complete their development. If super-/multiparasitism occurs, then parasitoid development will likely be disrupted or prevented (Quicke 1997). Solitary parasitoids often possess morphological and/or physiological means such as biting mandibles or secreted encapsulation compounds that can eliminate conspecifics within the host (Harvey et al. 2013). Because resident parasitoids are equipped to destroy later-arriving conspecifics within the host, oviposition into previously parasitized hosts may be inefficient and/or wasteful for foraging females. Many solitary parasitoids have thus developed methods of determining if a host is fit for parasitization. Examples include probing hosts before oviposition (Bai and Mackauer 1991) and detecting compounds deposited on the host by another female (Vinson and Guillot 1972). By
minimizing super-/multiparasitism, parasitoids reduce their own and may increase their effectiveness for control of the pest. Therefore, it is desirable that parasitoids chosen for biological purposes be able to discern suitable hosts to minimize super/multi-parasitism.

1.3 Parasitoid courtship sequences

The ease with which a parasitoid species can be reared for release should be analyzed during the parasitoid selection process. Some parasitoid Hymenoptera are thelytokous parthenogenic species, and can produce female progeny without male fertilization. However, the majority of species can only produce females from fertilized eggs (Quicke 1997). If females are to be produced, then the courtship and copulation sequences for these species should be analyzed. The courtship sequence for hymenopteran parasitoids is usually stereotypical (van den Assem 1986). The sequence most often begins when a courtship pheromone is released by either the male or female. These compounds are released so that adults from the other sex can orient to the emitting sex’s position (Quicke 1997). While male-produced pheromones have been documented (Cosse et al. 2012), orientation pheromones are most often produced by females (Yoshida 1978; Eller et al. 1984; McNeil and Brodeur 1995; Ruther et al. 2000). Parasitoid age and mating status may affect the receptivity of females to males (Tagawa et al. 1985; Perez-Lachaud and Campan 1994; Schworer et al. 1999; King et al. 2005). Identifying parasitoid pheromones is important to biological control programs as they may be useful for parasitoid detection or aggregation (Kainoh 1999). If pheromones are released by females, males will orient to the female, and initiate courtship sequences.

The male courtship sequence may be carried out in close proximity to the female. In some species however males will mount the female and carry out their courtship displays while
on the female’s dorsum (Barras 1960; van den Assem and Vernel 1979; Field and Keller 1993). Courtship displays can include swaying while walking, attention of the substrate near the female, or of the female herself, and wing fanning (Vinson 1972; Leonard and Ringo 1978; Hansen 1980; Ruther et al. 2000; Steiner et al. 2006). In several parasitoids, an important courtship display involves the male touching his head to the antenna of the female (Barras 1960; Bin et al. 1999; Ruther et al. 2000). This action is thought to result in the transfer of a contact pheromone from the male to the female (Isidoro and Bin 1995; Isidoro et al. 1996). The purpose of this pheromone is likely to quiet the female and prepares her for copulation.

The female signals her readiness for copulation by lowering her head and antenna, and raising her abdomen (van den Assem 1976; van den Assem and Vernel 1979). In doing this latter action, she exposes her genital pouch. The male at this time mounts the female if he has not done so already and inserts the tip of his abdomen where his aedeagus is located, and begins copulation. In many of the documented observations of courtship and copulation in parasitic wasps, actions such as mounting, wing-fanning, and swaying are consistent between species (Barras 1960; Leonard and Ringo 1978; Hansen 1980; Abdurahiman et al. 1983; van den Assem 1986; Field and Keller 1993). However, the duration and frequency of each behaviour are highly variable between species. This is likely to ensure that inter-species matings do not occur. Mating may also be highly dependent on parasitoid status, with both age and previous mating status being potentially important determinants of detectability and receptivity to mating (Ridley 1989; Ridley 1993). Therefore, during the development of an insect mass production program, it is important to determine the effects of parasitoid status on the occurrence of courtship and copulation.
1.4 Chemical ecology of wasp parasitoids

Hymenopterous parasitoids often use olfactory cues derived from semiochemicals as a means of locating hosts (Weseloh 1981; Vinson 1985; Turlings and Wackers 2004). Semiochemicals can be divided into two categories: host-produced kairomones, and host-plant-produced synomones (Weseloh 1981). Kairomones are substances that is produced by an organism that elicits a behavioural or physiological response that is beneficial to the receiver, but not to the emitter (Brown et al. 1970; Nordlund and Lewis 1976). Conversely, synomones are substances that are produced by an organism that elicits a behavioural or physiological response that is beneficial to both the receiver and emitter (Nordlund and Lewis 1976). In relation to hymenopterous parasitoids, kairomones are substances produced by the insect host that serve as olfactory cues for host location by the parasitoid, whereas synomones are substances produced by the host-plant that serve a similar host-location purpose (Vinson 1985).

1.4.1 Kairomones

Kairomones can be produced either by the target stage of the host, or by another non-target host stage (Vinson 1985). For example, a larval parasitoid could be attracted by kairomones produced by both the host larvae and the ovipositing female. There are several important kairomone sources that parasitoids exploit, including adult sex pheromones, frass of both the adult and larva, and ovipositional secretions associated with eggs (Weseloh 1981; Vinson 1985).

Sex pheromones from host adults are often used as olfactory cues by egg parasitoids (Vinson 1991; Godfray 1994) because the eggs are often inconspicuous (Bruce et al. 2009). This olfactory cue is very important to egg or egg-larval parasitoids, as high levels of sex pheromone
in the air attracts parasitoids to areas where oviposition is about to begin, or has already taken place (Godfray 1994; Colazza et al. 1997). Bruce et al. (2009) observed the responses of three egg parasitoids - *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae), *Telenomus isis* Polaszek, and *Trichogramma bournieri* Pintureau and Babault (Hymenoptera: Trichogrammatidae) - to sex pheromones produced by calling female *Busseola fusca* (Fuller) and *Sesamia calamistris* (Hampson) (Lepidoptera: Noctuidae). The authors observed that the egg parasitoids were significantly more attracted to calling females than to non-calling females and clean air. Similarly, Colazza et al. (1999) observed that females of *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), an egg parasitoid of *Nezara viridula* (L.) (Heteroptera: Pentatomidae), were attracted to volatiles produced by pre-ovipositional host adults. As well, Hilker et al. (2000) noted that females of *Chrysonotomyia ruforum* Krausse and *Dipriocampe diprioni* (Ferrière) (Hymenoptera: Eulophidae) spent significantly more time in test fields containing the major components of the sex pheromone of *Diprion pini* (L.) and *Neodiprion seritifer* Geoffrey (Hymenoptera: Diprionidae). The results of these studies illustrate that the kairomonal properties of host adult sex pheromones are used by different families of hymenopterous parasitoids for hosts from different insect orders and guilds, and it may be possible that *P. sulcata* may use either or both of the two recognised pheromones produced by *A. planipennis* adults in host location.

Kairomones associated with host oviposition have also been observed to attract egg and egg-larval parasitoids (Weseloh 1981; Vinson 1985; Vet and Dicke 1992). The kairomone that elicits parasitoid interest is sometimes derived from substances deposited by the female during oviposition. In a study by Bin et al. (1993), *T. basalis* was observed to be attracted to a proteinaceous material produced by the accessory gland in the host-adult female reproductive
tract that is used to cement eggs to the plant substrate. A similar substance used to cement the ootheca of *Periplaneta americana* (L.) (Blattodea: Blattidae) to surfaces also acted as a kairomone for *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) (Suiter et al. 1996). The egg-larval parasitoid *Chelonus texanus* Cresson (Hymenoptera: Braconidae) also exhibited ovipositional behaviour when presented with kairomones associated with the eggs of its host, the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Vinson 1975). This author indicated that the kairomone was either on or around the eggs, and was present in ovary tissue of the adult. Given the reliance of egg and egg-larval parasitoids on egg-associated kairomones, it is possible that similar substances associated with the eggs of *A. planipennis* may act as kairomones for *P. sulcata*.

Frass from both adults and the target host stage have also been known to aid parasitoids in host location (Weseloh 1981; Vet and Dicke 1992). Perhaps surprisingly, parasitoids of concealed hosts such as stem- and wood-borers can use larval frass kairomones to locate their hosts. For example, *Rhyssa persuasoria* (L.), an ichneumonid parasitoid of siricid woodwasps, locates its larval hosts using kairomones released from symbiotic fungi in the larval frass (Spradbery 1970). These kairomones allowed this parasitoid to locate its host though ~ 7.6 cm of wood (Weseloh 1981). Hailemichael et al. (1994) also observed that another ichneumonid, the solitary endoparasitoid *Xanthopimpla stemmator* (Thunberg), used frass-derived volatiles produced during the host’s larval stage to locate pupae of the stalk-boring *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae). The parasitized life stage was embedded in grass stems. Although adult frass has not been widely studied for its use as a kairomone for egg and egg-larval parasitoids, it has been observed in several instances that materials left behind by adults in regions nearby their eggs and larvae have served as olfactory cues (Vinson 1985). Perhaps the
most well-documented example of this involves the stimulation of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) in response to kairomones released from scales left behind by ovipositing *Heliothis* sp. (Lepidoptera: Noctuidae) (Lewis et al. 1971; 1972). Thus, it may be possible that frass deposited nearby eggs by adults could serve as a kairomone for parasitoids.

### 1.4.2 Synomones

*Fraxinus* spp. under attack by *A. planipennis* have been observed to produce two types of synomones: green leaf volatiles and bark terpenoids (Crook et al. 2008; de Groot et al. 2008). Green leaf volatiles include several C₆ alcohols, aldehydes, and acetates (Zhang and Schlyer 2004), and such compounds are produced in elevated levels by angiosperms that are being fed upon by insects (Rodriguez-Saona et al. 2006). Green-leaf volatiles induced in plants subjected to insect herbivory have been shown to attract parasitoids (Weseloh 1981). Reddy et al. (2002) noted that the egg parasitoid *Trichogramma chelonis* Ishii (Hymenoptera: Trichgrammatidae) and the larval parasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) were both responsive to particular chemicals in the green-leaf volatile mixture produced by cabbage that had been damaged by *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) larvae. These compounds, which included (Z)-3-hexenyl-acetate, (E)-2-hexenal, and (Z)-3-hexenol, were also found to attract parasitoids when produced by corn (Turlings et al. 1991). Du et al. (1998) and DeMoraes et al. (1998) observed that (Z)-3-hexenol produced by both insect-damaged broad bean, and tobacco plants, respectively, aided hymenopterous parasitoids in locating their herbivorous insect hosts. Rodriguez-Saona et al. (2006) and de Groot et al. (2008) both noted that (Z)-3-hexenyl-acetate, (E)-2-hexenal, and (Z)-3-hexenol are also produced by *Fraxinus* spp.
under attack by *A. planipennis*. These substances may elicit similar positive host-searching responses *P. sulcata*.

Bark terpenoids are released by various plants that are under insect herbivory (Turlings et al. 1991; Langenheim 1994; Engelberth et al. 2004), and are often used as olfactory cues for parasitoids (Turlings et al. 1991; Turlings and Tumlinson 1992). Camors and Payne (1972) showed that *Heydenia unica* Cook and Davis (Hymenoptera: Pteromalidae) was attracted to host tree terpenes produced in response to feeding by *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae). Similar terpenoid compounds produced by Scots pine under attack by *D. pini* were shown to attract *Chrysonotomyia ruforum* (Hymenoptera: Eulophidae) (Hilker et al. 2002). Among the terpenes produced in conifer terpene synthesis are sesquiterpenes (Trapp and Croteau 2001), which are also produced by *Fraxinus* sp. during periods of stress (Crook et al. 2008). Bark sesquiterpenes produced by stressed *Fraxinus* sp. might be used as synomones for hymenopterous parasitoids similar to the sesquiterpenes produced by various conifers. As with green-leaf volatiles, bark sesquiterpenes produced by ash trees under attack by *A. planipennis* could act as synomones for attracting *P. sulcata* adults.

### 1.4.3 Application of chemical ecology in biological control

Attractive compounds such as semiochemicals and pheromones may be used in retaining and/or aggregating parasitoids to a release site. In some cases, host density may not be high enough for parasitoids, released or natural populations, to exert appreciable amounts of mortality. Consequently, released parasitoids may disperse from the release site, while natural populations may not aggregate within the affected area. The application of semiochemicals to these sites may alleviate both of these problems.
Several studies on the improvement of *Trichogramma* spp.-parasitization on *H. zea* illustrate the potential of kairomones to improve enemy effectiveness. Lewis et al. (1972) first observed that adult *T. evanescens* Westwood displayed host-seeking responses to scales from adult *H. zea* in laboratory bioassays. The authors then applied a hexane-based moth scale extract to leaves of host plants and observed significant increases in egg parasitism by released *T. evanescens*. Lewis et al. (1975b) then applied a synthetic kairomone and hexane-extracts of moth scales to soybean plots, and observed that parasitism rates by released and natural *Trichogramma* spp. on *H. zea* eggs were significantly higher in treated plots as compared to untreated control plots. The authors concluded that the application of kairomones in treated plots caused an intensification of host-searching by *Trichogramma* spp., and thus increased egg parasitism. Lewis et al. (1975a) treated plots with low, intermediate, and high amounts of kairomones, and observed a positive correlation with application size and egg parasitization. The authors also determined that superparasitism was significantly lower in treated plots than in control plots. Gross et al. (1975) also showed that parasitism by *Trichogramma* spp. and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) on *H. zea* eggs and larvae was significantly higher when adult parasitoids were exposed to host kairomones before release. In these studies, the authors observed that parasitism was consistently higher and superparasitism was lower when kairomones were either applied to plots, or when exposed to parasitoids before release. It is possible that similar improvements to *A. planipennis* parasitoids might be made if beneficial semiochemicals are identified. This might be especially important if parasitism by native species was not consistent between sites. The identification of semiochemicals through bioassays and field tests to analyze their potential application should be investigated during the evaluation of all potential biological control agents.
Identified semiochemicals can also be incorporated into enemy detection protocols. Evaluation of biological control agent populations is critical to the success of the biological control program (Van Driesche and Bellows 1996). Firstly the resident enemy population must be quantified before releasing new natural enemies. This ensures that the optimal number of enemies is released at the site, and that the release of either too many or too few parasitoids is avoided. If too many parasitoids are released, then competition between enemies may reduce enemy effectiveness. Also, if parasitoids are difficult to produce, then an over-release of parasitoids could represent a significant waste of insects that could be released at other sites. Conversely, if too few parasitoids are released, then pest control will not be achieved. Thus, the identification of effective detection tools is a necessary undertaking in the development of a biological control program.

Semiochemicals that are used by parasitoids in host searching may also be employed as baits in detection traps. Traps baited with semiochemicals that are attractive to the target organism are among the most effective for determining the presence and abundance of low density populations (Coppel and Mertins 1977). Baited traps are commonly used for monitoring forest insects. For example, both *A. planipennis* (Crook and Mastro 2010), and *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) (Elkinton and Carde 1988) are monitored using detection traps baited with attractive compounds specifically tailored to the target insect. Baited traps are used in the detection of other forest insects including various scolytids (Turchin and Odendaal 1996; Flechtmann et al. 2000; Petrice et al. 2004), cerambycids (Nakamuta et al. 1997; Reddy et al. 2002), and sawflies (Anderbrant et al. 1989; Bergstrom et al. 1995; Staples et al. 2009). Baited traps are necessary in forests as destructive sampling of infested trees is often extremely labor intensive and costly (Elkinton and Carde 1988). Consequently, the identification of volatiles that
can be used in baited traps to determine establishment and dispersal is especially important for monitoring forest insects such as parasitoids.

Although effective baits are necessary in detection traps, the trap’s physical characteristics must also be investigated as these may influence trap catch. Parasitoid species have been shown to prefer different colors including yellow (Moreno et al. 1984; Ridgway and Mahr 1986; Ma et al. 1992; Udayagiri et al. 1997), white (Romeis et al. 1998), black (McClain et al. 1990), and green (Goff and Nault 1984). Color preference is thought to be related to the substrate on which the host is commonly found. For example, green and yellow traps are preferred by parasitoids whose hosts occur on exposed vegetation, while black traps are preferred by parasitoids of bark-associated insects (McClain et al. 1990). Such considerations should therefore be taken into account when selecting colors for detection traps. Trap shape also influences effectiveness. In studies of agricultural parasitoids, panel traps have been utilized (Weseloh 1986; McClain et al. 1990; Udayagiri et al. 1997). However, forest insects such as wood boring beetles have been shown to prefer cylindrical or tubular traps as these resemble the silhouette of a standing tree (Lindgren 1983; Chenier and Philogene 1989). Therefore, the niche in which the target species operates should be taken into account when choosing a potential shape for a trap.

Finally, the placement of the trap within the environment may also influence the number of insects collected. Pucci (2008) sampled parasitoids at both ground and canopy levels in temperate forests in Ohio, USA, and observed that ichneumonids, pteromalids, and some braconids were captured in significantly higher numbers in ground-level traps, while encyrtids were captured in significantly higher numbers in canopy-level traps. This is likely also related to
the habitat in which the host resides. Dyer and Landis (1997) also observed that trap placement influenced trap catch in of *Eriborus terebrans* (Gravenhorst) (Hymenoptera: Ichneumonidae), a parasitoid of the European cornborer (*Ostrinia nubialis* (Hübner) (Lepidoptera: Pyralidae)). The authors observed that Malaise traps placed on the wooded edge of infested cornfields captured significantly more parasitoids in some generations than traps placed either within the cornfield or on the herbaceous field edge. It was concluded that the availability of sugar resources provided by the herbaceous understory of the wooded area likely influenced the number of parasitoids caught at in this area. Both of these results indicate that both vertical and horizontal placement of traps may influence catches of the target species. Therefore, the selection process for a trap must also analyze the effect of placement within the environment on the effectiveness of the trap in catching the target insect.

### 1.5 Spatial and temporal distribution of enemies

An important obstacle in maximizing host mortality is the presence of host habitat refuges (Collier and van Steenwyk 2004). Host refuges can be either temporal or spatial. Temporal refuges occur if the seasonal phenology of the adult parasitoid does not synchronize with that of the preferred host. A spatial refuge may be an area of the host habitat, such as a specific depth within a substrate, which a parasitoid cannot reach. In both cases, the effectiveness of the parasitoid is diminished because a significant proportion of the host population is not available for parasitization. Lack of temporal synchronization has been cited as an important impediment to a parasitoid becoming an effective control agent for a pest (Coppel and Mertins 1977; Collier and van Steenwyk 2004). For example, recruitment of native parasitoid species on the exotic leaf miner *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae) in
Europe was hampered due to adult parasitoid emergence occurring 5 weeks prior to the presence of suitable host stages in the environment (Girardoz et al. 2006; Grabenweger et al. 2007). Because adult longevity was approximately 50 days, very few hosts were parasitized, and ultimately mortality by native parasitoid populations was low. Asynchrony between adult parasitoid emergence and the preferred host stage has also been implicated in the ineffectiveness of indigenous parasitoids of the pine false webworm (Acantholyda erythrocephala (L.), Hymenoptera: Pamphiliidae) (Lyons 1999), and the European pine sawfly (Neodiprion sertifer (Geoff.), Hymenoptera: Diprionidae) (Griffiths 1969). In these studies, parasitoids may have been able to exert higher amounts of parasitism on host populations if not for an asynchrony of the phenologies of the adult parasitoid and preferred host stage. Therefore, synchronization of phenologies must occur if control is to be attained.

Hosts may also be able to enter spatial refuges within the habitat that are not accessible by parasitoids. If a large subset of the pest population is able to avoid parasitization in these refuges, then effective control cannot be achieved. For example, Udayagiri and Welter (2000) observed that eggs deposited by Lygus hesperus Knight (Hemiptera: Miridae) in the fruit tissue of strawberries were parasitized significantly less than eggs deposited in the calyx, petiole, and leaflet by Anaphes iole Girault (Hymenoptera: Mymaridae). The authors concluded that the presence of achenes in the fruit tissue prevented the adult A. iole from accessing a large proportion of the L. hesperus eggs. Consequently, the effectiveness of A. iole populations against L. hesperus was severely reduced. Wang et al. (2009) noted similar results when they observed that Bactrocera oleae (Rossi) (Diptera: Tephritidae) larvae developing in large olive fruits were parasitized significantly less than those in smaller olive fruit by the larval parasitoid Psyttalia concolor (Szépligeti) (Hymenoptera: Braconidae). The authors determined that ovipositor length
limited parasitoids to host in small olives, while a large proportion of hosts residing in large olives were effectively invulnerable. In trees, areas of thick bark can provide wood-boring insects with a refuge that is inaccessible to foraging parasitoids. For example, Manojlevic et al. (2000) analyzed parasitism rates on five scolytid species from different height levels in elm (*Ulmus* spp.) by the braconid *Euphyus silesiacus* (Ratz.). The authors observed that species in regions where bark <3 mm was significantly higher than parasitism of species in regions where bark was >3mm. They concluded that the ovipositor of most *E. silesiacus* was not long enough to penetrate bark >3mm thick. Therefore, an important proportion of the pest population could not be parasitized. Wermelinger (2002) analyzed parasitism on bark beetles in conifers in Central Switzerland, and observed that parasitism by several pteromalid species was also significantly higher in the upper parts of trees than in the base. He also concluded that bark thickness was an important factor in the reduced parasitism observed in lower boles of sampled trees. Host refuges have also known to occur in the parasitoid complex of *A. planipennis*. Abell et al. (2012) determined that bark up 3.2 mm thick was not penetrable by the ovipositor of the larval parasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae). Therefore, *A. planipennis* larvae in large diameter trees that possessed bark thicker than 3.2 mm would be protected from parasitization. The authors indicated that releases of *T. planipennisi* adults should be restricted to sites with small DBH (diameter at breast height) ash trees otherwise effective control would not occur. Based on the important effect of bark thickness on parasitism rates, it is important to determine if host refuges exist in the habitat in order to ascertain if a potential biological control agent will be effective against a pest species.
1.6 Thesis objectives and chapter outlines

I have outlined the necessity for *A. planipennis* management in North America. Since eradication at this time is unlikely, long-term management that minimizes its spread into uninfested areas must be undertaken. The use of biological control agents has been identified as a potential strategy for slowing the spread of this pest. Several native and imported wasp parasitoids have shown promise as potential biological control agents. *Phasgonophora sulcata*, a native chalcidid wasp, has shown promise as a potentially successful biological control agent for *A. planipennis* populations in southwestern Ontario. In order for a parasitoid to be used successfully in a biological control program, a comprehensive knowledge of the insect’s biology and life history must be determined. Unfortunately, no such information on the interactions between *P. sulcata* and *A. planipennis* exists. The goal of my thesis is to provide information that can be used to evaluate the potential of this parasitoid for use as a biological control agent against *A. planipennis*. This information will also provide the basis for a biological control program using this parasitoid. Over the following four chapters, I have conducted analyses on several aspects of *P. sulcata*’s life history and ecology. The results of my work provide important information that is applicable to the evaluation of *P. sulcata* as an augmentative biological control agent for *A. planipennis*, and for the initiation of a production and liberation protocol for this parasitoid. I have written each chapter as a self-contained manuscript including introduction and methods sections for each. Consequently, some repetition in these sections specifically may occur. Efforts have been taken however to minimize this.

In Chapter 2, I analyzed several life history parameters of *P. sulcata* specific to *A. planipennis* that are necessary for ascertaining potential effectiveness and for optimizing its
effectiveness if used in a biological control program. These included adult longevity, sex ratio, flight period, emergence times, and age-specific potential fecundity. I also investigated the occurrence of super- and multi-parasitism in field-collected specimens, and documented the number of instars expressed during immature development. Based on *P. sulcata* being a koinobiont, and therefore most likely proovigenic, I hypothesized that several adult life history parameters would be influenced by the presence of mature eggs at emergence. I also predicted that, due to the success of *P. sulcata* at several sites in Southern Ontario, traits associated with effective enemies such as synchronization with the preferred host stage, female-biased sex ratios and low proportions of super- and/or multiparasitized individuals would be present. I observed that the presence of a complete mature egg complement at emergence, relatively short longevity compared to other parasitoids of *A. planipennis* and extended emergence times were present in *P. sulcata* adults. I also observed that: adult sex ratios were female-biased; synchronization between adults and the preferred host stage was present; and that super-/multi-parasitism was very low. Lastly, I observed that *P. sulcata* possesses three distinct larval instars in addition to a prepupal and pupal stage. The results of this chapter serve to provide information that is important to rearing insects, and provide evidence that *P. sulcata* possesses several characteristics that are observed in successful biological control agents. These results also provide important developmental information that may be useful in the detection of *P. sulcata* in parasitized hosts.

In Chapter 3, I analyzed the courtship and mating behaviours of adult *P. sulcata*. I predicted that courtship would be initiated by a female-emitted pheromone which would be detected by and initiate courtship behaviour in adult males. Males would then undertake a number of actions while females would remain passive until copulation occurred. I observed that females emitted compounds that were detectable to males, while males did not elicit responses in
any adults. No intra-sex compounds were observed. The courtship sequence also included several actions that were repeated in all mating pairs. These results illustrate the importance of a female-produced compound in initiating the courtship process. Determining the mating sequence is necessary if \textit{P. sulcata} is to be reared for release into \textit{A. planipennis}-infested areas. These results provide information that will assist in establishing a rearing protocol for \textit{P. sulcata}, and in the potential use of a pheromone-based bait that may be used in parasitoid aggregation and detection.

In Chapter 4, I analyzed the role of semiochemicals in host-habitat location by adult female \textit{P. sulcata}, and tested the effectiveness of several trap types for detecting native parasitoid populations. I predicted that adults would be attracted to one or more host and host-plant semiochemicals. Specifically, I predicted that because \textit{P. sulcata} is a larval parasitoid, adults would be attracted to bark sesquiterpenes elicited by the consumption of cambium tissue by the preferred host stage. I also predicted that traps resembling the silhouette of an infested tree and yellow-panel traps would be effective in collecting parasitoids. Although females were attracted to the kairomone 3-(Z)-lactone, and a green-leaf volatile, (Z)-3-hexenol, attraction to a bark terpenoid surrogate was not observed. This may be due the surrogate containing only a portion of all bark terpenoids released by ash trees during an \textit{A. planipennis}-infestation. I also conducted GC-EAD (gas-chromatography electroantennogram detection) analyses using the antennae of adult female \textit{P. sulcata}, but observed no responses to any of the three compounds. I observed that sticky band and prism traps were effective in collecting parasitoids, while yellow-panel traps were ineffective. The results provide the basis for several applications, including the development of detection traps, and the identification of compounds that could potentially be used for aggregating or retaining \textit{P. sulcata} to areas of low \textit{A. planipennis} densities.
In Chapter 5, I analyzed the within-tree and temporal distribution of all \textit{P. sulcata} developmental stages. Due to the high parasitism rates at the study site, I predicted that \textit{P. sulcata} parasitism would not be influenced by tree height. Additionally, I hypothesized that because \textit{P. sulcata} is a koinobiont, early-instar larvae would comprise the majority of the immature individuals present during the summer as well as be the overwintering stage. I observed that parasitism was not significantly related to tree height, and that, numerically, the early-instar stage was the most important immature stage for most of the developmental cycle. I also tested if parasitism within height levels was related to host density; however I observed no significant relationship. These results indicate that host refuges do not exist in ash trees of these sizes. As well, sampling of infested trees for immature \textit{P. sulcata} should be conducted while looking for early-instar parasitoids developing within \textit{A. planipennis} larvae.

The overall findings of my thesis indicate that \textit{P. sulcata} demonstrates several traits that are characteristic of successful biological control agents. These include: synchronization with the preferred host stage, high fecundity during synchronization; low super- and multiparasitism; absence of host refuges in ash trees samples in this study. The determination of the courtship sequence and semiochemicals which may be used in detection and manipulation of parasitoid populations are important to the establishment of a potential release program. My final chapter (Chapter 6) summarizes the results and conclusions of each chapter. Here I will discuss a potential augmentative biological control program that utilizes \textit{P. sulcata}, and directions for future research. These include: analyzing protocols for releasing \textit{P. sulcata} in \textit{A. planipennis} infested sites, examining interactions with native and imported biological control agents, and expanding our understanding of the chemical ecology of \textit{P. sulcata}.  

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Chapter 2: Life history traits and a description of the immature stages of *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), a North American parasitoid of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)

2.1 Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive buprestid pest of ash (*Fraxinus* spp.) in North America. Since its discovery in the Detroit/Windsor region in 2002 (Haack et al. 2002), *A. planipennis* has been detected in 22 American states and two Canadian provinces (USDA/MSU 2012). This pest likely arrived in North America via contaminated wood packing materials from Asia (Haack et al. 2002). Depending on latitude, *A. planipennis* adults emerge in mid-spring to late-summer and after mating deposit their eggs in bark crevices or under bark scales on trunks and branches of ash trees (Poland and McCullough 2006). Larvae hatch through the ventral surface of the egg and burrow down through the bark into the cambial layer of the tree. Larvae feed while developing through four instars (Cappaert et al. 2005), damaging the conductive tissues of the tree throughout the summer until mid- to late-fall (Haack et al. 2002). Continued feeding disrupts nutrient movement within the tree and eventually leads to tree death. Most 4th-instar larvae create pupal chambers in either the sapwood or the bark, and pupate within them the following spring, however 20-25% of *A. planipennis* overwinter as feeding stage larvae (Cappaert et al. 2005).

Ash is an important tree species for the production of wood pulp and products, and is a major component of both natural and planted forests in Canada and the Northern United States (Poland and McCullough 2006). Black ash (*F. nigra*) is common in wet sites such as swamps
and bogs, while green ash (*F. pennsylvanica*) is a significant constituent of forest canopies in riparian areas (Poland and McCullough 2006). The elimination of ash from these unique areas could lead to the extinction of rare organisms found only in these habitats, as well as negatively influence various other ecological and biogeochemical processes associated with these forests (Gandhi and Herms 2010). In urban areas where ash is often a significant component, elimination by *A. planipennis* would reduce the urban forest canopy. The loss of ash in urban areas would result in reduced property values, and wildlife habitats, as well as increased storm water runoff, and increased heating and cooling costs (Sydnor et al. 2007; McKenney et al. 2012). Consequently, there is an important need for methods to prevent the spread of *A. planipennis* and reduce population densities to non-damaging levels.

Management of *A. planipennis* in Canada has consisted of a ‘slow-the-spread’ strategy involving quarantines on areas positive for *A. planipennis* (Lyons 2008). By restricting the movement of untreated ash products including firewood, wood pallets, nursery stock, and wood chips, the rate of spread can be reduced, while also allowing time for the development and implementation of long-term management options such as chemical and biological control programs. Chemical controls are effective for protecting high-value trees; however widespread use in areas of high ash densities has proven to be difficult due to prohibitory costs associated with application (Poland and McCullough 2006). Pesticide application has therefore been restricted primarily to low density ash environments such as urban regions (Mota-Sanchez et al. 2009; McKenzie et al. 2010). Biological control using parasitoids may be a potentially effective option in areas of high ash densities. Surveys conducted in China shortly after the discovery of *A. planipennis* identified three hymenopteran parasitoids that have since been studied and released in the United States (Liu et al. 2003; Bauer et al. 2008; USDA-APHIS/ARS/FS 2012).
Surveys of North American parasitoids have also identified several potential candidates for augmentative biological control (Liu et al. 2003; Bauer et al. 2008; Marsh et al. 2009; Lyons 2010; Duan et al. 2011b). Some promising candidates for augmentation include several \textit{Atanycolus} spp. (Hymenoptera: Braconidae), \textit{Balcha indica} (Mani & Kaul) (Hymenoptera: Eupelmidae), and \textit{Phasgonophora sulcata} Westwood (Hymenoptera: Chalcididae). In southwestern Ontario, parasitisation on \textit{A. planipennis} by \textit{P. sulcata} was approximately 40\% in three sites over multiple years (Lyons 2010). Therefore, \textit{P. sulcata} may be a potentially effective augmentative biological control agent for \textit{A. planipennis}.

If a parasitoid species is to be used as biological control agent, a detailed understanding of its biology is necessary (DeBach and Rosen 1991). One important life history attribute is potential fecundity. To estimate the effect of a released number of parasitoids, the potential number of hosts parasitized per individual parasitoid must be known. By determining the fecundity of the agent, an estimation of the number of hosts that could be parasitized by an augmented population can be determined. Without this estimate, the number of parasitoids required for a release cannot be accurately judged. Determining age-specific fecundity is also necessary for the release of parasitoids that have maximized their mature egg load. By releasing parasitoids that have either achieved or are near their maximum egg complements, the likelihood for pest suppression is increased. Another important variable that should be understood is when adults will emerge into the environment. For effectiveness to be maximized, synchronization of the adult parasitoid with the preferred host stages must occur (Coppel and Mertins 1977). If not, then parasitoids will not be able to interact with the host and regulation of pest populations will not occur. Lastly, the occurrence of superparasitism and multiparasitism should also be quantified as high levels of either could reduce the effectiveness of the control agent (DeBach
and Rosen 1991). In analyzing these traits, the effectiveness of potential biological control agents can be evaluated. By choosing parasitoids that possess traits associated with successful biological control agents, the likelihood of managing damaging pests can be increased.

Several life history traits are influenced by the condition of the parasitoid at emergence. Parasitoids may be classified based on whether or not the host is paralyzed during oviposition. Koinobionts do not paralyze their prey during oviposition, allowing them to continue development until a point where the parasitoid rapidly undergoes its own development (Quicke 1997). This is opposite to idiobiont parasitoids which paralyze the host during oviposition. Most koinobionts are proovigenic, and emerge with a nearly complete mature egg complement. Idiobionts are synovigenic and do not emerge with a mature egg complement (Quicke 1997). Due to the sequestering of resources by the reproductive system during immature development, proovigenic parasitoids often possess distinct variations in life history as compared to synovigenic parasitoids (Jervis et al. 2001). The effects of egg maturation before emergence include extended emergence times, and relatively short lifespan, as well as the presence of a complete mature egg complement at emergence (Jervis et al. 2001; Jervis et al. 2005). These traits should be understood for predicting potential effectiveness in the field and for insect production. *Phasgonophora sulcata* is a koinobiont endoparasitoid of North American buprestid beetles. It has been recorded from across the United States and in eastern Canada (Boucek 1992) from several *Agrilus* spp. (Barter 1957; Haack et al. 1981; Haack and Benjamin 1982; Loerch and Cameron 1983; 1984). Although Haack et al. (1981) provided information on sex ratios and flight periods in relation to *A. bilineatus*, the life history of *P. sulcata* is largely unknown. Additionally, no information exists on its life history traits as they relate to *A. planipennis*. The goal of this paper is to describe the aforementioned life history traits as well as determine the sex
ratio, flight period, and the possible occurrence of protandry in *P. sulcata*. As well, I intend to describe the immature stages of *P. sulcata*. Because *P. sulcata* is a koinobiont, it is my prediction that its life history traits will be typical of proovigenic koinobiont parasitoids. These results will serve to assess the suitability of *P. sulcata* as a biological control agent of *A. planipennis*, and provide interesting information on this recent host-parasitoid relationship.

### 2.2 Methods

#### 2.1.1 Insects: Emergence, protandry, sex ratio, and longevity

Adult parasitoids were reared from *A. planipennis*-infested ash logs collected from sites in Essex, Lambton and Middlesex Counties, Ontario, Canada. The sites included: an urban site with planted ash sampled in 2008 (Sarnia Golf and Curling Club, Sarnia, Ontario; 42.99545, -82.39970); a high-density mixed woodlot with large ash sampled in 2010 (Highway 401 Service Centre at Dutton, Ontario; 42.65890, -81.55401); a planted ash woodlot near Harrow, Ontario sampled in 2008 ("Larry’s"; 42.06257, -82.89026); and a site where ash (*Fraxinus pennsylvanica* Marshall) had been planted approximately 25 years prior which was sampled in both 2009 and 2010 (W. Darcy McKeough Dam Floodplain, Duthill, Ontario; 42.41416, -81.55401). These sites were known to have large numbers of *P. sulcata* based on previous surveys. Logs were collected in the autumn of each sample year, and transported to the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario, Canada. Logs were held at 4°C in environmental chambers for at least six weeks. After this overwintering period, logs were placed in wire rearing cages and were incubated at approximately 25°C, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D). The emergence times for each sex of *A. planipennis* and parasitoid adults were recorded, as well as the relative abundances of each sex and species. Sex ratios were calculated as the ratio
of females:males. Parasitoids were kept in sex-specific groups in 12-oz. plastic cups with up to eight parasitoids per cup. Cups were covered with a plastic lid with the center removed and replaced with an aluminum mesh screen to facilitate air circulation. Insects were held at 26 ± 1°C, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D). Parasitoids were provided water from a 12 ml vial plugged with two cotton dental wicks and a synthetic strip of shammy, and honey that was smeared onto the wire mesh of the lid. Water and honey were both replaced every two or three days. Parasitoids were observed daily, and longevity was recorded.

2.1.2 Age-specific potential fecundity

Female parasitoids aged 0 (i.e., newly emerged), 5, 10, 15, 20, 25, 30, 45, and 50 days old were freeze-killed by placing them in a freezer at -20°C for 10 minutes. Between 11 and 15 females were examined for each age class. Parasitoids were then dissected underneath a dissecting microscope. To view the eggs within the ovaries, the abdomen was first removed from the female and then cut open dorsally using a pair of micro-scissors. The ovaries were removed from the abdomen using an insect pin and were placed on a glass microscope slide. A drop of 1% acetocarmine was applied to the ovaries using a bulb pipette. Acetocarmine stained immature eggs purple, while mature eggs remained white as their thicker chorions prevented absorption (Edwards 1954). After one minute of absorption, the ovaries were rinsed with saline solution on the microscope slide and a cover slip was placed over them. Mature eggs undergoing reabsorption were identified by the presence of degenerating chorions and were also counted. The sum of mature and reabsorbed eggs represented the total mature complement in each ovary. The counts for each ovary were summed together to determine the total mature egg load for the parasitoid.
2.1.3 Super-/multiparasitism

Observations on the occurrence of super-/multiparasitism were conducted material retrieved from two sites (W. Darcy McKeough Dam Floodplain, Duthill, Ontario; 42.41416, -82.24145; and Highway 401 Service Centre at Dutton, Ontario; 42.65890, -81.55401) collected throughout the summer of 2010. Infested ash trees were felled and cut into eight 60-cm long logs using a chainsaw. Trees were identified as *A. planipennis*-infested by the presence of one or more of the following signs and symptoms: reduced canopy, epicormic shoots, splits in bark, and ‘D’-shaped exit holes. Logs were de-barked on-site using drawknives (Veritas Carver’s Drawknife, Lee Valley Tools, Canada). All *A. planipennis* larvae and pre-pupae in either larval galleries or pupal chambers, respectively, were dissected. All associated ectoparasitoids and endoparasitoids were removed from their hosts and counted. The occurrences of superparasitism and multiparasitism were reported as percentages of the total incidences of *P. sulcata* parasitism.

2.1.4 Flight period

Flight period monitoring of parasitoids was conducted at the McKeough Floodplain beginning in May 2011. Sticky band traps consisted of 50 cm wide bands of plastic shrinkwrap packaging (Catalogue Number 498385, Staples, Business Depot, Markham, Ontario) wrapped around the trunks of 20 ash trees. The bottom edge of the band was 1.4 m above the ground. Bands were coated with Pestick (Catalogue Number 4002, Phytotronics Inc., Earth City, Missouri) applied with paint rollers. Traps were deployed in May 2011 and monitored bi-weekly from 6 June 2011 until 8 August 2011. Traps were last sampled and dismantled on 4 October 2011. Parasitoids were collected off the traps using forceps and placed into a vial containing a solvent (Histo-Clear II, National Diagnostics, Atlanta, Georgia) to dissolve the Pestick. Upon return to the laboratory, vials were immersed in a heated ultrasonic cleaner (Model FS30H,
Fisher Scientific Company, Ottawa, Ontario) to remove the glue on the insects. Insects were then pinned or placed in vials containing 70% ethanol.

2.1.5 Morphology of the immature stages of *P. sulcata*

To retrieve eggs, the ovaries of six newly emerged adult female *P. sulcata* were dissected and five mature eggs from each female were measured using an ocular micrometer attached to a compound microscope. Parasitoid larvae and pupae were obtained from dissections of parasitized *A. planipennis* larvae from the W. Darcy McKeough Dam Floodplain, Duthill, Ontario. Collections of material were completed over eight sampling events from May to August 2010, and from one sampling event in August 2012. Parasitized hosts were dissected in a Petri dish under a dissecting microscope. Upon discovery, both the host and parasitoid were both placed in a 1.5 ml microcentrifuge tube (Fisher Scientific, USA) with 70% ethanol. Immature parasitoids were viewed using a Nikon digital camera attached to a compound microscope. Images were captured and analyzed using an Archpix computer imaging package. Separation of instars was done through the comparison of reliable morphological characters such as the presence of exuviae on the body of the specimen, head and mandible structure, and cuticular segmentation. In addition, measurements of body length (excluding the tail if present), body width, mandible length (measured from the distal tip of the mandible to the anterior articulation point) and mandible width (measured from the anterior to posterior articulation points) were collected where possible. A two-sample t-test was carried out to determine if mean mandible widths and lengths were significantly different for first and second instar larvae.
2.1.5 Statistics

All analyses were conducted using SigmaPlot (V.12). Due to the non-normality of the data, emergence times for *Atanycolus* spp., *P. sulcata* and *A. planipennis* adults were compared using a Kruskall-Wallis One Way Analysis of Variance on Ranks Test. Means were then separated using a post-hoc Dunn’s Method analysis. To analyze if protandry existed in *P. sulcata*, male and female emergence times were compared using a Mann-Whitney Rank Sum Test. To determine if parasitoid age had an effect on different egg stage categories, a one-way analysis of variance (ANOVA) was conducted on the mean number of viable mature eggs, and mean number of total mature eggs. Means were compared using post-hoc Tukey tests. To determine if parasitoid age had an effect on the mean number of reabsorbed eggs, a Kruskall-Wallace One Way Analysis of Variance on Ranks test was used, with means being compared using Dunn’s Method. A significance level of $P < 0.05$ was assumed for all statistical results.

2.3 Results

2.3.1 Emergence, protandry, sex ratio, and longevity

A consistent sequence of emergence for all three insect groups was observed in all sites (Table 2.1, Figures 2.1, 2.2). *Atanycolus* spp. emerged first, followed by *A. planipennis*. *Phasgonophora sulcata* was always the last species to emerge. Mean (± S.E.) emergence dates were significantly different between all three insects for all sites. Mean *Atanycolus* spp. emergence after diapause ranged from 16.1 ± 0.4 days at the Sarnia Golf and Curling Club site in 2008 to 22.0 ± 1.4 days for the W. Darcy McKeough Dam Floodplain site in 2011. *Agrilus planipennis* emerged as early as 15.1 ± 0.5 days for the ‘Larry’s’ site in 2009. Emergence at the other sites ranged from 30.6 ± 0.3 days at W. Darcy McKeough Dam Floodplain in 2010 to 37.8 ± 0.1 days for the Sarnia Golf and Curling Club site in 2009. *Phasgonophora sulcata* adults
emerged as early as 37.4 ± 0.8 days for the McGregor site in 2009. Emergence ranged from 45.5 ± 0.7 days at the W. Darcy McKeough Dam Floodplain site in 2011 to 57.0 ± 0.9 days at the Dutton site in 2011.

Mean female *P. sulcata* emergence was always significantly later than mean male *P. sulcata* emergence (Mann-Whitney Ranks Sum Test, *P* < 0.05, Table 2.2, Figures 2.3, 2.4, 2.5). This was seen at all sites for all dates. In general, overlapping of emergences did occur but mean male emergence always preceded mean female emergence. Male emergence occurred 4.3 days before female emergence at the McGregor site 2008, 6.1 days at the McKeough Dam site in 2011, and 5.4 days at Dutton site in 2011. Sex ratios for female and male parasitoids varied from 2.2:1 to 2.5:1 (Table 2.3). Mean (± S.E.) longevity of both males and females were 29.2 ± 0.8 (*n* = 32) and 30.1 ± 1.1 (*n* = 31) days respectively.

### 2.3.2 Potential and age-specific fecundity

Mean total mature egg complement was statistically equivalent from emergence (0 days) to 25 days old (One-way ANOVA, *P* > 0.05, Fig. 2.6). Mean total mature egg complement (± S.E.) for adult female *P. sulcata* at emergence (0 days old) was 55.7 ± 2.9 eggs. Peak mean total mature egg complement (58.2 ± 2.7) occurred at 7.5 days, but decreased while remaining statistically equivalent to 47.8 ± 3.3 eggs on day 25 (Fig. 2.6). Mean total mature egg load at day 30 day was 41.9 ± 2.8 eggs, and was significantly lower than egg complements of 0 – 25 day-old females (One-way ANOVA, *P* < 0.05). Mean total mature egg load at 50 days was 34.6 ± 1.67 eggs. This was significantly less than that at 30 days, but was not significantly less than egg loads at 45 days.

The mean number of mature viable eggs at emergence was identical to the mean total number of mature eggs, and was not significantly different between 0 and 25 days old (One-way
ANOVA, $P < 0.05$, Fig. 2.7). Mean number of mature viable eggs was significantly less at 30 days ($31.0 \pm 3.3$ eggs) compared to $0 – 25$ days, but was not significantly different at 45 days. The mean number of mature viable eggs at 50 days ($18.0 \pm 1.3$ eggs) was significantly less than that at 30 days, but was not significantly different from 45 days.

Ooabsorption was not observed until parasitoids were 30 days old. Eggs undergoing ooabsorption were first observed at 30 days ($6.0 \pm 2.0$ eggs), but this was not statistically different from 0 (Kruskall-Wallace Analysis of Ranks, $P < 0.05$, Fig. 2.7). The mean number of reabsorbed eggs at 30 days was significantly less than the mean number at 45 ($16.5 \pm 1.5$ eggs) and 50 days ($15.9 \pm 0.7$ eggs) (Kruskall-Wallace Analysis of Ranks $P < 0.05$). There was no significant difference between egg loads at 45 and 50 days ($P < 0.05$).

2.3.3 Super-/multiparasitism

Superparasitism was rarely observed. At the McKeough Dam in 2010, 1.6% of parasitized $A. planipennis$ by $P. sulcata$ were superparasitized ($n = 180$). At the Dutton site in 2011, 1.1% of parasitized $A. planipennis$ parasitized were superparasitized ($n = 89$). Multiparasitism was never observed at any site.

2.3.4: Flight period

Adult $P. sulcata$ parasitoids were observed on sticky band traps from 4 July 2011 to 4 October 2011 (Fig. 2.8). Mean number of $P. sulcata$ adults caught per traps on 4 July was $3.5 \pm 0.6$. Mean $P. sulcata$ parasitoid catch peaked on 18 July 2011, with $5.7 \pm 1.3$ caught per trap. Mean $P. sulcata$ parasitoid catch only peaked once. Parasitoid catch on 4 October 2011 was $0.5 \pm 0.2$ parasitoids per trap.
Adult *Atanycolus* spp. were observed on sticky traps on all sampling dates (Fig. 2.8). Peak mean catch occurred on the first sampling date. From this sampling date onward, mean trap catch decreased from 4.5 ± 1.1 insects per trap on 6 June 2011 to 0.1 ± 0.1 and 0.2 ± 0.1 insects per trap on 8 August 2011 and 4 October 2011, respectively.

2.3.5 **Morphology of immature stages of** *P. sulcata*

2.3.5.1 The egg

Mature eggs of *P. sulcata* adult females were hymenopteriform (Clausen 1940). Mature eggs were oval-shaped and appeared to possess a chorion. When viewed under a dissecting microscope, the eggs appeared to be smooth. The mean (± S.E.) egg length was 417.7 ± 0.01 \( \mu m \) (n = 30). Eggs were approximately three times as long as they were wide. Eggs were white and opaque.

2.3.5.2 First instar

First instar larvae possessed a mandibulate form with a well developed head and jaws and a posteriorly tapered body (Fig. 2.9) (Clausen 1940). Mandibles were brownish in appearance and heavily scleratized throughout. Mandibles were also distinctly curved inwards. Mean (± S.E.) mandible length and base width were 127.5 ± 1.9 and 72.8 ± 4.8 \( \mu m \) respectively (n = 19). First-instar larvae were always found in the hind-gut region of the host. First-instar larvae were whitish and opaque, and consisted of 13 body segments that were widest near the head, and narrowed towards the posterior of the insect.

2.3.5.3 Second instar

Second instar larvae were a caudate-mandibulate form (Fig. 2.10). The body was not strongly tapered as in the first instar, but rather was straight and elongate. Larvae possessed well
developed jaws; however, rather than rectangular as in the first instar, the attachment site for the mandibles was round. These sites appeared to be less scleratized when compared to those of the first instar. The mandibles also appeared to be longer in relation to the articulation site, and were not as deeply curved. Mean (± S.E.) mandible lengths and base widths were 142.1 ± 1.63 (n = 41) and 81.5 ± 1.06 µm (n = 40), respectively. Both values were significantly higher than those of first instar larvae (t = -5.12, P < 0.001). Second instar larvae also usually possessed a distinct pleurostoma and distinct antennal lobes (Fig. 2.11). Larvae also possessed distinct palps on the posterior edge of the mandible. These palps were not observed in the previous instar. The body consisted of 13 segments. Second instar larva also occasionally possessed a distinct tail that may be indicative of gender (Onagbola and Fadamiro 2008). Second instar larvae occasionally possessed the exuvia of the first instar, including the distinct head structure. The second instar was the primary stage of P. sulcata in parasitized A. planipennis overwintering as mature larvae.

2.3.5.4 Third instar

Third instar larvae were hymenopteriform and were visually distinct from the previous two instars (Fig. 2.12). Mandibles were weakly sclerotized, with only the distal tips being pigmented (Fig. 2.13). Mandibles were weakly curved, and never overlapped with one another. The mandibles also ended in a blunt point with several small teeth. The pleurostoma is lightly pigmented, but was not as visible as in previous larva. Antennal lobes were occasionally visible. Larvae were often observed with the exuviae of one or more previous instars attached to them. As with previous instars, some individuals possessed a tail, while others did not. Larvae were white, and transparency varied; some larvae were largely transparent which allowed for the gut and tracheal systems to be seen, while others were opaque. When visible, the gut was usually opaque and white from the ingestion of host tissues. Larvae also possessed a pair of weakly
developed prolegs on the ventral surface of each of the three thoracic segments posterior to the head. Body consisted of 13 segments. Mean body length (± S.E.) was 4.06 ± 0.31 mm (range 3.16 – 5.95 mm). Mean body width (± S.E.) was 0.799 ± 0.08 mm (range 0.63 – 1.35 mm) (n = 21).

2.3.5.5 Fourth instar (The prepupa)

Prepupae were hymenopteriform (Fig. 2.14). Hypostoma, pleurostoma, and mandibles were sclerotized and easily visible (Fig. 2.15). Imaginal eyes were also visible. Nine pairs of lateral spiracles were visible between body segments. Prepupae possessed 13 well developed body segments, but segmentation in the posterior half of the larvae was less discernible in larger specimens. Body began as white and opaque, but gradually became yellower in larger specimens. Prolegs on thoracic segments were again visible, but were more strongly developed than in previous instars. Mean body length (± S.E.) was 9.12 ± 0.286 mm (range 6.69 – 11.8 mm). Mean body width (± S.E.) was 2.12 ± 0.184 mm (range 0.775 – 2.33 mm) (n = 31). During the overwintering period, third-instar larvae and prepupae were the sole form of *P. sulcata* in parasitized *A. planipennis* that had constructed and were residing in pupal chambers in the sapwood of infested trees. Prepupa formation was completed inside the remains of the host soon after the host had entered the pupal chamber.

2.3.5.6 Pupa

Formation of the pupa was completed within the cuticle of the parasitized host. The pupa is exarate with adult features such as the antennae, mouthparts, and wings visible. In the early stages of pupation, remnants of the final instar, such as the meconium and sclerotized head structures are present near the abdomen of the pupa. At the time, the pupa is white with no
pigmentation. As the pupa ages, pigmenting of the eyes, thorax, and abdomen occur. The thorax and head darken first, then finally the abdomen, which takes on a reddish pigment until the final crimson color observed in adults. Pupation of *P. sulcata* occurs in the spring months.

### 2.4 Discussion

Since its arrival in North America, *A. planipennis* has spread rapidly killing millions of ash trees in Canada and the United States. Eradication through destruction of infested trees has proven unsuccessful; therefore long-term management options are currently being investigated. A potential management strategy might incorporate augmentative and/or classical biological control agents to be released into areas of *A. planipennis* infestation. An important component of this plan is to identify hymenopterous parasitoids that show potential as control agents, followed by an evaluation of their potential effectiveness. Herein, I studied several important life history parameters of *P. sulcata*. These results indicate that *P. sulcata* may show promise as an effective natural enemy of *A. planipennis*. In exploring these life history parameters, I gained an understanding of their importance as potentially effective biological control agents.

#### 2.4.1 Emergence, protandry, sex ratio, and longevity

*Phasgonophora sulcata* parasitoids had a mean longevity of approximately 30 days. Compared to another native parasitoid *B. indica* (58.81 ± 5.93 days), *P. sulcata* can be considered short-lived (Duan et al. 2011b). The relatively host lifespan may be linked to *P. sulcata* being proovigenic. Proovigenic parasitoids are often shorter-lived than synovigenic parasitoids because trade-offs in resource partitioning during emergence (Ellers and van Alphen 1997). For example, Jervis et al. (2005) compared the proportion of the total egg complement available at emergence with the mean longevity for 34 Hymenoptera parasitoid species, and
observed a strong negative relationship between eggs present at emergence and adult lifespan. During the development of proovigenic species, nutrients sequestered from the host by the developing wasp are directed primarily to the reproductive system instead, rather than to somatic maintenance of the organism (Flanders 1950; Ellers and van Alphen 1997; Jervis et al. 2005). One aspect of somatic maintenance that is observed is reduced adult longevity. Trade-offs in reproductive effort and somatic maintenance at emergence have been observed also in Lepidoptera and Trichoptera (Jervis et al. 2005). Although *P. sulcata* does synchronize its emergence with *A. planipennis*, its shortened longevity does restrict it to attacking early-instar *A. planipennis* larvae for only a portion of the season. If *P. sulcata* were to be used in augmentative control program, it may be advantageous to release parasitoids in cycles of approximately 30 days in order to ensure constant enemy pressure on early instar larvae, which are available throughout the season. However, this may not be necessary as even with only one generation of 30 days, *P. sulcata* adults were still able to parasitize nearly 40% of *A. planipennis* in some sites (Lyons 2010). Therefore, the relatively short longevity may be compensated by other positive traits such as synchronization, pro-ovogenesis and foraging ability.

Adult male *P. sulcata* displayed protandrous emergence. Protandry occurs in most insects (Wiklund and Fagerstrom 1977; Hastings 1989), and is important in maximizing mating success (Ridley 1989; 1993). Following emergence, female parasitoids often begin host searching (van Alphen et al. 2003). However, the probability of finding a mate after leaving the emergence site is often very low (Waage and Ming 1984). Therefore, the presence of males at the emergence site would be necessary for females to produce fertilized eggs that will become females in the next generation. In possessing protandrous emergence and a female-biased sex ratio, *P. sulcata* populations will thus be inherently be more stable and cause higher levels of *A. planipennis*
mortality than species with male-biased sex ratios and/or non-protandrous emergence due to increased mating success and the presence of higher amounts of females.

*Phasgonophora sulcata* adults possessed a female-biased sex ratio. This result is consistent with observations of adults reared from *A. bilineatus* made by Haack et al. (1981), who observed a sex ratio of 1.4:1. Our results, however, showed a stronger bias towards females. This difference may be related to the hosts used in each study. Host quality has been shown to affect sex ratio, with daughters typically being deposited in larger hosts rather than smaller ones (King 1987; Godfray 1994; Ode and Heinz 2002). Although *A. bilineatus* is a common host for *P. sulcata*, *A. planipennis* larvae appear to be slightly larger based on urogomphi length (Haack and Benjamin 1980; Liu et al. 2007). Arrenotokous parasitoids, which require male sperm to fertilize and produce female eggs, are able to control the number of male and female eggs they produce (Hardy 1992). Because of the nutritional demands required by the development of the reproductive system, females require more nutrients during development than males (van den Assem et al. 1989). Therefore, larger hosts usually produce higher numbers of females than smaller hosts because they may provide the nutrient supply required by female development (Ueno 1999). An increased number of large hosts would allow female *P. sulcata* to lay more female eggs. Female-biased sex ratios are important to the potential success of a biological control agent because of increased population growth rates associated with larger numbers of females in the environment (Heimpel and Lundgren 2000). Males are still required to ensure the production of females in the population. However, high numbers of females in the environment associated with such a sex ratio are necessary in order to maximize pest mortality, and to ensure the stability of parasitoid populations.
2.4.2 Age-specific potential fecundity

My results indicate that adult female *P. sulcata* contain their mature egg complement at emergence. This agrees with our prediction that *P. sulcata* is proovigenic. I also observed no significant change in mean potential fecundity from emergence to 25 days old. In order for an enemy to cause the maximum amount of mortality against a host, synchronization while possessing maximum potential fecundity would be important. As previously indicated, *P. sulcata* emergence coincides with a high availability of their preferred host stage. During this time as well, their mature egg complement is fully developed. Therefore, *P. sulcata* should be able to exert the maximum amount of mortality on *A. planipennis* at all ages up to 25 days old. Although *P. sulcata* is an example of a proovigenic species, surveys of wasp parasitoids have indicated that a large majority of parasitoid species are synovigenic (Jervis et al. 2001). Ellers and Jervis (2004) concluded that this may be linked to the distribution of hosts throughout the environment in which parasitoids emerge. In many environments, hosts distribution is patchy rather than uniform. Consequently, synovigenic parasitoids, which can be flexible in the production and observation of eggs, would be selected for rather than those with fixed egg complements. Due to their shortened longevity and inability to produce eggs according to host availability, proovigenic parasitoids are not as successful under these conditions. However, proovigenic parasitoids would be selected for in an environment where hosts were in high densities and patchiness was minimized (Ellers and Jervis 2004). Interestingly, high levels of parasitization were restricted largely to the McKeough Dam and Dutton sites where *A. planipennis* infestations are well established. In such environments, *A. planipennis* distributions would be relatively uniform as compared to satellite populations, or areas of lower ash densities such as urban areas. Surveys of both types of environments have shown *P. sulcata* parasitization to be lower than at
the Dutton and McKeough Dam sites. This may indicate that for *P. sulcata* to be an effective enemy, it should be used in sites where hosts densities and distributions are high and uniform respectively. In areas of low densities and/or patchy distributions, releases of a synovigenic parasitoid such as *Atanycolus* spp. and *B. indica*, or chemical controls should be considered.

### 2.4.3 Super-/multiparasitism

Dissections of *A. planipennis* larvae parasitized by *P. sulcata* showed that both super- and multiparasitism were rare. For solitary parasitoids, successful development and emergence requires that only one parasitoid can be reared from an individual host (van Alphen and Visser 1990). If superparasitism does occur, one of the eggs may be destroyed by the other competitor either through physical combat (Bai and Mackauer 1991; Ueno 1997), or by physiological means (Jorgensen 1975; Hegazi et al. 1991). In some instances, multiple parasitoids may emerge from superparasitised hosts, albeit with negative impacts on fitness including reduced dry weight (Tunca and Kilincer 2009), prolonged emergence (Harvey et al. 1993; Sousa and Spence 2000), and increased mortality before emergence (Avni 1996) often occurring. Solitary parasitoids often possess methods for marking and avoiding parasitized hosts (Danci et al. 2011). It is likely that *P. sulcata* possesses a similar avoidance mechanism; however, further studies would be required to confirm this. Avoiding superparasitism has been regarded as an important attribute of an effective biological control agent as the aforementioned negative effects may hinder the overall effectiveness of the enemy (Coppel and Mertins 1977; DeBach and Rosen 1991). Therefore, the low levels of superparasitism observed in our study indicate that *P. sulcata* possesses this important attribute of an effective biological control agent.

These results indicate that multiparasitism may not be common between *P. sulcata* and the idiobiont ectoparasitoids *Atanycolus* spp. Like superparasitism, multiparasitism can be
detrimental to the success of a biological control agent, especially if the wasp of importance is a koinobiont endoparasitoid such as *P. sulcata*. Koinobionts allow the host to continue developing after parasitisation, while idiobionts paralyze the host during oviposition (Quicke 1997). If a host already contains an endoparasitoid, then it too will be paralyzed and consumed along with the rest of the host’s contents. Koinobionts are therefore intrinsically weaker competitors when faced with an idiobiont (Petters and Stefanelli 1983; Mitsunaga and Yano 2004; Harvey et al. 2013). In rare circumstances however, some koinobionts have developed methods of avoiding competition with idiobionts. Koinobionts may secrete substances during oviposition into the host that may alter the internal environment in order to promote the parasitoid’s development (Asgari 2006). In some cases, these substances include toxins that may eliminate competitors. For example, Uka et al. (2006) observed that *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae) secreted proteins that were toxic to other competitors feeding on the host. Consequently, *C. floridanum* endoparasitoids were able to prevent the establishment of other parasitoid larvae which may have impeded its development. Some koinobiont species have demonstrated a resistance to paralyzing compounds in idiobiont venom (Quicke 1997; Luna et al. 2010), and thus such species may be able to sequester a majority of the available host nutrients before the competing idiobiont. In some cases, both parasitoids may each complete their development and emerge successfully. This sharing of nutrition has been termed ‘resource sharing’ and has been observed occasionally in cases of multiparasitism (Magdaraog et al. 2012). It may be possible that one or potentially both of these tactics may be utilized by *P. sulcata*. Further laboratory assays with *P. sulcata* and other idiobiont competitors may explain how our results were possible. If *P. sulcata* possesses methods for overcoming competition by idiobionts, then the combined release of *P. sulcata* and an idiobiont parasitoid may be a possible option for an augmentative release program.
2.4.4 Flight period

This study provides evidence that synchronization likely exists between *P. sulcata* adults and their preferred *A. planipennis* host stage. An overlap of the preferred host stage is vital for the success of a biological control (Coppel and Mertins 1977; DeBach and Rosen 1991; Collier and van Steenwyk 2004). Parasitoids possess sensory and olfactory mechanisms that are specialized usually only to their preferred host stage (Vinson 1985). Therefore, if the preferred host stage is not available, then parasitoids will not be able to detect the host species. Even if oviposition in non-preferred host stages occurs, developing parasitoids often experience increased mortality while associated with the host (Harvey and Strand 2002; Henry et al. 2005). A temporal overlap of the preferred host stage and the adult parasitoid is thus vital if effective control is to be accomplished. Trap catches of *P. sulcata* adults indicated that emergence begins and peaks in early- to mid-July, and decreases into early-October. Phenology of *A. planipennis* immature stages in the previous year indicates that first instar larvae at the same site were most abundant during this time (Chapter 5). This indicates that *P. sulcata* adults are present in the environment when their preferred host stage is also present. This synchronization is likely an important factor in the high levels of parasitization of observed by Lyons (2010). For *Atanycolus* spp., synchronization between the adult parasitoid and the preferred host late-larval host stage likely does not occur, as *Atanycolus* spp. adults were shown to emerge before late-instar *A. planipennis* larvae are present in large numbers (Chapter 5). Although some *Atanycolus* spp. parasitism was observed at all of our study sites, the number of *Atanycolus* spp. emerging from *A. planipennis*-infested logs was always very much lower than the number of *P. sulcata*. It is likely that lower amounts of *Atanycolus* spp. parasitisation are related to the lack of strong temporal synchronization between adult wasps and late-instar larvae. Out of the two parasitoids,
it appears *P. sulcata* may be a more effective natural enemy of *A. planipennis* in sites where the two parasitoids co-exist due to their emergence when their preferred host is available.

### 2.4.5 Morphology of the immature stages of *P. sulcata*

The conclusion that *P. sulcata* has three distinct larval instars plus a pre-pupal and a pupal stage appears to be consistent with current literature on the immature development of chalcidoids. I found mature *P. sulcata* eggs to be smooth, oval-shaped, and likely hymenopteriform which is characteristic of most chalcidoid parasitoids (Clausen 1940). The number of larval instars in chalcidoids has been shown to vary (Clausen 1940). For example, *Hyssopus pallidus* (Askew) (Hym.: Eulophidae) has been observed to have five larval instars (Tschudi-Rein and Dorn 2001). Four larval instars have been reported for *Pteromalus cereallae* (Hym.: Pteromalidae) (Onagbola and Fadamiro 2008) and both *Chrysolampus schwarzi* Crawford and *Chrysolampus sisymbrii* (Ashmead) (Hym.: Perilampidae) (Darling and Miller 1991). Three instars have been reported in both *Macroglenes penetrans* (Kirby) (Hym.: Pteromalidae) (Doane et al. 1989) and *Entedon leucogramma* (Ratzeburg) (Hym.: Eulophidae) (Beaver 1966). Within Chalcididae, the number of larval instars varies, with *Spilochalcis side* (Walker) possessing four (Arthur 1958), while five instars were observed in *Brachymeria intermedia* (Nees) (Dowden 1935). In most cases, the first instar is often the most distinctive, with the remaining instars almost always moving towards hymenopteriform (Parker 1924; Clausen 1940). Indeed, I observed that the first instar larva was a distinctive mandibluate type, with the third instar and prepupa being hymenopteriform. Such specialization of the first instar is often due to the specialized environment in which the instar resides. A striking example is the planidial type larvae typical of perilampids. As this stage, rather than the adult female, is required to conduct the host searching, first instar perilampids are covered in hardened tergites,
possess long cerci, and are generally equipped to both move in the external environment and withstand dessication outside the host (Heraty and Darling 1984). Mandibulate-type larvae are typical of the first instars of solitary endoparasitoids (Hagen 1964). Because solitary parasitoids require the entire host to complete their development, early instars must eliminate any potential con specifics present in the host. By possessing large, well developed mandibles, these larvae are equipped to deal with competitors (Vinson and Hegazi 1998). As the larva matures, the need to fight other parasitoids for possession of the host is diminished. Consequently, later instars do not require the specialized structures observed in previous instars (Hagen 1964). The presence of hymenopteriform later instars confirms that this is likely the scenario in the development of *P. sulcata*. Lastly, I observed that the prepupal stage possessed characteristics such as imaginal eyes and an enlarged abdominal area. These traits are typical of other documented prepupae (Kazimirova and Vallo 1999; Onagbola and Fadamiro 2008).

### 2.4.6 Conclusions

In this chapter, I analyzed selected life history traits of *P. sulcata* as they related to *A. planipennis*, including adult longevity, sex-specific emergence, and emergence in relation to the host and *Atanycolus* spp., occurrence of super-/multiparasitism, age-specific fecundity, and adult flight period. The results of this work indicate that *P. sulcata* possesses several attributes that are consistent with those of effective biological control agents. The primary attribute is synchronization with the preferred host stage. I found that when the preferred early instar host stage, *P. sulcata* adults are present, have the potential to have been mated, and possess a large number of mature eggs. The synchronization of all these traits with the occurrence of the preferred host stage is important as it maximizes the chance that a large proportion of available hosts can be parasitized. I have also shown that *P. sulcata* exhibits low super-/multiparasitism
and a female-biased sex ratio, both important attributes present in successful biological control agents. My work has also shown that *P. sulcata* is proovigenic based on the presence of mature eggs at emergence. Therefore, in sites where host density is high, such as the site used in this study, high levels of parasitization can occur. However, the increased reproductive effort shown in developing proovigenic parasitoids results in a shorter longevity. This is apparent in the relatively short longevity of adult parasitoids when compared to synovigenic species. Due to this short longevity, proovigenic parasitoids like *P. sulcata* are likely to be ineffective at sites where host density is low or patchy. Indeed, *P. sulcata* densities have been shown to vary dramatically across sites. It is possible that their ovigeny condition upon emergence may be a reason for this. Therefore, any possible *P. sulcata* releases should be applied to sites where *A. planipennis* populations are high in order to maximize their effectiveness. In sites where natural populations of *P. sulcata* are present, the reproductive and temporal synchronization of *P. sulcata* adult females with the preferred host stage should allow these populations to exert high amounts of parasitization similar to the amounts shown by Lyons (2010) and in Chapter 5. Lastly, I observed that *P. sulcata* likely has three larval instars, plus a prepupal and pupal stage. These conclusions are consistent with current literature on chalcidoïd development. These results may assist in both the detection of *P. sulcata* individuals in dissected *A. planipennis*, and serve to expand the currently limited literature on chalcidid immature development.
Table 2.1: Mean (± S.E.) emergence times of *Agrilus planipennis*, *Atanycolus* spp., and *Phasgonophora sulcata* from *Fraxinus* logs collected from *A. planipennis*-infested sites in southwestern Ontario. A ‘n/a’ denotes that for that particular species group, not enough insects emerged to determine a reliable mean. All logs were collected in fall of the collection year.

<table>
<thead>
<tr>
<th>Year of collection</th>
<th>Site</th>
<th>Coordinates</th>
<th><em>Agrilus planipennis</em> emergence ((\bar{x} \pm \text{S.E.})) time (d)</th>
<th><em>Atanycolus</em> sp. emergence ((\bar{x} \pm \text{S.E.})) time (d)</th>
<th><em>Phasgonophora sulcata</em> emergence ((\bar{x} \pm \text{S.E.})) time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>‘Larry’s’</td>
<td>42.06257, -82.89026</td>
<td>15.1 ± 0.5 (n = 62)</td>
<td>n/a</td>
<td>37.4 ± 0.8 (n = 61)</td>
</tr>
<tr>
<td>2008</td>
<td>Sarnia Golf and Curling Club</td>
<td>42.99545, -82.39970</td>
<td>37.80 ± 0.1 (n = 1425)</td>
<td>16.2 ± 0.4 (n = 74)</td>
<td>50.5 ± 1.5 (n = 24)</td>
</tr>
<tr>
<td>2009</td>
<td>McKeough Dam</td>
<td>42.41416, -82.24145</td>
<td>30.6 ± 0.382 (n = 581)</td>
<td>19.3 ± 0.4 (n = 72)</td>
<td>53.4 ± 0.3 (n = 387)</td>
</tr>
<tr>
<td>2010</td>
<td>McKeough Dam</td>
<td>42.41416, -82.24145</td>
<td>n/a</td>
<td>22.0 ± 1.4 (n = 38)</td>
<td>45.5 ± 0.7 (n = 46)</td>
</tr>
<tr>
<td>2010</td>
<td>Dutton</td>
<td>42.65890, -81.55401</td>
<td>32.8 ± 0.1 (n = 1433)</td>
<td>n/a</td>
<td>57.0 ± 1.0 (n = 147)</td>
</tr>
</tbody>
</table>
Table 2.2: Mean (± S.E.) adult emergence times for *Phasgonophora sulcata* reared from *Agrilus planipennis*-infested *Fraxinus* sp. collected from sites in southwestern Ontario from 2009 to 2011 (Kruskall-Wallis One Way Analysis of Variance on Ranks, \( P = 0.05 \)).

<table>
<thead>
<tr>
<th>Year of emergence</th>
<th>Site</th>
<th>♂ emergence ((\bar{x}) ± S.E.) time (d)</th>
<th>♂ emergence ((\bar{x}) ± S.E.) time (d)</th>
<th>Mann-Whitney U Statistic</th>
<th>T</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>‘Larry’s’</td>
<td>34.3 ± 1.5 (n = 24)</td>
<td>38.7 ± 0.9 (n = 61)</td>
<td>412.00</td>
<td>712.0</td>
<td>0.002</td>
</tr>
<tr>
<td>2010</td>
<td>McKeough Dam</td>
<td>49.1 ± 0.4 (n = 112)</td>
<td>55.3 ± 0.3 (n = 271)</td>
<td>3373.0</td>
<td>9701.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2011</td>
<td>Dutton</td>
<td>52.6 ± 1.1 (n = 51)</td>
<td>58.1 ± 1.6 (n = 1112)</td>
<td>1931.0</td>
<td>3257.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 2.3: Sex ratios of *Phasgonophora sulcata* emerging from *Agrilus planipennis*-infested logs collected from three sites in southwestern Ontario from 2009 to 2011.

<table>
<thead>
<tr>
<th>Year of emergence</th>
<th>Site</th>
<th>No. of ♀ <em>Phasgonophora sulcata</em> adults</th>
<th>No. of ♂ <em>Phasgonophora sulcata</em> adults</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>‘Larry’s’</td>
<td>61</td>
<td>24</td>
<td>2.5:1</td>
</tr>
<tr>
<td>2010</td>
<td>McKeough Dam</td>
<td>271</td>
<td>112</td>
<td>2.4:1</td>
</tr>
<tr>
<td>2011</td>
<td>‘Dutton’</td>
<td>112</td>
<td>51</td>
<td>2.2:1</td>
</tr>
</tbody>
</table>
Figure 2.1: Emergence of overwintered *Atanycolus* spp., *Agrilus planipennis*, and *Phasgonophora sulcata* from *Fraxinus* sp. logs collected from the McKeough Floodplain in autumn of 2009.
Days after removal from cold storage

No. of emerged insects

Figure 2.2: Emergence of overwintered *Agrilus planipennis* and *Phasgonophora sulcata* from *Fraxinus* sp. logs collected from ‘Larry’s’ site in autumn of 2008.
Figure 2.3: Emergence of overwintered male and female *Phasgonophora sulcata* from *Fraxinus* sp. logs collected from ‘Dutton’ site, Southwestern Ontario in autumn of 2010.
Figure 2.4: Emergence of overwintered male and female *Phasgonophora sulcata* from *Fraxinus* sp. logs collected at the McKeough Floodplain, Southwestern Ontario in autumn of 2009.
Figure 2.5: Emergence of overwintered male and female *Phasgonophora sulcata* from *Fraxinus* sp. logs collected at ‘Larry’s’ site, Southwestern Ontario in autumn of 2008.
Figure 2.6: Effect of age on total mature egg count (± S.E.) in adult female *P. sulcata*. Different letters indicate significant (One-way ANOVA, *P* < 0.05) differences between age categories.
Figure 2.7: The effect of age on mature viable egg load ($\bar{x} \pm $ S.E.) and number of eggs ($\bar{x} \pm $ S.E.) undergoing oobsorption in adult female *P. sulcata*. Different letters indicate significant (One-way ANOVA, $P < 0.05$) differences between age categories. Capital letters represent significant differences between age categories for eggs undergoing oobsorption, and lower-case letters represent significant differences between age categories for the mean number of mature viable eggs.
Figure 2.8: Number of *Agrilus planipennis*, *Phasgonophora sulcata*, and *Atanycolus* spp. adults caught on sticky traps (No. of insects/trap; $\bar{x} \pm$ S.E.) at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario, from 6 June to 4 October 2011.
Figure 2.9: Ventral view of the first instar larva of *Phasgonophora sulcata.*
Figure 2.10: Ventral view of the second instar larva of *Phasgonophora sulcata*. Note the tail, which was not present in all specimens.
Figure 2.11: Ventral view of the head of second instar larva of *Phasgonophora sulcata*. Note the antennal lobes (*ant*) and palps (*plp*) on the posterior edge of the mandibles.
Figure 2.12: Ventral view of the third instar larva of *Phasgonophora sulcata.*
Figure 2.13: Ventral view of the head of a third instar *Phasgonophora sulcata* larva.
Figure 2.14: Ventral view of the prepupa/fourth instar larva of *Phasgonophora sulcata.*
Figure 2.15: Ventral view of the head of a prepupal *Phasgonophora sulcata*. Note the pleurostoma (*pls*) and hypostoma (*hyp*).
Figure 2.16: Pupae of *Phasgonophora sulcata*. From top to bottom, images represent pupae taken from host pupal chambers at three dates, with the first pupa representing the youngest, and last image the oldest.
Chapter 3: Courtship sequence and evidence of mating pheromones in

*Phasgonophora sulcata*, a North American parasitoid of *Agrilus planipennis*

3.1 Introduction

*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is a significant invasive pest of ash (*Fraxinus* sp.) in Canada and the United States. Depending on latitude, adults emerge from infested trees in early- or mid-summer and, following mating, lay their eggs in bark crevices and under bark scales of ash trees (Poland and McCullough 2006). Larvae hatch from the eggs and burrow through the bark into the conductive tissues (Haack et al. 2002). There, larvae feed on the phloem and xylem tissues causing disruption in nutrient and water transport within the tree that ultimately leads to tree death. Since its accidental introduction to North America in the early 1990’s, *A. planipennis* has killed millions of ash trees in infested areas. Potential costs of *A. planipennis* treatment in the United States has been estimated at $10.7 billion, while the potential combined costs of *A. planipennis* treatment in Canadian urban centers have approached $1.5 billion (Kovacs et al. 2010; McKenney et al. 2012). Ash is also a major component of forest regions throughout North America, including riparian corridors, and its elimination in these regions will lead to significant ecological disturbances (Poland and McCullough 2006). While regulatory efforts to contain the spread of *A. planipennis* are being conducted in both countries, difficulties associated with detection and human-mediated dispersal have meant that biological and chemical controls are required for the long-term management of this pest. In urban centers, where ash densities are low, insecticides such as TreeAzin have proven effective; however, applications in forested areas are not viable due to prohibitive costs associated with application
Consequently, the introduction or augmentation of natural enemies such as wasp parasitoids may be a potentially efficacious and cost-effective management option.

In the United States, two larval parasitoids - *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Spathius agrili* Yang (Hymenoptera: Braconidae) - and an egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), were identified in China, and have subsequently been studied, reared, and released in 14 states (Liu et al. 2003; Wang 2007; Bauer et al. 2008; Yang et al. 2008; Duan et al. 2009; 2010; Ulyshen et al. 2010b; Wang et al. 2010; Duan et al. 2011a; Dean et al. 2012; Duan and Oppel 2012; USDA-APHIS/ARS/FS 2012; Yang et al. 2012). Preliminary observations have shown that host parasitization and establishment has occurred in many sites and that released parasitoids show potential as effective enemies of *A. planipennis* in North America. Research on rearing, dispersal, and population establishment in release sites are ongoing (USDA-APHIS/ARS/FS 2012).

Surveys of *A. planipennis* populations conducted in Michigan from 2002-2004 indicated that <1% of *A. planipennis* were parasitized by native parasitoids (Bauer et al. 2008). However, recent surveys conducted in Michigan, Pennsylvania, and Ontario have shown parasitization by several North American parasitoid species has reached appreciable levels (Cappaert and McCullough 2009; Duan et al. 2010; Lyons 2010). Native parasitoids observed attacking *A. planipennis* included several *Atanycolus* spp. (Hymenoptera: Braconidae), *Balcha indica* (Mani and Kaul) (Hymenoptera: Eupelmidae), and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae). In southwestern Ontario, inferences from sticky band traps indicate that parasitism by *P. sulcata* on *A. planipennis* can be as high as 40% at some sites (Lyons 2010). Consequently,
investigations into the feasibility of incorporating *P. sulcata* into an augmentative biological control program are underway.

*Phasgonophora sulcata* is a koinobiont endoparasitoid of buprestid beetles in North America (Boucek 1992). It is among a unique group of chalcidids that attack wood-boring beetle larvae, rather than exposed Diptera and Lepidoptera (Boucek 1988). *Phasgonophora sulcata* has been reported parasitizing North American *Agrilus* spp., including *A. anxius* Gory (Loerch and Cameron 1983; 1984) and *A. bilineatus* (Weber) (Haack and Benjamin 1982). Although some life history parameters in relation to North American *Agrilus* spp. have been determined, a comprehensive knowledge of such parameters in relation to *A. planipennis* has yet to be compiled. Observing both the interactions of *P. sulcata* with *A. planipennis* and its basic biology are necessary in the evaluation of this parasitoid’s potential as an augmentative control agent of *A. planipennis*.

If a biological control agent is to be reared for augmentative release, it is necessary to understand its mating and courtship sequences. Parasitoid species often follow a species-specific mating process so that matings with other species are avoided (van den Assem 1986). Determining the courtship sequences of a biological control agent is necessary in the formulation of a biological control program if insect mass production is to occur. In the majority of mating pairs, the release of sex pheromones by the female initiates the sequence (Quicke 1997). These pheromones are required allow males to orient to the female’s location, and may work at close and/or long-ranges (McNeil and Brodeur 1995; Ruther et al. 2000; Collatz et al. 2009; Onagbola and Fadamiro 2011). Pheromone production is critical to the mating process particularly if spatial or temporal obstacles, such as protandrous emergence, exist. Pheromone detection by males is also required to initiate their courtship actions after locating the female (Vinson 1972;
Ruther et al. 2000). These actions may include wing-fanning, rocking of the head or the body, and antennal stroking (Barras 1960; van den Assem 1986; Field and Keller 1993; Bin et al. 1999; Ruther et al. 2000). Following orientation and location, mating pairs engage in a number of repeatable events before copulation. Pheromone production and mating receptiveness can, however, be affected by female status. Female age has been shown to influence the detectability of females by males (Collatz et al. 2009). In some species, older females are less attractive to males (Schworer et al. 1999; McClure et al. 2007), while in other species, newly emerged females are less attractive than older females (Tagawa et al. 1985; Kainoh 1986). Therefore, predicting when *P. sulcata* females are attractive to males may be difficult. Determining this period is necessary if insect production is to occur. Additionally, pheromones may be useful in quantifying *P. sulcata* populations in sites prior to parasitoid release as part of an insect-specific trapping protocol (Kainoh 1999). Consequently, the identification of when a female parasitoid is able to produce detectable sex pheromones and mate is important for both insect production and for ensuring the establishment of released populations.

This study was designed to analyze the mating processes of *P. sulcata*. Specifically, I sought to determine: 1) if an intraspecific pheromone exists within *P. sulcata*, and to determine if parasitoid age has an effect on production and/or detectability and 2) to describe the courtship and copulation sequences of adult *P. sulcata*. These results will contribute to the knowledge necessary to comprehensively evaluate *P. sulcata* as a viable *A. planipennis* biological control agent.
3.2 Methods

3.2.1 Parasitoids

Adult parasitoids were reared from *A. planipennis*-infested *Fraxinus* spp. logs retrieved from sites in Lambton and Middlesex counties in southwestern Ontario (McKeough Floodplain, 42.41416, -82.24145; Dutton, Ontario, 42.65890, -81.55401). All sampled logs were retrieved in October 2011, and held at 4°C in incubation chambers for at least six weeks. After this overwintering period, logs were placed in rearing cages and were incubated at 25°C with 60 ± 5% relative humidity (RH). Following emergence, adult *P. sulcata* were kept in sex-specific groups in plastic cups, with up to 8 parasitoids per cup, which were covered with a plastic mesh screen to facilitate air circulation. Adults were held at 26 ± 1°C and 70 ± 5% RH, and a photoperiod of 16:8 (light: dark). Adults were provided with water from a 12 ml vial plugged with two cotton dental wicks and a synthetic strip of shamy, and honey that was applied to the wire mesh of the lid. Both water and honey were replaced every two or three days.

3.2.2 Y-tube olfactometer bioassay

A Y-tube olfactometer (Analytical Research Systems Inc., Gainsville FL) was used to test the attraction of virgin 1-5 and 10-15 day old adult male and female *P. sulcata* to conspecifics. The Y-tube consisted of an 11 cm main stem that branched into two distal 9 cm arms. The internal diameter of the tube was 1.5 cm. Each arm was attached to connecting arm that either contained an insect (treatment) or was left empty (control). Air was passed over five parasitoids of a specific treatment group in the treatment connecting tube, while clean air was pumped through an identical but empty connecting arm that was attached to the other distal arm. A wire mesh screen was placed at either end of the connecting arms to prevent insects from escaping. Air was drawn into the olfactometer, humidified, purified, and passed through a charcoal filter...
into the connecting arms and into the Y-tube. Air travelled into each arm of the Y-tube at a rate of 900 ml/min. To prevent visual distractions for the parasitoids during the bioassay, the treatment arm was placed inside a paper sleeve. This prevented parasitoids travelling through the Y-tube from observing the parasitoids in the treatment arm. An office desk lamp with a 40W bulb that was placed approximately 30 cm above the Y-tube to provide illumination.

During the bioassay, a single parasitoid was released into the base of the central arm of the Y-tube and was observed for 10 minutes. A choice of either the treatment or control was recorded if the parasitoid reached the end of the distal arm and touched the wire sieve of the connecting tube. A non-response (NR) was recorded if the parasitoid did not make a choice within the allotted time. After three individual parasitoids had been tested, the entire Y-tube apparatus was flipped to prevent bias. After 6-8 parasitoids had been tested, the Y-tube and connecting tubes were washed with hot soapy water, rinsed with acetone, and air-dried. Parasitoids were used only once, and at least 24 parasitoids were used for each analysis. All bioassays were completed at ~26°C and ~60% RH. Bioassays were first analyzed using a $\chi^2$ goodness-of-fit test to test if the ratio of parasitoids choosing the treatment/control and ‘NR’ outcomes differed significantly from 1:1. In bioassays where a significant proportion demonstrated a treatment/control response, another $\chi^2$ goodness-of-fit test which determined if the ratio of parasitoids choosing either the control or treatment outcomes differed significantly from 1:1 was carried out. Statistics were carried out in SigmaPlot (V.12).

### 3.2.3 Mating sequences of *P. sulcata*

To determine the mating sequences for adult *P. sulcata*, males and females were observed in an arena consisting of a 9.0 cm circular filter paper (Cat. 1005 090, Whatman International Ltd., Maidstone, U.K.) with a 6.3 cm Pyrex glass lid that enclosed the parasitoid
mating pair. Preliminary results showed that males of various ages responded to females by sight and through olfaction. Therefore, males used varied in age from 1 to 30-days-old. Females were either newly emerged or >3-days-old. Preliminary observations indicated that four distinct behaviours occurred before copulation. These included 1) lateral swaying of the male (herein referred to as ‘swaying’) while walking (Fig. 3.1); 2) male swaying in front of the female while antennating; female remains still (Fig. 3.2); 3) swaying in front of female with erect antenna (Fig. 3.3); and 4) mounting and copulation (Fig. 3.4). For each bioassay, one male parasitoid was first introduced into the arena, followed by one female parasitoid. The mating pair was observed by eye for up to 20 minutes or until copulation occurred. The occurrence of each behaviour was recorded. If copulation occurred, the mating pair was placed alone in a plastic cup; otherwise parasitoids were put back into sex-specific containers. Parasitoids were only used in a bioassay once. Experiments were carried out at ~26°C and ~60% RH.

3.3 Results

3.3.1 Y-tube olfactometer bioassay

Significant differences in the proportions responders and non-responders were observed in the Y-tube bioassay. Only in bioassays where males were exposed to females did a significant proportion of the test insects respond to either the treatment or control (Table 3.1). In all other bioassays, including all bioassays where test insects were female and where males were exposed to males, there was either no significant difference between non-responders and responders, or a significant proportion were non-responders (Table 3.1).

In bioassays where a significant proportion of males responded, males were highly attracted to the female treatment over the control (Fig.). This result was consistent for all male and female age combinations.
3.3.2 Mating sequences of *P. sulcata*

Twenty seven male-female pairs were observed. Of these, twelve pairs were newly emerged females and 15 pairs were >3-day old females. Four pre-copulatory behaviours were identified: 1) male swaying with attention; 2) male swaying with erect antenna; 3) arresting of female movement; and 4) male mounts female and copulation occurs. Copulation only occurred in 3 out of 27 cases (Figure 3.9). One attempted copulation was observed, but the female ended the copulation shortly after it began. Behaviours 1-4 always occurred sequentially.

Behaviour (1) was observed when males first encountered females in the arena. The behaviour was observed in 92.6% of courting pairs. In mating pairs with newly emerged females, behaviour (1) was observed in 100% of courting pairs. In mating pairs with 3+ day-old females, behaviour (1) was observed in 86.7% of mating pairs. Males typically initiated this behaviour upon observing females; however, some would initiate this behaviour even if females were out of direct line of sight. Males would walk around the arena while conducting behaviour (1) while following the female directly. In some cases, males would also walk towards and antennuate areas of the arena where the female had recently been. The male would approach the female from the rear or side, and would then attempt to move in front of the female. While approaching from the rear or side, the male would occasionally tap the female with his antennae. Females would usually continue walking around the arena and ignore the male. This occurred in most cases. However, if the male successfully oriented itself in front of the female, behaviour (2) would follow.

Behaviour (2) only occurred in 29.6% of courting pairs. For newly emerged females, behaviour (2) was observed in 25.0% of courting pairs. In 3+ day-old females, behaviour (2) was observed in 33.3% of mating pairs. This behaviour would only occur if the male was directly in
front of and facing the female. Behaviours (2) and (3) did sometimes occur in quick succession; however females would usually ignore males and continue walking while the male was attempting behaviour (2). The swaying in this behaviour was different than in the previous, as males would stand upright, and occasionally stand on just their middle and posterior pairs of legs. This is different from their posture in behaviour (1), where males usually walked with their bodies very close to the substrate. Additionally, males usually walked around while swaying, whereas in behaviour (2) males were most often not walking or tracking but would remain stationary in front of the female.

Behaviour (3) only occurred in 18.5% of all courting pairs. This behaviour occurred in 16.7% and 20.0% of courting pairs with newly-emerged and 3+ day-old females, respectively. This behaviour would occur sometimes immediately after behaviour (2) began, but also could occur after several failed attempts by the male to move in front of the female. Besides arresting their own movement, females would also have their antennae extended out in front of their head in the direction of the male. While females were in behaviour (3), males would continue behaviour (2). While the female had her antenna extended, swaying males would alternately touch the tip of each antenna of the female with the front of their face.

Behaviour (4) occurred in 11.1% of courting pairs. For newly emerged females, behaviour (4) was observed in 8.33% of mating pairs. In 3+ day-old females, behaviour (2) was observed in 20.0% of courting pairs. Behaviour (4) only occurred if behaviour (3) had preceded it, and only in cases where touching of the female’s antennae by the male’s head took place. This behaviour would begin with the female lowering her head and antennae to the floor of the arena. At the same time, the female would raise the last segments of her abdomen to expose her genital pouch. The male would often briefly flick its wings, and mount the female via her head. While
mounting, the male would rotate his body and extend his aedeagus from the distal end of his abdomen. Upon reaching the female’s abdomen, the male would insert his aedeagus into the genital pocket of the female. Once inserted, the male would thrust himself forwards at a rate of about once every second. In one instance, a female had lowered her body and raised her abdomen, but the male did not attempt to copulate. This event was rare. Once the male began to mount the female, copulation nearly always followed.

Following copulation, males usually removed themselves from the female. In one case, the female removed the male from her by kicking him with one of her hind legs. Males and females showed no post-copulatory behaviours after copulation, and continued walking around the arena as they did before copulation. Males never swayed when in contact with females after copulation.

3.4 Discussion

In this study, I observed evidence that adult female *P. sulcata* produced detectable amounts of compounds that attracted adult males. I also found that female age did not affect male attraction, and that no intra-sexual communication compounds exist. The age of males did not influence their attraction to females. The courtship sequence for *P. sulcata* included several actions that were consistent between mating pairs; however, actual copulation events were rare for adult *P. sulcata* in the lab. Several actions seen in the mating sequence of *P. sulcata* are present in the mating sequences of other parasitic Hymenoptera; however, the unique sequence displayed by *P. sulcata* is necessary to reduce matings with other species.
3.4.1 Y-tube olfactometer bioassay

These results provided some important information about chemical signalling and mating in adult *P. sulcata*. A significant proportion of males oriented towards females exhibiting rocking behaviour during the olfactometer bioassay, suggesting that females produce an airborne volatile that is attractive to males. There appears to be no effect of age for either the male or the female, as females from both age groups elicited similar responses in both male age groups. The lack of significant responses of males detecting males, and females to either males or females suggests that intrasexual volatiles are not present. The frequent male response to females may provide the basis for population survey techniques and could be useful in a potential augmentative biological control program using *P. sulcata*.

The strong male response to females indicates that females likely produce a volatile sex pheromone as predicted. Although male-produced pheromones have been documented in other studies (Matthews et al. 1985; Cosse et al. 2012), sex pheromones are most often produced by females (Eller et al. 1984; Quicke 1997; Kainoh 1999). The production of pheromones by females is likely linked to whether or not the species is non-parthenogenic. In parthenogenic species, daughters can be produced without mating, while non-parthenogenic species require sperm to produce daughters (Godfray 1994). Consequently, extremely female-biased sex ratios are often indicative of parthenogenic species (Manzano et al. 2000). Because the production of daughters is essential to the fitness of the mother, non-parthenogenic parasitoids must mate. However, the encounter rate between male and female parasitoids is likely very low, especially for solitary parasitoids. Therefore, the selection for the production of sex pheromones that attract and initiate male courtship should occur in non-parthenogenic species (Godfray 1994). *Phasgonophora sulcata* is likely non-parthenogenic because of the relatively similar numbers of
males and females found in the population (Haack et al. 1981). Therefore, it should be expected that females produce a sex pheromone that attracts males. Our results suggest that females produce such a compound, and that it is vital for both male orientation and in the initiation the mating sequence.

The detection of female-produced volatiles by both age classes of males agrees with the theory that males can maximize their fitness by mating with multiple partners (Arnqvist and Nilsson 2000). By remaining receptive to females throughout their lives, males are able to increase the number of matings they can potentially secure in their lifetime. The lack of an age-effect on male detection abilities has been observed in another chalcid, Brachymeria lasus (Walker) (Hymenoptera: Chalcididae), where male age did not significantly affect responsiveness to female-produced pheromones (Simser and Coppel 1980). Similarly, both young and old P. sulcata males responded strongly to female volatiles during our bioassay. Therefore, it appears that P. sulcata males are maximizing their ability to locate females and thus maximizing their fitness. I also observed that both young and old females were readily detected; therefore female age likely has no affect on detectability by males. Female pheromone production has been observed to vary depending on age in other parasitoids (Tagawa et al. 1985; Schworer et al. 1999; McClure et al. 2007; Collatz et al. 2009). However, the constant detectability of pheromones in this case may be due to female mating status. Solitary parasitoids (i.e., those that only lay one egg at a time) are typically monandrous and usually only mate once in their lives (Ridley 1993). After mating, female parasitoids often stop producing detectible levels of pheromone (Quicke 1997; Ruther et al. 2000). In this study, all female parasitoids were virgin, and thus, because P. sulcata is a solitary parasitoid, would likely continue to produce volatiles unless mated. Additionally, female P. sulcata are proovigenic and maintain their mature
egg complement for most of their adult lives (Chapter 2). This adds further incentive to locating a mate if the female is to produce daughters. Further studies that compare virgin versus mated females would be required to confirm this. However, it appears that mating status rather than age likely influences whether or not volatile pheromone production continues.

The bioassay suggests that intrasexual attraction does not occur in *P. sulcata*. The attraction of adults to live conspecifics of the same sex has been observed in other studies (Kainoh 1999; Onagbola and Fadamiro 2011), with the reasoning being that mating opportunities may be increased through aggregation. A possible reason for this not occurring in *P. sulcata* is that females are likely monandrous. Because females are only receptive to mating once, it is critical that males be able to locate virgin females before other males in order to copulate. If males were to also release a pheromone that attracted other males, then males responding to the female pheromone would also be accompanied by males responding to intrasexual pheromones. This may result in high numbers of males competing for a very small number of females. This could hinder the ability of the individual male to copulate with the female. The production of female intrasexual pheromones may be advantageous for females, as males are usually always associated with females if they are producing pheromones, however these are very rare (Kainoh 1999).

### 3.4.2 Courtship sequences of *P. sulcata*

*Phasgonophora sulcata* courting pairs exhibited repeated and sequential behaviours during courtship. These stages were divided into four behaviours, the majority of which were undertaken by males, while some were carried out by the female. During the courtship process, the male takes an active role, while the female is largely passive. However, the few actions undertaken by the female were necessary to continue the courtship process through to copulation.
(i.e., arrestment, head-lowering). The examination of the courtship sequence of *P. sulcata* provides an interesting comparison to the mating sequences of other parasitoids. It illustrates that although many similar actions are observed across parasitoid species, the exact sequence and timing of behaviours varies, which prevent inadvertent mating between species.

The detection of airborne volatiles produced by the female is often interpreted as the first action of the courtship process (Leonard and Ringo 1978; van den Assem 1986; Field and Keller 1993). Upon detection, males initiate a specific action, such as perhaps wing-fanning and raising the abdomen (Vinson 1972; Field and Keller 1993; Ruther et al. 2000), or mounting of the female (van den Assem and Vernel 1979; Abdurahiman et al. 1983). In both *B. lasus* and *Spilochalcis albifrons* (Walsh) (Hymenoptera: Chalcididae), lateral swaying was observed as one of the first behaviours exhibited by the male after detecting the presence of the female (Leonard and Ringo 1978; Hansen 1980). Body swaying in *P. sulcata* was observed, and was interpreted as the first action of the courtship sequence. In the case of *B. lasus*, the authors concluded that lateral swaying assisted the males in detecting sex pheromones produced by the females (Leonard and Ringo 1978). This may be a factor in the occurrence of this behaviour in *P. sulcata* due to males swaying and walking while in the Y-tube olfactometer when exposed to female treatments. It has also been proposed that this behaviour assists the female in recognizing the approaching insect as a male (Leonard and Ringo 1978). Other authors have also suggested that swaying allowed the male to better observe a non-moving female (Wigglesworth 1950). Similar actions were observed in other chalcidids (Leonard and Ringo 1978; Hansen 1980). Again, the exact purpose of this is unknown, but a likely explanation is that the male is using receptors on its antenna to detect the female pheromone in both the air and the substrate so that it can better orient itself to its potential mate.

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Once the *P. sulcata* male has reached the female, it would usually approach from the rear or the side. Leonard and Ringo (1978) also observed that male *B. lasus* males would approach females from the rear, while Hansen (1980) noted that *S. albifrons* would approach from the side. While the male is searching for the female, the female is typically wandering and grooming. In the experiment, females would usually ignore approaching males and continue wandering. This was also observed by Leonard and Ringo (1978). Female arrestment only occurred if the male was able to move in front of the female and face her directly. At the same time, the male would straighten his antenna while continuing to sway. This continuation of the courtship process by directly facing the female was not observed in either *B. lasus* or *S. albifrons*. Rather, males would demonstrate from the rear or side of the female respectively, then mount the female, and continue the courtship sequence. Frontal approaches were, however, observed in *Lariophagus distinguendus* Först (Hymenoptera: Pteromalidae) males (Ruther et al. 2000). Possible reasons for frontal approaches may be so the female is able to recognize the male as a potential mate or so that the male can prevent the female from walking away. This latter reason may be the case in *P. sulcata*, as induction of female receptivity requires that the female directly face the male.

An important step in the courtship sequence that was consistent among many parasitoid mating pairs was the movement of the male’s head, either in very close proximity to the female’s head, or actually touching her antennae. This action has been seen in several species, including *S. albifrons* (Hansen 1980), *Antrocephalus hakonensis* (Ashmead) (Hymenoptera: Chalcididae) (Abdurahiman et al. 1983), *L. distinguendus* (Ruther et al. 2000), and *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) (Barras 1960; van den Assem and Vernel 1979). In all of the above mentioned parasitoids, the male mounts the female. While mounted, the male moves its head between the female’s antennae while touching them in succession. In *P. sulcata*, the
male sways its body so that its head is between the female’s antennae. While swaying, the male is also touching the female’s antennae with its head. It is believed that “antennal touch” behaviour (Onagbola and Fadamiro 2011) serves to transmit contact pheromones from the male to the female. Analysis of antennal morphology in other parasitoids has shown that glands exist on male antennae which may serve to transmit pheromones to the female (Isidoro and Bin 1995; Isidoro et al. 1996). Similar structures may exist on the antennae of male *P. sulcata*, and could serve a similar purpose in inducing female receptivity. Further analysis of antennal structure should be carried out to determine if these structures are present in *P. sulcata*.

After this antennal touching, receptive females typically lower their antenna and head, while simultaneously exposing their genitalia by raising their abdomen (Leonard and Ringo 1978; van den Assem and Vernel 1979; Abdurahiman et al. 1983; Field and Keller 1993; Ruther et al. 2000). In all of these species, the male mounts the female and copulation immediately follows. When *P. sulcata* females initiate these movements, males will mount the female from the front, rotate so they are facing the same direction as the female, and insert their aedeagus into the genitalia of the female. I observed males rhythmically and rapidly thrust their bodies forward while connected to the female by its genitalia. Similar movements were observed in *N. vitripennis* (Barras 1960), *S. albifrons* (Hansen 1980), and *A. hakonensis* (Abdurahiman et al. 1983). It is possible that these movements aid in the transfer of sperm to the female, but further analysis would be required to confirm this. Copulation in *P. sulcata* took approximately 1 minute. Times for other parasitoids varied from 28 to 36 seconds in *A. hakonensis* (Abdurahiman et al. 1983) to 3 minutes in *S. abifrons* (Hansen 1980). Following copulation, no specific post-copulatory behaviours were observed in *P. sulcata*. In some insects, post-copulatory behaviours may include the male remaining close to the female, likely as a way of preventing other males
from mating with her (Alcock 1994). Lack of post-copulatory behaviours was also observed by Leonard and Ringo (1978). It is possible that these monandrous species do not require mate guarding, as females usually are not detectable or receptive to further mating after copulation (Quicke 1997). Therefore, it is likely in the best interest of the male to quickly leave the female in search of another to female with whom to mate.

3.4.3 Conclusions

I observed that although the courtship process for *P. sulcata* included several actions that occur in other parasitoid species, the specific sequence of events in *P. sulcata* shared several important differences. Firstly, several parasitoid species conducted their courtship displays while mounted on the female. Conversely, *P. sulcata* males carried out their courtship actions directly in front of the female. Males only mounted the female immediately before copulation. This difference is likely important in preventing inappropriate matings with other species. It is likely though that the specific sex pheromones, which I believe there are evidence of based on the responses of males in the behavioural assay, mediate the courtship sequence. These would also be important in preventing any other species from participating in the mating process. The presence of a sex pheromone that is effective at attracting males would be an important tool for surveying parasitoids in potential release areas.
Table 3.1: Proportions of *Phasgonophora sulcata* adults either choosing a control/treatment or choosing neither (‘No response’) in a Y-tube olfactometer. ‘*‘ represents a significant proportion choosing either the control/treatment, or choosing neither (‘No response’). ‘---‘ represents no significant differences in proportions of parasitoids distributed across the two result categories (Chi-square test, df =1, \( P = 0.05 \)).

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>n</th>
<th>Choice</th>
<th>( X^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 1-5</td>
<td>Male 1-5</td>
<td>30</td>
<td>No response</td>
<td>5.56</td>
<td>0.018 *</td>
</tr>
<tr>
<td>Male 1-5</td>
<td>Male 10-15</td>
<td>20</td>
<td>No response</td>
<td>8.90</td>
<td>0.029 *</td>
</tr>
<tr>
<td>Male 1-5</td>
<td>Female 1-5</td>
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<td>Control/Treatment</td>
<td>10.8</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Male 1-5</td>
<td>Female 10-15</td>
<td>34</td>
<td>Control/Treatment</td>
<td>23.1</td>
<td>1.00*10^-4 *</td>
</tr>
<tr>
<td>Male 10-15</td>
<td>Male 1-5</td>
<td>24</td>
<td>No response</td>
<td>9.80</td>
<td>0.002 *</td>
</tr>
<tr>
<td>Male 10-15</td>
<td>Male 10-15</td>
<td>20</td>
<td>---</td>
<td>0.80</td>
<td>0.371</td>
</tr>
<tr>
<td>Male 10-15</td>
<td>Female 1-5</td>
<td>29</td>
<td>Control/Treatment</td>
<td>15.2</td>
<td>1.00*10^-4 *</td>
</tr>
<tr>
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<td>Female 10-15</td>
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<td>Control/Treatment</td>
<td>16.7</td>
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<td>Female 10-15</td>
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<td>---</td>
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<td>0.835</td>
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<tr>
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<td>---</td>
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</tr>
<tr>
<td>Female 10-15</td>
<td>Male 10-15</td>
<td>31</td>
<td>---</td>
<td>0.806</td>
<td>0.369</td>
</tr>
<tr>
<td>Female 10-15</td>
<td>Female 1-5</td>
<td>23</td>
<td>---</td>
<td>0.032</td>
<td>0.858</td>
</tr>
<tr>
<td>Female 10-15</td>
<td>Female 10-15</td>
<td>30</td>
<td>---</td>
<td>0.360</td>
<td>0.548</td>
</tr>
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</table>
Table 3.2: Responses of adult male *Phasgonophora sulcata* (1-5 and 10-15 days old) to airborne volatiles produced by female conspecifics in a Y-tube olfactometer. ‘*’ represents a significant proportion of adults responding to either the control (no volatile) or treatment (volatile) (Chi-square test, df =1, $P = 0.05$).

<table>
<thead>
<tr>
<th>Test (days old)</th>
<th>Treatment (days old)</th>
<th>n</th>
<th>Choice</th>
<th>$X^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 1-5</td>
<td>Female 1-5</td>
<td>30</td>
<td>Treatment</td>
<td>10.8</td>
<td>1.00*10^{-4} *</td>
</tr>
<tr>
<td>Male 1-5</td>
<td>Female 10-15</td>
<td>34</td>
<td>Treatment</td>
<td>27.3</td>
<td>1.00*10^{-4} *</td>
</tr>
<tr>
<td>Male 10-15</td>
<td>Female 1-5</td>
<td>29</td>
<td>Treatment</td>
<td>14.4</td>
<td>1.00*10^{-4} *</td>
</tr>
<tr>
<td>Male 10-15</td>
<td>Female 10-15</td>
<td>24</td>
<td>Treatment</td>
<td>18.2</td>
<td>1.00*10^{-4} *</td>
</tr>
</tbody>
</table>
Figure 3.1: Male \((m)\) and female \((f)\) *Phasgonophora sulcata* in a Petri dish mating arena. The male is moving towards the female while swaying laterally and antennating the air and substrate. These movements by the male constitute Behaviour 1.
Figure 3.2: Male ($m$) and female ($f$) *Phagomonopora sulcata* in Petri dish mating arena. Male has moved in front of female while continuing to sway and antennate. These actions by the male are referred to as Behaviour 2.
Figure 3.3: Male \((m)\) and female \((f)\) *Phasgonophora sulcata* in Petri dish mating arena. Male *P. sulcata* has moved in front of female and is swaying, but has straightened its antennae. This is referred to as Behaviour 3.
Figure 3.4: Male (m) and female (f) Phasgonophora sulcata in Petri dish mating arena. Male P. sulcata has crawled on top of the female via her head and thorax, and has now mounted on top of her. Female has lifted her abdominal segments exposing her genital pocket. Male turns 180°, inserts the tip of his abdomen into the female’s genital pocket, and begins copulating. These mounting and copulation are referred to as Behaviour 4.
Figure 3.5: Y-tube olfactometer responses of 1-5 day old virgin male *Phasgonophora sulcata* to airborne volatiles of male and female conspecifics aged 1-5 and 10-15 days old. Figure shows proportions of male 1-5 day old parasitoid test groups responding to either control or treatment (Responders) or not choosing within 10 minutes (Non-responders). Asterisks (*) represent a significant difference between proportions of responses (Chi-square test; $P < 0.05$).
Figure 3.6: Y-tube olfactometer responses of 10-15 day old virgin male *Phasgonophora sulcata* to airborne volatiles of male and female conspecifics. Figure shows proportions of male 10-15 day old parasitoid test groups responding to either control or treatment (Responders) or not choosing within 10 minutes (Non-responders). Asterisks (*) represent a significant difference between proportions of responses (Chi-square test; $P < 0.05$).
Figure 3.7: Y-tube olfactometer responses of 1-5 day old virgin female *Phasgonophora sulcata* to airborne volatiles of male and female conspecifics aged 1-5 and 10-15 days old. Figure shows proportions of female 1-5 day old parasitoids responding to either control or treatment (Responders) or not choosing within 10 minutes (Non-responders). Asterisks (*) represent a significant difference between proportions of responses (Chi-square test; $P < 0.05$).
Figure 3.8: Y-tube olfactometer responses of 10-15 day old virgin female *Phasgonophora sulcata* to airborne volatiles of male and female con-specifics aged 1-5 and 10-15 days old. Figure shows proportions of female 10-15 day old parasitoids responding to either control or treatment (Responders) or not choosing within 10 minutes (Non-responders). Asterisks (*) represent a significant difference between proportions of responses (Chi-square test; $P < 0.05$).
Figure 3.9: Y-tube olfactometer responses of 1-5 and 10-15 day old virgin male *Phasgonophora sulcata* to airborne volatiles of female conspecifics aged 1-5 and 10-15 days old. Figure shows proportions of male parasitoids responding to either clean air (control) or female conspecifics (treatment). Asterisks (*) represent a significant difference between proportions of responses (Chi-square test; \( P < 0.05 \)).
Figure 3.9: Young (newly emerged), old (3+ day old), and total (sum of young and old groups) proportions of adult *Phasgonophora sulcata* mating pairs exhibiting mating behaviours (1-4) during observational study.
Chapter 4: Chemical ecology of *Phasgonophora sulcata* in relation to the non-native *Agrilus planipennis* and an evaluation of trap type on the detection of *A. planipennis* parasitoids in southwestern Ontario

4.1 Introduction

*Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) is a koinobiont endoparasitoid of buprestid larvae in North America (Boucek et al. 1997). While most chalcidids attack exposed immature Lepidoptera and Diptera, *P. sulcata* and other members of the Tribes Phagonophorini and Cratocentrini attack wood-boring Coleoptera larvae exclusively (Boucek 1988). *Phasgonophora sulcata* is a known parasitoid of North American *Agrilus* spp., including the bronze birch borer, *A. anxius* Gory and the two-lined chestnut borer *A. bilineatus* (Weber) (Haack et al. 1981; Loerch and Cameron 1983). Recently, *P. sulcata* has been observed attacking the emerald ash borer (*A. planipennis* Fairmaire), an important non-native pest of native and imported ash (*Fraxinus* spp.) in the northeastern United States and Canada (Lyons 2010). Since its official discovery in 2002, *A. planipennis* has spread from the Lake St. Clair region to a range that now includes 22 states and 2 provinces (USDA-APHIS 2013). Within infested areas, *A. planipennis* has killed millions of native and imported ash trees (Kovacs et al. 2010). Larvae feed within the cambium of infested ash, damaging the conductive tissues and eventually causing tree death (Haack et al. 2002). As this life stage is hidden, detecting outbreaks is difficult. This and its ability to attack healthy ash trees has made *A. planipennis* a serious threat to ash trees in infested regions and beyond (Kovacs et al. 2010). Economic losses associated with ash loss are very high, with expected costs for managing *A. planipennis* in urban municipalities in Canada
totalling approximately $890 million (McKenney et al. 2012). Ecological damage due to *A. planipennis* is also significant. Ash comprises a significant portion of the canopy in hardwood forest ecosystems and specialized areas such as riparian corridors (Poland and McCullough 2006). Its elimination from these regions would cause significant ecosystem disruption. The management of *A. planipennis* outbreaks and prevention of future infestations from establishing is therefore of great importance in both affected and susceptible areas.

In Canada, long-term management of *A. planipennis* will involve the use of biological control agents to reduce current populations to non-epidemic levels (Lyons 2010). Autodissemination traps containing strains of pathogenic fungi have shown potential in field studies; however, widespread production and dissemination of virulent strains has not yet been accomplished (Lyons et al. 2012). The use of parasitic wasps in a biological control program is also being investigated. In the United States, a classical biological control program using three Chinese parasitoids - *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), *Spathius agrili* Yang (Hymenoptera: Braconidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) - has been undertaken. Since 2009, parasitoids have been produced at a USDA-APHIS facility in Brighton, Michigan and released in Illinois, Indiana, Kentucky, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, Wisconsin and Ontario. Research on improving rearing and detection methodology is currently underway (USDA-APHIS/ARS/FS 2012). Surveys in the United States and Canada have also identified several parasitoids of *A. planipennis* that may be effective augmentative biological control agents. These include several *Atanycolus* spp. (Hymenoptera: Braconidae), *Balcha indica* (Mani & Kaul) (Hymenoptera: Eupelmidae), and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) (Cappaert and McCullough 2009; Duan et al. 2010;
Of the parasitoids studied in Canada, *P. sulcata* exerted the highest estimated amount of parasitization at approximately 40% (Lyons 2010). Very little biological and ecological information is available about this parasitoid. As such, studies are underway to develop an understanding of its life history as it pertains to *A. planipennis* so that its use in an augmentative control plan can be evaluated.

Parasitoids of plant pests often rely upon chemical cues, or ‘semiochemicals’, produced by the host or host-damaged plant to orient themselves to and within the host-habitat (Vinson 1976; Weseloh 1981). Semiochemicals produced by the host-plant are called synomones, while those produced by the host itself are called kairomones (Nordlund and Lewis 1976). Synomones, such as green-leaf volatiles and bark terpenoids, elicit host searching responses in many parasitoids, including those of concealed hosts (Camors and Payne 1972; Mills et al. 1991; Turlings and Tumlinson 1992; Potting et al. 1995; Rutledge 1996; Pettersson et al. 2001). Kairomones produced by the host itself and occasionally by a non-preferred host stage may also important for host searching by some parasitoids (Prokopy and Webster 1978; Bin et al. 1993; Wiskerke et al. 1993; Colazza et al. 1999; Hilker et al. 2000). Both types of semiochemicals are vital to parasitoids for host searching as these chemicals may indicate both the spatial and temporal location of the host.

Several possible semiochemicals that may be beneficial to host searching in *P. sulcata* have already been identified. Two potential synomones include a seven-compound C$_6$ volatile solution than comprises a green-leaf volatile mixture, and mixture of sesquiterpenes than is derived from bark tissue (Crook et al. 2008; de Groot et al. 2008). Another identified compounds associated with *A. planipennis* is  Derivations of both of these semiochemicals elicit host
searching responses in *A. planipennis*, and are currently used in population detection protocols (Crook and Mastro 2010). Another potential semiochemical that may be used as a kairomone is (3Z)-dodecen-12-olide (3-(Z)-lactone). This volatile is produced by both male and female *A. planipennis*, but is emitted in larger amounts by females (Bartelt et al. 2007). Studies have shown that 3-(Z)-lactone elicits significant responses in GC-EAD analyses for *A. planipennis* (Silk et al. 2009), and improves trap catches in green prism traps when used in combination with phoebe oil and (Z)-3-hexenol (Silk et al. 2009; Ryall et al. 2012; Ryall et al. 2013).

Identified semiochemicals may be applied in several ways within a biological control program. Applications include aggregations compounds that be applied to sites of low enemy densities, and as lures in survey traps (Lewis et al. 1972; 1975b; James 2003b; 2003a; 2005). Effective survey traps in particular are necessary to determine the outcome of parasitoid liberations, and to determine present natural enemy population levels. Assessing the enemy population densities after liberation is required for determining the need for any future liberations. If not undertaken, further liberations cannot be carried out with any certainty of their potential effectiveness. Physical characteristics of the trap such as size, color, shape and orientation must also be determined. Trap type has been observed to influence trap catch. For example, traps that present a silhouette similar to a tree-trunk have been shown to attract significantly more wood-associated insects than other non-cylindrical traps (Chenier and Philogene 1989). Trap color has also been shown to significantly influence parasitoid catches (Dowell and Cherry 1981; Moreno et al. 1984; McClain et al. 1990). Due to the sensitivity of insect catches in relation to trap characteristics, it is thus necessary to determine the physical characteristics of a survey trap so that target catch is maximized. Once identified, semiochemicals used by the target organism can then be incorporated into these traps. If
effective survey techniques are not developed, then monitoring the effectiveness of enemy populations cannot be accomplished.

The goals of this research were to: 1) identify one or more semiochemicals that might be used in host location by *P. sulcata*, 2) determine if these semiochemicals elicit antennal responses in adults of *P. sulcata*, and 3) test several trap designs and lures in areas where natural populations of *P. sulcata* occur so that an effective lure/trap combination can be identified. The behavioural and antennal responses of adult female *P. sulcata* to two potential synomones and one kairomone were tested. Once identified, these lures were combined with several trap designs that are currently available for capturing wasp parasitoids and wood-associated insects and the ability of these lure-trap combinations to catch *P. sulcata* adults was compared. I predicted that *P. sulcata* adults would initiate host searching and positive antennal behaviour when in contact with one or more of these semiochemical treatments, which themselves are either produced by the host-plant during feeding by *A. planipennis* or are produced by the host itself. I also hypothesized that trap color and shape would have important effects on parasitoid trap catch, with traps possessing the colour and shape of host trees likely attracting more parasitoids. Finally, I hypothesized that traps baited with the most attractive compounds identified in the behavioural assay would catch more parasitoids than unbaited traps or those baited with other compounds due to the intensified searching behaviour elicited in parasitoids when in the presence of attractive semiochemicals.
4.2 Methods

4.2.1 Gas-chromatography electroantennogram detection (GC-EAD)

Extracts of manuka oil, green-leaf volatiles, and 3-(Z)-lactone were analyzed using gas chromatography-electroantennogram detection (GC-EAD) to determine if they elicited electrophysical responses in antennae of 1-10 and 15+ days-old adult virgin female *P. sulcata*. Chemical surrogates were used as treatments for the green-leaf volatiles and bark terpenoids. This took place in order to ensure uniform doses across replicates. Synthetic mixtures of bark volatiles were also costly to produce and difficult to acquire for these experiments. Green-leaf volatiles were represented by a mixture containing equal parts of seven foliar C₆ molecules produced by damaged *Fraxinus* sp.: hexenal (98% purity), (E)-2-hexenal (98% purity), (Z)-3-hexenol (98% purity), hexenol (99% purity), and (Z)-3 hexenyl acetate (98% purity) (Sigma Aldrich Canada); and (E)-2-hexenol (95% purity), hexyl acetate (99% purity) (Bedoukian Research Inc., Danbury, CT, USA). Manuka oil was used as a surrogate for naturally-derived bark terpenoids. Manuka oil is a steam distillate of the New Zealand tea tree *Leptospermum scoparium* J.R. and G. Forst (Myrtaceae). It contains four of the six *Fraxinus*-derived bark sesquiterpenes in that elicit antennal responses in adult *A. planipennis* (Crook et al. 2008). The volatile used in these analyses Manuka oil and 3-(Z)-lactone were diluted in hexane to a concentration of 10 ng/ul, while the GLV mixture was diluted to the same concentration but in methylene chloride.

GC-EAD bioassays were performed as described by de Groot et al. (2008). A two microliter (µl) sample of an extract was injected into a Varian 3400 GC that was fitted with a non-polar HP-1 column (25 m x 0.20 mm with a 0.33 µm film thickness, Hewlett-Packard). The GC temperature program for manuka oil and 3-(Z)-lactone extracts began at 60°C for one
minute, increased at 8°C/minute to 190°C, then increased at 35°C/minute to 265°C and held for five minutes. For the green-leaf volatile extract, the GC program began at 40°C, then increased at 8°C to 200°C and held for 6 minutes. Helium was used as the carrier gas. The column effluent was split 1:1 with one half going to the flame ionization detector of the GC, and the other through a transfer line (Syntech, Hilversum, Netherlands) heated to approximately 202°C. Within this line, the effluent was carried at approximately 650 ml/minute into a humidified airstream directed at an excised *P. sulcata* antenna attached to a Syntech portable INR-2 amplifier. Both ends of the antenna were cut, and inserted into droplets of electrode gel (Sigma Gel, Parker Labs, NJ, USA) held in loops on the ends of gold wire electrodes connected to the amplifier. Outputs from the GC and the amplifier were recorded and analysed using AutoSpike software (Syntech Hilversum, Netherlands). At least twelve female *P. sulcata* antennae were tested for each chemical blend.

### 4.2.2 Behavioural assay

To test for short-range attraction to potential semiochemicals, a Y-tube olfactometer (Analytical Research Systems Inc., Gainesville FL) was used. The Y-tube consisted of an 11 cm main stem that branched into two 9 cm arms. The internal diameter of the tube was 1.5 cm. Separate glass tubes each containing either a treatment or control volatile were connected the Y-tube arms to the olfactometer. Air was drawn into the olfactometer, humidified, and emitted through a charcoal filter into the arms of the Y-tube. Air travelled into each arm of the Y-tube at a rate of 1.2 L/min.

One µl of a treatment was applied on a strip of filter paper in the treatment arm (5 x 20 mm) using a micropipette, while one µl of hexane was applied on a strip of filter paper in the
control arm. The semiochemical treatments were manuka oil, 3-(Z)-lactone, and (Z)-3-hexenol, which was one of the seven green leaf volatiles used in the GC-EAD study. All treatments were diluted in hexane to 10 ng/µl. Each strip was then placed in their respective arm using separate clean forceps. New filter paper strips with either the treatment or control were placed in the respective arms every 25 minutes. The glass tube was flipped every three replicates to prevent bias to one side of the apparatus.

Insects were introduced into the main stem and were monitored for up to ten minutes. A choice was recorded when the insect reached a glass tube at the end of one of the arms; no response was recorded if the insect did not choose an arm. Each insect was only used once. For each treatment, 30-44 parasitoids were used. Following the experiment, all glass parts were cleaned with hot soapy water and rinsed with acetone. A Chi-Square test ($P = 0.05$) was used to compare the proportion of insects responding to either the treatment or the control. Analyses were carried out in Sigma Plot (v.12).

4.2.3 Field sampling

Sticky and purple prism traps were set up in mid-May 2010 at the W. Darcy McKeough Dam Floodplain (42°41′ 41.75″N, 82°24′ 14.44″W). The site possessed active populations of $P. sulcata$ and $Atanycolus$ spp. that were observed in surveys conducted in 2009. The site consisted of planted rows of green ash and silver maple ($Acer saccharinum$ L. (Aceraceae)) that were distributed in two sections separated by a central access route. Ash trees were planted within rows approximately 3.5 m apart. Rows were approximately 3.5 m apart. Ash trees were planted in 1984, and were approximately 15 cm DBH and 7 m tall.
Sticky band traps consisted of 50 cm wide clear plastic shrink wrap sheets (Catalogue Number 498385, Staples Business Depot Markham, Ontario) that were wrapped three times around the trunk of an ash tree. The bottom band of the plastic sheet was approximately 1 m above the ground. The band was coated in Pestick (Catalogue Number 4002, Phytotronics, Inc., Earth City, Missouri) using paint brushes. Thirty sticky band traps were randomly placed on thirty ash trees within the site. Sticky bands were not baited with any semiochemicals.

Purple prism traps (Synergy Semiochemicals, Burnaby, British Columbia) consisted of three sides measuring 36 cm wide by 61 cm tall. Each trap was fitted with a modified umbrella rig spreader (Midwest Wire, Ferndale, Michigan). Traps were hung from the lower canopy of ash trees by a metal limb hanger (Midwest Wire) that was attached to the spreader. In baited prism traps, a manuka oil pouch was attached to the center of the spreader and was allowed to hang from inside the prism trap. Manuka oil lures (Synergy Semiochemicals, Burnaby, British Columbia) released volatiles at a rate of approximately 50 mg/day (Grant et al. 2010). Lures were installed in baited traps during the initial set up of the experiment. Baited and unbaited purple prism traps were randomly placed on ash trees within the site at distances of approximately 15 – 20 m apart. Three replicates of each treatment were placed within the site.

Sticky band and prism traps were sampled bi-weekly from 2 June 2010 to 16-18 August 2010. Insects were removed from traps using forceps and placed in labelled vials containing HistoClear II (National Diagnostics, Atlanta, Georgia). Upon return to the laboratory, the vials were placed in a heated ultrasonic cleaner (Model FS30H, Fisher Scientific Company, Ottawa, Ontario). This cleaner dissolved the residual glue from the insects which were then placed in vials containing 70% ethanol.
Twenty yellow panel traps (AGS-121-10, Great Lakes IPM, Inc., Vestaburg, MI.) were placed each in two sites containing *P. sulcata* populations on 1 July 2012. The first site was a private mixed woodlot near Dutton, Ontario consisting largely of ash of various ages. The second site was a mixed hardwood woodlot located in Middlesex County. The stand consisted of trees of mixed species and ages, and contained an established population of *A. planipennis*. Bait treatments consisted of manuka oil, (Z)-3-hexenol, 3-(Z)-lactone, and a (Z)-3-hexenol/3-(Z)-lactone combination, and a blank (unbaited) control. Manuka oil and (Z)-3-hexenol were released from pouches (Synergy Semiochemicals, Burnaby, British Columbia) that emitted volatiles at a rate of 50 and 80 mg per day respectively (Grant et al. 2010). 3-(Z)-lactone was released from an impregnated red rubber septum with a release rate of 60-70 µg per day (Ryall et al. 2013). Within each site, four traps were assigned to each treatment and control category. Traps were placed at approximately 2 m from an *A. planipennis*-infested as tree. Traps were suspended at approximately 1.5 m from L-shaped wooden hangers that held the traps approximately 38 cm from a 2 m wooden stake. Traps were attached to the hanger by one corner using a plastic zip-tie. Lures on baited traps were attached to the corner from which the trap was attached to the hanger. One lure was placed on each trap, except for the (Z)-3-hexenol/3-(Z)-lactone combination treatment which possessed both a (Z)-3-hexenol pouch and a 3-(Z)-lactone septum. Insects were collected from traps on 28 July 2012 and traps were removed from the sites on the same day. Insects were collected using the same protocol used at the McKeough Dam in 2010.
4.3 Results

4.3.1 Gas-chromatography electroantennogram detection (GC-EAD)

EAD responses to manuka oil and lactone were not observed in any of the tested *P. sulcata* antennae. Five male and female parasitoids from age groups 1-5, 10-15, and 15+ days old were analyzed in this experiment. No consistent strong responses were found in antennae exposed to the GLV mixture. Two analyses showed a weak antennal response to (Z)-3-hexenol, however in both cases the response was not larger than normal variations in measured potential in the antennae. These results were consistent in both age classes for all treatments.

4.3.2 Behavioural assay

Adult *P. sulcata* females significantly preferred the treatment volatile over the hexane control for (Z)-3-hexenol (n = 30, $\chi^2 = 4.48$, $P = 0.034$, Fig. 4.1) and 3-(Z)-lactone (n = 42, $\chi^2 = 4.33$, $P = 0.037$, Fig. 4.1). Females were did not display a preference for manuka oil over the hexane control (n = 44, $\chi^2 = 0.027$, $P = 0.8694$, Fig. 4.1). In each of the three bioassays, three parasitoids were classified as ‘no response’.

4.3.3 Field sampling

Mean total male, female, and total *P. sulcata* and *Atanycolus* spp. adult trap catches were not significantly different between trap treatments at the McKeough Dam site (Table 4.1). However, mean *P. sulcata* trap catches were consistently higher for all three categories on purple traps than sticky band traps, albeit not significantly (Fig. 4.2, Table 4.2). Adult *P. sulcata* were first caught on sticky band traps and purple baited traps on 16 June 2010 (Table 4.2, Fig.4.4). No parasitoids were caught on purple unbaited traps until 29 June 2010. On this date, all traps caught parasitoids, and mean catches for all three traps were statistically equivalent (Kruskall-Wallis
One-Way Analysis of Ranks, $H = 1.195$, $P = 0.550$; Table 4.2). The mean trap catch of 21.7 ± 3.89 insects per trap on sticky bands was the highest mean value for this trap type throughout the sampling period. By 14 July, mean sticky band trap catch had dropped to 6.5 ± 1.1 insects per trap. Mean trap catches for purple baited and purple unbaited traps however had increased to 40.0 ± 35.5 and 41.7 ± 13.9 insects per trap, respectively. These values were statistically equivalent, however the mean trap catch for purple unbaited traps was significantly higher than that of sticky bands (Dunn’s Test, $P < 0.05$; Table 4.2). Mean trap catches for all three treatments dropped on 28 July 2010 and were all statistically equivalent on this sampling date and the final sampling date of 16 August 2010 ($P > 0.05$). Baited and unbaited yellow panel traps were largely ineffective, and did not catch any appreciable numbers of parasitoids.

4.4 Discussion

These results provide important information about the chemical ecology of *P. sulcata*. While adult females did not respond to a bark terpenoid surrogate during the behavioural assay, females did display a preference for a green-leaf volatile and a volatile produced by *A. planipennis* adults. The identification of these volatiles is important to the selection of potential baits for *P. sulcata*-specific detection traps. Both purple panel and sticky band traps were effective in capturing adult *P. sulcata* and *Atanycolus* spp. while baited and non-baited yellow panel traps were ineffective. Interestingly, I did not observe strong antenna responses to any of the three semiochemical treatments. The results of this study provide important information that may be used in potentially improving parasitoid effectiveness and in creating a sensitive detection trap for estimating parasitoid populations.
4.3.1 Gas-chromatography electroantennogram detection (GC-EAD)

No strong antennal responses by *P. sulcata* females to green-leaf volatiles, manuka oil, or 3-(Z)-lactone were observed. These results do not agree with our prediction that these treatments would elicit antennal responses of *P. sulcata* females, nor do they agree with the positive responses of females to (Z)-3-hexenol and 3-(Z)-lactone in the behavioural assays. This is an interesting result particularly with respect to the absence of response to the GLV mixture in the GC-EAD analysis. Green-leaf volatiles are used by a variety of parasitoids in host-habitat location, and often elicit strong antennal responses during GC-EAD analyses due to the presence of large numbers of chemoreceptors on the antennae of hymenopterous parasitoids (Gouinguene et al. 2005; Wei and Kang 2006; Ngumbi et al. 2009). One possible reason is that the volatile dose reaching the antenna may not have been large enough to elicit a response. Volatile dose has been shown to influence the response observed in GC-EAD analysis using parasitoids. For example, Baehrecke et al. (1989) analysed the GC-EAD responses of *Campoplelis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) to several volatiles released by cotton plants (*Gossypium hirsutum* L.). These authors observed that for one green-leaf volatile and several terpenes, the dose used in analysis significantly influenced the electrophysical response of *C. sonorensis* antennae. In all instances where responses were increased, a positive relationship between dose and response was observed. In several instances, the increase in dose was necessary to elicit some sort of response in antennae. Ngumbi and Fadamiro (2012) observed a similar effect in adult female *Costesia marginiventris* (Cresson) (Hymenoptera: Braconidae) where responses to linalool, a herbivore-induced plant volatile, occurred only when doses were increased from 1µg to 100µg. It is possible that an increase in dose concentration may be
required to elicit antennal responses in *P. sulcata*. Further studies should be undertaken to confirm this.

**4.4.2 Behavioural assay**

Adult females *P. sulcata* were attracted to (Z)-3-hexenol. (Z)-3-hexenol is one of six antennally active GLVs produced by ash trees under attack by *A. planipennis* (de Groot et al. 2008). Green-leaf volatiles include several carbon-6 aldehydes, acetates, and alcohols (Zhang and Schlyter 2004) that are released by angiosperms during periods of increased insect feeding (Rodriguez-Saona et al. 2006). Green-leaf volatiles have been shown to elicit host searching responses in several parasitoid species (Turlings et al. 1991; De Moraes et al. 1998; Pare and Tumlinson 1999; Reddy et al. 2002). In several instances where green-leaf volatiles were being used by parasitoids as synomones, the preferred host stage is often the direct causing their release. For example, volatiles released in response to feeding by lepidopteron larvae have been shown to act as long-range cues for their parasitoids (Whitman and Eller 1990; Turlings et al. 1991; Geervliet et al. 1994). However, parasitoids may be able to use volatiles generated by a non-target stage to locate a preferred host stage. For example, the egg parasitoid *Anaphes iole* Girault (Hymenoptera: Mymaridae) was attracted to cotton leaves (*Gossypium hirsutum* L., Malvaceae) that had been damaged by feeding by both male and female *Lygus hesperus* Knight (Hemiptera: Miridae) (Manrique et al. 2005). The specific synomone that was most attractive to *A. iole* was (Z)-3-hexenyl-acetate (Williams III et al. 2008). This volatile is also part of the green-leaf volatile spectrum produced by *Fraxinus* sp. damaged by *A. planipennis* feeding (Rodriguez-Saona et al. 2006). The authors speculated that this host-plant volatile may act as a long-range cue for *A. iole*, which then rely on other short range cues such as kairomones produced by the adults and eggs to locate their hosts. *Phasgonophora sulcata* adults may
similarly be using green-leaf volatiles released during *A. planipennis* adult feeding as a long-range cue for orientation to the host-habitat. Once parasitoids have entered the host-habitat, they then may rely upon other short-range cues such as kairomones or auditory cues to locate the *A. planipennis* host stage.

The attraction of *P. sulcata* females to green-leaf volatiles may be applicable to the improvement of a biological control program. Experiments have demonstrated that beneficial insects such as predators and parasitoids may be attracted to areas where green-leaf volatiles are artificially emitted (James 2003b; James and Price 2004). James (2005) observed that panel traps baited specifically with (Z)-3-hexenol caught significantly more microhymenoptera, including scelionids, encyrtids, and mymarids, than unbaited traps. The author concluded that (Z)-3-hexenol may be an effective compound for aggregating parasitoids to sites, and could be useful in developing sensitive detection traps. In a similar study, Lewis et al. (1972; 1975b) compared parasitism rates by both released and native *Trichogramma* spp. on *Heliothis zea* (L.) (Lepidoptera: Plutellidae) in plots treated with a kairomonal extract. The authors observed that parasitism by *Trichogramma* spp. on *H. zea* in treated plots was significantly higher than parasitism in untreated plots. The authors concluded that the increased presence of kairomonal substances within the treated plots increased searching time for individual parasitoids which allowed them to detect and parasitize more hosts. These studies illustrate the applicability of semiochemicals in potentially increasing the effectiveness of released and/or native parasitoid populations. The creation of a similar protocol using semiochemicals that are attractive to *P. sulcata* should be investigated, as *P. sulcata* parasitism levels have varied from nonexistent to substantial across surveyed sites in southern Ontario (Lyons 2010). The application of attractive
semiochemicals in sites where parasitism is low may be effective in increasing *P. sulcata* parasitism rates to levels where *A. planipennis*-management may be possible.

Females in this study did not preferentially choose manuka oil over the hexane control. This does not agree with my hypothesis that females would be attracted to manuka oil as it possesses several terpenoids that are produced by ash tree damaged by *A. planipennis*. Bark terpenoids released by plants affected by insect-damage have been shown as a commonly used synomone for parasitoids of wood-boring insects (Camors and Payne 1972; Pettersson et al. 2000; Pettersson et al. 2001). This is due to their release often being a direct result of insect damage to bark or woody tissue by a wood-boring insect (Mumm and Hilker 2006). Although manuka oil contains several important terpenes found in ash bark volatiles, it does not include all of the terpenes found in the complete chemical spectrum of ash-released bark volatiles (Crook et al. 2008). This substance was used here due to the prohibitive costs of synthesising *Fraxinus* spp. bark volatiles. It is possible that a terpene that was not included in manuka oil mixture may be attractive to *P. sulcata*. Due to this, it is impossible to rule out the use of terpenes by *P. sulcata* as a synomone. Continued testing using actual damaged bark tissue should be carried out to determine if *P. sulcata* is indeed not responsive to bark terpenoids.

The positive response of *P. sulcata* females to 3-(Z)-lactone is noteworthy because of the host stage being attacked and the concealment of the host. Adult-derived kairomones are used often by egg parasitoids such as *Trichogramma* sp. (Noldus et al. 1991; Van Huis et al. 1994; Rani et al. 2007), and scelionids (Colazza et al. 1997; Conti et al. 2003; Conti et al. 2004). This is because high-levels of adult pheromones such as aggregation and sex compounds are a temporal indicator of host mating and oviposition. By detecting these oviposition-associated
compounds, egg parasitoids are able to locate the host-habitat at the moment when the preferred host stage density is highest. Larval parasitoids have also been observed to use adult-derived volatiles to locate their hosts (Dweck et al. 2010), but examples of these are fewer. This discrepancy is due to the host larval stage often occurring significantly later than adult emergence and mating. Some adult-produced volatiles have been shown to remain in the environment for some time after being deposited (Colwell et al. 1978) and have demonstrated attractiveness to parasitoids (Lewis et al. 1971; Noldus et al. 1991). However, the volatiles studied in these articles were only detectable for up to 24 hours after application. Although the retention of 3-(Z)-lactone on a bark surface has not been measured, it is unlikely that the kairomone may remain detectable after this amount of time. A more possible explanation for our result is that the immature host stage may itself be producing the adult compound. It has been shown that immature stages of an insect sometimes contain the precursors to pheromones used by adults. For example, *Cyclocephala lurida* (Coleoptera: Scarabaeidae) larvae produce compounds that are similar enough to a pheromone produced by the adult female that adult males would attempt to mate with them (Haynes et al. 1992). As parasitoids can be attracted by volatiles produced by concealed hosts themselves (Xiaoyi and Zhongqi 2008), it is possible that *A. planipennis* larvae may possess compounds similar to 3-(Z) lactone that *P. sulcata* may use as kairomones. Further experiments on the presence of these compounds in *A. planipennis* larvae would be required to determine if this is indeed occurring.

The attraction of *P. sulcata* to 3-(Z)-lactone is also important as *A. planipennis* and *P. sulcata* have only recently been in contact with one another. Typically, if a pest enters a new range, then it is possible that the native enemies will not be able to detect the volatiles produced by it and therefore would neither be able to locate nor attack it. Thus non-native pests may have
few enemies, and would be able to reach high densities (Van Driesche and Bellows 1996). However, if the new host is ecologically and physiologically similar to ancestral hosts, then the parasitoid may be able to successfully parasitize it (Wiedenmann and Smith 1997). Ecological similarities include similar habitats, host plants, and temporal phenologies, while physiological similarities would include similar developmental trends and sizes. Ecologically, *A. planipennis* is similar to native hosts of *P. sulcata* including *Agrilus bilineatus* (Weber) and *Agrilus anxius* Gory (Haack et al. 1981; Loerch and Cameron 1983). *Agrilus planipennis, A. bilineatus,* and *A. anxius* attack *Fraxinus* spp., *Quercus* spp., and *Betula* spp. respectively (Haack et al. 1981; Loerch and Cameron 1983; Poland and McCullough 2006). All three species are native hardwood trees that occur together in naturally forested areas in Ontario and the northeastern United States (OMNR 1998). When stressed by insect attack, all three tree species also produce similar GLVs, including (Z)-3-hexenol and (Z)-3 hexenyl acetate (Arey et al. 1991; Zhang and Schlyter 2004; de Groot et al. 2008). Additionally, in areas where all three buprestids exist, peak emergence typically occurs during the late-spring and early-summer depending on latitude (Haack et al. 1981, Loerch and Cameron 1983, Lyons et al. 2009). Physiologically, *A. planipennis* is similar in larval dimensions for each instar, and undergoes the same number of instars as the native species (Loerch and Cameron 1983). All three larvae also live in the cambial region of the tree, and construct pupal chambers prior to overwintering (Barter 1957, Haack et al. 1982, Haack et al. 2002). It is also possible that all three species share compounds in their adult-pheromone suite that *P. sulcata* adults can detect. At this time however, this is only speculative, as the pheromone chemicals produced by both North American species are unknown. It may however explain the response of female *P. sulcata* to 3-(Z)-lactone.
It is likely that enough shared traits between the native and non-native species existed so that female *P. sulcata* females were able to attack and successfully parasitize *A. planipennis*. Because *P. sulcata* emerged from *A. planipennis*, it is likely that the adult parasitoid was conditioned to its host shortly after emergence. Studies on responses to volatiles present during emergence have shown that parasitoids will react preferentially to volatiles associated with materials present at that time. These can include host-plant volatiles (Herard et al. 1988, Bjorksten and Hoffman 2004, Gandolfi et al. 2003) and host products, such as frass and exuvia (Thorpe and Jones 1937, Caubet and Jaisson 1991). For example, Cortesero and Monge (1994) observed that *Eupelmus vuitteti* (Crw.) (Hymenoptera: Eupelmidae) were attracted significantly more to volatiles from both the seeds and the host, *Bruchidius atrolineatus* (Pic) (Coleoptera: Bruchidae), from which they emerged from rather than volatiles from another seed species and bruchid host. Because *P. sulcata* emerge within the exuvia of the *A. planipennis* host, and must chew their exit out of the pupal chamber through a tunnel packed with *A. planipennis* frass, it is possible that during these events an imprinting of the host may be created on the emerging parasitoid. This may lead to preferential parasitization of *A. planipennis* which may be reflected in the high amounts of parasitism observed by Lyons (2010). Further studies analyzing the preference of *A. planipennis*-reared *P. sulcata* in choice tests with native *Agrilus* spp. would be required to substantiate this hypothesis. If it is shown that *P. sulcata* specialize on *A. planipennis*, then *P. sulcata* would be demonstrating an important quality that is consistent with effective biological control agents (DeBach 1991).

### 4.4.3 Field sampling

These results indicate that dark coloured traps that resemble the silhouette of a tree are more effective in capturing *A. planipennis* parasitoid adults than panel traps. Various studies
have shown that wood-boring insects are often more attracted to cylindrical traps rather than flat panel traps (Lindgren 1983). This is likely due to the physical characteristics of the traps resembling those of a potential target tree. The tendency of wood-boring insects to prefer these types of traps is evident in established detection programs for wood-associated pests, as many programs utilize Lindgren, stove-pipe, and interception designs that mimic the host plant (Miller and Crowe 2011; Barnes 2012; Miller et al. 2013). It is possible that the cylindrical shape of both the purple traps and sticky traps was an important factor in the results observed.

This silhouette hypothesis may also explain why the yellow panel traps were completely ineffective in capturing any appreciable numbers of parasitoids. This was somewhat surprising as several studies had previously used yellow panel traps to capture parasitoids of various families (Weseloh 1986; McClain et al. 1990; Udayagiri et al. 1997; James 2005). In these cases however, the target parasitoid was itself locating hosts associated with vegetation, and not tree trunks. Because their host occurs within the tree’s bole, it would be likely that *P. sulcata* and *Atanycolus* spp. would prefer cues associated with a tree’s trunk and thus remain in close association with infested trees. Further evidence of this is the lack of adult *P. sulcata* and *Atanycolus* spp. caught in sweep-netting of vegetation around tree trunks at this site during peak parasitoid emergence (L. E. Roscoe, unpublished). Based on this information, it would thus be advantageous for *P. sulcata* traps to reflect the qualities of an *A. planipennis*-infested ash tree. It appears that both purple prism traps and sticky band traps may fulfill this role. As both of these are currently in use for sampling *A. planipennis* in the United States, their extended use for also sampling *P. sulcata* populations may be easily accomplished. Improving their effectiveness by perhaps using (Z)-3 hexenol and/or 3-(Z)-lactone lures should be investigated in future studies.
As well, testing of other trap types such as Lindgren funnels and stovepipe traps commonly used for other wood-associated insects in conjunction with attractive volatiles should be carried out.

4.4.4 Conclusions

In this chapter, I sought to determine the semiochemicals used by *P. sulcata* females while searching for *A. planipennis* hosts. The determination of these compounds is important to the construction of sensitive detection traps that are required for estimating parasitoid population sizes in sites both before and after liberations. Such steps are critical to the outcome of a biological control program. Using a Y-tube olfactometer, I found that adult females respond positively to a synomone, (Z)-3-hexenol, and a kairomone, 3-(Z)-lactone, however females did not respond to manuka oil, a chemical surrogate for ash bark terpenoids. While (Z)-3-hexenol is commonly used by various parasitoids of foliage-associated pests, the use of 3-(Z)-lactone is significant as *P. sulcata* and *A. planipennis* do not share an extensive evolutionary history. This attraction is also interesting as this compound is produced predominately by adult female *A. planipennis*. It is possible that *P. sulcata* can detect 3-(Z)-lactone because it may be similar in composition to semiochemicals produced by North American *Agrilus* spp. This substance may also be produced by *A. planipennis* larvae in amounts detectable to female *P. sulcata*. Further experiments on the composition of cuticular compounds in native *Agrilus* spp. and *A. planipennis* larvae would be required to determine if these scenarios are indeed true. I also evaluated the effectiveness of several commercially available detection methods at catching *P. sulcata* adults. I found that traps that possess the physical characteristics of ash trees, such as colour and shape, were more effective that commonly used yellow panel traps even when baited with attractive semiochemicals. Purple prism and sticky band traps were both effective in detecting both *P. sulcata* and *Atanycolus* spp. populations. It is recommended that *P. sulcata*
sampling should use traps with similar physical characteristics as these in conjunction with attractive semiochemicals identified in the first part of my study. By coupling these experiments, it may be possible to create an effective trap that can accurately measure *P. sulcata* population numbers at a variety of densities. Such an experiment is necessary for the successful release and/or augmentation of biological control agent populations.
Table 4.1: Number of adult *Phasgonophora sulcata* caught on traps (No. of insects/trap; $\bar{x} \pm$ S.E.) at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario from 2-4 June to 16-18 August 2010 (Kruskall-Wallis One-way Analysis of Ranks, $P = 0.05$).

<table>
<thead>
<tr>
<th>Insect group</th>
<th>Sex</th>
<th>Sticky band trap</th>
<th>Purple prism trap, unbaited</th>
<th>Purple prism trap, baited</th>
<th>$H$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phasgonophora sulcata</em></td>
<td>Males</td>
<td>4.0 ± 0.7</td>
<td>6.1 ± 2.7</td>
<td>5.78 ± 3.72</td>
<td>1.009</td>
<td>0.604</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>2.4 ± 0.4</td>
<td>6.67 ± 2.2</td>
<td>6.44 ± 2.85</td>
<td>3.526</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>Males + Females</td>
<td>6.46 ± 0.92</td>
<td>12.72 ± 4.44</td>
<td>12.22 ± 6.45</td>
<td>0.350</td>
<td>0.839</td>
</tr>
<tr>
<td><em>Atanycolus</em> spp.</td>
<td>Males</td>
<td>0.2 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>1.091</td>
<td>0.580</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.2</td>
<td>3.238</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>Males + Females</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>1.388</td>
<td>0.500</td>
</tr>
</tbody>
</table>
Table 4.2: Temporal distribution of *Phasgonophora sulcata* adults caught on sticky-band, unbaited, and baited purple prism traps (No. of insects/trap; \( \bar{E} \pm \text{S.E.} \)) at the W. Darcy McKeough Dam Floodplain, 2-4 June to 16-18 August, 2010 (Kruskall-Wallis One-way Analysis of Ranks, \( P = 0.05 \)).

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Sticky-band trap</th>
<th>Purple prism trap, unbaited</th>
<th>Purple prism trap, baited</th>
<th>H</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4 June</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>16 June</td>
<td>0.9 ± 0.4</td>
<td>0</td>
<td>0.3 ± 0.3</td>
<td>1.18</td>
<td>0.550</td>
</tr>
<tr>
<td>29 June</td>
<td>21.7 ± 3.9</td>
<td>21.3 ± 14.2</td>
<td>26.3 ± 5.3</td>
<td>0.803</td>
<td>0.669</td>
</tr>
<tr>
<td>14 July</td>
<td>6.50 ± 1.1</td>
<td>40.0 ± 35.5</td>
<td>41.7 ± 13.9</td>
<td>7.852</td>
<td>0.02*</td>
</tr>
<tr>
<td>28 July</td>
<td>6.90 ± 1.8</td>
<td>6.33 ± 3.5</td>
<td>8.0 ± 4.7</td>
<td>0.203</td>
<td>0.904</td>
</tr>
<tr>
<td>16 August</td>
<td>2.62 ± 0.6</td>
<td>5.67 ± 5.8</td>
<td>0</td>
<td>2.917</td>
<td>0.233</td>
</tr>
</tbody>
</table>
Figure 4.1: Response of female adult 1-5 day-old virgin *Phasgonophora sulcata* in a Y-tube olfactometer to host- and host-plant volatile treatments. ‘*’ represents a significant difference between responses to treatment and control volatiles. (Chi-Square test, $P < 0.05$).
Fig. 4.2: Number of (A) male, (B) female, and (C) male+female adult *Phasgonophora sulcata* adults (No. of insects/trap; ± S.E.) caught on traps at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario from 2-4 June to 16-18 August 2010.
Fig. 4.3: Number of (A) male, (B) female, and (C) male+female adult *Atanycolus* spp. adults (No. of insects/trap; $\bar{x} \pm$ S.E.) caught at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario from 2-4 June to 16-18 August 2010.
Figure 4.4: Number of *Phasgonophora sulcata* adults caught on sticky band traps (No. of insects/trap; $\bar{x} \pm $ S.E.) at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario from 2-4 June to 16-18 August 2010.
Chapter 5: Spatial and temporal distributions of *Phasgonophora sulcata*, a native endoparasitoid of the invasive *Agrilus planipennis* in southwestern Ontario

5.1 Introduction

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive wood-boring pest of ash (*Fraxinus* spp.) in North America. *Agrilus planipennis* was first discovered in the Detroit/Windsor region during the summer of 2002 (Haack et al. 2002). Dendrochronological evidence indicates it may have been introduced approximately 10 years earlier. The current range of *A. planipennis* includes 22 states and 2 provinces (USDA-APHIS 2013), and likely arrived in North America via untreated wooden packing materials from its native range in Asia (Haack et al. 2002). Females lay their eggs in bark crevices and under bark scales of ash trees and larvae emerge to burrow through the bark and into the trunk cambium. Larvae feed in the phloem and xylem until fourth-instar (Haack et al. 2002). In the early to late fall, fourth-instar larvae excavate pupal chambers, either in the bark or about 1.5 cm into the sapwood, and overwinter as prepupae (Poland and McCullough 2006). These prepupae begin pupation the following spring and emerge as adults usually in late spring; however adult emergence can vary with latitude. A one-year life cycle is most common, but a two–year life cycle may occur in more northerly latitudes and in new infestations (Poland and McCullough 2006). Larval feeding damages phloem and xylem tissues and prevents the transport of water nutrients between the roots and the canopy. Prolonged larval feeding will result in dieback and eventually tree death.
Agrilus planipennis is a significant pest of both native and imported ash species in North America (Yu 1992; Wei et al. 2004; Poland and McCullough 2006; Rodriguez-Saona et al. 2006; Czerwinski et al. 2007; Pureswaran and Poland 2009). It will readily attack healthy and stressed trees, and is able to move large distances undetected via untreated firewood. Detection of larvae of A. planipennis at low densities is also difficult as this stage is spent hidden underneath the bark of host trees. These attributes thus make the potential spread of this pest through natural and planted ranges of ash, followed by the subsequent destruction of millions of ash trees, a distinct possibility (Sydnor et al. 2007).

The loss of ash on the North American landscape will have substantial ecological and economic consequences. Ash is an important component of various forest types throughout the continent. The collective natural range of ash species in North America includes most of the United States, and southern Canada (MacFarlane and Meyer 2005). In the northeastern United States and eastern Canada, green ash (Fraxinus pennsylvanica Marsh) is a major canopy component of forests on wet soils (Poland and McCullough 2006). White ash (F. americanum L.) is a common component of eastern mesophytic forests especially those in early to intermediate stages of plant succession (OMNR 1998). Pure stands of black ash (F. nigra Marsh) regularly dominate many low-lying swampy regions in central Ontario (Strickland et al. 1987). The elimination of ash from these unique areas could lead severe ecological damage including the extinction of rare organisms found only in these habitats. The economic costs associated with the loss of ash are equally important with the compensatory value of ash timber in the United States ca. $282 billion dollars (Poland and McCullough 2006). Ash is also used to make a variety of goods, including baseball bats, furniture, paper products, and crating (Poland and McCullough 2006). The subsequent job losses associated with reductions in ash-dependent industries could
have large impacts on the overall economies of Canada and the United States. Ash is also a major component of urban forests in major cities throughout Canada and the United States (MacFarlane and Meyer 2005; McKenney et al. 2012). The loss of trees would have a major impact on urban landscape values, including reductions in property and aesthetic values, as well as increased costs associated with increased storm water runoff (Poland and McCullough 2006). Variation in temperature regulation, and reductions in associated wildlife may also be associated with ash loss in urban areas (Sydnor et al. 2007). Due to these significant economical and ecological effects of *A. planipennis* in North America, the management of this pest is a critical issue for both affected and potentially vulnerable areas.

Two potential management protocols for *A. planipennis* are chemical and biological controls. Chemical control using trunk injected pesticides has caused *A. planipennis* mortality in infested trees that were treated (Herms et al. 2009). However, wide-spread dissemination in areas of high ash densities such as naturally forested areas or woodlots is not possible due to prohibitive costs of production and application (Poland and McCullough 2006). Biological control with wasp parasitoids may be a more suitable strategy in such environments (Haack et al. 2002). Natural enemy surveys conducted across the native range of *A. planipennis* in China identified three wasp parasitoids that showed promise for use in North America (Liu et al. 2003). Two larval parasitoids, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Spathius agrili* Yang (Hymenoptera: Braconidae), and an egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) were identified and released in 2007 in Michigan (Bauer et al. 2008). Additional releases in Ohio and Indiana, as well as again in Michigan were conducted in the next year. In 2008, *S. agrilus* and *O. agrili* were recovered from dissected ash trees recovered from the previous year’s release sites, indicating that both of these species were able to
successfully attack *A. planipennis* and overwinter (Bauer et al. 2010). All three parasitoids are currently being reared in the United States, and have been subsequently released in a number of states (USDA-APHIS/ARS/FS 2012). This program has shown promise for long-term *A. planipennis* management, and continued improvements to rearing and release methodology may increase their potential for permanent and effective management for *A. planipennis* (USDA-APHIS/ARS/FS 2012).

Surveys of *A. planipennis* populations have also yielded several parasitoids that show potential as augmentative biological control agents (Lyons 2010). These include several *Atanycolus* spp. (Hymenoptera: Braconidae), including *A. cappaerti* (Marsh and Strazanac) and *A. hicoriae* Shenefelt (Cappaert and McCullough 2009), the eulophid *Balcha indica* (Many & Kaul) (Duan et al. 2009), and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) (Lyons 2010). Surveys in 2003 indicated that *A. planipennis* parasitism by all indigenous parasitoids was ~1% (Liu et al. 2003). Recent surveys in the United States (Cappaert and McCullough 2009; Duan et al. 2009), and Canada (Lyons 2010) however suggest that mortality by native parasitoids is high enough that warrant their potential use in future augmentation programs. In southwestern Ontario, *P. sulcata* has been observed to parasitize up to 40% of *A planipennis* larvae at some sites and thus may be a potentially effective biological control agent (Lyons 2010). Our knowledge about its interactions with its new host however is limited. If this parasitoid is to be considered as a biological control against *A. planipennis*, then a comprehensive understanding of its ecology and biology should be compiled.

For a biological control agent to be successful, it must be able to attack its host throughout the host-habitat. Collier and van Steenwyk (2004) analyzed the outcomes of a
number of augmentative biological control programs, and determined the presence of host refuges was an important determinant in the success of several programs. Host refuges are areas of the habitat where host may reside and be protected from predators or parasitoids. If large proportions of the pest population is able to access these refuges, then regulation of host populations may be impossible. In forests, phloem-feeding pests have been shown to congregate in the lower-bole of infested trees (Timms et al. 2006). Evidence shows however that parasitoids of phloem-feeding insects may be restricted to attacking hosts in the mid- to upper-bole of trees (Mills 1986; Van Laerhoven and Stephen 2002; Wermelinger 2002). Consequently, the lower-boles of infested trees may act as refuges for wood-boring pests. With large proportions of the pest population protected, population management would be unlikely. If such pests to be regulated, parasitoids must have the capacity to locate and parasitize hosts at all tree heights. A determination of the effect of tree height on parasitism by a potential biological control agent is thus necessary during parasitoid evaluation.

Determining the temporal distribution of life stages of a potential biological control agent is also necessary when developing a biological control program. Parasitoid development strategies may be difficult to predict as developmental rates may vary greatly between species (Quicke 1997). Determining the majority life stage at any given time during the sampling period is important if parasitoids are to be detected within host trees and their populations estimated. The development of a parasitoid within its environment must also be understood if the species is to be potentially reared in production facilities for release. Consequently, it is necessary to directly observe the phenology of potential biological control agents prior before considering their use in a management program.
The goal of this study is to analyze the spatial and temporal variations in *P. sulcata* parasitism and abundance in *A. planipennis*-infested ash trees in southern Ontario. I also analyzed the within-tree distribution of *A. planipennis* and that of another group of native parasitoids, *Atanycolus* spp. Due to high preliminary estimates of *A. planipennis* parasitisation by *P. sulcata* in the study site, I predicted that parasitism by *P. sulcata* would not be affected by tree-height. I also predicted that, because *P. sulcata* is a koinobiont parasitoid which allows its host to develop after parasitization, the majority immature stage for most of the sampling period will be an early- to mid-instar larva within the host. Such delayed development is common in koinobiont species (Quicke 1997). These results will provide important knowledge that can be used in detection protocols for *P. sulcata* and *Atanycolus* spp. populations and as an assessment of the potential effectiveness of *P. sulcata* as an augmentative biological control agent against *A. planipennis* in southern Ontario.

5.2 Methods

5.2.1 Study sites and sampling procedures

The study was carried out at the W. Darcy McKeough Dam Floodplain in Lambton County, Ontario. The site consisted of rows of planted ash, silver maple (*Acer saccharinum* L.), and poplar (*Populus* spp.). A central gravel access route running on a north-south bearing divided the site into two sectors. These sectors are herein identified as east- and west-sectors. In each sector, the rows of trees adjacent to the road were silver maple. The next four rows were comprised of ash, and rows beyond those were poplar. Each row of trees was planted on a north-south axis. The lengths of the rows in each sector were 131.9 m, and 120.5 m in the east- and west-sectors, respectively. Thirty ash trees were found in each row. The mean (± S.E.) distance between ash rows for both sectors was 3.5 ± 0.3 m. Trees were planted in 1984. The mean (±
S.E.) height of trees in the stand was 10.4 ± 0.3 m (n = 29), and mean (± S.E.) diameter at breast height (DBH) was 10.8 cm ± 0.5 (n = 24). Mean (± S.E.) tree bark thickness at 30 cm was 6.0 ± 0.2 mm (n = 29).

Tree sampling began on 28 May 2010 and was carried out bi-weekly up until the final sampling event on 28 August 2010. Three trees were selected for each sampling. Only trees containing an active *A. planipennis* population were selected. An active *A. planipennis* population was determined if the tree displayed *A. planipennis*-related symptoms such as epicormic shoots, canopy dieback, and 'D'-shaped exit holes (de Groot et al. 2008). Infestations were confirmed within the tree by creating a small bark window from which *A. planipennis* larval galleries could be seen. Sample trees were randomly selected from either sector. At least one tree from each sector was used on each sampling date. Prior to felling, the DBH were recorded for all selected trees.

Selected trees were felled and limbed using a chainsaw. Beginning with the base of the main stem, the selected tree was cut into eight 0.6 m segments labelled from 1 to 8. These numbers represented the standing height level of each segment for the tree. For each segment, the top and bottom diameters were recorded. Each segment was de-barked using a carver’s drawknife (Veritas Carver’s drawknife, Lee Valley Tools, Canada). Insects embedded within the sapwood of the segment were removed using a folding knife (Swiss Army ‘Recruit’, Victorinox, Switzerland) and collected using forceps.

For each segment, all *A. planipennis* larvae, pupae, and adults were collected. Larvae were found within their larval galleries in the cambium of the segment. Pupae and adults were in pupal chambers that were either in the sapwood or in the bark. Immature *P. sulcata* and
Atanycolus spp. were also collected from the *A. planipennis* larval galleries and pupal chambers. Exit holes generated by *A. planipennis, P. sulcata* and *Atanycolus* spp. by insects emerging in the current season were also counted. A ‘current’ exit hole was associated with a larval gallery that contained tightly-packed light-colored frass, and which displayed very little or no callous tissue generated by the tree (Timms et al. 2006). ‘Old’ exit holes containing loosely-packed dark frass and large amounts of callous tissue were not counted. The determination of exit holes as being either ‘current’ or ‘old’ was based on a visual inspection.

All specimens removed from *A. planipennis* larval galleries were immediately placed in 20 ml scintillation vials (Kimble Glass Inc., USA) containing 70% ethanol. Each vial was labelled with the date of collection and the tree-segment from which they were removed. All specimens collected from pupal chambers from a given segment were placed in one well within a 12-well culture plate to prevent mixing with samples from other segments (Becton Dickinson Labware, USA). The well was labelled with information indicating the tree and segment from which its contents came, as well as the date of collection.

All insects for each segment were classified and counted. The classifications used were: *A. planipennis* larvae, pupae, and adults; *Atanycolus* sp. larvae and cocoons; and *P. sulcata* early-/mid-instar larvae, ‘larval-pupae’, and ‘pupal-adults’. *Phasgonophora sulcata* early-/mid-instar larvae were found within *A. planipennis* larvae. *Phasgonophora sulcata* ‘larval-pupae’ and ‘pupal-adults’ were recovered from host pupal chambers. Specimens were designated as ‘larval-pupal’ and ‘pupal-adult’ based on the degree of sclerotization of the thorax and abdomen. Parasitoids that had no visual evidence of sclerotization were identified as ‘larval-pupal’. Parasitoids with obvious sclerotization were classified as ‘pupal-adult’. Separation between
developmental levels was done so that the temporal distribution of within-tree *P. sulcata* populations could be accomplished.

Instars of all *A. planipennis* larvae were determined based on the peristoma width (Loerch and Cameron 1983). Measurements were done using a micrometer. All *A. planipennis* larvae were dissected and any endoparasitoids found were removed and counted. Both the endoparasitoid and the *A. planipennis* larval host collected in dissections were then placed in a 1.5 ml microcentrifuge tube (Fisher Scientific, USA) containing 70% ethanol. Each microcentrifuge tube was labelled with information regarding the tree and segment which the parasitoid and host came from, and the date of collection.

5.2.2 Spatial distribution of *A. planipennis* and parasitoids

The total number of *A. planipennis* within a segment was calculated by summing together the total number of *A. planipennis* larvae, pupae, adults, and ‘current-year’ exit holes for that segment level. The total number of *P. sulcata* within a tree segment was calculated by summing the number of *P. sulcata* larvae from *A. planipennis* hosts, *P. sulcata* ‘larval-pupae’, *P. sulcata* ‘pupal-adults’, and *P. sulcata* exit holes. No distinction of life stages were used for *Atanycolus* spp. Insect species densities within a 0.6 m segment were calculated as the number of insects per 1 m² of available surface area of phloem. The available surface area of phloem was calculated by using the length of the bolt and diameters of the top and bottom ends for the formula for a quadrilateral. The mean insect density and standard error for each segment level was calculated based on the individual segments collected for each height level over the sampling period. For all trees with five or more segments containing *A. planipennis*, the presence of a correlation between height level and parasitization by *P. sulcata* was determined using a linear regression (*P* = 0.05).
Total parasitism within a height-level was determined with dividing the sum of all parasitoids recovered by the sum of all *A. planipennis* recovered within that segment level. To determine species-specific parasitism, the sums of all parasitoids of either *P. sulcata* or *Atanycolus* spp. were divided by the total number of *A. planipennis* (parasitized and non-parasitized) recovered from a specific segment.

### 5.2.3 Temporal distribution of *A. planipennis* and *P. sulcata*

The seasonal distributions of *A. planipennis*, and *P. sulcata* were also studied. For each sample date, all stages for each insect species recovered from all sampled trees were converted to a proportion of the total number of insects for each species. Exit holes were not taken into account for this analysis. The distribution of life stages within sample date were then compared with other sample dates.

### 5.2.4 Statistics

Due to the significant right skewness of our data even after transformation, spatial distributions of *A. planipennis* densities, mean total parasitism, and mean parasitism by *P. sulcata* and *Atanycolus* spp. were compared against height using a Kruskall-Wallis One-way Analysis of Ranks. Mean within-height level parasitism within height levels for each wasp species were compared using a Mann-Wilcox test. Again because of the high number of zeros in the data, unpaired t-tests were not applicable. To determine if species-specific parasitism was related to host density with height levels, a Spearman Rank test was undertaken. For this analysis, *A. planipennis* density within the height level was log-transformed, while the species-specific parasitism value was arc-sin transformed. Statistics and figures were completed in Sigma Plot (V.12). A ‘*P*’ value of 0.05 was assumed for all tests.
5.3 Results

5.3.1 *Spatial distribution of A. planipennis and parasitoids*

Average within-tree density of *A. planipennis* was significantly influenced by tree height (Kruskall-Wallis One-way ANOVA, \( H = 16.84 \), d.f. = 7, \( P = 0.018 \)). Overall, the mean density of *A. planipennis* within ash trees declined with tree height (Figure 5.1). Mean (± S.E.) within-tree *A. planipennis* density varied from 2.6 ± 2.0 insects/m\(^2\) in the 421–480 cm height level to 23.9 ± 3.8 insects/m\(^2\) in the 0–60 cm height level. Mean (± S.E.) *A. planipennis* density within each sampled height level was 12.7 ± 1.1 insects/m\(^2\).

Mean (± S.E.) total *A. planipennis* parasitism was observed at all heights (Figure 5.2). Parasitism varied from 23.1 ± 15.5% at 421-480 cm to 44.5 ± 8.0% at 241-300 cm; however, mean parasitism was not statistically different across heights (Kruskall-Wallis One-way ANOVA, \( H = 5.709 \), d.f. = 7, \( P = 0.259 \)). Total mean parasitism across all segments was 37.8 ± 2.6%.

Parasitism by *P. sulcata* was observed at all heights (Figure 5.2). Mean (± S.E.) *P. sulcata* parasitism varied from 15.1 ± 10.9% at 421-480 cm, to 39.3 ± 7.60% at 241-300 cm. Parasitism appeared to follow a parabolic trend with higher amounts of parasitism in the middle segments; however, there were no significant differences between mean *P. sulcata* parasitism rates across segments (Kruskall-Wallis One-way ANOVA, \( H = 11.6 \), d.f. = 7, \( P = 0.116 \)). Mean (± S.E.) total *P. sulcata* parasitism across all segments was 28.7 ± 2.3%. Of the twenty trees with five or more segments containing *A. planipennis*, nineteen did not display any correlation between parasitism and height level. One tree dissected on July 24 displayed parasitism by *P.*
that significantly correlated with increasing height ($r^2 = 0.740, P = 0.013$); however, this was the only instance of parasitism correlating with height level.

Parasitism by *Atanycolus* spp. was observed at all heights (Figure 5.2). Mean ($\pm$ S.E.) *Atanycolus* spp. parasitism varied from $8.0 \pm 5.2\%$ at 421-480 cm, to $16.5 \pm 6.0\%$ at 121-180 cm. Parasitism appeared to be peak in the bottom three height levels; however, differences between mean parasitism rates were not significant (Kruskall-Wallace One-way ANOVA, $H = 8.92$, d.f. = 7, $P = 0.259$). Mean ($\pm$ S.E.) total *Atanycolus* spp. parasitism was $9.1 \pm 1.6\%$.

Mean parasitism by *P. sulcata* was significantly higher than that *Atanycolus* spp. parasitism in five out of eight height levels (Mann-Wilcoxon Test, $P < 0.05$, Figure 5.2). These height levels were 0-60, 121-180, 181-240, 241-300, and 301-360 cm. *Phasgonophora sulcata* parasitism was nearly significantly higher at 61-120 cm. The greatest disparities in parasitism were at 181-240 cm and 241-300 cm. There was no significant difference in mean parasitism at 361-420 cm and 421-480 cm.

### 5.3.2 Temporal distribution of *A. planipennis* and *P. sulcata*

On 27 May, 89.6\% of *A. planipennis* were pupae residing in chambers constructed in the either the bark or sapwood (Figure 5.3). The other 10.4\% were second-, third-, and fourth-instar larvae that had overwintered. By 3 June, most of the pupae had emerged as adult *A. planipennis*. The within-tree population on this date were overwintering larvae, and some pupae which had yet to emerge. These proportions carried on until 12 July, when upon early-instar progeny of the adult population emerging in 2010 were first observed. From this time to the end of the summer, the proportion of early-instar *A. planipennis* larvae decreased as they grew into third and fourth instar larvae. By the final sampling event, 93.4\% of *A. planipennis* were third or fourth instar
larvae, with the remaining early-instar *A. planipennis* likely joining the late-instar larval cohort soon after.

On 27 May, 90.0% of *P. sulcata* were of the larval-pupal stage residing in the pupal chambers of their *A. planipennis* hosts (Figure 5.4). The remaining proportion was early-instar endoparasitoids of the overwintering *A. planipennis* larval cohort. Pupal-adult *P. sulcata* were first observed on 3 June. By 14 June, 67.5% of the within-tree *P. sulcata* population were pupal-adult. On 23 June, the progeny of the emerging *P. sulcata* were first observed as early-instar parasite in *A. planipennis* larval hosts. Similar proportions were also seen on 12 July. By 24 July, 80.4% of within-tree *P. sulcata* were early-instar endoparasitoids of *A. planipennis* larvae. The remaining proportion was pupal-adult *P. sulcata* that had yet to emerge. The early-instar proportion continued to grow in subsequent sampling events, until 23 August when they comprised 100% of the within-tree *P. sulcata* population.

5.3.3 Density-related *A. planipennis* parasitism

I observed that *A. planipennis* parasitism by both *P. sulcata* and *Atanycolus* spp. were largely independent of host density (Table 5.1). Parasitism by *P. sulcata* was never positively density-dependent; however, parasitism by *P. sulcata* was nearly significant at 241-300 cm (Spearman Rank Test, \( \eta = -0.3937, P = 0.0774 \)). A significant negative relationship between host density and parasitism by *Atanycolus* spp. was observed at 241-300 cm (Spearman Rank Test, \( \eta = 0.4501, P = 0.0406 \)). Parasitism by *Atanycolus* spp. was nearly significantly influenced by host density at 361-420 cm. Parasitism was never density-related for *Atanycolus* spp. within any other height level.
5.4 Discussion

My results indicated that *A. planipennis* within-tree density varies significantly with tree height. Overall, a negative correlation with *A. planipennis* density and tree height was observed with insects occurring in higher densities in the lower boles of sampled trees. I also observed that neither total parasitism nor species-specific parasitism by *P. sulcata* or *Atanycolus* spp. was influenced by tree height. Overall *P. sulcata* parasitism was significantly higher than overall *Atanycolus* spp. parasitism, as well as within a majority of height levels. Analysis of the temporal distribution shows that *P. sulcata* indeed possesses delayed larval development, and that the majority immature stage throughout the sampling season is an early-instar larva within the host larva. Due to the lack of effect by tree height on insect distributions, I was able to test whether parasitism within height levels was significantly affected by host density and found that species-specific parasitism was largely independent of host density. These results provide information that can assist in evaluating the effectiveness of *P. sulcata* as well as in creating detection methodologies for sampling parasitoid populations.

5.4.1 Spatial distribution of *A. planipennis* and parasitoids

My observations on the within-tree density of *A. planipennis* agree with previous studies on the distribution of *Agrilus* spp. in host trees of similar dimensions (Loerch and Cameron 1984; Timms et al. 2006; Abell et al. 2012). Timms et al. (2006) found increases in bark thickness towards the base of trees to be an important factor behind these observations. Increased bark thickness may result in a number of benefits, including protection from parasitism and/or predation (Wermelinger 2002). Most parasitoids must pierce through the bark of a host tree using their ovipositor in order to access their hosts (Quicke 1997). For parasitization to be successful,
the female’s ovipositor must be long enough to penetrate the bark of the host plant. If bark thickness exceeds their ovipositor length, then parasitization may be prevented. This has been shown to significantly affect parasitization in otherwise effective parasitoids. For example, Hanks et al. (2001) observed that two common braconid parasitoids of the eucalyptus longhorned borer (*Phoracantha semipunctata* F., Coleoptera: Cerambycidae) - *Syngaster lepidus* Brullé and *Callibracon limbatus* (Brullé) - were both ineffective in attacking hosts concealed by bark >17 mm thick. Abell et al. (2012) observed similar reductions in effectiveness in *T. planipennisi* Yang (Hymenoptera: Eulophidae), a larval parasitoid of *A. planipennis*, when confronted with hosts concealed by bark > 3.2 mm thick. In both studies, the authors concluded that the inability of these parasitoids to attack hosts underneath bark greater than their limit would negatively impact their effectiveness in regulating pest populations in areas of large trees.

Neither parasitism by *P. sulcata* or *Atanycolus* spp. was significantly affected by height in the trees sampled in this study. As bark thickness is highly correlated with tree height, it is unlikely that bark thickness was a limiting factor in regards to parasitoid effectiveness in trees sampled at this site. Comparisons of mean bark thickness and ovipositor lengths for *P. sulcata* confirm this. Measurements of a number of ovipositors excised from *P. sulcata* adults showed that their length was approximately 6.4 mm, and the maximum length was 7.1 mm (L. E. Roscoe, unpublished), while mean bark thickness for trees at this site was slightly less. The ability of female parasitoids to access their hosts in trees at this site is thus an important factor in the relatively high amounts of parasitism observed. Any proposed use of *P. sulcata* against *A. planipennis* should therefore be limited to areas of predominantly young ash trees similar to those used in this study so that the maximum number of hosts can be parasitized. In sites of larger trees possessing bark thicker than 6.50 mm, a parasitoid with a longer ovipositor, such as
Atanycolus spp., may be more effective. Abell et al. (2012) observed that Atanycolus spp. were able to attack A. planipennis through bark up to 8.8 mm thick. In stands containing predominately older ash trees with similar bark, the liberation or augmentation of Atanycolus spp. would likely be more effective than similar release strategies using other known parasitoids. In stands containing ash of various ages, it would be advantageous to utilize both parasitoids to ensure that the greatest proportion of A. planipennis is vulnerable to parasitization.

Both overall and height-specific levels of parasitism by P. sulcata were significantly higher than parasitism by Atanycolus spp. in this study. Given that Atanycolus spp. are able to attack A. planipennis at all heights, it may be somewhat surprising that this ectoparasitic species occurs less frequently than the endoparasitic P. sulcata. Competition between ecto- and endoparasitoids is highly skewed towards ectoparasitoids because almost all ectoparasitoids are idiobionts that paralyze their host during oviposition (Quicke 1997). This is done so that the attached ectoparasitoid is not damaged or dislodged by a live host during the parasitoid’s development. These paralytic toxins also incapacitate any endoparasitoids that may be associated with the host. Although some exceptions exist, endoparasitoids cannot defend themselves in this situation, and are thus severely handicapped when competing with ectoparasitoids. Consequently, I would expect that Atanycolus spp. would outcompete P. sulcata and thus occur more frequently. However, this was not observed, perhaps due to the better synchronization of P. sulcata adults with their preferred host stage, which may have allowed for increased parasitization. Synchronization is essential for maximizing host mortality, and is necessary for biological control agents to be effective at suppressing host populations (Coppel and Mertins 1977). In Chapter 4, peak flight period of P. sulcata adults for this site during the sampling period occurred between 29 June and 14 July. Here, I found that the highest proportion of first-
and second-instar *A. planipennis* occurred on 12 July. As early-instar larvae are the preferred hosts of *P. sulcata*, synchronization between the preferred host stage and peak flight period of adult *P. sulcata* seems to exist. Conversely, peak *Atanycolus* spp. emergence occurred when most *A. planipennis* were pupae (Chapter 2). As its preferred larval host stage was not present at this time, it would therefore have been difficult for *Atanycolus* spp. to cause high levels of mortality to *A. planipennis* populations. Although poor synchrony with the preferred host stage may be overcome by augmenting the population through releases while the preferred host stage was available, it would be an important hindrance to the effectiveness non-manipulated parasitoid populations. Therefore, if *Atanycolus* spp. were to be involved in an augmentative biological control program, augmentation of naturally occurring populations during the occurrence of the preferred host stage would be necessary. Due to the demonstrated synchronization between preferred host stage and *P. sulcata* adult emergence, this may not be necessary for natural *P. sulcata* populations in *A. planipennis*-infested sites.

### 5.3.2 Temporal distribution of *A. planipennis* and *P. sulcata*

I observed that the majority of immature *P. sulcata* observed during the final sampling periods were early-/mid-instar larvae. This agrees with my hypothesis that *P. sulcata* would possess delayed development as this is consistent with koinobiont parasitoids. In larval-pupal parasitoids, the immature wasp will usually remain as an early-instar larvae while the host is still in its larval form. When the host nears pupation, the parasitoid will quickly complete its own pupation and kill the host. These results indicate that this likely occurs for *P. sulcata*. Following the emergence of the adult generation, the sole representatives of *P. sulcata* in the sampled trees were early-instar larvae within *A. planipennis* second- and third-instar larvae. Late-instar *P. sulcata* larvae were not observed until the pupal chamber had been formed by the host. As these
are only formed by the *A. planipennis* fourth-instar immediately prior to the formation of the prepupae and pupae, it is likely that the *P. sulcata* larva quickly developed into late-instars after the host reached its final-instar. After formation of the host pupal chamber, *P. sulcata* likely consumed the host and began its own pupation within the chamber. Parasitoid pupation occurred before host pupation as pupal features such as head-thorax-abdomen segmentation, and eyes were not observed on the host exuviae. All parasitized host castes also possessed urogomphi, a larval structure that is lost in *A. planipennis* after pupation. Delayed development is a common developmental mode in koinobionts, and has been documented in another chalcid, *Chalcis canadensis* (Cresson) (Cowan 1979).

Delayed development has not been observed in any other studied parasitoids of *A. planipennis*. Based on this and emergence data from Chapter 2, the approximate length of a single generation of *P. sulcata* is likely ten months. This is much longer than the life cycles for *S. agrili* and *B. indica*, which require approximately 25 and 82 days respectively (Wang et al. 2007; Duan et al. 2011b). Delayed development precludes the possibility of multiple generations per year, thus making *P. sulcata* univoltine. Although multivoltine parasitoids are more effective in natural populations than univoltine species if multiple host stages can be attacked, univoltine species can still effective if they synchronize with their preferred host stage. As indicated in this and previous chapters, synchronization likely exists between *P. sulcata* and its preferred host. Therefore univoltinism should not be of hindrance to the effectiveness of naturally occurring populations. However, univoltine species such as *P. sulcata* are difficult to rear in large numbers in the laboratory due to their long life cycle. Thus, other methods such as the movement of material containing parasitoids should be considered if the release of *P. sulcata* between sites is required.
5.4.3 Density-related A. planipennis parasitism

I observed that parasitism by both *P. sulcata* and *Atanycolus* spp. was largely not influenced by host density. Density-dependent parasitism is believed to be important in the regulation of pest populations (DeBach and Rosen 1991). According to optimal foraging theory, parasitoids should aggregate in areas of high host densities where their oviposition rates can be maximized. However, observed density-dependent parasitism is rare in parasitic Hymenoptera (Walde and Murdoch 1988). Several limitations exist within parasitoid-host dynamics that commonly prevent density dependent attack rates. Females may be limited by their searching ability, the number of mature eggs they possess while in the high density environment, mutual interference between parasitoids, or by handling time for each host (Walde and Murdoch 1988). Density dependent parasitism for parasitoids of concealed hosts is also compounded by the occurrence of higher host densities in areas of high concealment. As indicated previously, wood-boring larvae are often found in trees where bark is thickest. Consequently, only parasitoids with suitably long ovipositors would be able to attack these hosts, and this refuge would thus potentially exclude a large proportion of the parasitoid community from attacking these hosts. However, non-density dependent parasitism may provide significant mortality to pest populations. Such mortality may be increased to levels where pests at high densities may be effectively suppressed if several parasitoid species are utilized. Therefore, although density-dependent parasitism is important to regulating pest populations, its absence may be overcome by the use of more than one parasitoid species in a biological control program.

5.4.4 Conclusions

In this chapter, parasitism by *P. sulcata* and *Atanycolus* spp. on A. planipennis were not influenced by either tree height or host density. This is important as it indicates that host refuges
do not exist for *A. planipennis* in trees of ages used in this study. However, it does not eliminate the possibility that host refuges exist for *A. planipennis* in larger trees where bark thickness exceeds ovipositor length. The lack of influence of host density on parasitism is important as it indicates that a single parasitoid species may not be effective in preventing host densities from reaching economically damaging levels. However, the combined mortality caused by several species of parasitoids indicates that native parasitoids in this site may be as effective as introduced classical biological control agents in parasitizing large numbers of *A. planipennis*.

Finally, I observed that *P. sulcata* displays delayed development, which is consistent with other koinobiont parasitoids and suggests that *P. sulcata* is univoltine. Although this does not reduce its effectiveness because *P. sulcata* is well synchronized with its preferred host stage, it means that the production of large numbers of laboratory reared insects may not be possible. Therefore, improvement of *P. sulcata* populations should be accomplished through the movement of material containing developing parasitoids, or perhaps through aggregation or retention using attractive semiochemicals (See Chapter 4).
Table 5.1: Within-height parasitism rates for two native parasitoids vs. *Agrilus planipennis* host density in young, green ash trees cut from the W. Darcy McKeough Dam Floodplain, Duthill, Ontario, Summer 2010 (Spearman Rank Test, \( P = 0.05 \)).

<table>
<thead>
<tr>
<th>Height (cm)</th>
<th>n</th>
<th>Wasp</th>
<th>Rho (( \eta ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 60</td>
<td>24</td>
<td><em>P. sulcata</em></td>
<td>0.9968</td>
<td>0.9968</td>
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<tr>
<td></td>
<td></td>
<td><em>Atanycolus</em> spp.</td>
<td>0.0294</td>
<td>0.8916</td>
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<tr>
<td>61 – 120</td>
<td>24</td>
<td><em>P. sulcata</em></td>
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<tr>
<td></td>
<td></td>
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<td>0.1583</td>
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<tr>
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<td></td>
<td><em>Atanycolus</em> spp.</td>
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<td>0.6443</td>
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<td>181 – 240</td>
<td>19</td>
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<td>0.2357</td>
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<td><em>Atanycolus</em> spp.</td>
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<td><em>Atanycolus</em> spp.</td>
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<td>0.0406</td>
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<td>0.1732</td>
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<td>361 – 420</td>
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<td></td>
<td></td>
<td><em>Atanycolus</em> spp.</td>
<td>0.6681</td>
<td>0.1009</td>
</tr>
</tbody>
</table>
Figure 5.1: Within-tree *Agrilus planipennis* densities vs. height in young, green ash (*Fraxinus pennsylvanica*) trees cut from the McKeough Dam Floodplain in southwestern Ontario from May-August 2010. Each bar represents the number of insects per m² of phloem of *A. planipennis* (± S.E.) for 21 to 24 samples of each height level. Bars with the same letter are not significantly different (*P > 0.05*, Dunn’s Test).
Figure 5.2: Mean (± S.E.) proportion of *A. planipennis* parasitized by *Phasgonophora sulcata* and *Atanycolus* spp. at different height levels in ash trees at the McKeough Floodplain in southwestern Ontario, May to August 2010. An ‘*’ represents a significant difference between parasitism rates for each species at a given height level (Mann-Wilcoxon Test, *P* < 0.05).
Figure 5.3: Proportion of *Agrilus planipennis* within ash by sample date at the McKeough Floodplain, Ontario in 2010. The number above each sample bar represents the total number of *Phasgonophora sulcata* individuals recovered from within sampled trees.
Figure 5.4: Proportion of *Phasgonophora sulcata* within *Agrilus planipennis*-infested ash by sample date at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario in 2010. The number above each sample bar represents the total number of *P. sulcata* individuals recovered from within sampled trees.
Chapter 6: General conclusions and avenues for future research

6.1 General Overview

In my thesis, I analyzed several aspects of the life history of *P. sulcata* that would aid in both the evaluation of this parasitoid as a biological control agent for *A. planipennis*, and in the development of an augmentative biological control program using this parasitoid species. In Chapter 1, I discussed the critical importance of the *A. planipennis*-problem in North America, and the potential use of native wasp parasitoids as part of a long-term management option in Canada. In this Chapter, the definition of biological control, several important characteristics of successful biological control agents, and the components of a biological control program were discussed. Based on this, I outlined several studies that could be carried out that would assess if *P. sulcata* may be used as an augmentative agent against *P. sulcata*. In Chapter 2, the life history of *P. sulcata* and immature stages in relation to *A. planipennis* were determined, followed in chapter 3 by an examination of the courtship and mating sequences of adult *P. sulcata*. In Chapter 4, I studied the chemical ecology of *P. sulcata* by observing the use of semiochemicals in host location by adult female *P. sulcata*, followed by an analysis of the spatial and temporal distribution of *P. sulcata* in *A. planipennis*-infested ash trees in Chapter 5. The results of these chapters provide important information that can be used to assess the potential of *P. sulcata* as an augmentative biological control agent for *A. planipennis*, and its use as part of a biological control program. The results also illustrate an intriguing relationship between two insects that share nearly no evolutionary history, and provide an important insight into a little known group of chalcidid parasitoids. As a whole, my work will be of significance to both the application of *P.
sulcata as a biological control agent for A. planipennis and in furthering the general knowledge of parasitoid-host dynamics and chalcidid biology.

6.2 Phasgonophora sulcata as a potential augmentative biological control agent for A. planipennis

The results indicate that the flight period of adult P. sulcata is well synchronized with the peak occurrence of the parasitoid’s preferred A. planipennis host stage. Synchronization is an important requirement if a biological control agent is to control its target host. Most parasitoids have methods and structures that are specific to locating their hosts only when the host is in a certain state. For example, parasitoids of wood-boring larvae often use auditory cues produced by the consumption of woody material made only by their target stage. Parasitoids of these hosts may also use synomones such as bark volatiles that are released as a direct result of larval feeding in host location. If these parasitoids were exposed to other life stages, such as non-feeding eggs or pupae, or free-living adults, which do not produce these cues, then it would be impossible for the parasitoid individual to locate and parasitize its host. Even if parasitization were to occur, parasitoid development would likely be disrupted or possibly not occur at all as the host life stage either may not be long enough or provide the required nutrition to ensure complete development. Synchronization is thus vital in determining the level of parasitoid effectiveness.

My results indicated that both flight period and peak reproductive output of P. sulcata females were synchronized with the maximum abundance of the preferred host stage. Based on trap catches at the W. Darcy McKeough Dam Floodplain in 2010, flight of adult P. sulcata is greatest from 29 June to 14 July (Chapter 4), and in Chapter 5, this period was shown to coincide
with the highest observed numbers of early instar *A. planipennis*. As this life stage is the preferred host stage for adult females, temporal synchronization between the host and parasitoid was present. Furthermore, *P. sulcata* adults possess much of their mature egg complement at emergence, and are thus proovigenic (Chapter 1, 2). This eliminates the requirement of a pre-oviposition period, which is necessary for synovigenic parasitoids that must develop their mature egg complement after emergence before parasitizing hosts. In addition to both temporal and reproductive synchronization, adult female wasp parasitoids must be mated if female eggs are to be laid. Two important factors that can influence mating success in emerging insects are protandry and age of receptiveness. Protandry is the emergence of male individuals prior to females. The presence of males in the environment during female emergence maximizes the opportunities for females to mate before dispersal (Ridley 1993). For this to be effective, females themselves must be receptive to mating immediately after emergence, and produce attractant that make them detectable by males. The occurrence of protandrous emergence (Chapter 2), and the consistent elicitation of mating sequences observed in newly emerged males and females (Chapter 3) indicates that the likelihood of parasitoids mating soon after emergence is high. Therefore, the presence of mated female parasitoids, each possessing a mature egg complement during the occurrence of the preferred host stage in the environment, is a strong possibility in natural populations of *P. sulcata*. This synchronization is likely an important factor in the high levels of parasitization by *P. sulcata* observed at the W. Darcy McKeough Dam Floodplain (Chapter 5).

The presence of a mature egg complement at emergence may also provide *P. sulcata* with an advantage in sites where *A. planipennis* densities are high. An important dichotomy in parasitic wasps is between species that are ‘egg-’ or ‘time-limited’ (see Chapter 1). During
development, some species can direct resources to egg development at a cost of somatic maintenance. At emergence, these parasitoids have developed most or all of their lifetime mature egg complement. An important trade-off of this developmental strategy, however is reduced longevity. Therefore, these parasitoids are ‘time-limited’. Conversely, ‘egg-limited’ parasitoids are those which do not develop their egg complement during larval development, but rather generate eggs based on host and nutrient availability during their lifetime. Because these parasitoids have directed nutrients during development towards longevity, while only producing comparatively few eggs at a given age, they are thus ‘egg-limited’. In order to maximize their reproductive output, ‘time-limited’ parasitoids must locate patches of high host densities shortly after emergence if they are utilize their high fecundity. Once at such sites, their high fecundities can allow these parasitoids to cause high levels of mortality in host populations. If ‘time-limited’ parasitoids cannot locate high-density sites quickly, then the likelihood of the parasitoid dying before using all of its eggs is high. In such areas, ‘egg-limited’ parasitoids would possess a distinct advantage in that they require only a small number of eggs at a given time, while possessing the longevity required to locate hosts within a low-density site or move to new patches if required. In high-density sites, ‘egg-limited’ parasitoids would not possess the large fecundity required to parasitize high number of hosts. Therefore, ‘egg-limited’ parasitoids would likely be more successful in sites of lower host densities.

In Chapter 2, *P. sulcata* was shown to be proovigenic, and can be considered ‘time-limited’, based on the possession of a mature egg load at emergence. Therefore, *P. sulcata* should be found in higher numbers at sites where *A. planipennis* densities are high. Indeed, I observed in Chapter 5 that overall rates of parasitization by *P. sulcata* were significantly higher than those of the ‘egg-limited’ synovigenic *Atanycolus* spp. at the McKeough Floodplain. This
site possessed a well established infestation of *A. planipennis*, which had been present for several years, and could thus be classified as a high-density site. Therefore, the host populations at this site would possess the attributes required for a ‘time-limited’ parasitoid such as *P. sulcata* to be successful. In the case of the synovigenic *Atanycolus* spp., parasitism may be limited by the potentially low fecundities of individual females. Lower parasitism by *Atanycolus* spp. may also be strongly influenced by asynchrony of adult parasitoid emergence (Chapter 2), which occurred both before *A. planipennis* adult emergence, and the presence of the preferred larval host stage that occurs from July onward (Chapter 5). However, it cannot be expected that *P. sulcata* would be the dominant parasitoid species for all sites. This includes sites where host densities are low such as in satellite populations, areas where classical biological control agents have suppressed host populations, or at recent introductions. In such sites, ‘egg-limited’ species such as *Atanycolus* spp. may be more effective. It is therefore necessary to take into account host density prior to parasitoid release in order to ensure the most effective parasitoid species is released.

### 6.3 Restrictions on *P. sulcata* use

An important attribute of a successful biological control program is the ease with which insects can be mass reared. For example, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are used globally against various pests and may be reared at rates of many millions per week in some facilities (Smith 1996). Consequently, large-scale release programs of many individuals at several sites can be undertaken. This is undoubtedly an important aspect of the continued use of this group in biological control programs around the world. In the United States, the Biological Control Production Facility (BCPF) in Brighton, MI has produced and released 720 000 *A. planipennis* parasitoids from January 2009 to February 2013. This has
allowed for the liberation of many thousands of Chinese parasitoids on multiple occasions in several states (USDA-APHIS 2013). Several parasitoid characteristics assist in the production of large numbers of insects. These include multiple generations per year, and gregariousness. At the BCPF, *S. agrili*, *T. planipennisi*, and *O. agrili* all produce multiple generations per year, and with the exception of *O. agrili*, are gregarious (USDA-APHIS/ARS/FS 2012). With multiple parasitoids being produced per host in multiple generations, the production of large numbers of parasitoids can be accomplished. The ability to produce large numbers of parasitoids allows for larger and more frequent releases to occur, and will increase the potential for effective pest regulation.

Based on this research, it appears that production of *P. sulcata* on a scale similar to that of the three parasitoids produced at the BCPF cannot be achieved. Firstly, *P. sulcata* likely only produces one generation per year. Figures presented in Chapters 2 and 4 illustrating the adult flight period indicated that adult emergence peaked only once. Additionally, dissections of *P. sulcata* populations in ash trees indicated that *P. sulcata* late-instar and pupae were only observed up until adult emergence (Chapter 2). If *P. sulcata* were multivoltine, then multiple peaks of both pupal and adult proportions would have been observed in tree dissections and sticky trap collections, respectively. According to my research, this was not the case. *Phasgonophora sulcata* is also a solitary parasitoid, with only one parasitoid being produced per parasitized host (Chapter 5). Lastly, *P. sulcata* attacks early instar hosts, and possesses delayed development. Therefore, if parasitoids were to be produced, it would be necessary to maintain parasitized *A. planipennis* throughout the duration of their larval and pupal development if *P. sulcata* were to be reared. This cycle also includes an overwintering period that likely requires at least six weeks. Based on the incidence of *P. sulcata* dissected from *A. planipennis* observed in
Chapter 5, and the first observations of adult *P. sulcata* in mid-June (Chapters 2 and 4), the generation time for this parasitoid is approximately ten months. This length of time precludes the possibility of rearing large numbers of this parasitoid at multiple times during the year. The combination of univoltinism, solitary parasitism, and delayed larval development thus prevents the rearing of large numbers of this parasitoid as is done for other parasitoids at the BCPF.

A potential method of distributing *P. sulcata* from site-to-site is through the dissemination of *A. planipennis*-infested material from *P. sulcata*-positive sites into sites where parasitoid populations were absent. This method, however, would require the site to already be infested by *A. planipennis* as adult beetles would emerge from the material. This is may not be an important limitation, however, as my work provides evidence that *P. sulcata* would only be effective in areas of mid- to high-densities of *A. planipennis*. In areas where *A. planipennis* infestations are low, injected pesticides and/or releases of synovigenic parasitoid species should be used. Such protocols already exist, as injected pesticides are currently being used in urban centres in both the United States and Canada. Additionally, the protocol for the release of Chinese parasitoids recommends that low- to medium-density sites be targeted (USDA-APHIS/ARS/FS 2012). Currently, there are no strategies beyond tree eradication for management of *A. planipennis* in high density sites. *Phasgonophora sulcata* may be useful in this role.

### 6.4 Future studies

The results of my work provide a necessary and useful basis for investigations into the potential use of *P. sulcata* as an augmentative biological control agent for *A. planipennis*. However, further research is required explicitly test some of the conclusions of these studies, as
well as directly measure the effectiveness of released and/or augmented parasitoid populations. Such studies are critical to the determination of whether or not *P. sulcata* may be useful against *A. planipennis*.

### 6.4.1 Release of parasitoids

The rearing of large numbers of laboratory reared populations of *P. sulcata* likely cannot be accomplished. Thus, one possible method of disseminating *P. sulcata* may be through the movement of material containing parasitoids into sites where populations are absent. A useful future study using this method would be to place material in sites possessing different characteristics, such as host density, basal density, and level of fragmentation. By comparing rates of establishment of *P. sulcata* populations between sites, it would be possible to determine the important site factors that influence parasitoid establishment. These factors could then be used in the selection of sites for future release and potentially in determining the number of parasitoids to be released and number of releases required to attain a specific level of management. It is important that conditions that may influence establishment be understood prior to the widespread and effective use of any biological control agent in order to increase the chances of success.

### 6.4.2 Interactions with other parasitoids

Although Chapter 5 provided some evidence that *P. sulcata* is a more effective biological control agent than *Atanycolus* spp. at the McKeough Floodplain, a comprehensive study of the interaction between co-existing parasitoids should be carried out. Although *P. sulcata* was shown as the most numerically important source of parasitism for *A. planipennis* in these studies, it is but one of several North American parasitoids that have been recorded from *A. planipennis* (see
Appendix A). A study such as this is necessary because some parasitoids, such as ectoparasitoids, are intrinsically better competitors than endoparasitoids based on their ability to feed externally on the host (see Chapters 1, 2). Consequently, endoparasitoid species may not be successful in a site where ectoparasitoids are present in high numbers. In such an event, endoparasitoids should be released in areas where competing ectoparasitoids are rare. Prior to release, it is therefore necessary to observe the competition strategies of all ecto- and endoparasitoid species involved in the biological control program. This should be done in a laboratory setting where conditions are controlled and specific attributes can be tested and observed. A potential line of study would be similar to the study completed by Ulyshen et al. (2010a), who analyzed the interactions between the *A. planipennis* parasitoids *S. agrili* and *T. planipennisi*. Potential experiments could include specifically testing if multi-parasitism occurs or if one parasitoid is consistently out-competed by the other. The results of my study would be important to understanding whether these parasitoids can coexist in the same site while providing effective pest management. Additional studies that observed the interactions between *P. sulcata* and other *A. planipennis* parasitoids such as *B. indica* and the three Chinese parasitoids - *S. agrili*, *T. planipennisi*, and *O. agrili* - would also be useful, and would again be important in determining if these parasitoids can coexist while also providing effective pest control.

### 6.4.3 Applied chemical ecology of *P. sulcata*

In Chapter 4, adult females of *P. sulcata* were attracted by a synomone, (Z)-3-hexenol, and a kairomone, 3-(Z)-lactone; however, I was not able to test whether males responded to these volatiles as well. A similar experiment that analyzed the response of males to semiochemicals would be advantageous for the identification of detection or aggregation volatiles. Furthermore, the response of both males and females to ash-bark volatiles should also be carried out to
determine if adults respond to bark sesquiterpenes. If a positive response were observed, then these semiochemicals might also be used for detection and retention purposes. Lastly, expansion of the trap design process would be useful, where more types of traps in combination with the responsive volatiles observed in my study were evaluated as to their ability to capture *P. sulcata*. It is possible that a trap baited with the pheromone observed in Chapter 3 might be most effective in detecting adult males due to the high response rates of males to active females. To complete such a study, the pheromone would have to be isolated and, if possible identified and synthesized. If this were not possible, then either polar or non-polar washes of live adult females should be undertaken to isolate compounds for use as part of a lure. An experiment where various traps differing in color, height, bait, and placement is also needed. The goal of such a study would be to identify the most effective trap for sampling parasitoid populations.

6.5 Final conclusions

Throughout this thesis, I have sought to analyze important characteristics of *P. sulcata* with the goal of determining if it showed potential as a biological control agent for *A. planipennis*. It appears that natural populations of *P. sulcata* represent an important source of mortality for high density populations of *A. planipennis* in wooded sites such as those in this study. However, difficulties in insect production related to the long development time and solitary nature of this endoparasitoid make mass rearing of this parasitoid unlikely. Further studies on the evaluation of detection and retention of parasitoids using semiochemicals should be conducted so that effective sampling methods could be developed to estimate field populations. As well, evaluations of site factors such as host density as they relate to parasitism should be conducted to determine if *P. sulcata* is an effective source of mortality for both low
and high-density pest populations. Although I did not conclusively prove that *P. sulcata* can be the primary long-term management option for *A. planipennis*, in Ontario, I have shown that a native species with a very short evolutionary history with a non-native species can be an effective source of natural mortality.
Literature cited


Asgari, S. 2006. Venom proteins from polydnavirus-producing endoparasitoids: Their role in host-parasite interactions. Archives of Insect Biochemistry and Physiology **61**: 146-156.


Bartelt, R.J., Cosse, A.A., Zilkowski, B.W. and Fraser, I. 2007. Antennally active macrolide from the emerald ash borer *Agrilus planipennis* emitted predominantly by females. Journal of Chemical Ecology **33**: 1299-1302.


green leaf volatiles (GLVs) emitted by host foliage. Journal of Chemical Ecology 34: 1170-1179.


Hansen, L.S. and Jensen, K.V. 2002. Effect of temperature on parasitism and host-feeding of


Ko, J.H. 1969. A list of forest pests in Korea. Forest Research Institute, Seoul


the emerald ash borer, *Agrilus planipennis*, to induced volatiles of Manchurian ash, *Fraxinus mandshurica*. Chemoecology **16**: 75-86.


Chrysopidae), and *Macrocentrus grandii* (Hymenoptera: Braconidae) trapped on colored sticky traps in corn habitats. Environmental Entomology 26: 983-988.


Chemoecology **1**: 69-76.


Yang, S., Duan, J.J., Lelito, J. and Van Driesche, R. 2013. Multiparasitism by Tetrastichus planipennisi (Hymenoptera: Eulophidae) and Spathius agrili (Hymenoptera: Braconidae): Implication for biological control of the emerald ash borer larvae (Coleoptera: Buprestidae). Biological Control 65: 118-123.


Appendix 1: Photographic key to the hymenopterous parasitoids of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in Ontario.

Introduction and Methods

The emerald ash borer (EAB) *Agrilus planipennis* Fairmaire, is an invasive destructive pest of *Fraxinus* spp. (ash) in North America. Since its initial discovery in 2002, EAB has killed millions of ash trees in Canada and the United States (Poland and McCullough 2006). First found in the Windsor/Detroit region, EAB has spread to 22 states and 2 provinces (USDA-APHIS 2013). Due to the ability of this insect to move undetected in contaminated wood, the potential spread of EAB to unaffected areas is a distinct possibility. Therefore, management plans for controlling EAB are in high demand.

One potential long-term management plan in Canada is the augmentation of native wasp parasitoid populations to provide biological control over EAB populations (Lyons 2008). Surveys conducted in both Canada and the United States have identified several indigenous parasitoids that can attack EAB (Liu et al. 2003; Lyons 2008). Some of these parasitoids have been observed to parasitize EAB in high enough numbers that further investigation into their use in a biological control plan is warranted.

In the following document, we provide a key for identifying native parasitoids of EAB in Ontario. We have identified ten indigenous wasp parasitoids that have been reared from EAB-infested material collected from near the cities of Sarnia and London, Ontario. This key has been designed to assist both trained and untrained personnel in the identification of EAB parasitoids.
Along with identification keys, we have also provided species pages that discuss the distribution, biology and distinguishing characters of these wasps.

Our key is based on the specimens within the reference collection of Dr. D. Barry Lyons of the Canadian Forest Service at the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario. The key to the species of *Atanycolus* is based on work undertaken by Dr. Paul M. Marsh, but is limited only to species recovered in Ontario.

It should be noted that this document presents only North American wasp parasitoids, and does not cover imported Chinese parasitoids that have been released in the United States and very recently Canada. Images were generated using a Nikon imaging system attached to a compound microscope. Images were compiled using Nikon processing software. All images were taken by Lucas Roscoe unless indicated otherwise.
A: Species Checklist

- Hym.: Ichneumonoidea: Braconidae
  - *Atanycolus cappaerti* Marsh and Strazanac
  - *Atanycolus disputabillis* Cresson
  - *Atanycolus hicoriae* Shenefelt
  - *Atanycolus longicauda* Kokujev
  - *Atanycolus tranquebaricae* Shenefelt
  - *Leluthia astigma* (Ashmead)
  - *Spathius floridanus* Ashmead

- Hym.: Chalcidoidea: Eupelmidae
  - *Balcha indica* (Mani and Kaul)
  - *Metapelma spectabile* Westwood

- Hym.: Chalcidoidea: Chalcididae
  - *Phasgonophora sulcata* Westwood
B: Key to the North American Hymenopterous parasitoids of *Agrilus planipennis* in Ontario

1 Abdomen red or yellow. Shade of red may vary from deep scarlet to bright crimson (Figure A1). Thorax black or largely black with some yellow patterning on dorsum and sides. ..................2

- Abdomen black, brown, or metallic (Figure A1). Thorax same colour as abdomen. ..................3

2 Hind femora swollen with distinct teeth of the interior margin (Figure A2)

*Phasgonophora sulcata*

- Hind femora without distinct teeth (Figure A2). ..................6

*Atanycolus* (5 spp.)

3 Cuticle metallic green (Figure A3).

..................4

- Cuticle drab; brown or black in colour (Figure A3). ..................5

4 Distal antennal segments thicker than proximal antennal segments (Figure A4).

*Metapelma spectabile*

- Distal and proximal segments approximately the same thickness (Figure A4).

*Balcha indica*

5 Propodeum (1st abdominal segment) width nearly uniform from thorax to 2nd abdominal segment. Body brown (Figure A5).

*Spathius floridanus*

- Propodeum narrow at thorax and wide at 2nd abdominal segment (Figure A5).

*Leluthia astigma*
C: Key to the *Atanycolus* spp. of Ontario.

6 Scape (first antennal segment) without a deep excavation on the anterior side. Last flagellomere blunt and bare of setae (Figure A6).

- Scape with deep excavation on the anterior side. Last flagellomere pointed and covered with dense setae (Figure A6). \(Atanycolus\) \(longicauda\)

7 Side and dorsum of thorax with distinct yellow markings throughout (Figure A7).

- Side of thorax completely black. Dorsum of thorax usually black, but sometimes with pale yellow bands running longitudinally on the scutellum (Figure A7). \(Atanycolus\) \(tranquebaricae\)

8 4\(^{th}\) abdominal tergite smooth. Occasionally rugose (rough) on basal edge (Figure A8).

- 4\(^{th}\) abdominal tergite entirely rugose (Figure A8). \(Atanycolus\) \(disputablis\)

9 Dorsum and ventral surface of abdomen yellow, but infused with red or pinkish-red (Figure A9).

- Dorsum and ventral side of abdomen nearly completely yellow. Very little, if any, red or pinkish red coloring (Figure A9). \(Atanycolus\) \(cappaerti\)
D. Species information

*Phasgonophora sulcata* Westwood (*Hymenoptera: Chalcidoidea: Chalcididae*)

_*Phasgonophora sulcata*_ is distinguishable from other EAB parasitoids by its swollen hind femora that are toothed on the interior edge and by a heavily punctated cuticle on the head and thorax. It is a solitary koinobiont endoparasitoid of North American buprestids and is the sole species in the genus. The preferred host stage is likely a neonate, and abundance is often highest in early- to mid-summer. This species has been found large numbers in EAB-positive sites near Sarnia and London, Ontario. It has also been found in the Northeastern United States, but in significantly fewer numbers (Liu et al. 2003). In terms of abundance, *P. sulcata* is the most important native parasitoid of EAB in Ontario.
Atanycolus spp. (Hymenoptera: Ichneumonoidea: Braconidae)

Five species of Atanycolus have been recorded from EAB in Ontario. They include A. longicauda, A. tranquebaricae, A. disputablis, A. hicoriae, and A. cappaerti. Based on the number of collected specimens, the latter two species are the most important in terms of abundance. Atanycolus spp. are distinguishable by their bright red abdomens, delicate appearance, and black wings. These species are all solitary idiobiont ectoparasitoids, and can produce multiple generations in a season (Cappaert and McCullough 2009). Atanycolus spp. are the second most important group of EAB parasitoids in the province after P. sulcata. In Michigan, A. cappaerti is the most abundant of all native parasitoids and has been known to parasitize over 70% of EAB in some sites (Cappaert and McCullough 2009). Atanycolus spp. are also an important indigenous EAB parasitoids in Pennsylvania (Duan et al. 2013b).
Metapela spectabile Westwood (Hymenoptera: Chalcidoidea: Eupelmidae)

Though comparatively rare when compared with P. sulcata and Atanycolus spp., Metapela spectabile has been reared in association with EAB in Ontario and in the Northeastern United States (Knight et al. 2009). This wasp may be confused with small examples of B. indica, but can be differentiated based on the clubbed appearance of its antennae. In Ontario, a single specimen was reared from EAB-infested logs from Essex County. This species is a documented parasitoid of Agrilus subcinctus, a North American relative of EAB (Petrice et al. 2009).
*Balcha indica* Mani and Kaul (Hymenoptera: Chalcidoidea: Eupelmidae)

This parasitoid is characterised by its metallic luster and long, tapered abdomen. *B. indica* is a solitary idiobiont ectoparasitoid and can successfully attack various stages of EAB larvae and pupae (Duan et al. 2011). This species is parthenogenic, and is originally from Southeast Asia (Gibson 2005). It likely arrived in North America while associated with a non-native wood-boring beetle. Compared to *P. sulcata* and *Atanycolus*, *B. indica* is relatively rare in Ontario, however it is the most important parasitoid in terms of abundance in EAB-positive sites in Pennsylvania (Duan et al. 2009).
**Spathius floridanus** Ashmead (*Hymenoptera: Ichneumonoidea: Braconidae*)

*Spathius floridanus* (=*Spathius similimus* Ashmead) is a gregarious idiobiont ectoparasitoid. It has been recorded from EAB-positive sites in the Northeastern United States and Ontario (Liu et al. 2003). This parasitoid is relatively rare in both Canada and the United States. A close relative, *Spathius agrili* Yang, is an important parasitoid of EAB in China and has recently been imported into the United States for use in a classical biological control program (Bauer et al. 2008).

*Note:* *Spathius agrili* will key out to this species. It is not possible to separate the two species based on this key alone. Specimens should be kept for identification by an expert. *Spathius* agrili individuals however are likely to be extremely rare in Ontario as they have only been released in the United States, but may occur in the extreme south of Ontario due to the proximity to Michigan.
Leluthia astigma (Ashmead) (Hymenoptera: Ichneumonoidea: Braconidae)

*Leluthia astigma* is an ectoparasitoid of several *Agrilus* and *Chrysobiothris* species in North America (Kula et al. 2010). It has been reared from EAB in Ohio, and is widely distributed in the United States. It has also been recorded from Quebec, Ontario, and Mexico, however it has yet to be collected from EAB-positive sites in Canada.
E. Figures

Figure A1: Lateral photos of A) *Phasgonophora sulcata*, B) *Atanycolus hicoriae*, C) *Spathius floridanus* and D) *Balcha indica.*
Figure A2: Hind femora of A) *Phasgonophora sulcata* and B) *Atanycolus cappaerti*.
Figure A3: Lateral views of A) *Metapema spectabile* and B) *Leluthia astigma*. 
Figure A4: Antennae of A) *Metapema spectabile* and B) *Balcha indica*.
Figure A5: Dorsal views of abdomens for A) *Spathius floridanus* and B) *Leluthia astigma*.
Figure A6: Head and antennae of A), B) *Atanycolus longicauda* and C), D) *Atanycolus* spp.
Figure A7: Lateral and dorsal views of A) C) *Atanycolus tranquebaricae* and B) D) *Atanycolus sp.*
Figure A8: Abdomens of A) *Atanycolus disputablis* and B) *Atanycolus hicoriae*. Abdominal segments are numbered 1 to 4.
Figure A9: Dorsal and ventral sides of A) B) *Atanycolus cappaerti* and C) D) *Atanycolus hicoriae*. 