Ecological and logistical considerations toward introducing *Heringia calcarata* to New Zealand

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Abstract

This thesis outlines research conducted as part of a collaborative project between Virginia Tech and Plant and Food Research New Zealand (PFRNZ) to introduce *Heringia calcarata* (Loew) (Diptera: Syrphidae) to New Zealand (NZ) for biological control of woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae). Ultimately, the introduction of *H. calcarata* to New Zealand will be contingent upon satisfying regulatory requirements and concerns, including documentation that it will not have an adverse effect on the existing biological control of WAA by *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae). As well, it will be critical to develop methods for sustained rearing of *H. calcarata* in captivity. Basic and applied studies were conducted toward providing essential information for advancing this project. Apple shoot sections with a WAA colony that did or did not contain mummified aphids parasitized by *A. mali* were deployed in pairs at the base of apple trees. There was no significant difference in the mean number of *H. calcarata* eggs deposited between shoots with parasitized (1.5 ± 0.34 SE) and non-parasitized colonies (1.75 ± 0.42 SE), although female *H. calcarata* laid eggs less frequently on colonies with a high percentage parasitization. In choice-test feeding studies, larvae were offered non-parasitized aphids in combination with aphids in an early stage of parasitization or mummified aphids. Larvae consumed significantly fewer aphids in an early stage of parasitization (10.8 ± 0.48 SE) than non-parasitized aphids (13.4 ± 0.42 SE) and very few mummies (0.4 ± 0.14 SE) compared with non-parasitized aphids (14.2 ± 0.4 SE). In no-choice feeding trials, larvae consumed significantly more non-parasitized aphids (25.3 ± 1.93 SE) than aphids in an early stage of parasitization (19.7 ± 1.85 SE) or mummified aphids (2.2 ± 0.71 SE) and significantly fewer mummified aphids were consumed than early parasitized aphids. WAA colonies *in situ* on the branches of potted apple trees were exposed to *H. calcarata*, *A. mali*, or both. Exposure to *H. calcarata* larvae independently and in combination with *A. mali* was shown to have a significant effect on the number of WAA compared with control colonies, and *H. calcarata* larvae did not affect the number of mummified aphids produced within colonies. *Heringia calcarata* eggs were collected by deploying excised apple shoot sections containing at least one WAA colony at the base of apple trees for 8-12 h. One or more eggs were laid on 29% of shoots (n = 233 shoots). On shoots with eggs, 2.4 ± 0.21 SE eggs per shoot were recorded. In 2012, four shipments of *H. calcarata* eggs and larvae (total of 178) were sent from Virginia to a quarantine containment facility in NZ. This demonstrated that juvenile *H. calcarata* could be successfully transported internationally. In total, 124 adult flies were generated in NZ, representing 69.9% of the number of eggs and larvae recovered upon delivery to quarantine. Field-collected gravid female *H. calcarata* oviposited on WAA colonies under captive conditions: 63% in 2011 (n = 8) and 80% in 2012 (n = 15). In 2012, 98% of the eggs deposited hatched. Virgin females reared from eggs in the laboratory developed mature oocytes regardless of access to pollen. The findings of this research offer valuable insights into the biology and ecology of *H. calcarata* that are directly relevant to the project goals and that will help guide the development of *H. calcarata* as a classical biological control agent for WAA in NZ.

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Dedication

In loving memory of my late grandfather, George (Hori) Underdown.

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1. Introduction and literature review

Introduction

The woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), is native to the USA and a pest of apples in many of the world's apple growing regions (Hatton 1937). Colonizing both the roots and above-ground parts of apple trees, WAA populations on branches and shoots can reach very high levels, causing economic effects including reduced tree vigor, yield, and fruit quality (Sherbakoff and McClintock 1935, Weber and Brown 1988, Brown and Schmitt 1990, Brown et al. 1995). At harvest, it can be a significant phytosanitary issue for export fruit and a nuisance pest for those harvesting the crop (Cockfield and Beers 2007). WAA is a common resident on apple trees in commercial orchards in the Mid-Atlantic region of the USA (Brown and Schmitt 1994), although a complex of natural enemies usually suppresses it effectively (Brown & Schmitt 1994, Short and Bergh 2004, Bergh and Short 2008, Bergh unpublished) and therefore intervention with insecticides is rarely needed.

The parasitoid wasp, *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae), has long been known to be an important, specialized natural enemy of WAA (Howard 1929, DeBach 1964) and was exported from its native North America to many apple-producing countries in the early 20th century (Hagen and van den Bosch 1968). Brown & Schmitt (1994) reported that *A. mali* and syrphids were the most abundant natural enemies in WAA colonies in West Virginia, USA, although the syrphid species were not identified. Subsequent research in Virginia showed that aphidophagous larvae of three hover fly (Diptera: Syrphidae) species, *Heringia calcarata* (Loew), *Eupeodes americanus* (Wiedemann), and *Syrphus rectus* Osten-Sacken, were the predominant predators of WAA (Short and Bergh 2004, Bergh and Short 2008).

In New Zealand, where the vast majority of fruit is grown for export to foreign markets, WAA has been a chronic phytosanitary concern for apple producers. In 2008, a widespread and severe outbreak of WAA followed an insecticide-based disruption of biological control by *A. mali*. This event highlighted the tenuous nature of WAA biological control in New Zealand, prompting scientists from Plant and Food Research New Zealand (PFRNZ) to seek other biological control agents to supplement the effects of *A. mali*. For reasons that will be discussed later, *H. calcarata* was deemed the most suitable candidate for this purpose. In 2010, the New Zealand Environmental Risk Management Authority, now the Environmental Protection Authority (EPA), granted permission to import *H. calcarata* into a PFRNZ quarantine facility in Auckland, and the first shipments of flies occurred in 2012. Ultimately, the release of *H. calcarata* into New Zealand orchards will be contingent upon satisfactorily addressing the criteria required by law under New Zealand's Hazardous Substances and Noxious Organisms Act 1996 (Barratt and Moeed 2005). According to the Act, the release of *H. calcarata* must not:

a. Cause any significant displacement of any native species within its natural habitat; or
b. Cause any significant deterioration of natural habitats; or
c. Cause any significant adverse effects on human health and safety; or
d. Cause any significant adverse effects to New Zealand's inherent genetic diversity; or
e. Cause non-target disease, be parasitic, or become a vector for human, animal, or plant disease (unless that is the purpose).

Furthermore, given that *A. mali* is effectively the sole natural enemy of WAA in NZ (Shaw and Walker 1996), the impact of introducing *H. calcarata* on *A. mali* populations is of high importance.

In practice, these questions must be addressed through combined research on *H. calcarata* in quarantine containment in New Zealand as well as in its native range. A key requirement for the success of studies on non-target effects in quarantine and for its ultimate release into New Zealand orchards will be the ability to rear *H. calcarata* in captivity, which will be most efficiently achieved via research in the USA.

Here, I address aspects of the biology and ecology of *H. calcarata* that relate directly to the potential introduction of *H. calcarata* as a biological control agent for WAA in New Zealand.

Specifically, the main objectives are:

1. Examine aspects of intraguild competition between *A. mali* and *H. calcarata*
2. Explore factors associated with collection, transport, and captive rearing of *H. calcarata*

Biology, pest status, and management of woolly apple aphid

Woolly apple aphid is a purplish-pink aphid with short antennae. Like other members of the sub-family Eriosomatinae, WAA derives its common name from its copious production of a white, waxy exudate of woolly appearance that often envelops colonies (Fig. 1.1). Although originally showing alternation of generations between American elm, *Ulmus americana* L., and other woody hosts (Venables 1929, Sandanayaka and Bus 2005), the demise of American elm from Dutch elm disease in the 20th century appears to have resulted in WAA adapting by maintaining asexual, anholocyclic reproduction on apple, as it does in other parts of the world where elm is absent (Baker 1915, Sandanayaka and Bus 2005). With the exception of a rare oviparous sexual morph, WAA is parthenogenically viviparous; adult females give live birth to daughters (Baker 1915). Like other aphid pests, WAA populations are capable of rapid, exponential growth.

Apterous viviparae on apple can produce up to 200 young under favorable conditions (Baker 1915). Asante et al. (1991) reported optimal temperatures for reproductive output and development ranging from 13-25^{o}C, and a mean lower development threshold of 5.2 ^{o}C. Baker (1915) reported that up to 11 generations per year could be produced in Washington, but 14 generations per season have been shown to occur in the Mid-Atlantic region (Brown and Schmitt 1994).

WAA most commonly overwinters in edaphic colonies on apple roots as adults. Beginning in early spring, first instar nymphs (crawlers) produced by overwintering aphids on the roots move upward, establish on semi-protected arboreal parts of the tree, such as pruning cuts or leaf axils, and initiate new colonies (Baker 1915, Venables 1929, Gautam and Verma 1983, Heunis and Pringle 2006, Cockfield and Beers 2007). Although upward movement of crawlers is highest in early spring, they also move downward and there is usually constant movement of crawlers throughout the tree during the growing season. Walker (1985) found that crawlers were active throughout the season from July to October in Washington orchards, while a more recent study in Washington found a single peak of crawler movement from roots between early spring until June or July (Beers et al. 2010). Arboreal populations tend to follow a seasonal pattern; colony numbers increase throughout the spring, peak in early summer, subside through mid-summer and rebound to some extent from late August through mid-October (Brown and Schmitt 1994, Heunis and Pringle 2006, Beers et al. 2010). Brown and Schmitt (1994) reported that the age structure of WAA colonies changes throughout the season, with a decreasing proportion of 1st instar aphids during mid to late summer.

Fig 1: Woolly apple aphid colonies on shoots of potted apple tree.

Although a common orchard resident, WAA tends to be an ephemeral and unpredictable pest, with outbreak populations showing highly aggregated spatio-temporal distributions (Asante et al. 1993). Its feeding on roots diverts carbohydrates and causes the formation of hypotrophic galls which can restrict water uptake, and therefore impact the growth of young apple trees (Brown and Schmitt 1990, Brown et al. 1995). Outbreak populations in the arboreal parts of trees can have severe and prolonged effects on yield by reducing fruit buds, splitting and weakening fruit-bearing wood, and stimulating premature defoliation. The "honeydew" exuded by aphids can accumulate on fruit and foliage, promoting the growth of sooty mold which can reduce light interception and photosynthesis (Pringle and Heunis 2001, Heunis and Pringle 2006). Honeydew residues and sooty mold also contaminate fruit for the fresh market and are a difficult post-harvest problem (Cockfield and Beers 2007). The presence of live aphids within the fruit calyx and stem bole at harvest creates a serious phytosanitary issue for fruit exporters (Cockfield and Beers 2007). Finally, the sticky "wool" and honeydew associated with outbreak populations at harvest is unpleasant to workers in large commercial orchards and to the public in pick-your-own orchards.

The three main factors that influence the population dynamics, pest status, and management of WAA in commercial orchards are resistant rootstocks, insecticide-based intervention, and

biological control. Historically, growers have relied on use of WAA-resistant rootstocks to limit the establishment of colonies on apple roots (Sandanayaka et al. 2003). A series of resistant rootstocks (Malling-Merton) were developed at the East Malling Research Center in England by crossing the varieties 'Malling' and 'Northern Spy' (Webster 2003). In particular, the MM.111 rootstock was widely used in eastern USA orchards for several decades in the mid-20th century. Subsequently, WAA biotypes that appeared to have overcome the resistance in the MM.111 rootstock were detected in the USA (Dozier et al. 1974, Rock and Zeiger 1974, Young et al. 1982), South Africa (Giliomee et al. 1968), and Australia (Sen Gupta and Miles. 1975, Cummins et al. 1981). The availability and adoption of clonal, size-controlling rootstocks (e.g. M.26, M.9, M.7) beginning in the 1970's diminished the importance of rootstock resistance in WAA management, since they were generally susceptible to the pest (Webster 2003). Recently, Cornell University has developed the Geneva™ series, a dwarfing rootstock with WAA resistant properties (Robinson et al. 2003), and horticulturists at PFRNZ are actively pursuing the same goal (Sandanayaka et al. 2005). However, the replacement of existing plantings with WAA-resistant rootstocks will, at best, be a long-term initiative, and the evolution of WAA biotypes that may overcome the resistance in these new rootstocks is not inconceivable.

In the eastern USA, insecticide-based intervention specifically targeting WAA is relatively rare and, if needed, usually occurs during the latter portion of the growing season. There are few effective chemicals for this purpose and none are rated "excellent" for WAA control (Virginia Cooperative Extension Service 2012). Pre-harvest intervals often preclude the use of the most effective products in late season. Furthermore, the waxy exudates covering WAA colonies and the fact that colony density is often highest in the interior canopy may well confer some protection from insecticides. Historically, organophosphates (e.g. methyl-parathion, diazinon)

and an organochlorine (e.g. endosulfan) were used for WAA control in orchards (Walker 1985), although they are either no longer available or being phased out of conventional programs (Cockfield and Beers 2007). In New Zealand, use of some of the products used to manage WAA in the USA is not permissible under the regulatory restrictions of their export markets.

Research in West Virginia (Brown and Schmitt 1994), Virginia (Bergh and Short 2008, Bergh unpublished), and Washington (Walker 1985, Beers et al. 2010, Gonjito 2011), has shown that natural enemies play a major role in maintaining WAA populations below economic levels in most growing seasons. Similarly, researchers from Australia (Asante 1995ab, Nicholas et al. 2005), Europe (Mols 1996, Beber and Miklavc 1999, Bribosia et al. 1999, Lemoine and Huberdeau 1999, Immik et al. 2002, Monteiro et al. 2004), and elsewhere (Bodenheimer 1947, Hafez 1978, Mueller et al. 1992, Singh 2003, Wearing et al. 2010) have also documented the significance of biological control in WAA suppression. The specialized parasitoid of WAA, *A. mali*, plays a very important role in most apple-producing countries, but recent research has also highlighted the significance of aphidophagous predators. Asante (1997) reviewed the literature on WAA predators and found that 73 predator species from 10 families have been observed in association with WAA colonies, including Coccinellidae (48%), Syrphidae (21%), and Chrysopidae (14%), although there are few published studies documenting their impact. In Australia and the Netherlands the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), has been shown to have a significant impact on WAA populations (Mueller 1988, Nicholas et al. 2005).

Biology and ecology of *Aphelinus mali*

Aphelinus mali (Fig. 2) is a solitary, koinobiont, endoparasitoid that specializes on WAA and, like its host, is native to the USA (Howard 1929). During the 1920's and 1930's, it was

introduced to over 40 countries for WAA biological control and was one of the first successful examples of classical biological control of an aphid using a parasitoid (Howard 1929). With some exceptions, introduction of *A. mali* usually resulted in successful establishment and effective suppression of WAA populations (DeBach 1964).

Fig. 2: *Aphelinus mali* adult on WAA colony

Aphelinus mali is synovigenic; nutrition for reproductive development is obtained from aphid honeydew, plant nectar, and occasionally from aphid haemolymph exuded from the oviposition puncture (Viggiani 1984). Oviposition rate and lifetime fecundity ranges from 2-11 eggs per day (Thompson 1934) and from 50-140 eggs per female (Lundie 1924), respectively. After a short pre-oviposition period, females lay a single egg within the abdomen of all aphid stages, but most frequently in 3rd instar nymphs (Hagen and van den Bosch 1968, Mueller et al. 1992). Eggs hatch in 1-3 d and larvae develop within its host over 10-12 d (Asante and Danthanarayana 1992). Aphids in an early stage of parasitism are pale yellow and the gut contents of the developing parasitoid larva are visible as a dark spot within the abdomen of the aphid (Bodenheimer 1947) (Fig. 3 A). Aphids continue to feed during the early stages of parasitism and adult aphids may be able to reproduce, although the *A. mali* larva will often consume ovaries and unborn young before killing their host (Bodenheimer 1947). Following aphid death, a black

"mummy" is formed (Fig. 3B) within which *A. mali* pupates. Subsequently, the adult wasp emerges by chewing a circular hole in the posterior portion of the mummy (Bodenheimer 1947).

Muller et al. (1992) showed that *A. mali* parasitized more aphids at the colony periphery than in its interior, resulting in a higher proportion of aphids parasitized in smaller, linear colonies than in large, clumped colonies. Parasitization levels can be very high in mid- and late season, with over 90% parasitization reported in Washington orchards (Beers et al. 2010). *A. mali* does not appear to be a highly capable flyer; adults usually move around the tree in short hopping, flights (Bodenheimer 1947). Following the disruption of *A. mali* in New Zealand orchards in 2008, re-colonization of orchards occurred over several years (Bergh, pers. comm.).

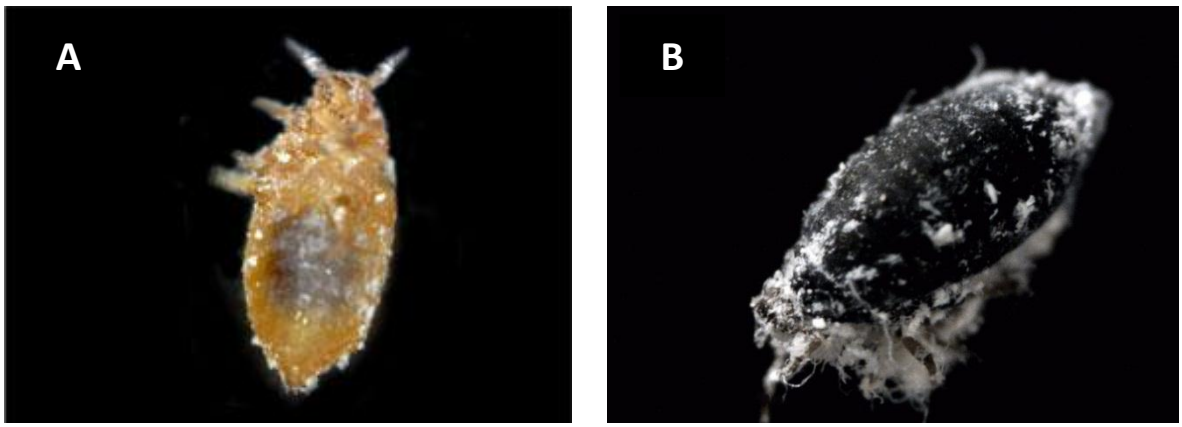

Fig. 3: Woolly apple aphid parasitized by *A. mali* in **(A)** early stage of parasitization and **(B)** mummified stage.

The two main abiotic factors that can limit the effectiveness of *A. mali* for biological control of WAA are climatic conditions and exposure to orchard insecticides. A number of studies have reported that *A. mali* populations have a slower growth rate than WAA (Bodenheimer 1947, Evenhuis 1958, Kuang et al. 1989) and that the effect of temperature on development rate differs between the two species (Bodenheimer 1947, Kuang et al. 1989, Asante and Danthanarayana 1992). Optimal conditions for development and activity of *A. mali* are 26.9ºC with medium

humidity (Bodenheimer 1947), while Asante (1995) reported an optimum growth range for WAA of 13-25ºC. WAA has a baseline developmental threshold of 5.2ºC (Asante et al. 1991), while the baseline developmental thresholds reported for different strains of *A. mali* range from 8.5ºC (Asante and Danthanarayana 1992) to 9.4ºC (Trimble et al. 1990). Consequently, WAA populations generally begin to increase earlier in the spring than *A. mali,* and WAA can complete 10-14 generations per year (Baker 1915, Brown and Schmitt 1994) compared with only 4-5 generations per year of the wasp (Mols and Boers 2001). A frequent consequence of this in some areas is that the level of parasitism of WAA by *A. mali* remains low early in the season, allowing development of arboreal WAA populations (Mols and Boers 2001). Percentage parasitization usually increases greatly by summer (Mueller et al. 1992, Brown and Schmitt 1994), but in some countries, often not before damage occurs (Mols and Boers 2001).

Aphelinus mali populations can also be adversely impacted by exposure to orchard chemicals (Cohen et al. 1996, Bradley et al. 1997, Zhao et al. 2005, Gonjito 2011). This was made evident in New Zealand in 2008, when multiple applications of a new insecticide for control of lepidopteran pests was linked to major disruption of *A. mali* populations and resulted in a serious WAA outbreak. Laboratory trials have identified a number of insecticides that are toxic to *A. mali* at field rates (Table 1). Although *A. mali* is affected by a range of chemicals, the general consensus is that management programs that rely heavily on broad-spectrum insecticides are most harmful to *A. mali,* whereas programs that minimize the use of these are most compatible with WAA biological control (Holdsworth Jr. 1970, Shaw and Walker 1996, Pringle and Heunis 2001, Heunis and Pringle 2003, Nicholas et al. 2005, Cockfield and Beers 2007, Shaw and Wallis 2009, Wearing et al. 2010).

Table 1: Relative toxicity of common orchard insecticides to *Aphelinus mali.*

Chemical	**Toxicity***	**Reference**
Carbaryl	++++	(Bradley et al. 1997, Gonjito 2011)
Diazinon	++++	(Bradley et al. 1997)
Imidacloprid	++++	(Cohen et al. 1996, Ateyyat 2012)
Spinetoram	++++	(Gonjito 2011)
Spinosad	++++	(Gonjito 2011, Rogers et al. 2011)
Thiacloprid	+[1] or +++[2]	(Rogers et al. 2011)[1] or (Gonjito 2011)[2]
Acetamiprid	++	(Gonjito 2011)
Lambda-cyhalothrin	++	(Gonjito 2011)
Novalum	+	(Gonjito 2011)
Spirotetramat	+	(Gonjito 2011)
Cyantranilipole	+	(Gonjito 2011)
Tebufenozide	+	(Shaw and Walker 1996, Bradley et al. 1997)
Primacarb	+	(Ateyyat 2012)
Methoxyfenozide	+	(Rogers et al. 2011)
Chlorantraniliprole	+	(Gonjito 2011, Rogers et al. 2011)
Emamectin benzoate	+	(Rogers et al. 2011)

* Toxicity rating adapted from Croft (1982) + (<10%) no or little toxicity; ++ (10-40%) low toxicity; +++ (50-70%) moderate toxicity; ++++ (75-99.99%)

Biology and ecology of *Heringia calcarata*

Heringia calcarata is a member of the syrphid tribe, Pipizini. Native to North America, its larvae are important members of a guild of WAA natural enemies. Adults (Fig. 4C) are black and vary in size, with an average wingspan and body length of 12 and 7 mm, respectively (Short 2003). Females are easily distinguished from males by the orientation of their eyes; males have holoptic eyes that meet along the dorsal midline of the head, whereas females have dichoptic eyes that are completely separated. First instar *H. calcarata* are small (1 mm length) and mostly transparent, with little external coloration (Short 2003). The dark, yellow-black gut contents and the sclerotized mouthparts can be seen within the body. Second instars have a pale, off-white

translucent to pink-purple opaque coloration that darkens with age. By the 3rd and final instar, their size increases to 6.5 mm in length and their coloration darkens to purplish-gray (Fig. 4B). Feeding cessation by late 3rd instar larvae is indicated when they void their greasy, black gut contents, after which they retract their mouthparts and become sessile. After 1-4 d a gray-brown, tear-drop shaped puparium is formed (Short 2003).

Short (2003) studied the developmental duration of *H. calcarata* at constant 25°C and a 15:9 h light:dark regimen. Eggs hatched after about 3 d and larvae fed on WAA throughout their development, consuming an average of 105 aphids (range = 100-150 aphids) over a 7-9 d period, with peak daily rate of consumption occurring during the 2nd instar. Adults emerged from the puparia after 8-9 d and their longevity (d) in captivity was 28 ± 2.3 SE for females and 20 ± 2.6 SE for males (Bergh and Short 2008).

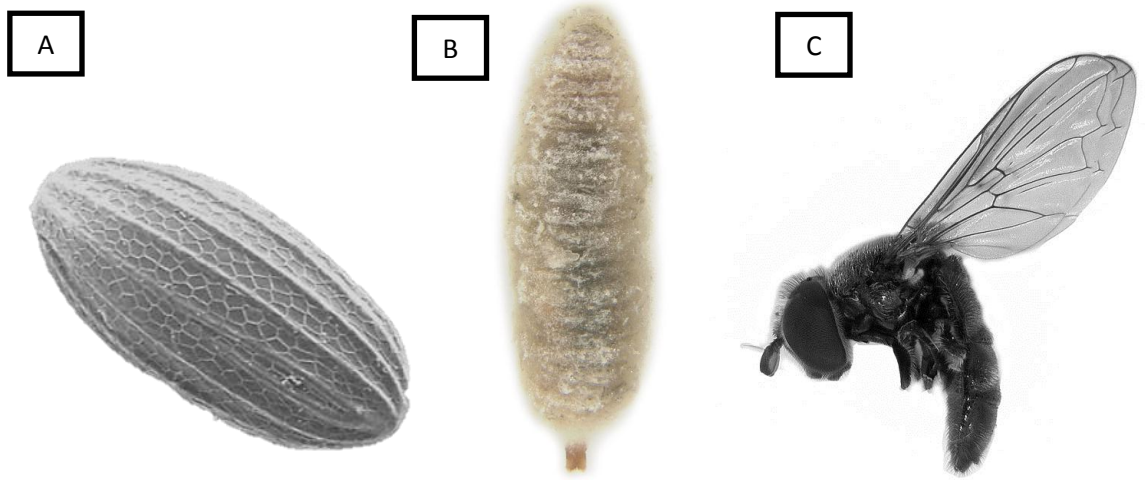

Fig. 4: *Heringia calcarata* (A) egg, (B) 2nd instar larvae, and (C) adult. (Images from Short 2003)

During a widespread outbreak of WAA in 2000, Bergh and Louque (2000) found that *H. calcarata* was the most abundant predator of WAA in Virginia orchards and that two other syrphid species, *Eupeodes americanus* (Wiederman) and *Syrphus rectus* Osten Sacken, were

relatively common (Short and Bergh 2005). The eggs of these three species can be readily differentiated, based on pronounced differences in their exochorionic sculpturing (Short and Bergh 2005). *Eupeodes americanus* and *S. rectus* are known to be generalist predators of soft-bodied insects, while *H. calcarata* appears to be a specialized predator of WAA within the apple ecosystem. Orchard surveys of arboreal aphid colonies revealed that eggs of *H. calcarata* were associated with WAA colonies only, while those of *E. americanus* and *S. rectus* were found in colonies of WAA, rosy apple aphid, *Dysaphis plantaginea* Passerini, and spirea aphid, *Aphis spirecola* Patch (Short and Bergh 2004). No-choice feeding studies showed that survival of *H. calcarata* larvae was significantly greater and their developmental duration was significantly shorter on a diet of WAA than on a diet of rosy apple aphid (Short and Bergh 2004). No larvae survived on a pure diet of spirea aphid. Furthermore, the final weight of larvae fed WAA was significantly greater than those fed rosy apple aphid. In choice tests using all possible pair combinations of the three aphid species, larvae consumed significantly more WAA than rosy apple aphid or spirea aphid (Short and Bergh 2004).

Field studies over three consecutive seasons using weekly, 48 h deployments of potted apple trees with arboreal WAA colonies showed that *H. calcarata* laid eggs beginning between mid-April and mid-May, and continued to lay eggs through early October (Bergh and Short 2008). In five consecutive seasons, Bergh (unpublished) recorded the number of adult female *H. calcarata* observed foraging at the base of apple trees. The daily period of peak activity occurred between 1100 and 1600. Flies were first observed in May and peaked in abundance between June and July, followed by a period of inactivity in mid-summer and resurgence in September and early October. Field observations have shown that female *H. calcarata* also oviposit in the soil at the base of trees (Bergh pers. comm.), and larvae are thought to prey on both arboreal and edaphic

WAA colonies. Walsh and Riley (1869) described finding larvae of *Pipiza radicum* Walsh & Riley commonly on the roots of apple trees in Illinois. An unpublished revision of the genus by F.C. Thompson, USDA Insect Systematics Laboratory, Washington, DC (retired) recognized this species as *H. calcarata*.

Based on work with other syrphid species, it is assumed that *H. calcarata* overwinters in a state of diapause as larvae or pupae on edaphic WAA colonies. Rojo and MarcosGarcia (1997) reported that two European species closely related to *H. calcarata, Heringia heringii* Zetterstedt and *Pipiza festiva* Meigen, overwinter as late-stage larvae in a state of diapause and noted that diapause in those species can be broken artificially in the laboratory by increasing humidity.

Exclusion cage studies using potted apple trees with arboreal WAA colonies showed that WAA populations were significantly greater on fully caged trees than on trees in partial cages or those completely exposed, suggesting that natural enemies played an important role in controlling WAA populations (Bergh unpublished). Subsequent studies using fully exposed, potted sentinel trees deployed in a commercial orchard confirmed that *H. calcarata* laid eggs on WAA colonies within the first 48 h of tree deployment, that it was the most abundant WAA predator, and that natural enemy activity caused the demise of colonies within about 2 wk. In 2009 and 2010, Bergh (unpublished) followed the fate of cohorts of WAA colonies developing on the branches of trees in an orchard in May and June. *H. calcarata* and *A. mali* were the predominant natural enemies and co-occurred in most colonies. Colony demise occurred within about 4 wk from the beginning of the study.

The effects of intraguild predation on biological control

Classical approaches to biological control have focused on single, specialized agents. However, there is an increasing recognition of the importance of natural enemy guilds in biological control (Muller and Brodeur 2002, Cardinale et al. 2003). In theory, increasing the diversity of a natural enemy guild may improve control of the target pest population via synergistic effects on pest suppression and improve the stability of predator-prey systems (Nakazawa and Yamamura 2006). However, interactions within natural enemy guilds are complex (Frechette et al. 2007) and competition among species may interfere with pest suppression and result in decreased levels of biological control (Rosenheim et al. 1995, Chang and Kareiva 1999, Snyder and Ives 2001).

Intraguild predation (IGP) is a specific type of competition wherein one species preys upon a competitor species that shares a common resource (Rosenheim et al. 1995). These interactions can have a significant impact on arthropod population dynamics and community structure in natural and managed systems (Muller and Brodeur 2002, Denno and Finke 2006, Mills 2006, Chong and Oetting 2007, Chacon et al. 2008). Hence, IGP is particularly important in biological control (Kindlmann and Houdkova 2006).

Predators will often prey upon the eggs and nymphs or larvae of competing predator species and endoparasitoids within their host prey (Rosenheim et al. 1995). IGP can negatively affect natural enemy populations and biological control through direct and indirect interference. Erbilgin et al. (2004) examined the combined effects of a parasitoid, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), and a predator, *Anthocoris nemoralis* Fabricius (Hemiptera: Anthocoridae), on the suppression of a psyllid pest of Eucalyptus trees in California, *Glycaspis brimblecombei* Moore (Hemiptera: Psylloidea). Biotic interference was observed when both

species were present, which decreased pest mortality rates despite higher total natural enemy densities. The presence of predators has been shown to impose an indirect fitness cost on parasitoids (Jazzar et al. 2008, Martinou et al. 2010) and predators (Putra et al. 2009) due to increased foraging time.

Conversely, multi-agent systems can be effective in controlling arthropod pests, and IGP can improve the efficacy of natural enemy control of pests (Rosenheim et al. 1995, Bampfylde and Lewis 2007). Theoretical models have shown that shared prey systems can result in higher predation of the target prey (the pest) because of the positive effects of alternative prey on predator reproduction and population stability (Chang and Kareiva 1999, Harmon and Andow 2004). Kindlmann and Houdkova (2006) used a simple stochastic model to show that IGP is not likely to occur at a high frequency and is therefore unlikely to have a detrimental effect on pest suppression. IGP can also enhance control through preventing local extinctions of natural enemy populations (Bampfylde and Lewis 2007) and can improve the stability of a parasitoid-host system (Martinou et al. 2010).

In Virginia, *H. calcarata* and *A. mali* are known to be temporally and spatially sympatric in WAA colonies, each contributing to WAA control. *Aphelinus mali* and native predators have been shown to have a significant impact on WAA colonies, maintaining populations below economic threshold in the Mid-Atlantic region (Brown 2004, Bergh unpublished), and elsewhere, including Washington (Walker 1985, Gonjito 2011), Europe (Mols 1996, Beber and Miklavc 1999, Bribosia et al. 1999, Lemoine and Huberdeau 1999, Immik et al. 2002, Monteiro et al. 2004), South Africa (Pringle and Heunis 2001) and Australia (Nicholas et al. 2005). Exclusion cage studies in Washington showed that predators in combination with *A. mali* resulted in greater biological control than *A. mali* alone (Walker 1985, Gonjito 2011). Gonjito

(2011) studied the relative effects of *A. mali* and predators, including syrphid species, on WAA control in Washington. He showed that predator exclusion decreased control by natural enemies and concluded that syrphids are an important part of the natural enemy guild. Although *A. mali* seems to be compatible with a wide range of predator species, the specific intraguild interactions between it and *H. calcarata* have not been examined.

Given that biological control of WAA is a very important component of its management in many of the world's apple growing regions and that the ultimate goal of this project is to release *H. calcarata* in New Zealand, addressing the potential effects of IGP between *H. calcarata* and *A. mali* is imperative. Knowledge of the interaction between these two natural enemies will provide critical evidence for the development of *H. calcarata* as a biological control agent for WAA in New Zealand.

Reproductive biology and behavior of aphidophagous syrphids

Despite their demonstrated importance as biological control agents of aphids in agricultural systems (Solomon et al. 2000, Gilbert 2005), there is much about the reproductive biology and behavior of syrphids that is relatively poorly understood. Knowledge of the anatomical and physiological aspects of syrphid reproduction is largely based on a few studies with economically important species such as *Episyrphus balteatus* de Geer and *Metasyrphus corollae* (Fabricus), both of which can be mass-reared in captivity. In this section, reproductive development in Diptera is summarized and the literature on reproductive biology and behavior of syrphids is reviewed.

Members of Diptera have a pair of meriostic-polytrophic ovaries within the abdomen (Heming 2003). Ovaries consist of one or more egg tubules (ovarioles), the number of which is variable

and often a function of larval nutrition (Heming 2003). Each ovariole is connected at the distal end to the lateral oviduct via the pedicel, and is terminated at the caudal end by the terminal filament. Terminal filaments connect to form the ovarial ligament which anchors the ovary within the body cavity (Chapman 1982). The ovariole is the functional unit of the polytrophic ovary, with two main regions, the germarium and the vitellarium. The germarium consists of stem cells and is associated with production of oocytes and trophocytes. The vitellarium is the region in which the egg forms and grows. Each ovariole is covered by an outer sheath and, within, by an extra-cellular basal laminar known as the tunica propria. The ovary is covered by an ovarial envelope in juveniles but is most often absent in adults (Buning 1994).

The development of the reproductive system in insects begins at the embryonic stage, with early differentiation of germ line cells. The ovaries continue to develop throughout a fly's life, with oogenesis (egg production) and vitellogenesis (egg growth) occurring at maturity (Heming 2003). Oocytes develop within the ovariole, arising from stem cells at the terminal filament, and form a follicle along with the trophocytes, or nurse cells (Heming 2003). The nurse cells act as a source and transport mechanism for vitellogenesis; they sequester and synthesize compounds, and deliver them to the growing oocyte via specialized channels. Hence oocytes in increasingly advanced stages of maturation can often be seen within each ovariole (Buning 1994). This process is regulated by a number of exogenous cues including pheromones and nutritional status. These cues in concert with endogenous developmental regulation are mediated by hormonal signaling, usually via juvenile hormone signaling (Buning 1994).

Syrphids are synovigenic and require nutrition as adults for optimal reproductive performance. The reproductive potential, rate of development, and requirements for reproductive maturity vary among species. The aphidophagous syrphid, *E. balteatus*, has been studied extensively, including

one study on ovary development (Branquart and Hemptinne 2000). Laboratory-reared, adult females were dissected at various ages to determine post-emergence ovary maturation and adult ovary development time was at least 7-d. The researchers divided the maturation of ovaries into four stages: (1) maturation; (2) early maturity with maximum of one mature oocyte per ovariole; (3) late maturity with one or more mature oocytes per ovariole; and (4) signs of reabsorption. Most females oviposited 8-10 d after emergence but some did so after 14 d. Eggs were reabsorbed in the absence of host aphids. The pre-oviposition period, or period required for ovary maturity, for reproductively active female *Pseudodorus clavatus* F. was 6 d, with the average lifespan (d) of 29.8 ±1.9 SE at 23^{o}C (Belliure and Michaud 2001). Fecundity and reproductive biomass was positively correlated with body size, which was thought to affect larval and adult nutrition (Belliure and Michaud 2001). Laboratory-reared *Syrphus luniger* Meigen mated frequently starting at 7 d following emergence when held within a cage with males, cut flowers, and honey solution (Dixon 1959). Their ovaries enlarged after 3 wk and oviposition occurred on the sixth week (Dixon 1959). Gravid females withheld eggs for up to 21 d when no aphid stimulus was provided. Barlow (1961) reported that female *S. corollae* held in small cages with males and provided honey solution and pollen began laying eggs 1 wk after emerging.

Adult syrphids are highly mobile and feed on pollen and nectar (Schneider 1969). Access to pollen and nectar or nectar substitutes has been shown to improve fertility and fecundity of syrphids (Dong and Xiong 1988, Branquart and Hemptinne 2000, Dong et al. 2004, Hong and Hung 2010) and pollen is considered to be an important dietary component for reproductively active females in the field (Holloway 1976, Gilbert 1981, Haslett 1989, Ssymank and Gilbert 1993, Hickman et al. 1995). Total fecundity of adult females varies greatly among species,

ranging from fewer than 100 to 1694 eggs per female (Schneider 1969). High light conditions, adequate ovariole maturation, mating, and access to hosts are important stimuli for oviposition (Schneider 1969). Successful captive breeding of syrphids is rare and difficult but has been achieved with *Syrphus torvus, S. rebesii, S. opinator, Metasyrphus sp., Scaeva pyrastri* (Frazer 1972), *S. corollae* (Barlow 1961), and *E. balteatus* (Medvey 1988, Hart and Bale 1997).

The development of a methodology for sustained rearing of *H. calcarata* in captivity will be essential to the ultimate success of species' introduction into New Zealand, affecting both the efficiency of studies on non-target effects of *H. calcarata* in quarantine and the capacity to mass-rear it for release. While raising adult flies from larvae is relatively simple, preliminary studies have confirmed that we do not yet understand the factors and conditions required for successful mating in captivity. Given that studies on eliciting mating by *H. calcarata* in captivity was deemed too high-risk as an objective within the context of a Masters project, I have focused on two other aspects of *H. calcarata* reproductive biology in captivity. The first step was to examine whether gravid females will deposit viable eggs under captive conditions. Deposition of fertile eggs by gravid females caught in the field will demonstrate the capacity for reproductively viable females to lay eggs under captive conditions. This will insure that captive reproduction is not limited by the conditions in which oviposition stimulus is offered. Second, we need to determine whether virgin females will produce mature oocytes in captivity, assuming that this is a prerequisite for their receptivity to mating and/or reproductive maturity.

References

Asante, S. K. 1995a. Funtional responses of the European earwig and two species of Coccinellids to densities of *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae). J. Australian Entomology Society 34: 105-1009.

Asante, S. K. 1995b. Biological and ecological studies on the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Pemphigidae), with reference to control. Ph.D. thesis. University of New England, Armidale, Australia.

Asante, S. K. 1997. Natural enemies of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae): a review of the world literature. Plant Protection Quarterly 12: 166-170.

Asante, S. K., and W. Danthanarayana. 1992. Development of *Aphelinus mali* an endoparasitoid of woolly apple aphid, *Eriosoma lanigerum* at different temperatures. Entomologia Experimentalis et Applicata 65: 31-37.

Asante, S. K., W. Danthanarayana, and H. Heatwole. 1991. Bionomics and population growth statistics of apterous virginoparae of woolly apple aphid, *Eriosoma lanigerum*, at constant temperatures. Entomologia Experimentalis et Applicata 60: 261-270.

Asante, S. K., W. Danthanarayana, and S. C. Cairns. 1993. Spatial and temporal distribution patterns of *Eriosoma lanigerum* (Homoptera: Aphididae) on apple. Environmental Entomology 22: 1060-1065.

Ateyyat, M. 2012. Selectivity of four insecticides to woolly apple aphid, *Eriosoma lanigerum* (Haus.) and its sole parasitoid, *Aphelinus mali* (Hald.). World Applied Sciences Journal 16.

Baker, A. C. 1915. The woolly apple aphis. Report No. 101. USDA, Washington, DC.

Bampfylde, C. J., and M. A. Lewis. 2007. Biological control through intraguild predation: case studies in pest control, invasive species and range expansion. Bulletin of Mathematical Biology 69: 1031-1066.

Barlow, C. A. 1961. On the biology and reproductive capacity of *Syrphus corollae* Fab. (SYRPHIDAE) in the laboratory. Entomologia Experimentalis et Applicata 4: 91 - 100.

Barratt, B. I. P., and A. Moeed. 2005. Environmental safety of biological control: policy and practice in New Zealand. Biological Control 35: 247-252.

Beber, K., and J. Miklavc. 1999. New experiences with oppresion of woolly apple aphid (*Eriosoma lanigerum*, Hausmann). In Proceedings of 4th Slovenian Conference on Plant Protection 413-417.

Beers, E. H., S. D. Cockfield, and L. M. Gontijo. 2010. Seasonal phenology of woolly apple aphid (Hemiptera: Aphididae) in Central Washington. Environmental Entomology 39: 286-294.

Belliure, B., and J. P. Michaud. 2001. Biology and behavior of *Pseudodorus clavatus* (Diptera : Syrphidae), an important predator of citrus aphids. Annals of the Entomological Society of America 94: 91-96.

Bergh, J. C., and B. D. Short. 2008. Ecological and life-history notes on syrphid predators of woolly apple aphid in Virginia, with emphasis on *Heringia calcarata*. BioControl 53: 773-786.

Bergh, J. C., and R. L. Louque. 2000. *Heringia* (Neocnemodon) *calcarata*: a specialist predator of the woolly apple aphid? In, Proceedings of 76th Cumberland-Shenandoah Fruit Workers Conf., Winchester, VA.

Bodenheimer, F. S. 1947. Studies on the physical ecology of the woolly apple aphis (*Eriosoma lanigerum*) and its parasite *Aphelinus mali* in Palestine. Bull. Rehovot. Agric. Res. Stn 23.

Bradley, S. J., V. C. Murrell, P. W. Shaw, and J. T. S. Walker. 1997. Effect of orchard pesticides on *Aphelinus mali*, the woolly apple aphid parasitoid. In Proceedings of the New Zealand Plant Protection Conference (pp. 218-222). New Zealand Plant Protection Society Inc.

Branquart, E., and J. L. Hemptinne. 2000. Development of ovaries, allometry of reproductive traits and fecundity of *Episyrphus balteatus* (Diptera: Syrphidae). European Journal of Entomology 97: 165-170.

Bribosia, E., R. Champagne, and D. Bylemans. 1999. Useful organisms in our orchards: the parasitoids of the woolly apple aphid and the aphids. Fruitteelt-nieuws 12: 8-10.

Brown, M. W. 2004. Role of aphid predator guild in controlling spirea aphid populations on appple in West Virginia. Biological Control 29: 189-198.

Brown, M. W., and J. J. Schmitt. 1990. Growth reduction in nonbearing apple trees by woolly apple aphids (Homoptera, Aphididae) on roots. Journal of Economic Entomology83: 1526-1530.

Brown, M. W., and J. J. Schmitt. 1994. Population dynamics of woolly apple aphid (Homoptera: Aphididae) in West Virginia apple orchards. Environmental Entomology 23: 1182-1188.

Brown, M. W., J. J. Schmitt, S. Ranger, and H. W. Hogmire. 1995. Yield reduction in apple by edaphic woolly apple aphid (Homoptera, Aphididae) populations. Journal of Economic Entomology 88: 127-133.

Buning, J. 1994. The insect ovary, University Press, Cambridge, UK.

Cardinale, B. J., C. T. Harvey, K. Gross, and A. R. Ives. 2003. Biodiversity and BioControl: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. Ecology Letters 6: 857-865.

Chacon, J. M., D. A. Landis, and G. E. Heimpel. 2008. Potential for biotic interference of a classical biological control agent of the soybean aphid. Biological Control 46: 216-225.

Chang, G. C., and P. Kareiva. 1999. The case for indigenous generalists in biological control. In B. A. Hawkins and H. V. Cornell (eds.), Theoretical Approaches to Biological Control. University Press, New York, NY.

Chapman, R. F. 1982. The insects: structure and function, 3rd ed. Harvard University Press, Cambridge, MA.

Chong, J. H., and R. D. Oetting. 2007. Intraguild predation and interference by the mealybug predator *Cryptolalemus montrouzier*i on the parasitoid *Leptomastix dactylopii*. Biocontrol Science and Technology 17: 933-944.

Cockfield, S. D., and E. H. Beers. 2007. Biology and management of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), in Washington State, pp. 37-42. *In*, IOBC WPRS Bulletin 30 (4) 37.

Cohen, H., A. R. Horowitz, D. Nestel, and D. Rosen. 1996. Susceptibility of the woolly apple aphid parasitoid, *Aphelinus mali* (Hym: Aphelinidae), to common pesticides used in apple orchards in Israel. Entomophaga 41: 225-233.

Croft, B. A. 1982. Arthropod resistance to insecticides: a key to pest control failures and successes on North American apple orchards. Entomologia Experimentalis et Applicata 31. 88-110.

Cummins, J. N., P. L. Forsline, and J. D. Mackenize. 1981. Woolly apple aphid colonization on *Malus* cultivar. J. Am. Soc. Hort. Sci. 106: 26-30.

DeBach, P. 1964. Biological control of insect pests and weeds, Reinhold Pub. Corp, New York.

Denno, R. F., and D. L. Finke. 2006. Multiple predator interactions and food-web connectance: implications for biological control, pp. 45-70. In J. Brodeur and G. Boivin (eds.), Trophic and Guild Interactions in Biological Control, vol. 3.

Dixon, T. J. 1959. Studies on oviposition behaviour of Syrphidae (Diptera) Transactions of the Royal Entomological Society of London 111: 57-80.

Dong, H., and H. Xiong. 1988. Rearing of the adults of *Metasyrphus corollae* (F.) (Dip: Syrphidae). Chinese Journal of Biological Control.

Dong, K., Y. Dong, and Y. Luo. 2004. Study on rearing of the adults of *Syrphus corollae* Fabricius in laboratory. Natural Enemies of Insects 03.

Dozier, W. A., A. J. Latham, C. A. Kouskolekas, and E. L. Mayton. 1974. Susceptibility of certain apple rootstocks to black root rot and woolly apple aphids. Hort Science 9: 35-36.

Erbilgin, N., D. L. Dahlsten, and P. Y. Chen. 2004. Intraguild interactions between generalist predators and an introduced parasitold of *Glycaspis brimblecombe*i (Homoptera : Psylloidea). Biological Control 31: 329-337.

Evenhuis, H. H. 1958. The influence of the winter on the parasitism of the woolly apple aphid, *Eriosoma lanigerum*, by its parasite, *Aphelinus mali*. Overdruk uit: Tijdschrift Over Planteziekten 64: 328-332.

Frazer, B. D. 1972. A simple and efficient method of rearing aphidophagous hoverflies (Diptera: Syrphidae). J. Entomological Society of British Columbia 69: 23-24.

Frechette, B., S. Rojo, O. Alomar, and E. Lucas. 2007. Intraguild predation between syrphids and mirids: who is the prey? Who is the predator? BioControl 52: 175-191.

Gautam, D. C., and L. R. Verma. 1983. Seasonal biology and reproductive behaviour of woolly apple aphid (*Eriosoma lanigerum* Hausmann). Indian Journal of Horticulture 40: 119 - 123.

Gilbert, F. 2005. Syrphid aphidophagous predators in a food-web context. European Journal of Entomology 102: 325-333.

Gilbert, F. S. 1981. Foraging ecology of hoverflies: morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species. Ecological Entomology 6: 245-262.

Giliomee, J. H., D. K. Strydom, and H. J. V. Zyl. 1968. Northern Spy, Merton and Malling-Merton rootstocks susceptible to woolly aphid, *Eriosoma lanigerum*, in the Western Cape. S. Afr. J. Agric. Sci. 11: 183-186.

Gonjito, L. M. 2011. Integrated biological control of woolly apple aphid in Washington state. Doctor of Philosophy, Washington State University, Pullman, WA.

Hafez, M. 1978. Introduction and establishment of *Aphelinus mal*i (Hald.) (Hym., Chalcidoidea), a wasp parasitic on the woolly apple aphid, in Saudi Arabia. Anzeiger fur Schadlingskunde Pflanzenschutz Umweltschutz 51: 29-30.

Hagen, K. S., and R. van den Bosch. 1968. Impact of pathogens, parasites, and predators on aphids. Annual Review of Entomology 13: 325-384.

Harmon, J. P., and D. A. Andow. 2004. Indirect effects between shared prey: predictions for biological control. BioControl 49: 605-626.

Hart, A. J., and J. S. Bale. 1997. A method of mass-rearing the hoverfly *Episyrphus balteatus* (Diptera, Syrphidae). Dipterist Digest 4: 1-3.

Haslett, J. R. 1989. Adult feeding by holometabolous insects: pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae). Oecologia 81: 361-363.

Hatton, R. G. 1937. The problems raised by the woolly aphis (*Eriosoma lanigerum*) of the apple- a case for team research. Annals of Applied Biology 24: 169-210.

Heming, B. S. 2003. Insect development and evolution, Cornell University Press, Ithaca, NY.

Heunis, J. M., and K. L. Pringle. 2003. The susceptibility of *Aphelinus mali* (Haldeman), a parasitoid of *Eriosoma lanigerum* (Hausmann), to pesticides used in apple orchards in the Elgin area, Western Cape Province, South Africa. African Entomology 11: 91-95.

Heunis, J. M., and K. L. Pringle. 2006. Field biology of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), and its natural enemy, *Aphelinus mali* (Halderman), in apple orchards in the Western Cape province. African Entomology 14: 77-86.

Hickman, J. M., G. L. Lövei, and S. D. Wratten. 1995. Pollen feeding by adults of the hoverfly *Melanostoma fasciatum* (Diptera: Syrphidae). New Zealand Journal of Zoology 22: 387 - 392.

Holdsworth Jr., R. P. 1970. Aphids and aphid enemies: effect of integrated control in an Ohio apple orchard. J. Economic Entomology 63: 530-536.

Holloway, B. A. 1976. Pollen-feeding in hover-flies (Diptera: Syrphidae). New Zealand Journal of Zoology 3: 339 - 350.

Hong, B. M., and H. Q. Hung. 2010. Effect of temperature and diet on the lifecycle and predatory capacity of *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae) cultured on *Aphis gossypii* (Glover). Journal ISSAAS 16: 98-103.

Howard, L. O. 1929. *Aphelinus mali* and its travels. Annals of the Entomological Society of America 22: 341-368.

Immik, E., K. Hetebrügge, J. Zimmer, and H. Holst. 2002. Studies on the biological control of woolly aphid (*Eriosoma lanigerum* Hausm.) with beneficial insects, pp. 15-20. Fördergemeinschaft Ökologischer Obstbau e.V. (FÖKO), Weinsberg.

Jazzar, C., R. Meyhofer, L. Ebssa, and H. M. Poehling. 2008. Two protagonists on aphidophagous patches: effects of learning and intraguild predation. Entomologia Experimentalis et Applicata 127: 88-99.

Kindlmann, P., and K. Houdkova. 2006. Intraguild predation: fiction or reality? Population Ecology 48: 317-322.

Kuang, R. P., F., Y. H. T. Shan, and N. Zhong. 1989. Evaluation of natural enemies of woolly apple aphid, *Eriosoma lanigerum* (Hausm.). Natural Enemies of Insects 11: 51-56.

Lemoine, J., and D. Huberdeau. 1999. The woolly apple aphid (*Eriosoma lanigerum* Hausmann): the come-back of a pest in apple orchards. Arboriculture Fruitière: 19-26.

Lundie, A. E. 1924. A biological study of *Aphelinus mali* (Hald.), a parasite of the woolly apple aphid, *Eriosoma lanigerum* (Hausm.). Memoir 79. Cornell University Agricultural Experiement Station, Ithaca, NY.

Martinou, A. F., B. Raymond, P. G. Milonas, and D. J. Wright. 2010. Impact of intraguild predation on parasitoid foraging behaviour. Ecological Entomology 35: 183-189.

Medvey, M. 1988. On the rearing of *Episyrphus balteatus* (Deg.) (Diptera: Syrphidae) in the laboratory. *In* E. Niemczyk and A. F. G. Dixon (eds.), Ecology and Effectiveness of Aphidophaga, 1987, Teresin, Poland. SPB Academic Publishing.

Mills, N. 2006. Interspecific competition among natural enemies and single versus multiple introductions in biological control, pp. 191-220. In J. Brodeur and G. Boivin (eds.), Trophic and Guild Interactions in Biological Control, vol. 3.

Mols, P. J. M. 1996. Do natural enemies control woolly apple aphid? Acta Hort. (ISHS) 422: 203-207.

Mols, P. J. M., and J. M. Boers. 2001. Comparison of a Canadian and a Dutch strain of the parasitoid *Aphelinus mal*i (Hald) (Hym., Aphelinidae) for control of woolly apple aphid *Eriosoma lanigerum* (Haussmann) (Hom., Aphididae) in the Netherlands: a simulation approach. Journal of Applied Entomology 125: 255-262.

Monteiro, L. B., A. Souza, and E. L. Belli. 2004. Parasitism on *Eriosoma lanigerum* (Homoptera: Aphididae) by *Aphelinus mali* (Hymenoptera: Encyrtidae) on apple orchards, in Fraiburgo County, State of Santa Catarina, Brazil. Revista Brasileira de Fruticultura 26: 550-551.

Mueller, T. F. 1988. Earwig (*Forficula auricularia*) predation on the woolly apple aphid, *Eriosoma lanigerum*. Entomologia Experimentalis et Applicata 47: 145.

Mueller, T. F., L. H. M. Blommers, and P. J. M. Mols. 1992. Woolly apple aphid (*Eriosoma lanigerum* Hausmann, Hemiptera: Aphididae) parasitism by *Aphelinus mali* Hal. (Hymenoptera: Aphelinidae) in relation to host stage and host colony size, shape and location. Journal of Applied Entomology 114: 143-154.

Muller, C. B., and J. Brodeur. 2002. Intraguild predation in biological control and conservation biology. Biological Control 25: 216-223.

Nakazawa, T., and N. Yamamura. 2006. Community structure and stability analysis for intraguild interactions among host, parasitoid, and predator. Population Ecology 48: 139-149.

Nicholas, A. H., R. N. Spooner-Hart, and R. A. Vickers. 2005. Abundance and natural control of the woolly aphid *Eriosoma lanigerum* in an Australian apple orchard IPM program. BioControl 50: 271-291.

Pringle, K. L., and J. M. Heunis. 2001. Woolly apple aphid in South Africa: biology, seasonal cycles, damage and control. Deciduous Fruit Grower 51: 22-23, 36.

Putra, N. S., H. Yasuda, and S. Sat. 2009. Oviposition preference of two hoverfly species in response to risk of intraguild predation. Applied Entomology and Zoology 44: 29-36.

Robinson, T., H. Aldwinckle, G. Fazio, and T. Holleran. 2003. The Geneva series of apple rootstocks from Cornell: performance, disease resitance, and comercialization. Acta Hort. (ISHS) 622: 513-520.

Rock, G. C., and D. C. Zeiger. 1974. Woolly apple aphid infests Malling and Malling-Merton rootstocks in propagation beds in North Carolina. Journal of Economic Entomology 67: 137-138.

Rogers, D. J., N. Sharma, D. C. Stretton, and J. T. S. Walker. 2011. Toxicity of pesticides to *Aphelinus mali*, the parasitoid of woolly apple aphid. New Zealand Plant Protection 64: 235-240.

Rojo, S., and M. A. MarcosGarcia. 1997. Syrphid predators (Dipt.: Syrphidae) of gall forming aphids (Hom.: Aphididae) in Mediterranean areas: implications for biological control of fruit trees pests. Entomophaga 42: 269-276.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological control agents - theory and evidence. Biological Control 5: 303-335.

Sandanayaka, W. R. M., and V. G. M. Bus. 2005. Evidence of sexual reproduction of woolly apple aphid, *Eriosoma lanigerum*, in New Zealand. Journal of Insect Science 5:27.

Sandanayaka, W. R. M., V. G. M. Bus, and P. Connolly. 2005. Mechanisms of woolly aphid [*Eriosoma lanigerum* (Hausm.)] resistance in apple. Journal of Applied Entomology 129: 534-541.

Sandanayaka, W. R. M., V. G. M. Bus, P. Connolly, and R. Newcomb. 2003. Characteristics associated with woolly apple aphid *Eriosoma lanigerum*, resistance of three apple rootstocks. Entomologia Experimentalis et Applicata 109: 63-72.

Schneider, F. 1969. Binomics and physiology of aphidophagous syrphidae. Annual Review of Entomology 14: 103-124.

Sen Gupta, G. C., and P. W. Miles. 1975. Studies on the susceptibility of varieties of apple to the feeding of two strains of woolly aphis (Homoptera) in relation to the chemical content of the tissues of the host. Journal Agricultural Research 26: 157-168.

Shaw, P. W., and J. T. S. Walker. 1996. Biological control of woolly apple aphid by *Aphelinus mali* in an integrated fruit production programme in Nelson. In Proceedings 49th New Zealand Plant Protection Conference (pp. 59-63) Zew Zealand Plant Protection Society.

Shaw, P. W., and D. R. Wallis. 2009. Early-season use of insecticides for management of woolly apple aphid (*Eriosoma lanigerum*) in Nelson apple orchards. New Zealand Plant Protection: 291-295.

Sherbakoff, C. D., and J. A. McClintock. 1935. Effect of crown gall, hairy root, and woolly aphis on apple trees in the orchard. Phytopathologist 25: 1099-1103.

Short, B. D. 2003. Inaugural studies of the life history and predator/prey associations of *Heringia calcarata* (Loew) (Diptera: Syrphidae), a specialist predator of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Eriosomatidae). Masters of Science, Virginia Tech Blacksburg, VA.

Short, B. D., and J. C. Bergh. 2004. Feeding and egg distribution studies of *Heringia calcarata* (Diptera: Syrphidae), a specialized predator of woolly apple aphid (Homoptera : Eriosomatidae) in Virginia apple orchards. Journal of Economic Entomology 97: 813-819.

Short, B. D., and J. C. Bergh. 2005. Separation of three common hover fly predators of woolly apple aphid based on the exochorionic sculpturing of eggs. Canadian Entomol. 137: 67-70.

Singh, S. S. 2003. Feeding potential of *Coccinella septempunctata* L. on woolly apple aphid, *Eriosoma lanigerum* Hausmann. Journal of Entomological Research 27: 213-216.

Snyder, W. E., and A. R. Ives. 2001. Generalist predators disrupt biological control by a specialist parasitoid. Ecology 82: 705-716.

Solomon, M. G., J. V. Cross, J. D. Fitz Gerald, C. A. M. Campbell, R. L. Jolly, R. W. Olszak, E. Niemczyk, and H. Vogt. 2000. Biocontrol of pests of apples and pears in northern and central Europe - 3. Predators. Biocontrol Science and Technology 10: 91-128.

Ssymank, A., and F. Gilbert. 1993. Anemophilous pollen in the diet of Syrphid flies with special reference to the leaf feeding strategy occurring in Xylotini. (Diptera, Syrphidae). Deutsche Entomologische Zeitschrift 40: 245-258.

Trimble, R. M., L. H. M. Blommers, and H. H. M. Helsen. 1990. Diapause termination and thermal requirements for post-diapause development in Aphelinus mali at constant and fluctuating temperatures. Entomologia Experimentalis et Applicata 56.

Thompson, W. R. 1934. The development of a colony of *Aphelinus mali* Hald. Parasitology 26: 449-453.

Venables, E. P. 1929. Observations on the woolly aphis of the apple, *Eriosoma lanigerum* (Hausm.). In Proceedings of British Colmbia Entomological Society 26: 28-33.

Viggiani, G. 1984. Bionomics of the Aphelinidae. Annual Review of Entomology 29: 257-276.

Virginia Cooperative Extension Service. 2012. Virginia spray bulletin for comercial tree fruit growers, Virginia Cooperative Extension Service, Blacksburg, VA.

Walker, J. T. S. 1985. The influence of temperature and natural enemies on population development of woolly apple aphid, *Eriosoma lanigerum* (Hausmann). PhD, Washington State University Pullman, WA.

Walsh, B. D., and C. V. Riley. 1869. The apple-root plant-louse *(Eriosoma (Pemphigus) pyri*, Fitch). American Entomologist: 81-84.

Wearing, C. H., B. A. Attfield, and K. Colhoun. 2010. Biological control of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), during transition to integrated fruit production for pipfruit in Central Otago, New Zealand. New Zealand Journal of Crop and Horticultural Science 38: 255-273.

Weber, D. C., and M. W. Brown. 1988. Impact of woolly apple aphid (Homoptera, Aphididae) on the growth of potted apple-trees. Journal of Economic Entomology 81: 1170-1177.

Webster, A. D. 2003. Breeding and selection of apple and pear rootstocks. Acta Horticulture (ISHS) 622: 499-512.

Young, E., G. C. Rock, D. C. Zeiger, and J. N. Cummins. 1982. Infestation of some Malus cultivars by the North Carolina woolly apple aphid biotype. Horticultural Science 17: 787-788.

Zhao, S., J. Zhong, T. Tang, X. Zhao, S. Gong, and A. Zhan. 2005. The effectiveness of several pesticides on the adults of woolly apple aphid parasite, *Aphelinus mali* (Hald.). Entomological Journal of East China 2005:03.

2. Intraguild interactions between *Aphelinus mali* and *Heringia calcarata*.

Introduction

Native to the USA, woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), is a chronic pest of apple trees in many apple-producing countries (Baker 1915). Colonizing both the roots and arboreal parts of apple trees, WAA can cause a range of adverse effects and injuries to trees and fruit (Weber and Brown 1988, Brown and Schmitt 1990, Brown et al. 1995). Its feeding on roots diverts carbohydrates and causes the formation of hypotrophic galls which can restrict water uptake, thus impairing the growth of apple trees, with most pronounced effects on young trees (Brown and Schmitt 1990, Brown et al. 1995). Feeding by outbreak populations in the arboreal parts of trees can have severe and prolonged effects on yield by reducing fruit bud formation, splitting and weakening fruit-bearing wood, and premature defoliation (Weber and Brown 1988, Brown et al. 1995). The "honeydew" exuded by aphids can accumulate on fruit and foliage, promoting the growth of sooty mold which can reduce light interception and photosynthesis (Pringle and Heunis 2001, Heunis and Pringle 2006). Honeydew residues and sooty mold may also create cosmetic issues in fresh market fruit. In countries that produce fruit primarily for export, such as New Zealand, WAA can be a major phytosanitary concern, by colonizing the stem bole or calyx of apples near harvest (Cockfield and Beers 2007).

In the Mid-Atlantic region of the USA, WAA is a common resident of commercial apple orchards, although it does not often reach economically damaging levels and is not usually targeted in spray programs. In most years, beginning relatively early in the growing season, a guild of natural enemies maintains effective suppression of arboreal WAA colonies. The

specialized, hymenopteran endoparasitoid, *Aphelinus mali* (Haldeman), plays an important role in biological control of WAA throughout the apple-producing regions of the USA (DeBach 1964, Hagen and van den Bosch 1968) and in many countries to which it was exported from the US early in the 20th century (Howard 1929, Yothers 1953). Various predators also contribute importantly to WAA biological control in the USA (Walker 1985, Brown and Schmitt 1990, Bergh and Short 2008, Gonjito 2011), Australia (Asante 1995ab, Nicholas et al. 2005), Europe (Mols 1996, Beber and Miklavc 1999, Bribosia et al. 1999, Lemoine and Huberdeau 1999, Immik et al. 2002, Monteiro et al. 2004), and elsewhere (Bodenheimer 1947, Hafez 1978, Mueller et al. 1992, Singh 2003, Wearing et al. 2010). In Virginia, the predominant members of the guild of WAA natural enemies consist of *A. mali* and three native syrphids, *Heringia calcarata* (Loew), *Eupeodes americanus* (Weidemann), and *Syrphus rectus* Osten-Sacken (Bergh and Short 2008).

Larval *E. americanus* and *S. rectus* are generalist predators of soft-bodied insects (Rojo et al. 2003), whereas *H. calcarata* is considered a specialized predator of WAA in the apple ecosystem (Short and Bergh 2004). Surveys of aphid colonies in the canopy of apple trees revealed that eggs of *H. calcarata* were associated only with WAA colonies while those of *E. americanus* and *S. rectus* were found in colonies of WAA, rosy apple aphid, *Dysaphis plantaginea* (Passerini), and spirea aphid, *Aphis spirecola* Patch (Short and Bergh 2004). *H. calcarata* larva that developed on a pure diet of WAA showed significantly greater survivorship and a significantly shorter developmental duration than those fed rosy apple aphid (Short and Bergh 2004). Furthermore, the mean weight of 3rd instar larvae at cessation of feeding was significantly greater on a diet of WAA than of rosy apple aphid. No larvae survived on a diet of spirea aphid. In

choice tests involving all possible pairs of the three aphid species, larvae consumed significantly more WAA than rosy apple aphid or spirea aphid (Short and Bergh 2004).

In New Zealand apple orchards, *A. mali* has been effectively the sole natural enemy of WAA, with predation by generalist predators not considered to be important (Dumbleton and Jeffreys 1938, Shaw and Walker 1996). Since its importation and release in NZ in 1922 (Howard 1929), *A. mali* has generally provided acceptable control of WAA. However, a severe, widespread, and prolonged WAA outbreak in New Zealand beginning in 2008 was attributed to insecticide-based disruption of *A. mali* populations, prompting a search for other potential agents to supplement WAA biological control. Entomologists from Plant and Food Research New Zealand (PFRNZ) deemed that *H. calcarata* was the most suitable candidate and received permission from New Zealand's Environmental Protection Authority (EPA) to import *H. calcarata* into quarantine in Auckland.

In Virginia, *A. mali* and *H. calcarata* are temporally sympatric, each contributing to WAA control in concert with other, less prominent natural enemies (Short and Bergh 2004, Bergh and Short 2008, Bergh unpublished data). During the early stages of development within WAA, eggs and larvae of *A. mali* may be at risk from predators feeding in aphid colonies. Later in the parasitization process, aphids die and become black and "mummified" (Asante and Danthanarayana 1992), at which point the developing parasitoid may be at less risk from predators.

Ultimately, permission to release *H. calcarata* into New Zealand orchards will depend upon satisfying the NZ EPA's key regulatory concerns, one of which is the potential impact on *A. mali* populations through intraguild predation. Syrphid larvae are known to prey on parasitized aphids

(Kindlmann and Ruzicka 1992, Meyhofer and Klug 2002, Pineda et al. 2007, Almohamad et al. 2008) and to feed more frequently on non-parasitized aphids than aphid mummies (Rosenheim et al. 1995, Almohamad et al. 2008). Using cages that excluded predators but enabled access of *A. mali*, Walker (1985) found that *A. mali* alone was often not sufficient to control WAA colonies. Similar studies conducted by Gonjito (2011) in Washington found that predator exclusion decreased biological control of WAA colonies. He also experimentally exposed WAA colonies to *A. mali* and syrphid larvae and found that the combined effect of syrphid larvae and *A. mali* on WAA control was greater than the control exerted by *A. mali* alone.

Here, I report the results of field and laboratory studies examining aspects of the intraguild interactions between *H. calcarata* and *A. mali*, including the effects of parasitization on *H. calcarata* oviposition and feeding, and the effect of *H. calcarata* larvae on *A. mali* within WAA colonies.

Materials and Methods

Insects

WAA colonies for rearing *H. calcarata* and for experimental trials were maintained on 1- to 4-yr-old apple trees of various cultivars growing in a 1:1 mix of peat moss and loamy soil in 10 L plastic pots. Trees were held in a greenhouse or outdoors in 3.6 x 1.8 x 1.8 m screened cages (BioQuip Products, Inc., Rancho Dominquez, CA) at Virginia Tech's Alson H. Smith, Jr. Agricultural Research and Extension Center (AHS-AREC), Winchester, VA. Spider mites, fungal pathogens, and other aphids were controlled as required, utilizing sanitation or chemistries known to have low insecticidal effects on WAA (Virginia Cooperative Extension Service 2012); no aphidicides were applied to the potted trees in 2012.

On some trees, WAA colonies were allowed to become parasitized by *A. mali*. On others, parasite-free colonies were initiated and maintained by transferring WAA crawlers from colonies with low levels of parasitization and holding the trees in a controlled environment chamber (4.2 x 2.7 x 2.7 m) (Environmental Growth Chambers, Chargrin falls, OH) at 20-22^{o}C, 40-70% RH, and a 15:9 h light:dark regimen with overhead fluorescent lights (900-1200 lux). Crawlers were unlikely to be parasitized, as *A. mali* most frequently parasitizes 3rd instar and larger aphids (Bodenheimer 1947). During the period of colony development early in the season (14-25 May), some of the trees were treated with Delegate^{TM} (Spinetoram) which is highly toxic to *A. mali* (Gonjito 2011). Aphids were not used in trials or rearing for at least two weeks following insecticide treatment. To maintain low levels of parasitization, yellow sticky cards were placed in half of the outdoor screened tents and in the greenhouse, and heavily parasitized colonies were removed by hand.

H. calcarata eggs were collected on short sections (5-15 cm) of apple shoot infested with a WAA colony that were pruned from potted trees. Shoots were placed individually in 50 ml vials with water and secured with a small strip of Parafilm M wrapped around the base of the shoot. Shoots were deployed between 08:00 and 09:00 h in holes created in the soil near the base of apple trees in experimental orchards at the AHS-AREC, so that the shoot was essentially perpendicular to the ground (Fig. 5). After 8-12 h, shoots were collected and examined under a dissecting microscope at 20X for *H. calcarata* eggs, which were identified based on their exochorionic sculpturing (Short and Bergh 2005). Shoots with eggs were pruned to ~2 cm sections that included the WAA colony and placed in Petri dishes (50 x 9 mm BD-Falcon Tight-Fit Lid Dishes, Fisher Scientific, Pittsburg, PA) that were held in translucent plastic boxes (30 x 20 x 8 cm) in a growth chamber (Percival Scientific Inc., Perry, IA) at 25^{o}C and15:9 h

(light:dark). After 3 d, the expected time of larval eclosion (Bergh and Short 2008), the dishes were provisioned with additional WAA, after which they were inspected daily and provisioned with new aphids as necessary. At 5 d intervals, larvae were transferred to new dishes with a fresh apple shoot section and aphids, using a moist, fine-tipped paintbrush.

Effect of parasitism by *A. mali* on oviposition by *H. calcarata*

Female *H. calcarata* commonly forage for oviposition sites at the base of apple trees, with two annual periods of peak abundance from about late May to early July and from early September to early October (Bergh unpublished). Apple shoot sections (5-15 cm long), each containing a WAA colony (~1-3 cm diam.), were pruned from potted trees and deployed in pairs at the base of mature apple trees (canopy width ~3-6 m) at the AHS-AREC. Shoots with parasitized or non-parasitized WAA colonies were selected and paired so that the colonies were of approximately equal size. Shoots were 30-50 cm from the base of the trunk and separated by 50-100 cm. Periodic herbicide treatments around the base of trees maintained a soil surface that was essentially free of vegetation. Between 31 May and 6 July, 2012, five pairs of shoots were deployed at the base of the same five trees on each of eight sunny, warm, and relatively calm days considered optimal for female *H. calcarata* foraging activity (Bergh pers. comm.). Mean (± SE) daily maximum and minimum temperatures and mean (± SE) wind speed on the days during which shoots were deployed were, respectively, 32.3

Fig. 5: Pair of apple shoot sections (10 cm) each with a woolly apple aphid colony parasitized or non-parasitized) in a vial of water placed into a hole in the soil near the base of an apple tree.

± 1.7 ^{o}C, 18.3 ± 1.4^{o}C, and 3.5 ± 0.6 kmh^{-1}. Shoots were deployed from 09:00 until 17:00, then retrieved and inspected under a dissecting microscope. The number of *H. calcarata* eggs, live and mummified aphids, and colony diameter was recorded for each colony.

Effect of parasitism by *A. mali* on feeding by *H. calcarata* larvae

Aphids were collected from colonies grown on potted trees by brushing them into a receptacle using a soft paint brush. Using a dissecting microscope, parasitized and non-parasitized aphids were separated based on their external appearance, following Bodenheimer (1947). Briefly, non-parasitized aphids were purplish-pink in color and were actively extruding waxy filaments from their abdomen. Aphids in an early stage of parasitism were pale yellow and often showed a dark spot internally, which were the gut contents of the developing parasitoid larva (Bodenheimer 1947) (Fig. 3 A). Black, "mummified" aphids in final stage of parasitism were very apparent (Viggiani 1984) (Fig. 3 B). *H. calcarata* larvae were reared individually from eggs for 8-9 d using methods described above, then placed individually in closed 1.5 ml plastic vials with damp tissue paper. Prior to the initiation of feeding experiments, larvae were starved for 8-12 h. Using a choice-test design, individual larvae (n = 25 per treatment) were transferred to the small glass dishes (1.5 cm diameter, 0.5 cm deep) used by Short and Bergh (2004), containing the following treatments, (A) 15 non-parasitized aphids and 15 aphids in an early stage of parasitism, and (B) 15 non-parasitized aphids and 15 mummified aphids. The dishes were sealed with Parafilm M and held in a growth chamber (Percival Scientific Inc., Perry, IA) at 25^{o}C and15:9 h (light:dark). After 24 h, the larvae were removed and the aphids in each dish that had not been preyed upon were counted.

Thirty-six larvae from the choice trial were used in a subsequent no-choice trial. Following cessation of the choice test, each larva was starved for 8-12 h, then placed individually into a

glass arena (described previously) with either, (a) 30 non-parasitized aphids, (b) 30 aphids in early stages of parasitism, or (c) 30 mummified aphids. The prior treatment allocation from the choice test (non-parasitized vs early-parasitized, or non-parasitized vs mummies) was taken into account in a balanced completely randomized 2 X 3 factorial design with 6 replications. The numbers of aphids in each dish that had not been preyed upon were counted after 24 h.

Intraguild interactions between larval *H. calcarata* and *A. mali* within *in situ* WAA colonies

Fig. 6: Custom-made sleeve cage used to encase woolly apple aphid colony *in situ*.

Woolly apple aphid nymphs were transferred to 20 potted 'Daybreak Fuji' apple trees held in a controlled environment chamber at 20-22^{o}C, 40-70% RH, and a 15:9 h light:dark regimen. Four colonies per tree were numbered and caged using customized sleeves (20 x 25 cm) made of fine (~280 micron holes) Dacron Chiffon Netting (Bioquip, Rancho Dominguez, CA) with Velcro™ strips attached along both sides so that the cages could be wrapped around the branch and secured with twist ties at both ends (Fig. 7). Prior to initiation of the test, the size of colonies was adjusted by removing aphids with a small brush so that colonies on the same tree were of approximately equal size (~1-2 cm diam). On each tree, the following treatments were randomly assigned to colonies within each tree; 1) *H. calcarata* (Hc), 2) *A. mali* (Am), 3) *H. calcarata* and *A. mali* (Hc+Am), and 4) neither (Con). For treatments Am and Hc+Am, five adult female *A. mali* were collected and introduced to the cages for 2 d, then removed. *H. calcarata* eggs were collected from the field using methods

Fig. 7: Two *H. calcarata* eggs pruned form apple shoot attached with a small amount of diluted Elmer's glue to a 3 cm twig.

described previously. Using a scalpel, the eggs were removed from collection shoots by carefully cutting away a section of bark under the egg. The bark containing the egg was attached with a small amount of diluted Elmer's glue (Glue-All® multipurpose glue, Elmer's Products Inc., Westerville, OH) to a 3 cm apple shoot section (Fig. 7). Eggs that became dislodged were glued directly to the shoot section using a very small amount of diluted glue at the posterior end of the egg. Following removal of *A. mali* from the cages, the shoots sections with *H. calcarata* eggs were introduced to colonies assigned to treatments "Hc" and "Hc+Am" within 24 h of collection by pinning them to the branch adjacent to the colony (Fig. 6). After 14 d, the cages were opened and inspected for *H. calcarata* larvae or pupae. The colonies were pruned from trees and placed individually in vials with 70% ethanol for subsequent counts of the number of non-parasitized and parasitized aphids per colony.

Data Analysis

The effect of parasitism on the mean number of eggs laid on shoots by *H. calcarata* deployed at the base of apple trees was analyzed using ANCOVA, with number of eggs as the response, parasitism as the treatment factor, and number of live WAA as a covariate. Spatio-temporal variation was accounted for by deploying treatments in pairs with WAA colonies of approximately equal size, although colony size (i.e. number of live WAA) was found to differ among replicates ($F_{1,78} = 13.96$, $P = 0.0004$). An initial assessment found that there was not a

significant parasitism x live WAA interaction ($F_{1, 76} = 0.342$; $P = 0.5604$) on the number of eggs laid, and variance appeared to be homogenous across treatment levels.

The frequency of shoots with ≥1 egg was compared between those with parasitized and non-parasitized colonies using 2 x 2 contingency table analysis, as the number of live WAA did not have a significant effect on presence/absence of *H. calcarata* eggs and was therefore omitted.

A post-hoc analysis of egg numbers from shoots with parasitized colonies was conducted using logistic regression analysis to assess the effect of percentage parasitization on the presence or absence of *H. calcarata* eggs.

For each choice-test feeding trial, the number of parasitized and non-parasitized aphids consumed was compared using separate paired *t*-tests.

The effect of (1) prior choice-test allocation (levels: (A) non-parasitized vs early-parasitized, or (B) non-parasitized vs mummies) and (2) aphid stage of parasitism (levels: (a) non-parasitized, (b) early parasitized, (c) mummified) on the number of aphids consumed in no-choice feeding trials was assessed as a 2 X 3 factorial design using a two-way ANOVA. Means were separated using Tukey-Kramer test.

The independent and combined effects of *H. calcarata* and *A. mali* on the number of WAA (log transformed) within *in situ* colonies were analyzed as an unbalanced randomized incomplete block design with trees as blocks using PROC GLM using SAS statistical sofware (SAS Institute 2009). Tukey-Kramer test was used to separate means. Initially, a randomized complete block design was employed; however eight of the Hc replicates and four of the Hc+Am replicates did not contain a *H. calcarata* larvae or pupa at the end of the trial and were excluded from the analysis, thus the data were analyzed as an incomplete block design.

The effect of *H. calcarata* larval presence on the number of *A. mali* mummies produced within *in situ* colonies exposed to *A. mali* was assessed with a one-way ANOVA.

The results of all statistical comparisons were considered significant at $\alpha < 0.05$ and were computed using JMP statistical software (SAS Institute 2010) unless otherwise stated.

Results

Effect of parasitism by *A. mali* on oviposition by *H. calcarata*

At least one egg per pair was laid on 75% of the pairs of shoots deployed (n = 40 pair total). There was not a significant difference in the mean number of eggs deposited on shoots with parasitized (1.5 ± 0.34 SE) compared with non-parasitized (1.75 ± 0.42 SE) colonies ($F_{1,77}$ = 0.28; P = 0.595). There was not a significant difference in the number of shoots with ≥1 egg(s) between parasitized and non-parasitized shoots (χ^2 = 0.05; DF = 1; P = 0.082). When the presence/absence of *H. calcarata* eggs data for shoots with parasitized colonies were analyzed (ie. shoots with non-parasitized colonies excluded), there was a significant negative effect of percentage parasitization on the presence of *H. calcarata* eggs (χ^2= 4.207; DF = 1; P = 0.040). For each one percent increase in parasitization, the likelihood of finding an egg decreased by 4.5% (likelihood odds ratio = 0.957).

Effect of parasitism by *A. mali* on feeding by *H. calcarata* larvae

Larvae consumed significantly more non-parasitized (14.2 ± 0.4 SE) than mummified aphids (0.4 ± 0.14 SE) (t = 30.7; DF = 24; P < 0.0001) (Fig. 8). Larvae consumed significantly more non-parasitized aphids (13.4 ± 0.42 SE) than aphids in an early stage of parasitization (10.8 ± 0.48 SE) (Fig. 2.4) (t = 6.4; DF = 24; P < 0.0001).

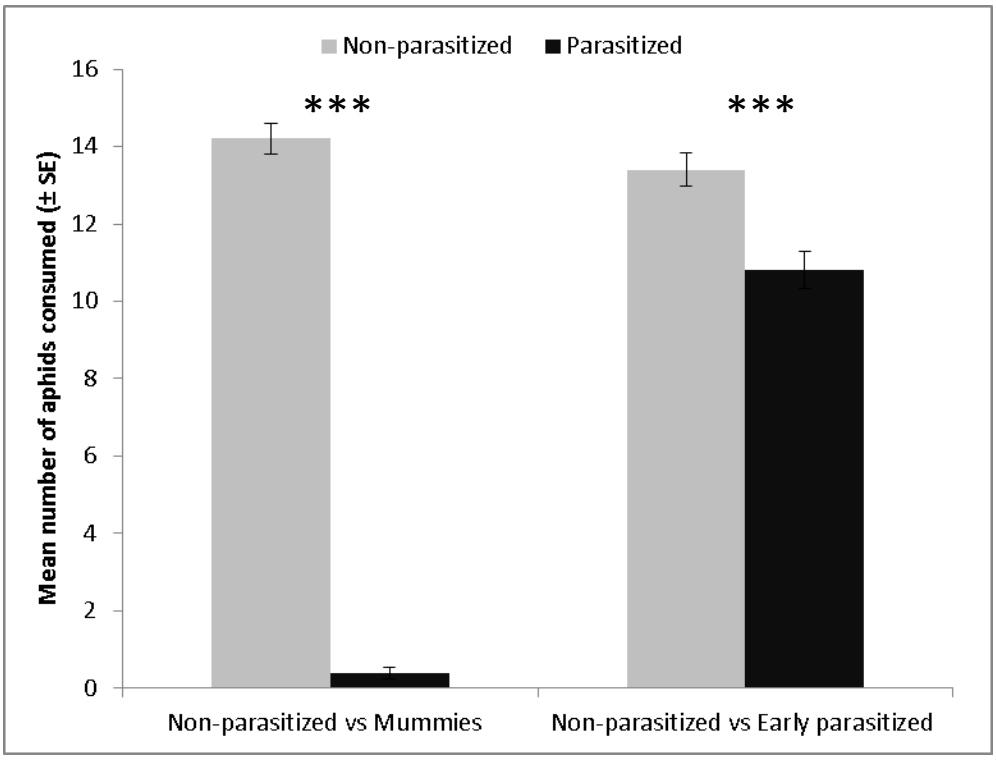

Fig. 8: Mean (± SE) number of woolly apple aphids consumed by *H. calcarata* larvae over 24 h in arenas with non-parasitized vs mummified aphids (n = 25) or non-parasitized vs early parasitized aphids (n = 25). (***) indicates significant difference, paired t-test ($P < 0.0001$).

The choice-test treatment allocation did not have a significant effect on the no-choice trial results. There was a significant treatment effect on the number of aphids consumed in the no-choice feeding trials ($F_{2,33} = 56.9$; $P < 0.0001$). Larvae consumed significantly more non-parasitized aphids (25.3 ± 1.93 SE) than aphids in an early stage of parasitization (19.7 ± 1.85 SE) or mummified aphids (2.2 ± 0.71 SE), and significantly more aphids in an early stage of parasitization than mummified aphids.

Intraguild interactions between larval *H. calcarata* and *A. mali* within *in situ* WAA colonies

There was a significant effect of *H. calcarata* and *A. mali* presence on the number of live WAA recovered from colonies on potted trees ($F_{3,44} = 3.39$; $P = 0.0262$). Significantly more live WAA were found in control colonies than in colonies exposed to *H. calcarata* only or to *H. calcarata* and *A. mali* together (Fig. 9). There was no significant difference in the number of live aphids between control colonies and colonies exposed to *A. mali* only (Fig. 9). However, there was considerable variation across colonies with block-wise variation contributing 18% of the variance.

There was not a significant effect of *H. calcarata* presence on the number of *A. mali* mummies recovered from the WAA colonies. However, overall levels of parasitization were low; the number of aphid mummies recovered from the "Am" and "Hc + Am" treatments were 37.0 ± 6.9 SE and 38.5 ± 7.6 SE mummies, respectively ($F_{1,32} = 0.0301$; $P = 0.8647$).

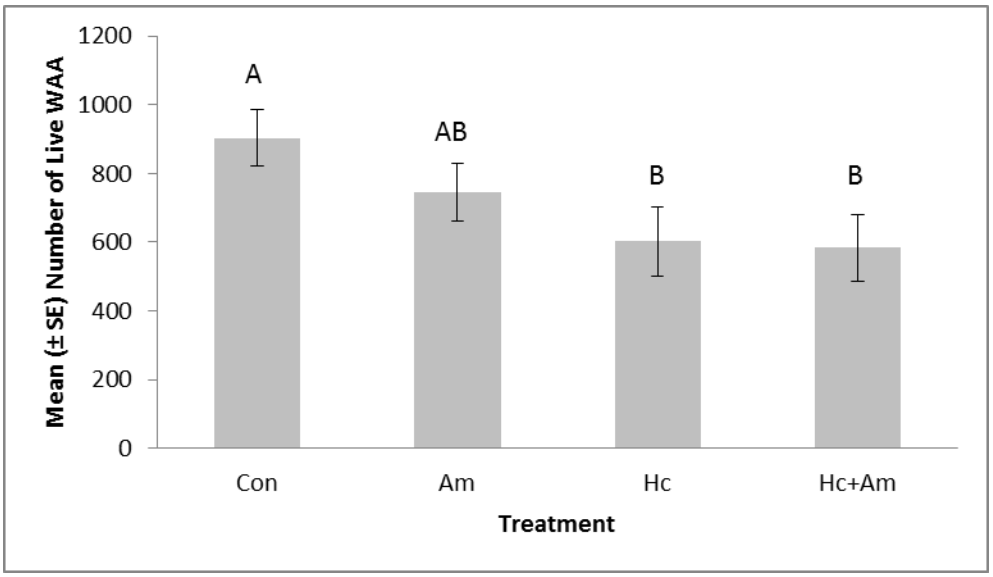

Fig. 9: Independent and combined effects of *H. calcarata* and *A. mali* on the number WAA in caged colonies on potted trees in a controlled environment chamber. Treatments were "Con" = control (n = 19); "Am" = *A. mali* only (n = 18); "Hc" = *H. calcarata* only (n = 11); and "Hc+Am" = both natural enemies (n = 15). Means with the same letter are not significantly different (Tukey-Kramer test; $P < 0.05$).

Discussion

These results provide new information on the interactions between two important natural enemies of WAA in Virginia and are directly relevant to considerations underlying the potential introduction of *H. calcarata* to New Zealand. Overall, our data indicate that although some level of intraguild predation by *H. calcarata* on *A. mali* is likely to occur, it is unlikely to adversely affect *A. mali* populations or its role in WAA biological control.

Field studies showed that *H. calcarata* will oviposit within WAA colonies with and without aphid mummies. These results conform to those from field studies (Bergh unpublished) showing that both un-hatched eggs of *H. calcarata* and aphids parasitized by *A. mali* were present in WAA colonies. In relative terms however, the percentage of mummies in parasitized colonies appeared to influence egg-laying; eggs were deposited less frequently in colonies that contained a higher percentage of mummies than in those with a smaller percentage. Therefore, WAA

parasitized by *A. mali* are likely to be exposed to *H. calcarata* larvae in many colonies, although heavily parasitized colonies are less likely to be exposed to IGP risk. Studies with other syrphid species have shown similar results. Female *E. balteatus* laid fewer eggs on aphid colonies, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), with a high proportion of mummies or mummy exuvia parasitized by *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) compared with non-parasitized aphid colonies (Almohamad et al. 2008). *E. balteatus* also showed avoidance behavior to green peach aphid colonies, *Myzus persicae* Sultzer (Hemiptera: Aphididae), parasitized by *Aphididus colemani* Viereck (Hymenoptera: Aphididae) (Pineda et al. 2007). Oviposition site selection was shown to be mediated by a number of cues, such as exudates and honeydew and that these cues, were produced by the live green peach aphid but not mummies (Pineda et al. 2007). Meyhofer and Klug (2002) showed that *E. balteatus* laid fewer eggs in colonies of *Aphis fabae* Scopoli (Hemiptera: Aphididae), that were in the late stages of parasitism by *Lysiphlebus fabrum* Marshall (Hymenoptera: Aphididae) compared with non-parasitized colonies, but this difference was not detected at early stages (2-4 d) of colony parasitism.

When *H. calcarata* larvae co-occur with WAA that have been parasitized by *A. mali*, the results of choice and no-choice feeding studies indicate that larvae will discriminate between parasitized and non-parasitized aphids, and that the extent to which this occurs depends upon the stage of parasitism. It is therefore expected that parasitized aphids will be preyed upon less frequently than non-parasitized aphids within WAA colonies. A search of the literature revealed only one previous study specifically pertaining to discrimination of parasitized and non-parasitized aphids by syrphid larvae (Akinlosotu 1978). It was shown that *E. balteatus* larvae consumed fewer cabbage aphid mummies parasitized by *Diaretiella rapae* McIntosh compared with non-

parasitized aphids, but the larvae did not appear to discriminate between aphids in earlier stages of parasitism and non-parasitized aphids. Almohamad et al. (2008) found that *E. balteatus* larval performance, measured by pupal weight and survival, was lower on a diet of *A. pisum* parasitized by *A. ervi* compared with a diet of non-parasitized *A. pisum.* Discrimination against mummified aphids was also shown with the predatory lady beetle, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), feeding on citrus mealy bug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), parasitized by an encyrtid parasitoid, *Leptomastix dactylopi* Howard (Chong and Oetting 2007). In choice tests, both adult and larval *C. montrouzieri* fed upon parasitized and non-parasitized mealy bugs, but consumed fewer late-stage mummies.

Results from exposing WAA colonies *in situ* on the branches of potted apple trees revealed that *H. calcarata* larvae did not affect the number of mummified aphids produced. Given the limitations associated with natural enemy exposure period, study duration, and variable levels of natural enemy pressure, the number of live WAA recovered at the end of the study was highly variable across replicates. Unfortunately, it is difficult to draw many conclusions about the relative levels of pressure exerted by each of the natural enemies because parasitization by *A. mali* was very low (5%) and did not seem to have a significant effect on aphid suppression.

In other agricultural systems, the combination of predators and parasitoids has been shown to have a greater impact on pest populations than parasitoids alone. In cotton fields, Colfer & Rosenheim (2001) evaluated the impact of introducing the convergent ladybird beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), on the biological control of cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), by the parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae). They found that although there was a high level of predation on the immature endoparasitoid by the lady beetle, biological control was

better in plots with both natural enemies compared with plots with the predator or parasitoid alone. Kindlmann and Ruzicka (1992) found that the addition of the aphidophagous hoverfly, *Metasyrphus corolla* (Fabricius), to systems containing the aphids, *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), and their parasitoid, *Diaeretiella rapae* (MacIntosh) (Hymenoptera: Braconidae), increased the percentage parasitization due to selective feeding (avoidance of aphid mummies) by the predator. Their findings showed that although there was some degree of IGP of the immature endoparasitoid by the hoverfly larvae, aphid populations were lower when both natural enemies were present. Experimental field studies in Washington orchards have shown that *A. mali* in concert with generalist predators, such as coccinellids, chrysopids, and syrphids, can effectively control WAA populations but that *A. mali* alone was unable to suppress WAA (Walker 1985, Gonjito 2011). Exclusion of predators reduced the impact of *A. mali* on WAA colonies, suggesting that generalist predators have an additive effect on biological control in orchards utilizing selective pesticides (Gonjito 2011). Syrphids, including *H. calcarata,* were found to be the most abundant predators in WAA colonies and were reported to suppress WAA colonies in concert with *A. mali* (Gonjito 2012). In Mid-Atlantic (Brown 2004, Bergh unpublished), and Australian (Nicholas et al. 2005) orchards, it has been shown that the combination of predators and *A. mali* has a significant impact on WAA colonies and in most years natural enemies regulate WAA populations sufficiently. It is therefore expected that introduction New Zealand *H. calcarata,* combined with *A. mali,* will result in enhanced biological control of WAA colonies.

References

Akinlosotu, T. A. 1978. The inter-relationship of the cabbage aphid parasite, *Diaeretiella rapae* McIntosh (Hym: Aphidiidae) and the entomophagous predators of the aphid. Nigerian Journal of Entomology 3: 5-9.

Almohamad, R., F. J. Verheggen, F. Francis, T. Hance, and E. Haubruge. 2008. Discrimination of parasitized aphids by a hoverfly predator: effects on larval performance, foraging, and oviposition behavior. Entomologia Experimentalis et Applicata *128: 73-80.*

Asante, S. K. 1995a. Funtional responses of the European earwig and two species of Coccinellids to densities of *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae). J. Australian Entomology Society 34: 105-1009.

Asante, S. K. 1995b. Biological and ecological studies on the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Pemphigidae), with reference to control. Ph.D. thesis. University of New England, Armidale, Australia.

Asante, S. K., and W. Danthanarayana. 1992. Development of *Aphelinus mali* an endoparasitoid of woolly apple aphid, *Eriosoma lanigerum* at different temperatures. Entomologia Experimentalis et Applicata 65: 31-37.

Baker, A. C. 1915. The woolly apple aphis. Report No. 101. USDA, Washington, DC.

Beber, K., and J. Miklavc. 1999. New experiences with oppresion of woolly apple aphid (*Eriosoma lanigerum,* Hausmann). In Proceedings of 4th Slovenian Conference on Plant Protection 413-417.

Bergh, J. C., and B. D. Short. 2008. Ecological and life-history notes on syrphid predators of woolly apple aphid in Virginia, with emphasis on *Heringia calcarata*. BioControl 53: 773-786.

Bodenheimer, F. S. 1947. Studies on the physical ecology of the woolly apple aphis (*Eriosoma lanigerum*) and its parasite *Aphelinus mali* In Palestine. Bull. 43, Rehovot. Agric. Res. Stn.

Bribosia, E., R. Champagne, and D. Bylemans. 1999. Useful organisms in our orchards: the parasitoids of the woolly apple aphid and the aphids. Fruitteelt-nieuws 12: 8-10.

Brown, M. W. 2004. Role of aphid predator guild in controlling spirea aphid populations on appple in West Virginia. Biological Control 29: 189-198.

Brown, M. W., and J. J. Schmitt. 1990. Growth reduction in nonbearing apple trees by woolly apple aphids (Homoptera, Aphididae) on roots. Journal of Economic Entomology 83: 1526-1530.

Brown, M. W., J. J. Schmitt, S. Ranger, and H. W. Hogmire. 1995. Yield reduction in apple by edaphic woolly apple aphid (Homoptera, Aphididae) populations. Journal of Economic Entomology88: 127-133.

Chong, J. H., and R. D. Oetting. 2007. Intraguild predation and interference by the mealybug predator *Cryptolalemus montrouzier*i on the parasitoid *Leptomastix dactylopii*. BioControl Science and Technology 17: 933-944.

Cockfield, S. D., and E. H. Beers. 2007. Biology and management of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), in Washington State, pp. 37-42. *In*, IOBC WPRS Bulletin 30 (4) 37.

Colfer, R. G., and J. A. Rosenheim. 2001. Predation on immature parasitoids and its impact on aphid suppression. Oecologia 126: 292-304.

DeBach, P. 1964. Biological control of insect pests and weeds, Reinhold Pub. Corp, New York.
Dumbleton, L. J., and F. J. Jeffreys. 1938. The Control of the Woolly Aphis by *Aphelinus mali*. New Zealand Journal of Science and Technology, Section A 20: 183A-190A pp.
Gonjito, L. M. 2011. Integrated biological control of woolly apple aphid in Washington state. Doctor of Philosophy, Washington State University, Pullman, WA.
Gontijo, L.M., Cockfield, S.D. and Beers, E.H. 2012. Natural enemies of woolly apple aphid (Hemiptera: Aphididae) in Washington State. Biological Control 41: 1364-1371.
Hafez, M. 1978. Introduction and establishment of *Aphelinus mal*i (Hald.) (Hym., Chalcidoidea), a wasp parasitic on the woolly apple aphid, in Saudi Arabia. Anzeiger fur Schadlingskunde Pflanzenschutz Umweltschutz 51: 29-30.
Hagen, K. S., and R. van den Bosch. 1968. Impact of pathogens, parasites, and predators on aphids. Annual Review of Entomology 13: 325-384.
Heunis, J. M., and K. L. Pringle. 2006. Field biology of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), and its natural enemy, *Aphelinus mali* (Halderman), in apple orchards in the Western Cape province. African Entomology 14: 77-86.
Howard, L. O. 1929. *Aphelinus mali* and its travels. Annals of the Entomological Society of America 22: 341-368.
Immik, E., K. Hetebrügge, J. Zimmer, and H. Holst. 2002. Studies on the biological control of woolly aphid (*Eriosoma lanigerum* Hausm.) with beneficial insects, pp. 15-20. Fördergemeinschaft Ökologischer Obstbau e.V. (FÖKO), Weinsberg.
Kindlmann, P., and Z. Ruzicka. 1992. Possible consequences of a specific interaction between predators and parasites of aphids. Ecological Modelling 61: 253-265.
Lemoine, J., and D. Huberdeau. 1999. The woolly apple aphid (*Eriosoma lanigerum* Hausmann): the come-back of a pest in apple orchards. Arboriculture Fruitière: 19-26.
Meyhofer, R., and T. Klug. 2002. Intraguild predation on the aphid parasitoid *Lysiphlebus fabarum* (Marshall) (Hymenoptera : Aphidiidae): mortality risks and behavioral decisions made under the threats of predation. Biological Control 25: 239-248.
Mols, P. J. M. 1996. Do natural enemies control woolly apple aphid? Acta Horticulture (ISHS) 422: 203-207.
Monteiro, L. B., A. Souza, and E. L. Belli. 2004. Parasitism on *Eriosoma lanigerum* (Homoptera: Aphididae) by *Aphelinus mali* (Hymenoptera: Encyrtidae) on apple orchards, in Fraiburgo County, State of Santa Catarina, Brazil. Revista Brasileira de Fruticultura 26: 550-551.
Mueller, T. F., L. H. M. Blommers, and P. J. M. Mols. 1992. Woolly apple aphid (*Eriosoma lanigerum* Hausmann, Hemiptera: Aphididae) parasitism by *Aphelinus mali* Hal. (Hymenoptera: Aphelinidae) in relation to host stage and host colony size, shape and location. Journal of Applied Entomology 114: 143-154.
Nicholas, A. H., R. N. Spooner-Hart, and R. A. Vickers. 2005. Abundance and natural control of the woolly aphid *Eriosoma lanigerum* in an Australian apple orchard IPM program. BioControl 50: 271-291.
Pineda, A., I. Morales, M. A. Marcos-Garcia, and A. Fereres. 2007. Oviposition avoidance of parasitized aphid colonies by the syrphid predator *Episyrphus balteatus* mediated by different cues. Biological Control 42: 274-280.
Pringle, K. L., and J. M. Heunis. 2001. Woolly apple aphid in South Africa: biology, seasonal cycles, damage and control. Deciduous Fruit Grower 51: 22-23, 36.

Rojo, S., F. Gilbert, M. A. MarcosGarcia, J. M. Nieto, and M. P. Mier. 2003. A world review of predatory hoverflies (Diptera, Syrphidae: Syrphinae) and their prey, CIBIO Ediciones, Alicante (Spain).

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological control agents - theory and evidence. Biological Control 5: 303-335.

SAS Institute 2009. SAS Version 9.2 SAS Institute, Cary, NC.

SAS Institute 2010. JMP Statistical Software Version 9. SAS Institute Institute, Cary, NC.

Shaw, P. W., and J. T. S. Walker. 1996. Biological control of woolly apple aphid by *Aphelinus mali* in an integrated fruit production programme in Nelson. (pp 59-63) *In* In Procceedings 49th Plant Protection Conference New Zealand Plant Protection Society.

Short, B. D., and J. C. Bergh. 2004. Feeding and egg distribution studies of *Heringia calcarata* (Diptera : Syrphidae), a specialized predator of woolly apple aphid (Homoptera : Eriosomatidae) in Virginia apple orchards. Journal of Economic Entomology 97: 813-819.

Short, B. D., and J. C. Bergh. 2005. Separation of three common hover fly predators of woolly apple aphid based on the exochorionic sculpturing of eggs. Canadian Entomol. 137: 67-70.

Singh, S. S. 2003. Feeding potential of *Coccinella septempunctata* L. on woolly apple aphid, *Eriosoma lanigerum* Hausmann. Journal of Entomological Research 27: 213-216.

Viggiani, G. 1984. Bionomics of the Aphelinidae. Annual Review of Entomology 29: 257-276.

Virginia Cooperative Extension Service. 2012. Virginia spray bulletin for comercial tree fruit growers, Virginia Cooperative Extension Service, Blacksburg, VA.

Walker, J. T. S. 1985. The influence of temperature and natural enemies on population development of woolly apple aphid, *Eriosoma lanigerum* (Hausmann). PhD, Washington State University Pullman, WA.

Wearing, C. H., B. A. Attfield, and K. Colhoun. 2010. Biological control of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), during transition to integrated fruit production for pipfruit in Central Otago, New Zealand. New Zealand Journal of Crop and Horticultural Science 38: 255-273.

Weber, D. C., and M. W. Brown. 1988. Impact of woolly apple aphid (Homoptera, Aphididae) on the growth of potted apple-trees. Journal of Economic Entomology 81: 1170-1177.

Yothers, M. A. 1953. An annotated bibliography on *Aphelinus mali* (Hald.), a parasite of the woolly apple aphid. In Bulletin E-861, USDA, Washington, DC.

3. Collection, Transport, and Captive Rearing of *Heringia calcarata*

Introduction

The predatory syrphid, *Heringia calcarata* (Loew), is native to the United States and an important, specialized natural enemy of woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann), in Mid-Atlantic apple orchards (Bergh and Short 2008, Bergh unpublished). *H. calcarata* gained the attention of entomologists in New Zealand (NZ) following recent outbreaks of WAA in NZ orchards that caused serious damage and threatened access to foreign markets for apple exporters. The outbreaks were thought to be the result of chemical and climatic disruption of populations of the hymenopteran parasitoid, *Aphelinus mali* (Haldeman), which is effectively the sole biological control agent of WAA in NZ (Shaw and Walker 1996). The sensitivity of this system to disturbance by external, abiotic factors highlighted the need for increased stability of WAA biological control, prompting scientists from Plant and Food Research New Zealand (PFRNZ) to search for additional natural enemies. Based on its apparent specialization on WAA in apple orchards in Virginia (Short 2003, Bergh & Short 2008), *H. calcarata* was selected for potential introduction to NZ apple orchards as a classical biological control agent for WAA. In 2010, permission was granted by NZ regulatory authorities to import *H. calcarata* from Virginia into quarantine facilities at PFRNZ in Auckland, NZ. Importation and release of novel organisms into NZ is restricted by the Hazardous Substances and Novel Organisms Act 1996. According to the Act, the potential release of *H. calcarata* into NZ apple orchards will be by permission of the Environmental Protection Authority, and contingent upon documentation that, among other considerations, it will not have a deleterious effect on non-target arthropods native to NZ.

Studies on non-target effects will be conducted in quarantine in NZ, and will form an important component of the case to release *H. calcarata* from quarantine.

Periodic shipments of *H. calcarata* from Virginia to NZ will be required for initial research. A search of the literature revealed no published information about collecting and transporting live syrphids. Therefore, the development of protocols and methods for efficiently collecting *H. calcarata* in Virginia and verification that eggs and larvae can withstand shipment to NZ were required. Since WAA is typically a sporadic and ephemeral pest, collecting *H. calcarata* eggs and larvae from colonies in orchards is labor-intensive and inefficient. Bergh and Short (2008) deployed young, potted apple trees infested with WAA in orchards for 48-h intervals and then destructively sampled colonies to search for *H. calcarata* eggs. Although this was an effective method for collecting eggs, maintaining, deploying and destructively sampling entire trees was considered very inefficient and resource-intensive (Bergh, pers. comm.). Since adult female *H. calcarata* are known to search for oviposition sites in the canopy and at the base of apple trees (Bergh and Short 2008), an alternative method to collect eggs was investigated. Additionally, methods for packing and transporting *H. calcarata* as eggs and larvae to NZ were tested via several shipments in spring 2012.

Ultimately, the success of this project will be contingent upon the ability to rear multiple generations of *H. calcarata* in captivity. With some notable exceptions (Barlow 1961, Frazer 1972, Medvey 1988, Hart and Bale 1997), syrphids are widely considered to be difficult to mate in captivity (Schneider 1969). Given that there is no information on the mating behavior of *H. calcarata* and that adult males have never been observed in the field, research on this aspect of their biology was considered too high-risk within the context of a Master's project. However,

other questions related to the development of protocols for mating and mass-rearing *H. calcarata* in captivity are equally relevant.

Mating in captivity by an insect species does not necessarily imply that females will oviposit readily or reliably under captive conditions, as was shown by Frank et al. (2010) for female dogwood borer. Therefore an important question related to this project is, "Will mated female *H. calcarata* deposit viable eggs in captivity?" Based on data from one female captured in the field, Bergh and Short (2008) provided preliminary evidence that oviposition in captivity can occur, but further documentation of the frequency with which this behavior will occur, and the viability of eggs deposited, was required.

Assuming that females having a complement of mature oocytes are in a physiological state of readiness to mate and lay eggs, a second highly relevant question is, "Will virgin female *H. calcarata* produce mature oocytes in captivity?" Female syrphids are synovigenic; they emerge with poorly developed ovaries and typically must obtain carbohydrates and amino acids to complete maturity (Schneider 1969, Chambers 1988, Thomson 1999). As the "flower fly" common name of the family Syrphidae suggests, females obtain nutrients from foraging on flowers for pollen and nectar (Schneider 1969, Gilbert 1993). Several studies have investigated the conditions required to successfully rear syrphids in captivity. Branquart and Hemptinne (2000) reported that oviposition by laboratory-reared *Episyrphus balteatus* de Geer was affected by the presence of a host stimulus; female flies developed but did not lay eggs in the absence of an oviposition stimulus. It was also found that fecundity and reproductive biomass were positively correlated with body size, which was thought to be affected by larval and adult nutrition. Laboratory-reared *Syrphus luniger* (Meig.) mated frequently after the first week following emergence when caged with cut flowers and honey solution (Dixon 1959). Their

ovaries enlarged after three weeks and oviposition occurred on the sixth week. Dixon (1959) also found that gravid females withheld eggs for up to 21 d when no aphid stimulus was provided. Barlow (1961) held female and male *S. corollae* together in small cages with honey solution and pollen and reported that females began to lay eggs about one week after emergence. Analyses of the gut contents of field-collected flies have shown that syrphids feed on a variety of flowers, consuming both pollen and nectar (Holloway 1976, Gilbert 1981, Ssymank and Gilbert 1993). The pollen to nectar ratio was shown to relate to reproductive development, with the highest pollen ratio coinciding with peak yolk production (Haslett 1989). Although there is a great deal of variation in the relative amount of pollen consumed, it seems that pollen consumption by females is common among syrphid species (Hickman et al. 1995). Therefore we expected that ovary maturation would be enhanced in flies provided pollen in their diet compared with flies provided only sugar and water.

We present here the research that addressed the wide-ranging objectives outlined previously. First we collected eggs throughout the season using sections of WAA-infested apple shoot deployed at soil level near the baser of apple trees and recorded the number of eggs collected. We also sent a number of trial shipments of *H. calcarata* from Virginia to NZ and present the results from those shipments. And finally we present findings form captive reproduction studies looking at oviposition of gravid females and development of ovaries in virgin adults in the laboratory.

Materials and methods

Collecting *Heringia calcarata* eggs in the field

Sections of apple shoot (10-15 cm) with at least one WAA colony (1-5 cm diam.) were pruned from potted trees grown at the Alson H. Smith Jr. Agricultural Research and Extension Center

Fig. 10: Apple shoot section (10 cm) with woolly apple aphid colony in a vial with water at the base of an apple tree.

(AHS-AREC) in screened field cages or in a greenhouse. The shoots were placed individually in 50 ml vials with water and secured with Parafilm M wrapped around the vial at the base of the shoot. Shoots were deployed between 0800 and 0900 on warm, relatively calm days from 8 June to 21 September, 2012. Mean (± SE) daily maximum and minimum temperatures and mean (± SE) wind speed on the days when shoots were deployed were, respectively, 30.0 ±0.75^{o}C, 15.8 ± 1.01^{o}C, and 3.3 ± 0.44 kmh^{-1}. Based on the results of a preliminary study in 2011, showing that eggs could be recovered from ground-deployed shoots in numbers similar to those from shoots tied to branches in the canopy, shoots were deployed at the base of apple trees. Each vial with shoot was inserted into a hole created in the soil near the base of apple trees in blocks at the AHS-AREC, so that the shoot was essentially perpendicular to the ground (Fig. 10). After 8-12 h, shoots were collected and examined for *H. calcarata* eggs under a dissecting microscope at 20X. Eggs were identified based on their exochorionic sculpturing (Short and Bergh 2005).

Transporting live *H. calcarata* from Virginia to New Zealand

Un-hatched eggs and larvae of *H. calcarata* were transported to New Zealand on four occasions between 8 June and 26 September, 2012. Three shipments were sent via DHL™ International

Fig. 11: Small plastic vials containing *Heringia calcarata* eggs or larvae with WAA packed into a plastic-lined polystyrene box with an ice pack for international courier transport.

Express courier service, while one was carried as hand luggage. Eggs were collected using methods described previously. Larvae were reared in the laboratory using methods described in Chapter 2 or were collected from WAA colonies in Virginia orchards. Eggs were separated individually or in pairs from the shoot using pruners or a scalpel, leaving a 1-3 cm section with the egg and live WAA attached. The shoot section containing the egg(s) was placed inside a plastic vial of suitable size. Larger shoot sections were wrapped in slightly dampened facial tissue and placed within a 30 ml plastic vial (Fisher Scientific, Pittsburg, PA) with a tight-fitting lid that had been punctured with a pin several times to provide ventilation. Smaller shoot sections (<2 cm) were placed into a 1.5 ml plastic vial (Fisher Scientific, Pittsburg, PA) with a small plug of facial tissue in the base and were sealed with a tight-fitting lid that had been punctured with a pin several times. Larvae were placed, with an apple shoot with an intact WAA colony within a Petri dish of appropriate size. If needed, additional WAA were added. The containers were packed in a polystyrene box (20 x 20 x 25 cm) lined with a plastic bag and containing an icepack (Fig. 11). The polystyrene box was taped closed and packed inside a sealed cardboard box. All relevant and necessary documentation was placed inside a clear folder taped to the outside of the box.

Oviposition by field-collected *H. calcarata* in captivity

Fig. 12: Cage for testing propensity of gravid females to lay eggs in captivity – a plastic container (1.7 L) (100 mm X 150 mm diameter) with screened lid provisioned with food, water, and a shoot (10 cm) with woolly apple aphid colony in a vial of water.

Female *H. calcarata* observed foraging at the base of trees in apple orchards were captured with an insect net and placed individually in translucent plastic containers (1.7 L) (100 mm high X 150 mm diameter) with a screened lid (Fig. 12). Each container was provisioned with: (1) a plastic Petri dish (2.5 cm diam.) containing ~ 1 g white, granulated sugar and ~ 0.5 g crushed fresh bee pollen (High Desert®, CC Pollen Co., Phoenix, AZ), (2) a vial of water containing a piece of dental wick (5 cm) and, (3) a vial of water containing a ~10 cm section of apple shoot with a WAA colony. The containers were held in a room with overhead fluorescent lights (450 lux) at 24-26^{o}C, 15:9 h (light:dark), and 50 - 80% RH for 24 h, after which the shoots were removed and inspected under a dissecting microscope at 15 to 30X. Eggs deposited were counted *in situ* and incubated with the shoot section in a growth chamber (Percival Scientific Inc., Perry, IA) at 25^{o}C and15:9 h (light:dark). After 5 d, egg viability was determined by examining each under a microscope and by gently prodding them with a small, fine-bristled paintbrush. Hatched eggs were empty of contents, had an exit slit on the ventral surface at the anterior end, and collapsed readily when prodded, whereas un-hatched eggs were turgid. Upon death the wings were removed and the wing length from axillary incision to wing tip was measured using a Dinolite-X-scope digital microscope (BigC, Torrance, CA). Upon death, flies that had not become overly degraded were dissected

using methods described in the following section and the mature oocytes were counted and the ovaries and oocytes measured using a Dinolite-X-scope digital microscope (BigC, Torrance, CA).

Ovary development in captive *H. calcarata* females

Adult flies used in this study were reared from as eggs in the laboratory, as described in Chapter 2. Upon emergence, adults were sexed and females were placed individually in screened-top, cylindrical plastic cages (16 x 7.5 cm diam) in a flower pot (10.2 cm diam) containing 100 ml of moist, coarse sand and provisioned with food, water, and a perch. The containers were held under overhead, fluorescent lighting (450 lux) in a rearing room at 24-26^{o}C, 50 – 80% RH and a 15:9 h light:dark regimen.

Initially, oocyte maturation in females was investigated using a 3-way factorial completely randomized design using the following factors: (1) male presence/absence, (2) pollen presence/absence plus water and sugar solution and, (3) time (5, 10, 15, or 20 d), with three replicates (n = 36 flies). The flies were monitored daily and removed if dead or euthanized by freezing after 5, 10, 15, or 20 d, then placed individually in 1.5 ml plastic vials with 70% ethanol and stored in a freezer at -20^{o}C for 1-20 d until dissection, described below. Given that ovary development of *Aedes rusticus* (Rossi) (Diptera: Culicidae) was examined using specimens that had been frozen for up to 90 days (Ungureanu 1972), freezing for this duration was not expected to have an effect on subsequent measurements.

In another study, the effect of diet (pollen or no pollen) on the size of ovaries in virgin female *H. calcarata* was assessed. Ten females were reared from eggs on WAA in the laboratory and upon emergence were placed in the plastic cylinder containers described previously and provisioned

with, (1) pollen + water + sugar solution (n = 5), or, (2) no-pollen + water + sugar solution (n = 5). The flies were maintained in a room with 24-26^{o}C, 50 – 80% RH and a 15:9 h light:dark regimen under overhead, fluorescent lighting (450 lux) for 7 d, after which they were euthanized, stored, and dissected as described previously. The wing length from axillary incision to wing tip and length and width of the dissected ovaries were measured using a digital microscope. Ovary volume was estimated based on the equation for an ovoid:

volume = $\frac{4}{3}\pi \times l \times w^2$

where l = longitudinal radius, and w = width (radius) at center

Dissections.

The flies were softened and rehydrated for 10-30 min in 10% potassium hydroxide, then rinsed and soaked for 30-60 min in 60% phosphate buffered saline (PBS). Flies were dissected on a glass microscope slide under a microscope (15 – 30X) in 60% PBS. The abdomen was removed from the thorax using fine-tipped forceps, and the sternal abdominal segments were removed to expose the internal organs, using number 0 insect pins in a pin clamp tool. One pin was inserted longitudinally inside the abdomen, along the intersegmental membrane, and the second was used to cut the membrane and remove the segments. The ovaries were then teased apart from the alimentary tract and surrounding tissues and stained with drops of 10% methylene blue for 10 min, rinsed in 60% PBS, and then photographed using a Dinolite-X-scope digital microscope at 50 – 200X.

Ovary development was assessed and graded on a four point scale which is described in detail in results: 1 = little to no development or swelling of oocytes; 2 = evidence of vitellogenesis, some

swollen and elongated follicles but no mature oocytes; 3 = some (<50%) oocytes with exchorionic sculpturing, most ovarioles enlarged to some degree; 4 = >50% of ovarioles developed with many oocytes with exchorionic sculpturing.

Data Analysis

Descriptive statistics were used for *H. calcarata* egg collection data. Due to adult longevity being much shorter than that experienced by NZ researchers and that shown by Short (2003), many individuals could not be included in the study and the ovary development data were not balanced. Consequently, the sample size was very small, treatments were not balanced and treatment effects became confounded; therefore, statistical tests were not valid. The ovary development data from flies that died or were euthanized 5-15 d after emergence were pooled and summarized with descriptive statistics. The length and width of oocytes from captive-reared females was compared with the length and width of oocytes dissected from gravid, field-collected females using a non-parametric median score test. The effect of pollen in the diet on the mean ovary volume 7-d-old *H. calcarata* was analyzed using a non-parametric median score test. The mean wing length was approximately equal between groups with pollen (4.8 mm ± 0.17 SE) and without pollen (4.9 mm ± 0.13 SE) and was omitted from the analysis. All statistical analyses used significance level of $\alpha < 0.05$ and were conducted using JMP statistical software (SAS Institute 2010).

Results

Collecting *Heringia calcarata* eggs in the field

In total, 233 shoots were deployed across 19 days between 08 June and 21 September, 2012 (5 - 18 shoots per day). During the deployment interval (8 – 12 h), female *H. calcarata* deposited ≥1 egg(s) on 29% of shoots (Table 2). Across all shoots, an average of 0.78 ± 0.01 SE eggs per

shoot were laid, while among those with ≥1 egg (n = 68), an average of 2.4 ± 0.21 SE eggs per shoot were laid (range = 1-9 eggs per shoot).

Table 2: *Heringia calcarata* eggs collected on WAA-infested apple shoot sections deployed at the base of apple trees for 8-12 h in 2012.

Date	**Shoots deployed**	**Shoots with ≥ 1egg**	**Total eggs**	**Mean (± SE) eggs per shoot**
8-Jun	20	6	25	4.1 (1.14)
10-Jun	13	11	31	2.8 (0.55)
11-Jun	14	7	18	2.8 (0.78)
13-Jun	8	2	3	1.5 (0.5)
14-Jun	17	15	33	2.2 (0.38)
15-Jun	18	7	10	1.4 (0.3)
20-Jun	17	9	23	2.6 (0.58)
21-Jun	5	2	5	2.5 (0.5)
25-Jun	10	0	0	0 (NA)
10-Jul	10	0	0	0 (NA)
24-Jul	10	0	0	0 (NA)
8-Aug	13	1	1	1 (NA)
16-Aug	0	0	0	0 (NA)
28-Aug	13	1	1	1 (NA)
31-Aug	10	1	1	1 (NA)
5-Sep	9	2	7	3.5 (0.5)
11-Sep	10	4	6	1.5 (0.5)
21-Sep	16	3	9	3 (1)

The proportion of shoots with ≥ 1 egg varied during the season (Fig. 13), being highest in June and September (45 and 26% of shoots, respectively) and lowest in July and August (0 and 6% of shoots, respectively).

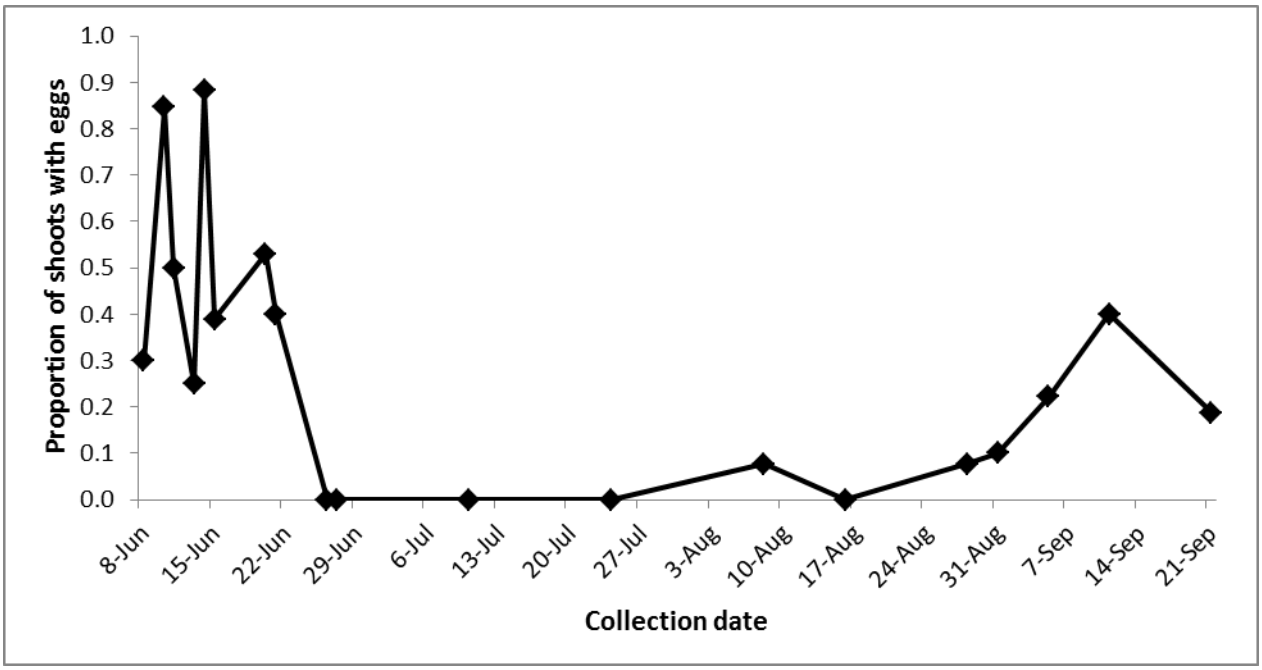

Fig. 13: Proportion of WAA-infested shoots deployed at the base of apple trees (n = 233) with ≥1 egg at each sampling date (sample size 5 to18 shoots) from 8 June to 21 September 2012.

Transporting live *H. calcarata* from Virginia to New Zealand

A total of 178 eggs and larvae were sent from Virginia to a quarantine facility in Auckland, New Zealand. Transportation time ranged from 3 to 11 d. Issues with customs in the US and NZ delayed delivery of one shipment, resulting in higher mortality than was observed with the others (Table 3). Upon arrival in NZ, larvae were immediately transferred to Petri dishes with WAA from NZ, whereas eggs were held with their substrate for several days before being inspected, to allow for larval growth. In total, 124 adult flies were generated in NZ, representing 69.9% of the number of eggs and larvae recovered upon delivery to quarantine.

Table 3: Details of shipments of *H. calcarata* eggs and larvae sent from Virginia to a quarantine containment facility in Auckland, New Zealand in 2012.

Date Sent[1]	Date Received[1]	Transport Method	Larvae	Eggs	Survival (%)[2]	Adults Emerged
09 Jun	12 Jun	DHL	15	22	84	31
16 Jun	26 Jun	DHL	6	20	58	14
24 Jun	25 Jun	hand luggage	30	27	100	36
27 Sep	02 Oct	DHL	37	21	100	43

[1] New Zealand Standard Time
[2] Percentage of *H. calcarata* recovered as live larvae at PFRNZ

Oviposition by female *H. calcarata* in captivity

In 2011 and 2012, 63% (n = 8) and 80% (n = 14) of field-collected adult female *H. calcarata* laid ≥ 1 egg on the WAA colony provided during the 24 h exposure period, respectively. The number of eggs laid per female was 3.6 ± 1.05 SE and 13.8 ± 3.5 SE in 2011 and 2012, respectively, with a maximum of 40 eggs. Based on observations of egg viability in 2012, 98% ± 1.0 SE (n = 11 flies) of the eggs hatched (Table 4).

Table 4: Wing length, eggs laid (after 24 h), eggs hatched, and number of mature oocytes within ovary of gravid females captured from the field in 2012 (n=14)

Date caught	Wing Length (mm)	Eggs laid	Eggs hatched	% hatch	Oocytes
11-Sep	4.21	0	NA	NA	
11-Sep	4.10	1	1	100	12
11-Sep	4.92	40	38	95	
12-Sep	4.32	14	14	100	24
12-Sep	-	22	22	100	
12-Sep	5.10	16	16	100	
13-Sep	4.80	2	NA	NA	15
13-Sep	5.37	2	2	100	55
17-Sep	4.55	32	31	96.9	
17-Sep	-	9	9	100	
17-Sep	-	17	15	88.2	
20-Sep	4.54	6	NA	NA	
25-Sep	4.81	6	6	100	
25-Sep	-	0	NA	NA	

Effect of nutrition on ovary development in captive females

Under conditions that were similar to those used here, Bergh and Short (2008) reported that the average longevity of adult female *H. calcarata* was 27.8 d (± 2.25 SE), and the experiment evaluating the effect of access to pollen and/or a male fly on oocyte maturation assumed this longevity. However, many females died before they were removed and euthanized. Despite efforts to minimize mortality, flies were often found dead in the dish containing pollen and sugar solution, suggesting that they had become trapped or drowned in the food. Subsequently, we were not able to show significant effects of diet and male presence on ovary development in this study. However, a summary of the ovary development scores showed that ovary development was similar across treatments (Table 5). Denying access to pollen did not seem to retard ovary development (Fig. 14); females without access to pollen developed mature oocytes (Fig. 15).

Table 5: Mean ± SE ovary development rating* for treatment combinations (n = 27)

		Male	
		Yes	**No**
Pollen	**Yes**	3.0 ± 0.32 (7)	2.4 ± 0.32 (7)
	No	2.6 ± 0.38 (5)	3.0 ± 0.30 (8)

* (1) early development with little growth of follicles; (2) follicles at the posterior end of the inner ovarioles elongate and grow in size; (3) some ovarioles containing mature oocytes, and most ovarioles with partially developed follicles; (4) almost all ovarioles with at least one mature oocyte and developing follicles visible distally.

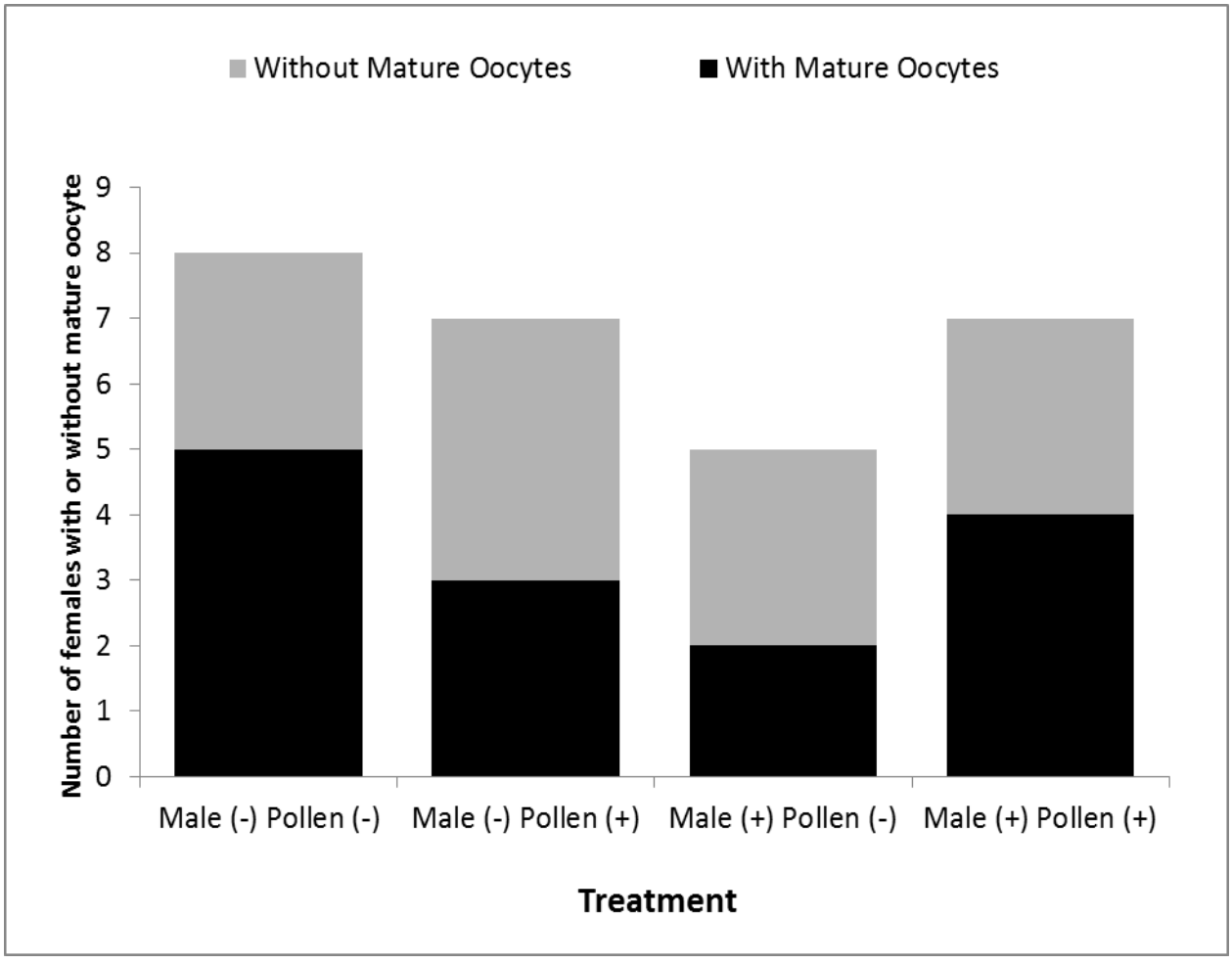

Fig. 14: Number of females (5- to 20-d-old) with and without mature oocytes.

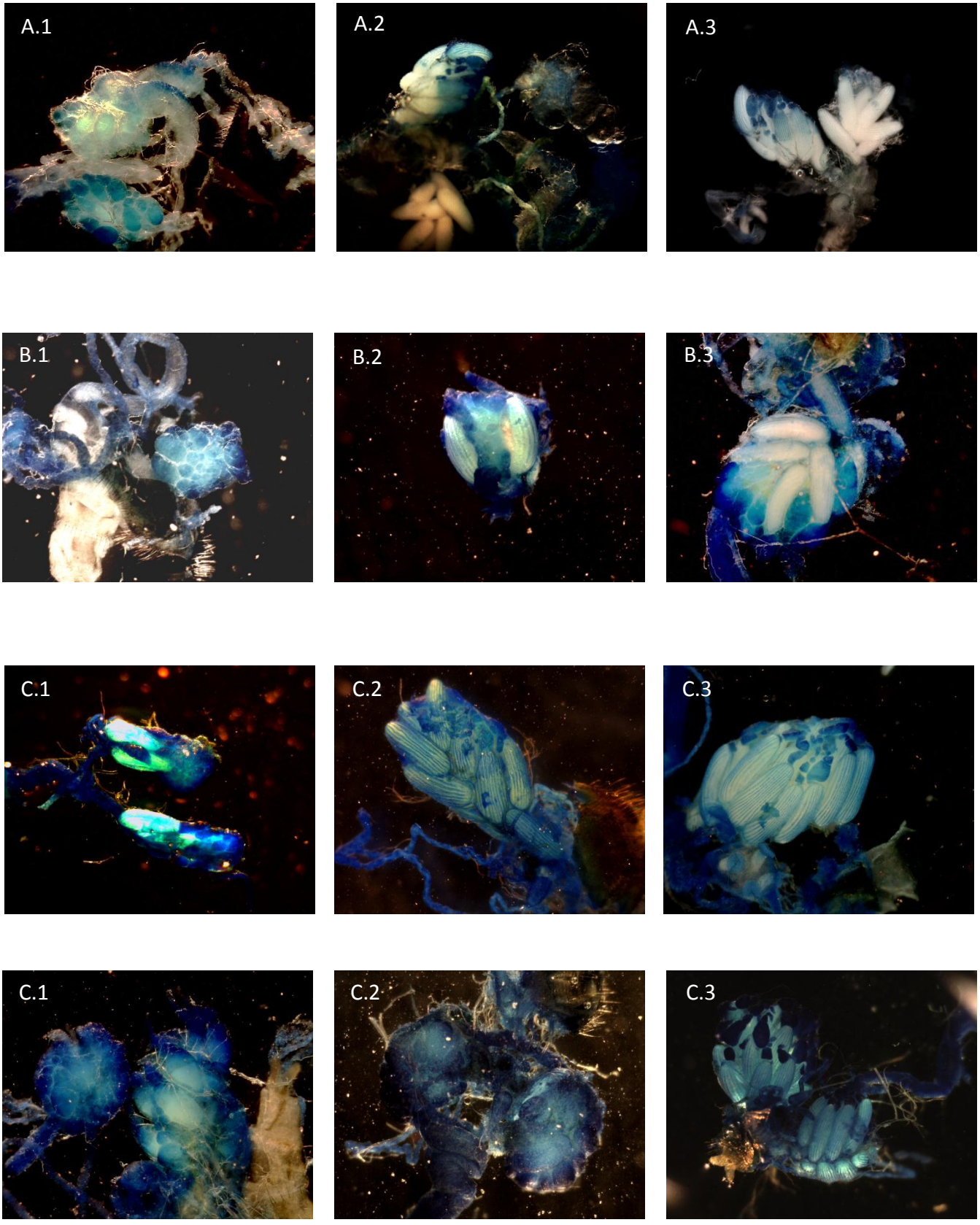

Fig. 15: Effect of male presence and pollen access on ovary development in *H. calcarata.* Treatments were: **(A .1-3)** male, no pollen; **(B. 1-3)** male, pollen; **(C. 1-3)** no male, no pollen; **(D. 1-3)** no male, pollen. Each photograph is of the ovaries from a different individual, at 150 – 200X.

In the second ovary development experiment, there was not a significant difference (df = 8; Z = -0.990; P = 0.3222) in ovary volume between 7-d-old female *H. calcarata* provided pollen, sugar, and water (n = 4) (0.23 mm^3 ± 0.07 SE) and those provided only sugar and water (n = 5) (0.23 mm^3 ± 0.06 SE).

Female *H. calcarata* emerged with a pair of partially developed ovaries which were located in the lower sections of the abdomen. The ovaries continued to develop after emergence, with the follicles increasing in number, elongating, increasing in size, and ultimately producing mature eggs. Development rate varied greatly among individuals, with mature oocytes observed within the ovaries of 5-d-old individuals, yet some individuals >15-d-old did not have mature oocytes. In general, mature oocytes were most commonly observed in 7- to 10-d-old individuals. The mean size (mm) of mature oocytes, with exochorionic sculpturing (n = 20 oocytes) was 0.201 ± 0.007 SE wide x 0.623 ± 0.006 SE long. The oocytes from the ovaries of gravid females (n = 9) were 0.256 mm (± 0.010 SE) wide x 0.646 mm (± 0.010 SE) long, and were significantly wider (Z = 3.56; P = 0.0004) and longer (Z = 2.1; P = 0.0338) than oocytes of captive-reared females.

Stage 1 ovaries were defined as those with little development. The ovaries of the newly emerged adult were small (0.3-0.6 wide x 0.6-0.8 long) (n = 4) ovoid bodies of off white appearance. They stained dark blue, revealing that each ovary was made up of a tightly clustered collection of spheroid ovarioles (Fig. 16A).

At stage 2 the ovarioles were somewhat enlarged, apparently resulting from growth of follicles. Follicles began to develop and enlarge at the posterior portion of the ovary, elongating to form ovoid bodies. As the ovaries increased in size, the ovarioles became more distinctive and

individual follicles were readily recognized within the ovariole sheath when stained (Fig. 16B). The posterior portion of the ovary, or the vittellarium, engorged first, while the distal section, or germarium remained approximately equal in diameter so that the ovary tapered towards the terminal filament.

At stage 3 some of the first follicles had produced oocytes with exochorionic sculpturing (Fig. 16C) but most of the ovarioles contained undeveloped follicles. The smaller, less developed follicles stained a darker blue than the mature oocytes. Ovariole development appeared to be asynchronous, with a greater proportion of the inner ovarioles often developing mature follicles earlier compared with the outer ovarioles. Multiple follicles developed within each ovariole progressively; the first follicle seemed to develop almost to completion before the more distal follicles begin enlarging considerably.

At stage 4 the majority of oocytes appeared to be mature. As each oocyte matured to form the distinctive ovoid shape, the nurse cells decreased in size until they became completely depleted. Mature oocytes were tightly clustered, parallel to each other (Fig. 16D). Mature eggs moved down, through the pedicel, into the lateral oviduct, and finally into the egg chamber. The number of ovarioles appeared to vary among individuals, with a maximum of 55 counted in one individual. In the later stages of maturity, an increasing proportion of the ovarioles contained multiple developing follicles and at least one mature oocyte.

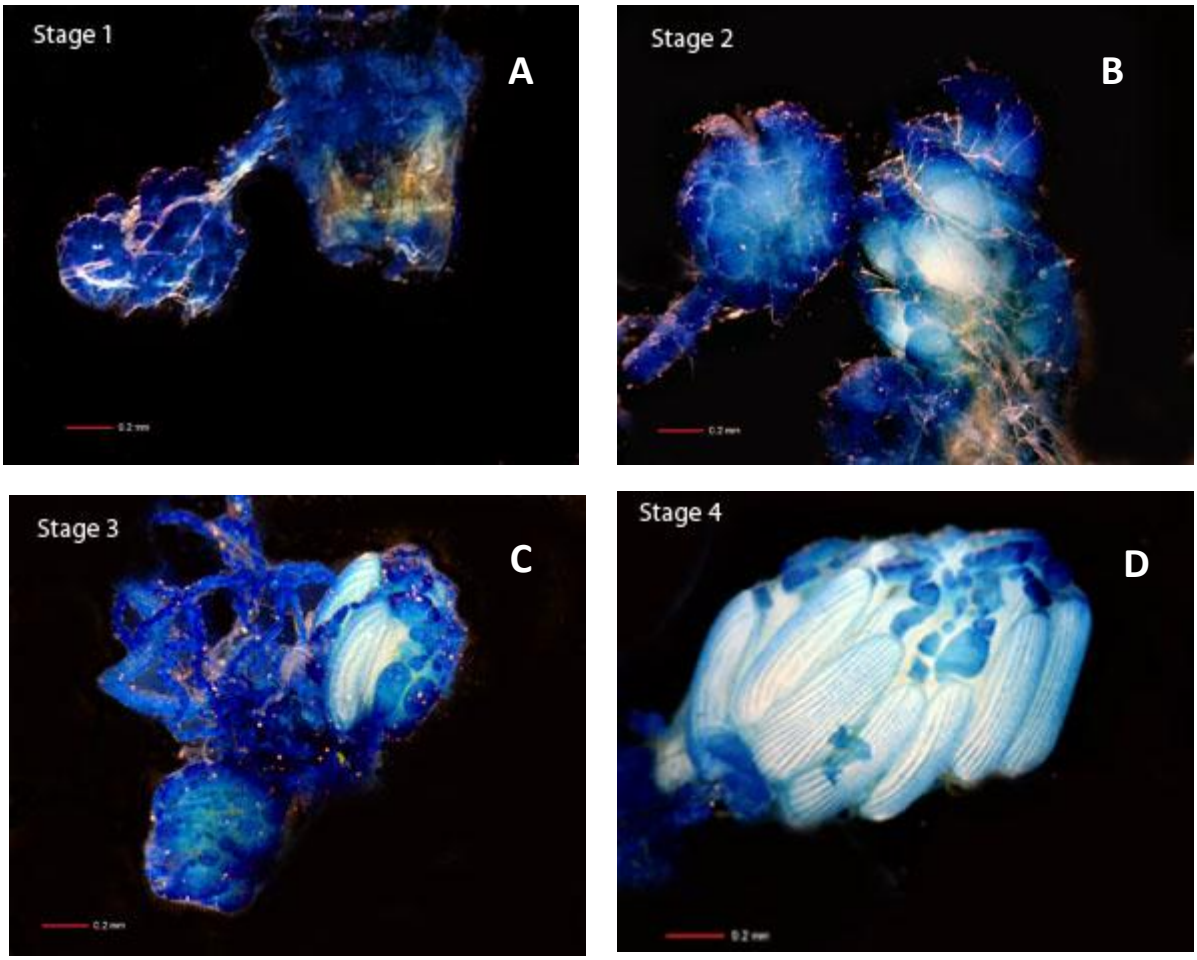

Fig. 16: Four stages of ovary development in *H. calcarata* reared in captivity: **(A)** early development with little growth of follicles; **(B)** follicles at the posterior end of the inner ovarioles elongate and grow in size; **(C)** some ovarioles containing mature oocytes, and most ovarioles with partially developed follicles; **(D)** almost all ovarioles with at least one mature oocyte and developing follicles visible distally. Photographs taken at 200X.

Discussion

These studies have provided important basic and applied information that should enhance further research and other activities related to rearing *H. calcarata* in captivity and to exporting it from Virginia to New Zealand.

Deploying WAA-infested apple shoot sections for collecting *H.* calcarata eggs was vastly more efficient than scouting WAA colonies in the field and much less demanding on plant resources than deploying and destructively sampling colonies on potted apple trees. Furthermore, we showed that WAA colonies can be readily cultivated within screened, sleeve cages on shoots of mature apple trees in orchards. In future, the use of colonies on shoot sections from orchard trees will reduce or eliminate the need to maintain potted trees. Field evidence suggests that *H. calcarata* spend considerable time searching around the base of apple trees, likely toward finding oviposition sites close to edaphic WAA colonies. My field observations in 2011 revealed that flies spent up to 5 min searching around the base of a single tree, with the majority of time within 0.5 m of the trunk, and often alighted upon and walked on the ground. A maximum of 6 flies were observed simultaneously searching at the base of the same tree. Bergh and Short (2008) reported observing a female *Heringia* that appeared to be in the process of ovipositing in the soil at the base of an apple tree. Upon further inspection, four un-hatched *H. calcarata* eggs were found in the soil above an edaphic WAA colony on a tree root. Furthermore, field inspections suggested that *H. calcarata* readily detected the ground-deployed shoots. *H. calcarata* regularly laid eggs on shoots that were present in the field for as little as 8 h and on one occasion in 2012 an individual was seen alighting upon a shoot 15 min after it was deployed.

Seasonal differences in the number of shoots upon which eggs were laid conform well to data from previous studies of seasonal patterns of *H. calcarata* oviposition and relative abundance.

Bergh and Short (2008) deployed potted apple trees infested with WAA in Virginia orchards at weekly intervals throughout the growing season from 2003-2005 and destructively sampled 5 colonies per tree to examine the seasonal distribution of un-hatched syrphid eggs. They found that weekly abundance of un-hatched *H. calcarata* eggs was variable within and across seasons and that its eggs were present throughout most of each growing season. In 2003, a relatively large peak occurred over several weeks in June, followed by a somewhat smaller peak in August, with smaller numbers recorded at other times. Numerous smaller peaks were recorded during the 2004 and 2005 seasons and the latest record of un-hatched *H. calcarata* eggs was on 1 October, 2004. From 2008-2012 Bergh (unpublished data) examined the seasonal activity of *H. calcarata* around the base of apple trees in a single orchard block by making weekly surveys on days deemed amenable to *H. calcarata* flight. It was shown that *H. calcarata* activity begins in May and peaks in June – early July, with little to no activity typically observed in August, and a that second peak of activity occurred between late August through early-October.

Overall, a high percentage of *H. calcarata* eggs and larvae survived transport to New Zealand by commercial courier. Bergh (unpublished data) showed that larval *H. calcarata* can withstand food deprivation for at least one week without adverse effects on their resumption of feeding and development to the adult stage. This suggested that *H. calcarata* should be particularly amenable to transport over multiple days, even if resources are depleted, which has been confirmed by our shipments. Future shipments to NZ are expected to focus primarily on un-hatched eggs and young larvae, since larvae will be required for non-target feeding studies in Auckland.

The ability to sustain *H. calcarata* in captivity over multiple generations will be critical to the success of our long-term objective to release it in NZ orchards. At this time, the main impediment to sustained rearing is our poor understanding of the factors required to elicit mating

by this species in captivity. However, we have demonstrated two key factors that will influence future research on this goal. First, we showed that field-collected gravid females readily laid eggs on WAA-infested apple shoots in small plastic cages in the laboratory and that nearly all of the eggs laid were viable. Preliminary trials have been conducted with virgin *H. calcarata* males and females placed in pairs inside cages with food and an oviposition stimulus (WAA-colony). Although eggs have been found on the WAA colonies in several instances, none have hatched (Charles unpublished; Short 2003). Therefore, assuming that it is possible to successfully mate *H. calcarata* in captivity, we can now be assured that deposition of viable eggs is possible under captive conditions. Indeed, the deposition of fertile eggs by females paired with males in captivity may provide the initial indication of successful mating in captivity. Oviposition by field-collected females on shoots in captivity can also serve as an effective alternative method for collecting eggs.

Our studies have also revealed that virgin adult female *H. calcarata* will produce mature oocytes in captivity. Ovaries with apparently mature oocytes were dissected from female *H. calcarata* reared from eggs in the laboratory. Mortality in captivity inhibited our ability to conduct a statistical analysis of the results, but it was shown that females not provided pollen in their diet were able to mature oocytes. This suggests that pollen is not an essential dietary component for reproductive development of *H. calcarata*. These data are in contrast to the conventional assumption that pollen is essential for reproductive development in syrphid females (Schneider 1969, Chambers 1988, Thomson 1999). Gut content analysis of field collected syrphids found that the amount of pollen consumed, as measured by the proportion of the gut volume as pollen, varied seasonally (Haslett 1989), by sex (Hickman et al. 1995), and across species (Holloway 1976, Gilbert 1981, Ssymank and Gilbert 1993). It was shown that the proportion of pollen

within females was greater than males with the assumption that females consumed more pollen in order to obtain sufficient protein to mature ovaries (Hickman et al. 1995). Laboratory studies have shown that pollen is an important dietary component for adult syrphids, flies fed pollen had greater longevity, fertility and fecundity than flies fed only sugar and water (Dong and Xiong 1988, Branquart and Hemptinne 2000, Dong et al. 2004, Hong and Hung 2010). The fecundity and percentage of viable eggs increased in *Metasyrphus corollae* provisioned with dried bee-pollen compared with individuals provided sugar and water only (Dong and Xiong 1988). The same species was shown to perform better when provided fresh flowers compared with dried pollen (Dong et al. 2004). A similar study with *E. balteatus* showed that provision of honey solution and bee pollen or fresh pollen increased the longevity and reproductive output of female flies (Hong and Hung 2010). Pollen is therefore an important dietary component for reproductive development, but may not be essential for egg maturation.

Understanding the development of ovaries in *H. calcarata* will help researchers better understand the conditions required to optimize ovary development and, assuming captive mating is achievable, will improve the ability to enhance fertility and fecundity of captive females. Although the development of oocytes in captive-reared *H. calcarata* were observed and recorded, the primary objective was not to give a detailed assement of development or physiology, but was to analyse the influence of environmental factors on ovary development. There is evidence from other studies on the ultrastructure, anatomy, and development of ovaries in other dipterans that assist our understandings. Adham et al. (2009) examined ovary development in adult *Culex pipiens quinquefasciatus* (Say) (Diptera: Culicidae) and reported seven distinct stages within two main periods, previttellogenesis (which consisted of 3 stages of cellular differentiation), and vitellogenesis (which began immediately following a blood meal,

was associated with the enlargement and maturation of the follicles, and culminated in the formation of mature eggs). Chaiwong et al. (2012) examined the ultrastructure and development of the ovaries in *Chrysoma megacephala* (F.) (Diptera: Calliphoridae) and reported eight distinct stages of development over ten days, with the oocyte increasing in size and progressively becoming differentiated from the surrounding cells. A study of another Calliphorid described ten stages of ovary development (Avancini and Pires do Prado 1986), with each stage distinguished by the relative proportion of the follicle occupied by the oocyte compared with nurse cells and the morphology of the follicular epithelium.

This research has provided important insights that have advanced the logistical capacity of the project and has provided essential basic knowledge of the reproductive biology of *H. calcarata* which will assist in the development of captive rearing methods.

References

Adham, F. K., H. Mehlorn, Z. M. El-Basheir, and A. S. Yamany. 2009. Light and electron microscopic studies on the developement of the ovaries of *Culex pipiens quinquefasciatus* (Say) (Diptera: Culicidae). Parasitology Research 105: 939-948.

Avancini, R. M. P., and A. Pires do Prado. 1986. Oogenesis in *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae). International Journal of Insect Morphology and Embryology 15: 375-384.

Barlow, C. A. 1961. On the biology and reproductive capacity of *Syrphus corollae* Fab. (SYRPHIDAE) in the laboratory. Entomologia Experimentalis et Applicata 4: 91 - 100.

Bergh, J. C., and B. D. Short. 2008. Ecological and life-history notes on syrphid predators of woolly apple aphid in Virginia, with emphasis on *Heringia calcarata*. BioControl 53: 773-786.

Branquart, E., and J. L. Hemptinne. 2000. Development of ovaries, allometry of reproductive traits and fecundity of *Episyrphus balteatus* (Diptera: Syrphidae). European Journal of Entomology 97: 165-170.

Chaiwong, T., K. Sukantason, U. Chaisri, B. Kuntalue, R. C. Vogtsberger, and K. L. Sukontason. 2012. Ovarian ultrastructure and development of the blow fly, *Chrysomya megacephala* (Diptera: Calliphoridae). International Journal of Parasitology Research 4: 65-70.

Chambers, R. 1988. Syrphidae, pp. 259-270. In A. K. Minks and P. Harrewijn (eds.), World Crop Pests: Aphids, Their Biology, Natural Enemies, and Control. Elsevier, Amsterdam, Netherlands.

Dixon, T. J. 1959. Studies on oviposition behaviour of Syrphidae (Diptera) Transactions of the Royal Entomological Society of London 111: 57-80.

Dong, H., and H. Xiong. 1988. Rearing of the adults of *Metasyrphus corollae* (F.) (Dip: Syrphidae). Chinese Journal of Biological Control.

Dong, K., Y. Dong, and Y. Luo. 2004. Study on rearing of the adults of *Syrphus corollae* Fabricius in laboratory. Natural Enemies of Insects 03.

Frank, D. L., T. C. Leskey, and J. C. Bergh. 2010. Development of a rearing methodology for the dogwood borer (Lepidoptera: Sesiidae). Annals of the Entomological Society of America 103: 50-56.

Frazer, B. D. 1972. A simple and efficient method of rearing aphidophagous hoverflies (Diptera: Syrphidae). J. Entomological Society of B In Proc. 49th Plant Protection Conference New Zealand Plant Protection Society Inc., pp 59-63. In Proc. 49th Plant Protection Conference New Zealand Plant Protection Society Inc., pp 59-63.ritish Columbia 69: 23-24.

Gilbert, F. S. 1981. Foraging ecology of hoverflies: morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species. Ecological Entomology 6: 245-262.

Gilbert, F. S. 1993. Hoverflies, 2nd ed. Richmond Press, Slough, UK.

Hart, A. J., and J. S. Bale. 1997. A method of mass-rearing the hoverfly *Episyrphus balteatus* (Diptera, Syrphidae). Dipterist Digest 4: 1-3.

Haslett, J. R. 1989. Adult feeding by holometabolous insects: pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae). Oecologia 81: 361-363.

Hickman, J. M., G. L. Lövei, and S. D. Wratten. 1995. Pollen feeding by adults of the hoverfly *Melanostoma fasciatum* (Diptera: Syrphidae). New Zealand Journal of Zoology 22: 387 - 392.

Holloway, B. A. 1976. Pollen-feeding in hover-flies (Diptera: Syrphidae). New Zealand Journal of Zoology 3: 339 - 350.

Hong, B. M., and H. Q. Hung. 2010. Effect of temperature and diet on the lifecycle and predatory capacity of *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae) cultured on *Aphis gossypii* (Glover). Journal Int. Soc. Southeast Asian Agric. Sci. 16: 98-103.

Medvey, M. 1988. On the rearing of *Episyrphus balteatus* (Deg.) (Diptera: Syrphidae) in the laboratory. *In* E. Niemczyk and A. F. G. Dixon (eds.), Ecology and Effectiveness of Aphidophaga, 1987, Teresin, Poland. SPB Academic Publishing.

SAS Institute 2010 JMP Statistical Software Version 9. SAS Institute Institute, Cary, NC.

Schneider, F. 1969. Binomics and physiology of aphidophagous syrphidae. Annual Review of Entomology 14: 103-124.

Shaw, P. W., and J. T. S. Walker. 1996. Biological control of woolly apple aphid by *Aphelinus mali* in an integrated fruit production programme in Nelson. In Proceedings 49th New Zealand Plant Protection Conference (pp. 59-63) Zew Zealand Plant Protection Society.

Short, B. D., and J. C. Bergh. 2005. Separation of three common hover fly predators of woolly apple aphid based on the exochorionic sculpturing of eggs. Can. Entomol. 137: 67-70.

Ssymank, A., and F. Gilbert. 1993. Anemophilous pollen in the diet of Syrphid flies with special reference to the leaf feeding strategy occurring in Xylotini. (Diptera, Syrphidae). Deutsche Entomologische Zeitschrift 40: 245-258.

Thomson, S. N. 1999. Nutrition and culture of entomophagous insects. Annual Review of Entomology 44: 561 - 592.

Ungureanu, E. M. 1972. Methods for dissecting dry insects and insects preserved in fixative solutions or by refrigeration. Bulletin of the World Health Organization 47: 239-244.

4. Conclusion

The research presented in this thesis was conducted as part of a collaborative project between Virginia Tech and Plant and Food Research New Zealand, the ultimate goal of which is to introduce *H. calcarata* into New Zealand (NZ) orchards to complement biological control of woolly apple aphid by the hymenopteran parasitoid, *A. mali*. In 2010, NZ regulatory authorities granted permission to import *H. calcarata* from Virginia into quarantine in Auckland. Any future decision and approval to release *H. calcarata* from quarantine, and subsequently to introduce it to NZ orchards, will be contingent upon proof that it will not have a negative impact on the NZ environment, with particular regard to the potential impact on the native arthropod fauna. The potential impact of *H. calcarata* on *A. mali* populations is also highly relevant, as it is currently the only effective natural enemy of WAA in NZ. As one component of satisfying these regulatory requirements, researchers in NZ will conduct studies on the potential non-target effects of *H. calcarata* on a selection of native arthropod species. In the first instance, these studies will rely on periodic shipments of *H. calcarata* from Virginia, but in future it will be necessary to maintain and sustain *H. calcarata* in quarantine in Auckland. Other information that will influence a decision to release *H. calcarata* from quarantine will be derived from this thesis and from the published literature.

I have addressed two key issues pertaining to the project, (1) intraguild competition between *H. calcarata* and *A. mali* (Chapter 2), and (2) collecting, transporting, and rearing *H. calcarata* (Chapter 3). The findings of this research offer valuable insights into the biology and ecology of *H. calcarata* that are directly relevant to the project goals and that will help guide the development of *H. calcarata* as a classical biological control agent for WAA in NZ.

I showed that *H. calcarata* adults will lay eggs on WAA colonies parasitized by *A. mali* and that *H. calcarata* larvae preyed upon parasitized WAA, confirming that *A. mali* are exposed to intraguild predation (IGP) by *H. calcarata*. However, we also showed that the effects of IGP on *A. mali* populations are likely mitigated via discrimination by adult female and larval *H. calcarata*. In oviposition studies in the field, female flies laid eggs less frequently on WAA colonies with higher numbers of mummified aphids than on those with fewer mummies. Furthermore, in choice and no-choice feeding studies in the laboratory, larvae preyed on mummified aphids and aphids in an early stage of parasitization at a lower rate than non-parasitized aphids, with most pronounced differences between mummified and non-parasitized aphids. When *in situ* WAA colonies on the branches of potted apple trees were exposed to *H. calcarata* and/or *A. mali*, there was no difference in the number of mummies produced by colonies exposed to *A. mali* only or to both *A. mali* and *H. calcarata* (although parasitization rates were very low across treatments). *H. calcarata* larvae had a significant effect on the number of WAA in colonies with and without *A. mali*.

The likelihood of adverse effects of predation by *H. calcarata* on *A. mali* populations appears to be mitigated by two factors, exposure risk and predation risk. Exposure to the risk of predation is lower for heavily parasitized colonies, due to the apparent effects of higher percentages of mummified aphids on oviposition site selection by adult female *H. calcarata*. The risk that a parasitized aphid will be preyed upon by *H. calcarata* larvae appears to be lower than would be expected by chance alone, since larvae largely avoided preying on mummies and preyed on early parasitized live aphids less frequently than non-parasitized aphids. Furthermore, field observations suggest that the abundance of *A. mali* is greater than for *H. calcarata*, *A. mali* tend to occupy WAA colonies more frequently and have a higher density within colonies than *H.*

calcarata. Therefore *H. calcarata* should have a greater impact on populations of its aphid host than on those of *A. mali.* Field observations have demonstrated repeatedly that *A. mali* is spatially and temporally sympatric with *H. calcarata* and other syrphid predators in Mid-Atlantic apple orchards, providing strong evidence that *A. mali* and *H. calcarata* co-exist compatibly. By extrapolation, although introduction of *H. calcarata* to the NZ apple orchard ecosystem is likely to result in some level of IGP between it and *A. mali*, it is unlikely that the fly will negatively impact the overall effects of *A. mali* on WAA biological control. Rather, based on historical field observations from Virginia and the Mid-Atlantic region and the results from this thesis, I contend that *H. calcarata* and *A. mali* are likely to have complementary effects in NZ.

To begin non-target impact testing, the fly must be transported from Virginia to NZ, and reared in quarantine. From a logistical perspective, we have advanced the ability to move this project forward to this stage by developing a more efficient method for collecting *H. calcarata* eggs, utilizing excised shoots containing at least one WAA colony. In addition, we have successfully transported juvenile *H. calcarata* with WAA from Virginia to New Zealand, and methods and protocols for future shipments were developed. These methods will be used for future collection and transport, required for research in NZ and eventual introduction of *H. calcarata.*

Maintaining multiple generations of *H. calcarata* in captivity will become an essential aspect of the project. My research has advanced our understanding of the reproductive biology of *H. calcarata* which will assist in future efforts to achieve reproduction of *H. calcarata* in captivity. Successful captive breeding of syrphids is rare and difficult but has been achieved with *Syphus torvus, S. rebesii, S. opinator, Metasyrphus sp, Scaeva pyrastri* (Frazer 1972), *Syrphus corolla* (Barlow 1961), and *Episyrphus balteatus* (Medvey 1988, Hart and Bale 1997). High light conditions, adequate ovariole maturation, mating, and access to host aphids are important

conditions for oviposition by captive females (Schneider 1969). Thus far, captive reproduction of *H. calcarata* appears to be limited by mating and development of fertile eggs. Field-captured gravid *H. calcarata* laid viable eggs on WAA colonies under captive conditions, assuring that if mating and maturation of fertile eggs is possible, oviposition in captivity should result. I examined the ovaries of adult females reared from eggs in the laboratory and found that mature oocytes can develop, although premature mortality and retarded development was common. Interestingly, females reared in individual cages and given a diet of only sugar and water were able to develop mature oocytes similarly to females caged individually with a male, and provided pollen as crushed bee pollen. This finding is in conflict with the conventional assumption that pollen is an essential dietary component for reproductive development in syrphids. Although pollen may enhance the fertility and fecundity of *H. calcarata* and would likely be provided in any attempt to maintain and mate virgin females, it has been shown that it is not an essential dietary component for reproductive development. Further work to delineate the conditions necessary for captive reproduction of *H. calcarata* is essential to the success of the project. Captive reproduction is not only necessary for culturing *H. calcarata* but production of gravid females will be necessary for non-target studies in New Zealand. Non-target impact assessment will be based on *H. calcarata* larval feeding tests in addition to an assessment of the propensity for *H. calcarata* females to lay eggs near non-target hosts. This best reflects the host range of the predator. Short and Bergh (2004) found that *H. calcarata* larvae would prey upon rosy apple aphid, *Dysaphis plantaginea* Passerini, when provided a diet of rosy apple aphid only, but surveys found no *H. calcarata* eggs, larvae, or pupae in any aphid colony other than WAA colonies.

If *H. calcarata* is eventually released in NZ orchards, a consideration of the factors that may affect its establishment and the success of the project suggest that NZ conditions should be highly suitable. Neither the availability of larval or adult food appears to be limiting. WAA is a common pest in NZ orchards, colonizing both the above- and below-ground portions of trees, as it does in Virginia. Surveys of the diversity and relative abundance of flowering weed species in and around NZ orchards at monthly intervals during the summer (Bergh and Walker, unpublished) revealed many species that are also found in Virginia, most of which are native to Europe. The primary apple-producing regions of NZ (Hawkes Bay and Nelson) experience much more moderate winter and summer conditions than are typical in the Mid-Atlantic region of the US, and therefore should be suitable to *H. calcarata*. Importantly, arthropod pest management programs in NZ are much less aggressive than in the eastern US, and therefore highly compatible with biological control. Finally, belonging to the tribe Pipizini, members of which show a preference for wax-producing (i.e. "woolly") insect species (Rojo et al. 2003), *H. calcarata* is unlikely to negatively impact native arthropods in NZ. The native NZ arthropod fauna is relatively limited, especially in aphid species richness (Dixon et al. 1987).

To realize the full potential of this project, further research is needed to better understand the biology and ecology of *H. calcarata*. As discussed previously, mating and reproduction in captivity remains an elusive challenge that must be addressed. Understanding how adult *H. calcarata* utilizes the floral resources in NZ will be important to ensure that the appropriate resources are available through conservation or augmentation in orchards. Determining the geographic range of *H. calcarata* in North America and its phenology and developmental rate at various temperatures will allow for more accurate climate matching with conditions in New

Zealand and other countries that have also expressed interest in this species, including South Africa and Australia.

References

Barlow, C. A. 1961. On the biology and reproductive capacity of *Syrphus corollae* Fab. (Syrphidae) in the laboratory. Entomologia Experimentalis et Applicata 4: 91 - 100.

Dixon, A. F. G., P. Kindlmann, J. Leps, and J. Holman. 1987. Why are there so few species of aphids, especially in the tropics. American Naturalist 129: 580-592.

Frazer, B. D. 1972. A simple and efficient method of rearing aphidophagous hoverflies (Diptera: Syrphidae). J. Entomological Society of British Columbia 69: 23-24.

Hart, A. J., and J. S. Bale. 1997. A method of mass-rearing the hoverfly *Episyrphus balteatus* (Diptera, Syrphidae). Dipterist Digest 4: 1-3.

Medvey, M. 1988. On the rearing of *Episyrphus balteatus* (Deg.) (Diptera: Syrphidae) in the laboratory. *In* E. Niemczyk and A. F. G. Dixon (eds.), Ecology and Effectiveness of Aphidophaga, 1987, Teresin, Poland. SPB Academic Publishing.

Rojo, S., F. Gilbert, M. A. MarcosGarcia, J. M. Nieto, and M. P. Mier. 2003. A world review of predatory hoverflies (Diptera, Syrphidae: Syrphinae) and their prey, CIBIO Ediciones, Alicante (Spain).

Schneider, F. 1969. Binomics and physiology of aphidophagous syrphidae. Annual Review of Entomology 14: 103-124.

Short, B. D., and J. C. Bergh. 2004. Feeding and egg distribution studies of *Heringia calcarata* (Diptera : Syrphidae), a specialized predator of woolly apple aphid (Homoptera : Eriosomatidae) in Virginia apple orchards. Journal of Economic Entomology 97: 813-819.