INVESTIGATIONS OF THE BIOLOGY OF THE PEST APHID *AULACORTHUM SOLANI* (KALTENBACH) (HEMIPTERA: APHIDIDAE) AND OF BIOLOGICAL CONTROL AGENTS FOR CONTROL OF MULTI-SPECIES APHID OUTBREAKS IN GREENHOUSE FLORICULTURE CROPS.

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

By

Sarah Elizabeth Jandricic

August 2013

© 2013 Sarah Elizabeth Jandricic

INVESTIGATIONS OF THE BIOLOGY OF THE PEST APHID *AULACORTHUM SOLANI* (KALTENBACH) (HEMIPTERA: APHIDIDAE), AND OF BIOLOGICAL CONTROL AGENTS FOR MULTI-SPECIES APHID OUTBREAKS IN GREENHOUSE FLORICULTURE CROPS.

Sarah Elizabeth Jandricic, Ph.D.

Cornell University 2013

Foxglove aphid, *Aulacorthum solani* (Kaltenbach), has recently become a significant pest of greenhouse crops in the north eastern U.S., Canada, and the U.K. Given its previous status as an occasional pest, little was known about its biology or ecology. Using a North American population, development time and life table statistics of *A. solani* were investigated at 6 temperatures. *Aulacorthum solani* developed fastest (6.9 ± 0.29 d) and had the highest intrinsic rate of increase (r_m = 0.25) at 25 °C; limited development was seen at higher temperatures (r_m = -0.24 at 30 °C). A study of 10 different greenhouse crops showed that these aphids generally distribute to bottom leaves of vegetative plants, but move upwards when plants are reproductive. Biological control of *A. solani* using the generalist aphid predator *Aphidoletes aphidimyza* (Rondani) was assessed in a series of greenhouse experiments. Here, the green peach aphid, *Myzus persicae* (Sulzer), was also included because aphid pests can co-occur. Experiments showed that *A. aphidimyza* perceives aphid colonies located on new growth of plants (meristems or top leaves) to be of higher value as oviposition sites compared to other plant locations. *Aulacorthum*

solani-infested plants, with aphids primarily present on lower leaves or flowers, received fewer eggs than *M. persicae*-infested plants. In trials using a single innundative release of the predator, this translated to more variable control of *A. solani* compared to *M. persicae* (12-80% vs. 78-95%, respectively; tested across several stages of plant growth). This is likely partially attributable to apparent competition, since control of *A. solani* was significantly improved in the absence of alternate prey. Entomopathogenic fungi were assessed as another biocontrol option against aphids, including the melon aphid, *Aphis gossypii* Glover. Novel isolates of fungi originally collected from aphid hosts were sought to potentially increase pathogenicity. However, no isolate tested, commercial or novel, resulted in acceptable mortality of 1st instar aphid nymphs, with all LC_{50} values >700 conidia/mm^2 under ideal lab conditions. Control options for *A. solani* and multi-species aphid infestations are discussed in light of the results presented in this thesis.

BIOGRAPHICAL SKETCH

Sarah Jandricic grew up thinking she was going to be a Zoologist, a Marine Biologist, *and* an Environmental Lawyer. At 22, she decided she should maybe settle on just one profession, and picked Entomologist after falling in love first with honeybees, then with all other insects at the University of Guelph in Ontario, Canada during her Honors B.Sc. After completing a M.Sc. in Environmental Biology and Toxicology at U of G, she made the switch to industry. She worked for 2 years as Director for Research for a small company offering pest management consulting for greenhouse crops. Realizing she had so much more to learn about insect pests and biological control, she returned to academia to begin her Ph.D. at Cornell in 2007 with Dr. John Sanderson, a leading expert in floriculture integrated pest management. After 6 years, she finally got her Ph.D. She hopes to continue in the field of IPM back in her native Canada, and to one day own an alpaca farm (or at least live visit one regularly).

This Ph.D. thesis is dedicated to the memory of my wonderful mother,

Mrs. Brenda Marguerite Pulsifer McCann,

who supported me whole-heartedly in all things, and whom I think about each and every day.

ACKNOWLEDGEMENTS

To properly thank everyone involved in this process, this section would have to be longer than my thesis itself. But as we have it, I have only a few pages, so I'll try my best.

My utmost thanks to my advisor, Dr. John Sanderson, for his all his guidance and expertise; for always treating me as a colleague; for his amazing abilities as a word-smith and extension expert; and for his patience and understanding when things went from being a safe-bet to a gamble. John, I really can't thank you enough for giving me this amazing opportunity and for seeing me through. Equal thanks must go to Dr. Steve Wraight, my "co-advisor", for all the research, statistical, and life experience he brought to the table; for spending countless hours helping me analyze (and re-analyze) my data; and for being a general calming force. My deep gratitude for my other committee members as well: Drs. Dave Gillespie, Jan Nyrop, and Jennifer Thaler. Each one of you brought wisdom and inspiration to my scientific experience, and I appreciate that your doors (or phone-lines) were always open.

Special thanks to two especially wonderful people: to K.C. Bennett for all her help staring at aphids with me, and to Mark Ramos for all his hard work in the greenhouses and at the compound scope. Work was always more fun with one of you around! Thanks also to Dylan Beal, Allison Box, Taylor Fitzpatrick, Liza White, Dr. Neil Mattson, Tim Putzke, Merritt Compton and the greenhouse staff at the Insectary for their assistance (often when it wasn't even their job).

The past 5 years would not have been in any way possible without the support and comaraderie of the following people. Thank you to the McCann and Jandricic families for giving unconditional encouragement and understanding while I was putting myself through this process again. Thank you to best friends, old and new (Heather Ritchie and Patricia Davidson), for always giving me someone to count on at the other end of a phone (or a cribbage board). To my cohort friends (especially Calum Russell, Erik Smith and Leo Stellwag): what can I say, except it was a privilege to share the Ph.D. experience with you? Sincere gratitude goes to my office mates, past and present (Lauren Cator, Sarah Short, Nick Ledesma, Moria Chambers and David Duneau) for the endless hours of friendship, support, and entertainment -- I couldn't have gotten through some days without you. Special thanks to all the Girl's Night girls (Drs. Lauren Cator, Madeline Galac, Melissa Hardstone, Michelle Helinski, Punita Juneja, Sarah Short, and Susan Villarreal) -- our friendship and once-a-week get togethers meant more to me than I can say. Thanks also to Erin Morris, who was single-handedly responsible for making me incredibly productive at least one day a week this past 8 mo. Super special thanks to the Bug Kids (Adrienne & Ian Brewster, Drs. Heather Mattila & Justin Armstrong, Nicole McKenzie & Sean Sepulis, Phil Careless, and our honorary Bug Kid, Dr. Gard Otis) for all our fun-filled BAWGs and other life adventures, and for giving me people to aspire to be like.

Last, is the most important person: my rock, my stability, my hope. Spreadsheet Wizard, palmier supplier, giver of world-class hugs, bringer of Totoro. The man who is my everything, and for whom there are not enough words of appreciation. Thank you, thank you, thank you to my amazing, saint of a husband,

Mark Jandricic, who (already having survived a M.Sc. with me) was ready and willing for round 2 and handled it all with an abundance of love and patience. You are the best person I know.

TABLE OF CONTENTS

BIOGRAPHICAL SKETCH iii
DEDICATION iv
ACKNOWLEDGEMENTS v
TABLE OF CONTENTS viii
LIST OF FIGURES ix
LIST OF TABLES xi

CHAPTER 1: LITERATURE REVIEW 1

CHAPTER 2: DEVELOPMENTAL TIMES AND LIFE TABLE STATISTICS OF *AULACORTHUM SOLANI* (HEMIPTERA: APHIDIDAE) AT SIX CONSTANT TEMPERATURES, WITH RECOMMENDATIONS ON THE APPLICATION OF TEMPERATURE-DEPENDENT DEVELOPMENT MODELS 38

CHAPTER 3: PATHOGENICITY OF CONIDIA-BASED PREPARATIONS OF ENTOMOPATHOGENIC FUNGI AGAINST THE GREENHOUSE PESTS GREEN PEACH APHID (*MYZUS PERSICAE* (SULZER)), COTTON APHID (*APHIS GOSSYPII* GLOVER) AND FOXGLOVE APHID (*AULACORTHUM SOLANI* (KALTENBACH)) (HEMIPTERA: APHIDIDAE) 76

CHAPTER 4: OVIPOSITION BEHAVIOR OF THE BIOLOGICAL CONTROL AGENT *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOMYIIDAE) IN ENVIRONMENTS WITH MULTIPLE PEST APHID SPECIES (HEMIPTERA: APHIDIDAE) 130

CHAPTER 5: BIOLOGICAL CONTROL OUTCOMES USING THE APHIDOPHAGOUS PREDATOR *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOYMYIIDAE) UNDER MULTI-PREY CONDITIONS: EFFECTS OF CROP STAGE, WITHIN-PLANT DISTRIBUTION AND APPARENT COMPETITION 172

CHAPTER 6: WITHIN-PLANT DISTRIBUTION OF THE FOXGLOVE APHID, *AULACORTHUM SOLANI* (KALTENBACH) (HEMIPTERA: APHIDIDAE) ON VARIOUS GREENHOUSE CROPS AND IMPLICATIONS FOR CONTROL 219

CHAPTER 7: GENERAL SUMMARY 252

LIST OF FIGURES

Figure 2.1. Constant-temperature-dependent developmental rate for *A. solani* based on the Lactin model (solid line) and the Logan model (dashed line). 58

Figure 2.2. Kaplan-Meier survival curves for *A. solani* reared at 6 different constant temperatures. 60

Figure 2.3. Age-specific daily fecundity of *A. solani* reared at constant temperatures (x-axis is not indicative of longevity). Error bars show + SE only. 64

Figure 4.1. A. Effect of the number of aphid nymphs of *A. solani* and *M. persicae* on the number of *Aphidoletes aphidimyza eggs* per plant when aphids were confined to a single location (middle leaves of plants). Though aphid species was not significant, both species are shown for comparison. For linear regressions on each species, R^2= 0.46 for *A. solani* and R^2= 0.38 for *M. persicae*. B. Effect per patch (treatment leaf); aphid species was not significant. For linear regressions on each species, R^2 =0.15 for *A. solani* and R^2= 0.13 for *M. persicae*. The linear regression for both species combined was significant ($F_{(1,46)}$=7.16, P=0.0103, R^2 =0.14). 146

Figure 4.2. Number of *A. aphidimyza* eggs on bottom leaf vs. top leaf aphid -infested patches, untransformed data and trendlines shown (data for aphids that migrated to middle leaves and meristems not shown). For the linear regresssions on log-log transformed data, the correlation between the number of eggs laid in a patch and the number of aphids per patch was higher on infested top leaves (R^2 = 0.82) than bottom leaves (R^2 = 0.33). 150

Figure 4.3. Number of *A. aphidimyza* eggs on aphid-infested bottom leaves vs. meristems, untransformed data and trendlines shown. Though aphid species was not significant, both species are shown for comparison. The correlation between the number of eggs laid in a patch and the number of aphids per patch was greater for meristems (R^2 = 0.75) than bottom leaves (R^2 = 0.17) (linear regression on log-log transformed data). 152

Figure 4.4. Number of *A. aphidimyza* eggs per patch, when only the bottom leaves of plants were infested with either *A. solani* or *M. persicae*. Though aphid species was not significant, both species are shown for comparison. For linear regressions on each species, R^2=0.42 *for M. persicae* and R^2 = 0.20 for *A. solani*. 154

Figure 4.5. A. Effect of aphid density (all stages) of *A. solani* or *M. persicae* on the number of *A. aphidimyza* eggs per plant, untransformed data and trend lines shown. Aphids were allowed to distribute naturally on plants. For linear regressions on log-log transformed data, R^2= 0.39 for *A. solani* and R^2 = 0.43 for *M. persicae*. B. Effect of aphid density on *Aphidoletes aphidimyza* eggs per patch, ntransformed data and trend lines shown. For linear regressions on log-log transformed data, R^2=0.13 for *A. solani* and R^2=0.62 for *M. persicae*. 156

Figure 4.6. A. Proportion of the total aphid population (across all treatment plants within two greenhouse compartments) found at each location on plants for *A. solani* (black bars) and *M. persicae* (white bars). Number of plants sampled was n=15 for *A. solani*, n=16 for *M. persicae*. B. Proportion of total *A. aphidimyza* eggs deposited at each plant location for each species. 158

Figures 5.1-3. Plants were in the following stages of growth: Figure 1) vegetative, Figure 2) budding, Figure 3) flowering. A) Mean aphid density ± SE of *A. solani* or *M. persicae* infested plants with no control measure (square symbols) or with releases of *Aphidoletes aphidimyza* (circle symbols). Different letters or symbols represent significant differences within aphid species. B) Mean number ± SE of *A. aphidimyza* eggs and larvae found on aphid infested plants 2d, 6d or 9-11d after release of *A. aphidimyza* adults. Different symbols represent significant differences between aphid species. Any eggs deposited around day 6 likely did not contribute to overall control during the experiment time frame, thus data are not shown. Primarily, large larvae were present on days 9-11 as the population awas ageing. 188

Figure 5.4. Distribution (average % ± SE) of *Myzus persicae* (*M.p.*) or *Aulacorthum solani* (*A.s.*) on vegetative plants with no predator vs. in the presence of *Aphidoletes aphidimyza* (*A.a.*). Aphid distributions are shown as dark grey bars; distribution of *A. aphidimyza* eggs/larvae on treatment plants are shown as white bars. Total aphid, predator egg or larval numbers for each sampling day are given. Results are shown from day 2, 6 and 9 after release of 100 *A. aphidimyza adults*/compartment (data combined over 3 compartments). On day 2, only *A. aphidimyza* eggs are present (no larvae). 191

Figure 5.5. Distribution (average % ± SE) of *Myzus persicae* (*M.p.*) or *Aulacorthum solani* (*A.s.*) on budding plants with no predator vs. in the presence of *Aphidoletes aphidimyza* (*A.a.*). Aphid distributions are shown as dark grey bars; distribution of *A. aphidimyza* eggs/larvae on treatment plants

are shown as white bars. Total aphid, predator egg or larval numbers for each sampling day are given. Results are shown from day 2, 6 and 9 after release of 100 *A. aphidimyza adults*/compartment (data combined over 3 compartments). On day 2, only *A. aphidimyza* eggs are present (no larvae). 196

Figure 5.6. Distribution (average % ± SE) of *Myzus persicae* (*M.p.*) or *Aulacorthum solani* (*A.s.*) on flowering plants with no predator vs. in the presence of *Aphidoletes aphidimyza* (*A.a.*). Aphid distributions are shown as dark grey bars; distribution of *A. aphidimyza* eggs/larvae on treatment plants are shown as white bars. Total aphid, predator egg or larval numbers for each sampling day are given. Results are shown from day 2, 6 and 11 after release of 100 *A. aphidimyza adults*/compartment (data combined over 2 compartments). On day 2, only *A. aphidimyza* eggs are present (no larvae). 200

Figure 5.7. A. Mean and SE of *Aulacorthum solani* and *Myzus persicae* with either no treatment (square symbols) or in the presence of the predator *Aphidoletes aphidimyza* (round symbols). B. Mean and SE of *Aulacorthum solani* with either no treatment or in the presence of the *A. aphidimyza* when present as the only aphid prey. Experiments were run concurrently. Results are shown from day 2, 6 and 9 after release of 120 *A. aphidimyza* adults/ compartment (data pooled over 2 compartments). 205

Figure 6.1. Frequencies of adult *Aulacorthum solani* occurring per patch (*i.e.* leaf, meristem, flower or bud). 238

LIST OF TABLES

Table 2.1. Duration (mean ± standard error) of each stadium for *A. solani* incubated at constant temperatures. 52

Table 2.2. Mean total developmental times (± SE), intrinsic rate of increase (r_m), net reproductive rate (R_o), total fecundity and doubling times (DT) for *A. solani* reared on various crops. 53

Table 2.3. Temperature dependent nymphal mortality of *A. solani* reared at 6 constant temperatures. 55

Table 2.4. Linear regression equations for temperature-dependent development of *A. solani*. 56

Table 2.5. Parameter estimates (mean ± SE) and estimated temperature thresholds of the Logan and Lactin models for the development of *A. solani.* 57

Table 2.6. Kaplan-Meier survival curves for *A. solani* reared at six different constant temperatures. 60

Table 2.7. Mean (± SE) pre-larviposition period, larviposition period, total fecundity, daily fecundity and longevity for adult *A. solani* females reared at constant temperatures. 62

Table 2.8. Life table statistics for *A. solani* reared at differing temperatures; r_m = intrinsic rate of increase; R_o = net reproductive rate; GT = mean generation time; DT = doubling time. 65

Table 3.1 Fungal strains/isolates included in single-dose screening assays against *Aphis gossypii* and *Myzus persicae*. 98

Table 3.2 Mortality (%) for *B. bassiana*, *M. anisopliae* and *I. javanica* (sensu lato) isolates screened against 1st instar (12±12 h old) *M. persicae* and *A. gossypii*. 101

Table 3.3 LC_{50} and LC_{25} estimates from 5-dose bioassays against 1st instar nyphs of A. *Myzus persicae*, B. *Aphis gossypii* and C. *Aulacorthum solani* exposed to isolates of *B. bassiana* and *M. anisopliae* (sensu lato). Isolates are compared within each species only. 105

Table 3.4. Comparison of *Beauveria bassiana* strain GHA in its original,

technical powder form vs. as a single spore resisolate from an aphid host (*A. gossypii*) against 1st instar nymphs (12 ± 12h old) of *M. persicae* and *A. gossypii*. 106

Table 3.5. Single assay directly comparing the virulence of *B. bassiana* strain JW-1 (Naturalis®) against 1st instar nymphs (12 ± 12h old) of *M. persicae* and *A. solani*. 108

Table 3.6. Comparative virulence of two isolates against 1-2 day old adults and 1st instar nymphs (12 ± 12h old) of *M. persicae* and *A. gossypii* and *A. solani*. 110

Table 3.7. Effects of fungal infection on aphid survival and reproduction. 111

Table 3.8. Effects of fungal disease progression on aphid reproduction. 114

Table 4.1. Effects of prey patch species and location on *Aphidoletes aphidimyza* (*A.a.*) oviposition. Aphids were allowed to naturally distribute and reproduce on plants. 160

Table 4.2. Effects of prey patch density, location and species on *Aphidoletes aphidimyza* (*A.a.)* oviposition. Aphids were allowed to naturally distribute and reproduce on plants. 161

Table 5.1. F-test statistics and P values for ANOVAs evaluating the effect of aphid density and aphid species on *A. aphidimyza* egg or larval numbers per plant on three different sampling days. 189

Table 5.2. Statistics for control outcomes using *A. aphidimyza* for multiple aphid species across several stages of plant growth. 193

Table 6.1. Planting dates for plant species used in experiments. 232

Table 6.2. Influence of plant species and growth stage on within-plant distribution of *Aulacorthum solani* (all ages). 234

Table 6.3. Influence of plant species and growth stage on within-plant distribution of adult *Aulacorthum solani* only. 240

Table 6.4 Influence of plant species and growth stage on feeding of *Aulacorthum solani* on different plant organs. 238

CHAPTER 1: LITERATURE REVIEW

BACKGROUND ON THE GREENHOUSE FLORICULTURE INDUSTRY

Economics

Greenhouse floral crops are among the most valuable commodities in U.S. agriculture and in the state of New York. In 2011, wholesale value for floriculture crops in the United States was $4.1 billion from the top 15 states (USDA, 2012). Of this, $1.9 billion was attributed to bedding and garden plants and $1.2 Billion was attributed to potted plants; cut flowers, foliage plants, propagation material and hanging baskets rounding out the rest of the net value (USDA, 2012). New York State is currently ranked as the 8th highest state in floral crop sales, with $1.7 million worth of sales in 2011 (a 3% increase from 2010). NY growers tend to grow a wide variety of bedding and potted plants which were typically sold wholesale in the past, but retail sales have increased dramatically in recent years due to competition with large out-of-state producers who supply of "big box" chain stores. This is in contrast to larger producers in many other areas of the world (*e.g*. Ontario, Canada; western Europe; Central and South America; Africa) which tend to grow large monoculture crops (J.P. Sanderson, personal communication).

Insect Control in Greenhouses

Cosmetic damage by pests can occur quickly and easily lead to crop loss in floriculture crops, so greenhouse flower growers apply more pesticides per square meter than in any other commodity (Smith 1998). Since flowers are not consumed,

there is no testing of pesticide residues on ornamental plants, but such intense pesticide use is an unsustainable approach and can cause several pesticide-related problems. Pesticide resistance problems are so serious that manufacturers have added instructions for resistance management to greenhouse pesticide labels, and concerns for worker safety (due to the greenhouse being an enclosed environment) have prompted the EPA to add sections to its Worker Protection Standards specifically for greenhouse workers and applicators (U.S. Federal Register, 40 CFR Part 170). Alternatives to pesticides which are safe, efficacious, cost effective, and relatively easy to use are greatly needed in this industry.

Although many NY growers employ some method of integrated pest management (IPM), including sanitation, cultural control and the use of sticky cards and basic scouting, less than 13% of growers release biological control agents (*i.e.* predators or parasitoids to control insect pests through natural mechanisms), according to a 2000 survey (Lamboy 2002). Less than 3.5% of growers made releases on a weekly basis. Most IPM was implemented by larger operations (> 1 acre), likely because they have more resources to spend on dedicated IPM scouts, etc. However, 66% of NY growers indicated they would like to learn more about IPM. Thus, it is likely that if an effective and relatively simple method of implementing biological control of a specific pest was demonstrated to growers, the chances of successful adoption could be significant.

Aphid Pests in Greenhouse Crops

Aphids are soft, pear-shaped insects that are typically slow-moving and aggregate in dense groups to feed (Marshall 2006). Of the approximately 4700 total species of aphids (Aphididae) worldwide, 450 of these have been recorded from crop plants, but only ca. 100 of these are considered pest species of economic importance (Blackman and Eastop 2007). Aphids contribute greatly to pesticide use in greenhouses: in a 1996 survey of pesticide application in Massachusetts floriculture operations, the number of pesticide applications for aphids (at 3/crop) was second only to thrips (Smith 1998). Although a wide variety of aphids can potentially infest greenhouse crops, there are several key species of aphids that pose the greatest problems in North American floriculture. These include (but are not limited to) green peach aphid / peach potato aphid (*Myzus persicae* (Sulzer)), melon aphid / cotton aphid (*Aphis gossypii* Glover), potato aphid (*Macrosiphum euphorbiae* (Thomas)), foxglove aphid /glasshouse potato aphid (*Aulacorthum solani* (Kaltenbach)), rose aphid (*Macrosiphum rosae* (Linnaeus)), and chrysanthemum aphid (*Macrosiphoniella sanborni* (Gillette)) (Gill and Sanderson 1998). In a 2004 survey on the presence of aphids in NY and MA floriculture greenhouses (Van Driesche et al. 2008), the 3 most common aphid species found were *M. persicae* (53% of infestations), *A. solani* (28%), and *A. gossypii* (6%). These 3 species, with the addition of *M. euphorbiae*, are generally considered by North American floriculture growers to be the species of most serious concern, with the exception that *A. solani* is not known to be a pest in the southern U.S. (S. Jandricic, personal observation; L. Osborne, personal communication). Detailed biology of the two aphid pests currently of the most concern in the northeastern U.S. and Canada (*M. persicae* and *A. solani*) are given here, and are the main focus of this

thesis. However, descriptions of *A. gossypii* and *M. euphorbiae* can be found in (Blackman and Eastop 1984).

MYZUS PERSICAE (GREEN PEACH APHID)

Identification

Myzus persicae (Aphididae; Aphidinae, Macrosiphini) is one of the smaller aphid species found in greenhouse ornamental crops, and is usually found on plant terminals and flowers (Harrington and Taylor 1990; Guldemond et al. 1998). This pest has two color morphs, green and red, of which the former is much more common (Blackman 1987). Apterous adult females of *M. persicae* have well-developed lateral frontal tubercles at the base of their antennae, which gives the impression of an indentation in the frons or vertex when viewed under a microscope. Body sizes range from 1.62-2.10 mm long and 0.82-1.04 wide. Antennae are 6-segmented and are ca. 0.82 to 0.92 times their body length. The rostrum is darker at the tip and reaches the hind coxae. The abdominal dorsum is smooth and without pigmentation, and the siphunculi are cylindrical and exhibit a flange (this flange is absent in nymphal stages). The cauda is short, triangular and ca. 0.20 mm in length and has 6 hairs (Devi and Singh 2007). In contrast, alate adult females are brownish-black (including legs, siphunculi, rostrum and antennae), more elongated than the apterous adults and tend to be longer in length and less wide (ca. 1.78-2.18 mm in length by 0.85-0.98 wide). Their wings are glass-like with brownish veining. Their siphunculi are cylindrical (without a flange as in the apterous adults; Devi and Singh 2007). For extensive descriptions of *M. persicae* nymphal stages and adult morphs, see Devi and Singh (2007).

Apterous *M. persicae* can be distinguished from other common greenhouse aphids most easily by i) confirming the presence of distinct antennal tubercles, ii) determining that antennal tubercles are at a convergent angle (vs. *A. solani*, and *M. euphorbiae*, which are parallel and divergent, respectively) (see Gill and Sanderson 1998 for illustrations); iii) observing that their antennae are not quite as long as their body, (vs. *A. solani*, where the antennae are longer than the body); and iv) noting a uniformly pale-greenish (or pinkish), non-shiny body color with the absence of any darker green lines on their abdomen or darker patches near the siphunculi.

Identification of this pest is complicated by the fact that *M. persicae* is most likely made up of a complex of sub-species, each of which is adapted to a limited host range. An example of this is *M. persicae nicotianae* which feeds almost exclusively on tobacco (Nikolakakis et al. 2003). It is entirely possible that some of these sub-species may evolve into species in the near future, as in the case of *Myzus antirrhinii* on snapdragon (Blackman and Paterson 1986), which, although it is almost morphometrically identical to *M. persicae* (Blackman and Paterson 1986), has been given new species status on the basis of some consistent differences detected by multivariate morphometric analysis and molecular differences such as allozyme and rDNA characteristics (French-Constant et al., 1988; Fenton et al., 1998).

Biology

Host Plants:

Myzus persicae is thought to be of Asian or possibly European origin (Blackman and Eastop 2007), but today is cosmopolitan (Vorburger 2006) and has a documented host

range of over 400 plant species in more than 50 families (Weber 1985). Indeed, *M. persicae* is considered the most polyphagous aphid in the world (Blackman and Eastop 1984), and is a major pest of many agricultural and greenhouse crops, including potato (van Toor et al. 2008), tobacco (Nikolakakis et al. 2003), peppers (Perdikis and Lykouressis 2004), chrysanthemum (Guldemond et al. 1998) and many other crops. Interestingly, several studies have demonstrated high genetic variation between *M. persicae* lines in terms of host plant adaptation (Weber 1985, 1986; Edwards 2001; Vorburger et al. 2003 and others). For example, Weber (1985) moved more than 1000 *M. persicae* clones onto various plant species. Even after 10 generations of habituation to the new plant, he saw higher performance of clones when they were placed back onto their original host plant, suggesting that host plant adaptation is a genetically fixed trait (Weber 1985). In a review by Blackman (1990), over half of the published studies on aphid-plant interactions indicated the presence of host-adapted genotypes (Blackman 1990). Thus, polyphagy in *M. persicae* may be an attribute of the species as a whole, but is not necessarily an attribute of all clones (Weber 1985).

Reproduction and Morphs:

Myzus persicae, like other aphids, exhibits what is known as telescoping generations. Specifically, females are parthenogenic and give rise to viviparous daughters that already have developing embryos inside them (von Burg et al. 2008). Their short generation time and overlapping generations resulting from vivipary dramatically increases their reproductive potential during the parthenogenic phase (Blackman and Eastop 1984; Capinera 2005).

Myzus persicae has a heteroecious (*i.e.* requiring more than one plant host) and holocyclic (*i.e.* having an annual sexual phase which interrupts parthenogensis) life cycle in areas with cold winters (see van Emden et al. 1969 for a detailed description of the life cycle). During the winter, when suitable secondary hosts cannot persist, holocyclic populations overwinter on peach (*Prunus persicae*) and other *Prunus* spp. (*e.g. Prunus nigra* in the north-eastern USA) in the egg stage (Vorburger 2006). In the spring, the eggs hatch and, after several generations, winged dispersants deposit nymphs on the summer (secondary) hosts. The fundatrix is the term given to the first parthenogenic female produced from the fertilized egg, and each gives birth to a line of clones (all viviparous parthenogenic females, but they can be alate or apterous) which ends when sexuals appear again in the fall due to environmental stimuli (*e.g.* shorter days) (Miyazaki 1987). The presence of the sexually reproductive mode of *M. persicae* is influenced by the availability of the primary host, as well as the severity of the winter in the area; *e.g.* in more temperate regions, the species remains parthenogenic on the secondary host (Miyazaki 1987), and this is true within the sheltered environment of greenhouses as well.

As mentioned previously, two color morphs can be produced within *M. persicae*: green and red. Color morph has been shown to be genetically controlled by a pair of alleles, with red being dominant. Although the biological function of the change in color itself is not known, it appears that certain aphid clones displaying the red phenotype show differences in characteristics such as reproductive rate, host preference, and behavior (Miyazaki 1987), and this may be important for biological

control in terms of insecticide resistance and susceptibility to parasitoids (Gillespie et al. 2009).

Damage Caused by M. persicae

The damage caused by this aphid is due to i) direct damage from feeding, resulting in wilting, reduced growth rate, distortion of plant tissue, and significant reductions in yield, as well as ii) indirect damage from exuviae and honeydew production (*e.g.* growth of sooty molds). However, in many agricultural crops, it is the ability of *M. persicae* to transmit more than 100 plant viruses that makes it especially damaging (Devi and Singh 2007). Indeed, *M. persciae* is considered the most important vector of plant viruses in the world (Kennedy et al. 1962; Capinera 2005). Agriculturally important viruses transmitted by this aphid include potato leafroll virus, beet yellows virus, lettuce mosaic virus, and cucumber mosaic virus (Kennedy et al. 1962).

The Role of M. persicae in the Aphid Pest Complex

Myzus persicae tends to be less noticeable to growers than other pest aphid species (such as *A. gossypii*) due to its pale green color and its tendency to be less aggregated within the plant canopy (Vehrs et al. 1992). It also tends to be highly mobile, and its populations can quickly disperse throughout the greenhouse (*e.g. M. persicae* can move an average of 131 cm/day in potted chrysanthemum) (Heinz 1998). Due to its high dispersal rate relative to other aphid species, it may initially occur at a lower density per plant. This can give *M. persicae* the opportunity to increase in number and spread throughout the crop before it is noticed by pest managers (Heinz 1998).

Furthermore, founding populations of *M. persicae* have faster growth rates than established populations (Heinz 1998), leading to what can seem like sudden outbreaks. Complicating the control of this pest in greenhouses is the fact that it is considered the most insecticide resistant insect in the world (Vasquez 1995), being resistant to 71 different insecticides across the majority of insecticide classes, including organophosphates, carbamates and pyrethroids (Devonshire et al. 1998), as well as low level resistance to neonicitinoids (van Toor et al. 2008).

AULACORTHUM SOLANI (FOXGLOVE APHID)

Identification

Aulacorthum solani has had a confusing taxonomic history, being placed in several different genera, and with several populations named as distinct species or subspecies (Hille Ris Lambers 1949; Mueller 1976). Originally described by Kaltenbach from potato as *Aphis solani* (Kaltenbach 1843), it has also been periodically included in the genus *Myzus* (*e.g.* Mason 1940), *Macrosiphum* (*e.g*. Bartholomew 1932), and *Acyrthosiphon* (*e.g.* Russell 1963), mostly by North American taxonomists, but was recognized as belonging to the genus *Aulacorthum* by European scientists (Wave et al. 1965). Much of the confusion in taxonomy is likely a result of the large morphometric variation this species can display due to differing climatic and biological conditions. Damsteegt and Voegtlin (1990) showed the body lengths of specimens within the same population of *A. solani* are significantly different when reared on different host plants, and that *A. solani* from soybean can be distinguished using the length of the cauda. Damsteegt and Voegtlin (1990) suggest that these

morphometric differences are evidence for recognizable biotypes, or possibly even subspecies. Current genetic analysis indicates that *A. solani* is a single species (Miller et al. 2009), but biotypes likely exist.

Identifying characteristics of *A. solani* are as follows: apterous adult females vary in color from pale green to yellow (there may be 2 distinct color morphs; Damsteegt and Voegtlin 1990), with a body length of 1.8-3.0 mm, making them larger than *M. persicae*. They have 6-segmented antennae, with dark apices. The antennal tubercles are well developed and their inner faces are parallel (vs. *M. persicae*, which has convergent inner faces). The siphunculi are pale with dark tips, gradually tapering but with a distinct large apical flange and 2 rows of reticulations. Typically, dark green blotches can be seen near the base of the siphunculi. The cauda is pale and elongate. Alate females are yellow-green with a brown head, and a dark thorax and abdomen with pale to dark transverse bands. The body shape is similar to wingless females, but is 2.0-3.0 mm long (Miller and Stoetzel 1997). In practice, the foxglove aphid is easily distinguished from other aphid species due its i) larger body size than green peach or melon aphid, ii) dark joints of the antennae and legs, iii) parallel antennal tubercles, iv) the usual presence of darker green patches at the base of the siphunculi (Gill and Sanderson 1998), and v) overall "shiny" appearance (vs. the more matte appearance of *M. persicae*) (Murphy and Shipp, 2006). Behaviorally, *A. solani* is also known to engage in defensive dropping behavior (Gillespie and Acheampong 2012), which also distinguishes it from other floriculture pest aphids.

Biology

Host Plants:

Native to Europe (Blackman and Eastop 1984), *A. solani* is now cosmopolitan. Known as one of the most agriculturally important pests affecting potatoes since the 1960's (Wave et al. 1965), in recent years it has gone from an occasional pest to a major pest of many agricultural and greenhouse crops, including pepper (Down et al. 1996; Sanchez et al. 2007) and lettuce (Palumbo 2003; Lee et al. 2008a). It is also an important pest of soybean in Japan and Korea, where it has been known to defoliate soybean plants as well as significantly lower yield and quality (Kim et al. 1991; Takada et al. 2006). It is not known from this crop in North America.

Although originally described from potato (*Solanum tuberosum*) (Blackman and Eastop 1984), *Digitalis purpurea* L. (common foxglove) and *Hieracium* spp. (common perennial hawkweed) are the important primary hosts for *A. solani* in North America (Patch 1928; Wave et al. 1965). However, *A. solani* can overwinter and produce sexuals on several other plant species (Hille Ris Lambers 1947; Hille Ris Lambers 1949), and thus has a wide variety of primary hosts throughout its distribution (Blackman and Eastop 1984). Holocyclic *A. solani* also differ from *M. persicae* in that they migrate between herbaceous plants within the same habitat, rather than between woody and herbaceous plants (Blackman and Eastop 2000). *Aulacorthum solani* uses a wide variety of plants as secondary hosts, and is a reported pest on 95 different plant species from 25 families (Kim et al. 1991), including mono- and dicotyledonous, herbaceous and woody plants (Blackman and Eastop 1984; Blackman and Eastop 1994; Blackman and Eastop 2006). However, the actual number of plant hosts may be much higher than reported. In greenhouse crops, for

example, this aphid is known to infest nearly every plant that is attacked by *M. persicae* or melon aphid (*A. gossypii*) (Gill and Sanderson 1998). *Aulacorthum solani* has been reported from important ornamentals such as carnations, lilies, gladiolas, tulips, orchids (Blackman and Eastop 1984), carnation, dahlia, geranium, gloxinia and nasturtium (Gill and Sanderson 1998).

Reproduction:

Reproductive cycles of foxglove aphid are similar to other aphid species. Populations of this species can be holocyclic or entirely anholocyclic (Mueller 1970). Considering the males and gynoparae of this species are often apterous and that this aphid can live on the primary host all year long, foxglove aphid is considered to have a lower migratory ability compared to *M. persicae* (Hille Ris Lambers 1949; Ishitani et al. 1971).

Damage Caused by A. solani

Along with the usual suite of problems caused by aphids as described above, *A. solani* also secretes salivary toxins that can cause vein yellowing in leaves, severe twisting and curling of plant tissue, as well as localized tissue necroses (Miles 1990, Sanchez et al. 2007). Tolerance for this aphid in ornamental crops may be lower compared to other aphids due to its tissue-distorting feeding damage. Like other aphids, they are also able to transmit various plant viruses. Currently, 45 different plant viruses are known to be transmitted by this aphid (Miller and Stoetzel 1997), including leaf roll

and mosaic viruses (Wave et al. 1965) and tomato aspermy virus, which can affect chrysanthemums (Govier 1957).

Role of A. solani in the Aphid Pest Complex

Greenhouse floriculture growers are finding *A. solani* to be an increasing problem. A 2006 survey of floriculture greenhouses in MA and NY found *A. solani* to be the second most common aphid species infesting floriculture crops, surpassing both *A. gossypii* and *M. euphorbiae* and second only to *M. persicae* (van Driesche et al. 2008). It has been suggested that the change in pest status of *A. solani* may be due to recent widespread reduction of pesticide sprays for other pests due to increasing adoption of IPM practices in various agricultural and greenhouse crops (Sanchez et al. 2007), although this is unlikely to be the case in greenhouse floriculture crops in the United States, where insecticides are still heavily used.

Previous reports in the literature sugges *A. solani* feeds on the lower leaves of plants (Wave et al. 1965; Robert 1979; Down et al. 1996; Verider 1999). Further, *A. solani* are often anecdotally reported to be predominately "stem feeding" aphids (vs. leaves). However, these reports are generally unverified experimentally (Verider 1999), conducted on only one species of plant (*i.e.* potato: Robert 1979, Down 1996), or in relation to weeds or field crops (Wave et al. 1965; Robert 1979; Down 1996), which are grown for much longer periods of time than ornamentals. In contrast, *M. persicae*, is known to generally feed on new growth of ornamental plants (Vehrs et al 1992; Bethke 2010). Additionally, adult *A. solani* tend to be found along the midribs of leaves and along the primary veins, whereas *M. persicae* tend to be found on the

secondary veins and the lamina (Lowe 1967; Gibson 1972). This is thought to be because foxglove aphids have longer stylets than *M. persicae* (avg. 0.55 mm vs. 0.41 mm, respectively) (Gibson 1972).

CHEMICAL CONTROL OF APHID PESTS IN GREENHOUSE CROPS

Common insecticides in the US for aphid control currently include several neonicitinoids such as Marathon ® (imidacloprid), Tristar® (acetamiprid), and Aria® (flonicamid), as well as abamectins such as Avid®, azadirachtins such as Aza-direct® and pyriproxifen (Distance®), a juvenile hormone analouge. Endeavor® (pymetrozine) works especially well for aphids, has a novel (yet not well understood) mode of action that prevents aphids from inserting their stylets and leads to starvation (Harrewijn and Kayser 1997). Horticultural oils and insecticidal soaps are also registered for use against aphids. In general, systemic/translaminar insecticides are more effective for aphids than contact insecticides, due to their phloem-feeding. Aphids are easiest to kill when infesting the upper canopy, whereas those in the lower canopy can be missed by contact insecticides and act as a reservoir for infestation. Systemic insecticides are most effective against aphids feeding on new growth.

Chemical control of *M. persicae* has become notoriously difficult in the past decade, as this species has become strongly resistant to several chemical classes of insecticide (organophosphates, pyrethroids and carbamates, with some tolerance to neonicitinoids) (Foster et al. 2000). The ability of *M. persicae* to resist this variety of insecticides is due to several resistance mechanisms. These include overproduction of two related carboxylesterases, which sequester and degrade organophosphates, a

modified acetylcholinesterase target site (MACE), which provides an insensitive binding site for dimethyl carbamates, and a knock-down resistance (kdr) mechanism based on changes in voltage-gated sodium channel proteins, which affects pyrethroids and DDT, with a lesser effect on neonicitinoids (Foster et al. 2000). But resistant *M. persicae* have shown signs of reduced fitness, including reduced reproductive success, reduced overwintering ability, and maladaptive behaviors, including a decreased response to alarm pheromone and a slower rate of movement off of senescing leaves (Foster et al. 2000). As a result of these fitness costs, there seems to be a fluctuating polymorphism of susceptible and resistant strains existing in many populations (Foster et al. 2000). Thus, if greenhouse growers can curb their chemical control of aphids with alternate tools (see Biological Control below), or eliminate their use in normal production all together, then these pesticides will be effective when serious outbreaks of *M. persicae* occur.

Foxglove aphid does not have the common resistance to insecticides that *M. persicae* does. Using gel electrophoresis, the carboxylesterase enzymes that confer organophosphate resistance to *M.persicae* were not present in *A. solani* (Pozarowska 1987). In a study by Takada et al. (2006), only 1 out of the 8 clones tested was found to be weakly resistant to acephate (an organophosphate) and all 8 clones were susceptible to fenvalerate (a pyrethroid). This corroborates work done by Ueno et al. (2002) who demonstrated that *A. solani* was highly susceptible to 5 organophosphorous and 3 pyrethroid insecticides. Although the previous 2 studies were done in Japan, there are no references to date showing insecticide resistance of this species to common agricultural insecticides from North America.

Given the plant response to the saliva of *A. solani,* which can include necrosis of leaf tissue, pesticides are usually applied as soon as *A. solani* is detected (Sanchez et al. 2007), but insecticides are often not compatible with natural enemies used in biocontrol programs for aphids or other pests. Thus, despite the good control that can be achieved with pesticides, alternative control strategies for *A. solani* control need to be developed and implemented in order to keep IPM programs in the greenhouse intact.

COMMERCIALLY AVAILABLE BIOCONTROL AGENTS FOR APHIDS

Aphidius Parasitoids

Aphidius colemani (Hymenoptera: Aphidiidae) were originally of Indian or Pakistani origin, but are now found pan-tropically (Messing and Rabasse 1995). They are small (4-5 mm long) parasitoid of aphids (Helyer et al. 2003). This parasitoid has become a widely-used aphid biocontrol agent in European and North American floriculture production. The optimal temperature for development for *A. colemani* is ca. 25-27 °C, which makes them an ideal candidate for biological control of aphid pests in the spring and summer months (Zamani et al. 2007), though their efficacy is reduced at temperatures above 30 °C (Helyer et al. 2003)

Aphidius colemani has dozens of reported hosts (Messing and Rabasse 1995) but is sold commercially for control of smaller-sized greenhouse aphid pest species only (specifically, *M. persicae* and *A. gossypii*). Research suggests that this parasitoid prefers *A. gossypii* over *M. persicae* when given a choice. This is true even if reared on *M. persicae* for several generations, indicating a possible innate genetic preference

for *A. gossypii* (Messing and Rabasse 1995). The preference of *A. colemani* for smaller aphid species means that larger sized species such as *A. solani* and *M. euphorbiae* are left uncontrolled if this biocontrol agent is used on its own (J. Sanderson, personal communication). Narrow host preference is also reported in another commercially available Aphidiine wasp, *A. matricariae*, which prefers *M. persicae* as a host, and is reportedly not an effective biocontrol agent for *A. gossypii*, *M. euphorbiae*, or presumably, *A. solani* (Mahr, 2001). Furthermore a study by Henry et al. (2010) demonstrated that *A. matricariae* can trigger defensive dropping of *A. solani*, resulting in the spread of this pest in the crop.

Aphidius ervi, originally introduced into North America from Europe and originally for the control of pea aphid (Mackauer and Campbell 1972), may be difficult for the lay person to tell apart from *A. colemani A. ervi* is used for the biological control of larger-sized aphid species such as *A. solani*, but are not considered effective parasitoids for *M. persicae* or other smaller-sized aphids. A study by Henry et al. (2005) showed that although *A. ervi* develops best in 2nd instar foxglove aphids, they prefer to parasitize 4th instars. It is recommended that *A. ervi* be released before aphid populations build up, with weekly introductions of ca. 1.5 adults/ft^2. Higher rates of release (5.5 adults/ft^2) should be used if aphid populations are high (Mahr, 2011).

Despite the availability of 3 different parasitoid species that could attack all aphid pests found in a floriculture greenhouse, parasitoids alone may not be a realistic approach. The use of an *Aphidius* sp. requires that growers are able to correctly identify the aphid species present in their crop. This is complicated by the fact that

simultaneous outbreaks of multiple aphid species can be common in greenhouses, either within the same crop or within different crops in the same compartment. Moreover, continuous releases of multiple *Aphidius* spp. would prove too costly for most operations, especially considering that *A. ervi* is currently 4x the cost of *A. colemani*, making it's regular use cost-prohibitive to many growers. The use of banker plants in this situation (a plant offering a non-pestiferous aphid as a host to sustain/increase parasitoid populations at times of low pest-aphid infestations), though potentially reducing the cost of multiple releases, may inadvertently offer a false sense of security to the grower. Anecdotal reports suggest that *A. ervi* can take over banker plant systems intended for open-rearing of *A. colemani*. This would likely go unnoticed by the grower and outbreaks of smaller-sized aphid species could follow. Thus, in terms of ease of use, host range and cost, more general aphid biocontrol agents may be a preferable approach.

Aphidoletes aphidimyza

Aphidoletes aphidimyza (Rondani) (Diptera: Cecidoymiidae) is a predatory midge that is distributed throughout the Northern hemisphere (Hagen et al. 1999). The larvae are generalist aphid predators (Harris 1973), while the adults consume honeydew for energy (Kuo-Sell 1987). A detailed description of *A. aphidimyza* biology is given in Markkula et al. (1979) and Harris (1973). Adults are long-legged flies, approximately 2-3mm in length. Males have very plumose antennae, which distinguishes them from females (Markkula et al. 1979). Adults are reported to be crepuscular/nocturnal, live for an average of 1 week (Uygun 1971; Madahi et al. 2013) and canproduce more than

100 eggs (Uygun 1971). These are laid singly or in small clusters (Hagen et al. 1999). Eggs laid by a single female are either all male or all female (monogenic) (Sell 1976). Most oviposition takes place within the first 2-4 days after mating (Uygun 1971). Eggs are laid directly within aphid colonies, as the newly hatched larvae only search a small leaf area and are sensitive to hunger and low humidity (Hagen et al. 1999). An adult female is able to find a single aphid infested plant among uninfested plants (El Titi 1972/73) using honeydew as an attractant and oviposition cue (Choi et al. 2004). Females also have the ability to distinguish between fresh and old honeydew (Choi et al. 2004). Furthermore, adult females can distinguish between aphid densities, and lay more eggs with increasing aphid density (El Titi 1972/73; Stewart and Walde 1997; Choi et al. 2004). Miesner (1975) reports that they tend to deposit more eggs near adult aphids than near nymphs. Adult females also have the capacity to distinguish between host plants, according to Mansour (1975), who showed that more eggs were deposited on *M. persicae* colonies infesting the original host plant (upon with *A. aphidimyza* were reared) compared to *M. persicae* on novel host plant choices.

Eggs of *A. aphidimyza* hatch in ca. 3-4 days, and the larvae immediately begin to forage. Larvae are orange colored and are 0.3 mm (1st instar) to 3mm (3rd instar) in size (Markkula et al. 1979). There are 3 larval instars. Larvae are considered “furtive predators”, and cause little to no disruption or alarm in the aphid colonies they prey on (Lucas and Brodeur 2001). This may be partially attributable to their feeding strategy, which involves paralyzing aphid prey by injecting venom into the leg joint and subsequently sucking out the contents (Laurema et al. 1986; Harris 2004). Larvae have been observed to place empty aphid carcasses on their backs, possibly to further

protect themselves from detection (Lucas and Brodeur, 2001; S. Jandricic, personal observation), although there may be other biological explanations for this behavior. Larvae complete development in 5-13 days, depending on temperature (*e.g.* 5d at 25 ^{o}C, 7.4d at 20 ^{o}C and 13 d at 15 ^{o}C) (Kim and Kim 2004a). Larvae can consume between 5-10 large aphids or 40-80 small aphids in total, but kill more aphids than they consume (Uygun 1971). A single larvae need only consume 5 large aphids to complete its life cycle (Uygun 1971; Harris 2004). *Aphidoletes aphidimyza* generally pupate in the soil (though some may pupate on leaves), with adults emerging after ca. 1-3 weeks (Hagen et al. 1999). The entire life cycle takes ca. 3-4 weeks to complete, depending on temperature. The intrinsic rate of increase of *A. aphidimyza* ranges from 0.11-0.17 when reared on the melon aphid, *Aphis gossypii*, and increases with increasing prey density (Madahi et al. 2013).

Aphidoletes aphidimyza has been used in biological control in greenhouse crops since 1973 (Asyakin 1973). They are commercially available from biological control companies in the pupal stage. Reported effective release rates for this natural enemy vary from a ratio of 1 predator: 200 aphids for *Aphis gossypii* in the USSR, to 1:10 for *M. persicae* on peppers (Gilkeson and Hill 1987), to as high as 1:3 for *M. persicae* on peppers and tomatoes at 14 day intervals (Meadow et al. 1985). The use of *A. aphidimyza* can be incredibly successful at controlling aphid populations. For example, in sweet pepper, Markkula and Tittanen (1982) found that the addition of a single application of *A. aphidimyza* cocoons (ratio = 1:3) provided better control of *M. persicae* than 6 applications of the pesticide Mevinphos (an organophosphate). According to Hansen (1987) *A. aphidimyza* works best for controlling *M. persicae* in

greenhouse pepper crops if it is established before aphid infestation, by means of banker plants (Hansen 1987). The use of *A. aphidimyza* has also been attempted previously for control of *A. solani* in lettuce greenhouses. However, this natural enemy is usually not effective at the climatic conditions common for greenhouse lettuce (*i.e.* 5-15 ^{o}C) (Quentin et al. 1995), and is generally not recommended for use below 20 ^{o}C (Lee et al. 2008b) due to the much slower development rate of *A. aphidimyza* vs. aphids below this threshold (Alotaibi 2008). This being said, *A. aphidimyza* was found to provide sufficient control of *M. persicae* on sweet peppers under winter greenhouse conditions (at a release rate of 1:10, a nightly min. temp of 15 ^{o}C and a daily max. temp of 21 ^{o}C; Gilkeson and Hill 1987). Furthermore, *A. aphidimyza* are considered the only aphid predator that can maintain populations in the greenhouse throughout the season (Ramakers 1988), as long as supplemental light is provided to prevent diapauses. Greater than 16 h day length is needed for this, but even low intensity light from a 60 Watt bulb every 10m throughout the greenhouse will suffice (Gilkeson and Hill 1986).

As with most biocontrol strategies for aphids, the use of *A. aphidimyza* for aphid control theoretically has the best chance of working if they are introduced prophylactically, or at least at low aphid levels, rather than as a curative approach. This was seen by Bennison (1992), who observed insufficient control of *A. gossypii* with curative releases of *A. aphidimyza* and *Aphidius matricariae* in cucumber, but was able to keep aphids at acceptable levels when natural enemies were introduced concurrently with planting (via banker plants; Bennison 1992).

Other Commercially Available Arthropods for Aphid Biocontrol

Although many ladybird beetles (Coleoptera: Coccinellidae) are important aphid predators in nature, only a few species are commercially available. These include *Adalia bipunctata* in Europe and *Hippodamia convergens* in North America. The fourth instar larvae of *H. convergens* are capable of consuming ca. 50 aphids/day and adults consume an average of 22 aphids/day (Balduf 1935). Typically, *H. convergens* are field collected for commercial sale from the Sierra Nevada mountains in California from overwintering aggregation sites. Because of this, these beetles have a strong dispersal instinct when they emerge from overwintering, usually migrating before feeding or laying eggs, and thus can be a waste of money for consumers (Weeden et al. 2010).

Chrysoperla (Chrysopa) rufilabris and *C. carnea* (Neuroptera: Chrysopidae) are commercially available green lacewings that occur naturally in much of North America. Sometimes called “aphid lions”, the larvae are capable of eating between 100-600 aphids over their life span; most prey are consumed during the 3rd instar (ca. 50 aphids/day). They are sometimes recommended for release into aphid “hot spots” (Biobest 2011a). Adult lacewings need nectar and/or pollen as food for egg laying, and therefore have better survival when released into flowering crops. However, it is recommended that they be released as larvae or eggs, as newly emerging adults will likely disperse before laying eggs (Weeden et al. 2010). A benefit to using lacewings is that they are active across a range of temperatures (12-35 ° C) (Biobest 2011a). Generally, though, lacewings are not widely used in greenhouse floriculture IPM (S. Jandricic, personal observation), likely due to a combination of cannabilistic behavior

and lack of successful pupation in the greenhouse environment (J. Sanderson, personal communication).

Aphelinus abdominalis (Hymenoptera: Aphelinidae) is a parasitic wasp used for larger aphid species, such as *M. euphorbiae* as well as *A. solani*. The adult is 3mm long, with a black thorax and a yellow abdomen. They rarely fly, and instead walk on the leaf to find aphid hosts. They can parasitize any aphid stage, including alates. Positive attributes of *A. abdominalis* include i) a long oviposition period (ca. 8 weeks, with 5-10 aphids parasitized/day), ii) host feeding on non-parasitized aphids, and iii) a lower tendency to induce defensive dropping behavior in *A. solani* (Henry et al. 2010). However, they are considered much slower-acting than *Aphidius* spp. (Biobest 2011b), and have a long life cycle relative to aphids and are therefore not ideal as the primary biocontrol agent for aphids in floriculture crops.

Entomopathogens

Entomopathogens represent another possible "generalist" aphid biological control measure for use in greenhouses. According to Volkl et al. (2007), the most significant entomopathogens of aphids are in the "true" fungi. Most fungi that have been isolated from aphid populations are in the order Entomophthorales (*e.g. Pandora* spp.). Although these are known to cause epizootics that can practically eliminate aphid populations locally, they are difficult to culture *in vitro*, and therefore are not produced commercially. Instead, most commercial mycoinsecticides are formulations of fungi within the order Hypocreales (Ascomycetes: Sardiomycetes) (*e.g. Beauveria, Metarhizium*, *Isaria*, and *Lecanicillium* species) due to their general ease of culture.

Of these, only *Lecanicillium (Verticillium) longisporum (lecanii)* (Hypocreales: Cordycipitaceae) is known to cause occasional natural epizootics in aphids in nature. As a formulated product, it is effective against aphids (including *M. persicae* and *A. solani*; Kim et al. 2007), but, unfortunately, is only registered for commercial use in Europe (as Vertalec®). *Beauveria bassiana* (Hypocreales: Cordycipitaceae) does not commonly infect aphids in the field (Volkl et al. 2007), but due to its ease of culturing, it has been formulated into two commercial products available in North America for aphid control: BotaniGard® (strain GHA) and Naturalis® (strain JW-1). Although *B. bassiana* can be highly effective for other phloem feeding hemipterans in greenhouses (*e.g.* whiteflies: Hemiptera: Alyerodidae), it is not considered particularly effective against aphids in practice (S. Wraight, personal communication). The same is true for the other mycoinsectides registered in the U.S. for phloem-feeding insects (including *Metarhizium brunneum* F52 as Met52® and *Isaria javanica* Apopka strain 97 and strain FE 9901 as PFR-97™ and NoFly™, respectively). Moreover, few large-scale trials investigating the efficacy of this and other entomopathogenic fungi against aphids in greenhouse floriculture crops exist (but see Hall and Burges 1979; Olson and Oetting 1999). Indeed, achieving acceptable control in ornamental crops with a mycoinsecticide alone may be questionable, given that susceptibility of aphids to funal infection is decreased the closer the application time is to a nymphal molt (Yin-Quan et al. 2003). And, the low humidity levels often found in greenhouses (e.g. as low as 10% in the winter months, S. Jandricic. personal observation) are not conducive to germination of fungi (Shipp et al. 2003). Although it is possible to artificially increase % RH with misters, growers may be hesitant to do this, even for short periods, due to

the common problem of plant pathogens such as downy mildews and *Botryitis* (M. Daughtrey, personal communication).

Despite these drawbacks, research on entomopathogenic fungi for aphid control should not yet be abandoned. Given the extensive culture collections of entomopathogenic fungi that have been amassed, it is probable that strains more efficacious against aphids than the current commercial products have been collected, but have not yet been tested. Entomopathogenic fungi are generally composed of diverse assemblages of genotypes, and an isolate collected from a target host should theoretically be more virulent than a one isolated from a non-related species (Inglis et al. 2001). As currently registered fungal products generally come from non-hemipteran sources (see Chapter 3 for more detail), the possibility remains of finding a highly efficacious novel fungal isolate for aphids. Moreover, mycoinsecticides have been demonstrated to have good compatibiltiy with natural enemies such as *Aphidius* spp. when timing of sprays are considered (e.g. spraying when the wasps are in the mummy stage, the least susceptible life stage; Rashki et al. 2009). Therefore, entomopathogenic fungi are considered by many to have the potential to be a useful tool within an IPM program (Shipp et al. 2003).

RESEARCH GOALS

Despite the plethora of commercially available natural enemies, control of aphids using biological control alone is still difficult to achieve in ornamental greenhouse crops. A serious challenge is the recent emergence of *A. solani* as a primary aphid pest. Until recently, biological control research has focused predominately on *M.*

persicae and *A. gossypii*, previously considered the top two aphids of concern in ornamentals. Though understandable, this focus has resulted in a paucity of information about effective controls for *A. solani*. Even basic biology and behavior is not well characterized for *A. solani*, information that can aid in the development of a biocontrol program for this pest.

Success of aphid biocontrol in greenhouse crops is also limited by the general focus of researchers on single-species pest outbreaks, despite the simultaneous occurrences of multiple aphid species. Coinciding with this is a general lack of information on the behavior and efficacy of commercially-available natural enemies under multi-prey conditions.

To address these issues, research was undertaken to 1) determine the development rate, life table statistics, and within-plant dispersal behavior of *A. solani*, information previously lacking on ornamental crops in North America; 2) investigate the effect of aphid species on oviposition choices of *A. aphidimyza*, the most promising of the generalist aphid predators, when presented with two of the most important aphid pests of greenhouse crops (*M. persicae* and *A. solani*); 3) determine the efficacy of *A. aphidimyza* for controlling multiple aphid species in longer term greenhouse experiments with the goal of developing a biocontrol program using only a single natural enemy; and 5) investigate the potential effectiveness of a mycoinsecticide an additional tool in an IPM program for aphids.

REFERENCES

Alotaibi, S. 2008. Mass production and utilization of the predatory midge, *Aphidoletes aphidimyza* Rondani for controlling aphids. Global J. Biotechnol. Biochem. 3: 01-07.

Asyakin, B. P. 1973. The use of aphidophagous gall-midge *Aphidoletes aphidimyza* (Rondani) (Dipt., Cecidomyiidae) in biological control of aphids in glasshouses. Zapiski LSHI: 212.

Balduf, W. V. 1935. The bionomics of entomophagous Coleoptera. John S. Swift Co., St. Louis, MO.

Bartholomew, P. S. 1932. Six new species of aphids, with records of other species new to California. Ann. Emer. Ent. Soc. 25: 713-729.

Bennison, J. A. 1992. Biological control of aphids on cucumbers: use of open rearing systems or "banker plants" to aid establishment of *Aphidius matricareae* and *Aphidoletes aphidimyza*. Med. Fac. Landbouww. Univ. Gent. 57: 457-466.

Bethke, J.A. 2010. Insects and mites. UC IPM Pest Management Guidelines: Floriculture and Ornamental Nurseries: UC ANR Publication 3392.

Biobest. 2011a. Biological control: Beneficial insects and mites: *Chrysopa* MC-500-System. Accessed February 2011, from http://www.biobest.be/producten/118/3/0/0/.

Biobest. 2011b. Biological control: Beneficial insects and mites: *Aphelinus* System. Accessed February 2011 from, http://www.biobest.be/v1/en/producten/nuttig/aphelinus.htm.

Blackman, R. L. 1990. Specificity in aphid/plant genetic interactions, with particular attention to the role of the alate colonizer, pp. 251-274. In R. K. Campbell and R. D. Eikenbary (eds.), Aphid-plant genotype interactions. Elsevier, New York.

Blackman, R. L., and V. F. Eastop. 1984. Aphids on the world's crops: an identification guide. John Wiley & Sons, New York, NY.

Blackman, R. L. 1987. Morphological discrimination of a tobacco-feeding form of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (Nectarosiphon) species. Bull. Entomol. Res. 77: 713-730.

Blackman, R. L., and V. F. Eastop. 1994. Aphids on the world’s trees: an identification and information guide. CAB International, Wallingford, UK.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the world's crops: an identification guide, 2nd ed. John Wiley & Sons, Chichester, England.

Blackman, R. L., and V. F. Eastop. 2006. Aphids on the world's herbaceous plants and shrubs. Wiley Ltd., Chichester, England.

Blackman, R. L., and V. F. Eastop. 2007. Taxonomic issues, pp. 1-30. In H. F. van Emden and R. Harrington (eds.), Aphids as crop pests. CAB International, Oxfordshire UK.

Blackman, R. L., and A. J. C. Paterson. 1986. Separation of *Myzus (Nectarosiphon) antirrhinii* (Macchiati) from *Myzus persicae* (Sulzer) and related species in Europe (Hemiptera: Aphididae). System. Entomol. 11: 267-276.

Capinera, J. L. 2005. Featured Creatures: green peach aphid - *Myzus persicae* (Sulzer). www.entnemdept.ufl.edu/creatures/veg/aphid/green_peach_aphid.htm.

Choi, M. Y., B. D. Roitberg, A. Shani, D. A. Raworth, and G. H. Lee. 2004. Olfactory response by the aphidophagous gall midge, *Aphidoletes aphidimyza* to honeydew from green peach aphid, *Myzus persicae*. Entomol. Exp. Appl. 111: 37-45.

Damsteegt, V. D., and D. J. Voegtlin. 1990. Morphological and biological variation among populations of *Aulacorthum solani* (Homoptera: Aphididae), the vector of soybean dwarf virus. Ann. Entomol. Soc. Am. 83: 949-955.

Devi, P. B., and T. K. Singh. 2007. Studies on the morphometry of the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). J. Entomol. Res. 37: 81-85.

Devonshire, A. L., L. M. Field, S. P. Foster, G. D. Moores, M. S. Williamson, and R. L. Blackman. 1998. The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. Phil. Trans. R. Soc. Lond. B. 353: 1677-1684.

Down, R. E., A. M. R. Gatehouse, W. D. O. Hamilton, and J. A. Gatehouse. 1996. Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani)* when administered *in vitro* and via transgenic plants both in laboratory and glasshouse trials. J. Insect Physiol. 42:1035-1045.

Edwards, O. R. 2001. Interspecific and intraspecific variation in the performance of three pest aphid species on five grain legume hosts. Entomol. Exp. Appl. 100:21–30.

El Titi, A. 1972/73. Influences of prey density and morphology of the host-plant on the egg-distribution of the aphidophagous gall midege *Aphidoletes aphidimyza* (Rond.) (Diptera: Itonididae). Z. Angew. Entomol. 72: 400-415.

Fenton, B., G, Malloch and F. Germa. 1998 A study of variation in rDNA ITS regions shows that two haplotypes coexist within a single aphid genome. Genome 41, 337–345.

ffrench-Constant, R.H., F.J. Byrne, M.F. Stribley and A. L. Devonshire. (1988) Rapid identification of the recently recognized *Myzus antirrhinii* (Macchiati) (Hemiptera:Aphididae) by polyacrylamide gel electrophoresis. Entomologist 107, 20–23.

Foster, S. P., I. Denholm, and A. L. Devonshire. 2000. The ups and downs of insecticide resistance in peach-potato aphids (*Myzus persicae*) in the UK. Crop Prot. 19: 873-879.

Gibson, R. W. 1972. The distribution of aphids on potato leaves in relation to vein size. Entomol. Exp. Appl. 15: 213-233.

Gilkeson, L. A., and S. B. Hill. 1986. Diapause prevention in *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) by low intensity light. Environ. Entomol. 15: 1067-1069.

Gilkeson, L. A., and S. B. Hill. 1987. Release rates for control of green peach aphid (Homoptera: Aphidae) by the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) under winter greenhouse conditions. J. Econ. Entomol. 80: 147-150.

Gill, S., and J. Sanderson. 1998. Ball identification guide to greenhouse pests and beneficials. Ball Publishing, Batavia, Illinois.

Gillespie, D. R., D. M. J. Quiring, R. G. Foottit, S. P. Foster, and S. Acheampong. 2009. Implications of phenotypic variation of *Myzus persicae* (Hemiptera: Aphididae) for biological control on greenhouse pepper plants. J. Appl. Entomol. 133: 505-511.

Govier, D. A. 1957. The properties of tomoato aspermy virus and its relationship with cucumber mosaic virus. Ann. Appl. Biol. 45: 62-73.

Guldemond, J. A., W. J. van den Brink, and E. den Belder. 1998. Methods of assessing population increase in aphids and the effect of growth stage of the host plant on population growth rates. Entomol. Exp. Appl. 86: 163-173.

Hagen, K. S., N. J. Mills, G. Gordh, and J. A. McMurtry. 1999. Terrestrial arthropod predators of insect and mite pests, pp. 1046. In T. S. Bellows and T. W. Fisher (eds.), Handbook of Biological Control: Principles and Applications of Biological Control. Academic Press, San Diego.

Hall, R. A., and H. D. Burgess. 1979. Control of aphids in glasshouses with the fungus, *Verticillium lecanii*. Ann. Appl. Biol. 93: 235-246.

Hansen, L. S. 1987. Biological control in Danish glasshouses - Extent of use, application and success, pp. 45-50. In R. Cavalloro (ed.), Integrated and biological control in protected crops. Proceedings of a meeting of the EC experts' group / Heraklion 24-26 April 1985. A.A.Balkema, Netherlands.

Harrewijn, P. and H. Kayser. 1997. Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. In-situ studies with electronic monitoring of feeding behaviour. Pestic. Sci. 49: 130 -140.

Harrington, R., and L. R. Taylor. 1990. Migration for survival: fine-scale population redistribution in an aphid, *Myzus persicae*. J. Anim. Ecol. 59: 1177-1193.

Harris, K. M. 1973. Aphidophagous Cecidomyiidae (Diptera): taxonomy, biology and assessments of field populations. Bull. Entomol. Res. 63: 305-325.

Harris, K. M. 2004. Specialist and generalist cecidomyiid predators on aphids, mites, scale insects and other invertebrates. Entomologica 38: 29-40.

Heinz, K. M. 1998. Dispersal and dispersion of aphids (Homoptera: Aphididae) and selected natural enemies in spatially subdivided greenhouse experiments. Biol. Control 27: 1029-1038.

Helyer, N., K. Brown, and N. D. Cattalin. 2003. A color handbook of biological control in plant protection. Timber Press, Inc, Portland, Oregon.

Henry, L. M., J.A. Bannerman, D. R. Gillespie, and B.D. Roitberg. 2010. Predator identity and the nature and strength of food web interactions. J. Anim. Ecol. 79: 1164-1171.

Henry, L. M., D. R. Gillespie, and B. D. Roitberg. 2005. Does mother really know best? Oviposition preference reduces reproductive performance in the generalist parasitoid *Aphidius ervi*. Entomol. Exp. Appl. 116: 167-174.

Hille Ris Lambers, D. 1947. Contributions to a monograph of the Aphididae of Europe III. Temminckia 7: 179-319.

Hille Ris Lambers, D. 1949. Contributions to a monograph of the Aphididae of Europe IV. Temminckia 8: 182-323.

Inglis, G.D., M.S. Goettel, T.M. Butt, and H. Strasser. 2001. Use of hypomycete fungi for managing insect pests. In T. M. Butt, C.W. Jackson, and N. Magan (eds.), *Fungi as biocontrol agents. Progress, problems and potential*. CABI Publishing, Wallingford, UK.

Ishitani, M., K. Honda, and S. Miyai. 1971. Interclonal differences in the wing form of gynoparae of *Aulacorthum solani*. Annu. Rep. Plant Prot. Soc. North Japan 48: 223.

Kaltenbach, J. H. 1843. Monographie der familien der pflanzenlause (Phytophthires). Aachen 1843: 1-223.

Kennedy, J. S., M. F. Day, and V. F. Eastop. 1962. A conspectus of aphids as vectors of plant viruses. Commonwealth Institute of Entomology, London.

Kim, D. H., G. H. Lee, J. W. Park, and C. Y. Hwang. 1991. Occurrence aspects and ecological characteristics of foxglove aphid, *Aulacorthum solani*, Kaltenbach (Homoptera: Aphididae) in soybean. Res. Rept. RDA (C.P.) 33: 28-32.

Kim, J. J., M. S. Goettel, and D. R. Gillespie. 2007. Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. Biol. Control 40: 327-332.

Kim, T., and J. Kim. 2004a. Development and adult life span of *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) fed on the melon aphid, *Aphis gossypii* Glove or the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Korean J. Appl. Entomol. 43: 297-304.

Kuo-Sell, H. L. 1987. Some bionomics of the predacious aphid midge, *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidomyiidae), and the possibility of using the rose grain aphid, *Metopolophium dirhodum* (Wlk.), as an alternative prey in an open rearing unit in greenhouses, pp. 151-161. In R. Cavealloro (ed.), Integrated and biological control in protected crops. Proceedings of a meeting of the EC experts' group / Heraklion 24-26 April 1985. A.A.Balkema, Netherlands.

Lamboy, J. S. 2002. Level of adoption of IPM in New York greenhouses. IPM Publication # 417.

Laurema, S., G. B. Husberg, and M. Markkula. 1986. Composition and functions of the salivary gland of the larvae of the aphid midge *Aphidoletes aphidimyza*, pp.

113-118. In I. Hodek (ed.), Ecology of Aphidolophaga. Dr. W. Junk, N.V., Dordrecht, The Netherlands.

Lee, S. G., H. H. Kim, T. H. Kim, G. Park, K. H. Kim, and J. S. Kim. 2008a. Development model of the foxglove aphid, *Aulacorthum solani* (Kaltenbach) on lettuce. Korean J. Appl. Entomol. 47: 359-364.

Lee, S. G., H. H. Kim, T. H. Kim, G. Park, K. H. Kim, and J. S. Kim. 2008b. Longevity and life table of the foxglove aphid (*Aulacorthum solani* K.) adults on lettuce (*Lactuca sativa* L.). Korean J. Appl. Entomol. 47: 365-368.

Lowe, H. J. B. 1967. Interspecific differences in the biology of aphids (Homoptera: Aphididae) on leaves of *Vicia faba* II: growth and excretion. Entomol. Exp. Appl. 10: 413-420.

Lucas, E., and J. Brodeur. 2001. A fox in sheep's clothing: furtive predators benefit from the communal defense of their prey. Ecology 82: 3246-3250.

Mackauer, M., and A. Campbell. 1972. The establishment of three exotic aphid parasites (Hymemoptera:Aphidiidae) in British Columbia. J. Entomol. Soc. Brit. Col. 69: 54-58.

Madahi, K., A. Sahragard and R. Hosseini. 2013. Influence of *Aphis gossypii* Glover (Hemiptera: Aphididae) density on life table parameters of *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) under laboratory conditions. J. Crop. Prot. 2: 355-368.

Mahr, S. Aphidius wasps. 2001. Midwest Biocontrol News Vol. V, no. 2. Accessed February 2011, from http://www.entomology.wisc.edu/mbcn/kyf502.html.

Mansour, M.H. 1975. The role of plants as a factor affecting oviposition by *Aphidoletes aphidimyza* (Dipt. Cecidomyiidae). Entomol. Exp. Appl. 18: 173–179.

Markkula, M., and K. Tiittanen. 1982. Possibilities of biological and integrated control on vegetables. Acta Entomol. Fennica 40: 15-23.

Markkula, M., K. Tiittanen, M. Hamalainen, and A. Forsberg. 1979. The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. Ann. Entomol. Fennica 45: 89-98.

Marshall, S. A. 2006. Insects: Their natural history and diversity. Firefly Books Ltd., Richmond Hill, Ontario.

Mason, P. W. 1940. A revision of the North American aphids of the genus *Myzus*. U.S. Dept. Agr. Misc. Pub. 371.

Meadow, R. H., W. C. Kelly, and A. M. Shelton. 1985. Evaluation of *Aphidoletes aphidimyza* (Dip.: Cecidomyiidae) for control of *Myzus persicae* (Hom.: Aphididae) in greenhouse and field experiments in the United States. Entomophaga 30: 385-392.

Messing, R. H., and J. M. Rabasse. 1995. Oviposition behaviour of the polyphagous aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). Agr. Ecosyst. Environ. 52: 13-17.

Miesner, H. 1975. Einfluß unterschiedlicher Beuteverteilung auf den Sucherfolg von *Aphidoletes aphidimyzu* (Rond.) (Diptera, Cecidomyiidae). Disseration, Univ. Gottingen.

Miles, P. W. 1990. Aphid salivary secretions and their involvement in plant toxicoses., pp. 131-147. In R. K. Campbell and R. D. Eikenbary (eds.), In Aphid-Plant Genotype Interactions. Elsevier, Amsterdam.

Miller, G. L., C. Favret, A. Carmichael, and D. J. Voegtlin. 2009. Is there a cryptic species within *Aulacorthum solani* (Hemiptera: Aphididae)? J. Econ. Entomol. 102: 398-400.

Miller, G. L., and M. B. Stoetzel. 1997. Aphids associated with chrysanthemums in the United States. Fl. Entomol. 80: 218-239.

Miyazaki, M. 1987. Forms and morphs of aphids, pp. 27-50. In A. K. Minks and P. Harrewijn (eds.), Aphids: Their biology, natural enemies and control Volume A. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

Mueller, F. P. 1970. Zucht und übertregungsversuche mit populationen and klonen der grünfleckigen kartoffelblattlaus *Aulacorthum solani* (Kalthenbach, 1843) (Homoptera: Aphididae). Dtsch. Entomol. Z. 17: 259-270.

Mueller, F. P. 1976. Hosts and non-hosts in subspecies of *Aulacorthum solani* (Kalthenbach) and intraspecific hybridizations (Homoptera: Aphididae). *In* T. Jermy [ed.], The host-plant in relation to insect behavior and reproduction, pp. 187-190. *In* Anonymous Symp. Biol. Hung. 16, Plenum, New York.

Murphy, G., and L. Shipp. 2006. Aphids in greenhouse Crops. OMAF Factsheet No. 06/081. Accessed February 2011 from http://www.omafra.gov.on.ca/english/crops/facts/06-081.htm.

Nikolakakis, N. N., J. T. Margaritopoulos, and J. A. Tsitsipis. 2003. Performance of *Myzus persicae* (Hemiptera: Aphididae) clones on different host-plants and their host preference. Bull. Entomol. Res. 93: 235-242.

Olson, D. L., and R. D. Oetting. 1999. Compatibility of insect growth regulators and *Beauveria bassiana* (Balsamo) vuillemin in controlling green peach aphid (Homoptera: Aphididae) on greenhouse chrysanthemums. J. Entomol. Sci. 34: 286-294.

Palumbo, J. C. 2003. The emergence of the foxglove aphid, *Aulacorthum solani* as an economic pest of lettuce in the desert Southwest. In D. N. Byrne and P. Baciewicz (eds.), 2003 Vegetable Report. The University of Arizona College of Agricultural and Life Sciences.

Patch, E. M. 1928. The foxglove aphid on potato and other plants. Bull. Maine Agric. Expt. Stn. 346: 49-60.

Perkikis, D. C., and D. P. Lykouressis. 2004. *Myzus persicae* (Homoptera: Aphididae) as suitable prey for *Macrolophus pygmaeus* (Hemiptera: Miridae) population increase on pepper plants. Environ. Entomol. 33: 499-505.

Pozarowska, B. J. 1987. Studies on low temperature survival, reproduction and development in Scottish clones of *Myzus persicae* (Sulzer) and *Aulacorthum solani* (Kalthenbach) (Hemiptera: Aphididae) susceptible and resistant to organophosphates. Bull. Entomol. Res. 77: 123-134.

Quentin, U., M. Hommes, and T. H. Basedow. 1995. Studies on the biological control of aphids (Hom., Aphididae) on lettuce in greenhouses. J. Appl. Entomol. 119: 227-232.

Ramakers, P. M. J. 1988. Biological control in greenhouses, pp. 199-208. In A. K. Minks and P. Harrewijn (eds.), World Crop Pests. Aphids. Their biology, natural enemies and control, vol. 2C. Elsevier, New York.

Rashki, M., A. Kharazi-pakdel, H. Allahyari, and J. J. M. van Alphen. 2009. Interactions among the entomopathogenic fungus, *Beauveria bassiana* (Ascomycota: Hypocreales), the parasitoid, *Aphidius matricariae* (Hymenoptera: Braconidae), and its host, *Myzus persicae* (Homoptera: Aphididae). Biol. Control 50: 324-328.

Robert, Y. 1979. Recherches ecologiques sur les pucerons *Aulacorthum solani* Kltb., *Macrosiphum euphorbiae* Thomas et *Myzus persicae* Sulz. dans l'Ouest de la France. III. - Importance du parasitisme par Hymenopteres *Aphidiidae* et par Entomophthora sur pomme de terre. Ann. Zool. Ecol. anim. 11: 371-388.

Russell, L. M. 1963. Changes in the scientific names of some common aphids. U.S. Agr. Res. Serv. Coop. Econ. Insect Rpt. 13: 84.

Sanchez, J. A., F. Canovas, and A. Lacasa. 2007. Thresholds and management strategies for *Aulacorthum solani* (Hemiptera: Aphididae) in greenhouse pepper. J. Econ. Entomol. 100: 123-130.

Sell, P. 1976. Monogenie bei *Aphidoletes aphidimyza* Rond. (Diptera, Cecidomyiidae). Z. Angew. Entomol. 82: 58-61.

Shenghua, Y., F. Mingguang, X. Shoutao, and M. Zaibo. 2003. Field efficacy of emulsifiable suspensions of *Beauveria bassiana* conidia for control of *Myzus persicae* population on cabbage. Yingyong Shengtai Xuebao 14: 545-548.

Shipp, J. L., Y. Zhang, D. W. A. Hunt, and G. Ferguson. 2003. Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. Environ. Entomol. 32: 1154-1163.

Smith, T. 1998. Survey of integrated pest management for bedding plants: Part II. Floral Notes 10: 4-5.

Stewart, H. C., and S. J. Walde. 1997. The dynamics of *Aphis pomi* de Geer (Homoptera: Aphididae) and its predator, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), on apple in Nova Scotia. Can. Entomol. 129: 627-636.

Takada, H., T. Ono, H. Torikura, and T. Enokiya. 2006. Geographic variation in esterase allozymes of *Aulacorthum solani* (Homoptera: Aphididae) in Japan, in relation to its outbreaks on soybean. App. Entomol. Zool. 41: 595-605.

Ueno, K., T. Saito, R. Chuba, O. Abe, T. Sato, and S. Goto. 2002. Bionomics and control of the foxglove aphid *Aulacorthum solani* (Kaltenbach), on soybean. III. Susceptibility of the foxglove aphid to insecticides and control effects of Ethofenprox. Annu. Rep. Plant Prot. Soc. North Japan 53: 206-208.

United States Department of Agriculture. 2012. USDA Floriculture crops 2011 Summary. USDA National Agriculture Statistics Service.

United States Federal Register. 40 CFR Part 170. Date of last access: June 5/2013: www.epa.gov/oppfead1/safety/workers/PART170.htm

Uygun, N. 1971. Der einfluss der nahrungsmenge auf fruchtbarkeit und lebensdauer von *Aphidoletes aphidimyza* (Rondani 1847) (Diptera: Itonididae). Zeitschr. Angew. Ent. 69: 234-258.

van Driesche, R., S. Lyon, J. P. Sanderson, K. C. Bennett, E. J. Stanek III, and R. Zhang. 2008. Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker plants for control of aphids (Hemiptera: Aphididae) in greenhouse sping floral crops. Fla. Entomol. 94: 583-590.

van Emden, H. F., V. F. Eastop, R. D. Hughes, and M. J. Way. 1969. The ecology of *Myzus persicae*. Ann. Rev. Entomol. 14: 197-270.

van Toor, R. F., S. P. Foster, J. A. Anstead, S. Mitchinson, B. Fenton, and L. Kasprowicz. 2008. Insecticide resistance and genetic composition of *Myzus persicae* (Hemiptera: Aphididae) on field potatoes in New Zealand. Crop Prot. 27: 236-247.

Vasquez, B. L. 1995. Chapter 15: Resistant to most insecticides. In T. J. Walker (ed.), University of Florida Book of Insect Records, 2001. Accessed November 2010, from http://ufbir.ifas.ufl.edu/.

Vehrs, S. L. D., G. P. Walker, and M. P. Parrella. 1992. Comparison of population growth rate and within-plant distribution between *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae) reared on potted chrysanthemum. J. Econ. Entomol. 85: 799-807.

Volkl, W., M. Mackauer, J. Pell, and J. Brodeur. 2007. Predators, parasitoids and pathogens, pp. 187-234. In H. F. van Emden and R. Harrington (eds.), Aphids as crop pests. CAB International, Oxfordshire, UK.

von Burg, S., J. Ferrari, C. B. Muller, and C. Vorburger. 2008. Genetic variation and covariation of susceptibility to parasitoids in the aphid *Myzus persicae*: no evidence for trade-offs. Proc. R. Soc. Biol. Sci. 275: 1089-1094.

Vorburger, C. 2006. Temporal dynamics of genotypic diversity reveal strong clonal selection in the aphid *Myzus persicae*. J. Evol. Biol. 19: 97-107.

Vorburger, C., P. Sunnucks, and S. A. Ward. 2003. Explaining the coexistence of asexuals with their sexual progenitors: no evidence for general-purpose genotypes in obligate parthenogens of the peach-potato aphid, *Myzus persicae*. Ecology Letters 6: 1091-1098.

Wave, H. E., W. A. Shands, and G. W. Simpson. 1965. Biology of the foxglove aphid in the Northeastern United States. US. Dep. Agr. Tech. Bull. 1338: 1-40.

Weber, G. 1985. Genetic variability in host plant adaptation of the green peach aphid, *Myzus persicae*. Entomol. Exp. Appl. 38: 49-56.

Weeden, C. R., A. M. Shelton, and M. P. Hoffman. Biological control: a guide to natural enemies in North America. Accessed December 2010, from http://www.nysaes.cornell.edu/ent/biocontrol.

Yin-Quan, L., Z. Fa-Cheng, and L. Shu-sheng. 2003. Effects of moulting in *Myzus persicae* on the virulence of the entomopathogenic fungus *Beauveria bassiana*. Acta Entomol. Sinica 46: 441-446.

Zamani, A. A., A. Talebi, Y. Fathipour, and V. Baniameri. 2007. Effects of temperature on life history of *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Braconidae), two parasitoids of *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae). Environ. Entomol. 36: 263-271.

CHAPTER 2

DEVELOPMENTAL TIMES AND LIFE TABLE STATISTICS OF *AULACORTHUM SOLANI* (HEMIPTERA: APHIDIDAE) AT SIX CONSTANT TEMPERATURES, WITH RECOMMENDATIONS ON THE APPLICATION OF TEMPERATURE-DEPENDENT DEVELOPMENT MODELS

Jandricic, S.E.,[1,2] Wraight, S.P.,[3] Bennett, K.C.,[1] and Sanderson, J.P.[1]

ABSTRACT

Aulacorthum solani (Kaltenbach) (known as foxglove aphid or glasshouse potato aphid) is a pest of increasing economic importance in several agricultural crops worldwide, including greenhouse vegetables and ornamentals. Developmental rates and age-specific life tables for a North American population of *A. solani* on pansy (*Viola* × *wittrockiana*) (Gams.) were determined at 6 constant temperatures, and comparisons were made to previous studies of *A. solani* from differing geographic regions and host crops. On pansy, *A. solani* developed fastest at 25 °C, passing through the four nymphal instars in an average of 6.9 d. The highest intrinsic rates of population increase (0.239 and 0.248) and shortest population doubling times (2.90 and 2.80 days) were recorded at 20 and 25 °C, respectively. Average total fecundity remained high from 10–20 °C (74-68 nymphs/adult); a significant decrease to 39

[1] Department of Entomology, Comstock Hall, Cornell University, Ithaca NY, USA 14853
[2] Corresponding Author: Email: sej48@cornell.edu Fax: 607-255-0940 Tel: 607-255-9321
[3] USDA-ARS Robert W. Holly Center for Agriculture and Health, Tower Road, Cornell University, Ithaca NY, USA, 14853

nymphs/adult occurred at 25 °C. For calculating developmental thresholds, we present here a method of adjusting the lower developmental threshold (t_{min}) using estimates from non-linear models in order to provide an improved estimate of the thermal constant (K, in degree days). We also call attention to the necessity of employing a simulation method to estimate the true upper developmental threshold (T_{max}) and optimum developmental temperature (T_{opt}) from the Lactin-2 model of temperature-dependent development.

INTRODUCTION

Greenhouse floriculture growers are finding foxglove aphid (*Aulacorthum solani*) to be an increasing problem in many areas of the northeastern United States (JPS, pers. obs.; Van Driesche et al. 2008). Native to Europe (Blackman and Eastop 1984), *A. solani* is now a cosmopolitan pest. In recent years, this aphid has gone from an occasional pest to a major pest of many agricultural and greenhouse crops world-wide, including pepper (Down et al. 1996; Sanchez et al. 2007), potato (Down et al. 1996), and lettuce (Palumbo 2003; Lee et al. 2008a). It is also an important pest of soybean in Japan and Korea, but not in North America (Kim et al. 1991; Takada et al. 2006). In a 2006 survey of floriculture greenhouses conducted in Massachusetts and New York state, *A. solani* was found to be the second most common aphid species infesting floriculture crops, more common than both melon aphid (*Aphis gossypii*) and potato aphid (*Macrosiphum euphorbiae*) and second only to the green peach aphid, *Myzus persicae* (Van Driesche et al. 2008). It has been suggested that the change in pest

status of *A. solani* in some crops may be due to recent widespread reduction of pesticide sprays for other pests due to increasing adoption of IPM practices (Sanchez et al. 2007), although this is unlikely to be the case in greenhouse floriculture crops in the United States, where insecticides are still heavily used.

Although originally described from potato (*Solanum tuberosum*) (Blackman and Eastop 1984), *Digitalis purpurea* L. (common foxglove) and *Hieracium* spp. (common perennial hawkweed) are the important primary hosts for *A. solani* in North America (Patch 1928; Wave et al. 1965). The anholocyclic stage of *A. solani* uses an extremely wide variety of secondary hosts, including mono- and dicotyledonous, herbaceous and woody plants (Blackman and Eastop 1984, 1994, 2006). Some populations are entirely anholocyclic (Müller 1970). *Aulacorthum solani* is known as a pest on 95 different plant species from 25 families (Kim et al. 1991), but the actual number of plant hosts may be much higher than this. For example, to date, we have successfully maintained colonies of *A. solani* on pansy (*Viola* × *wittrockiana),* Victoria blue salvia (*Salvia farinacea),* scarlet sage *(Salvia splendens*), garden chrysanthemums (*Chrysanthemum morifolium),* potted mums (*Dendranthema* × *grandiflora*), million bells (*Calibrachoa hybrida*), pentas (*Pentas lanceolata) and poinsettia (Euphorbia pulcherrima). Our population of A. solani has successfully reproduced on every floral crop species we have provided. Aulacorthum solani has also been reported from* other important ornamentals such as carnations, lilies, gladiolas, tulips, and orchids (Blackman and Eastop 1984).

The extreme polyphagy of *A. solani* is of concern to floriculture growers, considering the damage this pest can cause. *Aulacorthum solani* is responsible for the

usual suite of problems caused by aphids, including the growth of sooty molds as a result of honeydew excretion (Miller and Stoetzel 1997), the unacceptable appearance of aphids and their cast skins in crops grown for aesthetic beauty (Heinz 1998), leaf discoloration (Okubu 2001), plant defoliation at high aphid densities (Okubu 2001; Sanchez et al. 2007), and transmission of 45 different plant viruses (Miller and Stoetzel 1997), including leaf roll viruses (Wave et al. 1965), soybean dwarf virus (Tamada 1970) (both readily transmitted), mosaic viruses (Wave et al. 1965), and tomato aspermy virus (which can affect chrysanthemums) (Govier 1957). In addition to this, *A. solani* also secrete salivary toxins that can cause leaf vein yellowing, local tissue necroses (which can result in leaf death), as well as severe twisting and curling of plant tissue (Wave et al. 1965; Miles 1990; Sanchez et al. 2007). Tolerance for this aphid in ornamental crops may be lower compared to other aphids due to its tissue-distorting feeding damage.

Recent genetic studies have provided no evidence that *A. solani* includes cryptic species (Valenzuela et al. 2007; Miller et al. 2009) despite morphological variability within the species (Müller 1976; Damsteegt and Voegtlin 1990). However, observations of damaging infestations on soybean in Asia (Kim et al. 1991; Nagano et al. 2001) but not in North America suggest that the species comprises multiple biotypes (Miller et al. 2009), a phenomenon known to occur in other aphid species (ex. *Myzus persicae, Acyrthosiphon pisum*) (Mittler and Wilhoit 1990; Peccoud et al. 2008). Other than these apparent host range differences, however, the biological variability of *A. solani* populations worldwide remains largely uncharacterized. To date, only one multi-temperature life table study has been reported for this aphid,

based on a Korean population reared on lettuce (Lee et al. 2008a, b). The objective of the present study was to estimate developmental times and life table statistics for a North American population of *A. solani* reared on a greenhouse ornamental crop and to compare these statistics to those reported by Lee et al. (2008a, b) and others. Given the importance of *A. solani* as a pest in the greenhouse vegetable and floriculture industries in the northeastern U.S., we sought to describe the development of this aphid over a range of temperatures common to greenhouse production systems in temperate climes.

MATERIALS AND METHODS

Plant Material

Pansies (*Viola × wittrockiana)* (var. Majestic Giant, Stokes Seeds, Buffalo, NY) were chosen as the host plant due to their popularity as a bedding plant in greenhouse ornamental production. Pansies were grown from seed in a Cornell University greenhouse at ca. 15-22 ^{o}C and transplanted into 10 cm pots filled with Pro Mix 'BX' (Premier Horticulture Inc., Quakertown, PA). Plants were fertilized 3 to 4 times weekly with Excel 21:5:20 (N-P-K) at 300 ppm (Scotts-Sierra Horticultural Products, Marysville, OH), and supplemental lighting was used to ensure a 12 h day length. After 4-6 weeks, the pansy leaves were large enough to be used for experiments. New pansies were planted every 2-4 weeks as needed.

Source and Maintenance of Insects

Aulacorthum solani were collected from blue saliva and pentas from a garden center in Ithaca, NY and reared on pansy for >5 generations prior to starting experiments. Aphid infested plants were kept in screened cages ("BugDorms" (Bioquip Products, Rancho Dominguez, CA)), 60 x 60 x 60 cm,104 x 26 mesh/2.54 cm) in a greenhouse compartment (temperature range: 20-30 ^{o}C, L:D = 16:8; RH = 30-50%). New plants were introduced approximately twice a week. The colony consisted mainly of apterous aphids, but some alates (~10-20% of adults) were present at all times, regardless of aphid density.

Temperature-Dependent Development and Mortality of A. solani Nymphs

Embedded leaves were used as the experimental arena to enable comparisons with previous *A. solani* studies, which were conducted on excised leaves. To embed, single leaves (abaxial side up) were pressed gently into 2.5% Difco agar (Fisher Scientific, Pittsburg, PA) before it solidified in a Petri dish. Nymphs (<8 h old) were obtained for experiments by placing 6-10 apterous, adult *A. solani* onto an excised, embedded pansy leaf in a 60 mm Petri dish. Dish lids had 1 cm diameter ventilation holes covered with thrips-proof screening. Dishes were placed in an incubator at 25 ± 1 ^{o}C, 16:8 L:D and ca. 40-50% RH. After 8 h, the newly-born nymphs were transferred to embedded pansy leaves in new dishes (1 aphid per dish) for experiments using a fine camel-hair brush. Two tests were conducted (1 week apart), with a slight modification in methods between them. Test date 1 used 90 mm Petri dishes with ventilated lids (2 x 1 cm diameter holes covered with the above-described mesh); slightly bigger leaves

were used in these dishes, and they were sealed with Parafilm "M" (Pechiney Plastic Packaging, Chicago, IL) to prevent aphid escape. Test date 2 used the 60 mm dishes described above, which had tight-fitting lids making Parafilm unnecessary. Dishes with individual aphids (14 replicates per temperature treatment in test date 1; 18 in test date 2) were placed in an incubator set at one of six temperatures: 10, 15, 20, 25, 30 or 35 ^{o}C (± 1 ^{o}C for all treatments). Chamber temperature was recorded every 2 h using a Hobo electronic data logger (Onset Computer, Bourne, MA). Nymphs were observed every 12 h (07:00 and 19:00 h) for molting (as evidenced by the presence of a cast skin) until adult emergence. Mortality was also recorded; if a nymph carcass could not be found the replicate was recorded as "missing". Leaves were changed as needed at each temperature. Typically, this was every 12-24 h at 35 ^{o}C, every 24-48 h at 30 ^{o}C, 48-96 h at 25 ^{o}C, 72-96 h at 20 ^{o}C, and 96-120 h at 15 and 10 ^{o}C. The above methodology was used for all trials involving embedded leaves.

As would be expected, we observed much more rapid declines in the quality of the excised, embedded leaves under the high vs. low temperature conditions of our tests. Due to concerns over possible effects of high temperatures on excised leaf quality, and thus aphid development and survival, we conducted tests using embedded leaves vs. leaves on whole plants to confirm the validity of the embedded leaf results. First, longevity was determined at 35 ^{o}C using whole pansy plants (4-6 weeks; 10 cm pots). An individual aphid nymph (< 8 h old) was placed on the underside of a leaf and confined to the leaf by a clip cage (n = 16). Simultaneously, an individual nymph (<8 h old) was confined on an embedded pansy leaf for the control treatment (n = 7). To eliminate the possibility that 1st-instar aphids died at 35 ^{o}C because they were too

fragile to survive the heat shock, we also placed 7–12 d old adult aphids (n=13) (reared at 25 °C) into the 35 °C chamber on embedded pansy leaves (1 aphid per leaf) to determine adult longevity at this temperature. All aphids were checked every 12 h until death. Second, we used clip cages and whole plants at 30 °C (using the same methods as above) and followed aphid developmental time from 1st instar (< 8 h old) until 3rd instar (n=16). Observations were made every 12 h; the presence of a cast skin on the leaf or within the cage indicated that a molt had taken place. Again, embedded pansy leaves were used as the control (n=7).

***Fecundity, Larviposition and Longevity of* A. solani**

Observations of survival and reproduction for each aphid that became an adult in the developmental tests were continued at the same temperature regime. Observations were made every 24 h (at 16:00 h) until death and leaves were changed as needed. Offspring were counted and removed daily.

Statistical Analyses

All analyses were done in SAS v. 9.13 (SAS Institute 2003). Analyses of variance (ANOVAs) to determine the effects of temperature and test date on the development of each life stage were conducted on all aphids that completed that life stage. In all cases, time of molt was estimated as the midpoint of the time interval during which the molt was observed. Developmental time data, an example of time-to-event data (whose distributions are commonly skewed to the right), were ln (x+1) transformed to better meet the assumption of normality for the parametric ANOVAs. ANOVAs were

also conducted to determine effects of temperature on fecundity and longevity; daily fecundity data were ln (x+1) transformed and adult longevity data were ln transformed prior to analysis. Additionally, results from the parametric analyses for developmental time, total reproduction and adult longevity were confirmed by ANOVA of the data following rank transformation, a nonparametric approach essentially equivalent to the Kruskal-Wallis test (Conover 1999; Stokes et al. 2001). To accommodate two-way designs that included test date as a factor, we opted to apply the aligned rank transformation technique (Mansouri 1999) using the PROC RANK function in SAS. Significance of main effects and interactions were compared between the parametric and nonparametric ANOVAs, and if in agreement, the results were accepted (see Conover 1999, Zar 1999) and the F-test results from the parametric analyses are reported herein. In the parametric ANOVAs, Tukey-Kramer tests on multiple means were used to determine differences in development times, total reproduction and adult longevity across temperatures, and therefore the least squared means (i.e. adjusted means) are presented for all data.

Two nonlinear equations were used to model developmental rate (1/development time) across temperature using the PROC NLIN procedure in SAS, which generates the best-fit model by iterating initial parameters. The Logan model is given as $r(T) = e^{\rho T} - e^{[\rho Tmax-(Tmax-T)/\Delta]}$, where ρ (rate of increase at optimal temperature), T_{max} (upper developmental threshold), Δ (difference between optimal and upper temperature threshold) are fitted parameters (Logan et al. 1976); the redundant Ψ parameter was removed as suggested by Lactin et al. (1995). The second model used was the Lactin-2 model (Lactin et al. 1995), which will be referred to

henceforth as the Lactin model. Given as $r(T) = e^{\rho T} - e^{[\rho T_{max} - (T_{max} - T)/\Delta]} + \lambda$, the Lactin model is simply the Logan model with an additional parameter λ that forces the curve to intercept the x-axis, allowing the estimation of a low-temperature developmental threshold (Lactin et al. 1995). Initial parameter values for both models were based on previously reported aphid developmental time data (i.e. from Diaz et al. (2007), who used the Lactin model for the lettuce aphid *Nasonovia ribisnigri*, and from Lamb (1992), who used the Logan model for the pea aphid *Acyrthosiphon pisum*). To determine the goodness-of-fit of each model, the residual sum of squares (RSS) and the pseudo-R^2 of each model were compared (Roy et al. 2002). The pseudo- R^2 is calculated as $R^2 = 1 - (S_r/S_m)$, where S_r is the variance of the residuals and S_m is the mean squared error of developmental rate (Medeiros et al. 2004).

Although T_{max} is a parameter in the Lactin equation, it does not actually represent the upper temperature at which the growth rate equals zero (the upper developmental threshold) as in the underlying Logan model (see discussion). The true developmental threshold predicted by the model can be obtained only via simulation: the temperature parameter in the models was iterated using R statistical software (v. 2.9.0) (Crawley 2007) until $r(T) = 0$ (identifying the upper point at which the model crossed the x-axis). Optimum temperature for development (T_{opt}) can be calculated for both models as $T_{max} - \Delta$. However, because of the above-described problem with T_{max} from the Lactin model, an additional estimate of T_{opt} was obtained from the Lactin equation by iterating the temperature parameter until the developmental rate was maximized. The lower developmental threshold (T_{min}) was estimated from the Lactin equation by iterating the temperature parameter to determine the lower point at

which the model crossed the x-axis. In the case of the Logan model, the lower threshold cannot be calculated as the function approaches zero asymptotically.

The linear model y = a + bx (Campbell et al. 1974) was used to provide an additional estimate of the lower developmental threshold (t_{min} = -a/b) as well as the thermal constant (K = 1/b) for all developmental stages of *A. solani*. The standard error for K was calculated as in Campbell et al. (1974). Developmental rates were regressed against temperature for 10, 15, 20 and 25 ^{o}C (a range of temperatures over which the response was approximately linear) (Kontodimas et al. 2004; Diaz et al. 2007) using the PROC REG procedure in SAS. Estimation of K using the linear model is the accepted method given that nonlinear models cannot estimate K (Kontodimas et al. 2004). However, this linear approach disregards the estimate of the lower developmental threshold estimated from the nonlinear model. We propose that an improved estimate of K can be obtained by adding the value of the lower threshold predicted by the Lactin model (T_{min}) to the data set used for the linear regression. We included this derived data point in a linear regression to produce a new estimate of the y-intercept. Then, we removed the derived data point and repeated the regression of the empirical data constrained to the new y-intercept to generate an adjusted slope and standard error for determination of an adjusted K value and its standard error. This approach provides a simple mechanism by which the lower threshold predicted by the nonlinear Lactin model contributes toward estimation of the thermal constant.

Product limit (Kaplan-Meier) survival estimates and median survival times (ST_{50}) were generated for each temperature using PROC LIFETEST in SAS (Allison 1995). All data were censored for aphids that went missing (0-6 per treatment; most

were lost within the first 48 h) or were eliminated because they became alate (only 2 alate were encountered). To determine if the main effects of test date and temperature significantly affected survival, and if it was appropriate to pool the data between test dates, data were submitted to proportional hazard analysis (PROC PHREG in SAS) (Allison 1995). ST_{50} values were also regressed on temperature (PROC REG).

ANOVAs and Tukey-Kramer tests were conducted to determine differences between temperatures for pre-larviposition period, larviposition period, total fecundity, daily lifetime fecundity (= total offspring produced divided by age at death) and adult longevity. As before, least squared means (i.e. adjusted means) were presented for all data due to an unbalanced design. Effect of test date was also determined for total fecundity and adult longevity.

To generate a graph of age-specific fecundity (i.e. a visual representation of fecundity based on the age of adult aphids), we used the (arithmetic) mean number of offspring produced per surviving female based on *day of adulthood*. We would like to clarify that this is not a graph of m_x (m_x being cohort-based fecundity, or, the mean number of offspring of surviving aphids based on *age from birth*). However, m*x* was used in the calculation of the Euler equation (below), despite our choice not to present a graph of m*x*.

To calculate life table statistics, we used the Euler equation, given as $\Sigma e^{-rx} l_x m_x = 1$, where x is the time in days (including immature stages), l_x is the proportion of individuals in the original cohort alive at time x (including immature mortality), and m_x (as stated before) is the mean number of offspring produced per surviving aphid during time interval x (1 d) (Davis et al. 2006). Additionally, any missing or

discarded aphids were simply ignored in the calculation of l_x for the Euler equation (contrary to the use of censoring in the survival analysis) (H. Chi, personal communication). Intrinsic rate of increase was determined by iterating r in the Euler equation until $\Sigma e^{-rx} l_x m_x = 1$ (see Southwood 1978). Net reproductive rate ($R_o = \Sigma l_x m_x$), generation time ($GT = \ln R_o/r$) and doubling time ($DT = \ln 2/r$) for each temperature were also calculated as per Birch (1948).

In the tests comparing longevity and developmental time on whole plants vs. embedded leaves, non-parametric t-tests (i.e. the Wilcoxon-Mann-Whitney test; PROC NPAR1WAY in SAS) were used to compare treatment means. Replicates where aphids went missing were removed from the data prior to analysis. The same test was used to compare longevity of adult aphids and nymphs at 35 ^{o}C on embedded leaves.

RESULTS

Comparison of Data between Test Dates

The proportional hazard analysis indicated no significant effect of test date on aphid survival ($\chi^2_{1df} = 0.099$ P = 0.75) and there was no test date x temperature interaction ($\chi^2_{1df} = 0.006$ P = 0.94). Data for the two test dates were therefore pooled for determination of the Kaplan-Meier survival curves.

In the parametric ANOVAs, test date was not a significant factor in developmental time for any of the four instars (P values from 0.08-0.86), total developmental time ($F_{1,105} = 2.27$ P =0.14), total reproduction ($F_{1,105} = 0.17$ P =0.68), or adult longevity ($F_{1,105} =0.03$ P=0.86). The nonparametric ANOVAs confirmed that test date was not a significant effect in total reproduction ($F_{1,105} = 2.28$ P =0.14), adult

longevity ($F_{1,105}$ =1.07 P=0.31), or developmental time of first ($F_{1,133}$ = 0.07 P = 0.79) and third instars ($F_{1,114}$ = 1.47 P = 0.229). However, test date was a statistically significant factor in total developmental time ($F_{1,105}$ = 4.85 P =0.03) and developmental time of second ($F_{1,125}$ = 4.42 P = 0.04) and fourth instars ($F_{1,105}$ = 10.67 P =0.002).

There were no significant test date x temperature interactions in any of the parametric analyses of duration of each stadium (P values ranging from 0.16–0.70 for the four instars), total developmental time (P =0.20), total reproduction (P = 0.88), or adult longevity (P = 0.95). The non-parametric tests supported these findings (P = 0.15–0.97) with the single exception of a marginally significant interaction detected in the third-instar data ($F_{4,114}$ = 2.5, P = 0.048).

Because results from the two test dates were generally similar and consistent across temperatures, data were pooled for determination of life table statistics. In the ANOVA/Tukey-Kramer tests of temperature effects, test date was retained in the model statements as a blocking factor to potentially reduce error variance. All presented results are means (± standard errors) expressed in the original (untransformed) scale.

Temperature Dependent Development and Mortality of* A. solani *Nymphs

Temperature had a significant effect on developmental rate ($F_{4,105}$ =253.10 P < 0.0001). Total developmental times were comparable to those found in previous research (Tables 2.1 and 2.2). From 10 to 25 ^{o} C, developmental time of *A. solani* significantly decreased as temperature increased (Table 2.1). *Aulacorthum solani*

Table 2.1. Duration (mean ± standard error) of each stadium for *A. solani* incubated at constant temperatures.

		Duration of each stadium (Days) [a]				
Temperature	Initial n	First instar	Second instar	Third instar	Fourth instar	Nymph to adult
10	32	6.20 ± 0.173a (24)	5.18 ± 0.172a (24)	5.02 ± 0.177a (22)	5.91 ± 0.160 a (22)	21.81 ± 0.360a (22)
15	32	3.32 ± 0.143b (29)	2.36 ± 0.142b (29)	2.51 ± 0.129b (29)	3.19 ± 0.117b (29)	11.37 ± 0.262b (29)
20	32	2.72 ± 0.162c (26)	1.61 ± 0.161c (26)	2.14 ± 0.146c (26)	1.92 ± 0.132c (26)	8.37 ± 0.296c (26)
25	32	2.02 ± 0.157d (26)	1.31 ± 0.155c (26)	1.44 ± 0.143d (25)	2.11 ± 0.130c (25)	6.88 ± 0.291d (25)
30	32	2.22 ± 0.145d (29)	1.74 ± 0.196c (21)	2.38 ± 0.230bc (13)	3.74 ± 0.368b (4)	9.48 ± 0.825bc (4)
35	32	– [b]	–	–	–	–

[a] Means within columns followed by same letter are not significantly different (Tukey-Kramer test, α = 0.05). The numbers of aphids that completed each stadium (vs. dead or missing) are presented in parentheses for each temperature.
[b] All aphids died within 48 h at 35ºC.

Table 2.2. Mean total developmental times (± SE), intrinsic rate of increase (r_m), net reproductive rate (R_o), total fecundity and doubling times (DT) for *A. solani* reared on various crops.

Temp. (°C)	Crop	*n*	Total Devel. Time (days)	r_m	R_o	Total Fecundity	DT (days)	Ref.
2.0	Potato	41	0 [a]	—	—	—	—	5
5.0	Potato	50	63.15 ± 1.08	—	—	45.3	—	5
10.0	Pansy	32	21.8 ± 0.36	0.0955	60.8	74.4	7.26	1
	Pepper	100 [b]	16.7 ± 0.24	0.1240	59.2	—	5.59	6
	Soybean	20	20.2 ± 4.50	N/A	N/A	—	—	2
	Lettuce	20 [c]	23.7 ± 0.43	0.078	29.8	—	8.89	7
	Eggplant	20 [c]	21.8 ± 0.62	0.089	42.0	—	7.79	7
	Pea	20 [c]	18.8 ± 0.58	0.079	10.2	—	8.76	7
	Fennel	20 [c]	23.8 ± 0.50	0.083	30.7	—	8.37	7
12.5	Lettuce	30	16.9 ± 0.15	0.1292	36.3	—	5.37	3
15.0	Pansy	32	11.4 ± 0.26	0.1820	75.6	74.9	3.81	1
	Lettuce	30	10.3 ± 0.15	0.2284	58.7	—	3.04	3
	Soybean	20	13.4 ± 2.6	—	—	—	—	2
17.5	Lettuce	30	7.9 ± 0.13	0.2631	35.4	—	2.63	3
20.0	Pansy	32	8.4 ± 0.30	0.2394	64.5	68.4	2.90	1
	Lettuce	30	7.2 ± 0.13	0.2747	33.8	—	2.52	2
	Potato	50	7.9 ± 0.06	—	—	84.8	—	5
	Soybean	20	7.8 ± 1.20	—	—	—	—	2
22.5	Lettuce	30	6.6 ± 0.14	0.2625	17.9	—	2.64	3
avg. 22.6	Potato	37	9.3 [d]	—	—	60.3	—	4
25.0	Pansy	32	6.9 ± 0.29	0.2478	37.2	39.1	2.80	1
	Lettuce	30	7.4 ± 0.30	0.1794	8.2	—	3.86	3
	Soybean	20	7.0 ± 1.0	—	—	—	—	2
27.5	Lettuce	30	0 [a]	—	—	—	—	3
30.0	Pansy	32	9.5 ± 0.83 [e]	-0.2367	0.07	1.89	— [f]	1
	Soybean	20	0 [a]	—	—	—	—	2
35.0	Pansy	32	0 [a]	—	—	—	—	1

[a] All nymphs died before reaching adulthood at this temperature.
[b] Data for 1 cohort (out of 4) were used, as there were no significant differences among cohorts.
[c] Data were chosen from the best performing cohort (out of 2 cohorts).
[d] No standard error available.
[e] Based on 4 nymphs that developed into adults.
[f] Doubling time at 30 °C is not reported since a negative r_m value would yield a negative doubling time.

Table 2.2 (Continued)
1 = This study
2 = Kim et al. 1991
3 = Lee et al. 2008 a,b
4 MacGillivray and Anderson 1958
5 = Pozarowska 1987
6 = Vasicek et al. 2001
7 = Vasicek et al. 2003

nymphs developed significantly faster at 25 °C than at all other temperatures, growing from newly-laid nymph to adult in an average of 6.9 d. Developmental time increased to 9.5 d at 30 °C; however, only 4 aphids out of an original 32 were able to complete development at this temperature. No *A. solani* incubated at 35 °C were able to complete even the first molt. The 1st and 4th stadia and the 2nd and 3rd stadia tended to have similar duration at the non-lethal temperatures of 10–25 ° C, with the 1st and 4th stadia being longer than the middle stadia (Table 2.1). An exception occurred at 20 ° C, at which temperature the 3rd stadium exceeded the 4th by > 10%.

Temperature dependent nymphal mortality is shown in Table 2.3. Nymphal mortality was highest at 35 °C, where all aphids died within the first 48 h, followed by 30 °C and 10 °C, with 82.0 and 18.52 % mortality, respectively. Nymphal mortality was lowest at 15 °C (0%).

Using linear regression, the lower developmental thresholds for instars one to four were estimated at between 2.3 and 5.8 °C (Table 2.4), with the lower threshold for total development estimated as 3.1 °C (Table 2.4). The thermal constant (K) for nymph to adult development is estimated as 141.0 degree days. The Logan and

Table 2.3. Temperature dependent nymphal mortality of *A. solani* reared at 6 constant temperatures.

Temp. (°C)	Stage	Number observed at start of each stage	Number dying in each stage	Number missing or discarded in each stage	Stage specific percent mortality (minus missing aphids)	Percent of original cohort (minus missing aphids) dying in each stage
10	1st	32	3	5	11.11	11.11
	2nd	24	0	0	0.00	0.00
	3rd	22	2	0	9.09	7.41
	4th	22	0	0	0.00	0.00
	Total					**18.52**
15	1st	32	0	3	0.00	0.00
	2nd	29	0	0	0.00	0.00
	3rd	29	0	0	0.00	0.00
	4th	29	0	0	0.00	0.00
	Total					**0.00**
20	1st	32	2	4	7.14	7.14
	2nd	26	0	0	0.00	0.00
	3rd	26	0	0	0.00	0.00
	4th	26	0	0	0.00	0.00
	Total					**7.14**
25	1st	32	1	5	3.70	3.70
	2nd	26	0	0	0.00	0.00
	3rd	26	0	1	0.00	0.00
	4th	25	0	0	0.00	0.00
	Total					**3.70**
30	1st	32	0	3	0.00	0.00
	2nd	29	7	1	25.00	25.00
	3rd	21	7	1	35.00	25.93
	4th	13	9	0	69.23	31.03
	Total					**81.96**
35	1st	32	32	0	100.00	100.00
	2nd	—	—	—	—	—
	3rd	—	—	—	—	—
	4th	—	—	—	—	—
	Total	—	—	—	—	**100.00**

Table 2.4. Linear regression equations for temperature-dependent development of *A. solani*.

Life Stage	Regression Equation [a]	R^2	P value [b]	t_{min} [c]	K [d]
First instar	r(T) = -0.0527 + 0.0232 (T)	0.702	0.0001	2.27	43.0 ± 2.8
Second instar	r(T) = -0.2947 + 0.0512 (T)	0.246	0.0001	5.76	19.5 ± 3.4
Third instar	r(T) = -0.1324 + 0.0355 (T)	0.610	0.0001	3.73	28.2 ± 2.3
Fourth instar	r(T) = -0.0810 + 0.0280 (T)	0.441	0.0001	2.89	35.7 ± 4.0
Nymph to Adult	r(T)= -0.0217 + 0.0071 (T)	0.874	0.0001	3.06	140.8 ± 5.3
Adjusted Nymph to Adult [e]	r(T) = -0.0277 + 0.0075 (T)	0.983	0.0001	3.69	133.3 ± 1.4

[a] r(T) = growth rate (1/development time) at temperature T.
[b] P value from the test of significance of the regression coefficient.
[c] t_{min} = -intercept/slope; t represents the lower temperature threshold, expressed in ^{o} C.
[d] K = 1/slope; K represents the thermal constant, expressed in degree days.
[e] Linear regression was re-calculated incorporating the lower threshold from the nonlinear model to provide adjusted t and K estimates.

Table 2.5. Parameter estimates (mean ± SE) and estimated temperature thresholds of the Logan and Lactin models for the development of *A. solani.*

		Fitted model parameter estimates				Simulation estimates [d]					
Model	df	ρ	T_{max}, $T_{max\ modified}$ [c]	Δ	λ	T_{min}	T_{max}	T_{opt}	T_{opt} ($T_{max} - \Delta$)	Pseudo R^2	RSS
Logan [a]	138	0.1245 ± 0.0027	34.9791 ± 0.0506	7.9887 ± 0.1693	–	– [e]	– [f]	– [g]	27	0.977	0.030
Lactin [b]	137	0.0813 ± 0.0080	37.5759 ± 0.8036 [c]	11.8971 ± 1.0085	- 0.1316 ± 0.0414	4.0	35.0	25.5	25.7	0.945	0.025

[a] Logan model: $r(T) = e^{\rho T} - e^{[\rho Tmax-(Tmax-T)/\Delta]}$.
[b] Lactin model: $r(T) = e^{\rho T} - e^{[\rho Tmax-(Tmax-T)/\Delta]} + \lambda$.
[c] T_{max} in the Lactin model is not the temperature at which $r(T) = 0$ and thus does not fit the definition of T_{max} in the Logan model; we designate it here as $T_{max\ modified}$.
[d] Estimates derived by running a simulation of the model; T_{min} = lower development threshold; T_{opt} = optimal development temperature, T_{max} = upper development threshold.
[e] T_{min} cannot be calculated using the Logan equation, as the model asymptotically approaches zero.
[f] Equals parameter T_{max} from fitted model.
[g] Equals $T_{max} - \Delta$.

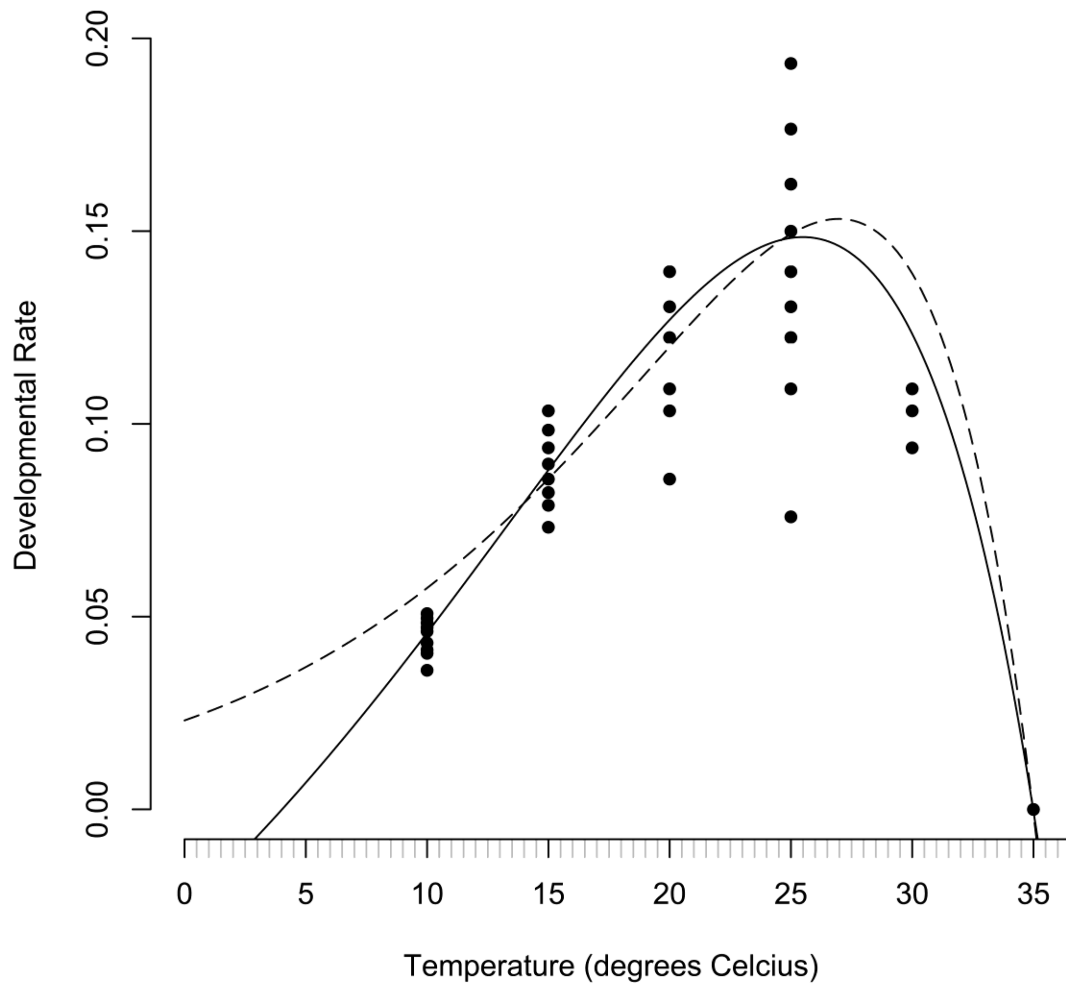


Figure 2.1. Constant-temperature-dependent developmental rate for *A. solani* based on the Lactin model (solid line) and the Logan model (dashed line).

Lactin models for developmental rate of *A. solani* are depicted in Figure 2.1 and parameter values are given in Table 2.5. In general, both models seemed to fit the data well, having high pseudo-R^2 values (0.98 and 0.95 for the Logan and Lactin models, respectively). In the Lactin model, the parameter T_{max} (which we refer to as $T_{max\ modified}$; see discussion) is given as 37.6 ^oC. This parameter value appears to be an overestimation of the upper development threshold, as 37.6 ^oC is not the point at which the model crosses the x-axis (see Figure 2.1). However, the simulation method with the Lactin model (described previously) produced an estimated T_{max} of 35.0 ^oC, which is identical to the $T_{max.}$ predicted by the Logan model (Table 2.5). This estimate is confirmed by the experimental results: no aphids were able to complete development at constant 35 ^oC. The optimal temperature for development (T_{opt}) was estimated between 25.5 and 27 ^oC using the various methods and models. The lower developmental threshold estimated by simulation of the Lactin model is 4.0 ^oC, which is ca. 1 ^oC higher than that estimated by the linear regression.

Adjusting for the higher T_{min} estimate from the non-linear regression (as previously described), the adjusted lower developmental threshold was estimated to be 3.7 ^oC and K was reduced from 141 to 133 degree days (Table 2.4).

Temperature Dependent Survival of A. solani

Survivorship curves for *A. solani* at the 6 temperatures tested are shown in Figure 2.2 and ST_{50} values are given in Table 2.6. A proportional hazards analysis shows that

Table 2.6. Median survival times (ST_{50}) of *A. solani* at six temperatures (Kaplan-Meier estimates).

Temperature (^{o} C)	ST_{50} (95% CI) in days
10	96.66 (88.67 - 113.67)
15	89.67 (80.67 - 94.67)
20	56.67 (53.67 - 60.67)
25	37.92 (32.67 - 39.92)
30	10.92 (8.42 - 11.92)
35	0.42 (0.42-0.92)

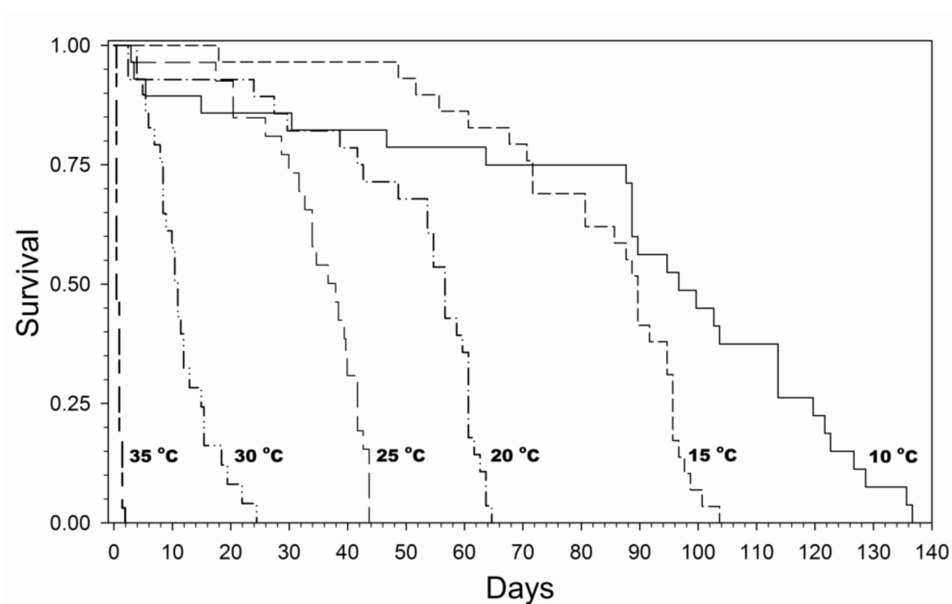


Figure 2.2. Kaplan-Meier survival curves for *A. solani* reared at 6 different constant temperatures.

temperature had a significant effect on survival ($\chi^2_{1df} = 70.75$, $P < 0.0001$). Median survival time decreased from 96.7 d to 0.4 d as temperature increased from 10 to 35 °C. Aphids survived up to a maximum of 136 d total at 10 °C. A linear regression of ST_{50} vs. temperature revealed a highly significant model, with temperature explaining nearly all of the variation ($F_{1,5} = 178.0$, $P = 0.0002$, $R^2 = 0.98$). The regression equation is given as $ST_{50} = 143.36 - 4.21$ (temperature).

Longevity and Development on Whole Plants vs. Embedded Leaves

In tests comparing the two experimental arenas at 35 °C, there was no significant difference in longevity of nymphs held on embedded leaves vs. whole plants (18.6 ± 2.2 h vs. 27.5 ± 3.7 h, respectively; Wilcoxon-Mann-Whitney test, $z = -1.66$, $P = 0.096$). In both cases, all aphids were dead within 48 h. Adults survived significantly longer than nymphs on embedded leaves at 35 °C (27.2 ± 1.5 h vs. 18.6 ± 2.2 h, respectively, $z = -2.57$, $P = 0.01$), but none of the aphids survived past 36 h. At 30 °C, time spent in the first and second stadia was not significantly different whether embedded leaves or whole plants were used ($z = -0.78$, $P = 0.44$ and $z = -0.96$, $P=0.34$ for first and second instars, respectively).

Fecundity, Larviposition Period, Adult Longevity and Population Dynamics

Mean pre-larviposition time, larviposition time, total fecundity, daily fecundity and adult longevity for aphids reared at each temperature are presented in Table 2.7. Each of these five parameters was significantly affected by temperature ($F_{4,102} = 21.13$ $P < 0.0001$; $F_{4,105} = 61.25$ $P < 0.0001$; $F_{4,10)} = 31.54$ $P < 0.0001$; $F_{4,105} = 19.59$, $P <$

Table 2.7. Mean (± SE) pre-larviposition period, larviposition period, total fecundity, daily fecundity and longevity for adult *A. solani* females reared at constant temperatures.

Temperature (° C)	n [a]	Pre-larviposition Period [b]	Larviposition Period [b]	Total Fecundity [b]	Daily Fecundity [b, c]	Adult Longevity [b]
10	22	4.37 ± 0.22 a	53.31 ± 2.01 a	74.38 ± 4.05 a	0.91 ± 0.12 a	82.98 ± 4.64 a
15	29	2.62 ± 0.16 b	32.70 ± 1.46 b	74.89 ± 2.94 a	1.11 ± 0.08 ac	70.08 ± 3.37 a
20	26	1.93 ± 0.18 c	25.89 ± 1.66 c	68.35 ± 3.34 a	1.58 ± 0.10 b	45.47 ± 3.82 b
25	25	2.48 ± 0.18 bc	18.77 ± 1.63 d	39.07 ± 3.28 b	1.48 ± 0.09 bc	28.16 ± 3.75 c
30	4	– [d]	–	–	–	11.39 ± 10.63 d
35	0	– [d]	–	–	–	–

[a] n represents the number of aphids that reached adulthood (out of 32 original replicates per temperature).
[b] Means within columns followed by same letter are not significantly different (Tukey-Kramer test, $\alpha = 0.05$). The least squared means presented differ slightly from the arithmetic means (which were 74.59, 75.55, 69.42, 38.64 and 0.50, respectively, for 10, 15, 20, 25 and 30°C).
[c] Daily lifetime fecundity = total fecundity/adult longevity.
[d] Parameters were not estimated because only 1 aphid reproduced at 30 °C and no aphids reached adulthood at 35 °C.

0.0001; $F_{4,105} = 33.99$ $P < 0.0001$, respectively). Aphids at 10, 15, and 20 °C had similar total fecundity (with 74, 75 and 68 offspring/female on average, respectively). Total fecundity was markedly lower at 25 °C, with an average of 39 offspring/adult, a 57 % reduction in fecundity from the 20 °C treatment. Daily fecundity was nominally highest at 20 °C, with 1.58 nymphs/day; however, this rate was not significantly higher than the rate of 1.48 nymphs/day recorded at 25 ° C (Table 2.7). Of the 4 aphids that survived to adulthood at 30 °C, only 1 reproduced, bearing 2 offspring on one day. The pre-larviposition (pre-reproductive) period decreased with increasing temperature between 10 and 20 ° C; this trend was reversed at 25ºC, although the increase between 20 and 25 ° C was not significant. The larviposition period decreased with increasing temperature. Adult longevity increased as temperature decreased. Age-specific fecundity per surviving aphid showed an expected decrease in the number of offspring produced over time (Figure 2.3). Maximum larviposition occurred at days 12, 7, and 6 of adulthood for temperatures 15, 20 and 25 °C, respectively. At 10 °C, the mean number of offspring per day was fairly consistent from day 5 to day 49 of adulthood; maximum offspring production occurred on day 37 of adulthood.

The life table statistics for *A. solani* are presented in Table 2.8. The intrinsic rate of increase (r_m) was highest for aphids reared at 25 °C ($r_m = 0.248$). Similarly, generation and doubling time were fastest for aphids reared at 25 °C (14.6 d and 2.80 d, respectively), while net reproductive rate (R_o) was highest at 15 °C. Life table statistics from other studies of *A. solani* are presented in Table 2.2 for comparison.

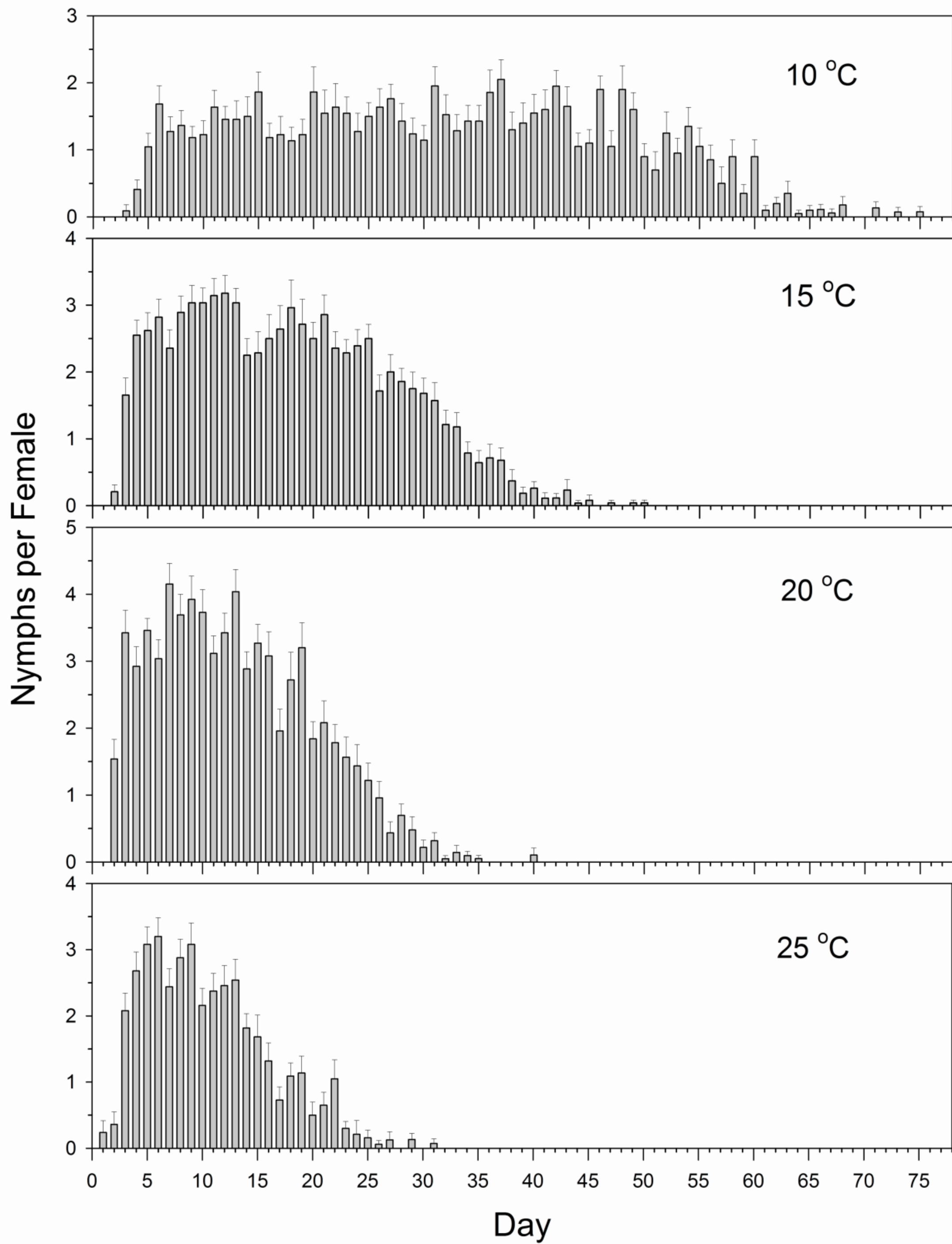


Figure 2.3. Age-specific daily fecundity of *A. solani* reared at constant temperatures (x-axis is not indicative of longevity). Error bars show + SE only.

Table 2.8. Life table statistics for *A. solani* reared at differing temperatures; r_m = intrinsic rate of increase; R_o = net reproductive rate; GT = mean generation time; DT = doubling time.

Temperature	r_m	R_o	GT	DT
10	0.0955	60.778	43.021	7.260
15	0.1820	75.551	23.763	3.809
20	0.2394	64.464	17.402	2.895
25	0.2478	37.154	14.589	2.797
30	-0.2367	0.0704	– [a]	– [a]
35	– [b]	– [b]	– [b]	– [b]

[a] Only 1 aphid was able to reproduce at 30 °C, resulting in a negative r_m value; thus, GT and DT could not be calculated.
[b] Values could not be calculated because no aphids developed at 35 °C.

DISCUSSION

The average developmental times for *A. solani* in this study were comparable to previous reports on other crops across a moderate temperature range (i.e. 10-25 °C). However, differences occurred at high temperatures between our study and the two studies conducted in Korea. In Lee et al. (2008a), only 3.3% of the 30 aphids tested developed at 27.5 ° C (a temperature that is only 0.5 °C above the estimated T_{opt} for our population calculated using the Logan model) and none of these produced nymphs. Similarly, Kim et al. (1991) reported that no aphids were able to develop or reproduce at 30 °C. However, in our study, 12.5% of nymphs developed into adults at 30 ° C, and 1 of these aphids was able to reproduce. Using the mean developmental data given by Lee et al. (2008a) and Kim et al. (1991), we used the Logan model to estimate the T_{max}

of their populations to be 28.0 ^{o}C and 30.0 ^{o}C, respectively, with optimum temperatures of 22.6 ^{o}C and 24.2 ^{o}C, respectively. In contrast, the T_{max} calculated for our population of *A. solani* was 35 ^{o}C, a much higher estimate, but one which is similar to other aphid species in several studies (e.g. 34.2 ^{o}C for green peach aphid at constant temperatures and 35.3 ^{o}C for lettuce aphid (*Nasonovia ribisnigri*)) (Davis et al. 2006; Diaz et al. 2007). Thus, our *A. solani* population appears to be more heat tolerant than those tested previously in Korea, and may be more likely to survive high temperatures that sometimes occur in floriculture production. Furthermore, Davis et al. (2006) showed that green peach aphid has a higher T_{max} (up to 3 ^{o}C higher) when reared under fluctuating temperature regimes; therefore, our North American *A. solani* population, when reared under natural, fluctuating conditions, may be able survive in greenhouses that reach ca. 37-38 ^{o}C for a short periods during the day, especially if *A. solani* engage in shade-seeking behaviors, which has been reported in other aphids (ex. Gish and Inbar 2006).

With regard to low temperatures, at 10 ^{o}C we observed extremely long survival of some *A. solani* individuals (up to 136 d from birth to death). However, this is probably not biologically relevant in nature. Older aphids (those past their reproductive period) were more often observed on the sides of the Petri dish than on the embedded leaf. In nature, they likely would have left the plant at this point and perished.

Upon comparing the lower developmental thresholds of *A. solani* populations, we noted that our adjusted t_{min} value of 3.7 ^{o}C was 1 ^{o}C higher than that calculated by Kim et al. (1991) (at 2.7 ^{o}C), but was much higher than the extremely low value calculated by Lee et al. (2008a) (0.08 ^{o}C). The value obtained by Lee et al. (2008a) is

surprising considering that Pozarowska (1987) provided empirical evidence that a selected population of *A. solani* did not develop at a temperature as low as even 2 ^{o}C. Because this estimate seemed biologically unrealistic, and because of the relatively low R^2 value of their linear regression ($R^2 = 0.75$), we suspected that Lee et al. (2008a) used their entire data set in their linear model in obtaining this $t_{min.}$ For the proper calculation of t_{min} and K, however, only those data that fit a straight line should be used (Kontodimas et al. 2004). To confirm this possible miscalculation, we conducted a linear regression of the mean data given in Lee et al. (2008a) using the Campbell method described earlier. Using their entire data set (12.5-25 ^{o}C) we obtained an extremely low t_{min} as well (= 0.15 ^{o}C). Using the approximately linear portion of the data set (i.e. 12.5-22.5 ^{o}C, as estimated by visual inspection of the developmental rate graph provided in their paper) yielded a t_{min} of 4.8 ^{o}C (with an R^2 of 0.94), a value which is much more realistic, though higher than our estimated t_{min} of 3.7 ^{o}C.

Due to the higher t_{min} estimate in this study vs. Kim et al. (1991), our thermal constant estimate is slightly lower. Using the method described by Campbell et al. (1974), we calculated K (for development from nymph to adult) to be 141 degree days and adjusted it to 133 by taking the estimate of T_{min} from the non-linear model into account; Kim et al. (1991) calculated K as 142 degree days. Lee et al. (2008a) originally calculated K to be 165 degree days, but this is based on their application of a linear regression to an inappropriate data set.

As defined by Logan et al. (1976), T_{max} is the upper temperature point at which the line describing development intersects the x-axis (temperature). This point, at which r(T) equals zero, is most precisely referred to as the upper development threshold

(UDT). In fitting data to the Lactin model, one finds that the parameter T_{max} clearly does not fit this definition. The T_{max} generated by fitting the Lactin equation to our data, for example, corresponds to a negative developmental rate, which translates to an overestimation of UDT. Although not always the case, this overestimation does appear consistently in the literature of insect developmental times. For example, in their original paper, Lactin et al. (1995) noted that their modifications of the Logan model produced estimates of T_{max} that were sometimes as much as 5 ^{o}C higher than those from the original Logan model. With respect to aphids, Diaz et al. (2007) obtained a T_{max} parameter from the Lactin equation that was 1.4 ^{o}C higher than their estimate of UDT. A proper estimate of UDT is obtainable from the Lactin model only via simulation (substituting values into the equation to identify the point of intersection). Given that T_{max} in the Lactin model does not actually represent UDT, we would argue that researchers fitting a Lactin model should refrain from referring to this parameter as an estimate of UDT. Furthermore, to avoid confusion in the literature when using non-linear models for developmental rates, it is important to clearly define true T_{min} and T_{max} as the temperatures at which $r(T) = 0$. These lower and upper development thresholds could be identified as T_{LDT} and T_{UDT}, respectively. A more fitting alternative, however, would be to rename the parameter T_{max} in the Lactin model; in Table 2 we refer to it as $T_{max\ modified}$. Finally, it should also be noted that with the Lactin-2 model the estimate of optimum developmental temperature (T_{opt}) obtained from simulation also differs slightly from the calculation of T_{max}-Δ.

In our study, *A. solani* reared at constant 25 ^{o}C had significantly lower fecundity than aphids reared at 10-20 ^{o}C. Studies with other aphid species show that a higher

fecundity and a higher r_m may be achieved at more natural, fluctuating temperatures vs. constant temperatures (Siddiqui et al. 1973; Elliott and Kieckhefer 1989; Kieckhefer and Elliott 1989; Davis et al. 2006). Thus, under greenhouse conditions, *A. solani* may possibly have a higher r_m and doubling time than indicated by our study.

The important differences in life table characteristics observed between our population and other populations of *A. solani* support the hypothesis of multiple biotypes. For example, the *A. solani* in Lee et al. (2008a, b) were originally collected from a commercial organic lettuce producer and developed more slowly at 25 ^{o}C than at 20 ^{o}C, which contrasts with both our study and that of Kim et al. (1991), who used a population collected from soybean in Korea. Also, our population developed and reproduced at warmer temperatures than those used by either Lee et al. (2008b) or Kim et al. (1991). These life history differences may have resulted from selection under different in environmental conditions such as temperature (ex. lettuce is typically grown at cooler temperatures of ca. 15.5-18.3 ^{o}C (Sanders 2001)). However, these differences (Table 2) may also be attributable to the various host plants used among these studies.

This paper provides the first report of multi-temperature life table statistics and developmental time modeling for a North American population of *A. solani*. Intrinsic rates of increase are highest and doubling times are shortest for this species at 20–25 ^{o} C, which is consistent with the observation that this species is most abundant in northeastern U.S. greenhouses on spring bedding crops during the cooler spring crop production temperatures. The poor survival/nearly complete lack of reproduction at 30 ^{o} C and the upper development threshold of 35 ^{o} C are also consistent with the observed decline of these aphids during greenhouse production temperatures of the summer

months (JPS, personal observation). The information gathered in this study increases our knowledge of the biology of this pest and may lead to better predictions of *A. solani* outbreaks and improvements in the timing of greenhouse pest management practices.

REFERENCES

Allison, P.D. 1995. Survival analysis using the SAS system: a practical guide. SAS Institute, Cary, NC.

Birch, L.C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15-26.

Blackman, R.L. and V.F. Eastop. 1984. Aphids on the world's crops: an identification and information guide. Wiley, New York, NY.

Blackman, R.L. and V.F. Eastop. 1994. Aphids on the world's trees: an identification and information guide. CAB International, Wallingford, UK.

Blackman, R.L. and V.F. Eastop. 2006. Aphids on the world's herbaceous plants and shrubs, Vol. 1&2. Wiley Ltd., Chichester, England.

Campbell, A., B.D. Frazer, N. Gilbert, A.P. Guiterrez, and M. Mackauer. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11: 431-438.

Conover, W.J. 1999. Practical Nonparametric Statistics, 3rd ed. Wiley, New York, NY.

Crawley, M.J. 2007. The R Book. Wiley, New York, NY.

Davis, J.A., E.B. Radcliffe, and D.W. Ragsdale. 2006. Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). Environ. Entomol. 35: 1461-1468.

Diaz, B.M., M. Muniz, L. Barrios, and A. Fereres. 2007. Temperature thresholds and thermal requirements for development of *Nasonovia ribisnigri* (Hemiptera: Aphididae). Environ. Entomol. 36: 681-688.

Down, R.E., A.M.R. Gatehouse, W.D.O. Hamilton, and J.A. Gatehouse. 1996. Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered *in vitro* and via transgenic plants both in laboratory and glasshouse trials. J. Insect. Physiol. 42: 1035-1045.

Elliott, N.C. and R.W. Kieckhefer. 1989. Effects of constant and fluctuating temperatures on immature development and age-specific life tables of *Rhopalosiphum padi* (L.) (Homoptera: Aphididae). Can. Entomol. 121: 131-140.

Gish, M. and M. Inbar. 2006. Host location by apterous aphids after escape dropping from the plant. J. Insect. Behav. 19: 143-153.

Govier, D.A. 1957. The properties of tomato aspermy virus and its relationship with cucumber mosaic virus. Ann. Appl. Biol. 45:62-73.

Heinz, K.M. 1998. Dispersal and dispersion of aphids (Homoptera: Aphididae) and selected natural enemies in spatially subdivided greenhouse environments. Environ. Entomol. 27: 1029-1038.

Kieckhefer, R.W. and N.C. Elliott. 1989. Effect of fluctuating temperatures on development of immature Russian wheat aphid (Homoptera: Aphididae) and demographic statistics. J. Econ. Entomol. 82: 119-122.

Kim, D-H., G-H. Lee, J-W. Park, and C-Y. Hwang. 1991. Occurrence aspects and ecological characteristics of foxglove aphid *Aulacorthum solani* Kaltenbach Homoptera Aphididae in soybean. Res. Rept. RDA (Crop. Prot.) 33 : 28-32 (in Korean).

Kontodimas, D.C., P.A. Eliopoulos, G.J. Stathas and L.P. Economou. 2004. Comparative temperature-dependent development of *Nephus includens* (Kirsch) and *Nephus bisignatus* (Bohenman) (Coleoptera: Coccinellidae) preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): evaluation of a linear and various nonlinear models using specific criteria. Environ. Entomol. 33: 1-11.

Lactin, D.J., N.J. Holliday, D.L. Johnson, and R. Craigen. 1995. Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24: 68-75.

Lamb, R.J. 1992. Developmental rate of *Acyrthosiphon pisum* (Homoptera: Aphididae) at low temperatures: implication for estimating rate parameters in insects. Environ. Entomol. 21: 10-19.

Lee, S.G., H.H. Kim, T.H. Kim, G-J. Park, K.H. Kim, and J.S Kim. 2008a. Development model of the foxglove aphid, *Aulacorthum solani* (Kaltenbach) on lettuce. Kor. J. Appl. Entomol. 47: 359-364.

Lee, S.G., H.H. Kim, T.H. Kim, G-J. Park, K.H. Kim, and J.S Kim. 2008b. Longevity and life table of the foxglove aphid (*Aulacorthum solani K.*) adults on lettuce (*Lactuca sativa* L.). Kor. J. Appl. Entomol. 47: 365-368.

Logan, J.A., D.J. Walkind, S.C. Hoyt, and L.K. Tanigoshi. 1976. An analysis model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5: 1133-1140.

Mansouri, H. 1999. Multifactor analysis of variance based on the aligned rank transform technique. Comput. Stat. Data Anal. 29: 177-189.

MacGillivray, M.E., and G.B. Anderson. 1958. Development of four species of aphids (Homoptera) on potato. Can. Entomol. 90:148-155.

Medeiros, R., F.S. Ramalho, J.E. Serrao, and J.C. Zanuncio. 2004. Estimative of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) development time with non linear models. Neotrop. Entomol. 33: 141-148.

Miles, P.W. 1990. Aphid salivary secretions and their involvement in plant toxicoses, pp. 131-147. In R.K. Campbell and R.D. Eikenbary (eds.), Aphid-plant genotype interactions . Elsevier, Amsterdam, Ther Netherlands.

Miller, G.L, C. Favret, A. Carmichael, and D.J. Voegtlin. 2009. Is there a cryptic species within *Aulacorthum solani* (Hemiptera: Aphididae)? J. Econ. Entomol. 102: 398-400.

Miller, G.L. and M.B. Stoetzel. 1997. Aphids associated with chrysanthemums in the United States. Fla. Entomol. 80:218-239.

Mittler, T.E., and L. Wilhoit. 1990. Sexual morph production by two regional biotypes of *Myzus persicae* (Homoptera: Aphididae) in relation to photoperiod. Environ. Entomol. 19: 32-35.

Müller, F.P. 1970. Zucht und übertregungsversuche mit populationen and klonen der grünfleckigen kartoffelblattlaus *Aulacorthum solani* (Kalthenbach, 1843) (Homoptera: Aphididae). Dtsch. Entomol. Z. 17: 259-270 (in German).

Müller, F.P. 1976. Hosts and non-hosts in subspecies of *Aulacorthum solani* (Kalthenbach) and intraspecific hybridizations (Homoptera: Aphididae). Symp. Biol. Hung. 16: 187-190.

Nagano, T., Y. Umetsu, N. Hoshi, and T. Kidokoro. 2001. Outbreak of the foxglove aphid, *Aulacorthum solani* (Kalthenbach), in soybean fields of Miyagi Prefecture. II. Effect on soybean yield. Ann. Rep. Plant Prot. North Japan. 52:168-171 (in Japanese).

Okubu, T. 2001. Suppression of reproduction of the foxglove aphid (*Aulacorthum solani*) by high temperature – a thought on the outbreak of the foxglove aphid in Tohoku District in 2000. Ann. Rept. Plant Prot. North Japan 52: 168-171 (in Japanese).

Palumbo, J.C. 2003. The emergence of the foxglove aphid, *Aulacorthum solani*, as an economic pest of lettuce in the desert Southwest. In D.N. Byrne and P. Baciewicz (eds.), 2003 Vegetable report, The University of Arizona College of Agricultural and Life Sciences. http://ag.arizona.edu/pubs/crops/az1323.

Patch, E.M. 1928. The foxglove aphid on potato and other plants. Maine, Agric. Exp. Stn., Bull. 346: 49–60.

Peccoud, J., C.C. Figueroa, A.X. Silva, C.C. Ramirez, L. Mieuzet, J. Bonhomme, S. Stoeckel, M. Plantegenest, and J.C. Simon. 2008. Host range expansion of an introduced insect pest though multiple colonizations of specialized clones. Mol. Ecol. 17: 4608-4618.

Pozarowska, B.J. 1987. Studies on low temperature survival, reproduction and development in Scottish clones of *Myzus persicae* (Sulzer) and *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae) susceptible and resistant to organophosphates. Bull. Entomol. Res. 77: 123-134.

Roy, M., J. Brodeur, and C. Cloutier. 2002. Relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and its prey *Tetranychus mcdanieli* (Acarina: Tetranychidae). Environ. Entomol. 31: 177-187.

SAS Institute. 2003. SAS/STAT user's guide. SAS Institute, Cary, NC.

Sanders, D.C. 2001. NC State University horticulture information leaflets: lettuce production. http://www.ces.ncsu.edu/depts/hort/hil/hil-11.html.

Sanchez, J.A., F. Canovas, and A. Lacasa. 2007. Thresholds and management strategies for *Aulacorthum solani* (Hemiptera: Aphididae) in greenhouse pepper. J. Econ. Entomol. 100: 123-130.

Siddiqui, W.H., C.A. Barlow, and P.A. Randolph. 1973. Effects of some constant and alternating temperatures on population growth of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae). Can. Entomol. 105: 145-156.

Southwood, T.R.E. 1978. The construction, description and analysis of age-specific life-tables, p. 367. In T.R.E. Southwood, Ecological methods: with particular reference to the study of insect populations, 2nd ed.,. Chapman & Hall, London, UK.

Stokes, M.E, C.S. Davis and G.G Koch. 2001. Categorical data analysis using the SAS system, 2nd ed. SAS Institute Inc., Cary, NC.

Takada, H., T. Ono, H. Torikura and T. Enokiya. 2006. Geographic variation in esterase allozymes of *Aulacorthum solani* (Homoptera: Aphididae) in Japan, in relation to its outbreaks on soybean. Appl. Entomol. Zool. 41: 595-605.

Tamada, T. 1970. Aphid transmission and host range of soybean dwarf virus. Ann. Phytopathol. Soc. Japan 36: 266-274.

Valenzuela, I., A.A. Hoffmann, M.B. Malipatil, P.M. Ridland, and A.R. Weeks. 2007. Identification of aphid species (Hemiptera: Aphididae: Aphidinae) using a rapid polymerase chain reaction restriction fragment length polymorphism method based on the cytochrome oxidase sub-unit I gene. Aust. J. Entomol. 46: 305-312.

Van Driesche, R.G., S. Lyon, J.P. Sanderson, K.C. Bennett, E.J. Stanek III, and R. Zhang. 2008. Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker plants for control of aphids (Hemiptera: Aphididae) in greenhouse spring floral crops. Fla. Entomol. 94: 583-590.

Vasicek, A., F. De La Rossa and A. Paglioni. 2001. Aspectos biológicos y poblacionales de *Aulacorthum solani*, *Myzus persicae* y *Macrosiphum euphorbiae* (Homoptera: Aphidoidea) en Pimiento (*Capsicum annum* L.). Bol. San. Veg. Plagas 27: 439:446 (in Spanish).

Vasicek, A., F. La Rossa, A. Paglioni and P. Mendy. 2003. Biological and populational functionality of *Aulacorthum solani* (Kalthenbach) (Homoptera: Aphididae) on vegetable hosts under laboratory conditions. Bol. San. Veg. Plagas 29: 359-365 (in Spanish).

Wave, H.E., W.A. Shands and G.W. Simpson. 1965. Biology of the foxglove aphid in the northeastern United States. Tech Bull.- U.S. Dep. Agric. 1338: 1-40.

Zar, J.H. 1999. Biostatistical Aaalysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

CHAPTER 3

PATHOGENICITY OF CONIDIA-BASED PREPARATIONS OF ENTOMOPATHOGENIC FUNGI AGAINST THE GREENHOUSE PEST APHIDS GREEN PEACH APHID (*MYZUS PERSICAE* (SULZER)), COTTON APHID (*APHIS GOSSYPII* GLOVER) AND FOXGLOVE APHID (*AULACORTHUM SOLANI* (KALTENBACH)) (HEMIPTERA: APHIDIDAE).

Jandricic, S.E.,[4,5] Filotas, M.,[2,6] Sanderson, J.P., [1] and Wraight, S.P.[7,8]

ABSTRACT

For the purpose of identifying strains of entomopathogenic fungi with greater pathogenicity than commercially available strains for agriculturally-important pest aphids (Hemiptera: Aphididae), single-dose screening tests of 38 novel isolates and 4 commercially available isolates were performed against nymphs of the pest aphids *Myzus persicae* and *Aphis gossypii*. More than half of the fungal isolates tested were originally collected from Hemipteran pests in the hopes of increasing target specificity. Of the 3 species of fungi tested, several novel isolates of *Beauveria bassiana* s.l. and

---

[4] Department of Entomology, Comstock Hall, Cornell University, Ithaca NY, USA 14853
[5] OMAFRA, Simcoe Office, Box 587, Blueline Road & Highway # 3 Simcoe, ON N3Y 4N5
[6] Experiments on *A. solani* and *M. persicae* conducted from 2010-2012, all statistical analyses and the writing of the Introduction and Discussion sections were contributed by the author of this thesis. Experiments on *M. persicae* and *A. gossypii* conducted prior to 2010 and the bulk of the Methods section were contributed by M. Filotas.
[7] Corresponding Author: Email: steve.wraight@ars.usda.gov Fax: (607) 255-1132 Tel: (607) 255-2458
[8] USDA-ARS Robert W. Holly Center for Agriculture and Health, Tower Road, Cornell University, Ithaca NY, USA, 14853

Metarhizium anisopliae s.l. showed promise as control agents. However, *Isaria javanica* s.l. isolates generally performed poorly (< 31% mortality in all cases; even when doses exceeded 1000 conidia/mm^2). In dose-response assays comparing selected novel isolates vs. commercial strains, *Beauveria* ARSEF 5493 proved the most pathogenic of the novel isolates against both *M. persicae* and *A. gossypii*; however, LC_{50} values for this isolate were not statistically different from *B. bassiana* JW-1 (a commercial strain). Dose-response assays were also conducted with *Aulacorthum solani*; these are the first reported evaluations of *Beauveria* and *Metarhizium* against this pest. The novel isolate *M. anisopliae* ARSEF 5471 showed virulence ≥ that of *Beauveria* ARSEF 5493 in terms of LC_{25} and LC_{50}, but 5493 produced a steeper dose response (slope). Given that a minimum dose of 726 conidida/ mm^2 was needed to cause 50% mortality of nymphal aphids in our assays, virulence of all fungal isolates/strains (novel or commercial) against greenhouse pest aphids is considered low. Additional tests showed that adult aphids are more susceptible than nymphs to fungal infection, but confirmed that fungal infection has a limited pre-mortem effect on aphid reproduction, even on the day of death. Effects of assay techniques and the practicality of entomopathogenic fungi as control agents for greenhouse aphid pests are discussed.

INTRODUCTION

Aphids (Hemiptera: Aphididae) are some of the most agriculturally important insect pests. Of the pestiferous aphid species, green peach aphid (*Myzus persicae* (Sulzer); Aphidinae: Macrosiphini), melon aphid (*Aphis gossypii* Glover; Aphidinae: Aphidini), and more recently, foxglove aphid (*Aulacorthum solani* (Kaltenbach); Aphidinae:

Macrosiphini), are three of the most common and important aphid pests found in greenhouse crops worldwide (Kocourec et al. 1994; Down et al. 1996; Sanchez et al. 2007; Lee et al. 2008; van Driesche et al. 2008). Novel control measures are constantly being sought for aphids, especially given that resistance to all classes of traditional chemical insecticides can occur in these pests (Devonshire 1989) and may be more prevalent in 'closed' greenhouse environments. Additionally, 'softer' pesticides are becoming more and more desirable in greenhouse production as the use of biological control increases (see Parrella et al. 1999; van Lenteren 2000; Pilkington 2010), and effects on non-target arthropods (pollinators and natural enemies) have become an important consideration in the control measure of choice. Also, with the rise in popularity of organically produced produce, especially in Europe and the U.S. (Lohr 2001), organically acceptable "biorational pesticides" or "biopesticides" (*i.e.* pest control materials with limited toxicity and environmental side effects; Grubinger 1999) are of increased need in the industry.

Entomopathogenic fungi have been mass-produced as biopesticides for control of arthropods since the 1970's. Much research has focused on control of aphids, as this group of insects is more susceptible to natural fungal epizootics than perhaps any other (Milner 1997). However, the most successful fungal pathogens of aphids in nature, the Entomophthorales, have proven difficult to mass-produce and formulate as biopesticides (see Leite et al. 2003). Thus, most aphid microbial biocontrol efforts with fungal pathogens have focused on species that are more readily mass produced and formulated as biopesticides, primarily ascomycetous species of the anamorphic genera *Beauveria*, *Metarhizium, Isaria*, and *Lecanicillium* (Sordariomycetes: Hypocreales).

Recent studies based on nucleotide sequencing have supported the long-held view that the anamorphic genera of insect pathogenic fungi comprise diverse genetic types difficult or impossible to differentiate on the basis of phenotype. Indeed, many new cryptic species have been described (Bischoff et al. 2009; Rehner et al. 2011; Cabanillas et al. 2013), creating a challenge for applied researchers seeking species identifications. Herein we provide specific names only to those isolates for which molecular determinations are available. Isolates that have not been genetically characterized are identified broadly on the basis of the classical descriptions (colony characteristics and micromorphology) of the species they most closely resemble, and are designated sensu lato.

Several fungi have been commercialized for use against aphids and other phloem-feeding pests in greenhouse crops. In North America, these currently consist of *Beauveria bassiana* (Balsamo) Vuillemin strain GHA registered as BotaniGard®; *B. bassiana* strain JW-1 (ATCC 74040) registered as Naturalis-L®, *Isaria javanica* (Friederichs & Bally) Samson & Hywel-Jones strain Apopka 97 (ATTC 20874) registered as PFR-97® and Preferal® (formerly identified as *Paecilomyces fumoroseus/Isaria fumosorosea;* see Cabanillas et al. 2013), *Isaria javanica* sensu lato strain FE 9901 registered as NoFly™ (labeled as *Paecilomyces fumosoroseus*), and *Metarhizium brunneum* Petch registered as Met52®. Products based on *Lecanicillium* spp. (e.g. Vertalec®) are not currently registered in the U.S., though they are approved in Europe.

Despite these registrations, the market for fungus-based biopesticide products is still a small percentage of insecticide sales (Wraight et al. 2010). In the context of

aphid control, the problem is two-fold. The first is that although most of these products are labeled for control of these pests, in practice they tend to exhibit only moderate to low efficacy. The second is that growers often must deal with aphids as part of an aphid pest complex, being confronted with simultaneous or successive outbreaks of more than one aphid species. Thus, the ideal fungus-based biopesticide for aphid control would have the combined attributes of greater, more consistent, and broader activity against these pests than existing products.

Given the extensive culture collections of entomopathogenic fungi that have been amassed, it is probable that strains of fungi with substantially greater virulence against aphids are available, though not yet identified. Species of entomopathogenic fungi are composed of diverse genotypes, and it is generally the case that strains isolated from a target host are more virulent against that host than those isolated from a non-related species (Inglis et al. 2001). Thus, one would expect novel strains isolated directly from aphids or their close relatives to be more virulent than currently registered fungi, which originate, in most cases, from coleopteran or lepidopteran hosts. Specifically, *B. bassiana* strain JW-1 (Naturalis®) is described as having been isolated from the boll weevil *Anthonomus grandis* (Coleoptera: Curculionidae) (although recent molecular analysis indicates it is very similar to ARSEF strain 252, originally from a Colorado potato beetle (L.A. Castrillo, personal communication)). Strain F52 of *M. brunneum* (Met52®) was isolated from the coddling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Beauveria bassiana* strain GHA (BotaniGard®) was isolated from the spotted cucumber beetle *Diabrotica undecimpunctata* (Coleoptera: Chrysomelidae) (though it was re-isolated through the whitefly *Bemisia tabaci*

(Hemiptera: *Aleyrodidae*) prior to commercialization (S.P. Wraight, unpublished)). *Isaria javanica* Apopka strain 97 (PFR-97® and Preferal®) and *Isaria* sp. strain FE 9901 (NoFly®) are the exceptions, being originally isolated from the Solanum mealybug *Phenacoccus solani* (Hemiptera: Pseudococcidae) and the whitefly *Bemisia tabaci*, respectively.

The primary objective of this research was to identify novel isolates of entomopathogenic fungi with greater virulence against greenhouse aphids than strains currently registered for control of these pests. Isolates for screening were selected primarily from the USDA-ARS collection of entomopathogenic fungi (ARSEF); all were from the three genera of entomopathogenic fungi that have been most extensively developed for pest control in North America (*Beauveria*, *Metarhizium*, and *Isaria*), and most were originally isolated from aphids or other Hemiptera. After identifying the most promising agents, we investigated the dose-responses (LC_{50}s) of these pathogens against the three important greenhouse pest aphids described above. A secondary objective was to investigate the effect of aphid life stage on virulence/pathogenicity assessments, including effects of fungal infection on reproduction by individual aphids prior to death. Pre-mortem effects of *Beauveria* and *Lecanicillium* infection on aphid reproduction have been reported (Hall 1976; Wang and Knudsen 1993; Liu et al. 2003; Baverstock et al. 2006), but to our knowledge, effects of *Metarhizium* and *Isaria* infections have not been investigated. We discuss repercussions of differences in screening techniques of entomopathogenic fungi, as well as impacts of our findings within greenhouse integrated pest management programs.

.

MATERIALS AND METHODS

Source and preparation of fungal isolates

Commercially produced fungal isolates will henceforth be referred to by their strain designations, rather than the product trade-name (*i.e. B. bassiana* JW-1 for Naturalis®; *B. bassiana* strain GHA for BotaniGard®; *M. brunneum* strain F52 for Met52®), with the exception of *I. javanica* Apopka strain 97 (ATTC 20874). This fungus will be referred to as *I. javanica* PFR-97 for greater ease of reference.

Fungal isolates selected for screening originated from a variety of sites in North and South America, Europe, Asia and Africa (Table 3.1). Most isolates were obtained from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF) maintained in Ithaca, NY. More than half of the novel strains originated from various species of Hemiptera, with the rest originating from a variety of orders (Table 3.1). Amoung *Beauveria* isolates, half were isolated or re-isolated directly from aphids (Table 1). Interestingly, aphids are rarely found infected with *M. anisopliae* s.l. The ARSEF collection contains a single isolate from a *Pemphigus* root aphid, and no *Metarhizium* isolates were acquired for testing.

Assays utilized dry conidia obtained from 14-day-old cultures on 90-mm diam. plates of barley agar (30 g barley flour plus 15 g agar/L distilled water incubated at room temperature (ca. 24°C)). Conidia were brushed off the agar plates and passed through a sterile 125 µm sieve to produce fine powders, which were then dried over silica gel for 24 h. Dry conidia were then stored in sealed 50 ml polypropylene tubes at -20°C. This protocol was followed for all fungi except *B. bassiana* GHA, in which case,

dry technical conidial powders were obtained directly from the manufacturer (Emerald BioAgriculture, Butte, MT; production facility now operated by Laverlam International).

For bioassays, stock suspensions were prepared by adding conidia to 15 ml deionized water in 50 ml plastic centrifuge tubes. Dry conidial weights of between 1 and 15 mg/ml were used, depending on the concentration of conidia desired for each assay and differences in numbers of viable conidia/mg of the different preparations across years. For assays with *A. gossypii* and *M. persicae*, Tween® 80 (Fisher Scientific, Fair Lawn, NJ), was added to the suspensions at a concentration of 0.02% as a surfactant to suspend the conidia; Silwet® L-77 (Setre Chemical Company, Memphis, TN), at a concentration 0.01%, was used as the surfactant for all assays with *A. solani*. All tubes also received one gram of glass beads (2mm-diameter), and were then agitated on a wrist action shaker (Model BT, Burrell Scientific, Pittsburgh, PA) set at maximum speed (6.7 oscillations/s) for 15 min. After agitation, a 5 ml aliquot of each suspension was removed for application to insects.

Experimental insects and assay arenas

A laboratory colony of melon aphids, *A. gossypii*, was maintained on potted cucumber plants (*Cucumis sativus* L. cv. Marketmore 76; Seedway, Hall, NY). The colony originated from a single clone obtained from an *A. gossypii* colony maintained at Cornell University (Ithaca, NY) since 1991. Green peach aphids (*M. persicae*) were obtained from the Entomology Research Lab, University of Vermont (Burlington, VT), and were maintained on potted pepper plants (*Capsicum annuum* cv. Sweet Banana;

Stokes Seeds, Buffalo, NY); it is unknown if this colony was mono- or polyclonal. Both aphids were maintained at 25 ± 5°C and a 14:10h L:D regime. *Aulacorthum solani* was originally collected from a garden center in Ithaca, NY, and were a mixed-clonal population. *Aulacorthum solani* was maintained on pansy (*Viola* × *wittrockiana)* (var. Majestic Giant, Stokes Seeds, Buffalo, NY) at 21 ± 2 °C with 16:8h L:D. All assays on *A. gossypii* and *M. persicae* were conducted from 2004-2006 (with the single exception discussed below); those using *A. solani* were conducted in 2010-12. Any methodological differences between aphid assays are described below.

Experimental arenas used in assays were modeled after those described by Vandenberg (1996), with modifications. For assays with *A. gossypii* and *M. persicae* conducted in 2004-2006, leaf disks (4.5 cm in diameter) were cut from the leaves of 3-4 week old cucumber or pepper plants. For assays with *A. solani* (2010-2012), whole excised pansy leaves were used. Leaves of all types were embedded in molten (45-50°C) 2.5-3% Difco agar (Fisher Scientific, Pittsburg, PA) in 60-mm Petri dishes with the abaxial surface exposed.

To obtain adult aphids for use directly in bioassays, adult aphids (collected directly from aphid colonies) were placed on excised cucumber (for *A. gossypii*), excised pepper (for *M. persicae*) or embedded pansy leaves (for *A. solani*) in 60-mm Petri dishes and allowed to larviposit for 24h. *Aphis gossypii* and *M. persicae* were maintained at 25 ± 5°C and 14:10h L:D; *A. solani* were kept at 25 ± 1°C and 16:8h L:D. Adults were then removed from the leaves and, for *A. gossypii* and *M. persicae*, excised leaves containing young nymphs were placed on un-infested three-week-old cucumber or five-week-old pepper plants (maintained under the same light and temperature

regime). Nymphs were allowed to feed on plants until they developed into adults of appropriate age for use in the bioassay (6-8d total). *Aulacorthum solani* nymphs were allowed to develop on embedded pansy leaves for a total of 9 days. Nymphs were transferred to new leaves every 3 d using a fine paint brush. After these respective time periods, aphids of all species were 1-2 d old adults, which were then removed from plants or embedded leaves and placed on freshly embedded leaves for assay. Each dish was randomly assigned to the treatments associated with each assay.

To obtain nymphs for use in bioassays, a fine paint brush was used to transfer 4-5 *A. gossypii* adults to each cucumber leaf, 6-7 *M. persicae* adults to each pepper leaf, or 8-10 *A. solani* adults per pansy leaf (all embedded leaves or leaf discs in 60 mm Petri dishes). Adults were left on dishes for 24 hours and then removed. This generally resulted in 15-30, 12 ± 12 hr-old, first-instar nymphs of each species per dish, although numbers varied among dishes. Excess nymphs (> ca. 25) were removed with a fine brush under a stereomicroscope. Excess *A. solani* nymphs were successfully transferred to sparsely populated dishes to meet the minimum number of 15, as individuals of this species were easily removed from leaves by probing with the brush (inducing a defensive dropping behavior; Gillespie and Acheampong 2012). For the other aphid species, only dishes with a minimum of 15 aphids were used, as nymphs of these species were easily injured during transfer, being slow to extract their sylets.

Additionally, a different population of *M. persicae* was used in the single assay directly comparing the virulence of *B. bassiana* JW-1 against *A. solani* and *M. persicae* in 2012. This population of *M. persicae* was collected, reared, and used in assays with

identical methodology as for *A. solani* above. Use of a new population was necessary because the original *M. persicae* colony from 2004-2006 was discontinued in 2007.

General bioassay methodology

Conidial preparations were applied to embedded leaf disks containing aphids using a Burgerjon spray tower (Burgerjon 1956). The tower was fitted with an air-atomizing nozzle (Fluid Cap 2850 + Air Cap 70) mounted in a 1/4 J nozzle body (Spraying Systems, Wheaton, IL) connected to a regulator valve providing constant airflow of 10 liter/m. Petri dishes containing leaf disks and aphids were positioned on a rotating turntable (33 rpm) during application, and spray deposition at the level of the target surface was approximately 0.01 $\mu l/mm^2$ (resulting from spraying 5 ml aliquots). After treatment application, leaves were allowed to "dry" for approximately 5 min, until no large spray droplets could be seen on the surface (restricting the test to conidia deposited directly on aphids). Petri dishes were then lidded and sealed with Parafilm (Pechiney Plastic Co., Chicago, IL) for 24 h to optimize humidity within the dishes and promote germination of the conidia.

For all assays with *A. gossypii* and *M. persicae*, the Parafilm seal was removed from the Petri dish after 24h to limit development of condensation. Also at this time, aphids were transferred to freshly prepared, unsprayed, embedded leaf disks to limit contact with fungal inocula produced saprophytically on the leaf disks. For the assays comparing adult vs. nymphal stages of *A. solani* the same methodology was used. However, methodology in dose-response assays with *A. solani* differed in that aphids were left on treated leaves for 3 days.

In tests of all aphid species, any dead aphids observed at 24 h were considered handling losses and omitted from the assay. After 3 d, all aphids were again transferred to fresh agar-embedded leaf disks due to leaf senescence. Aphids were incubated at 25 ± 1°C and a 14:10 h L:D regime for 6 days (7 days for *A. solani*), after which mortality was assessed.

Numbers of conidia deposited on aphids and leaf disks during each spray application were estimated by either placing three plastic cover slips in the center of a 60 mm Petri dish, or 5 cover slips on a 90 mm Petri dish (for *A. solani* assays); these dishes were placed on the rotating platform adjacent to aphid dishes. After each spray, cover slips were allowed to dry for at least 24 h, then placed onto a glass slide with a drop of lactic acid (85%) containing acid fuchsin (1 mg/ml) and viewed at 400x magnification. Numbers of conidia per mm^2 were determined using the procedure described by Wraight et al. (1998), with two replicate counts made per coverslip on three coverslips per spray application with the 60 mm dishes, or 1 count made per coverslip on 5 coverslips per application with the 90 mm Petri dishes.

To assess viability, a Petri dish with yeast extract agar (0.5%) was sprayed along with the leaf disks for each isolate in each assay. These inoculated plates were sealed with Parafilm and incubated for 16-18 h at 25±1°C. Spray deposits were then stained with acid fuchsin and the first 100 conidia encountered under phase contrast microscopy (400 x) were scored for germination. This procedure was conducted at three randomly chosen locations per Petri dish, and numbers of conidia in all assays were corrected for viability.

Single-dose screen against immature aphids: M. persicae and A. gossypii

A total of 42 (38 novel, 4 commercially available) isolates were screened against *A. gossypii* and *M. persicae* nymphs in a series of assays. Assays were conducted in groups of five to eight (testing 5-8 isolates); each group of assays was carried out on a different day. Isolates assayed on any given day were of the same genus. One commercial isolate of each genus (*i.e. B. bassiana* GHA, *M. brunneum* F52 or *I. javanica* PFR-97) was included in each group of assays to generate a commercial product baseline for comparisons. For each test, three replicate plates of each aphid species, with 15-25 nymphs per plate, were sprayed with each isolate. Additionally, each group of assays included three plates of aphids sprayed with a solution of 0.02% Tween as a carrier control. Assays were conducted on 17 days over a three-month period. All isolates were assayed on at least two different dates. Each fungal isolate was applied at a concentration of 1-3 mg condia/ml. Resulting suspensions, containing ca. 1–2 x 10^8 conidia/ml were chosen to give a deposition of ca. 1500 conidia/mm^2. Ultimately, however, the doses (viable conidia/mm^2) varied considerably among isolates (Table 3.2), and assays receiving a dose of <500 conidia/mm^2 were discarded (thus the results from these isolates are not presented in Table 3.2).

Multiple dose bioassays of selected isolates against aphid pests

Assays against *M. persicae* and *A. gossypii* (2004-2006):

One novel isolate of *B. bassiana* (5493) and *M. anisopliae* (5471) were selected, based on results of the single dose screen, for more detailed multiple dose bioassays against 12 ± 12h old nymphs of *A. gossypii* and *M. persicae* in 2004. Both isolates produced

relatively high mortality of both aphid species at relatively low doses compared to other novel isolates. Three commercial strains (*B. bassiana* GHA and JW-1 and *M. brunneum* F52) were included in the tests. Although re-isolation of entomopathogenic fungi through specific hosts can produce variable results (Vandenberg and Cantone 2004), all isolates (except GHA) were passed through a melon aphid and re-isolated as a single-spore isolate according to the protocol of Castrillo et al. (2003) in an attempt to standardize the isolates. Dry conidia were then produced as described in section 2.3. *B. bassiana* strain GHA was not re-isolated in this manner because the desire was to test the novel isolates against a commercially available product (at the time the original assays were conducted, GHA was the only widely accepted commercially available fungal product).

The comparative virulence of the five isolates against first-instar *A. gossypii* and *M. persicae* aphids was determined in a series of five-dose bioassays. Aqueous suspensions were prepared at starting concentrations designed to achieve approx. 1.5–2 x 10^4 conidia/mm^2, with lower doses based on a series of 4-fold dilutions. Doses were always applied in the Burgerjon spray tower from lowest to highest dose, with a total of 3 replicate plates with 15 aphids per plate (12 ± 12h old) sprayed for each dose and aphid species (*i.e.* 45 aphids per dose). Additionally, 10 replicate plates with 15 aphids per plate were sprayed with 0.02% Tween as a control. All isolates were assayed against one aphid species at a time. The entire experiment was conducted on four different dates for each aphid species, resulting in a total of four replicate assays with ca. 900 aphids exposed to each isolate. However, in one assay of *M. brunneum* strain

F52 against *M. persicae* and *M. anisopliae* isolate 5471 against *A. gossypii*, maximal mortality was extremely low (< 30%). These replicates were excluded from analyses.

To address the concern that lack of re-isolation of *B. bassiana* GHA through an aphid host could result in under-performance of this commercially available isolate compared to other strains, we also conducted a five-dose assay comparing the original isolate of GHA vs. a single-spore re-isolate (see methodology above). The assays were conducted in March-April 2006. Methodology was identical to that above, with a total of 3 replicate assays and ca. 650 insects exposed to each fungus.

Assays against *A. solani* and *M.persicae* (2010-2012):

In 2010-2012, *B. bassiana* (5493) and *M. anisopliae* (5471) were also tested against 12 ± 12 h-old nymphs of the aphid pest *A. solani* in a series of five-dose bioassays. As with the assays against *M. persicae* and *A. gossypii*, the commercially registered *M. brunneum* F52 and *B. bassiana* JW-1 were included for comparison. We chose to omit *B. bassiana* GHA due to its poor performance in the previous assays (see Results section). As in the assays with *M. persicae* and *A. gossypii* above, the novel isolates *B. bassiana* 5493 and *M. anisopliae* 5471 were first re-isolated through an aphid host (in this case, *M. persicae*). At the time of the assays with *A. solani*, *B. bassiana* JW-1 and *M. brunneum* F52 were widely used commercial products, thus we chose not to re-isolate these strains (similar to the decision made with *B. bassiana* GHA in the previous section).

Aqueous suspensions were prepared at starting concentrations designed to achieve ca. 1.5×10^4 conidia/mm^2. Lower doses were based on a series of four 2.5-fold

dilutions. Spray methodology was identical to trials with *M. persicae* and *A. gossypii* in 2004-2006, with the following exceptions: i) depending on the availability of *A. solani* first-instar nymphs during a replicate of the experiment, 7-21 nymphs were used per dish (vs. 15/dish for all replicate assays with *A. gossypii* and *M. persicae*), and ii) 5-10 replicate dishes per replicate experiment were sprayed with Silwet (0.01 %) as the carrier control (instead of 0.02% Tween). Additionally, the entire assay, including all four isolates tested simultaneously, was run a total of five times (with the exception of 2 assays that did not include *M. anisopliae* 5471).

Due to exceedingly poor results with the pathogen *B. bassiana* strain JW-1 against *A. solani* (see Results Section), we also chose to run a direct comparison of this isolate against *A. solani* and a new population of *M. persicae* (see Methods Section) to eliminate the chance that some change in the isolate between 2006 and 2010 had reduced its efficacy against all aphid species, rather than being a result specific to *A. solani*. A five-dose assay was conducted with identical methodology to that above (using embedded pansy leaves as the experimental arena for both aphids), with the exception that dishes of *A. solani* and *M. persicae* nymphs were placed under the spray tower at the same time for all doses. An initial weight of 3.33 mg/ml of *B. bassiana* strain JW-1 was used for the high dose, resulting in spore depositions ranging from 360-5499 conidia/mm^2.

Virulence against adult and immature stages of M. persicae, A. gossypii, and A. solani

Two isolates (*B. bassiana* strain GHA and *M. brunneum* strain F52, Table 3.1), both commercially registered as biopesticides in the United States, were used to compare virulence against adult and immature stages of aphids. All chosen concentrations were based on preliminary sprays that produced depositions of ca. 2500 conidia/mm^2. For *M. persicae* and *A. gossypii*, a total of three replicate plates containing 10-12 adults of each species and three replicate plates containing 15-17 nymphs were sprayed for each isolate. Additionally, three plates each of adults and nymphs were sprayed with 0.02% carrier controls (Tween). For each isolate, adults and nymphs were sprayed simultaneously. Methodology for assays with *A. solani* were identical with the following exceptions: due to a high percentage of control mortality seen in preliminary trials, we used 3 adult aphids per dish instead of 10, with a total of 10 replicate dishes (equaling the same number of aphids/assay as for the initial trials with *M. pericae* and *A. gossypii*), and Silwet (0.01%) was used as the carrier control. All assays were repeated on two different dates, for a total of two replicate assays per species. For all aphid and pathogen treatment combinations, leaf dishes were changed 24h after application, and again after 3 days. Mortality of all aphids was determined 6d after application.

Reproduction of infected aphids: M. persicae and A. gossypii

To determine effects of fungal infection on aphid fecundity, we treated adult aphids of both *M. persicae* and *A. gossypii* with the commercially registered products (*B. bassiana* strain GHA, *M. anisopliae* strain F52, or *I. javanica* strain PFR-97) and compared their reproduction to aphids treated with the carrier control only (Tween). *A.*

solani was not tested, as the other two aphid species tend to be more fecund, and therefore represent a more important target for reduction in offspring. Adult aphids were obtained as described previously and maintained on leaves (embedded in 60mm Petri dishes) of their respective host plants. Adults were 1-day old at the time of treatment and were placed in batches of 10 aphids/dish for treatment (with 2 dishes used per fungal treatment and 3 for the control treatment). After being sprayed with either a 'high' dose of fungi (range: 1249-1970 conidia/mm^2, depending on the fungal species) or a 'low' dose (range: 5-101 conidia/mm^2), 15 aphids per fungal treatment (30 for the control treatment) were randomly selected for immediate transfer to individual dishes containing fresh leaves. This was done in order to avoid contamination of any newly larviposited nymphs by spores on leaf surface. Dishes were sealed with Parafilm and placed in an incubator at 25°C. After 24h, the Parafilm was removed. Mortality and fecundity of each adult aphid was determined every 24h after treatment. Surviving aphids were transferred to fresh leaves daily, while those that had died were placed on water agar in order to check for sporulation; only aphids which had actually sporulated to confirm that death was from infection were used in the final data analysis. All nymphs produced were held for 6 d in order to ensure they were viable, and their survival rate was determined at this time. Survival and fecundity of adult aphids in the control treatment were recorded until all fungus-treated individuals had died.

Statistical Analyses

For the results from the single dose studies, treatment mortalities were corrected for control mortality using Abbott's formula (Abbott 1925), and mean percent mortalities

are presented. Given the variation in doses and the highly unbalanced experimental design of the primary screening tests, no statistical tests were performed on these data; data were compared qualitatively. Similarly, given the general lack of variation in the results against adult aphids, no statistical tests were conducted, but confidence intervals around the means are presented for comparison. To obtain more appropriate means and CIs, corrected data were transformed using the empirical logit model, as recent publications have suggested this is superior to the more commonly applied arcsine transformation (Warton and Hui 2011). Results presented are back-transformed. Given that samples sizes are needed to calculate the back-transformation of the mean of the empirical logit, we chose to apply the mean of the sample sizes for all replicates.

For multiple dose studies a probit analysis (PROC PROBIT; SAS v. 9.3; SAS 2011) was used to estimate slope and median lethal concentration (LC_{50}) values (in $\log_{10}$) for each isolate for each replicate assay. $LogLC_{25}$ values were also calculated because for some isolates maximal mortality was <50% and $logLC_{50}$ values were obtainable only via extrapolation. Treatment mortalities were corrected for control mortalities by the SAS program (OPTC option). Once values were obtained for each replicate assay, an ANOVA was conducted using PROC MIXED in SAS to calculate the mean $logLC_{50}$, mean $logLC_{25}$ and mean slopes across the trial replicates (see Wraight et al. 2010 for discussion of this procedure). The Kenward-Rodger method was used to calculate degrees of freedom. Trial replicate was included in the model as a random effect to control for trial-to-trial differences, and accounted for a similar amount of total variation in the analyses for all three aphid species (20-28% for the ANOVAs on LC_{50} values). The Tukey-Kramer test was used to test for differences among isolates

in lethal concentrations and slopes. Rigorous comparisons among aphid species were not made because the aphids were generally tested at different times. Least-squares (LS) means of the lethal concentrations (back-transformed) and slopes of the dose-responses are presented to account for the unbalanced design due to different numbers of replicate assays for different isolates. Log_{10} of the lethal concentrations, including the standard errors, are presented in parentheses.

In the case of assessing reproduction of infected and non-infected adult aphids, analysis was performed using JMP version 10 (SAS Institute 2012). Length of survival and length of the reproductive periods of infected aphids were not normally distributed, thus data were rank-transformed and the ANOVA was conducted on ranks. Because testing of interactions following rank transformation may be problematic (Zar 1999), data were analyzed with and without rank transformation and results were compared as recommended by Conover (1999). In each case the hypothesis test results from the alternative tests did not differ, and results from the standard ANOVA are reported.

Numbers of total offspring produced per infected aphid were $\log_{10}$ (n+1) transformed to stabilize variance prior to ANOVA. Effects of disease progression on daily aphid reproduction were assessed by mixed-model ANOVA of the differences in offspring production between fungus-treated and untreated aphids—no data transformation was needed to stabilize residuals in this analysis. The daily numbers of offspring produced by each treated aphid were compared directly to the corresponding daily numbers produced by a randomly selected control aphid. As total replicates for analysis were limited by overall numbers of treated and control aphids available for independent pairing, we used aphids treated with low doses of the fungi as additional

control insects since they did not exhibit signs of fungal infection and their life spans and reproduction did not differ from control insects. Offspring production on the day of death of an infected aphid was roughly estimated by assuming death at the midpoint of the final daily observation period and a constant rate of reproduction throughout the day. Based on these assumptions, offspring production by each treated aphid recorded on the day of death was compared to the same-day production by the paired control aphid divided by two (a half day of reproduction).

Repeated measures of reproduction by each aphid were accounted for in the analysis by inclusion of treated vs. control aphid pair as a random effect. A preliminary analysis in the greater SAS system revealed that compound symmetry (assumed by JMP) was an acceptable covariance structure (see Littell et al. 2006). Mean differences were determined for each treatment combination. Baseline numbers of offspring (numbers produced by the healthy control aphids) were included in the overall ANOVA as a random covariate to account for the unequal offspring production between aphid species over time. A preliminary analysis confirmed the fundamental covariance assumption of equal regression slopes among treatment groups; there was no significant treatment group x covariate interaction ($F_{35,190.9} = 1.1$, $P = 0.35$). The final mixed model was as follows: aphid species, fungal species, dose, and time before death as fixed effects (with only ≤3 days before death included, as there was no discernible effect at earlier time points). Aphid pair (nested within aphid species) and the baseline numbers of offspring were the random effects. Difference in offspring production between paired control and treated aphids was the dependent variable. In presenting results, differences

were ultimately expressed as percent change (increase or decrease) in offspring production relative to the controls.

RESULTS

Single dose screen against immature aphids: M. persicae and A. gossypii

Information on the identity and origin of each fungal isolate/strain are presented in Table 3.1. Control mortalities in the initial screening assays ranged from 0–23% across all replicate assays (avg. = 6.2%). Average Abbott's corrected mortality of *M. persicae* was < 62 % from all *Beauveria* isolates, < 47% from *Metarhizium* and < 24% from *Isaria* isolates (Table 3.2). Isolates causing nominally highest mortality of *M. persicae* were *Beauveria* 5493, *Metarhizium* 2517, and *Isaria* 2749. Average mortality of *A. gossypii* caused by all isolates of each of the respective genera was < 57%, < 49%, and < 31%. Isolates causing highest mortality of *A. gossypii* were *Beauveria* 5494, *Metarhizium* 3738 and *Isaria* 2749.

Mortalities were quite variable within isolates, as evidenced by the relatively large standard errors (Table 3.2). Additionally, the dose received by test aphids varied considerably among isolates, in part because of variation in spore viability (Table 3.2). Viability was greatest for *Beauveria* isolates, with 13 of 20 of these showing > 90% viability, whereas spores of *Metarhizium* (viabilities of 62–95%) and *Isaria* (viabilities of 59–95%) lost viability more readily under the described handling conditions. It warrants note that these tests were conducted prior to our knowledge of the susceptibility of dried *Metarhizium* conidia to imbibitional damage (Faria et al. 2009), which likely contributed to the low viability of some isolates.

Table 3.1. Fungal strains/isolates included in single-dose screening assays against *Aphis gossypii* and *Myzus persicae*.

Isolate/strain (current holding)	Species identification[a]	Host origin	Geographic origin and date of isolation
		Beauveria bassiana sensu lato	
252 (ARSEF)	*B. bassiana*	Coleoptera: Chrysomelidae: *Leptinotarsa decemlineata*	USA, Maine, 1978
717 (ARSEF)	–	Hemiptera: Delphacidae: *Nilaparvata lugens*	China, Hubei, 1981
2336 (ARSEF)	–	Hemiptera: Aphididae: *Schizaphis graminum*	USA, Idaho, 1986
2402 (ARSEF)	–	Hymenoptera: Formicidae: *Solenopsis invicta*	Brazil, Mato Grosso, 1986
2430 (ARSEF)	–	Hemiptera: Lygaeidae: *Blissus leucopterus*	USA, Kansas, 1980
2880 (ARSEF)	–	Hemiptera: Aphididae: *S. graminum*	USA, Idaho, 1986
2882 (ARSEF)	–	Hemiptera: Aphididae: *Diuraphis noxia*	USA, Idaho, 1986
3167 (ARSEF)	–	Hemiptera: Aphididae: *D. noxia*	Turkey, Ankara, 1988
3285 (ARSEF)	–	Hymenoptera: Formicidae: *Atta* sp.	Mexico, Monterrey, 1987
3528 (ARSEF)	*B. pseudo-bassiana*	Lepidoptera: Lymantriidae: *Lymantria dispar*	USA, Pennsylvania, 1991
3543 (ARSEF)	–	Lepidoptera: Pyralidae: *Galleria mellonella*	USA, Vermont, 1991
4100 (ARSEF)	–	Hemiptera: Aphididae: *D. noxia* (ARSEF 2879) [b]	USA, Idaho, 1988
4523 (ARSEF)	–	Hemiptera: Aphididae	USA, New York, 1994
5493 (ARSEF)	–	Hemiptera: Aphididae: *Aphis gossypii*	USA, Pennsylvania, 1997
5494 (ARSEF)	–	Hemiptera: Aphididae: *A. gossypii*	USA, Pennsylvania, 1997
5705 (ARSEF)	–	Hemiptera: Aphididae: *D. noxia*	South Africa, W. Cape, 1997
5978 (ARSEF)	–	Hemiptera	USA, Florida, 1988
7060 (ARSEF)	–	Lepidoptera: Pyralidae: *G. mellonella*	USA, California, 2001
GHA (Larverlam)	*B. bassiana*	Coleoptera: Chrysomelidae, *Diabrotica undecimpunctata* (ARSEF 201) [b, c]	USA, Oregon, 1977
JW-1 (Troy Bio Sciences)	*B. bassiana*	Coleoptera: Curculionidae: *Anthonomous grandis* [d]	USA, Texas

Table 3.1 (Continued)

Isolate/strain (current holding)	Species identification [a]	Host origin	Geographic origin and date of isolation
		***Metarhizium anisopliae* sensu lato**	
683 (ARSEF)	*M. guizhouense*	Coleoptera: Scarabaeidae	China, Guangdong, 1981
727 (ARSEF)	*M. robertsii*	Orthoptera: Tettigoniidae	Brazil, Goias, 1982
759 (ARSEF)	–	Hemiptera: Cercopidae: *Deois flavopicta*	Brazil, Goias, 1982
2106 (ARSEF)	*M. pingshaense*	Hemiptera: Cicadellidae: *Nephotettix virescens*	Indonesia, West Java, 1986
2153 (ARSEF)	*M. anisopliae*	Hemiptera: Cicadellidae: *N. virescens*	Indonesia, Sulawesi Selatan, 1986
2421 (ARSEF)	*M. anisopliae*	Hemiptera: Delphacidae: *N. lugens*	Indonesia, West Java, 1987
2517 (ARSEF)	*M. anisopliae*	Hemiptera: Cercopidae: *D. flavopicta*	Brazil, Joquapita, 1987
3307 (ARSEF)	–	Hemiptera: Cercopidae	Mexico, Colima, 1990
3340 (ARSEF)	–	Coleoptera: Scarabaeidae: *Popillia japonica*	USA, New York, 1989
3738 (ARSEF)	*M. brunneum*	Hymenoptera: Formicidae: *S. invicta*	USA, Texas, 1992
3822 (ARSEF)	*M. anisopliae*	Hemiptera: Cicadellidae: *N. virescens* (ARSEF 2153) [b]	Indonesia, Sulawesi Selatan, 1986
4123 (ARSEF)	*M. robertsii*	Coleoptera: Scarabaeidae, *Rhizotrogus majalis*	USA, New York, 1994
4556 (ARSEF)	–	Acari: Ixodidae: *Boophilus* sp.	USA, Florida, 1993
4824 (ARSEF)	–	Coleoptera: Curculionidae: *Otiorhynchus ligustici*	USA, New York, 1994
4901 (ARSEF)	–	Hemiptera	Location not recorded, 1995
5197 (ARSEF)	*M. pingshaense*	Coleoptera: Curculionidae: *Diaprepes abbreviata*	USA, Florida, 1996
5471 (ARSEF)	*M. anisopliae*	Lepidoptera: Pyralidae: *Eoreuma loftini*	USA, Texas, 1997
5624 (ARSEF)	–	Coleoptera: Tenebrionidae: *Tribolium castaneum*	Finland, Uusimaa, 1985
F52 (Novozymes)	*M. brunneum*	Lepidoptera: Tortricidae: *Cydia pomonella*	Austria
		***Isaria javanica* sensu lato**	
614 (Laverlam)	–	Hemiptera: Aleyrodidae: *Bemisia tabaci*	USA, Texas, 1993
2749 (ARSEF)	–	Lepidoptera: Plutellidae: *Plutella xylostella*	Philippines, Benguet, 1989
3889 (ARSEF)	–	Hemiptera: Aleyrodidae: *B. tabaci*	USA, Hawaii, 1993
4459 (ARSEF)	–	Hemiptera: Aleyrodidae: *B. tabaci* (ARSEF 3699) [b]	India, Tamil Nadu, 1992
4461 (ARSEF)	–	Hemiptera: Aleyrodidae: *B. tabaci* (ARSEF 3699) [b]	India, Tamil Nadu, 1992
4482 (ARSEF)	–	Hemiptera: Aleyrodidae: *B. tabaci* [e]	Nepal, Kathmandu, 1992
4491 (ARSEF)	–	Hemiptera: Aleyrodidae: *B. tabaci*	India, Tamil Nadu, 1992
5462 (ARSEF)	–	Hemiptera: Aleyrodidae: *B. tabaci*	USA, Texas, 1993
Apopka Strain 97 (Certis)	*I. javanica*	Hemiptera: Pseudococcidae: *Phenacoccus solani*	USA, Florida

Table 3.1 (Continued)

[a] Species determinations based on available nucleotide sequencing data or identification in the ARFEF catalog as sensu stricto.

[b] Original isolate from which the test isolate was derived.

[c] Strain GHA was originally isolated from ARSEF 201 (Bradley et al. 2001), but recent molecular analysis indicates that GHA and the currently held ARSEF 201 are distinct (L.A. Castrillo, pers. comm.).

[d] Molecular analysis indicates that strain JW-1 is very similar to ARSEF 252 (L.A. Castrillo, pers. comm.)

Table 3.2. Mortality (%) for *B. bassiana*, *M. anisopliae* and *I. javanica* (sensu lato). isolates screened against 1st instar (12±12 h old) *M. persicae* and *A. gossypii*.

Isolate	Avg. % Viability ±SE	Avg. Dose ±SE (conidia/mm^2)	*Myzus persicae* No. Assays	*Myzus persicae* No. Aphids	*Myzus persicae* Avg. % Mortality (±SE)[a]	*Aphis gossypii* No. Assays	*Aphis gossypii* No. Aphids	*Aphis gossypii* Avg. % Mortality (±SE)[a]
			Beauveria bassiana sensu lato					
5493	97.2 ± 0.7	2121 ± 142	2	130	61.6 ± 10.8	2	148	55.6 ± 7.2
5494	81.2 ± 4.4	1591 ± 33	2	138	59.4 ± 6.9	2	143	56.9 ± 10.4
3543	87.7 ± 1.9	1667 ± 129	2	134	51.2 ± 11.7	2	131	29.9 ± 9.3
JW-1[b]	**98.7 ± 0.8**	**1883 ± 17**	**2**	**133**	**31.0 ± 3.8**	**2**	**146**	**39.0 ± 5.6**
2430	97.3 ± 1.1	2017 ± 64	2	147	26.6 ± 5.3	2	143	25.1 ± 6.2
252	95.0 ± 1.0	3030 ± 129	2	132	22.0 ± 4.9	2	140	25.6 ± 7.2
GHA	**96.8 ± 0.4**	**1115 ± 87**	**9**	**601**	**21.7 ± 3.0**	**9**	**648**	**17.6 ± 3.0**
4100	94.6 ± 2.1	1748 ± 204	3	187	20.4 ± 6.3	3	232	1.38 ± 0.4
3285	93.3 ± 1.3	1333 ± 3	2	137	19.1 ± 5.0	2	149	24.0 ± 4.9
5705	93.5 ± 0.7	2549 ± 230	2	134	16.8 ± 4.7	2	159	10.6 ± 3.1
7060	97.0 ± 10	2396 ± 262	2	128	16.5 ± 5.5	2	144	12.3 ± 6.9
3167	81.3 ± 2.6	1458 ± 68	2	139	16.4 ± 7.1	2	144	29.7 ± 5.9
5978	93.2 ± 2.0	2684 ± 113	2	143	15.1 ± 4.7	2	151	15.7 ± 5.5
717	86.8 ± 3.0	1455 ± 21	2	150	13.1 ± 4.1	2	147	8.4 ± 4.1
2880	89.7 ± 1.9	1562 ± 202	2	142	11.6 ± 5.4	2	141	13.4 ± 2.5
2336	95.3 ± 1.1	1397 ± 29	2	139	10.5 ± 4.4	2	140	10.7 ± 4.5
4523	93.8 ± 1.5	2230 ± 246	2	150	8.8 ± 2.7	2	139	14.6 ± 6.1
2882	96.3 ± 0.5	1167 ± 93	2	126	6.4 ± 2.3	2	143	14.6 ± 4.2
2402	88.3 ± 1.7	947 ± 37	2	135	5.1 ± 2.7	2	148	3.0 ± 1.9
3528	77.0 ± 3.5	2407 ± 239	2	149	3.8 ± 0.9	2	139	5.5 ± 3.6
			Metarhizium anisopliae sensu lato					
2517	87.3 ± 3.2	1285 ± 60	2	132	46.9 ± 5.5	2	145	21.1 ± 8.1
759	83.5 ± 5.4	1107 ± 94	2	143	37.0 ± 6.4	2	154	26.2 ± 8.9
5471	89.4 ± 4.0	887 ± 104	2	150	33.1 ± 11.3	2	124	37.7 ± 9.8
F52	**88.7 ± 2.3**	**1176 ± 109**	**7**	**466**	**27.6 ± 5.1**	**7**	**518**	**38.5 ± 5.4**
3738	94.7 ± 1.7	1476 ± 251	2	131	26.1 ± 6.4	2	149	48.2 ± 10.1
4901	62.0 ± 4.7	774 ± 27	2	124	21.8 ± 7.0	2	126	14.0 ± 7.4
2421	95.0 ± 0.8	1210 ± 60	2	145	18.7 ± 4.9	2	147	25.7 ± 9.6
4556	76.9 ± 3.5	1342 ± 78	2	131	17.1 ± 6.6	2	138	30.6 ± 9.0
3307	68.8 ± 1.9	599 ± 20	2	109	12.5 ± 2.7	2	136	12.8 ± 3.4
3822	68.7 ± 2.8	678 ± 126	2	141	11.9 ± 5.0	2	144	18.3 ± 5.0
683	62.4 ± 4.0	503 ± 6	2	135	9.6 ± 4.6	2	154	24.2 ± 6.5
5197	89.8 ± 2.8	871 ± 55	2	138	7.3 ± 1.4	2	130	20.6 ± 0.4
5624	84.1 ± 3.5	1364 ± 59	2	131	5.9 ± 2.8	2	126	20.1 ± 7.4

Table 3.2 (Continued)

Isolate	Avg. % Viability ±SE	Avg. Dose ±SE (conidia/mm^2)	*Myzus persicae*			*Aphis gossypii*		
			No. Assays	No. Aphids	Avg. % Mortality (±SE)[a]	No. Assays	No. Aphids	Avg. % Mortality (±SE)[a]
Isaria javanica sensu lato								
2749	89.4 ± 1.1	837 ± 120	2	132	23.2 ± 7.6	2	146	30.1 ± 6.4
3889	68.0 ± 10.2	1305 ± 2.5	2	161	11.3 ± 3.1	2	157	11.7 ± 3.7
4459	89.5 ± 0.6	1295 ± 3	2	131	10.8 ± 5.4	2	149	23.7 ± 7.9
4461	84.0 ± 6.1	808 ± 4	2	150	6.1 ± 2.3	2	151	11.2 ± 4.1
612	59.2 ± 7.8	1049 ± 244	2	141	4.1 ± 2.4	2	155	5.1 ± 2.3
4491	88.3 ± 4.2	1291 ± 12	2	140	3.0 ± 2.0	2	147	10.3 ± 3.9
4482	95.3 ± 0.9	1876 ± 20	2	149	2.0 ± 1.4	2	154	19.5 ± 6.8
PFR97	**89.5 ± 1.2**	**1576 ± 120**	**4**	**281**	**1.5 ± 0.7**	**4**	**281**	**12.7 ± 3.3**
614	73.7 ± 3.9	585 ± 64	2	142	1.0 ± 0.7	2	281	8.1 ± 2.3

[a] Data are corrected for control mortality (aphids sprayed with 0.02% Tween) using Abbott's formula. Isolates sorted from highest to lowest mortality of *M. persicae*, regardless of dose of conidia sprayed.

[b] Commercially available isolates are indicated in bold.

The results of this screening assay led to the choice of isolates in the LC_{50} trials. *Beauveria* isolate 5493 was chosen, as it was highly effective against both aphid species (62% mortality of *M. persicae* and 56% mortality of *A. gossypii*), although 5494 (likely of the same genotype) would have been an equally valid choice. From the *Metarhizium* strains, 5471 was selected, as it exhibited comparably high activity against both *M. persicae* and *A. gossypii* (33 and 38% mortality). *Metarhizium* 3738, which was nominally most virulent against *A. gossypii*, produced only 26% mortality of *M. persicae*, while 2517, which showed greatest virulence against *M. persicae*, produced only 21% mortality of *A. gossypii*. It seems noteworthy that among isolates identifiable to species, the highest-ranking isolates against both aphids were *M. brunneum* and *M. anisoplae*. *Isaria* isolates were omitted from further testing due to their relatively poor performance.

Multiple dose bioassays of selected isolates: M. persicae, A. gossypii and A. solani

Beauveria 5493 and *M. anisopliae* 5471 were selected for head-to-head comparisons against commercially registered strains. The high doses used in these assays were the maximum that could be readily suspended, sprayed, and quantified using the described methods and equipment. These ranged from ca. 3,500 to > 20,000 viable conidia/mm^2 (Table 3.3). Despite this, maximal mortality for some of the isolates tested (particularly *B. bassiana* strain GHA) was < 50%, meaning that estimates of LC_{50} were obtainable only via extrapolation. Consequently, estimates of LC_{25} were also used to compare isolates (Table 3.3). Control mortalities were < 20% in all cases (average % control mortality = 4%).

With respect to assays against *M. persicae*, although LC_{50} varied significantly among isolates ($F_{4, 11.2} = 4.2$, $P = 0.027$), a significant difference was observed only between *Beauveria* 5493 (the most virulent isolate) and GHA (the least virulent) (Table 3.3). LC_{25} also varied significantly among isolates ($F_{4, 11} = 6.0$, $P = 0.008$), with all isolates other than *M. anisopliae* 5471 being significantly more virulent than *B. bassiana* strain GHA. In assays against *A. gossypii,* LC_{50} ($F_{4, 10.9} = 18.0$, $P < 0.0001$) and LC_{25} ($F_{4, 14} = 24.9$, $P < 0.0001$) varied significantly with isolate as well. Unlike the results for *M. persicae*, *B. bassiana* strain JW-1 was the most virulent strain against this aphid — more virulent than all other fungi except *Beauveria* 5493 (Table 3.3). Again, *B. bassiana* GHA was again the poorest performing strain.

In the assays comparing *B. bassiana* strain GHA in its original form (as was used for the results above) vs. a single spore reisolate (passed through an aphid host), there was no indication that aphid passage increased virulence (Table 3.4). With respect to the LC_{25} and LC_{50} values, there was no statistical effect of aphid passage, aphid species or their interaction ($F_{1,8} < 4.67$, $P > 0.063$ for all tests). In the case of the slope of the dose response, aphid passage and its interaction with aphid species were again non-significant ($F_{1,8} < 0.71$, $P > 0.43$ for all tests), though there was a significant main effect of aphid species ($F_{1,8} < 7.71$, $P = 0.03$). The steeper average slope for both forms of GHA against *M. persicae* (1.75 ± 0.28) suggests this species may be generally more susceptible than *A. gossypii* (avg. slope = 0.86 ± 0.28) to this strain. However, this was not necessarily reflected in dose response assay presented in Table 3.3.

Table 3.3. LC_{50} and LC_{25} estimates from 5-dose bioassays against 1st instar nyphs of A. *Myzus persicae*, B. *Aphis gossypii* and C. *Aulacorthum solani* exposed to isolates of *B. bassiana* and *M. anisopliae* (sensu lato). Isolates are compared within each species only.

Isolate	No. assays[a]	Max. mortality achieved (dose)	No. insects	Concentration range[b]	Back-transformed mean LC_{25} ($LogLC_{25} \pm SE$)[c]		Back-transformed mean LC_{50} ($LogLC_{50} \pm SE$)[c]		LS mean slope ± SE		X^2 range[d]
A.					*Myzus persicae* (12 ± 12 h old)						
Beauveria bassiana s. l.											
ARSEF 5493	4	91% (16,155)	888	26 - 17,741	479 (2.680 ± 0.2395)	a	2286 (3.359 ± 0.2312)	a	1.03 ± 0.262	a	2.13 - 3.91
JW-1	4 (1)	100% (21,954)	954	32 - 21,954	614 (2.788 ± 0.2395)	a	4121 (3.615 ± 0.2312)	ab	0.88 ± 0.262	a	3.88 - 20.83
GHA	4 (3)	56% (9,100)	890	11 - 13,904	5047 (3.703 ± 0.2395)	b	24,889 (4.396 ± 0.2312)	b	1.31 ± 0.262	a	0.36 - 6.87
Metarhizium anisopliae s.l.											
ARSEF 5471	4 (3)	53% (16,258)	917	3 - 21,648	1603 (3.205 ± 0.2395)	ab	12,106 (4.083 ± 0.2312)	ab	0.88 ± 0.262	a	0.79 - 8.00
F52	3	94% (18,323)	667	7 - 18,323	593 (2.773 ± 0.2647)	a	8492 (3.929 ± 0.2652)	ab	0.68 ± 0.302	a	1.22 - 12.32
B.					*Aphis gossypii* (12 ± 12 h old)						
Beauveria bassiana s. l.											
ARSEF 5493	4	86% (10,069)	894	10 - 16,372	201 (2.304 ± 0.1151)	ab	944 (2.975 ± 0.1677)	ab	1.05 ± 0.208	a	4.54 - 17.78
JW-1	4	98% (17,244)	894	21 - 20,528	130 (2.113 ± 0.1151)	a	726 (2.861 ± 0.1677)	a	1.01 ± 0.208	a	2.56 - 8.57
GHA	4 (4)	37% (13,904)	886	6 - 13,904	4009 (3.603 ± 0.1151)	c	19,907 (4.299 ± 0.1677)	c	1.23 ± 0.208	a	1.09 - 4.11
Metarhizium anisopliae s.l.											
ARSEF 5471	3	64% (12,466)	695	7 - 12,466	519 (2.715 ± 0.1329)	b	4037 (3.606 ± 0.1923)	bc	0.73 ± 0.237	a	0.14 - 7.16
F52	4 (1)	76% (10,057)	927	5 - 10,057	501 (2.700 ± 0.1151)	b	7261 (3.861 ± 0.1677)	c	0.70 ± 0.208	a	0.50 - 8.21
C.					*Aulacorthum solani* (12 ± 12 h old)						
Beauveria bassiana s. l.											
ARSEF 5493	5 (1)	69% (12,001)	1415	115-12,001	1062 (3.026 ± 0.1314)	b	4150 (3.618 ± 0.2148)	ab	1.21 ± 0.135	b	3.68-6.68
JW-1	5 (5)	23% (8,456)	1231	257-14,239	Not calculable	–	Not calculable	–	Not calculable	–	Not calculable
Metarhizium anisopliae s.l.											
ARSEF 5471	3	87% (3,493)	834	141-4569	82 (1.915 ± 0.1652)	a	1033 (3.014 ± 0.2870)	a	0.61 ± 0.180	a	2.11-8.77
F52	5 (4)	64% (8,685)	1319	67-10,298	637 (2.804 ± 0.1314)	b	10,093 (4.004 ± 0.2148)	b	0.69 ± 0.135	ab	0.18-12.42

[a] Number in parentheses indicates number of assays in which maximum mortality was < 50% and consequently estimated LC_{50} values are a projection. No parantheses included for those isolates in which maximum mortality always exceeded 50%.
[b] Range of concentrations (viable conidia/mm^2).
[c] Lethal concentrations estimated from replicated five-rate bioassays (20-54 aphid nymphs per rate). Mortality was recorded after 6 days incubation at 25°C for *M. persicae* and *A. gossypii*, and 7 days for *A. solani*.
[d] Heterogeneity χ^2 value with 2 or 3 degrees of freedom from probit analysis.

Table 3.4. Comparison of *Beauveria bassiana* strain GHA in its original, technical powder form vs. as a single spore resisolate from an aphid host (*A. gossypii*) against 1st instar nymphs (12 ± 12h old) of *M. persicae* and *A. gossypii*.

Isolate	No. assays[a]	No. insects	Concentration Range[b]	Back-transformed LS mean LC_{25} ($LogLC_{25}$ ± SE)[c]	Back-transformed LS mean LC_{50} ($LogLC_{50}$ ± SE)[c]	LS mean Slope ± SE	X^2 range[d]
				Myzus persicae			
GHA	3 (2)	667	14 - 6,584	4624 (3.665 ± 0.2557) a	14,093 (4.149 ± 0.3238) a	2.00 ± 0.48 a	2.02 - 3.07
GHA reisolate	3 (3)	626	36 - 13, 069	10,765 (4.032 ± 0.2557) a	31,333 (4.496 ± 0.3238) a	1.51 ± 0.48 a	1.22 - 3.40
				Aphis gossypii			
GHA	3 (2)	645	14 - 6,584	1795 (3.254 ± 0.2358) a	12,735 (4.105 ± 0.3294) a	0.89 ± 0.16 a	0.06 - 12.23
GHA reisolate	3 (2)	638	36 - 13, 069	8906.4 (3.950 ± 0.2358) a	75,336 (4.877 ± 0.3294) a	0.84 ± 0.16 a	2.95 - 6.41

[a] Number in parentheses indicates number of assays in which maximum mortality was < 50% and consequenjtly estimated LC^{50} value is a projection.
[b] Range of concentrations (viable conidia/mm^2)
[c] Lethal concentrations estimated from replicated five-rate bioassays (42-48 aphid nymphs per rate). Mortality was recorded after 6 days of incubation at 25°C
[d] Heterogeneity X^2 value with 3 degrees of freedom given by probit analysis.

For the pest aphid *A. solani*, LC_{25} again varied significantly with isolate ($F_{2, 6.5}$ = 21.7, P = 0.0013), although isolate was only weakly significant when it came to the LC_{50} ($F_{2, 7.2}$ = 4.5, P= 0.055). Tukey-Kramer pair-wise comparisons supported the conclusion of weak significance (Table 3.3). With this aphid species, *M. ansiopliae* 5471 was the most virulent isolate at the lower end of the dose responses (LC_{25}); however, *Beauveria* 5493 produced a greater regression coefficient (slope), and virulence of the two isolates did not differ at the LC_{50}. Unlike the assays with the previous two aphid species, *B. bassiana* strain JW-1 was the poorest performing strain (though GHA was not tested against this aphid), causing extremely low mortalities against *A. solani*, even at high doses (>14,000 spores/mm^2). It was not possible to calculate even an LC_{25} for this strain. The direct comparison of *B. bassiana* strain JW-1 against *A. solani* and *M. persicae* nymphs simultaneously in 2012 confirmed that this result was due to differences in aphid susceptibility (Table 3.5), rather than differences in the conidial powders tested during 2010-2012. The resulting LC_{50} and slope of *B. bassiana* strain JW-1 against *M. persicae* in 2012 was was similar to the results from 2004-2006 (4121 conidia/mm^2, slope = 0.88; Table 3.3), and once again, an LC_{50} could not be calculated for *A. solani* due to low mortalities at the same doses (max. 15%).

Slope estimates for the dose relationships against all three aphid species ranged from 0.61 to 1.31 (Table 3.3). Estimates generally did not vary significantly among isolates within aphid species ($F_{4, 14}$ = 0.71, *P* = 0.60 for *M. persicae*; $F_{4, 10.9}$ = 1.6, *P* = 0.25 for *A. gossypii*) except in the case of *A. solani*, with the above-noted significant effect of isolate ($F_{2,6.6}$ = 6.7 P = 0.026) (Table 3.3). Interestingly, if slope is analyzed

Table 3.5. Single assay directly comparing the virulence of *B. bassiana* strain JW-1 (Naturalis®) against 1st instar nymphs (12 ± 12h old) of *M. persicae* and *A. solani*.

Aphid species	Max. Mortality Achieved (Dose)	No. Assays (No. Insects)	Concentration Range[a]	Back-transformed mean LC_{50} ($LogLC_{50}$)	LS Mean Slope
M. persicae	61% (5,449)	1 (214)	329-5,449	2553 (3.407)	0.9
A. solani	15% (5,449)	1 (132)	329-5,449	Not calculable	Not calculable

[a] Viable conidia/mm^2

over all assays with *M. persicae* and *A. gossypii*, a significant difference can be seen for fungal species ($F_{1,34}$ = 4.3, P= 0.046), with *B. bassiana* strains having a steeper mean slope (1.08 ± 0.09) than *M. anisopliae* strains (0.77 ± 0.11). This was also true with the inclusion of the *A. solani* data ($F_{1,45}$ = 10.2, P=0.0026), but with the important caveat that this analysis excludes *B. bassiana* strain JW1, which exhibited such low virulence against this aphid that slopes were not calculable.

Virulence against adult and immature stages: M. persicae, A. gossypii and A. solani

Nymphal control mortality ranged from 3–21% across all treatments (mean 10.3%). Adult control mortality was higher, at 5.0 - 33.3% (mean 18.7 %), although the incident of > 30% control mortality occurred in just 1 replicate of 1 aphid species (*A. gossypii*). Adult control mortality was lowest for *A. solani* adults (avg. = 5%); the lower density per dish (3 individuals vs. 10) for this species may have contributed to better survival. Despite the high control mortality (and the lower doses achieved with *A. solani*), similar

trends were evident for all three aphid species. Corrected mortality of adults was greater than for first-instar nymphs in all fungal species/aphid species combinations tested (Table 3.6). Mortality of first-instar nymphs was a minimum of 35% lower than for adults, with the average mortality being 51% lower across all treatments.

Reproduction of infected aphids: M. persicae and A. gossypii

Overall effects of fungal infection on aphid reproduction are presented in Table 3.7. Untreated *M. persicae* and *A. gossypii* produced equal numbers of total offspring (59.5 vs. 58.3), but *A. gossypii* produced these offspring in a shorter time (9.8 vs. 13.7 days; $F_{1,99} = 29.0$, $P < 0.0001$). Initial analysis revealed a significant interaction between fungal species and dose as factors affecting mean offspring of infected aphids ($F_{2,117} = 5.0$, $P = 0.008$), thus data from the high vs. low dose categories were analyzed separately. In all subsequent within-dose analyses of all responses (survival time, reproductive period, and offspring production), we found no significant interactions between fungal species and aphid species (mean P value = 0.51, range = 0.15–0.88), and the insignificant interaction term was removed from each model.

At low doses, responses of the two aphid species did not differ among the fungal species (no significant main effect of fungal species on survival time ($F_{2,42} = 1.9$, $P = 0.16$), reproductive period ($F_{2,42} = 2.3$, $P = 0.12$), or total offspring production ($F_{2,42} = 1.4$, $P = 0.25$)). Across fungal species, *M. persicae* succumbed to infection in shorter time than *A. gossypii* (LS mean = 6.8 vs. 8.2 days; $F_{1,42} = 4.9$, $P = 0.032$), and infection had a greater negative effect on total offspring production by *M. persicae* than *A.*

Table 3.6. Comparative virulence of two isolates against 1-2 day old adults and 1st instar nymphs (12 ± 12h old) of *M. persicae*, *A. gossypii* and *A. solani*.

Isolate	Adults				Nymphs			
	No. assays	No. Insects	Avg. Dose ± SE (conidia/mm^2) [b]	Avg. % Mortality ± CI[a]	No. assays	No. Insects	Avg. Dose ± SE (conidia/mm^2) [b]	Avg. % Mortality ± CI[a]
				Myzus persicae				
B. bassiana GHA	2	63	2599 ± 170	100 (99.4-100%)	6	96	2599 ± 170	30.4 (14.8-51.2%)
M. brunneum F52	2	55	2759 ± 15	99.8 (98.0-100%)	6	94	2759 ± 15	43.6 (30.4-57.5%)
				Aphis gossypii				
B. bassiana GHA	2	73	2599 ± 170	100 (99.5-100%)	6	96	2599 ± 170	49.1 (32.2-66.3%)
M. brunneum F52	2	76	2759 ± 15	98.4 (92.6-100%)	6	90	2759 ± 15	52.2 (19.3-77.6%)
				Aulacorthum solani				
B. bassiana GHA	2	60	1996 ± 163	70.9 (51.4-87.1%)	6	77	1996 ± 163	29.2 (19.3-41.1%)
M. brunneum F52	2	60	1994 ± 143	99.9 (99.0-100%)	6	83	1994 ± 143	67.4 (44.6-84.8%)

[a] Mortality corrected for control mortality using Abbott's formula; data back-transformed from the empirical logit transformation (using the average n across replicates).
[b] Viable conidia/mm^2

Table 3.7. Effects of fungal infection on aphid survival and reproduction.

Treatment	Dose (conidia per mm^2)	Percent infected (n) [a]	Post-treatment survival time (days) [b, c, d]	Reproductive period (days) [b, c, d]	Total offspring per aphid [b, c, d] (% reduction)
Controls					
Myzus persicae	0	0 (43)	16.1 ± 0.92	13.7 ± 0.69	58.3 ± 2.43
Aphis gossypii	0	0 (58)	22.3 ± 0.82	9.8 ± 0.35	59.5 ± 1.63
High doses					
Myzus persicae					
Bb GHA	1491	100 (15)	3.3 ± 0.14 a	3.0 ± 0.17 a	18.0 ± 1.07 (69.1) ab
Mb F52	1323	100 (14)	3.2 ± 0.29 a	2.8 ± 0.24 a	15.1 ± 1.44 (74.1) a
Ij PFR-97	1701	100 (13)	4.1 ± 0.21 b	3.7 ± 0.15 b	20.3 ± 1.00 (65.2) b
LS Mean	–	–	3.6 [3.2-3.9] A	3.2 [2.9-3.4] A	17.2 [15.9-18.6] (70.5) A
Aphis gossypii					
Bb GHA	1249	80.0 (15) [e]	3.5 ± 0.17 a	3.3 ± 0.13 a	30.5 ± 1.59 (48.7) ab
Mb F52	1286	100 (15)	3.6 ± 0.15 a	3.0 ± 0.17 a	25.8 ± 1.87 (56.6) a
Ij PFR-97	1970	93.3 (15)	5.2 ± 0.55 b	4.7 ± 0.43 b	38.3 ± 3.04 (35.6) b
LS Mean			4.1 [3.8-4.4] B	3.7 [3.4-4.0] B	30.3 [28.0-32.7] (49.1) B
Low doses					
Myzus persicae					
Bb GHA	9	73.3 (15)	7.4 ± 0.53 a	7.1 ± 0.49 a	37.2 ± 2.35 (36.2) a
Mb F52	10	86.7 (15)	6.2 ± 0.62 a	5.5 ± 0.58 a	30.8 ± 3.18 (47.2) a
Ij PFR-97	96	35.7 (14)	6.7 ± 1.24 a	6.5 ± 1.34 a	37.0 ± 7.18 (36.5) a
LS Mean			6.8 [5.9-7.6] A	6.4 [5.6-7.1] A	32.4 [28.8-36.3] (44.3) A
Aphis gossypii					
Bb GHA	5	28.6 (14)	9.3 ± 1.55 a	8.0 ± 0.65 a	53.5 ± 2.96 (10.1) a
Mb F52	7	73.3 (15)	7.5 ± 0.54 a	7.0 ± 0.58 a	51.1 ± 3.18 (14.1) a
Ij PFR-97	101	13.3 (15)	8.0 ± 1.5 a	7.5 ± 2.0 a	36.5 ± 3.5 (38.7) a
LS Mean			8.2 [7.1-9.4] B	7.6 [6.6-8.7] B	49.8 [42.6-58.1] (16.3) B

[a] Infected aphids defined as those in which infection was confirmed by observation of fungal sporulation after death (n = number of aphids with confirmed infections).

[b] Response of carrier control vs. fungus-infected aphids (mean ± standard error or LS mean [95% confidence interval]).

[c] Within high dose treatments and within low dose treatments within columns, means or LS means (representing main effects of aphid species across fungi) followed by same uppercase letter are not significantly different (ANOVA F-tests, alpha = 0.05).

[d] Within each dose-aphid species treatment combination within column, means followed by same lowercase letter are not significantly different (Tukey-Kramer HSD test, alpha = 0.05).

[d] Number in parentheses is the percent reduction in offspring production relative to the controls.

[e] Three aphids apparently succumbed to infection (died on day 4 post-treatment), but did not support fungal sporulation and thus were not tallied as infected.

gossypii (LS means = 32.4 vs. 49.8 offspring/infected aphid, representing 44 vs. 16% reductions relative to the respective controls). In contrast to the low-dose treatments, there were highly significant differences among responses of both aphids to the three fungi applied at the high doses (survival time: $F_{2,79} = 12.5$, $P < 0.0001$; reproductive period: $F_{2,79} = 15.4$, $P < 0.0001$; and total offspring production: $F_{2,79} = 15.4$, $P < 0.0001$). For both aphid species, strains *B. bassiana* GHA and *M. brunneum* F52 had a significantly greater effect on survival time and reproduction compared to *I. javanica* PFR-97 (Table 3.7). Although tests conducted within each aphid species lacked power to completely separate the three fungi (Table 3.7), when averaged across both aphid species, total offspring production differed significantly with each fungal species (Tukey–Kramer HSD test, alpha = 0.05). The back-transformed LS means of total offspring for each strain can be arranged in the following order: F52 (18.9) < GHA (23.1) < PFR-97 (27.3). These correspond to mean offspring reductions of 67.9, 60.8, and 53.7%. Consistent with observations at the low doses, the main effects of fungal infection were greater against *M. persicae* than *A. gossypii*. LS mean survival times were 3.6 vs. 4.1 days, and LS mean numbers of offspring/infected aphid were 17.2 vs. 30.3 (a 71% reduction vs. 49%, relative to the respective controls).

Analysis of disease progression revealed that the reductions in aphid reproduction reported above were almost entirely the result of aphid mortality. Pre-mortem effects of fungal infection over the three days prior to death are presented in Table 3.8. Ultimately, day 3 before death was removed from the final ANOVA, as a preliminary analysis revealed no significant negative effects of infection on this day, and responses to the low vs. high doses were combined for presentation in Table 3.8, as

dose was not significant ($F_{1,102.2} = 1.9$, $P = 0.17$). The overall mixed-model, repeated measures ANOVA (described in the Methods section) revealed that only fungal species ($F_{2,91.7} = 3.9$, $P = 0.024$) and day before death ($F_{2,208.9} = 13.1$, $P < 0.0001$) were significant, with no significant interactions (all P values > 0.12). As expected, there was a significant decrease in offspring production over time (days) approaching death, but even on the day before death, the grand mean reduction was only 8%, and this increased to just 29% on the day of death. Differences among fungal species detected by the Tukey-Kramer test (Table 3.8) were few, and found only with *A. gossypii*. Here, the reduction in offspring production from day 2 until death of F52-infected aphids differed significantly from GHA-infected aphids (a significant 24% reduction vs. no significant effect). However, Tukey-Kramer comparisons of the main-effect means (across both aphid species) revealed significantly greater negative effects of F52 compared to GHA. PFR-97 was intermediate and not significantly different from the other fungi. Mean differences translated to changes of only -21.5, -11.6, and +6.7% relative to controls for F52, PFR-97, and GHA, respectively. In all cases, offspring produced from infected aphids exhibited normal (low) natural mortality, reached full reproductive maturity, and had healthy offspring themselves (data not shown).

DISCUSSION

One of the primary goals of this paper was to assess current commercial fungal-based strains against the 3 main aphid pests in greenhouse crops, and to hopefully identify novel isolates of entomopathogenic fungi with greater virulence than available commercial strains. In several cases (*e.g. I. javanica* PFR-97, *B. bassiana* strain

Table 3.8. Effects of fungal disease progression on aphid reproduction.

Treatment	n [a]	Mean offspring produced by treated vs. control aphids, difference [upper, lower 95% confidence limits], and percent change [b] on:				
		Day 3 before death	Day 2 before death [c]	Day 1 before death [c]	Day of death [c]	Day 2 until death [c,d]
		Myzus persicae				
Bb GHA	13	5.7 ± 0.5	5.0 ± 0.2	5.1 ± 0.4	2.1 ± 0.6	12.2 ± 0.8
Controls		4.8 ± 0.4	4.9 ± 0.4	4.9 ± 0.3	2.4 ± 0.3	12.2 ± 0.6
Difference		0.8 [-0.1, 1.7]	0.1 [-0.6, 0.9]	0.2 [-0.8, 1.2]	-0.3 [-1.7, 0.1]	0.03 [-1.9, 2.0]
% Change [a]		**+17.5**	**+2.7**	**+4.1**	**-12.3**	**+0.3** ab
Mb F52	11	5.4 ± 0.4	5.5 ± 0.5	4.3 ± 0.4	2.1 ± 0.7	11.9 ± 0.9
Controls		5.9 ± 0.4	5.5 ± 0.3	5.4 ± 0.5	3.0 ± 0.3	13.9 ± 0.8
Difference		-0.5 [-2.0, 0.9]	0 [-1.3, 1.3]	-1.1 [-2.5, 0.3]	-0.9 [-2.2, 0.5]	-2.0 [-4.2, 0.2]
% Change		**-9.2**	**0**	**-20.9**	**-28.9**	**-14.4** ab
Ij PFR-97	13	5.2 ± 0.3	5.7 ± 0.4	4.5 ± 0.5	2.2 ± 0.6	12.4 ± 0.8
Controls		5.2 ± 0.3	5.4 ± 0.4	5.0 ± 0.3	2.6 ± 0.2	13.0 ± 0.6
Difference		0.1 [-1.1, 1.3]	0.3 [-0.6, 1.2]	-0.5 [-1.6, 0.7]	-0.5 [-1.9, 1.0]	-0.6 [-3.1, 1.8]
% Change		**+1.5**	**+5.7**	**-9.2**	**-17.6**	**-4.7** ab
		Aphis gossypii				
Bb GHA	14	8.1 ± 0.9	7.1 ± 0.7	7.4 ± 0.8	3.4 ± 0.8	17.8 ± 1.6
Controls		8.1 ± 1.0	6.2 ± 0.8	6.6 ± 0.7	3.2 ± 0.5	16.0 ± 2.0
Difference		0 [-1.3, 1.3]	0.9 [-0.6, 2.4]	0.8 [-0.7, 2.2]	0.2 [-1.3, 1.7]	1.8 [-0.6, 4.3]
% Change		**0**	**+14.1**	**+11.3**	**+5.9**	**+11.3** a
Mb F52	24	8.5 ± 0.6	5.7 ± 0.5	6.4 ± 0.6	1.2 ± 0.3	13.3 ± 1.0
Controls		7.6 ± 0.7	7.3 ± 0.4	7.1 ± 0.6	3.2 ± 0.3	17.6 ± 1.1
Difference		0.8	-1.7 [-2.6, -0.7]	-0.7 [-1.7, 0.4]	-2.1 [-3.1, -1.1]	-4.4 [-6.2, -2.5]
% Change		**+10.9**	**-22.5 ***	**-9.2**	**-64.1 ***	**-24.4 *** b
Ij PFR-97	16	8.1 ± 0.5	6.3 ± 0.6	5.4 ± 0.9	2.4 ± 0.8	14.1 ± 1.7
Controls		8.1 ± 0.8	6.6 ± 0.6	7.0 ± 0.7	3.1 ± 0.4	16.7 ± 1.4
Difference		0.1 [-1.6, 1.7]	-0.3 [-1.5, 0.9]	-1.6 [-3.1, -0.1]	-0.7 [-2.4, 1.0]	-2.7 [-5.6, 0.3]
% Change		**+0.8**	**-4.7**	**-23.2 ***	**-23.2**	**-15.9** ab
		Overall Means				
Fungus-Treated	91	7.1 ± 0.3	5.9 ± 0.2	5.6 ± 0.3	2.1 ± 0.3	
Controls		6.8 ± 0.3	6.1 ± 0.2	6.1 ± 0.3	3.0 ± 0.1	
Difference		0.3	-0.3 [-0.7, 0.2]	-0.5 [-1.0, -0.01]	-0.8 [-1.4, -0.3]	
% Change		**+4.3**	**-4.5** a	**-8.2 *** a	**-28.5 *** b	

Table 3.8 (Continued)

[a] Means and standard errors for day 3 means are based on samples of 13, 11, 14, 16 or 24 aphids, as indicated.
[b] Percent change in offspring production by treated aphids relative to production by control aphids (each treated aphid paired with a randomly selected control aphid). Negative values indicate a reduction in offspring production relative to the controls.
[c] Within-treatment differences marked with an asterisk are statistically significant based on 95% confidence interval (interval does not include zero).
[d] Repeated measures analysis. Percentages of change within column or within row followed by same letter are not significantly different based on ANOVA/Tukey Kramer pair-wise comparisons at alpha = 0.05 (data from day 3 excluded).

GHA) commercial strains performed poorly, and unreasonably high doses were needed to achieve high mortality. Given that re-isolation of *B. bassiana* strain GHA from *A. gossypii* and *M. pericae* did not improve the LC_{50} value against either aphid, the lack of re-isolation in trials comparing multiple isolates does not explain these results with this strain. Although a novel isolate with a lower LC_{50} against *M. persicae* and *A. gossypii* was identified (*Beauveria* 5493), it was not statistically more pathogenic than the commercial strain JW-1 (Naturalis®). We also identified important differences in susceptibility of the different aphid species tested, with *B. bassiana* strain JW-1, for example, being quite virulent against *A. gossypii* nymphs, showing low but quantifiable virulence against *M. persicae* nymphs, and being almost completely non-virulent against nymphs of *A. solani*. Lastly, our results with *Beauveria*, *Metarhizium,* and *Isaria* isolates indicated that although adult aphids were far more susceptible to fungi than first-instar nymphs, there was little pre-mortem effect of fungal infection on aphid reproduction.

To be a successful candidate for use in greenhouse crops, isolates should provide effective control of *M. persicae*, *A. gossypii*, and *A. solani.* *Beauveria bassiana* 5493, a yet un-commercialized strain, was the most promising candidate in this regard. The fact that this strain was originally isolated from an aphid host supports the theory that strains isolated from closely related hosts should have greater virulence than isolates from phylogenetically distant hosts. However, several issues complicate further development of this isolate. First, *B. bassiana* ARSEF 5493 is very difficult to mass-produce, at least with respect to conidia (unpublished data). Secondly, even if it were more amenable to mass production (*e.g.* as blastospores), its LC_{50}s against all 3

aphid species were quite high in the context of previous bioassays with entomopathogenic fungi and sucking greenhouse pests. For example, in Wraight et al. (1998), the median lethal doses of the most virulent isolates against nymphs of the silverleaf whitefly (*Bemisia argentifolii*; Hemiptera: Aleyrodidae) ranged between 50-150 conidia/mm^2. Given these ranges, control of *B. argentifollia* in field applications in cucurbit crops was possible (Wraight et al. 2000). The fact that a dose > 900 conidia/mm^2 was needed to kill 50% of a nymphal aphid population with this *B. bassiana* 5493 (in Petri dishes with very high humidities) is indicative of the problem facing fungal-based pesticides for aphid control: isolates of *Beauveria*, *Metarhizium*, and *Isaria* tested to date are just not very virulent against these pests.

Several physiological and morphological characteristics help explain why aphids may be less susceptible to many fungal pathogens compared to other hemipteran pests. Their fast development time (*e.g.* 5.5 d at 24.7°C for *M. persicae*; Liu and Meng 1999) and multiple nymphal stadia mean that molts are occurring every 1-2 days. Liu et al. (2003) observed that the mortality of inoculated aphid nymphs was closely related to the time interval between inoculation and the next molting period. Specifically, the earlier the molt occurred, the lower the observed mortality. Thus, an aphid molt taking place ca. 24 after inoculation represents a successful mechanism for preventing entomopathogen infection. Secondly, unlike whitefly nymphs, many aphids (*e.g. M. persicae*, *A. solani*) are highly mobile, with long, stilt-like legs that minimize body contact with the leaf surface and, thus, the more humid leaf boundary layer. This limited contact also reduces the likelihood of aphids acquiring a lethal dose of fungal conidia from treated leaf surfaces (Hall 1979).

It should be pointed out that the poor efficacy seen in our assays are not consistent with all previous studies. For example, Loureiro and Moino (2006) reported 100% mortality of third-instar *A. gossypii* and *M. persicae* with 1.0 x 10^8 conidia/ml suspensions of non-commercial strains of *B. bassiana* and *M. anisopliae*. Less clearly, Vu et al. (2007) reported "control values" of 100% following treatment of 4-day old *A. gossypii* with 1.0 x 10^7 conidia/ml suspensions of *B. bassiana*, *M. anisopliae*, and *I. javanica;* control value was based on population increase of treated insects compared to untreated. Similar results were achieved with *M. persicae* (though only ca. 70% control with *M. anisopliae*). As nymphal stadia tend to be similar in duration, one would not expect later nymphal stages to be more susceptible to fungi than the first instar (the immature stage treated in our assays). On the other hand, as smaller targets of fungal applications, first-instars would be expected to receive a lower dose. Following spray applications of *Lecanicillium* vs. *A. gossypii*, Kim and Roberts (2012) observed 70% fewer conidia on the dorsal surfaces of first-instar nymphs and slower germination of these conidia than observed on third-instars. Other differences in assay techniques also may be important. Recent research suggests that conidia may germinate and penetrate the aphid cuticle most efficiently on the less-resistant intersegmental membranes at the proximal end of the legs (close to the body's ventral surface) (Amnuaykanjanasin et al. 2013). We used a relatively low-volume spray (0.01 µl/mm^2), compared, for example, to the high volume of 20 ml applied to a single leaf by Vu et al. (2007). High volume applications are likely to deliver more conidia to these more vulnerable areas of the aphid body. Additionally, aphids in the above-cited studies were exposed to fungal inocula on treated leaves for the duration of the incubation period, whereas the aphids is

our study were, in most cases, exposed for just 24 h (max. 72 h) before being transferred to clean leaf disks. Our more conservative assay technique may more closely mimic conditions in the field or ventilated commercial greenhouses (see below) as opposed to assay methods that provide optimal conditions for fungal activity. Further investigation is needed to identify sources of variability between our results and those from other studies.

Given the clonal nature and high reproductive capacity of aphids, assessing the impact of entomopathogenic fungi on aphid adults and their reproduction is of paramount importance in screening trials. Our results concur with previous results by Hesketh et al. (2008), Shan and Feng (2010) and Tesfaye and Syoum (2010), demonstrating high (>75%) mortality of adult *M. persicae* or *A. gossypii* exposed to *B. bassiana* GHA and/or other non-commercial *Beauveria* and *Metarhizium* isolates. As aphids have long reproductive periods, death due to mycosis, even if relatively slow compared to other control agents, can still significantly affect total reproduction of an aphid population. Our observations of reductions ranging from 36–74% following high dose applications are in accord with previous studies of *M. persicae* and *A. gossypii* (He and Li 2008; Gurulingappa et al. 2011). Such reductions, however, have little effect on the intrinsic rate of increase of an infected aphid population. This statistic is largely determined by the first few days of reproduction (Wyatt and White 1977, Baverstock et al. 2006), and fungi normally exhibit an initially slow, terminally abrupt mode of action. Our observations mirror several previous studies showing that fungal infected aphids continue to produce normal numbers of healthy offspring until near death (Hall 1976; Wang and Knudsen 1993; Liu et al. 2003; Baverstock et al. 2006). We also found no

effect of fungal infection of the mother aphid on the viability of her offspring. Hall (1976) similarly observed that adult *Macrosiphoniella sanborni* in "the last stages of infection…produced uninfected nymphs, which remained so if removed carefully at birth from the environment of the adult and placed at high humidity."

Although the results presented in Table 3.7 suggest a marked difference in fungal infection on the reproduction of the two aphid species (*A. gossypii* appearing be to less affected than *M. persicae*, particularly at low doses), this was the result of differences in both survival times and reproductive periods. Specifically, the highest offspring production by untreated *A. gossypii* (10.6 offspring/aphid) was recorded after day 1, and the mean reproductive period was < 10 days. In contrast, maximum reproduction of *M. persicae* (5.8 offspring/aphid) occurred on day 4, and reproduction continued for nearly 2 weeks. Thus, by day 5 of the experiment (within which time nearly all aphids treated at the high doses succumbed to infection), untreated *A. gossypii* had already produced 68% of total offspring, compared to just 44% for *M. persicae.* These patterns of reproduction also explain the apparent contradiction in Table 3.7 vs. Table 3.8. While Table 3.7 shows greater pathogenicity of *B. bassiana* GHA vs. *I. javanica* PFR-97, and Table 3.8 shows PFR-97 as more pathogenic than GHA, this is likely due to the faster speed of kill by GHA at high doses (survival time having a great effect on total offspring production). Overall, treatment with *M. brunneum* strain F52 had the highest effect on aphid reproduction of any fungus in our study. This was the first time the impact of this fungal species on aphid reproduction has been assessed to our knowledge. But, even the 24% reduction caused by F52 during the last few days before death is inconsequential in the context of aphid control, considering they were

still able to produce 15-25 offspring before they succumbed to infection. Unfortunately, this illustrates a further barrier to effective control using entopathogenic fungi: aphids clearly need to be sprayed in the greenhouse before they become reproductive in order to prevent population growth, given their short pre-oviposition period and the long period between treatment and death with fungal conidia.

A further complicating factor in greenhouse crops is humidity of the ambient greenhouse environment. Conidia of the entomopathogenic hypocrealean fungi generally require near-100% RH conditions for approximately for 24 hr to germinate. Although it would seem that a greenhouse would be an ideal environment, unless it is a tropical greenhouse, the reverse is often true. With the constant use of fans and ventilation systems to circulate air and cool, and especially with heating systems used in winter in the northern latitudes, the greenhouse environment can be quite dry. For example, in our research greenhouses at the USDA in Ithaca NY, the relative humidity rarely exceeds 60%, even in the summer months and when full of plant material. Reports from other countries are closer to 50% (*e.g.* Vu et al. 2007), and, unlike field crops, there is rarely significant dew accumulation in the evening and overnight period that would facilitate germination and infection (personal observations). In fact, high humidity conditions are actively avoided by growers to prevent the occurrence of foliar plant pathogens such as powdery mildews and *Botrytis* blight (M. Daughtrey, personal communication). However, humidity levels can reach up to 81-85% in more poorly regulated commercial greenhouses during the warmer months (Shipp et al. 2003; M. Daughtrey, personal communication), and humidity is higher within the leaf boundary layer (Wraight et al. 2000). Thus, as demonstrated by Shipp et al. (2003), germination

of conidia and a high percentage of infection of pests are possible, but this likely depends on time of year and ambient humidity levels. Further complicating matters is the fact that *A. solani* is considered a "cool-weather pest" (Jandricic et al. 2010), and thus may be present in the greenhouse when humidity conditions are near their lowest (with lower temperatures likely also decreasing efficacy; see Vu et al. 2007, Tesfaye and Seyoum 2010, and others). The use of misting systems to increase RH temporarily (*i.e.* 24h) have been explored, but it is unclear currently what level of manipulation of conditions is needed support activity of insect-pathogenic fungi without promoting plant pathogens. Further research into this area is needed.

Given the short windows of opportunity for fungal infection resulting from rapid nymphal development (short intermolt periods) and the desire on the part of growers to avoid extended periods of high humidity in greenhouse cultures, new technologies are needed to improve the speed of action of entomopathogenic fungi. Humectant (hygroscopic) materials added to formulations can prevent or slow desiccation, supporting more rapid germination. Most hypcrealean fungi also can be mass-produced as yeast-like hyphal bodies (blastospores) via liquid fermentation. These propagules are less amenable to formulation and shelf storage and, in some cases, less efficiently mass-produced than conidia, traits that have constrained their commercial development. On the other hand, they possess the capacity to germinate more rapidly than conidia, making them potentially more efficacious against nymphal stages of insect pests (Jackson et al. 1997; Vega et al. 1999; Kim and Kim 2008). Hall (1979), however, observed no difference in efficacy of *Lecanicillium* blastospores vs. conidia applied against aphids infesting chrysanthemums under open-bed greenhouse conditions. There

have been significant advances in development of blastospore-based biopesticides in recent years (*e.g.,* Jackson et al. 2003); the *Isaria*-based biopesticides registered in North America (PFR-97, Preferal, and NoFly) are formulations of blastospores.

Given that Kim et al. (2007) reported 100% mortality of 1st instar nymphs of *M. persicae* and *A. solani* using Vertalec® (*Lecanicillium longisporum*; similar methodology to our study), we suggest that identification of barriers surrounding the registration of the potentially more efficacious *Lecanicillium* isolates in the U.S are of primary importance. In contrast to *Beauveria*, *Metarhiziuma* and *Isaria* spp., some *Lecanicillium* spp. are common, naturally occurring pathogens of aphids (Hall 1981), and generally exhibit greater virulence against these insects (*e.g.* Hayden et al. 1992; Vu et al. 2007; Kim and Kim 2008). As with the Entomophthorales, however, commercial development has been slowed by mass production difficulties (Hall 1981). Conidia are produced in association with slime, and at lower densities on solid substrates than the conidia of *Beauveria*, *Metarhizium*, and *Isaria*. However, mass production as blastospores has been successful, and aphid-control products based on these propagules (including Vertalec) are registered in Europe.

This paper has added to knowledge surrounding the use of novel fungal isolates against greenhouse aphid pests, and is the first to document results of entomopathogenic fungi in the genera *Metarhizium* and *Beauveria* against the pest aphid *A. solani*. Unfortunately no novel fungal isolates with exceptionally high virulence were identified from within the *Metarhizium*, *Beauveria* or *Isaria* isolates selected for screening. This paper also highlights some of the significant challenges that continue to exist for the use of entomopathogenic fungi against these very important greenhouse pests. Based on

demonstrated differences in susceptibility between adults and nymphs of all three major greenhouse pest aphids, as well as limited fungal effects on pre-mortem reproduction, we strongly suggest that that multiple aphid stages (including adults) be included in all future assays testing the inherent pathogenicity/virulence of fungi against aphids. Additional research into methods of improving control with existing products and continued development of novel products will also be needed to make entomopathogenic fungi successful components of integrated pest management programs for aphids in greenhouses.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J Econ. Entomol. 18, 265-267.

Amnuaykanjanasin, A., Jirakkakul, J., Panyasiri, C., Panyarakkit, P., Nounurai, P., Chantasingh, D. Eurwilaichitr, L. Cheevadhanarak, S. Tanticharoen, M. 2013. Infection and colonization of tissues of the aphid *Myzus persicae* and cassava mealybug *Phenacoccus manihoti* by the fungus *Beauveria bassiana*. BioControl 58, 379-391.

Baverstock, J., Roy H.E., Clark S. J., Alderson P.G., Pell, J.K. 2006. Effect of fungal infection on the reproductive potential of aphids and their progeny. J. Invert. Path. 91: 136-139.

Bischoff, J.F., Rehner, S.A., Humber, R.A. 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101, 512-530.

Burgerjon, A. 1956. Pulvérisation et poudrage au laboratoire par des préparations pathogèns insecticides. Ann. Epiphyt. 7, 675-683.

Cabanillas H.E., de León, J.H., Humber, R.A, Murray, K.D., Jones, W.A. 2013. *Isaria poprawskii* sp. nov. (Hypocleales: Cordycipitaceae), a new entomopathogenic fungus from Texas affecting sweet potato whitefly. Mycoscience 54, 158-169.

Castrillo, L.A., Vandenberg, J.D., Wraight, S.P. 2003. Strain-specific detection of introduced *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers. J. Invertebr. Pathol. 82, 75-83.

Conover, W.J. 1999. Practical Nonparametric Statistics. Third Edition, John Wiley & Sons, New York. 584 pp.

Devonshire, A.L. 1989. Resistance of aphids to insecticides. In: Minks, A.K., Harrewijn, P. (Eds.), Aphids, Their Biology, Natural Enemies and Control, Volume C. Elsevier Science Publishers BV, Amsterdam, The Netherlands, pp. 123-135.

Down, R.E., Gatehouse, A.M.R., Hamilton, W.D.O., Gatehouse, J.A. 1996. Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani)* when administered *in vitro* and via transgenic plants both in laboratory and glasshouse trials. J. Insect Physiol. 42, 1035-1045.

Faria, M. Hajek, A.E., Wraight, S.P. 2009. Imbibitional damage in conidia of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium acridum*, and *Metarhizium anisopliae*. Biol. Control 51, 346-354.

Gillespie, D.R., Acheampong, S. 2012. Dropping behavior in *Aulacorthum solani* (Hemiptera: Aphididae) following attack by *Aphidius ervi* (Hymenoptera: Braconidae): are sticky stem bands a useful integrated pest management method? Can. Entomol. 144, 589-598.

Grubinger, V. 1999. Biorational pesticides. University of Vermont Extension. Accessed Mar 25/2011 at http://www.uvm.edu/vtvegandberry/factsheets/biorationals.htm.

Gurulingappa, P., McGee, P.A., Sword, G. 2011. Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. Crop Prot. 30, 349-353.

Hall, R.A. 1976. *Verticillium lecanii* on the aphid *Macrosiphoniella sanborni*. J. Invertebr. Pathol. 28, 389-391.

Hall, R.A. 1979. Pathogenicity of *Verticillium lecanii* conidia and blastospores against the aphid *Macrosiphoniella sanborni*. Entomophaga 24, 191-198.

Hall, R.A. 1981. The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In: Burges, H.D. (Ed.), Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, NY, pp. 483-498.

Hayden, T.P., Bidochka, M.J., Khachatourians, G.G. 1992. Entomopathogenicity of several fungi toward the English grain Aphid (Homoptera: Aphididae) and enhancement of virulence with host passage of *Paecilomyces farinosus*. J. Econ. Entomol. 85, 58-64.

He, H.-G., Li., Z.-Y. 2008. Effects of *Beauveria bassiana* on the fecundity and population parameters of *Myzus persicae* at different temperatures. Chinese Bull. Entomol. 45, 101-104.

Hesketh, H., Alderson, P.G., Pye, B.J., Pell, J.K. 2008. The development and multiple uses of a standardized bioassay method to select hypocrealean fungi for biological control of aphids. Biol. Control 46: 242-255.

Inglis, G.D., Goettel, M.S., Butt, T.M., Strasser H. 2001. Use of hypomycete fungi for managing insect pests. In: Butt, T.M., Jackson, C.W., Magan, N. (Eds.), Fungi as biocontrol agents. Progress, problems and potential. CABI Publishing, Wallingford, UK., pp. 23-69.

Jackson, M.A., Cliquet, S. Iten, L.B. 2003. Media and fermentation processes for the rapid production of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. Bioconrol Sci. Technol. 13, 23-33.

Jackson, M.A., McGuire, M.R., Lacey, L.A., Wraight, S. P. 1997. Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. Mycol. Res. 101, 35-41.

Jandricic, S., Wraight, S., Gillespie, D.R., Sanderson, J.P. 2013. Oviposition behavior of the biological control agent *Aphidoletes aphidimyza* (Diptera:Cecidomyiidae) in environments with multiple pest aphid species (Hemiptera: Aphididae). Biol. Control 65, 235-245.

Kim, J.J., Goettel, M.S., Gillespie, D.R. 2007. Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. Biol. Control 40, 327-332.

Kim, J.J., Kim, K.C. 2008. Selection of a highly virulent isolate of *Lecanicilliu attenuatum* against cotton aphid. J. Asian-Pacific Entomol. 11, 1-4.

Kim, J.J., Roberts, D.W. 2012. The relationship between conidial dose, moulting and insect developmental stage on the susceptibility of cotton aphid, *Aphis gossypii*, to conidia of *Lecanicillium attenuatum*, an entomopathogenic fungus. Biocontrol Sci. Technol. 22, 319-331.

Kocourek, E., Havelka J., Beránková J., Jarosik V. 1994. Effect of temperature on development rate and intrinsic rate of increase of *Aphis gossypii* reared on greenhouse cucumbers. Entomol. Exp. Appl. 71, 59-64.

Lee, S.G., Kim H.H., Kim T.H., Park G., Kim K.H., Kim J.S. 2008. Development model of the foxglove aphid, *Aulacorthum solani* (Kaltenbach) on lettuce. Korean J. Appl. Entomol. 47, 359-364.

Leite, L.G., Alves, S.B., Batista Filho, A., Roberts, D.W. 2003. Effect of salts, vitamins, sugars and nitrogen sources on the growth of three genera of Entomophthorales: Batkoa, Furia, and Neozygites. Mycol. Res. 107, 872-878.

Littell, R.C, Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O. 2006. SAS for Mixed Models, Second Edition. SAS Institute Inc., Cary, NC.

Liu, S, Meng X. 1999. Modeling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. Bull. Entomol. Res. 89, 53-63.

Liu, Y.-Q., Zhang, F.-C., Liu, S.-S. 2003. Effects of moulting in *Myzus persicae* on the virulence the of entomopathogenic fungus *Beauveria bassiana*. Acta Entomol. Sinica 46, 441-446.

Lohr, L. 2001. Factors affecting international demand and trade in organic food products. In: Regmi, A (Ed.), Changing Structure of Global Food Consumption and Trade. USDA-ERS Washington, D.C.

Loureiro, E. de S., Moino A. Jr. 2006. Patogenicidade de fungos hifomicetos aos pulgões *Aphis gossypii* Glover e *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Neotropical Entomology 35, 660-665.

Milner, R.J. 1997. Prospect for biopesticides for aphid control. Entomophaga 42, 227-239.

Parrella, M.P., Hansen, L.S., van Lenteren, J.C. 1999. Glasshouse environments. In: Fisher, T.W., Bellows, T.S., Caltagirone, L.E., Dahlstein, D.L., Huffaker, C.B., Gordh, G. (Eds.), Handbook of Biological Control. Academic Press, NY, pp. 819-839.

Pilkington, L.J., Messelink, G., van Lenteren, J.C., Le Mottee, K. 2010. "Protected Biological Control" – biological pest management in the greenhouse industry. Biol. Control 52, 216-220.

Rehner, S.A., Minnis, A.M., Sung, G.-H., Luangsa-ard, J.J., Devotto, L., Humber, R.A. 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103, 1055-1073.

Sanchez, J. A., Canovas, F., Lacasa, A.. 2007. Thresholds and management strategies for *Aulacorthum solani* (Hemiptera: Aphididae) in greenhouse pepper. J. Econ. Entomol. 100, 123-130.

SAS Institute Inc. 2011. SAS Software Release 9.3. SAS Institute Inc., Cary, NC.

SAS Institute Inc. 2012. JMP® version 10. SAS Institute Inc., Cary, NC.

Shan, L.T., Feng, M.G. 2010. Evaluation of the biocontrol potential of various *Metarhizium* isolates against green peach aphid *Myzus persicae* (Homoptera: Aphididae). Pest Manag. Sci. 66, 669-675.

Shipp, J.L., Zhang, Y., Hunt, D.W.A., Ferguson, G. 2003. Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. Environ. Entomol. 32, 1154-1163.

Tesfaye, D., Seyoum, E. 2010. Studies on the pathogenicity of native entomopathogenic funal isolates on the cotton/melon aphid, *Aphis gossypii* (Homoptera: Aphididae) Glover under different temperature regimes. African Entomol. 18, 302-312.

Vandenberg, J.D., Cantone F.A. 2004. Effect of serial transfer of three strains of *Paecilomyces fumosoroseus* on growth in vitro, virulence and host specificity. J. Invertebr. Pathol. 85, 40-45.

van Driesche, R.G., Lyon S., Sanderson J.P., Bennett, K.C., Stanek, E.J. III, Zhang, R. 2008. Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker

plants for control of aphids (Hemiptera: Aphididae) in greenhouse spring floral crops. Fla. Entomol. 94, 583-590.

van Lenteren, J.C. 2000. A greenhouse without pesticides: fact or fantasy? Crop Prot. 19, 375-384.

Vega, F.E., Jackson, M.A., McGuire, M.R. 1999. Germination of conidia and blastospores of *Paecilomyces fumosoroseus* on the cuticle of silverleaf whitefly, *Bemisia argentifolii*. Mycopathologia 147, 33-35.

Vu, V.H., Hong, S.I., Kim, K. 2007. Selection of entomopathogenic fungi for aphid control. J. Biosci. Bioeng. 104, 498-505.

Wang, Z.G., Knudsen, G.R. 1993. Effect of *Beauveria bassiana* (Fungi: Hyphomycetes) on fecundity of the Russian wheat aphid (Homoptera: Aphididae). Environ. Entomol. 22, 874-878.

Warton, D.I., Hui, F.K.C. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92, 3-10.

Wraight, S.P., Carruthers, R.I., Bradley, C.A., Jaronski, S.T., Lacey, L.A., Wood, P., Galaini-Wraight, S. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. J. Invertebr. Pathol. 71, 217-226.

Wraight, S.P., Carruthers, R.I., Jaronski, S.T., Bradley, C.A., Garza, C.J., Galaini-Wraight, S. 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. Biol. Control 17, 203–217.

Wraight, S.P., Ramos, M.E., Avery, P.B., Jaronski, S.T., Vandenburg, J.D. 2010. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. J. Invertebr. Pathol. 103, 186-199.

Wyatt, I.J., White, P.F. 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. Appl. Ecol. 14, 757-766.

Zar, J.H. 1999. Biostatistical Analysis, Fourth Edition. Prentice-Hall, Inc., Upper Saddle River, N.J.

CHAPTER 4

OVIPOSITION BEHAVIOR OF THE BIOLOGICAL CONTROL AGENT *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOMYIIDAE) IN ENVIRONMENTS WITH MULTIPLE PEST APHID SPECIES (HEMIPTERA: APHIDIDAE)

Jandricic, S.E.,[1,2] Wraight, S.P.,[3] D.R. Gillespie,[4] and Sanderson, J.P.[1]

ABSTRACT

We investigated the oviposition behavior of the aphidophagous midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) when faced with multiple prey choices, *i.e.* plants infested with *Myzus persicae* or *Aulacorthum solani* (Hemiptera: Aphididae). When within-plant location of aphid patches was controlled for, aphid density was a significant factor in *A. aphidimyza* oviposition, but species was not. When location was uncontrolled, aphid species and location of aphid patches on plants (and 2 and 3-way interactions with location) became significant, along with density. Aggregations of *Myzus persicae* on plant meristems received the largest number of *A. aphidimyza* eggs, while *A. solani*-infested plants received significantly fewer eggs (this aphid species being generally distributed among lower leaves). Upon giving *A. aphidimyza*

[1] Department of Entomology, Comstock Hall, Cornell University, Ithaca NY, USA 14853
[2] Corresponding Author: Email: sej48@cornell.edu Fax: 607-255-0940 Tel: 607-379-1127
[3] USDA-ARS Robert W. Holly Center for Agriculture and Health, Tower Road, Cornell University, Ithaca NY, USA, 14853
[4] Agriculture and Agri-Food Canada, Research Centre, Agassiz, BC, V0M 1A0, Canada.

a choice between two patch locations, aphid species was again unimportant in oviposition decisions, while a greater correlation with aphid density was seen in aphid colonies located on young plant tissue vs. old. These results suggest that, for *A. aphidimyza,* perceived quality of an aphid patch as an oviposition site is influenced more by density and location of the aphid patch on the plant than by the species of aphid within the patch. Given that within-plant distribution of pest aphid species can differ, this oviposition behavior could have important implications for the efficacy of *A. aphidimyza* as a biocontrol agent for aphids in multi-species environments.

INTRODUCTION

Insect natural enemies spend much of their time as adults making decisions critical to fitness of their offspring. A key step in foraging by many predacious insects involves the ability of adult females to assess the overall quality of prey patches as suitable habitats for their young. Factors such as resource richness, presence of other natural enemies, preferences for prey species or their microhabitats can all influence perceived patch quality and affect oviposition decisions (Dixon 1959; Kan 1988; Sarmento et al. 2007; Almohamad et al. 2007). Elucidation of these factors is especially important for natural enemies used as biological control agents in agricultural crops, as preference of a predator for a certain type of prey patch over another has the potential to negatively influence biocontrol outcomes (see Holt and Lawton 1994; Abrams and Matsuda 1996; Bergeson and Messina 1997; Hardwood and Obrycki 2005). Greater understanding of complex oviposition decisions of natural enemies is important if failures of biocontrol programs are to be avoided.

Aphidoletes aphidimyza Rondani (Diptera: Cecidomyiidae) is a commercially available natural enemy used against aphid pests (Hemiptera: Aphididae) in greenhouse crops in North America and Europe. Predacious in its larval stage, *A. aphidimyza* can prey on a wide variety of aphid species (Harris 1973). Given the relatively limited dispersal capacity of *A. aphidimyza* larvae, choice of oviposition sites by adult flies is paramount for offspring survival and development. Thus, it is not surprising that this insect has an impressive ability to detect oviposition sites, being able to find a single aphid-infested plant among 75 un-infested plants (El-Titi 1974a) using honeydew as a cue (Choi et al. 2004). It has also been well established that females demonstrate a positive correlation between aphid density and oviposition (El Titi 1972/73; Stewart and Walde 1997; Choi et al. 2004; Lucas and Brodeur 1999). However, much less is known about the species-related preferences of *A. aphidimyza*. Numerous investigators have assessed the suitability of various aphid species as prey for *A. aphidimyza* (Markkula and Tiitanen 1976; Havelka and Ruzicka 1984; Kuo-Sell 1989; Popov and Belousov 1987; Belousov and Popov 1989; Kim and Kim 2004). However, these studies have generally aimed to optimize mass rearing techniques, focusing on prey species effects on larval development and/or fecundity of *A. aphidimyza*. To date, no published work has investigated oviposition decisions of *A. aphidimyza* in the presence of more than one pest aphid species in the context of biocontrol efficacy. Given that aphid species (even within a single crop pest complex) may differ markedly in biology, physiology, and behavior, it is reasonable to hypothesize that *A. aphidimyza* may assign differential value among prey species when confronted with a mixed aphid infestation (patch value being measured by

number of predator eggs received). Unbalanced oviposition among aphid species could result in inadequate control of the less valued species (an unexpected outcome for growers relying on a control agent marketed as a generalist aphid predator).

Thus, in this study we investigate the effects of different pest aphid species on *A. aphidimyza* oviposition decisions in detail. The aphid species chosen were *Myzus persicae* (Sulzer) and *Aulacorthum solani* (Kaltenbach), two of the most common aphid pests found in greenhouses in the U.S., U.K. and Canada (van Driesche et al. 2008). These species can co-occur in the same greenhouse, depending on the crop, but exhibit marked species differences: e.g. late-instar and adult *A. solani* are physically larger than *M. persicae*; the highest intrinsic rates of increase (r_m) reported for *A. solani* range between 0.25-0.28, depending on the crop (Jandricic et al. 2011), but r_m's of up to 0.36 have been reported for *M. persicae* (Davis et al. 2006 & 2007); *A. solani* is thought to more readily engage in defensive dropping behavior; their honeydews differ in sugar composition (Hogervorst et al. 2007); and *A. solani* is commonly reported to feed on lower leaves of plants (Wave et al. 1965; Robert 1979; Verider 1999) while *M. persicae* often feeds on new growth (ex. Hodgson 1978; Vehrs et al. 1992).

Given these multitude of differences, we first chose to focus on the effect of aphid species alone on *A. aphidimyza* oviposition, while controlling for other factors (*i.e.* aphid size; location of prey patches on plants). Secondly, to examine effects of aphid species along with effects of within-plant location of aphid patches on *A. aphidimyza* oviposition, we simultaneously presented patches of either species on a) top leaves vs. bottom leaves of different plants, b) growing points vs. bottom leaves of

the same plant, as well as c) patches present on bottom leaves only (no-choice). Finally, we investigated the response of *A. aphidimyza* under more natural conditions: all aphid stages were present and aphids were allowed to distribute to their preferred feeding locations on plants. Using this series of five experiments, we present and discuss the effects of not only prey species, but also patch density, location of prey patches, and their interactions on oviposition site selection by *A. aphidimyza* in multi-prey environments.

MATERIALS AND METHODS

Source and Maintenance of Insects

A. solani and *M. persicae* were collected in Ithaca, NY in 2009, and reared on pansies (*Viola* × *wittrockiana* Gams.) as polyclonal colonies, as in Jandricic et al. 2010. Adult aphids for all experiments were selected directly from colonies, and were therefore of unknown age.

A. aphidimyza pupae were obtained from Applied Bio-Nomics Ltd. (Victoria, BC, Canada) for all experiments. Upon receipt of shipment, pupae were placed in 46 cm^3 cages (plastic on 2 sides, mesh on 2 sides) to emerge. Cages were kept in an incubator (21±1°C, 16:8 L:D cycle; 40-50% RH). A Petri dish (90 mm) containing cotton batting soaked in a 5% unpasteurized honey solution until saturation was provided in the cage as a source of carbohydrates. Cotton strands were provided on the ceilings of the cages as a mating substrate (serving as a proxy for cob webs; see van Schelt and Mulder 2000). Adult midges used in experiments were collected ca. 60 h post emergence for each experiment, because in commercial strains of this

insect, few eggs (< 5%) are laid within the first two days after mating (Havelka and Zemek 1999).

Plant Material

Source and Maintenance:

For all experiments, pansies (var. Majestic Giant II, Yellow Blotch, Stokes Seeds, Buffalo, NY) were grown as in Jandricic et al. 2010. Plants were maintained in 10 cm pots for 4-6 weeks prior to experimental use; at this age, all plants were non-flowering. Plants received fertilization 3-4 times a week with a 20:10:20 fertilizer at ca. 200 ppm (Scotts- Sierra Horticultural Products, Marysville, OH).

Description of Within-Plant Locations:

During destructive sampling of plants in each experiment, aphid patches were characterized as being in one of four locations on the plant. Mature (fully expanded) leaves were categorized as bottom, middle, or top leaves, based on heights of ca. 0–2, 2–5, or >5 cm above the soil surface, respectively (overall plant height was 6- 8 cm). The fourth location consisted of the central growing point of the plant, *i.e.* the meristematic tissue (henceforth referred to only as the 'meristem'). Specifically, this location consisted of the plant material left when all mature leaves were removed from the plant; *i.e.* small, immature leaves and, sometimes, the beginnings of flower buds (green tissue only) developing at the end of the apical meristem and at the ends of small, lateral meristems. Together, these formed a dense, terminal cluster. In terms of height, the meristem reached a point most often slightly below the top leaves.

Greenhouse Compartment Experiments: General Set up

All experiments were conducted in two identical but separate (partitioned) greenhouse compartments (2.75 x 7.30 m each) at the USDA-ARS agricultural research center in Ithaca, NY. Four benches (blocks) per compartment were used, each measuring 0.92 x 2.44 m. On each bench, 28 pansy plants (4 rows of 7 plants) were set up with ca. 15 cm plant spacing to prevent movement of insects between plants. Four or five plants per bench were randomly selected as treatment plants (with the two aphid species being assigned to separate plants in all cases); the rest were considered "background plants" and were not sampled. Four plants in the center of each bench were excluded from random selection and left untreated (see below). Temperature (set to approx. constant 20°C) and relative humidity (RH) were monitored in the greenhouse compartments using HOBO data loggers (Onset Computer, Bourne, MA). Supplemental lighting from 400 W high-pressure sodium lamps (3/bench) was used each late afternoon (16:00h), when required, to maintain a minimum 15-h photoperiod. Water mist emitters (situated underneath and between each bench) were used to help increase RH. Emitters were activated just prior to *A. aphidimyza* releases and continued for the duration of each experiment (emitters were not used during the pre-release period of aphid reproduction). Emitters were on for 20 min every 4h in Experiment 2.4 and 5-10 min every hour for all subsequent experiments. This increased RH above ambient by ca. 40% on average. Ventilation fans were turned off in the greenhouse compartments for 12 h on the first night of release to promote settling of *A. aphidimyza* in the crop. Adult midges were released at dusk, as per

commercial recommendations. Adults (unsexed) were collected from emergence cages with a mouth aspirator using glass vials (20–28/vial) to minimize risk of midge injury due to static electricity; inspection under a dissecting scope (40x magnification) confirmed that aspiration did not damage their antennae. The average sex ratio over all experiments was 1 male: 1.8 females (range = 1:1.5 to 1:1.9). Midges were released from a single vial placed beneath four 4 central, un-infested pansy plants on each bench (4 benches/greenhouse compartment); these plants were moved closer together on the night of release to provide a canopy of leaves above the vials, buts were moved back into position the following morning.

Prey Patch Selection in Experimentally Manipulated Prey Populations

The test objective was to determine if aphid species was a significant factor in prey patch selection by *A. aphidimyza*. Two aphid densities (low vs. high) were also included to determine if there was an interaction between species and density. Other variables, including aphid size and location on the host plant were held constant to the extent possible.

Nymphs of aphids were used, as preliminary data indicated that 3–4-day old nymphs of each species were similar in size based on aggregate sample weights (20 aphids/sample). Nymphal infestations were achieved by confining adult aphids on the abaxial surfaces of the largest middle leaves of each selected plant (leaves ca. 3-4 cm from the soil surface) using clip cages. The two aphid species were added to separate plants. The adults were allowed to reproduce for 24h, after which time the cages were removed and adults were picked off the leaves using a fine paintbrush. The nymphs

were counted and numbers per leaf were adjusted if necessary (by removing some individuals) so that treatment leaves were of similar density. The cages were then returned and left in place until the time of the experiment, when the nymphs were 60 ± 24h old. Two aphid densities (low vs. high) per plant were established. “Low density” treatment plants consisted of 15-30 nymphs on a single leaf produced by caging of 15 adult *A. solani* or 12 adult *M. persicae* per leaf. “High density” treatment plants consisted of 80-140 nymphs per plant, distributed across 2 leaves (40-80 nymphs per leaf). This range was produced by caging 35 *A. solani* or 30 adult *M. persicae* per leaf. Initial adult aphid densities were chosen to result in approximately equal densities of both aphid species after 24h of reproduction. Ranges rather than exact numbers of nymphs were targeted to minimize manipulation of nymphs. Each test plant ultimately presented one of four treatment combinations: *A. solani* at low density, *A. solani* at high-density, *M. persicae* at low density, or *M. persicae* at high density. There were four plants (replicates) of each species/density treatment combination per compartment (e.g., 1 “high” density *A. solani* plant on each of the 4 greenhouse benches). The entire experiment was replicated simultaneously in a second greenhouse compartment (resulting in a total of n=8 for each treatment combination at the whole-plant level). On the level of prey patch (i.e. individual leaves), this resulted in n = 8/species for “low” density patches of aphids and n=16/species for “high” density patches of aphids.

Twenty adult *A. aphidimyza* were released per bench (80 midges per compartment) as described in the general methodology for greenhouse experiments (above). Supplemental lighting was omitted the day of release, but resumed the next

day as usual (16:00–22:00h). After 2 nights, all treatment plants were collected and destructively sampled, and the number of aphids and *A. aphidimyza* eggs per leaf were counted under a dissecting scope (10-20x magnification). Both treatment and non-treatment leaves within plants were counted to determine if aphids migrated away from the original sites. Five background plants per bench were also sampled (1 plant randomly selected per bench, plus the 4 center plants which made up the release site for each bench) to confirm that *A. aphidimyza* did not oviposit on un-infested plants. This experiment was conducted in late April, 2010. Temperatures in the compartments (recorded at 10-min intervals) ranged from 15-30 °C over the course of the experiment. The average daily temperature was 19.9 °C in the first compartment and 19.7 °C in the second. The average daily RH was 57% across both compartments (range = 12-100%). Average RH over the evenings (*i.e.* 7pm-7am; when most oviposition by *A. aphidimyza* is thought to take place) was 91%.

Prey Patch Selection and Within-Plant Location

Prey Patches on Top vs. Bottom Leaves (Different Plants):

To investigate the effect of aphid species vs. within-plant location of prey patches on *A. aphidimyza* oviposition, we artificially inflated aphid numbers on either top or bottom leaves of plants (see above for a description of within-plant locations). Top leaves represented younger tissue close to the meristem, while bottom leaves represented the oldest plant tissue. Treatments (1 plant/treatment combination/bench) consisted of: i) *A. solani* on 2 top leaves/plant, ii) *A. solani* on 2 bottom leaves/plant, iii) *M. persicae* on 2 top leaves/plant, and iv) *M. persicae* on 2 bottom leaves/plant.

Treatment plants were replicated across 4 benches in each of 2 compartments for a total of n=8 for each treatment at the whole plant level, and a total of n=16 for each treatment at the prey patch level. For each of the treatments, adult aphids were confined to their assigned location using clip cages and allowed to reproduce for 6d prior to the experiment (after which time cages were removed). Initially, 3 *M. persicae* or 5 *A. solani* were added to each leaf in an attempt to obtain equal densities of the two species after 1 week of reproduction. As adult aphids were not removed in this experiment, all aphid ages were present. A greater number of *A. aphidimyza* adults were released in this experiment (i.e. 55 adults/bench, or a total of 220 per compartment) and they were only allowed to oviposit for 12 hours in an attempt to minimize the time that aphids could migrate from the initial treatment leaf to any other location on the plant. Data collection was the same as in previous experiments. The experiment was conducted in early June, 2011. Average temperatures over the course of this experiment (including the period of aphid reproduction) were 23.6 °C (16.7–38.0 °C) for compartment 1 and 22.9 °C (16.7–35.9 °C) for compartment 2. RH averaged 77% in compartment 1 and 73% in compartment 2, never falling below 42% RH. Average RH over the evenings was >90% for both compartments.

Despite the brief time period, the percentage of aphids that migrated from the treatment leaves in this experiment was relatively high (21%). However, the number of *A. aphidimyza* eggs deposited on these aphids was low (see Results Section), suggesting the aphids migrated after the majority of the oviposition by *A. aphidimyza* was completed. Thus, we continued with comparisons between top vs. bottom leaves.

Prey Patches on Meristems vs. Bottom Leaves (Same Plants):

This experiment was originally designed as a no-choice experiment to determine the capacity of *A. aphidimyza* to seek out and oviposit in patches confined to the bottom leaves of plants. The objective was altered, however, when significant numbers of aphids (22% of the total population) migrated from the bottom leaves after removal of the clip cages and established colonies in other locations. These colonies ultimately attracted 60% of all eggs deposited by *A. aphidimyza*, strongly suggesting that oviposition did not commence as quickly and/or the aphids migrated more rapidly than in the experiment conducted on top vs. bottom leaves. As the greatest numbers of migrant aphids of both species were found on meristems (16% of the total population), we chose to use these plants in a post-hoc investigation of *A. aphidimyza* oviposition on meristems vs. bottom leaves. As no eggs were deposited in aphid patches on middle leaves and only 12 eggs were found on 4 top leaves, these locations were ultimately excluded from data analysis.

Initial infestation of the pansy plants was achieved by adding adult aphids to either 1 bottom leaf (i.e. “low” aphid density plants) or 2 bottom leaves (i.e. “high” aphid density plants) and confining them using clip cages. Aphids were allowed to reproduce for 6 days prior to the experiment, after which time the cages were removed. Treatments included: i) 3 adult *M. persicae* per plant (added to 1 leaf), ii) 6 *M. persicae* per plant (2 leaves infested with 3 *M. persicae* adults each), iii) 7 *A. solani* adults per plant (added to 1 leaf), and iv)14 *A. solani* per plant (2 leaves infested with 7 *A. solani* each). There were 4 replicates per treatment (1 plant/bench). Aphid densities were chosen in an attempt to achieve similar aphid densities across aphid

species after 1 week of reproduction. The release rate of *A. aphidimyza* and experiment duration were the same as in the experiment on top vs. bottom leaves. The experiment was conducted in June 2011 in a single greenhouse compartment. The average temperature and RH for the experiment were 22.4 °C (14.9–31.9 °C) and 78% (38–100%; avg.= 92% over the evening).

Prey Patches on Bottom-Leaves Only (No-Choice):

To determine if *A. aphidimyza* could be forced to search for/attack aphid patches present on bottom leaves, a no-choice experiment was done with aphids confined to this location only. Methodology was identical to the experiment immediately preceding (meristems vs. bottom leaves). Unlike that experiment, however, far fewer aphids (< 8% in both experimental replicates) migrated from the bottom leaves after cage removal and received few *A. aphidimyza* eggs. The experiment was conducted twice: once in December 2011 (Avg. temp. = 17.6°C, range = 13.2-24.0 °C; Avg. RH= 70%, range = 39-95%) and again in May 2012 (Avg. temp. = 21.6 °C, range = 17.5-31.1°C; Avg. RH= 69%, range = 16-97%). Average RH over the evening of the experiment (7pm-7am) was 78% for December 2011 and 89% for May 2012.

Prey Patch Selection in Naturally Distributed Aphid Populations

In order to investigate *A. aphidimyza* oviposition choices under more natural conditions, whole-plant experiments were again conducted, but this time adult aphids were placed in the center of the plant and allowed to distribute themselves naturally and reproduce (vs. being caged at a particular location). Treatments included i) a low

density of *M. persicae* (2 adults per plant), ii) a high density of *M. persicae* (8 adults per plant), iii) a low density of *A. solani* (3 adults per plant), and iv) a high density of *A. solani* (16 per plant). There were 4 replicate plants per treatment combination (1/bench), and the experiment was replicated simultaneously in 2 greenhouse compartments (total n=8 infested plants per aphid species/density treatment combination). Numbers of adult aphids initially added were chosen to provide similar whole-plant population densities of both aphid species after 6 days of reproduction; the initially transferred adults were not removed. The release rate of *A. aphidimyza* and experimental duration was the same as when experimentally manipulated prey populations were tested. During destructive sampling, plants were divided into the 4 possible within-plant locations described in the "Plant Material" section above.

This experiment was conducted in mid June 2010. The average temperature in the first compartment was 22.4 °C (range: 15-32 °C). The average RH was 78% (range = 38-100%). Average RH over both evenings of the experiment (7pm-7am) was 91%. The data logger in the second compartment failed, but subsequent temperature measurements in that compartment indicated its average daily temperature fell within 1 °C of the first compartment.

Statistical Analyses

In all experiments, a preliminary analysis was conducted to determine the effects of aphid presence on *A. aphidimyza* egg presence. Aphid and egg counts were converted to presence/absence data and these binomial data were analyzed with a logistic regression, using the Proc Genmod procedure in SAS (v. 9.2, 2008). A Type 3

analysis was used to determine the importance of main effects in the model. For all experiments, aphid presence had a statistically significant effect on egg presence ($P \leq 0.0282$ for all analyses), and few eggs were found in patches (i.e. leaves or meristems) without aphids (<5 % of eggs in all experiments). Thus patches/plants without aphids were eliminated from further analyses.

Effects of aphid density, species, and location of aphid patches on the plant (where appropriate) on the number of *A. aphidimyza* eggs were analyzed using a mixed model with all possible interactions (Proc Mixed, SAS). The Kenward-Roger method of calculating degrees of freedom was applied to all models (Little et al. 2002). For all experiments, aphid density data were analyzed as a continuous variable. In analyses at the whole plant level, greenhouse compartment and bench (nested within compartment) were included as random effects. In most cases, compartment and bench accounted for a small fraction of the total variation seen in experimental outcomes (mean= 20% (range: 1-25%) for compartment; mean =10 % (range: 0-27%) for bench). Nevertheless, both were retained in all models to control for these sources of variation. For counts on individual patches, we also included plant (nested within block) as a random effect to control for between-plant differences; plant contributed an average of 9% (range: 0-20%) to the total variation. Statistical analyses at the patch-level also include plant meristems as data points as well as leaves. Statistical differences between patch locations were determined using the Tukey-Kramer test in all experiments. In the experiment on naturally distributed aphid populations, straightforward chi-square tests were used to examine whether the distribution of

aphids differed among plant locations (although migration and larviposition responses were likely not strictly independent).

In all tests, where aphid and egg counts data did not meet the assumptions of normality and variance homogeneity, the $\log_{10}(x + 1)$ transformation was applied to both of these variables prior to analysis (referred to henceforth as the log-log transformation). In all cases, untransformed, arithmetic means and standard errors are presented first, with least-squares (LS) means and standard errors or back-transformed least-squares means given in parentheses (for untransformed and transformed data, respectively). In cases where aphid density had a significant effect on *A. aphidimyza* egg deposition, data were further analyzed using linear regression (Proc Reg in SAS).

RESULTS

Prey Patch Selection in Experimentally Manipulated Prey Populations

At the whole plant level (Figure 4.1A), only aphid density affected the oviposition choice of *A. aphidimyza* ($F_{(1,21.6)}$ =32.4, P<0.0001); species and the species × density interaction were not significant ($F_{(1,21.4)}$ =0.32, P=0.5791 and $F_{(1,21.6)}$ =0.02, P=0.8777, respectively). The mean number of *A. aphidimyza* eggs per *A. solani* infested plant was 48.3 ± 8.82 (LSmean = 46.8 ± 12.75), with the mean number of aphids infesting each plant being 76.2 ± 13.00. For *M. persicae*, the mean number of eggs per plant was 39.6 ± 7.34 (LSmean = 41.2 ±12.75), with a mean of 69.2 ± 11.50 aphids per plant (average aphids/plant for each species were not significantly different; t_{30}=0.40, P=0.6474).

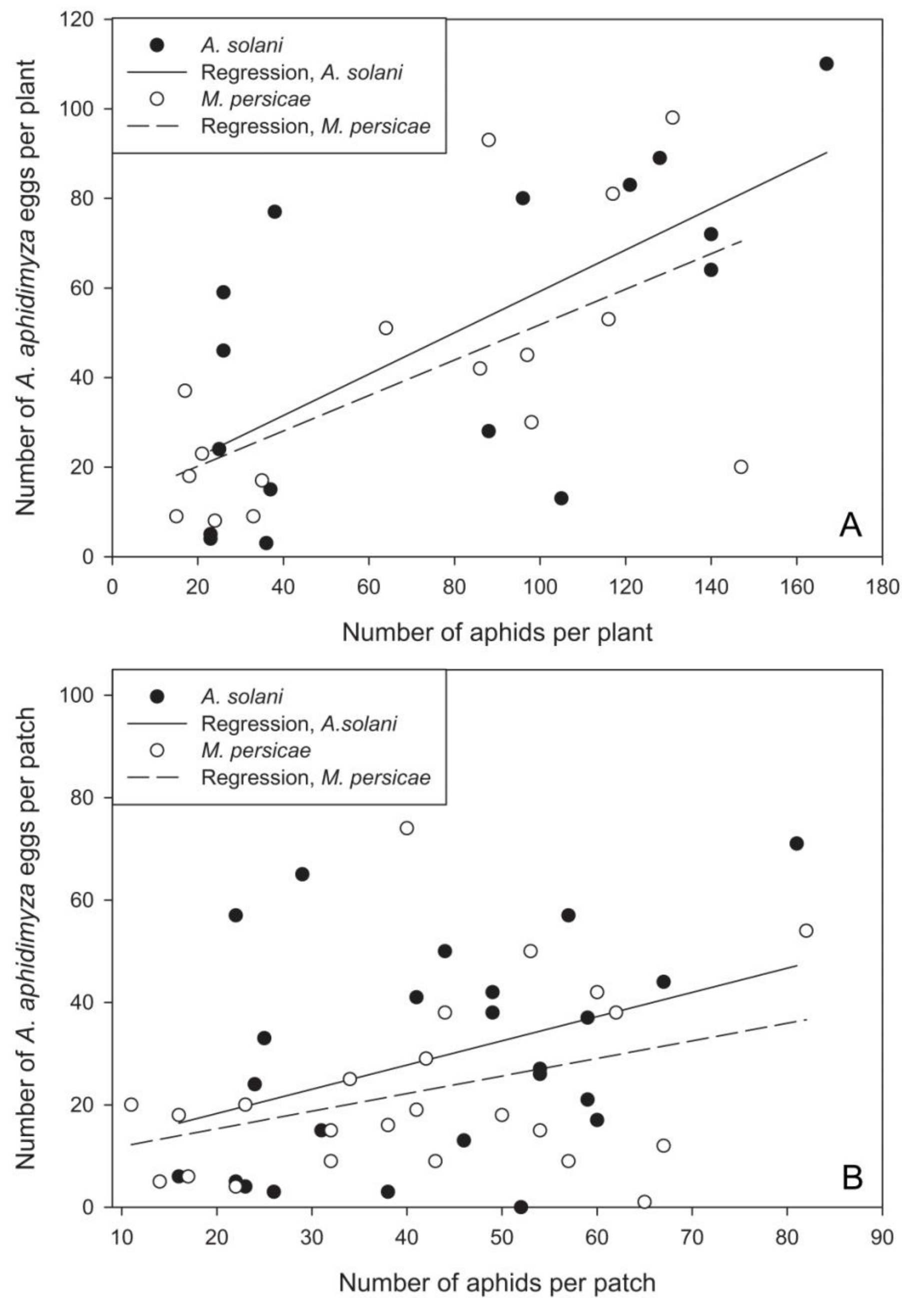


Figure 4.1. A. Effect of the number of aphid nymphs of *A. solani* and *M. persicae* on the number of *Aphidoletes aphidimyza eggs* per plant when aphids were confined to a single location (middle leaves of plants). Though aphid species was not significant, both species are shown for comparison. For linear regressions on each species, R^2= 0.46 for *A. solani* and R^2= 0.38 for *M. persicae*. **B**. Effect per patch (treatment leaf); aphid species was not significant. For linear regressions on each species, R^2 =0.15 for *A. solani* and R^2= 0.13 for *M. persicae*. The linear regression for both species combined was significant ($F_{(1,46)}$=7.16, P=0.0103, R^2 =0.14).

During the experiment, 12.9% of aphids migrated from the treatment leaves, and aphid patches on non-treatment leaves received < 10% of the total midge eggs. Due to this relatively low amount of aphid migration, only treatment leaves (those initially infested with aphids) were analyzed at the patch level (though an analysis including all aphid-infested patches confirmed the results for main effects and interactions). Analysis at the leaf level showed the same trends as at the whole plant level (Figure 4.1B): only aphid density had an effect on oviposition ($F_{(1,40.2)}$ =7.5, P=0.0093); aphid species and the density x species interaction were insignificant ($F_{(1,39)}$ =0.24, P=0.6289 and $F_{(1,39.3)}$ =0.01, P=0.977, respectively). The average number of eggs deposited on *A. solani* infested leaves was 29.1 ± 4.34 (LSmean = 28.9 ± 7.75), with 22.8 ± 3.70 on *M. persicae* infested leaves (LSmean = 23.0 ± 7.75). The average numbers of aphids per leaf for the two species were not significantly different, with 42.8 ± 3.50 for *A. solani* vs. 41.6 ± 3.84 for *M. persicae* (t_{46}=0.23, P=0.8171).

Regressions of *A. aphidimyza* eggs vs. aphids per plant were significant for both aphid species ($F_{(1,14)}$=12.03, P = 0.0038 for *A. solani*; $F_{(1,14)}$ = 8.74, P =0.0104 for *M. persicae*) (Figure 3.1A). Coefficients of determination (R^2) indicated that 38–46% of total variation in egg numbers was attributable to aphid density. Regressions were weaker (not significant) on the per leaf basis for each species separately ($F_{(1,22)}$ = 3.76, P=0.065 for *A. solani*, $F_{(1,22)}$ = 3.18, P=0.088 for *M. persicae*; Figure 4.1B). Regression was significant, however, when data for the two species were pooled ($F_{1,46}$=7.16, P=0.0103, R^2 =0.14).

Prey Patch Selection and Within-Plant Location

Prey Patches on Top Leaves vs. Bottom Leaves (Different Plants):

Two plants from this trial were removed from analysis due to excessive drooping of top leaves (possibly due to the weight of clip cages). As mentioned previously, though this experiment was run over a shortened time frame (12 h) to try to decrease aphid dispersal on plants, 21% of the aphids still migrated (with 53% of these migrants settling on plant meristems). Aphids at non-treatment locations received 10.2 % of the *A. aphidimyza* eggs deposited, suggesting that aphids moved after most of the oviposition took place. However, due to the high amount of wandering (almost double of that of our other experiments where location was controlled for), we included all aphid-infested patches at all plant locations in the analysis (vs. treatment leaves alone). Data were log-log transformed to better meet the assumptions of ANOVA.

In the resulting analysis, both aphid density and location of the patch on the plant had a significant effect on *A. aphidimyza* oviposition ($F_{(1,205)} = 69.25$, $P<0.0001$; $F_{(3,204)} = 6.52$, $P=0.0003$, respectively). The interaction of density × location was also significant ($F_{(3,204)} = 29.92$, $P<0.0001$). Species did not have a significant effect on oviposition ($F_{(1,199)} = 0.02$, $P=0.8889$). Patches infested with *M. persicae* (mean = 16.0 ± 2.28 aphids/patch) received an average of 6.9 ± 1.77 eggs/patch (back-transformed LSmean =0.6). *Aulacorthum solani* infested patches (mean = 13.5 ± 1.94 aphids/patch) received 3.0 ± 0.90 eggs/patch (back-transformed LSmean = 0.6). Density × species and the 3-way interaction including species also had no effect on oviposition ($F_{(1,205)} = 0.04$, $P=0.8485$; and $F_{(3,204)} = 1.14$, $P =0.3321$, respectively).

When numbers of eggs received by aphids at all plant locations were compared, aphid patches on top leaves (mean=28.7 ± 4.58 aphids/patch; back-transformed LSmean=9.9) received a higher number of eggs (mean = 16.2 ± 3.63 eggs/patch, back-transformed LSmean = 1.8) than all other plant locations ($t_{203} \geq 2.75$, $P \leq 0.0330$ for all comparisons; Tukey-Kramer test). Despite that bottom leaves were also highly infested with aphids (mean=16.5 ± 2.32 aphids/patch; back-transformed LSmean = 7.4), they received far fewer eggs (mean = 1.4 ± 0.43 eggs/patch; back-transformed LSmean = 0.4). The number of eggs received by bottom leaf patches was not statistically different from eggs received by aphids at migrant locations (i.e. mean =0.04 ± 0.39 eggs/patch, back-transformed LSmean= 0 for middle leaves; mean= 3.7 ± 0.93 eggs/patch, back-transformed LSmean = 0.63 for meristems) ($t_{204} \leq 2.29$, $P \geq 0.1043$ for all comparisons; Tukey-Kramer test).

Coefficients of determination (R^2) for regression analyses investigating the density × location interaction revealed that density explained 82% ($F_{(1,54)}$=244.79, $P<0.0001$) of the variation in egg deposition on top leaf patches, but only 29% of the variation for bottom leaf patches ($F_{(1,68)}$=33.49, $P<0.0001$; Figure 4.2). Only 0.7% and 12 % of the variation was explained for middle leaves and meristems, respectively, and the regression was not significant ($P \geq 0.0562$) for either of these locations (thus data for these locations were not shown in Figure 4.2). Additionally, out of 27 aphid-infested top leaves with >10 aphids each (considered a high density patch), only 2 aphid patches (7%) received no eggs by *A. aphidimyza*. In contrast, out of 30 bottom leaves with >10 aphids each, 14 aphid patches (47%) received no eggs (7 *M. persicae* infested, 7 *A. solani* infested) (Figure 4.2). This further supports the F-

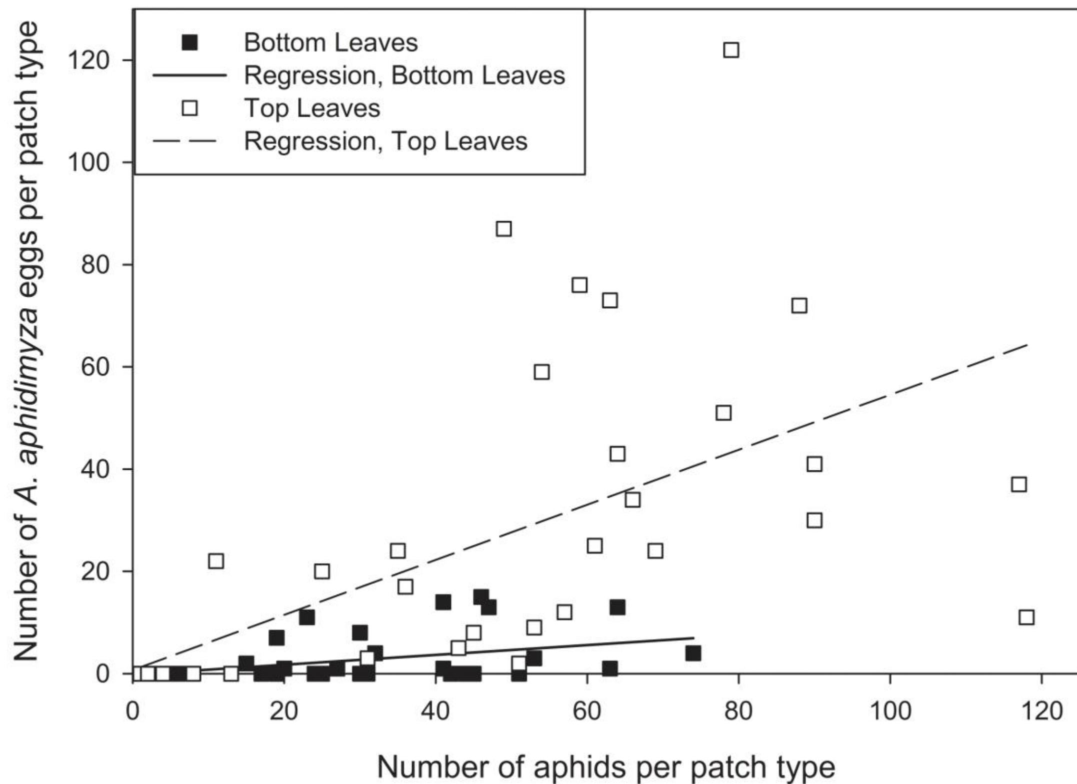


Figure 4.2. Number of *A. aphidimyza* eggs on bottom leaf vs. top leaf aphid-infested patches, untransformed data and trendlines shown (data for aphids that migrated to middle leaves and meristems not shown). For the linear regresssions on log-log transformed data, the correlation between the number of eggs laid in a patch and the number of aphids per patch was higher on infested top leaves ($R^2 = 0.82$) than bottom leaves ($R^2 = 0.33$).

test for the main effect of location in the model, and indicates that these bottom-leaf patches were not found or were rejected as oviposition sites by *A. aphidimyza*, even at a high release rate of this natural enemy.

Prey Patches on Meristems vs. Bottom Leaves (Same Plant):

Although a high percentage of wandering was seen in this trial (21%), the majority of the aphids (of both species) that migrated ended up on the plant meristems (16% of all

aphids in the experiment). Thus, only aphids present on the meristems and bottom leaves were considered in the analysis (though an analysis retaining other locations yielded similar results). Data were log-log transformed to better meet assumptions of ANOVA. As the interactions of density × species, species × location, and the 3 way interaction were all highly non-significant (i.e. $P \geq 0.72$), these were removed to provide a better fitting model.

As with previous results, aphid density had a significant effect on *A. aphidimyza* oviposition ($F_{(1, 42.3)} = 18.16$, $P=0.0001$). While *M. persicae*-infested patches received a higher number of eggs (mean=10.8 ± 3.15 eggs/patch; LSmean = 4.6) than *A. solani*-infested patches (mean = 4.8 ± 1.94 eggs/patch; LSmean = 3.0), aphid species was not a significant factor in the model ($F_{(1, 8.36)} = 1.29$, $P= 0.2876$). Despite that aphid patches on meristems received a greater number of *A. aphidimyza* eggs (mean = 16.7 ± 4.03 eggs/patch; LSmean = 1.6) compared to bottom leaves (mean = 4.5 ± 1.99 eggs/patch; LSmean = 0.2), location as a main effect was not significant ($F_{(1, 41.4)} = 0.42$, $P=0.522$). However, a significant interaction between density and patch location was observed ($F_{(3, 41.3)} = 5.65$, $P=0.0222$), which is apparent in Figure 4.3.

Although meristems held only 16.2% of the aphid population in the experiment (mean = 17.4 ± 2.66 aphids/patch), 86.7% of these patches were found by *A. aphidimyza*, receiving 59.6% of the eggs. The regression analysis for this location was significant ($F_{(1,13)}=38.95$, $P<0.0001$, Figure 4.3), with aphid density explaining 75% of the variation in egg deposition at this location. Although regression was also significant for aphid patches located on bottom leaves ($F_{(1,33)}=6.55$, $P=0.0153$), the

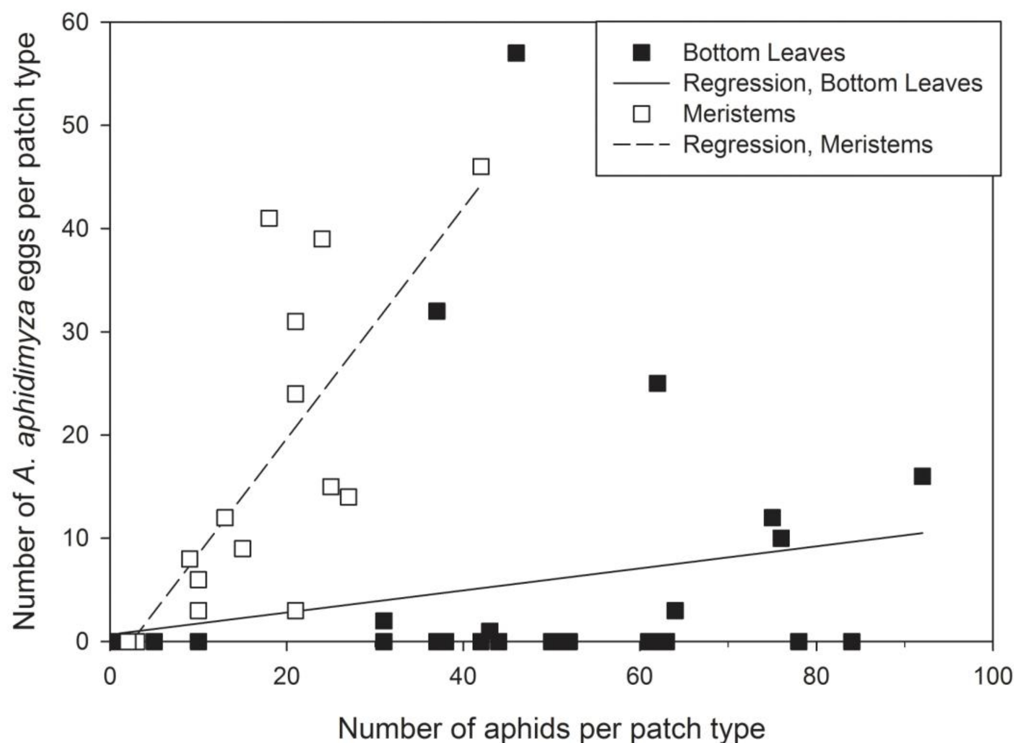


Figure 4.3. Number of *A. aphidimyza* eggs on aphid-infested bottom leaves vs. meristems, untransformed data and trendlines shown. Though aphid species was not significant, both species are shown for comparison. The correlation between the number of eggs laid in a patch and the number of aphids per patch was greater for meristems (R^2 = 0.75) than bottom leaves (R^2 = 0.17) (linear regression on log-log transformed data).

correlation between aphid density and egg density was much weaker (R^2=0.17; Figure 3.3). Of the bottom-leaf patches, only 9 out of 35 patches (25.7%) were found and selected as oviposition sites by *A. aphidimyza*. Despite comprising 78% of the aphids in the experiment (mean = 35.9 ± 4.94 aphids/patch), bottom patches received only 37.5% of *A. aphidimyza* eggs.

Prey Patches on Bottom Leaves Only:

For both replicates of this experiment, aphids generally stayed on the bottom leaves during the 12h period when cages were removed. In the first replicate of the experiment, the migration rate was 7.2% (with these aphids receiving <5 % of total deposited *A. aphidimyza* eggs). In the second replicate, 6.8% migrating was observed (with these aphids receiving <7% of eggs). Due to the low level of migration and egg deposition on non-treatment locations, only the treatment leaves were analyzed for this experiment. Data approximately met the assumptions of ANOVA, therefore no transformation was necessary.

Here, only density had a significant effect on *A. aphidimyza* oviposition on aphid-infested bottom leaves ($F_{(1,40.1)} = 7.21$ $P = 0.0105$). According to the coefficients of determination (R^2) for the linear regressions, density explained 42% of the variance for *M. persicae*, and 20% of the variance for *A. solani* (Figure 4.4; $F_{(1,22)}=15.90$, $P=0.0006$ for *M. persicae*; $F_{(1,20)}=5.03$, $P =0.0364$ for *A. solani*). Species and the interaction of density × species were not significant ($F_{(1,35)} = 3.22$, $P = 0.0814$ and $F_{(1,36.3)} = 3.11$, $P=0.0861$, respectfully). Out of the 46 aphid patches present, 36 (78%) received eggs, indicating that female midges will find and accept the majority of bottom leaf patches as oviposition sites if no other choices are present. Out of the 10 patches not found/selected, 6 of these were *A. solani* and 4 were *M. persicae*. The average number of eggs/patch for *A. solani* was 18.3 ±4.53 (LSmean = 20.0 ± 9.57), with an average of 46.3 ± 5.84 aphids/patch. The average eggs/patch for *M. persicae* was 18.8 ±3.48 (LSmean = 18.1 ± 9.53), with an average of 45.7 ± 2.75 aphids/patch

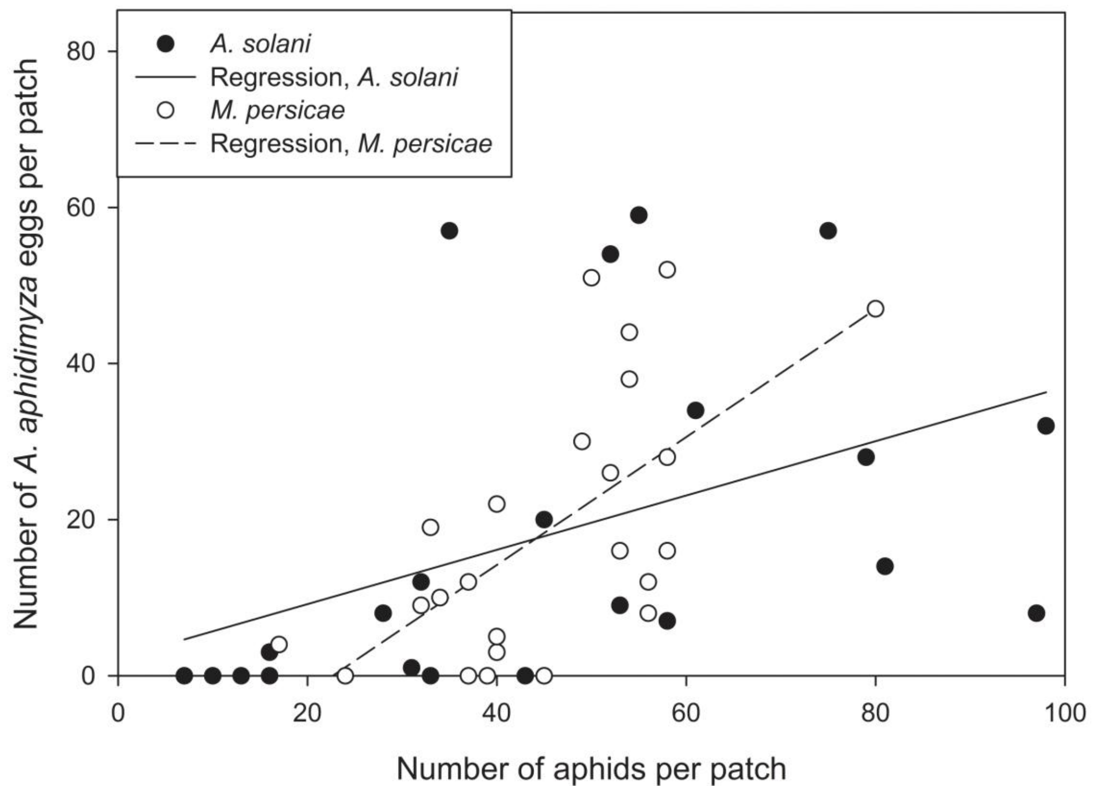


Figure 4.4. Number of *A. aphidimyza* eggs per patch, when only the bottom leaves of plants were infested with either *A. solani* or *M. persicae*. Though aphid species was not significant, both species are shown for comparison. For linear regressions on each species, R^2=0.42 *for M. persicae* and R^2 = 0.20 for *A. solani*.

(average aphid densities were not significantly different between the two species; t_{44}=0.10, P=0.9236).

Prey Patch Selection in Naturally Distributed Aphid Populations

In this experiment, one *A. solani* infested plant (in the "high-density" treatment) was removed due to significant development of a flower at the growing point, which

greatly affected the aphid distribution on that plant. Data from all other plants were retained; data were log-log transformed prior to analysis.

Aphid density again had a significant effect on *A. aphidimyza* oviposition ($F_{(1,20.6)}$ =21.8, P<0.0001) at the whole plant level (Figure 4.5A), but there was no density × species interaction ($F_{(1,21.1)}$ =0.10, P=0.7604). Aphid density accounted for 39–43% of total variability in oviposition (for *A. solani* ($F_{(1,13)}$ =8.21, P=0.0130) and *M. persicae* ($F_{(1,14)}$=10.33, P=0.0062, respectively). Unlike all previous experiments, however, species also affected oviposition ($F_{(1,20.9)}$ =8.17, P=0.0095). The average number of *A. aphidimyza* eggs laid on *M. persicae*-infested plants was 48.6 ± 6.56 (back-transformed LSmean = 35.4), vs. only 3.9 ± 1.78 egg/plant (back-transformed LSmean = 1.8) for *A. solani*. This was despite the fact that the average number of aphids per plant was not significantly different between the two species (*M. persicae* =55.4 ± 9.22 aphids/plant, *A. solani* = 43.5 ± 10.91 aphids/plant; t_{29}= 1.48, P =0.150, log-log transformed data).

At the patch level, aphid density was again a highly significant factor in oviposition ($F_{(1,207)}$ =40.9, P<0.0001). However, while aphid density accounted for 62% of the variability in *A. aphidimyza* oviposition in *M. persicae* patches, it only accounted for 13% in *A. solani* patches (Figure 4.5B; $F_{(1,121)}$=195.87, P<0.0001 for *M. persicae*; $F_{(1,98)}$ = 15.21, P=0.0002 for *A. solani*).

Location of the aphids on the plant again had a significant effect on midge oviposition at the patch level ($F_{(3,207)}$ = 3.7, P= 0.0119), but so did aphid species in this experiment ($F_{(1,207)}$ = 24.0, P<0.0001). Number of *A. aphidimyza* eggs averaged 6.0 ± 1.54 eggs/patch for *M. persicae* (back-transformed LSmean = 1.0), which was higher

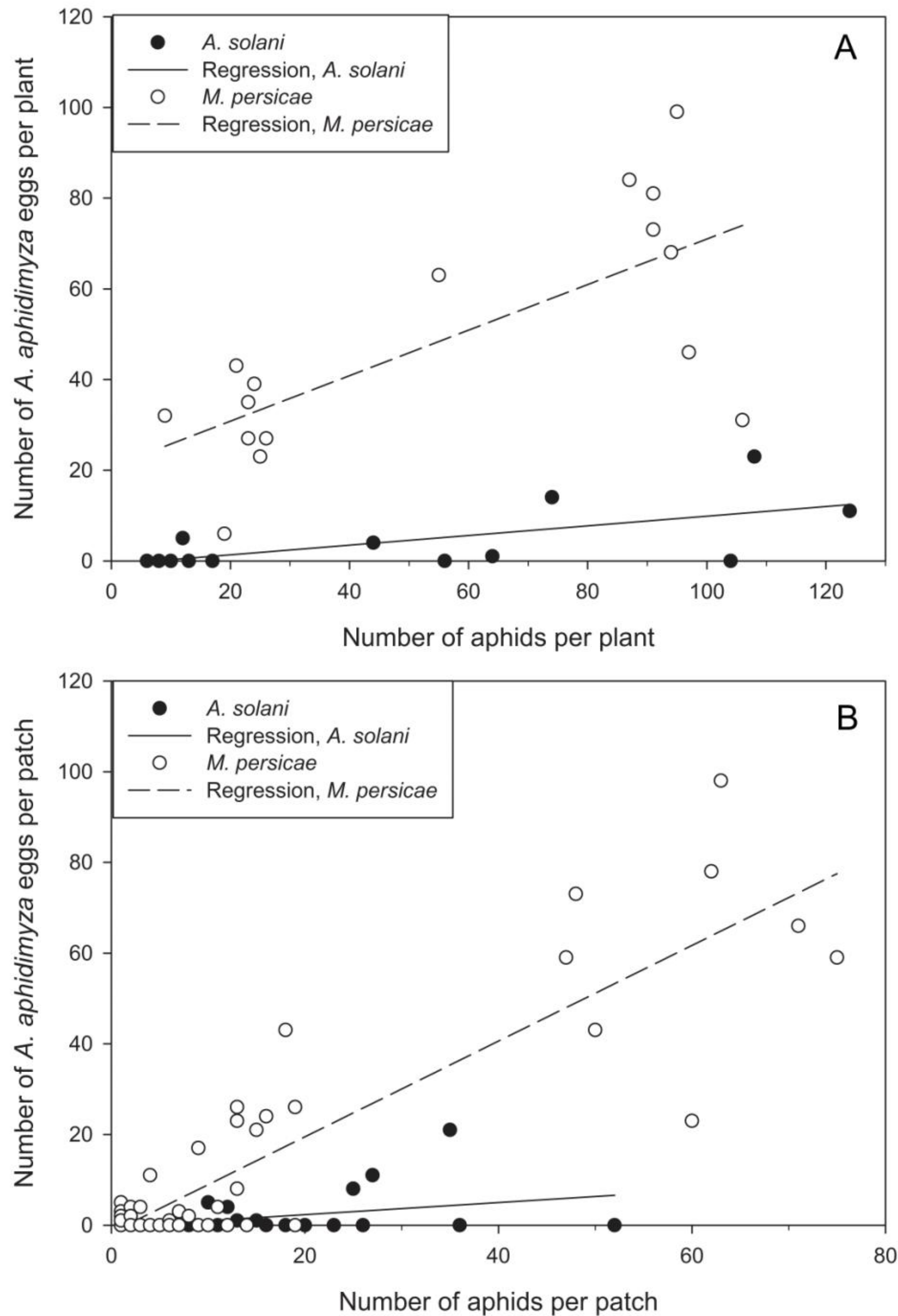


Figure 4.5. **A**. Effect of aphid density (all stages) of *A. solani* or *M. persicae* on the number of *A. aphidimyza* eggs per plant, untransformed data and trend lines shown. Aphids were allowed to distribute naturally on plants. For linear regressions on log-log transformed data, R^2= 0.39 for *A. solani* and R^2 =0.43 for *M. persicae*. **B.** Effect of aphid density on *Aphidoletes aphidimyza* eggs per patch, ntransformed data and trend lines shown. For linear regressions on log-log transformed data, R^2=0.13 for *A. solani* and R^2=0.62 for *M. persicae*.

than the 0.6 ± 0.26 eggs/patch (back-transformed LSmean = 0.0) for *A. solani* (t_{177}= 7.39, P<0.0001). Concerning the effect of patch location, a statistically greater number of eggs was found on aphid-infested meristems (mean=25.3 ± 5.19 eggs/patch; back-transformed LSmean=1.4) compared to all other plant locations (t_{207}≥2.87, P≤0.0231 for all comparisons). Top leaves also received more eggs on average than middle or bottom leaves (mean for top leaves = 1.0 ± 0.29 eggs/patch, back-transformed LSmean = 0.5; t_{207} ≥3.45, P≤ 0.0027 for all comparisons). However, it is important to note that in the model there were also highly significant interactions between aphid species and location ($F_{(3,207)}$ = 11.5, P <0.0001), density and location ($F_{(3,207)}$ = 32.9, P < 0.0001), as well as a 3-way interaction of density, species, and location ($F_{(3,207)}$ = 5.5, P = 0.0013) (which are examined further below). The species × density interaction was not significant ($F_{(1,207)}$ = 1.3, P=0.2476).

For each species, marked differences were observed in the proportion of aphids found at each plant location (*A. solani* $\chi^2_{(3)}$ = 353.3, P<0.0001; *M. persicae* $\chi^2_{(3)}$ =826.1, P<0.0001) (Figure 3.6A). The greatest number of *M. persicae* (66.6% of the population) was found on the meristems and the lowest (7.1%) on bottom leaves. In contrast, the greatest number of *A. solani* (55%) was found on the bottom leaves and the lowest (7.4%) on the middle leaves. In the case of both aphid species, *A. aphidimyza* egg deposition varied across patch location (Figure 4.6B), with meristems receiving the highest proportion of total eggs, regardless of prey species. This nearly identical oviposition response to the two aphid species was unexpected in view of the highly significant species × location interaction in the mixed model. To further

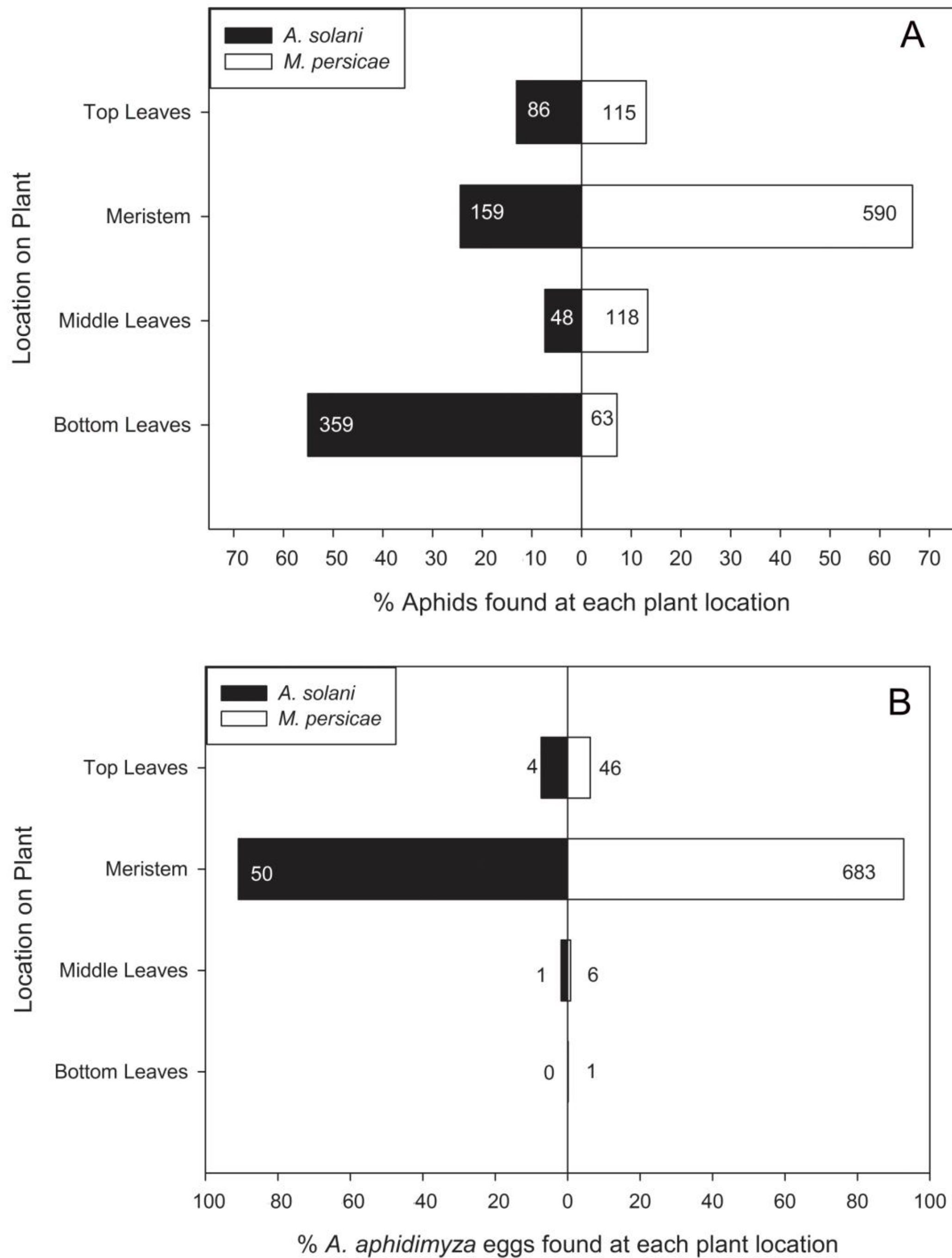


Figure 4.6. **A**. Proportion of the total aphid population (across all treatment plants within two greenhouse compartments) found at each location on plants for *A. solani* (black bars) and *M. persicae* (white bars). Number of plants sampled was n=15 for *A. solani*, n=16 for *M. persicae*. **B.** Proportion of total *A. aphidimyza* eggs deposited at each plant location for each species.

investigate this interaction, data were pooled across densities to produce Table 4.1. Here, the significant species × location interaction is evident. The LSmeans for *A. aphidimyza* eggs/patch (back-transformed from the log-log transformation and thus adjusted from the unbalanced design) are much smaller than the simple means (Table 4.1). A Tukey-Kramer test of LSmeans revealed differing patterns of oviposition in patches of each aphid species: *Aulacorthum solani* attracted statistically equivalent numbers of eggs at all locations ($t_{207} \leq 2.05$, $P \geq 0.0920$ for all tests), whereas *M. persicae* received a statistically greater number of eggs on meristems vs. other prey patch locations ($t_{207} \geq 5.61$, $P < 0.0001$ for all tests; Table 4.1). However, despite the above-described interaction, it is clear that mean egg deposition in each location, expressed as a percentage of the total, is similar for each aphid (Table 4.1). Though very few eggs were laid in *A. solani* patches on meristems, the number still represented 92% of all eggs laid in attacks against this host (Figure 3.6B, Table 3.1). Similarly, patches of each species on bottom leaves received virtually no eggs, despite several high-density patches of *A. solani* at this location (see Table 4.2).

To further investigate interactions between aphid species, density, and location at the patch-level, data were also tabulated with density categorized as low vs. high (< 10 vs. ≥ 10 aphids/patch) based on patterns observed in the data (Table 4.2). The density × location interaction is not immediately evident in the tabulated data, but this is largely due to the anomaly of a single low-density patch of *M. persicae* (with 9 aphids) occurring at the location of plant meristems – all other *M. persicae*-infested meristems were considered high density patches (Table 4.1). Omitting this datum reveals that exceedingly few eggs were deposited in any low-density patches,

Table 4.1. Effects of prey patch species and location on *Aphidoletes aphidimyza (A.a.)* oviposition. Aphids were allowed to naturally distribute and reproduce on plants.

Aphid species	Aphid patch location	Number of patches (% of total)	Mean aphids per patch	Total number of *A.a.* eggs laid per patch	Mean *A.a.* eggs per patch (% of total within each aphid species)	LSmean eggs per patch [a]	% of patches attacked
M. persicae	Meristems	16 (7.2)	36.9 ± 6.1	683	42.7 ± 6.6 (97.0)	7.1 A	100
	Top Leaves	39 (17.5)	2.9 ± 0.5	46	1.2 ± 0.4 (2.7)	0.7 B	38.5
	Middle Leaves	43 (19.3)	2.7 ± 0.4	6	0.1 ± 0.1 (0.2)	0.1 C	4.7
	Bottom Leaves	25 (11.2)	2.5 ± 0.7	1	0.04 ± 0.04 (0.1)	0 C	4.0
A. solani	Meristems	13 (5.8)	12.2 ± 2.8	50	3.9 ± 1.7 (91.8)	0 C	46.2
	Top Leaves	12 (5.4)	7.2 ± 3.1	4	0.3 ± 0.2 (7.2)	0.2 C	25.0
	Middle Leaves	25 (11.2)	1.9 ± 0.3	1	0.04 ± 0.04 (1.0)	0 C	4.0
	Bottom Leaves	50 (22.4)	7.2 ± 1.3	0	0 (0)	0 C	0
Totals		223		791			

[a] Least squares means back-transformed from log n+1. Means followed by same letter are not significantly different (Tukey-Kramer test, alpha = 0.05).

Table 4.2. Effects of prey patch density, location and species on *Aphidoletes aphidimyza* (*A.a.)* oviposition. Aphids were allowed to naturally distribute and reproduce on plants.

Patch Density	Patch location	Aphid species	Number of patches (% of total)	Mean Aphids per patch	Total number of *A.a* eggs per patch	Mean *A.a* eggs per patch (% of total mean egg production)	% of patches attacked
High (≥10 aphids)	Meristems	*M. persicae*	15 (35.7)	38.7 ± 6.3	666	44.4 ± 6.9 (77.9)	100
	Meristems	*A. solani*	6 (14.3)	20.3 ± 4.1	50	8.3 ± 2.9 (14.6)	100
	Top Leaves	*M. persicae*	2 (4.8)	12.5 ± 0.5	8	4.0 ± 4.0 (7.0)	50.0
	Top Leaves	*A. solani*	3 (7.1)	23.0 ± 6.6	1	0.33 ± 0.33 (0.6)	33.3
	Mid. Leaves	*M. persicae*	2 (4.8)	12.0 ± 2.0	0	0 (0)	0
	Mid. Leaves	*A. solani*	0	–	–	–	–
	Btm. Leaves	*M. persicae*	1 (2.4)	19.0 ± –	0	0 (0)	0
	Btm. Leaves	*A. solani*	13 (31.0)	18.2 ± 3.2	0	0 (0)	0
Totals			42	1077	725	57.03	
Low (<10 aphids)	Meristems	*M. persicae*	1 (0.5)	9.0 ± –	17	17.0 ± – (91.6)	100
	Meristems	*A. solani*	7 (3.8)	5.3 ± 0.6	0	0 (0)	0
	Top Leaves	*M. persicae*	37 (20.3)	2.4 ± 0.3	38	1.0 ± 0.4 (5.4)	34.0
	Top Leaves	*A. solani*	9 (4.9)	1.9 ± 0.7	3	0.33 ± 0.24 (1.8)	22.2
	Mid. Leaves	*M. persicae*	41 (22.5)	2.3 ± 0.3	6	0.15 ± 0.11 (0.8)	4.9
	Mid. Leaves	*A. solani*	25 (14.3)	1.9 ± 0.3	1	0.04 ± 0.04 (0.2)	4.0
	Btm. Leaves	*M. persicae*	24 (13.2)	1.8 ± 0.3	1	0.04 ± 0.04 (0.2)	4.2
	Btm. Leaves	*A. solani*	37 (20.3)	3.2 ± 0.4	0	0 (0)	0
Totals			181	461	66	18.56	

regardless of location or species. This contrasts sharply with the pattern seen within high-density patches, where a distinct location effect was seen (with 91% of all eggs in this density category being deposited on meristems). Also at high aphid densities, while we see that 100% of meristem patches were attacked for both aphid species, *A. solani*-infested meristems received a much lower proportion of the total eggs (7% vs. 92% for *M. persicae*). Although the mean density of *A. solani* on meristems was almost half of that of *M. persicae* (20 vs. 39 aphids/patch), even the *M. persicae*-infested meristems with fewest aphids (i.e. 7 patches with a mean of 15 aphids; data not shown) still attracted 3x more eggs than high-density *A. solani* patches on average (i.e. 23.9 vs. 8.3 eggs/patch for *M. persicae* and *A. solani*, respectively), further supporting the F-test result of a main effect of species.

DISCUSSION

Our results indicate that, under highly manipulated conditions (i.e. when a single location on a plant is infested with aphids), the density of aphids in a patch is the primary driving force behind oviposition decisions of the natural enemy *A. aphidimyza*, while prey species is un-important. Under more natural (uncontrolled) conditions, while density is still important, prey species also appears to become a factor. Given that *A. aphidimyza* is reared on *M. persicae* commercially in North America, a preference for its original prey would not be surprising (see Havelka and Ruzicka, 1984). As our study showed a higher average number of *A. aphidimyza* eggs deposited in *M. persicae* colonies vs. *A. solani* colonies in 3 out of 5 experiments, this suggests at least a weak species preference for *M. persicae* is likely. However, in

our only trial where a statistically significant effect of species was seen, aphid location on the plant also was significant when analyzed on the prey-patch level. Despite differing within-plant distributions of un-manipulated aphid populations (*A. solani* preferring to colonize bottom leaves; *M. persicae* preferring meristems), the highest proportion of total *A. aphidimyza* eggs within each species was found on meristem tissue for both aphids. This demonstrates an important effect of patch location in the assessment of patch quality by this natural enemy. Further results from location choice experiments, where a greater number of eggs (and a greater density-dependent response) was seen on patches located on top leaves and meristems vs. bottom leaves -- regardless of aphid species-- suggest that prey location may actually trump prey species in terms of perceived patch quality by *A. aphidimyza.*

However, discussions of species vs. location of prey in terms of importance in oviposition decisions of an insect predator are likely irrelevant. In natural infestations, prey species would not be decoupled from the preferred feeding location of that species. This was highlighted in our experiment where the oviposition response of *A. aphidimyza* was observed in naturally distributed aphid populations. Here, a highly significant interaction between aphid species and location within the plant was observed, with *Myzus persicae* patches located on meristems receiving 91% of all *A. aphidimyza* eggs. Although *A. solani*-infested meristems received the next highest total number of eggs, these patches could not compete with the high densities at which *M. persicae* colonized this plant location in the experiment. The phenomenon of an increase in density of one prey leading to reduced predation on (and thus increased fitness of) an alternative prey has received considerable attention in the ecological

literature as an important predator-mediated indirect interaction (Holt, 1977; Holt and Lawton 1994; Abrams and Matsuda 1993 & 1996). This usually short-term interaction is often referred to as "apparent mutualism", and is likely occurring within our system, with high-density colonies of *M. persicae* on plant meristems being attacked disproportionately. Thus, we predict that *A. solani* may generally be attacked to a lesser extent by *A. aphidimyza* in the presence of *M. persicae* (if the respective within-plant distributions of each species seen in this study hold up across crop plants). If this hypothesis were to be shown true, with a persistent focus of predator attacks leading to a decline in the *M. persicae* population, the long-term interaction would likely become one of apparent competition.

The overall purpose of oviposition site selection by any insect is to optimize the potential fitness of their offspring. Results from 3 separate trials in our study strongly suggest that *A. aphidimyza* ranks aphid colonies on the meristem and/or top leaves of pansies as higher quality patches for their offspring than other locations. This distinction between within-plant locations by *A. aphidimyza* was somewhat surprising, given the low growing, compact nature of this variety of pansy. Our results, though conflicting with Mansour (1975), concur with El Titi (1972/73), Lucas and Brodeur (1999), as well as with recent unpublished research by Messelink (G. Messelink, Pers. Comm.), who observed approx. 6x as many *A. aphidimyza* eggs on top leaves of greenhouse pepper plants infested with *M. persicae* vs. middle leaves, despite similar aphid densities at both strata. Exact cues for this preference for new tissue remain unknown. However, for many aphid species, the nutrient-rich new growth of plants represents the feeding location best suited for nymphal development. Aphid colonies

in this location should reproduce faster and provide more food for developing *A. aphidimyza* larvae, optimizing their fitness. This reproductive strategy -- where adult natural enemies prefer to lay their eggs in "young", developing prey colonies vs. degenerating colonies -- is referred to as "buy-futures" by Kan (1988). This behavior has been observed previously in hoverflies (Diptera: Syrphidae), another aphidophagous Dipteran species (Kan 1988; Scholz and Poehling 2000), and was first suggested as a possible mechanism behind *A. aphidimyza* oviposition site selection by Lucas and Brodeur (1999). Our results advance the idea that *A. aphidimyza* is engaging in the "buy futures" reproductive strategy, with the microhabitat of young leaves being the optimal oviposition site for this species. Other hypotheses, such as a decreased risk of predation to *A. aphidimyza* eggs by other predators when laid in the upper canopy, seem less likely, given that two studies on the subject have determined that *A. aphidimyza* does not discriminate between plants with and without other predators when selecting egg-laying sites (Lucas and Brodeur 1999; Messelink et al. 2011).

Our study consisted of short-term observations of *A. aphidimyza* oviposition choices in small research greenhouse compartments. In biological control, it is often unclear whether such results can be extrapolated to predict longer-term control outcomes under real-life conditions (Ives et al. 1993). Although our study strongly suggests *A. aphidimyza* will oviposit to a greater extent on whichever aphid species more heavily colonizes newer growth of plants, it is yet unclear whether this will actually result in unequal control of multiple aphid species. In our experiment that most closely resembled a natural infestation of aphids, we achieved a predator to pest

ratio of ca. 1: 1 on *M. persicae* infested plants over a 2-day period, assuming all eggs laid were viable (previous egg viabilities of >90% are reported for *A. aphidimyza*; Gilkeson 1987). At the release rate used, our single release of *A. aphidimyza* would more than likely have been sufficient for aphid control if we had let the eggs hatch (see Markkula et al. 1979; Gilkeson and Hill 1987). Conversely, only 55 *A. aphidimyza* eggs were laid over 652 *A. solani*, a predator to pest ratio of 1:12. Given this ratio, it seems doubtful that a single release of *A. aphidimyza* would have been sufficient to reduce *A. solani* pest pressure, especially given that 9 out of 15 *A. solani*-infested plants in this trial received no eggs at all over 48h (a time period which corresponded to the period of highest egg laying for *A. aphidimyza*).

This study is the first to show that, under multi-prey conditions, oviposition decisions of the predator *A. aphidimyza* are strongly influenced by prey patch location, and this can lead to differential oviposition between aphid species. The fact that the within-plant location of the preferred feeding sites of aphids has the ability to affect their attack by a natural enemy is an important consideration in the biological control of these pests. This is especially true given that distribution differences between aphid species has been seen in other species combinations besides the one presented here (see Vehrs et al. 1992). Further testing is needed to see how (under longer term conditions) oviposition choices of *A. aphidimyza* in response to varying aphid distributions on plants affects the ability of this natural enemy to control simultaneous outbreaks of two pest aphid species.

REFERENCES

Abrams, P. A. and H. Matsuda. 1993. Effects of adaptive predatory and anti-predator behavior in a two-prey-one-predator system. Evol. Ecol 7: 312-326.

Abrams, P.A., and H. Matsuda. 1996. Positive indirect effects between prey species that share predators. Ecology 77(2): 610-616.

Almohamad, R., F.J. Verheggen, F. Francis and E. Haubruge. 2007. Predatory hoverflies select their oviposition site according to aphid host plant and aphid species. Ent. Exper. Appl. 125: 13-21.

Belousov, Y.V. and N. A. Popov. 1989. Rearing of *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) on grain aphids. Acta Entomol. Fenn. 53: 3-5.

Bergeson, E. and F. J. Messina. 1997. Resource- vs. enemy-mediated interactions between cereal aphids (Homoptera: Aphididae) on a common host plant. Ann. Ent. Soc. Amer. 90: 425-432.

Choi, M. Y., B.D. Roitberg, A. Shani, D.A. Raworth and G.H. Lee. 2004. Olfactory response by the aphidophagous gall midge, *Aphidoletes aphidimyza* to honeydew from green peach aphid, *Myzus persicae*. Ent. Exper. Appl. 111: 37-45.

Davis, J.A., E.B. Radcliffe and D.W. Ragsdale. 2006. Effects of high and fluctuating temperatures on Myzus persicae (Hemiptera: Aphididae). Environ. Entomol. 35:1461-1468.

Davis, J.A., E.B. Radcliffe and D.W. Ragsdale. 2007. Resistance to green peach aphid, *Myzus persicae* (Sulzer) and potato aphid, *Macrosiphum euphorbiae* (Thomas) in potato cultivars. Am. J. Potato Res. 84:259-269.

Dixon, A.F.G. 1959. An experimental study of the searching behavior of the predatory coccinellid beetle *Adalia decempunctata* (L.). J. Anim. Ecol. 28: 259-281.

El Titi, A. 1972/73. Influences of prey density and morphology of the host-plant on the egg-distribution of the aphidophagous gall midge *Aphidoletes aphidimyza* (Rond.) (Diptera: Itonididae). Z. Angew. Entomol. 72: 400-415*(in German)*.

El Titi, A. 1974. Auswirkung von der rauberischen Gallmucke *Aphidoletes aphidimyza* (Rond.) (Itonididae: Diptera) aur Blattlauspopulationen unter Glas. Z. Angew. Entomol. 76: 406-417*(in German)*.

Gilkeson, L.A. 1987. A note on fecundity of the aphid predator, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Can. Ent. 119: 1145-146.

Gilkeson, L. A. and S.B. Hill. 1987. Release rates for control of green peach aphid (Homoptera: Aphidae) by the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) under winter greenhouse conditions. J. Econ. Entomol. 80:147-150.

Harris, K.M. 1973. Aphidophagous Cecidomyiidae (Diptera): taxonomy, biology and assessments of field populations. Bull. Ent. Res. 63: 305-325.

Harwood, J. D. and J. J Obrycki. 2005. The role of alternative prey in sustaining predator populations, pp. 453-462. In M. S. Hoddle (Ed.), Proceedings of the Second International Symposium on the Biological Control of Arthropods , Vol. II. USDA Forest Service, Morgantown, WV.

Havelka, J. and Z. Ruzicka. 1984. Selection of aphid species by ovipositing females and effects of larval food on the development and fecundity in *Aphidoletes aphidimyza* (Rondani) (Diptera, Cecidomyiidae). Z. Angew. Entomol. 98: 432-437.

Havelka, J. and R. Zemek. 1999. Life table parameters and oviposition dynamics of various populations of the predacious gall-midge *Aphidoletes aphidimyza*. Ent. Exper. Appl. 91: 481-484.

Hogervorst, P.A.M., F. L. Wackers and J. Romeis. 2007. Effects of honeydew sugar composition on the longevity of *Aphidius ervi*. Ent. Exper. Appl. 122: 223-232.

Hodgson, C .J. 1978. The distribution and movement of apterous *Myzus persicae* on rapidly growing turnip plants. Ecol. Entomol. 3: 289-298.

Holt, R.D. 1977. Predation, apparent competition and the structure of prey communities. Theor. Pop. Biol. 12: 197-229.

Holt, R.D. and J. H. Lawton. 1994. The ecological consequences of shared natural enemies. Ann. Rev. Ecol. Syst. 25: 495-250.

Ives, A.R., P. Kareiva and R. Perry. 1993. The ecological consequences of shared natural enemies. Ann. Rev. Ecol. Systs. 25: 495-520.

Jandricic, S.E., S. P. Wraight, K.C. Bennett and J. P. Sanderson. 2010. Developmental times and life table statistics of the *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models. Environ. Entomol. 39(5): 1631-1642.

Jandricic, S.E., S. P. Wraight, K.C. Bennett and J. P. Sanderson. 2011. Correction for the article "Developmental times and life table statistics of the *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models". Environ. Entomol. 40(3): iv-vi.

Kan, E. 1988. Assessment of aphid colonies by hoverflies. II. Pea aphids and 3 syrphid species; *Betasyrphus serarius* (Wiedemann), *Metasyrphus frequens* Matsumura and *Syrphus vitripennis* (Meigen) (Diptera: Syrphidae). J. Ethology 6(2): 135-142.

Kim, T. and J. Kim. 2004. Development and adult life span of *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) fed on the melon aphid, *Aphis gossypii* Glover or the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Korean J. Appl. Entomol. 43: 297-304 *(in Korean)*.

Kuo-Sell, H.L. 1989. Cereal aphids as prey species for mass rearing of *Aphidoletes aphidimyza* (Rond.) (Dipt., Cecidomyiidae) in the biological control of *Myzus persicae* (Sulz.) in greenhouses. J. Appl. Entomol. 107(1): 58-64 *(in German)*.

Littell, R.C., Stroup, W.W., Freund, R.J. 2002. SAS for Linear Models. SAS Institute, Cary, NC.

Lucas, E. and J. Brodeur. 1999. Oviposition site selection by the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). Environ. Entomol. 28(4): 622-627.

Mansour, M. H. 1975. The role of plants as a factor affecting oviposition by *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). Ent. Exper. Appli. 18:173-179.

Markkula, M. and K. Tiittanen. 1976. A method for mass rearing of *Aphidoletes aphidimyza* (Rond). SROP/wprs Bulletin 1976/4: 183-184.

Markkula, M., M.K. Tiittanen, M. Hamalainen, A. Forsberg. 1979. The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. Ann. Ent. Fenn. 45: 89-98.

Messelink, G.J. C.M.J. Bloemhard, J.A. Cortes, M.W. Sabelis and A. Janssen. 2011. Hyperpredation by generalist predatory mites disrupts biological control of aphids by the aphidophagous gall midge *Aphidoletes aphidimyza*. Biol. Control 57:246-252.

Popov, N.A. and Y.V. Belousov. 1987. Breeding *Aphidoletes aphidimyza*. Zashch. Rast. Moskva 10: 24 *(in Russian)*.

Robert, Y. 1979. Recherches écologiques sur les pucerons *Aulacorthrum solani* Kltb., *Macrosiphum euphorbiae* Thomas et *Myzus persicae* Sulz. dans l'Ouest de la France. II. Evolution spatio-temporelle de leurs populations sur la pomme de terre. Ann. Zoo. Ecol. Anim. 11: 67-83 *(in French)*.

Sarmento, R.A., M. Venzon, A. Pallini, E.E. Oliveira and A. Janssen. 2007. Use of odours by *Cycloneda sanguine* to assess patch quality. Ent. Exper. Appl. 124: 313-318.

SAS Institute. 2008. Version 9.2. SAS/STAT user's guide. SAS Institute, Cary, NC.

Scholz, D. and H.M. Poehling. 2000. Oviposition site selection by *Episyrphus balteatus*. Ent. Exper. Appl. 94: 149-158.

Stewart, H. C. and S.J. Walde. 1997. The dynamics of *Aphis pomi* de Geer (Homoptera: Aphididae) and its predator, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), on apple in Nova Scotia. Can. Ent. 129: 627-636.

van Driesche, R., S. Lyon, J.P. Sanderson, K.C. Bennett, E. J. Stanek III, and R. Zhang. 2008. Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker plants for control of aphids (Hemiptera: Aphididae) in greenhouse spring floral crops. Fla. Entomol. 91(4): 583-591.

van Schelt, J. and S. Mulder. 2000. Improved methods of testing and release of *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) for aphid control in glasshouses. Eur. J. Entomol. 97: 511-515.

Vehrs, S.L.C., G.P. Walker and M.P. Parella. 1992. Comparison of population growth rate and within-plant distribution between *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae) reared on potted chrysanthemums. J. Econ. Entomol. 85(3): 799-807.

Verider, K. 1999. Improvement of the biological control of the aphid *Aulacorthum solani* in glasshouses. Master's thesis, Horticultural Science Program, Swedish University of Agricultural Sciences, Uppsala Sweden: 1999: 6.

Wave, H. E., W.A. Shands and G.W. Simpson. 1965. Biology of the foxglove aphid in the Northeastern United States. US. Dep. Agr. Tech. Bull. 1338: 1-40.

CHAPTER 5

BIOLOGICAL CONTROL OUTCOMES USING THE APHIDOPHAGOUS PREDATOR *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOMYIIDAE) UNDER MULTI-PREY CONDITIONS: EFFECTS OF CROP STAGE, WITHIN-PLANT DISTRIBUTION, AND APPARENT COMPETITION

Jandricic, S.E.,[1,2] Wraight, S.P.,[3] Gillespie, D.R.,[4] and Sanderson, J.P.[1]

ABSTRACT

Previous short-term trials with the aphidophagous midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) indicated that this natural enemy prefers to oviposit among aphids colonizing new growth of plants, leading to differential attack rates for aphid species that differ in their within-plant distributions. Here, we used longer-term greenhouse trials to determine biological control outcomes using *A. aphidimyza* under multi-prey conditions and during different crop stages. When both *Myzus persicae* and *Aulacorthum solani* were present, control of *M. persicae* by *A. aphidimyza* was consistent at all stages of plant growth, with 78 - 95% control achieved. In contrast, control of *A. solani* was inconsistent in the presence of *M. persicae*, with 12 - 80 % control achieved. Highest control rates of this aphid were consistently seen when plants

[1] Department of Entomology, Comstock Hall, Cornell University, Ithaca NY, USA 14853
[2] Corresponding Author: Email: sej48@cornell.edu Fax: 607-255-0940 Tel: 607-379-1127
[3] USDA-ARS Robert W. Holly Center for Agriculture and Health, Tower Road, Cornell University, Ithaca NY, USA, 14853
[4] Agriculture and Agri-Food Canada, Research Centre, Agassiz, BC, V0M 1A0, Canada

were in the budding stage, which may be a result of a larger proportion of aphids moving onto growing points. Additionally, control of *A. solani* by *A. aphidimyza* was significantly greater in the absence of *M. persicae* than in its presence. This study illustrates how within-plant distribution of aphid pests can change with stage of plant growth, and thereby affect the level of biological control, and that apparent competition between prey species can negatively influence biological control programs in systems where pest complexes are common.

INTRODUCTION

The ability of a natural enemy to control a given pest species in an agricultural system can be affected by alternative prey. Although multiple prey species can have positive outcomes for biological control (e.g. Settle et al. 1996), alternative prey can also negatively impact control programs, especially if both prey species are significantly pestiferous. One way this can occur is through preferential attack of one herbivore, which can deflect predation away from a focal pest (Holt and Lawton, 1994). This phenomenon has been previously recorded in aphid pests and their natural enemies. For example, the presence of the bird-cherry oat aphid, *Rhopalosiphum padi*, is known to decrease the efficacy of lacewings for controlling the Russian wheat aphid, *Diuraphids noxia* as a direct result of the distribution of *R. padi* in more predator-accessible locations on the plant (Bergeson and Messina 1997; Bergeson and Messina 1998). Over the longer-term, such unbalanced predation due to plant distribution differences of aphid species is likely to lead to apparent competition. Specifically, repeated attacks on

the more preferred pest lower the fitness of the first pest species, while simultaneously resulting in reduced control (and increased fitness) of the second pest species.

Aphidoletes aphidimyza (Diptera: Cecidomyiidae) is a biological control agent for aphid pests commonly used in greenhouse vegetable and ornamental crops in North America and Europe. Although the larvae are described as generalist aphid predators (Kuo-Sell 1987), research has shown that location of aphid prey on plants is an important factor in *A. aphidimyza* oviposition decisions. Specifically, *A. aphidimyza* populations show a distinct preference for aphid colonies present on new growth of plants (especially meristematic tissue) over other plant locations for their oviposition sites (El Titi 1972/73; Lucas and Brodeur 1999; Jandricic et al. 2013), even when aphid densities are similar across locations, and regardless of aphid species (Jandricic et al. 2013). This presents a potential problem for control of foxglove aphid (*Aulacorthum solani* Kaltenbach). An important pest of such greenhouse crops as ornamentals, peppers and lettuce (Sanchez et al. 2007; Lee et al. 2008a; Jandricic et al. 2010), *A. solani* often uses of lower leaves of plants as its primary feeding site (Robert 1979; Vehrs 1992; Jandricic et al. 2013), depending on the crop. Jandricic et al. (2013) showed that this resulted in *A. solani*-infested plants receiving fewer eggs from *A. aphidimyza* than did plants infested with green peach aphid (*Myzus persicae* Sulzer), which aggregated on plant meristems. Thus, effective control of *A. solani* by *A. aphidimyza* may be reduced in the presence of *M. persicae* or another aphid species that aggregates on new growth of plants at high densities.

The oviposition studies by Jandricic et al. (2013) were conducted over 1-2 days. Given that *A. aphidimyza* adults continue to lay eggs over their lifetime (generally 5-7

d, though survival up to 19d has been reported under ideal lab conditions) (Havelka and Zemek 1999; Madahi et a. 2013), this can only be considered a "snapshot" of their oviposition behavior when confronted with multiple prey species. Here, we evaluated the effectiveness of *A. aphidimyza* in three ways. First, all studies herein used longer duration trials, in which females were allowed to continuously oviposit and eggs were allowed to hatch into predaceous larvae, to evaluate whether previous oviposition results would translate to reduced control of *A. solani* when *M. persicae* is also present. Second, given that within-plant distribution of aphids can significantly affect *A. aphidimyza* oviposition decisions (Jandricic et al. 2013), and that aphid within-plant distributions can change with plant growth stage, trials were conducted on vegetative, budding, and flowering plants to see whether plant stage affects aphid distributions and, potentially, aphid control. Third, *A. aphidimyza* adults were also presented with infestations of *A. solani* alone, as well as mixed-species infestations, to test the hypothesis that the lower oviposition rates on *A. solani* in previous experiments were a result of apparent competition instead of merely a lower attraction to *A. solani* in general. Additionally, we also conducted a laboratory bioassay to compare the amount of honeydew produced by both *M. persicae* and *A.solani* to evaluate whether differential oviposition rates are caused in part by differential honeydew production, honeydew being the main long-distance cue in prey location for *A. aphidimyza* (Choi et al. 2004).

MATERIALS AND METHODS

Insects

Mixed clonal populations of both aphid species (*M. persicae* and *A. solani*) were collected in Ithaca, NY in 2009 and were continuously reared on pansy (*Viola* × *wittrockiana* Gams.), as in Jandricic et al. 2010. Adult aphids for all experiments were selected directly from colonies, and were therefore of unknown age.

A. aphidimyza pupae were obtained from Applied Bio-nomics Ltd. (Victoria, BC, Canada) for all experiments. Upon receipt, pupae were placed in emergence cages as described in Jandricic et al. (2013). Adult midges were used instead of pupae, which provided better management of the actual number of adult flies released in each experiment. Adult midges were not used in experiments until ca. 60h post emergence to ensure mating, and that female midges had passed their period of no/low egg production (Havelka and Zemek 1999). For each experiment, adult midges were collected from emergence cages with a mouth aspirator using glass vials to prevent midges from being damaged due to static electricity. A subsample of 50-100 individuals was also taken from the *A. aphidimyza* emergence cage at the time of each experiment to determine sex ratio. The average sex ratio over experiments in Sections 2.4-2.6 was 1 male: 1.8 females (range = 1:1.5 to 1:1.9). However, a lower ratio of females was seen in the experiment assessing apparent competition, with an average of 1 male: 0.9 females.

Plant Material

For all experiments, pansy (*Viola* × *wittrockiana* Gams, var. Majestic giant II; Stokes Seeds, Buffalo, NY) was used as the host plant for aphid populations. Plants were grown as in Jandricic et al. (2010). Plants were used in experiments after 5-8 weeks of

growth in 10 cm pots, depending on the crop stage being tested, and the time of year the experiments were conducted.

Effect of Plant Stage on the Efficacy of A. aphidimyza for Controlling Mulitple Aphid Species

Plant stages tested:

A series of experiments was done to evaluate the effect of the stage of plant growth on control of multiple aphid species by *A. aphidimyza* over 9-11 day periods. The pansy crop stages included vegetative, budding, and flowering. Plants were kept under natural day length. Plants were considered vegetative as long as new growth at the meristem was not producing buds at the time of *A. aphidimya* oviposition, though the beginnings of buds (composed of entirely green tissue) may have been present on some plants. Most plants had developed fully formed buds by the last day of data sampling, however. To be considered budding, plants had to have at least 1 distinct bud forming at the apical meristem, raised on a small stem, with buds being large enough to have distinct petal tissue developing inside at the time of *A. aphidimyza* oviposition. In the first replicate experiment at this plant stage, buds had grown on tall stalks over the course 9d, but none fully opened into flowers. In the second replicate, 15 out of 32 plants had 1 open flower on the last day of data collection. However, no plant in the experiment had more than 1 open flower, thus plants were still considered to be in the budding phase. To test the flowering stage, plants (potted for ca. 8 weeks) had at least 1 fully open flower and 1 other flower bud on a tall stalk that was close to opening, and continued to flower over the course of the experiment.

All plant stages were tested separately due to logistical constraints. Experiments on vegetative plants were conducted twice. The first replicate was conducted on April 19, 2011. Plants had been potted for ca. 5 weeks at the start of the experiment. The second replicate of the trial was conducted on May 1. The initial replicate of experiments on budding pansies was conducted in 1 greenhouse compartment on April 4, 2012. A second replicate of the trial was conducted in late May 2012 (replicated across 2 compartments). The trial on flowering plants was conducted in early October, 2011 (across 2 greenhouse compartments). Here, the last day of data collection was 11 d after *A. aphidimyza* release (vs. 9d in previous trials) to account for a potentially longer development time of the predator at cooler temperatures at this time of year.

Experimental Methodology:

All experiments were conducted in separated, identical greenhouse compartments (2.75 x 7.30 m each) at the USDA Agriculture Research Service Station in Ithaca, NY. Four benches, used as blocks, per compartment were used, each measuring 0.92 x 2.44 m. In all experiments, environmental controls for the compartments were set to 24 °C day time temp. and 18 °C night time temp. Environmental conditions in all compartments were monitored with HOBO data loggers (Onset Computer, Bourne, MA).

Pansy plants used in experiments were initially infested with one of two treatments: either 3 adult *M. persicae* or 5 adult *A. solani* per plant. Aphids were added to the center of plants by fine brush and allowed to naturally distribute and reproduce for 1 week. Initial numbers of the two aphid species were chosen to ensure that

densities of each species would be similar at the time of *A. aphidimyza* release. Based on previous experiments, these numbers would result in ca. 40-50 aphids per plant on Day 1 of the experiment, a number that represents a moderate aphid infestation. Three plants per aphid species were placed on each bench for use in the "predator treatment". These were replicated on all 4 benches (giving a total of 24 aphid infested plants out of 112 plants total). Treatment plants were placed randomly among uninfested plants to force *A. aphidimyza* to search for prey, for a total of 28 plants/bench (6 infested, 22 un-infested). For plants in the "control treatment" (no exposure to the predator *A. aphidimyza*), 3 aphid-infested plants per species per bench were also used (replicated on the 4 benches). For the first experiment (vegetative pansies), we attempted to house these control treatment plants in a different greenhouse compartment, set at the same environmental conditions. However, some *A. aphidimyza* contaminated the control compartment in the first trial, which resulted in 2 plants having to be removed from the experiment. Thus, for all additional experiments (including the second replicate trial on vegetative pansies), control plants were instead kept in $61cm^3$ cages (BugDorm 2, BioQuip Products, Rancho Dominguez, CA) within the treatment compartment and placed at the end of each bench. Environmental conditions within cages themselves were extremely similar to those of the surrounding greenhouse compartments; thus, only conditions from compartments are reported. Five days after *A. aphidimyza* release, control plants were removed from cages and randomized amoungst the remaining treatment and background plants in order to decrease any potential sources of variabiltiy. At this point in time, it was likely that the majority of the adult *A.*

aphidimyza population was deceased or no longer laying eggs (based on preliminary cage trials), and thus would not affect aphid numbers in the control treatment.

On Day 0 of all experiments, 100 adult *A. aphidimyza* midges were released in the compartments just prior to dusk, as per commercial recommendations (see Jandricic et al. 2013 for details on release procedures). Although the release rate used was 2.5x the high-release rate of 2 midges/m^2 suggested by commercial biocontrol companies, their rate is based on unit area, not pest density. We based our release rate on a midge density that would approximately yield a predator: prey ratio of 1:10 in each experiment, a moderate release ratio that has shown to be successful in previous control trials in greenhouse crops (see Gilkeson and Hill 1987; Markkula 1976). Ventilation fans were turned off overnight to promote midges settling within the crop. To increase relative humidity (RH) to promote oviposition (Gilkeson 1987), mist emitters, located beneath each bench, as well as between each bench, were operated for 5 min of every 60 min for the duration of the experiment.

One aphid infested plant/bench/treatment/compartment (n=8 for each aphid species/treatment combination) was destructively sampled on each of three sample dates: day 2 after *A. aphidimyza* release, in order to assess number and location of the majority of *A. aphidimyza* eggs (with most oviposition taking place within 3-5 d after mating (Madahi et al. 2013), and flies were 60h old at the time of release), as well as to determine the initial aphid density around the time of oviposition; day 6 after *A. aphidimyza* release, to assess numbers and location of small *A. aphidimyza* larvae (given that eggs take ca. 3 d to hatch, larvae on this day would be ca. 2-3 days old, depending on the date of oviposition), as well as of eggs from any additional

ovipostion; and day 9 after release, to make final counts of aphids on treatment and control plants at a point when the oldest of the *A. aphidimyza* larvae (5-6 days old at this point) had potentially begun to pupate (therefore reducing control potential). Counts of large larvae still foraging on the plant were made (and also of any small larvae present from later oviposition). Though larval sizes were not distinguished during counts, the majority of larvae sampled on days 9-11 were large (S. Jandricic; personal observation).

As in Jandricic et al. (2013), all aphid-infested plants were destructively sampled. Aphids, predator eggs and larvae were recorded from leaves assigned to several possible within-canopy "locations". For vegetative plants, these locations consisted of bottom, middle or top leaves (based on height from the soil surface, i.e. bottom leaves = ca. 0-2cm from the soil surface, middle leaves = ca. 2-5cm, and top leaves = ca. >5cm, with plants generally being 6-8cm tall), or the center growing point of plant, henceforth referred to only as the meristem. Specifically, the meristem is defined as the plant material left when all mature leaves were removed from the plant, and consisted of many small, immature leaves on several small, under-developed (<2 cm) lateral meristems. For the initial replicate on budding plants, although each apical meristem, and some of the lateral meristems, also included buds, aphids at these locations were included in counts of meristems as a type of "new growth". However, during the second replicate, aphid and *A. aphidimyza* numbers were recorded from buds as a separate possible plant location to characterize attractiveness of this plant organ. For flowering plants, locations of plant buds, fully open flowers, and senescing flowers (*i.e.* visibly wiling and many having dropped petals) were included along with leaf locations and the meristem described previously.

Investigation of Apparent Competition

Apparent competition occurs when one prey species is preferentially attacked by a predator, subsequently resulting in lesser attack (and improved fitness) of a second prey species within the same system. The following experiment was conducted examine control of *A. solani* by *A. aphidimyza* in the presence and absence of *M. persicae*. If poorer control of *A. solani* results in the presence of *M. persicae* vs. in its absence, this would suggest that apparent competition was indeed occurring. The experiment was conducted from December 7-17th, 2012. The experimental set up was similar to the above, except that one greenhouse compartment contained both *M. persicae* and *A. solani* infested plants (on separate plants), while another contained plants infested only with *A. solani*. The number of *A. solani*-infested plants was doubled in the second compartment to present the predator with the same initial aphid densities in both treatments (i.e. 24 aphid-infested plants for each compartment). The experiment was repeated simultaneously in space using a total of 4 greenhouse compartments. Additionally, we released a separate population of *A. aphidimyza* that was collected from separate rearing areas by the commercial producer in each of the two experimental replicates in order to test results across different *A. aphidimyza* populations.

Aphids were added to plants as in previous experiments. This initial density was designed to yield similar aphid densities for both species after 1 week (ca. 40-50 aphids/plant). However, in this particular experiment, the emergence of *A. aphidimyza* adults was delayed by 3 days for unknown reasons. Thus, flies were not released until day 10 of aphid reproduction. Despite efforts to slow reproduction of *M. persicae* by reducing greenhouse temperatures to ca. 15 °C between days 7-10 of aphid

reproduction, this delay still resulted in initial numbers of *M. persicae* per plant being somewhat higher than for *A. solani* (see Results section). However, given that the goal of this experiment was to assess control of *A. solani* in the presence/absence of *M. persicae* as a distraction, this experimental set up still achieved this purpose. In real-world infestations, *M. persicae* populations would almost certainly be higher than *A. solani* if outbreaks occurred at the same time, given that *M. persicae* generally has a higher intrinisic rate of increase (see Davis et al. 2007; Jandricic et al. 2011).

Lab Assay Comparing Honeydew Production

Honeydew was collected from nymphs of both aphid species to evaluate whether the volume of honeydew produced differed by species, which would perhaps cause adult *A. aphidimyza* to be differentially attracted to one aphid species over the other. To obtain aphid nymphs of the same age, 4-6 adult *M. persicae* or 10-12 adult *A. solani* were selected from aphid colonies and placed onto excised pansy leaves embedded abaxial side up in 2.0% Difco agar (Fisher Scientific, Pittsburg, PA) in 60 mm Petri dishes. Dishes were placed in an incubator at 25 ±1° C and 16:8h L:D. After allowing 24h for larviposition, adult aphids were removed. Nymphs were maintained on the embedded pansy leaves and moved to fresh leaf dishes every 48h.

On the day of the assay (when nymphs of both species were 4-5 d old), 20 nymphs of either *M. persicae* or *A. solani* were placed on freshly embedded leaf dishes. This age of nymph was chosen to prevent aphids of both species from having reached adulthood, so reproduction resulting in additional extraneous nymphs would not complicate the assay. Methodology was similar to that of Gündüz and Douglas 2009.

Once the aphids had settled, 20 replicated dishes of both species were placed upside-down in plastic weigh boats containing hydrated mineral oil. As the agar holding the embedded leaf only filled approximately ¾ of the Petri dish depth at most, this provided a way of suspending the aphids above the oil in order to collect any honeydew droplets that were produced and fell into the oil.

Aphid nymphs were allowed to feed on leaves and produce honeydew for 24h, after which the Petri dishes were removed from the oil, and the number of aphids remaining on the leaf were counted (in some cases, up to 3 aphids had fallen from the leaf and into the mineral oil). Any aphid not feeding on the leaf was ignored in the total count. All honeydew droplets from a single replicate dish were then collected from the mineral oil using a 200 µL pipette under a dissecting microscope. 200 µL pipette tips which had been previously coated with Sigmaote® (Sigma-Aldrich, St. Louis, MO) were used to prevent honeydew from sticking to the side of the plastic pipette tip. Samples (honeydew + mineral oil that was inevitably pippetted up) were then transferred to centrifuge tubes, and the honeydew was allowed to settle to the bottom of the oil. Samples were then frozen for 4 d, upon which time they were thawed at room temperature for 20 min, and the honeydew was extracted and measured using a graduated, calibrated glass capillary tube.

Prior to the assay, 7 replicates of 20 aphids of each species were weighed on a microbalance to determine average aphid weight for each at 4-5 d old nymph. Nymphal weights of *M. persicae* were higher, likely because this species has a higher intrinsic rate of increase than *A. solani* (Davis et al. 2007; Jandricic et al. 2011; Dewhirst et al. 2012), and thus were in a later instar at the time of the experiment. Thus, the same *A.*

solani nymphs used in the initial assay were used again in a second replicate when they were 6-7 d old, in order to provide samples of aphids at both equal ages, and at approximately equal average weights (*M. persicae* were not re-tested in this second replicate). There were 15 replicates of *A. solani* at this age; these nymphs were also weighed in groups of 20 immediately before the assay.

Statistical Analysis

For each sampling date in each greenhouse experiment, the effect of treatment, aphid species, and their interactions on the response of aphid density per plant was analyzed using a mixed model ANOVA (Proc Mixed, SAS). If data did not meet the assumptions of the ANOVA, data were $\log_{10}(x+1)$ transformed before analysis. For all results, untransformed arithmetic means and standard errors are presented first, with back-transformed least-squared (LS) means or LSmeans and standard errors given in parentheses. In the mixed model, trial replicate and greenhouse compartment (where applicable), as well as greenhouse bench (nested within compartment), were included as random effects to control for compartment to compartment (and block to block) differences. When we assessed control outcomes on the last day of the experiment, trial replicate only contributed to 14-27% of the variance component, and greenhouse replicate 0-32%. Thus, we combined replicate experiments for each plant growth stage, although we retained trial and greenhouse in the model. As the mixed-model ANOVA showed a significant treatment × species interaction in all cases, treatment effects were analyzed within each aphid species separately using planned contrasts. Regression analyses were also conducted on aphid numbers per plant from Day 6 to the last day of

the experiment in all cases (aphid density data were log(x+1) transformed), with slopes of the line indicating positive or negative aphid population growth in the presence/absence of the predator. Day 2 was not included, as the predator was in the egg stage at this point.

To analyze the predator response, number of eggs and larvae per plant were modelled against aphid density, species and their interaction (with the same random effects included as above). If data did not meet assumptions of the ANOVA in this case, both aphids and eggs were $\log_{10}(x+1)$ transformed (hereafter referred to as a log-log transformation). Numbers of eggs and larvae of *A. aphidimyza* were compared on *A. solani*-infested and *M. persicae*-infested treatment plants using t-tests. In the case of both aphid and predator densities, chi-square tests were used to examine whether the distributions differed between plant locations within each aphid species. A sequential Bonferroni correction was used for multiple comparisons; the resulting cut-off for significance (alpha level) is reported if it differed from 0.05.

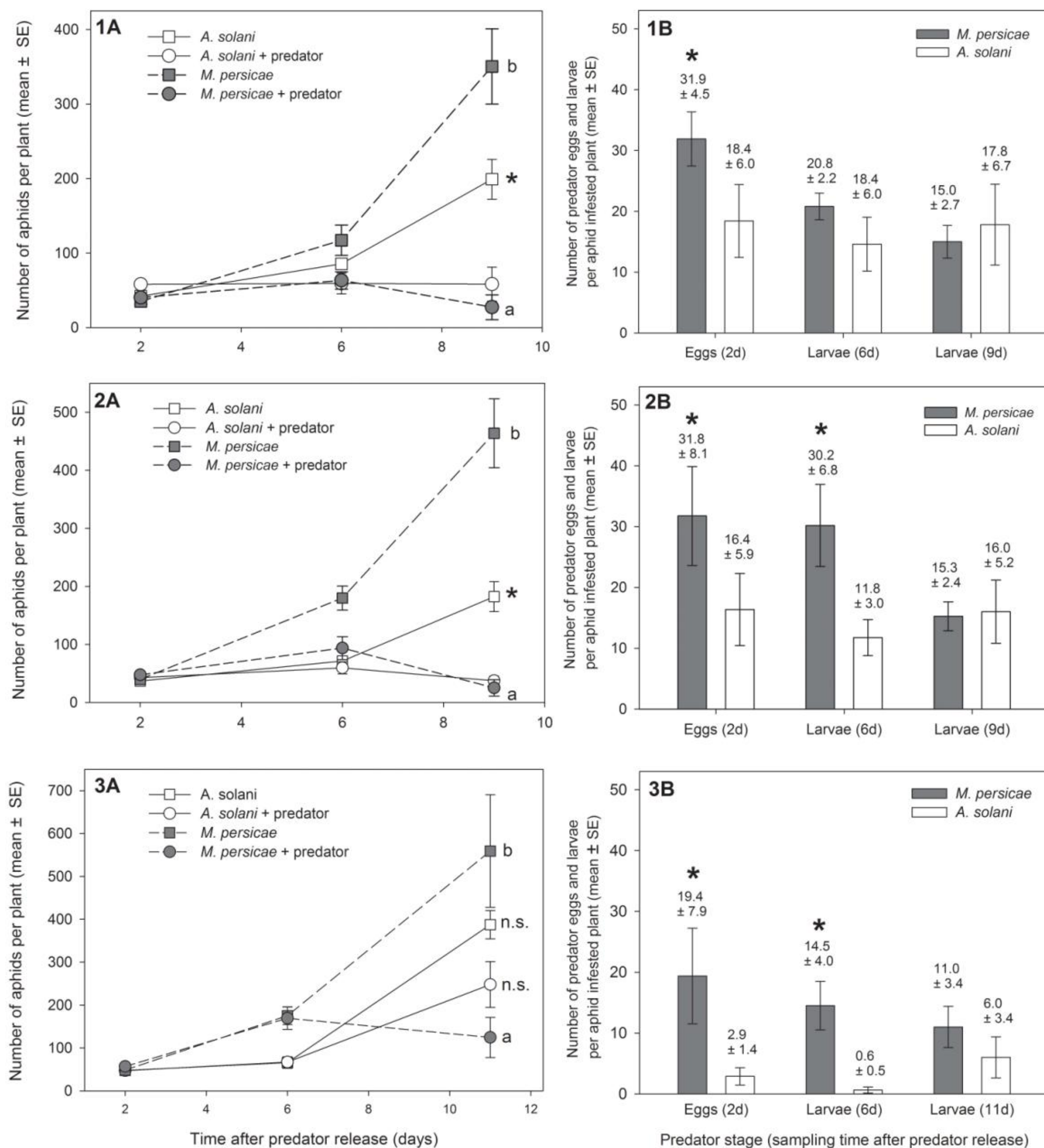
A simple analysis of variance was used to compare the amount of honeydew produced by 4-5 d old aphids of different species. No data transformation was needed to meet assumptions of variance. Although older *A. solani* were tested on a separate day, we chose to proceed with statistical comparisons to younger aphids because these were the same individuals used for the initial test. However, because we did not keep identical replicates (aphids were re-distributed on fresh pansy leaves in batches of 20 aphids/leaf), a repeated measures analysis was not applicable.

RESULTS

Aphid Control on Vegetative Pansies

The average temperature from the point of *A. aphidimyza* release to the end of the experiment in the treatment compartment for Replicate 1 was 19.6 °C (range: 14.5-27.6 °C). The average RH was 86% (38-100%). In the compartment where control plants were kept for the first 5 d, the average temperature and % RH were comparable (21.4 °C, 75% RH). Here, a limited number of *A. aphidimyza* contaminated the control compartment and 2 of the *M. persicae*-infested plants in the control treatment had to be removed from the analysis due to the presence of larvae. In Replicate 2, temperatures in both greenhouse compartments and cages (where control plants were now kept) were similar (avg. temp = 21.4 °C (12.1-30.8); average RH = 73 (18-100%)). One *M. persicae*-infested plant in the predator treatment was removed from analysis of day 2 data, due to its too-close proximity to a mist emitter.

Despite efforts to achieve equal aphid densities at the beginning of the experiment (Figure 5.1A), the average number of *A. solani*/plant (58.1 ± 6.81, back-transformed LSmean = 54.5) was statistically higher than the number of *M. persicae*/plant (40.3 ± 3.33; back-transformed LSmean = 38.9) (t_{42}=2.18, P = 0.0349). Despite this, more *A. aphidimyza* eggs were laid on *M. persicae* infested plants (Figure 5.1B). Although this difference was not significant using the full mixed model ($F_{1,18.9} < 2.85$, P > 0.10 for all tests), when the non-significant species × treatment interaction term was removed, aphid species became significant (back-transformed LSmean for *M. persicae* and *A.solani* = 34.0 and 10 eggs/plant, respectively), as did density (Table



Figures 5. 1-3. Plants were in the following stages of growth: Figure **1)** vegetative, Figure **2)** budding, Figure **3)** flowering. **A)** Mean aphid density ± SE of *A. solani* or *M. persicae* infested plants with no control measure (square symbols) or with releases of *Aphidoletes aphidimyza* (circle symbols). Different letters or symbols represent significant differences within aphid species. **B)** Mean number ± SE of *A. aphidimyza* eggs and larvae found on aphid infested plants 2d, 6d or 9-11d after release of *A. aphidimyza* adults. Different symbols represent significant differences between aphid species within sampling day. Any eggs deposited around day 6 likely did not contribute to overall control during the experiment time frame, thus data are not shown. Primarily large larvae were present on days 9-11 as the population was aging.

Table 5.1. F-test statistics and P values for ANOVAs evaluating the effect of aphid density and aphid species on *A. aphidimyza* egg or larval numbers per plant on three different sampling days.

Model Effects (Fixed) [a]	Sampling Date (Predator Life Stage Sampled)					
	Day 2 (Eggs)		Day 6 (Larvae)		Day 9 or 11 (Larvae)	
Vegetative Plants	F-Statistic	P-value	F-Statistic	P-value	F-Statistic	P-value
Aphid density	$F_{1,20} = 4.59$	0.0447	$F_{1, 20} = 1.31$	0.2652	$F_{1, 16.8} = 0.52$	0.4799 [b]
Aphid species	$F_{1,20} = 12.04$	0.0024	$F_{1, 20} = 0.19$	0.6716	$F_{1, 19.5} = 0.77$	0.3909
Density × species	Removed	–	$F_{1, 20} = 0.14$	0.7134	$F_{1, 19} = 0.68$	0.4205
Budding Plants						
Aphid density	Removed	–	$F_{1,18.6} = 0.12$	0.7323	$F_{1,19} = 0.73$	0.4035 [b]
Aphid species	$F_{1,14} = 4.71$	0.0476	$F_{1,19.4} = 7.0$	0.0158	$F_{1,19.1} = 2.59$	0.1239
Density × species	Removed	–	$F_{1,20} = 1.66$	0.2128	$F_{1,19} = 5.64$	0.0283
Flowering Plants						
Aphid density	Removed	–	Removed	–	$F_{1,9.1} = 0.07$	0.8036 [b]
Aphid species	$F_{1,7} = 10.01$	0.0158 [b]	$F_{1,7} = 20.47$	0.0001 [b]	$F_{1,9.7} = 0.01$	0.9600
Density × species	Removed	–	Removed	–	$F_{1,18.9} = 0.09$	0.7708

[a] In all cases, non-significant interaction terms were removed and models were re-tested. If removing these effects resulted in significance of the remaining terms, results from the reduced model are reported. If removing the non-significant terms did not change outcomes, results from the full model are given.

[b] Data were log-log transformed to better meet assumptions of variance.

5.1). We found that 73% of all *A. aphidimyza eggs* deposited over the course of the experiment (a total of 782 eggs over 3 compartments) were laid in the first 2 days. More eggs were laid on plant meristems than any other location for both aphid species ($\chi^2_1 \geq 20.4$, $P \leq 0.0001$ for all tests; Fig. 5.4). Meristems were the location of highest initial colonization by *M. persicae* (Fiure 5.4; $\chi^2_1 \geq 88.4$, $P \leq 0.0001$ for all tests); average aphid density at this location was 23.3 ± 3.53 aphids/plant. As in Jandricic et al. (2013), a statistically greater proportion of *A. solani* were found on bottom leaves (Figure 5.4; $\chi^2_1 \geq 112.4$, $P<0.0001$ for all tests; average density = 13.1 ± 1.73), with only

25.3 ± 3.65 % found on meristems (average density = 12.9 ± 2.37 aphids/plant). Unlike Jandricic et al. (2013), however, a considerable percentage of eggs were also observed on bottom leaves of *A. solani*-infested plants (28.9 ± 9.3%); this location received a higher percentage of eggs than the middle or top strata ($\chi^2_1 \geq 28.1$, $P \leq 0.0001$ for all tests). Eggs deposited on day 6 followed similar patterns as on day 2 for both *A. solani*- and *M. persicae*-infested plants, with most eggs (71.9 ± 10.45% and 91.5 ± 6.19%, respectively) deposited on plant meristems, and *A. solani* colonies on bottom leaves receiving eggs (11.5 ± 5.80%).

By day 6, eggs deposited on day 2 had hatched and larvae were now present; a higher number of larvae were seen on *M. persicae* infested plants vs. those infested with *A. solani* (Fig. 5.1B). However, there was not a significant difference (Table 5.1). An effect of predator treatment was already evident on this date ($F_{1,43} = 8.04$, $P = 0.0069$), but aphid numbers were only significantly reduced for *M. periscae*-infested plants compared to their controls ($t_{43} = 2.72$, $P = 0.0093$ for *M. persicae*; $t_{43} = 1.30$, $P = 0.1195$ for *A. solani*). On the last day of the experiment (9 days after *A. aphidimyza* release), there were arithmetically more larvae on *A. solani*-infested plants,but not significantly, even when non-significant effects were removed from the model; Table 6.1). We hypothesize that this may be due to pupation of some of the *A. aphidimyza* larvae attacking *M. persicae*-infested plants, as 7 out of 12 plants had effectively no food left, with ≤ 4 aphids/ plant. For *A. solani*-infested plants, a similar percentage of larvae as there was eggs were found on bottom leaves on day 9 (Figure 5.4). However, despite an absence of eggs on bottom leaves for *M. persicae* on any previous sampling date, larvae were found there on day 9 (along with aphid colonies).

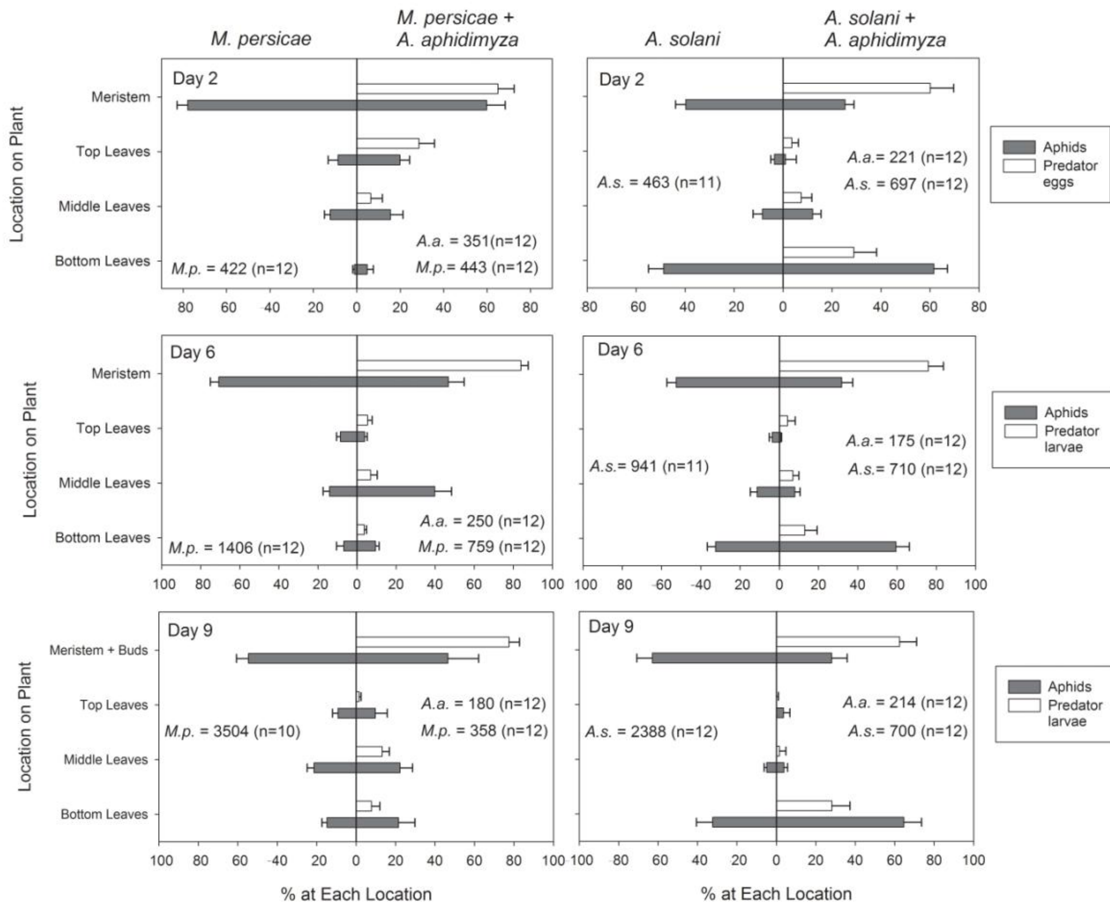


Figure 5.4. Distribution (average % ± SE) of *Myzus persicae* (*M.p.*) or *Aulacorthum solani* (*A.s.*) on vegetative plants with no predators vs. in the presence of *Aphidoletes aphidimyza* (*A.a.*). Aphid distributions are shown as dark grey bars; distribution of *A. aphidimyza* eggs/larvae on treatment plants are shown as white bars. Total aphid, predator egg or larval numbers for each sampling day are given. Results are shown from day 2, 6 and 9 after release of 100 *A. aphidimyza adults*/compartment (data combined over 3 compartments). On day 2, only *A. aphidimyza* eggs are present (no larvae).

As trial replicate contribute little to the variance component (14%) in the mixed model, and the general trends for both aphid species were similar, we combined results from both replicates when assessing control outcomes (Figure 5.1A). There was a significant treatment effect, and a significant species × treatment effect but no main effect of species (Table 5.2). The final number of aphids per plant for *M. persicae* exposed to *A. aphidimyza* was 27.3 ± 16.8 (back-transformed LSmean = 5.3 aphids/plant; Figure 5.1A). This was significantly lower than for the control treatment, (Fig. 5.1A; back-transformed LSmean = 389.8; $F_{1,41.2} = 55.4$, $P < 0.0001$). Overall, 92% control of this species was achieved, and the slope of the regression line from day 6 to day 9 indicates a significant reduction in the population growth (Table 5.2) Control results with *A. solani*-infested are more difficult to interpret. On one hand, only 70% control of *A. solani* was achieved, and there was no significant reduction in the population growth of *A. solani* from day 6-9 (Table 5.2). However, *A. solani*-infested plants exposed to *A. aphidimyza* had statistically fewer aphids (58.3 ± 22.67 aphids/plant; back-transformed LSmean = 22.2 aphids/plant) compared to those with no predators (199.0 ± 26.59 aphids/plant; back-transformed LSmean = 202.9; $F_{1,41} = 17.2$, $P = 0.0002$). Also, the number of *A. solani* left on predator-treated plants was not significantly higher than for *M. persicae* ($t_{41} = 2.49$, $P = 0.0769$). Additionally, all plants sampled on day 9 had large larvae present, suggesting that more control may have been seen had we sampled at a later date.

Examining the aphid patches left on *A. aphidimyza*-treated plants, the majority of *A. solani* remaining were found on bottom leaves (Fig. 5.4). Of these 32 remaining patches, 21 (65%) of these had no predator present (eggs or larvae) on day 9. For *M.*

Table 5.2. Statistics for control outcomes using *A. aphidimyza* for multiple aphid species across several stages of plant growth.

Effect/Species Tested	Vegetative Plants	Budding Plants	Flowering Plants
	ANOVA F-tests		
Model Effects			
Treatment	$F_{1,41.1} = 68.23$ $P < 0.0001$	$F_{1,41.5} = 116.69$ $P < 0.0001$	$F_{1,20.3} = 18.86$ $P = 0.0003$
Species	$F_{1,41.1} = 0.073$ $P = 0.3965$	$F_{1,41.5} = 1.70$ $P = 0.2210$	$F_{1,20.3} = 0.14$ $P = 0.7108$
Treatment x Species	$F_{1,41.1} = 6.56$ $P = 0.0142$	$F_{1,41.5} = 21.56$ $P <0.0001$	$F_{1,20.3} = 5.05$ $P =0.0359$
	Regression Equation and Statistics for Plants Treated with *A. aphidimyza*[a]		
Aphid Species			
M. persicae	Log (density +1) = – 0.27 (d) + 3.23 $F_{1,22} = 7.29$, $P = 0.0131$, $R^2 = 0.22$	Log(density +1) = – 0.352(d) + 3.88 $F_{1,22} = 14.00$, $P = 0.0011$, $R^2 = 0.39$	Log(density+1) = – 0.0814(d) + 2.680 $F_{1,14} = 2.65$, $P = 0.1257$, $R^2 = 0.10$
A. solani	Log(density +1) = –1.46 (d) + 2.37 $F_{1,22} = 2.14$, $P = 0.1574$, $R^2 = 0.05$	Log(density +1) = – 0.092(d) + 2.26 $F_{1,22} = 4.25$, $P = 0.0513$, $R^2 = 0.16$	Log(density+1) = 0.1012 (d) + 1.1837 $F_{1,14} = 12.22$, $P = 0.0036$, $R^2 = 0.47$

[a] Aphid density was regressed on day after *A. aphidimyza* release (d). Sampling day 6 and day 9 were included in the regression only, as only *A. aphidimyza* eggs were present on day 2 after release, which would not affect aphid numbers.

persicae, most of the remaining aphids were found on plant meristems; however, 100% of these patch types had larvae present (range: 2-24 larvae). But of the 10 bottom leaf patches remaining for this species (across 5 out of 12 plants), 6 of these had no predators. Thus, this bottom leaf location could potentially act as a reservoir for future outbreaks.

Aphid Control Trials on Budding Pansy using A. aphidimyza

In Replicate 1, the average temperature and RH in the research compartment was 21.3 °C (range=16.6-33.7°C) and 69% (28-94%). For Replicate 2, the average temperature was comparable in both compartments, with an overall average of 25.1 °C (19.8-35.5 °C) and 62.5% RH (22 -91%). Only results from Replicate 2 are reported for aphid/*A. aphidimyza* distributions within plants (Results below; Figure 5.5), as we did not record data separately from flower buds in Replicate 1. However, data from both replicates is shown i) for mean aphid and *A. aphidimyza* egg/larval numbers per plant, and ii) for the analysis of biocontrol outcomes. One *A. solani* infested plant was removed from the analysis of day 2 data for Replicate 2 due unexplained low aphid numbers (only 17 aphids/plant). One *M. persicae* plant in the control treatment was removed from analysis on day 9 due to significant contamination with *A. aphidimyza*.

Aphid numbers on treatment plants were similar for both species at the start of the experiment: an average of 47.6 ± 4.94 aphids/plant (LSmean = 46.5 ± 5.70) for *M.persicae*-infested plants; 45.1 ± 2.71 aphids/plant (LSmean = 44.2 ± 5.81) for *A. solani*-infested plants (Figure 5.2A). These were statistically similar to the staring densities for plants in the control treatments ($t_{42} < 1.96$, $P > 0.056$ in all cases). A total

of 723 eggs of *A. aphidimyza* were laid across all experimental replicates (3 greenhouse compartments total), with 78% of these laid in the first 2 days after *A. aphidimyza* release. Higher numbers of eggs were once again seen on *M. persicae*-infested plants (Fig. 5.2B), but using the whole mixed model, aphid species and other model effects were not signficant ($F_{1,15.1} \geq 0.04$, $P \geq 0.84$ in all cases). However, after removing the non-significant density and interaction terms, aphid species becomes significant (Table 5.2)

Aphid distributions (recorded from Replicate 2 of the experiment only) again differed across plant locations for both aphid species (*A. solani*: $\chi^2_4 = 252.07$, $P \leq 0.0001$; *M. persicae:* $\chi^2_4 = 288.22$, $P \leq 0.0001$), but more closely resembled each other than for any other plant stage tested (Fig. 5.5). For *M. persicae*-infested plants in the highest percentage of aphids were once again found on plant meristems ($\chi^2_1 \geq 74.5$, $P \leq 0.0001$ for all tests)(52.0% ± 4.36 aphids/plant at this location; avg. density = of 26.8 *M. persicae*/meristem). An additional 16.0% ± 3.47 were found on flower buds (in close proximity to meristems). Although the highest percentage of *A. solani* on treatment plants were still found on bottom leaves (44.1% ± 7.22), this was lower than the previous experiment on vegetative pansies. A higher proportion of *A. solani* were found near the growing point of the plant than in the previous experiment on vegetative plants, with 39.1% ± 4.47 on meristems and an additional 11.2% ± 3.10 on flower buds (Fig. 5.5). There was an average density of 19.8 ± 3.30 *A. solani* on meristems and buds together. The proportion of *A. solani* on bottom leaves and meristems was not significantly different ($\chi^2_1 = 0.94$, $P=0.3329$). The highest proportion of eggs were laid

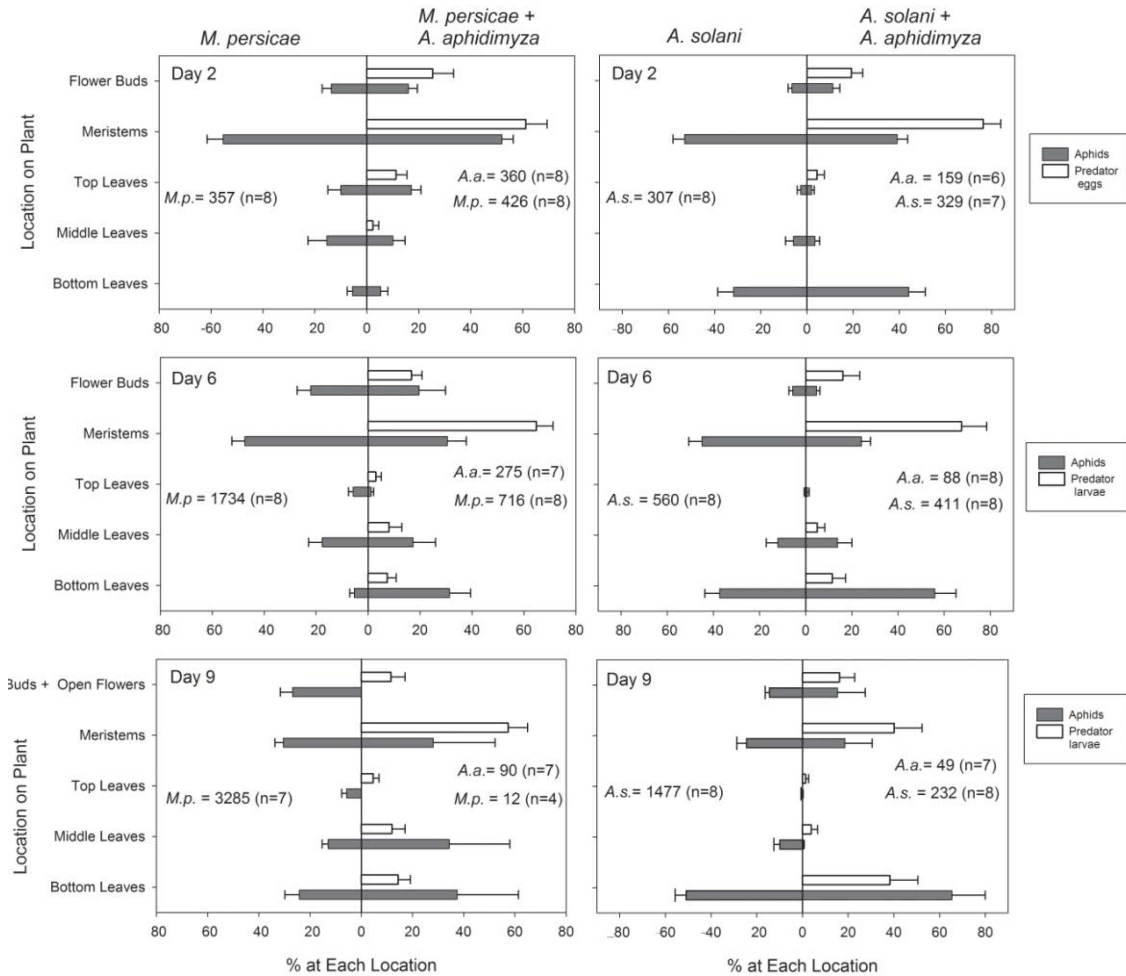


Figure 5.5. Distribution (average % ± SE) of *Myzus persicae* (*M.p.*) or *Aulacorthum solani* (*A.s.*) on budding plants with no predators vs. in the presence of *Aphidoletes aphidimyza* (*A.a.*). Aphid distributions are shown as dark grey bars; distribution of *A. aphidimyza* eggs/larvae on treatment plants are shown as white bars. Total aphid, predator egg or larval numbers for each sampling day are given. Results are shown from day 2, 6 and 9 after release of 100 *A. aphidimyza adults*/compartment (data combined over 3 compartments). On day 2, only *A. aphidimyza* eggs are present (no larvae).

by *A. aphidimyza* were again found on plant meristems, regardless of aphid species (*A. solani*, $\chi^2_1 \geq 6.8$, $P \leq 0.0091$ for all comparisons; *M. persciae*, $\chi^2_1 \geq 53.41$, $P \leq 0.0001$ for all comparisons), with flower buds receiving the next highest percentage of eggs (19-25%).

On sampling day 6, there was not a significant effect of aphid density, or its interaction with species, on the number of young *A. aphidimyza* larvae per plantHowever, there was a main effect of aphid species (Table 5.1) with more larvae on *M. persicae*-infested plants (Fig. 2B). Regarding aphid densities per plant as a response to predator treatment on day 6, there was a significant effect of treatment, aphid species, and a treatment × species interaction ($F_{1,43} \geq 5.65$, $P \leq 0.0220$ for all tests). As with the previous experiment on vegetative pansies, a reduction in the number aphids in the predator treatment compared to the control could only be seen for *M. persicae*-infested plants (Figure 5.2A) as a result of feeding by small larvae ($t_{43} = 3.89$, $P = 0.0003$ for *M. persicae*-infested plants; $t_{43} = 0.53$, $P = 0.6012$ for *A. solani*-infested plants). Again, egg laying on day 6 (6.8 ± 2.54 eggs/plant on *M. persicae*-infested plants; 6.7 ± 3.25 eggs/plant for *A. solani*-infested plants) was of a similar distribution as initial egg laying, with most found on meristems and buds: i.e. 67.9 ± 14.28% on *A. solani*-infested plants and 75 ± 25.00% on *M. periscae*-infested plants. A small number of eggs were laid on bottom leaves (*i.e.* 25 ± 25% for *M. persicae*-infested plants; 20.1 ± 6.89 % for *A. solani*-infested plants).

On day 9, some pansies had open flowers, but as this was not uniform across all plants, data from this location was combined with buds (Figure 5.5). Aphid density or species did not have a significant effect on the number of larvae per plant, though there

was a significant density x species interaction (Table 5.1). Looking at this interaction, the number of larvae on *M. persicae*-infested plants was fairly uniform across aphid densities. However, for *A. solani*, plants with a higher aphid density tended to have lower numbers of larvae. This suggests these plants received low numbers of eggs initially, resulting in higher aphid numbers on day 9 For both aphid species, a higher proportion of larvae where found on lower leaves of plants on day 9 compared to the number of eggs deposited in this location on day 2 (Fig. 5.5) (with the few eggs deposited on day 6 likely having little impact).

In terms of biocontrol outcomes (for both replicates combined), levels of *M. persicae* in the *A. aphidimyza* treatment averaged only 25.1 ± 14.17 aphids/plant (back-transformed LSmean = 4.76 aphids/plant), with 7 out of 12 plants sampled reduced to ≤ 2 aphids/plant. This was significantly lower than the average of 463.9 ± 59.49 aphids/plant on control plants (back-transformed LSmean = 462.7 aphids/plant; $F_{1,41.5}$ = 116.44, $P < 0.0001$; Figure 5.2A). This was the best control outcome of any of our experiments, at 95% control. Similarly, average *A. solani* per plant in the *A. aphidimyza* treatment was 37.4 ± 7.91 aphids/plant (back-transformed mean = 29.18 aphids/plant), which was significantly lower than the 182.3 ± 25.87 aphids/plant on control plants (back-transformed LSmean = 178.51 aphids/plant; $F_{1,41.4}$ = 20.22, $P < 0.0001$). This represented 80% control, the best control outcome seen for *A. solani* across all experiments (with outcomes being consistent across experimental replicates, *i.e.* 70% control in Replicate 1, 84% control in Replicate 2). Despite this, final aphid densities per plant were still significantly lower on plants infested with *M. periscae* vs. *A. solani* in the *A. aphidimyza* treatment, with a significant species × treatment effect (Table 5.2).

Negative slopes in population growth were seen over the last two sampling dates for both species treated with *A. aphidimyza* (Figure 5.1); however, for *A. solani*, the regression was only weakly significant (Table 5.2) The largest proportion of aphids left in the *A. aphidimyza* treatment in Replicate 2 could be found on bottom leaves of plants for both species (*A. solani*: 65 ± 14.6 %; *M. persicae*: 38 ± 23.9 %), though this was only significant for *A. solani* (Fig. 5.5) ($\chi^2_1 \geq 78.8$, $P \leq 0.0001$ for all comparisons). Given this shift towards bottom leaves, we looked at the aphid-infested bottom leaves remaining across both experimental replicates: of the 48 bottom leaf patches infested with *A. solani* (spread across 10 plants), 79% lacked any predator; for *M. persicae* 8 of the 14 (57%) remaining patches on bottom leaves lacked a predator (with these present on 5 out of 12 plants sampled).

Aphid Control on Flowering Pansy using A. aphidimyza

Average daily temperatures and RH were 20.0 C (min=15.5; max=25.2) and 67.0% (min=23.1; max=100) across both compartments. Numbers of aphids at the start of the experiment were similar per plant for all treatments ($t_{27} \leq 0.99$, $P \geq 0.7544$ for all comparisons; Figure 5.3A). Distributions of the two aphid species on plants once again differed (Figure 5.6). On day 2 the greatest proportion of *A. solani* were found on open flowers ($\chi^2_1 \geq 47.7$, $P \leq 0.0001$ for all comparisons); for *M. persicae*, the greatest portions were found on meristems ($\chi^2_1 \geq 30.9$, $P \leq 0.0001$ for all comparisons). Over

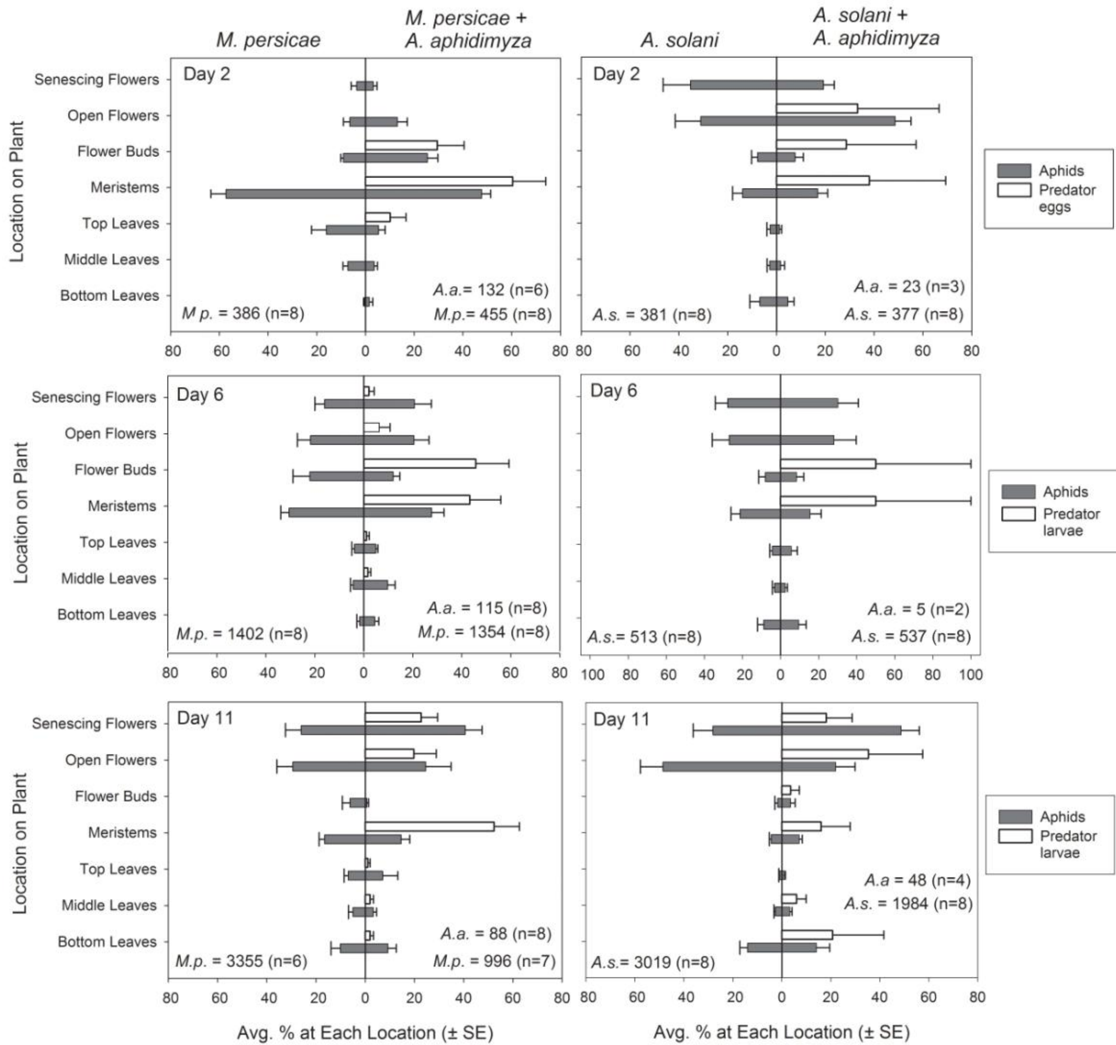


Figure 5.6. Distribution (average % ± SE) of *Myzus persicae* (*M.p.*) or *Aulacorthum solani* (*A.s.*) on flowering plants with no predator vs. in the presence of *Aphidoletes aphidimyza* (*A.a.*). Aphid distributions are shown as dark grey bars; distribution of *A. aphidimyza* eggs/larvae on treatment plants are shown as white bars. Total aphid, predator egg or larval numbers for each sampling day are given. Results are shown from day 2, 6 and 11 after release of 100 *A. aphidimyza adults*/compartment (data combined over 2 compartments). On day 2, only *A. aphidimyza* eggs are present (no larvae).

the 11 day course of the experiment, *M. persicae* gradually moved up the plant onto flowers (likely because few un-opened buds remained by day 11), while *A. solani* remained on these structures in similar percentages (Figure 5.6).

As in previous trials, 74% of *A. aphidimyza* eggs deposited in this trial were laid by day 2 of the experiment, and their distribution differed on the different plant locations ($\chi^2_6 = 31.6$, $P \leq 0.0001$ for *A. solani*-infested plants; $\chi^2_6 = 345.8$, $P \leq 0.0001$ for *M. persicae*). Eggs were generally laid on meristems and flower buds on both aphid species, with over 90% found here on *M. persicae*-infested plants, although statistically more eggs were distributed on meristems vs. buds ($\chi^2_1 = 16.4$, $P \leq 0.0001$). *For A. solani*-infested plants*,* 67% of eggs were found on these structures, with no statistical difference between the two ($\chi^2_1 = 0.31$, $P = 0.5751$); only 1 *A. solani*-infested plant in this experiment received eggs on an open flower. Again, more eggs were deposited on *M. persicae*-infested plants, though 2 out of the 8 plants sampled on day 2 were unfound or rejected by *A. aphidimyza;* for *A.solani*, this was 5 out of 8 plants. As none of the model effects were significant on this day ($F_{1,9} \leq 0.49$, $P \geq 0.5013$ for all tests); ,we removed density and its interaction and re-ran the model: here, there was a statistically higher number of eggs on *M. persicae*-infested plants vs. *A. solani* (Table 5.1).

However, the total number of eggs laid in this experiment was only 209 (across 2 compartments), which was much lower compared to all other plant stages tested in this study. As these trials were not run concurrently, time of year or batch effects may have had an effect on quality of the *A. aphidimyza* received from the commercial supplier. Furthermore, plants at this stage of growth generally had 3-4 lateral meristems

developing along with the apical meristem (although flowers and buds were only present at the apical meristem at the start of the experiment). We chose to pool data across these locations, due to what we perceived as their extremely close proximity on compact pansy plants once mature leaves were removed. However, it is possible that aphid colonies distributed on lateral meristems may have had a diluting effect in terms of prey-locating abilities of *A. aphidimyza*. Although separate data are not available, average density of aphid colonies on meristems may be closer to 7 *M. persicae*/ meristem, for example, vs. 28 ± 2.91 *M. persicae*/meristem if lateral and apical meristems are pooled. Thus, we cannot rule out that plant growth stage may have had a direct effect on attack rates by *A. aphidimyza*.

Looking at the presence of young larvae on day 6, these were also generally found on the meristems and buds of plants, with only a very small percentage on open flowers (6.3 ± 4.38% on *M. persicae* only; Fig 5.4). The average number of larvae per plant was higher on *M. persicae*-infested plants (Fig. 5.3B). Once again, there were no significant main effects for interactions with the full model, ($F_{1,6.2} \leq 3.16$ $P \geq 0.1242$ for all tests), but there was a significant difference in species when density and the interaction were removed. Interestingly, on day 11, a larger percent of the larval population was found on open/senescing flowers for both aphid species (65% for *M. persicae*; 61% for *A. solani*). Larval numbers were still higher on *M. persicae* plants vs. *A. solani* (Fig. 5.3B) but there was not statistical difference, even when species was the only effect in the model (Table 5.1).

Despite the lower number of eggs seen in this experiment, 78% control of *M. persicae* was still achieved (Figure 5.3A), and the final density of *M. persicae* on plants

exposed to *A. aphidimyza* differed significantly from those receiving no predators ($F_{1,19}$ = 20.32, P = 0.0002). However, it should be noted that *M. persicae* numbers in the *A. aphidimyza* treatment more than doubled over initial densities (with a final average of 124.5 ± 46.6 aphids/plant, LSmean = 124.5 ± 64.35 aphids/plant; Figure 5.1), which would likely be unacceptable to growers. For *A. solani*, only 36% control was achieved, and plants exposed to *A. aphidimyza* did not differ statistically from the control in terms of aphid density ($F_{1,19}$ = 2.41, P= 0.1364). Final *A. solani* density per plant in the *A. aphidimya* treatment was quite high, averaging 248.0 ± 53.62 aphids/plant (Lsmean = 248 ± 64.35 aphids/plant). The slope for aphid population growth for *M. persicae*-infested plants was negative when *A. aphidimyza* was present, although the regression was not significant in this experiment (Table 5.2). The population growth for *A. solani*, however, was significantly positive (Table 5.2).

Investigation of Apparent Competition

Temperature and humidity were similar in all 4 compartments from the time of *A. aphidimyza* release to the end of the experiment, with an average of 21 °C (range =17-24 °C) and 84% RH (range = 51-99%). One *A. solani* plant in the *A. aphidimya* treatment was removed from analysis on day 2 because it contained only 16 aphids. Additionally, 1 *A. solani* plant was removed from the analysis of control plants on day 6, and 3 plants were removed from analysis on day 9, due to contamination with either spider mites, *M. persicae* or larvae of *A. aphidimyza*, all of which could have affected *A. solani* numbers. No *M. persicae*-infested plants needed to be removed.

The 3 day delay in release of *A. aphidimyza* led to differing initial aphid densities/plant for the two aphid species. In the 2 compartments with a mixed-aphid species population, there was an average of 157.3 ± 23.14 (LSmean = 157.2 ± 20.38) aphids on *M. persicae*-infested treatment plants vs. only 102.6 ± 19.44 (LSmean = 102.6 ± 20.38) for *A. solani* treatment plants (Fig. 5.7); however, these starting densities were not statistically different ($t_{18.7} = 2.04$, $P = 0.2093$), nor were they significantly different from their control treatments ($t_{19} = < 1.04$, $P > 0.7258$ for all tests). In the *A. solani* only compartments, a slightly lower average initial aphid density was observed, at 87.7 ± 11.96 *A. solani/*plant. However, this was not statistically different from the starting density of *A. solani* in the mixed-aphid compartments ($t\ 39.9 = 0.65$, $P = 0.9164$).

As with the previous experiment on budding plants, the majority of *M. persicae* in the predator treatment were found on meristem tissue (53.9 ± 4.29%; $\chi^2_1 \geq 132.1$, $P \leq 0.0001$ for all tests). This was also true for *A. solani* in both the mixed-species compartments, (44.7 ± 4.13 %) and in the *A. solani*-only compartments (42.3 ± 3.08%) (with $\chi^2_1 \geq 31.1$ and $P \leq 0.0001$ for all tests). As expected, the next highest distribution was on bottom leaves for plants in both treatments (30.1 ± 4.49 % in the mixed-species compartment and 37.3 ± 7.46% for *A. solani* alone). Looking at *A. aphidimyza* oviposition by day 2 of the experiment, in the *A. solani* only compartments, there was an average of 22.0 ± 5.91 eggs/plant laid on treatment plants. The number of eggs laid on *A. solani*-infested plants in the mixed aphid compartments was slightly lower, at 18.5 ± 6.91 eggs/plant. In a mixed model comparing the number of eggs laid on *A. solani* alone (back-transformed LSmean = 8.8 eggs/plant) or in the presence of *M. persicae*

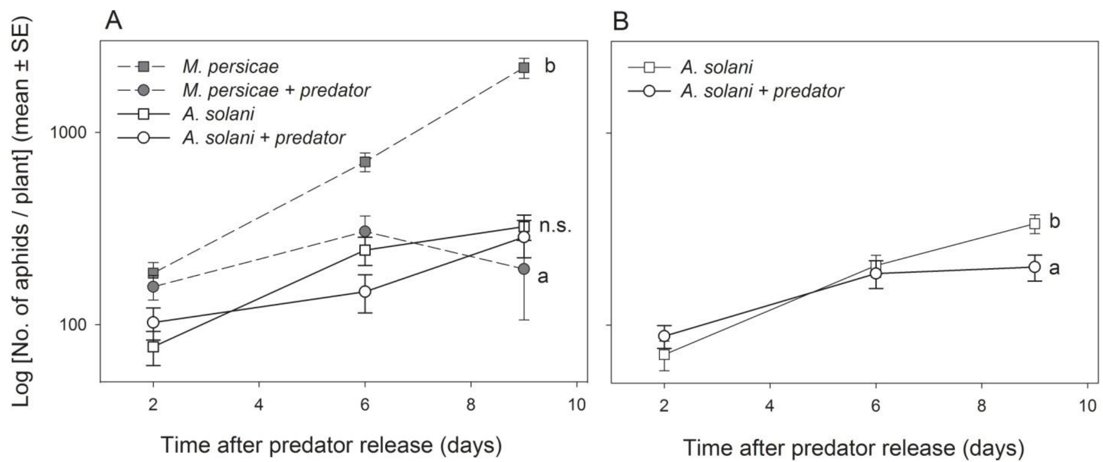


Figure 5.7. A. Mean and SE of *Aulacorthum solani* and *Myzus persicae* with either no predator (square symbols) or in the presence of the predator *Aphidoletes aphidimyza* (round symbols). **B**. Mean and SE of *Aulacorthum solani* with either no treatment or in the presence of the *A. aphidimyza* when present as the only aphid prey. Experiments were run concurrently. Results are shown from day 2, 6 and 9 after release of 120 *A. aphidimyza* adults/compartment (data pooled over 2 compartments).

(back-transformed LSmean = 7.0 eggs/plant), the presence/absence of *M. persicae* (or interactions with this factor) were not significant ($F_{1, 17.3} = 0.03$, P= 0.8548 and $F_{1, 15.5} = 0.05$, P = 0.8207, respectively) -- only aphid density per plant was a factor ($F_{1,16.5} = 12.48$, P = 0.0027). This was true even when the non-significant interaction was removed. The number of eggs laid on *M. persicae*-infested plants in the mixed aphid population was 64.0 ± 11.19 eggs/plant on day 2 (back-transformed LSmean = 58.1 eggs/plant), which was statistically higher than the number of eggs laid on *A. solani* plants in that compartment (back-transformed LSmean for *A. solani* = 12.3; $t_{4.6} = 4.75$, P = 0.0063). Along with species ($F_{1, 4.7} = 10.23$, P = 0.0266), there was also a significant effect of aphid density x species ($F_{1, 4.7} = 7.45$, P = 0.0448), but no effect of aphid

density per plant as a main effect ($F_{1,8} = 2.02$, $P = 0.1933$). Overall, there were more eggs laid per aphid in the compartments with a mixed aphid population on the first two days of oviposition (i.e. 1 egg: 3.2 aphids) vs. *A. solani* alone (i.e. 1 egg: 4.0 aphids). The predator: prey ratio on *A. solani*-infested plants only, however, was lower in the *A. solani* only compartments (1 egg: 4 aphids) vs. *A. solani* in the presence of *M. persicae* (1 egg: 5 aphids).

The average number of *A. aphidimyza* larvae on *M. persicae* infested plants in this experiment was impressively high, at 80.9 ± 15.14 larvae/plant on day 6 (with as many as 172 on one plant). Aphid species had a significant effect on the number of larvae per plant once the non-significant factors of aphid density and species x density were removed from the model ($F_{1,6.4} = 29.09$, $P = 0.0014$), with more larvae on *M. persicae*-plants (back-transformed LSmean = 71.1 larvae/plant) vs. *A. solani* (back-transformed LSmean = 4.0 larvae/plant). Number of larvae on *M. persicae*-infested plants dropped to an average of 17.6 ± 5.14 larvae/plant on day 9 (back-transformed LSmean = 11.6 larvae/plant), as mature larvae began to pupate, although this was still higher than the number found on *A. solani*-plants on this date (back-transformed LSmean = 1.5 larvae/plant; $F_{1,12} = 9.94$, $P = 0.0083$ for aphid species when density and density x species was removed from the model due to non-significance).

In the *A. solani* only compartments, there was an average of 7.4 ± 2.01 larvae/plant (LSmean = 7.4 ± 2.07) on day 6, which was not significantly different from the 9.1 ± 3.67 larvae/plant (LSmean = 8.5 ± 3.37 larvae/plant) found on *A. solani* plants in the mixed compartments ($F_{1,20} = 0.08$, $P= 0.7790$; aphid density and it's interaction with presence/absence of *M. persicae* were removed from the model due to non-

significance). Larval numbers were also similar on Day 9, with 2.9 ± 1.2 larvae/plant (LSmean = 2.8 ± 0.65 larvae/plant) in the mixed compartments, and 3.1 ± 0.65 larvae/plant (LSmean = 3.5 ± 1.00 larvae/plant) for *A. solani* alone ($F_{1,20} = 0.53$, P = 0.6004 for the effect of presence/absence of *M. persicae* on larvae abundance). Despite the similarities in predator densities per plant, the percentage of plants attacked by *A. aphidimyza* differed between the two treatments. On Day 6, 38% of *A. solani* plants sampled in the mixed-aphid compartments had no predators, with this percentage being lower (25%), in the *A. solani* only compartments. This trend was more pronounced on day 9, where 43% of *A. solani* plants in the mixed aphid compartment showed no evidence of *A. aphidimya* activity (no larvae present; no presence of sucked-out aphid carcasses), while every plant in the *A. solani* –only compartments showed evidence of *A. aphidimyza* attack. All *M. persicae* plants showed signs of attack.

Control of *M. persicae* was consistent with previous experiments, with a 91% reduction compared to predator-free controls, an outcome which was somewhat surprising given the higher initial densities than in previous trials (Figure 5.7). *Aphidoletes aphidimyza* had a significant effect on *M. persicae* density per plant ($F_{1,19.3} = 52.91$, $P < 0.0001$), and kept this pest from increasing much past initial densities (with a final density of 194.4 ± 88.6 aphids/plant), while plants with no control increased to an average of >2100 aphids per plant. In these same compartments, the predator had no significant impact on the density of *A. solani* per plant ($F_{1,19.3} = 0.85$, P = 0.4073), with only a 12% reduction (i.e. 323.0 ± 48.6 aphids/plant in the control treatment vs. 285.4 ± 62.6 aphids/plant in the predator treatment) (Fig. 5.7). However, when *A. solani* was presented alone, there was a significant effect of *A. aphidimyza* ($F_{1,22.6} = 9.54$, P =

0.0053), resulting in 40% control of the population (i.e. 337.2 ± 37.9 aphids/plant vs. 200.6 ± 31.0 aphids/plant in the control and predator treatments, respectively) (Fig. 5.7).

Lab Bioassay Comparing Honeydew Production

The average weight of a *M. persicae* nymph at 4-5 d old was 258.6 ± 75.47 µg. Given their lower intrinsic rate of increase, *A. solani* were much smaller at this age, at 85.0 ± 2.24 µg/aphid. Despite this, honeydew production was similar, at 246.8 ± 35.15 nL/aphid (LSmean = 246.8 ± 30.96 nL/aphid) for *M. persicae*, and 260.4 ± 35.15 nL/aphid (LSmean = 260.4 ± 31.81 nL/aphid) for *A. solani*. These were not significantly different at $t_{35} = 0.31$ and $P = 0.7610$. Older *A. solani* (6-7d) were closer in weight to *M. persicae* at 4-5 d old, at 275.8 ± 16.30 µg, but produced only slightly more honeydew (with 334.6 ± 43.60 nL/aphid; LSmean = 334.6 ± 42.30 nL/aphid). Production was not statistically different from *A. solani* or *M. persicae* at the younger age ($t_{29} = 1.34$, $P = 0.1917$ and $t_{30} = 1.79$, $P = 0.0832$, respectively).

DISCUSSION

Our experiments demonstrated that *A. aphidimyza* can reliably control *M. persicae* on all stages of plant growth when present as part of a mixed-aphid species outbreak (near or greater than 80% control in all cases). However, control of *A. solani* under these conditions was less reliable, even at the high release rates used. Acceptable control (80%) was seen when plants were in the budding stage, but lesser control was seen when plants were vegetative, and poor control (<40%) was observed when plants were in flower. Our findings revealed that control of *A. solani* was greater in the absence of

alternative prey, confirming our hypothesis that predator-mediated apparent competition is acting within this system. Additionally, the two aphids in our study produced similar volumes of honeydew, suggesting that differences in honeydew production is not responsible for the apparent differential attraction of *A. aphidimyza* to the two aphid pests. These results provide useful information for using *A. aphidimyza* for control of mutli-species aphid outbreaks in greenhouses, a common problem for growers, but one receiving little study.

As in Jandricic et al. 2013, *A. aphidimyza* females preferred to oviposit on new growth of plants – regardless of plant stage or aphid species infesting the plant. These areas of new growth corresponded to the most prevalent feeding locations for *M. persicae* colonies (at all stages of plant growth), but generally not for *A. solani*. Control outcomes for *M. persicae* were therefore relatively more consistent across all experiments (78-95%), while for *A. solani*, results were variable (12-80%). Our results support the hypothesis first stated in Jandricic et al. 2013: that different within-plant dispersal of aphid pest species can result in differential control outcomes of these pests using *A. aphidimyza* in a multi-species prey environment.

Consistent control of *M. persicae* by *A. aphidimyza* was accomplished across a variety of plant stages using a single release of *A. aphidimyza* at a rate of 1 adult predator: 10 aphids. In many cases, aphid populations on individual plants were completely eliminated. Previous reports of effective release rates for this natural enemy against *M. persicae* have varied somewhat, from a predator: prey ratio of 1:10 in peppers (Gilkeson and Hill 1987) to as high as 1:3 at 14 day intervals (also in peppers; Markkula and Tiittanen 1982). With the exception of a single study on roses (release

rate = 1:10; Markkula et al. 1979), literature on efficacy of *A. aphidimyza* in ornamental crops is lacking; thus our paper adds to this knowledge. Unfortunately, growers currently release biological control agents based on square footage, not as a response to pest densities, a factor which may lead to common misperceptions by some growers that biological control is not as effective as pesticide applications.

Our study provides the first information on effective release rates of *A. aphdimyza* for *A. solani* control, though control was variable. On vegetative pansy, control varied from 35%-80% across experimental replicates using this 1:10 rate (= 100 *A. aphidimyza*/compartment), suggesting possible variability in quality or searching behavior of this commercial natural enemy over time, given that almost identical aphid densities and sex ratios of the predator were used across replicates, and testsand done at the same time of year. Unlike Jandricic et al. 2013, we observed some oviposition on *A. solani* colonies on lower leaves (although this was highly disproportionate to the number of aphids there). Given that *A. aphidimyza* prefer to oviposit on aphid colonies on plant meristems, the high release rate used here may be required to obtain oviposition on lower-valued aphid colonies (with only 80 midges/compartment used in Jandricic et al. 2013). Somewhat surprisingly, control of *A. solani* was poorest on flowering plants (35%). On flowering plants, *A. solani* had a higher vertical distribution and higher aggregation on flowers. Thus, aphids on flowers might be more attractive to *A. aphidimyza* females than those on lower leaves. Although this seemed to be true of flower buds, which received the majority of eggs attributed to flowering structures across both aphid species, already open flowers received few eggs. However, poor control of both aphid species was observed in this experiment, and thus it is possible

that results may have been confounded by unknown factors such as time of year (rather than exclusively due to plant morphology). Highest control of *A. solani* (as well as *M. persicae*) occurred on plants in the budding stage (which was the only trial where > 50% control of *A. solani* was achieved in both replicates). This was likely due to the higher proportion and density of *A. solani* found on flower buds/ meristems compared to meristems only in the vegetative phase.

These longer-term experiments were beneficial as they provided us a view of *A. aphidimyza* behavior over time. Many studies with aphid predators demonstrate that females avoid laying their eggs in aphid colonies in the presence of eggs/larvae of conspecifics in order to mediate effects of competition and/or cannibalism (e.g. Ruzicka 1996; Fréchette et al. 2003). A previous laboratory study by Ruzicka and Havelka (1998) indicated that *A. aphidimyza* also demonstrates this behavior, with larval tracks deterring further oviposition. However, translation of oviposition deterring phereomones from lab to field studies must be done with care. Our results strongly suggest that *A. aphidimyza* females do not adjust for conspecific females, continuing to oviposit on plants in the same distribution patterns over time, with large numbers of conspecific larvae present in those locations. A criticism of our study could be that our insects were held as adults for ca. 48 h before release, which may have lead to less discrimination in oviposition choices by females as they aged (see Mangel 1989; Frechette 2004). However, our results confirm those of Sentis et al. (2012), where *A. aphidimyza* was released in the pupal stage in a large field study. Thus, lack of oviposition deterrence would seem to be a consistent characteristic of this natural enemy, regardless of age or space constraints. This finding has important real-world

applications, as it means that higher release rates should afford growers higher rates of control.

Sentis et al. (2012) also state that larvae of furtive predators must develop in the aphid colony chosen by the adult female, as larvae are not sufficiently mobile to change colonies. Although this may be true within canopies of large tree fruit crops, this may not be true for compact plants, such as those found in ornamental production. On the last sampling day of our experiments at every plant stage, we found numerous large *A. aphidimyza* larvae on bottom leaves of plants infested with *M. persicae* even though few eggs were ever observed to be laid in this location on the previous two sampling dates. However, direct observation of individual larvae over time is needed to confirm this. We also observed larvae on open/senescing flowers of plants, despite little oviposition at these locations as well. However, active movement of larvae up the long flower stalks and on to petals is less likely. It is more realistic that larvae located on buds are carried up with the flower as it opens (a relatively quick process in pansies).

The results in this paper also provide and example of apparent competition in a multiple aphid species system. Control of *A. solani* by *A. aphidimyza* was 26% greater in the absence of alternative prey. Our results revealed that the increased control of *A. solani* was not a direct result of increased *A. aphidimyza* oviposition/plant -- the average number of eggs and larvae on *A. solani*-infested plants was similar with and without the distraction of *M. persicae*. Instead, the primary contributing factor to the final control outcome appeared to be the number of plants attacked: *i.e.* all plants in the *A. solani*-only treatment showed some evidence of *A. aphidimyza* activity, while >40% of plants in the mixed aphid species treatment had not sign of *A. aphidimyza*. Although merely

40% control of *A. solani* in the absence of alternative prey was achieved, this experiment was done in late December. Had it been done at a more optimal time of year, it's possible that much higher control rates could have been achieved.

Lastly, although differences in honeydew production have been recorded previously from various aphid species feeding on the same food source (e.g. Mitlter and Meikle 1991; Volkl et al. 1999), we saw little difference in honeydew production between *A. solani* and *M. persicae*. These results confirm our hypothesis that disproportionate honeydew production between these two pests on a per aphid basis is likely not the cause of higher *A. aphidimyza* oviposition on *M. persicae* colonies over *A. solani* colonies. A potential criticism of our study is that we used excised leaves instead of whole plants. However, Mitler and Meikle (1991) were able to discern volumetric differences in honeydew output between *Acyrthosiphum pisum* and *M. persicae* reared on artificial diet. Fresh, excised leaves should serve as a more realistic proxy for plants than artificial diet. Despite these results, even with equal aphid densities per plant, honeydew would still be concentrated at the patch-level on meristems of *M. persicae*-infested plants due to aphid aggregation there. This would subsequently result in greater attraction of *A. aphidimyza*, given the dose-dependent response to honeydew of this predator (Choi et al. 2004). In contrast, honeydew produced from *A. solani* feeding on lower leaves is likely falling to the soil surface, and may be lost as a cue for *A. aphidimyza*.

This is the first paper assessing the ability of *A. aphidimyza* to control two aphid pest species together under different crop growth stages, as well as separately to help quantify the impact of apparent competition. Our results illustrate the challenge in

controlling multi-aphid species outbreaks, even with a “generalist” aphid predator. Additionally, our results demonstrate that prey microhabitats and crop phenology can play a significant role in predicting control outcomes, even with innundative releases of natural enemies. Although our research suggest that some control of *A. solani* is possible with *A. aphidimyza*, the presence of other aphids and crop stage have the ability to significantly affect the degree of control; thus, careful monitoring is prudent.

REFERENCES

Bergeson, E. and F. J. Messina. 1997. Resource- vs. enemy-mediated interactions between cereal aphids (Homoptera: Aphididae) on a common host plant. Ann. Ent. Soc. Amer. 90: 425-432.

Bergeson, E. and F. J. Messina. 1998. Effect of a co-occurring aphid on the susceptibility of the Russian wheat aphid to lacewing predators. Ent. Exper. Appl. 87: 103-108.

Carter, M. C., D. Sutherland & A. F. G. Dixon, 1984. Plant structure and the searching efficiency of coccinellid larvae. Oecologia 63: 394–397.

Choi, M. Y., B.D. Roitberg, A. Shani, D.A. Raworth and G.H. Lee. 2004. Olfactory response by the aphidophagous gall midge, *Aphidoletes aphidimyza* to honeydew from green peach aphid, *Myzus persicae*. Ent. Exper. Appl. 111: 37-45.

Davis, J.A., E.B. Radcliffe and D.W. Ragsdale. 2007. Resistance to green peach aphid, *Myzus persicae* (Sulzer) and potato aphid, *Macrosiphum euphorbiae* (Thomas) in potato cultivars. Am. J. Potato Res. 84:259-269.

Dewhirst, S.Y., M.A. Birkett, E. Loza-Reyes, J. L. Martin, B. J. Pye, L. E. Smart, J. Hardie, and J.A. Pickett. 2012. Activation of defence in sweet pepper, *Capsicum annum*, by *cis*-jasmone, and its impact on aphid and aphid parasitoid behavior. Pest Manag Sci 68: 1419-1429.

El Titi, A. 1972/73. Influences of prey density and morphology of the host-plant on the egg-distribution of the aphidophagous gall midge *Aphidoletes aphidimyza* (Rond.) (Diptera: Itonididae). Z. Angew. Entomol. 72: 400-415*(in German)*.

Fréchette, B., C. Alauzet and J-L. Hemptinne. 2003. Oviposition behavior of the two-spot ladybird beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinelidae) on plants with conspecific larval tracks. Aruipel Life Mar. Sci. Suppl. 5: 73-77.

Fréchette, B., A.F.G. Dixon, C. Alauzet and J-L. Hemptinne. 2004. Age and experience influence patch assessment for oviposition by an insect predator. Ecol. Entomol. 29(5):578-583.

Gilkeson, L.A. 1987. A note on fecundity of the aphid predator, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Can. Ent. 119: 1145-146.

Gilkeson, L. A. and S.B. Hill. 1987. Release rates for control of green peach aphid (Homoptera: Aphidae) by the predatory midge *Aphidoletes aphidimyza* (Diptera:

Cecidomyiidae) under winter greenhouse conditions. J. Econ. Entomol. 80:147-150.

Gross, P. 1991. Influence of target pest feeding niche on success rates in classical biological control. Environ. Entomol. 20: 1217–1227.

Gündüz, E.A. and A.E. Douglas. 2009. Symbiotic bacterial enable insects to use a nutritionally inadequate diet. Proc. R. Soc. B. 276: 987-991.

Havelka, J. and R. Zemek. 1999. Life table parameters and oviposition dynamics of various populations of the predacious gall-midge *Aphidoletes aphidimyza*. Ent. Exper. Appl. 91: 481-484.

Holt, R.D., and J. H. Lawton. 1994. The ecological consequences of shared natural enemies. Ann. Rev. Ecol. Syst. 25: 495-250.

Jandricic, S.E., S. P. Wraight, K.C. Bennett and J. P. Sanderson. 2010. Developmental times and life table statistics of the *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models. Environ. Entomol. 39(5): 1631-1642.

Jandricic, S.E., S. P. Wraight, K.C. Bennett and J. P. Sanderson. 2011. Correction for the article "Developmental times and life table statistics of the *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models". Environ. Entomol. 40(3): iv-vi.

Jandricic S.E., S.P. Wraight, D.R. Gillespie and J.P. Sanderson. 2013. Oviposition behavior of the biological control agent *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) in environments with multiple pest aphid species (Hemiptera: Aphididae). Biol. Contr. 65: 234-245.

Kuo-Sell, H. L. 1987. Some bionomics of the predacious aphid midge, *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidomyiidae), and the possibility of using the rose grain aphid, *Metopolophium dirhodum* (Wlk.), as an alternative prey in an open rearing unit in greenhouses, pp. 151-161. In R. Cavealloro (ed.), Integrated and biological control in protected crops. Proceedings of a meeting of the EC experts' group / Heraklion 24-26 April 1985. A.A.Balkema, Netherlands.

Lucas, E. and J. Brodeur. 1999. Oviposition site selection by the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). Environ. Entomol. 28(4): 622-627.

Lee, S.G., H.H. Kim, T.H. Kim, G-J. Park, K.H. Kim, and J.S Kim. 2008. Development model of the foxglove aphid, *Aulacorthum solani* (Kaltenbach) on lettuce. Kor. J. Appl. Entomol. 47: 359-364.

Markkula, M., M.K. Tiittanen, M. Hamalainen, A. Forsberg. 1979. The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. Ann. Ent. Fenn. 45: 89-98.

Markkula, M., and M.K. Tiittanen. 1982. Possibilities of biological and integrated control on vegetables. Acta Entom. Fenn. 40:15-23.

Milbrath, L.R., M. J. Tauber and C.A. Tauber. 1993. Prey specificity in *Chrysopa*: comparison of larval feeding and defensive behavior. Ecology 74: 1384–1393.

Mittler, T. E. and T. Meikle. 1991. Effects of dietary sucrose concentration on aphid honeydew carbohydrate levels and rates of excretion. Entomol. Exp. Appl. 59: 1-7.

Robert, Y. 1979. Recherches ecologiques sur les pucerons *Aulacorthum solani* Kltb., *Macrosiphum euphorbiae* Thomas et *Myzus persicae* Sulz. dans l'Ouest de la France. III. - Importance du parasitisme par Hymenopteres *Aphidiidae* et par Entomophthora sur pomme de terre. Ann. Zool. Ecol. anim. 11: 371-388.

Ruzicka, Z. 1996. Oviposition-deterring pheromone in Chrysopidae (Neuroptera): Intra- and interspecific effects. Eur. J. Entomol. 93: 161-166.

Ruzicka, Z., Havelka, J. 1998. Effects of oviposition-deterring pheromones and allomones on *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). Eur. J. Entomol. 95: 211-216.

Sanchez, J.A., F. Canovas, and A. Lacasa. 2007. Thresholds and management strategies for *Aulacorthum solani* (Hemiptera: Aphididae) in greenhouse pepper. J. Econ. Entomol. 100: 123-130.

Sentis, A., E. Lucas, and W.L. Vickery. 2012. Prey abundance, intraguild predators, ants and the optimal egg-laying strategy of a furtive predator. J. Insect Behav. 25: 529-542.

Settle, W.H., H. Ariawan, E.T. Astuti, W. Cahyana, A.L. Hakim, D. Hindayana and A.S. Lestari. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. Ecology 77: 1975-1988.

Völkl, W., J. Woodring, M. Fischer, M.W. Lorenz, K.H. Hoffmann. 1999. Ant-aphid mutualism: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia 118: 483-491.

Vehrs, S.L.C., G.P. Walker and M.P. Parella. 1992. Comparison of population growth rate and within-plant distribution between *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae) reared on potted chrysanthemums. J. Econ. Entomol. 85(3): 799-807.

CHAPTER 6

WITHIN-PLANT DISTRIBUTION OF THE FOXGLOVE APHID (*AULACORTHUM SOLANI* (KALTENBACH)) (HEMIPTERA: APHIDIDAE) ON VARIOUS GREENHOUSE PLANTS AND IMPLICATIONS FOR CONTROL

Jandricic, S.E.,[17,18] Mattson, N.S.,[19] Wraight, S.P.,[20] and Sanderson, J.P.[1]

ABSTRACT

Given its relatively recent status change from an occasional pest to a serious pest of greenhouse crops in North America and the UK, little non-anecdotal information exists on the ecology of foxglove aphid (*Aulacorthum solani* (Kaltenbach); Hemiptera: Aphididae). To help inform integrated pest management (IPM) decisions for this pest, we explored the within-plant distribution of *A. solani* on a variety of common greenhouse crops. Results indicate this aphid generally prefers to feed on lower leaves when plants are in the vegetative stage, but prefer to feed higher up in the canopy once plants become reproductive, though aphids were not necessarily found in high numbers on flowers themselves. This finding was not correlated with plant biomass within each plant stratum. Therefore, this upward movement is likely a direct result of nutritional

[17] Department of Entomology, Comstock Hall, Cornell University, Ithaca NY, USA 14853
[18] Corresponding Author: Email: sej48@cornell.edu; Tel: (607) 379-1127
[19] Department of Horticulture, Plant Science, Cornell University, Ithaca NY, USA 14853
[20] USDA-ARS Robert W. Holly Center for Agriculture and Health, Tower Road, Cornell University, Ithaca NY, USA, 14853

changes within the plant. Despite anecdotal references as a “stem-feeding aphid”, *A. solani* were almost never found feeding on stems at the densities used in our trial, with the exception of racemes of scarlet sage (*Salvia farniacea*). Previous reports suggest that *A. solani* prefers to feed on new growth of plants, but our study indicated that mature leaves are preferred over meristem tissue. The implications of the within-plant feeding preferences of *A. solani* populations on both biological and chemical control are discussed.

INTRODUCTION

The pest aphid *Aulacorthum solani* (Kaltenbach), also known as the foxglove aphid or glasshouse potato aphid, is known to infest a wide variety of plants, being a reported from 95 different plant species from 25 families (Kim et al. 1991). In greenhouse crops, particularly, *A. solani* is considered quite polyphagous, and is known to infest nearly every plant that is attacked by green peach aphid (*Myzus persicae*) or melon aphid (*Aphis gossypii*) (Gill and Sanderson 1998). Despite its previous status as an “occasional pest” in greenhouse crops, greenhouse floriculture growers worldwide are finding *A. solani* to be an increasing problem in recent years. A 2006 survey of floriculture greenhouses in MA and NY found *A. solani* to be the second most common aphid species infesting floriculture crops, surpassing both *A. gossypii* and *Macrosiphum euphorbiae* (potato aphid), and second only to *M. persicae* (van Driesche et al. 2008). In Southern Ontario, Canada, *A. solan*i is currently second only to *M. persicae* in terms of aphid pest status in ornamentals, though in recent years it was the primary aphid pest (G. Murphy, personal communication). Additionally, *A. solani* is the main pest of

several ornamental bedding crops (*e.g.* verbena, pelargonium) in greenhouses in the United Kingdom, and is also a significant pest of fuchsia and greenhouse-grown peppers (J. Bennison, personal communication). It has been suggested that the change in pest status of *A. solani* may be due to recent widespread reduction of pesticide sprays for other pests due to increasing adoption of IPM practices in various agricultural and greenhouse crops (Sanchez et al. 2007). Or, the increased incidence may be an indirect result of greenhouse facilities growing ornamentals at cooler temperatures to save on costs of heating (Jandricic et al. 2010). Cooler temperatures provide ideal developmental conditions for *A. solani*, which has its highest intrinsic rates of increase (r_m) between temperatures of 20-25°C (Lee et al. 2008; Jandricic et al. 2011).

Regardless of the reason, that *A. solani* has gone from an occasional pest to a serious pest in a relatively short time has meant there is a lack of information surrounding this pest in terms of its biology, ecology, and effective control measures. One such important consideration is information on the within-plant distribution of this pest. Previous literature has shown this to be an important consideration in pest management programs against aphids using biopesticides (Hall and Burges 1979) and natural enemies (Jandricic et al. 2013), and it may likely also have repercussions for pesticide applications. In previous (limited) reports in the literature, reports of *A. solani* feeding patterns are contradictory. Some report that this pest feeds on the lower leaves of plants (Robert 1979; Verider 1999; Jandricic et al. 2013), but can move up the flower stalk as the plant becomes reproductive (Jandricic et al. 2013). Others report that *A. solani* feed either in the top of the canopy or on succulent growing tips of leaves of younger plants (Wave et al. 1965; Down et al. 1996), but move to the underside of older

leaves adjacent to the ground of as the plant matures (Wave et al. 1965). Further, *A. solani* are often anecdotally reported to be predominately "stem feeding" aphids (vs. leaves). However, these reports are generally unverified experimentally (Verider 1999), conducted on only one species of plant (*i.e.* potato: Robert 1979, Down 1996; pansy: Jandricic et al. 2013), or in relation to weeds or field crops (Wave et al. 1965; Robert 1979; Down 1996), which are grown for much longer periods of time than ornamentals.

Thus, this paper sought to qualify the distribution of *A. solani* across various ornamental plants. We hypothesize that this pest generally prefers to feed on lower leaves of plants in the vegetative stage, but moves up towards flowers in the reproductive stage. Confirmation of this hypothesis would not only improve scouting practices for *A. solani,* but also pest management strategies. Specifically, predictions about the ability of the predator *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) to control this pest on specific plant species could be made. This predator is strongly influenced by within-plant location of aphid colonies, and considers colonies on growing points (meristems) as higher quality patches for its offspring than other plant locations (Lucas and Brodeur 1999; Jandricic et al. 2013). This behavior has been shown to subsequently affect biological control outcomes of *A. solani* (Jandricic et al., unpublished data) on a low-growing bedding crop (*i.e.* pansy, *Viola x wittrockiana* Gams.), but information from other crops is needed. Influences of within-plant distribution of this aphid pest on other biological and chemical controls in greenhouse production are also discussed.

MATERIALS AND METHODS

Source and Maintenance of Insects

A. solani were collected in Ithaca, NY in 2009, and reared continuously on pansies (*Viola* × *wittrockiana* Gams., var. Majestic Giant; Stokes Seeds, Buffalo, NY) as polyclonal colonies, as in Jandricic et al. 2010. Adult aphids for all experiments were selected directly from colonies, and were therefore of unknown age. Aphids from which the colony was descended had previous experience with Scarlet sage (*Salvia splendans*; the original host plant aphids were collected from), poinsettia (*Euphorbia pulcherrima*) and pansy. The aphid population was naïve to all other plant species to the best of our knowledge.

Source and Maintenance of Plant Material

General plant care is as follows: Seeds were obtained from the following sources: Harris Seeds (Rochester, NY), Stokes Seeds (Buffalo, NY) or Syngenta Flowers-GoldFisch® Vegetative (Boulder, CO). Planting dates for the various plants stages (as well the specific varieties tested) are given in Table 6.1. All plants were sown and grown in seedling trays for ca. 3-4 weeks and then transplanted into appropriate sized pots (4 inch, 6 inch, or 15 inch depending on the size of the plant species). For trials in 2010, Pro-Mix 'BX' was used as the growing media (Premier Horticulture Inc., Quakertown, PA); Lambert Professional Growing Mix (Lambert Peat Moss Inc., Rivière-Ouelle, QC) was used for plants grown in 2012. All plants were grown in a research greenhouse at Cornell University at constant 24°C with a 16:8 L: D period

using supplental light, unless otherwise specified. All plants received 150 ppm N 5 days a week from a 21:5:20 formulation (J. R. Peters, Inc., Allentown, PA).

Further details of note for separate species are as follows: Petunia (*Petunia x hybrida*) seedlings received mist for ca. 3 weeks in a propagation house, at which time the plants were brought into the greenhouse. Plants were generally exposed to ambient light only, but did receive 50% shade on sunny days (through October 1, 2010). Rooted poinsettia cuttings (*Euphorbia pulcherrima* cv. Freedom Red, ca. 1 month old) were obtained from Paul Ecke Ranch (Encinitas, CA) on September 2, 2010. Cuttings were immediately planted into 15 cm pots and were kept in a greenhouse with ambient light only. The flowering stage of poinsettia was not tested. Marigolds (*Tagetes patula*) and zinnias (*Zinnia marylandica*) were grown under a 9 hour photoperiod, as they are short-day flowering plants. Two varieties of snapdragons were tested due to their different canopy architectures. Though both *Antirrhinum majus*, var. Rocket yellow is a tall variety grown for cut flowers (henceforth referred to as "tall" snapdragon), and var. Montego Yellow is a dwarf variety used as a bedding (henceforth referred to as "dwarf" snapdragon). Scarlet sage (*Salvia splendans* var. Salsa red) was also planted in 2012 to provide a vegetative comparison for the flowering plants tested in 2010.

Basil (*Ocimum basilicum* var. Ceasar), tomato (*Solanum lycopersicum* var. Panzer) and dianthus (*Dianthus chinensis* var. Super Parfait Raspberry; Harris Seeds, Rochester, NY) were also investigated. Both the vegetative and flowering stage were tested for dianthus; the vegetative stage only was tested for basil and tomato. However, no aphids were recovered after 1 week on basil or dianthus, and less than 14 aphids/plant were recovered from tomato. Thus, data from these greenhouse crops are

not included in any data anlalyses or presentation.

Within-Plant Distribution of A. solani on Greenhouse Plants

The plant species/varieties in Table 6.1 were chosen based on a) popularity in greenhouse production, b) availability at the time of the experiment and c) estimated acceptability as a host for *A. solani*. Some plants (*i.e.* dwarf snapdragons, marigolds, scarlet sage) had a very short vegetative phase (with little plant architecture at this point in growth), and developed buds when quite small. Thus, these plants were tested when in the "budding" stage instead. The experiment was conducted across several dates: see Table 6.1. Although attempts were made to generate several plant species/stage combinations at the appropriate stage on the same date, this did not always occur. Thus, plants were tested as each species/variety reached the appropriate stage, regardless of the availability of other combinations. If possible, vegetative and flowering plants of the same species were tested on the same date.

For each experiment, 12 apterous adult *A. solani* were placed on plants at a point ca. half way up the plant canopy (not including the flower, if applicable). This was chosen over placing adults on the soil and allowing them to walk up the plant for two reasons. First, aphid infestations and transfer between plants in greenhouses most likely occurs from infested foliage (from cuttings or other materials), or from alate adults, which would be unlikely to land on the soil surface. Second, we did not want to bias adult aphids towards lower leaves by providing them with a much further walking distance from upper leaves/flowers. There were 6 replicates for each plant type. Any aphids that fell from the plant upon placement were considered "disturbed" and were

removed from the experiment, being replaced with a different adult (though this rarely happened). Given that plants did not always reach the appropriate stage for testing on schedule, preparing aphid cohorts of a known age for all tests was considered too impractical; thus, all adult aphids used for tests were collected directly from aphid colonies, and thus were of unknown age. Once placed on the plant, aphids were allowed to naturally distribute themselves and reproduce for 1 week, at which time plants were destructively sampled and the number of *A. solani* found at different within-plant locations were recorded. Adult and immature aphids were recorded separately for all plants on all dates, with the exception of tests on marigolds, where total aphid numbers were coutned.

In all cases, aphid colonies were categorized as being found on one of 3-4 possible plant strata. Vegetative plants were split into approximately equal thirds (based on height from the soil surface), and thus aphids could be found in the bottom, middle or top stratum. Bottom leaves can generally be considered the oldest leaves on the plant, and top leaves the youngest. Height of plants at the time of data collection can be found in the Results section. The same categories were used for flowering plants, except that an additional stratum consisting of reproductive organs (flowers and unopened flower buds) was also included (henceforth referred to only as the "flower" stratum). The flower stratum generally existed vertically higher in height than the top leaf canopy, although this could vary greatly with plant type. For example, for zinnia, flowers were only slightly higher than top leaves, while for species within the *Salvia* and *Antirrhinum*, flowering racemes were atop tall stalks. Dwarf snapdragon were an exception, as buds could be found in each stratum (except for bottom) of the leaf

canopy due to the high degree of lateral branching. For this plant, only the open flower (raceme) was placed in the "flower" stratum; buds found elsewhere on the plant were included in their respective stratum (*i.e.* top or middle). Generally, data from flowers and buds were recorded separately to assess the aphid numbers on each of these structures individually, except in the case of plants which produced racemes with unopened florets at the apex (*i.e.* flowering sage, scarlet sage, and snapdragons).

For both vegetative and flowering plants, data were further divided into the plant organs aphids were found on within each stratum. For vegetative plants, this consisted of either mature leaves or growing points (both apical and lateral meristems). For reproductive plants, these were mature leaves, meristems, or flowers/buds. Data from senescing flowers were also recorded (only applicable to marigolds and pansies) and combined with data on open flowers; trends on senescing flowers are discussed in the Results section. If high numbers of aphids were found on flowers/buds of a particular plant species, aphid presence was further categorized as being on petals, stems or sepals and calyx of the flowers/buds. Although *A. solani* are anecdotally reported to feed on plant stems, this was a rare occurrence in our experiments. Thus, stem was not included as an organ category, but any occurrence is discussed in the Results section.

To investigate whether the number of aphids per strata was not simply a function of the amount of biomass present in each (with bottom leaves generally being larger), we counted the total number of leaves in each stratum per plant and estimated leaf area. Leaf counts were taken on the day of the experiment. An average leaf area/stratum/species was estimated by destructively sampling a minimum of 3 plants/species (to obtain at least 20 leaves per stratum) and using the calculation in

Pandy and Singh (2011). Leaf measurements were taken from one growth stage only for each plant species tested and used as the leaf area estimate for both vegetative and flowering plants. Leaf estimates were not taken for zinnia or petunia, thus these plant species were left out of our initial analysis. To obtain the final estimate of biomass used in the statistical analysis, number of leaves per stratum was multiplied by the estimated leaf area.

Statistical Analyses

All analyses were done in SAS v. 9.3 (SAS Institute, 2011). To analyze overall effects of within-plant location on aphid distribution across plant species, a mixed model ANOVA was conducted on the proportion of total aphids found within each strata (bottom, middle or top third, based on height from the soil surface). Flowers/buds were combined into the top stratum to facilitate comparisons between vegetative, budding, and flowering plants. Because proportions would sum to 100% for all three strata, thus making estimates incalculable, we omitted data from the middle stratum from this global analysis. The middle stratum was chosen because a preliminary analysis revealed it contained the lowest number of aphids, and was also the stratum of least biological significance to our question of interest. Within-plant strata, plant species, stage of plant growth, and the estimated biomass per strata (see above) were included as fixed effects. All two- and three-way interactions with these factors were tested. Plants for which we lacked an estimate of biomass were omitted from this broader analysis. Plant replicate (nested within plant species) was the random effect. Proportional data were arcsine-square root transformed to better meet the assumptions of the ANOVA,

and the Kenward-Roger method of calculating degrees of freedom was applied (Littell et al. 2006). We also included the use of the REPEATED statement in SAS, specifying plant (nested within species) as the repeated measure. We also specified the use of the Autoregressive (AR (1)) model for the covariance structure, as recommended by Littell et al. 2006. This was done because, in our experiment, plant was the whole-plot experimental unit within which different "treatments" (strata) were sampled. Thus, errors are likely correlated between strata within plant. Additionally, the Akaike Information Criterion (AIC) statistic was lower in all tests using the AR(1) model compared to the Variance Components model (the default in SAS), indicating a better fit. Thus, the REPEATED statement and the AR (1) covariance structure were used in all further analyses. As a reference, however, all two, three and 4-way interactions were similar using the AR(1) model as with the Variance Components model (no repeated measures specified), with the exception of a non-significant species x stage interaction using the default model, as well as non-significant main effects of strata and species. Lastly, the entire analysis was repeated with data subject to the empirical logit transformation, as recent discussions have suggested they may be more appropriate for proportional data (Warton and Hui 2011). However, as we detected no significant differences in outcomes between the two transformations, we opted to stay with the more conventional arcsine-square root transformation, which has a less cumbersome back-transformation.

In addition to the analysis by stratum, a secondary analysis was done with data grouped by organ type. Specifically, the proportion of aphids feeding on leaves were compared to meristems (apical or lateral) and flower buds and flowers (open and

senescing) if applicable. Organ types were grouped together regardless of their location height-wise on plants. An estimate of biomass was not included in this model as we did not have area measurements of meristems, buds or flowers. Data were arcsine-square root transformed prior to analysis.

To assess aphid locations within each plant species and stage separately, we conducted an ANOVA on the number of aphids per stratum, or the number of aphids per organ type. Aphid numbers per location were log (n+1) transformed prior to analysis to better meet the assumptions of variance. The Autoregressive (1) model was used as the covariance structure, although no difference in outcomes was seen with the Variance Components model. The Tukey-Kramer test was used to determine differences between strata or organ types. Given that adult aphids were placed at the same starting location, and that only the nymphs that were larviposited on the first day of the experiment would have the potential to develop into adults by day 7, we analyzed the number of adult aphids in the same patch (leaf, meristem, bud or flower) as a measure of adult aphid dispersal. Frequencies of adult numbers are presented in the Results.

RESULTS

Temperature in the greenhouse for the week of the experiment averaged 20.2 °C (min. =18.7 °C, max. = 24.0 °C) for the plants tested in December 2010 (Table 6.1). For the plants tested in December 2012 - January 2013, temperatures were similar, at 19.1 °C (min. = 14.0 °C, max. = 23.0 °C) (Table 6.1). The replicate of flowering dwarf snapdragons and budding scarlet sage (conducted in late March 2013) had a similar

average temperature (20.1 °C), although the maxiumum temperature reached in the compartment was slightly higher (min. = 17.8 °C, max. = 27.2 °C).

After placing adult aphids on plants, aphids were observed to generally stay on or near the place of placement for 20-30 minutes (at which time infested plants were moved into the greenhouse). This suggests that aphid movement to locations in the following results were due to feeding/distribution preferences, rather than dispersal due to disturbance and the production of alarm pheromone.

As no/few aphids were recovered from basil, tomato and dianthus, this suggests these plants were not appropriate hosts for our population of *A. solani*. Thus, no further results are reported for these plant species. Results of the global analysis conducted on proportions of *A. solani* per stratum indicated significant interactions between biomass and stratum, biomass and species, as well as all 3 and 4-way interactions with biomass ($F_{5, 59.7} > 2.48$, $P < 0.0419$ in all cases), with the single exception of the interaction between biomass, stratum and stage ($F_{1,58.4} = 0.44$, $P = 0.5108$). To further understand these interactions, an ANOVA was conducted using biomass as the independent variable. Here, stratum, stage, plant species, and all 2 and 3-way interactions were highly significant ($F_{5,59} > 38.14$, $P < 0.0001$ in all cases). As this indicated a high degree of variability within this effect, we removed this factor from the global analysis on aphid proportions.

The results of the final ANOVA indicated that only stage and species were significant as main effects (strata: $F_{1,59} = 1.36$, $P = 0.2475$; stage: $F_{1, 59} = 36.65$, $P < 0.0001$; species: $F_{5,59} = 4.88$, $P = 0.0008$). However, all two-way interactions were

Table 6.1. Planting dates for plant species used in experiments.

Common name (species name, variety)	Vegetative/Budding Stage		Flowering Stage	
	Planting Date	Experiment Date	Planting Date	Experiment Date
Dwarf snapdragon (*Antirrhinum majus* var. Montego yellow)	Nov. 2, 2012	Jan. 5, 2013	Jan. 30, 2013	Mar. 30, 2013
Flowering sage (*Salvia farinacea* var. Victoria blue)	Nov. 2, 2012	Dec. 21, 2012	Oct. 5, 2012	Dec. 21, 2012
Marigold (*Tagetes patula* var. Disco yellow)	Nov. 5, 2012	Dec. 5, 2012	Oct. 5, 2012	Dec. 5, 2012
Pansy (*Viola × wittrockiana* var. Majestic giant)	Oct .10, 2010	Dec. 9, 2010	Sept. 26, 2010	Dec. 9, 2010
Pepper (*Capsicum annuum* var. Lady bell)	Oct 5. 2012	Dec. 5, 2012	Oct. 5, 2012	Dec. 27, 2012
Petunia *Petunia x hybrida* var. Bravo blue)	Aug. 15, 2010	Dec. 9, 2010	Aug. 15, 2010	Dec. 9, 2010
Poinsettia (*Euphorbia pulcherrima* var. Freedom red)	Sept. 2, 2010	Dec. 9, 2010	–	–
Scarlet sage (*Salvia splendans* var. Salsa red)	Feb. 23, 2013	Mar. 30, 2013	Sept. 10, 2010	Dec. 9, 2010
Tall Snapdragon (*Antirrhinum majus* var. Rocket yellow)	Nov. 30, 2012	Dec. 21, 2012	Nov. 2, 2012	Jan. 5, 2013
Zinnia (*Zinnia marylandica* var. Zahara yellow)	Nov. 2, 2012	Dec. 5, 2012	Oct. 5, 2010	Dec. 5, 2012

significant (strata × stage: $F_{1,59}$ = 129.67, P < 0.0001; strata × species: $F_{5, 90}$ = 15.27, P < 0.0001;species × stage: $F_{5,59}$ = 15.24, P < 0.0001). The 3-way interaction was also significant, at $F_{5,59}$ =7.08 and P < 0.0001. This analysis confirmed that more aphids were found feeding in the bottom stratum vs. the top stratum of vegetative plants across all varieties tested (t_{59} = 7.29, P < 0.0001; Tukey-Kramer test), and that this trend was reversed when plants were flowering (t_{59} = 8.81, P < 0.0001; Tukey-Kramer test).

We examined this interaction between plant strata and stage in greater depth in Table 6.2; results are arranged from the strongest effect of flowers (pansy), to the weakest (dwarf snapdragons). Analyses were conducted on log (n+1) transformed aphid numbers per stratum within each plant species. Here, a significant stratum x stage interaction was seen in all cases except marigolds and petunias (Table 6.2). This interaction was usually characterized by aphid selection of feeding sites near the bottom of the plant when vegetative, with a shift towards the top stratum when the plant was flowering, confirming the results of our analysis on all plant species together.   One exception to this trend was dwarf snapdragon, where a greater number of aphids was seen feeding in the middle stratum when flowering. However, this was the only plant variety tested that produced buds and flowers in the middle stratum, as well as top, due to a high degree of lateral branching. Aphids, placed in the middle of the plant initially, likely chose these more convenient buds/flowers here over those in the top stratum. Marigolds were the other exception. However, the replicate of this plant species took place when buds were well developed (compared to other species such as scarlet sage and dwarf snapdragons, which had just begun to bud). These well-developed buds may

Table 6.2 Influence of plant species and growth stage on within-plant distribution of *Aulacorthum solani* (all ages).

Plant species and stratum	Avg. aphids/stratum/plant of indicated growth stage ± standard error [a] (percent of total population). Avg. height of whole plant is given in parenthesis beside plant stage.		Percentage-point difference between stages	ANOVA: [b] Stage x Stratum interaction F-test
Pansy	Vegetative (7.3 ± 0.33 cm)	Flowering (12.0 ± 0.64 cm)		
Top [c]	2.0 ± 1.8 (3) a	80.5 ± 8.7 (86) A	+ 83	$F_{2,19.9}$ =
Middle	16.8 ± 5.9 (22) b	5.7 ± 1.2 (6) B	– 16	47.2
Bottom	56.8 ± 26.3 (75) b	7.0 ± 1.7 (8) B	– 67	P < 0.0001
Flowering sage	Vegetative (11.5 ± 0.44 cm)	Flowering (44.0 ± 1.21 cm)		
Top	0.2 ± 0.2 (< 0.3) a	74.7 ± 18.0 (81) A	+ 81	$F_{2,20}$ = 69.1
Middle	62.3 ± 8.3 (68) b	5.8 ± 3.0 (6) B	– 62	P < 0.0001
Bottom	28.8 ± 13.7 (31) b	11.3 ± 6.7 (12) B	– 19	
Scarlet sage	Budding (13.5 ± 0.56 cm)	Flowering (30.0 ± 0.33 cm)		
Top	77.2 ± 16.5 (41) a	192.2 ± 43.9 (98) A	+ 57	$F_{2,19}$ = 12.7
Middle	62.2 ± 15.8 (33) a	2.8 ± 1.7 (1) B	– 32	P = 0.0004
Bottom	50.5 ± 29.6 (26) a	2.8 ± 1.9 (1) B	– 25	
Zinnia	Vegetative (10.3 ± 0.56 cm)	Flowering (14.4 ± 0.24 cm)		
Top	3.5 ± 2.4 (3) a	36.0 ± 9.9 (44) A	+ 41	$F_{2,19.9}$ =
Middle	38.8 ± 12.2 (35) b	23.0 ± 8.5 (28) A	– 7	17.9
Bottom	68.0 ± 7.2 (62) b	23.2 ± 6.8 (28) A	– 34	P < 0.0001
Peppers	Vegetative (45.0 ± 0.89 cm)	Flowering (43.5 ± 1.96 cm)		
Top	7.5 ± 4.8 (12) a	54.8 ± 10.0 (44) A	+ 32	$F_{2,14.4}$ = 6.8
Middle	15.2 ± 6.2 (24) a	18.5 ± 4.3 (15) A	– 9	P = 0.0085
Bottom	41.0 ± 11.1 (64) a	50.0 ± 22.1 (41) A	– 23	
Tall snapdragon	Vegetative (38.3 ± 1.15 cm)	Flowering (70.2 ± 1.65 cm)		
Top	3.0 ± 0.8 (4) a	39.5 ± 7.2 (33) A	+ 29	$F_{2,14.3}$ =
Middle	9.0 ± 2.0 (14) a	27.2 ± 4.3 (23) A	+ 9	14.7
Bottom	54.2 ± 12.0 (82) a	52.8 ± 15.6 (44) A	– 38	P = 0.0003

Table 6.2 (Continued)

Plant species and stratum	Avg. aphids/stratum/plant of indicated growth stage ± standard error [a] (percent of total population). Avg. height of whole plant is given in parenthesis beside plant stage.		Percentage-point difference between stages	ANOVA: [b] Stage x Stratum interaction F-test
Petunia	Vegetative (17.0 ± 0.44cm)	Flowering (21.0 ± 0.65cm)		
Top	12.8 ± 6.1 (44) a	9.3 ± 5.8 A (51) A	+ 7	$F_{2,19.7}$ =
Middle	11.3 ± 5.3 (38) a	8.5 ± 5.0 A (47) A	+ 9	0.37
Bottom	5.2 ± 2.2 (18) a	0.3 ± 0.2 A (2) A	– 16	P = 0.6983
Marigold	Budding (9.8 ± 0.34 cm)	Flowering (13.8 ± 0.60cm)		
Top	44.4 ± 8.8 (88) a	62.8 ± 9.2 (85) A	– 3	$F_{2,18.9}$ =
Middle	2.2 ± 0.8 (4) b	1.3 ± 0.6 (2) B	– 2	3.92
Bottom	4.0 ± 0.9 (8) b	9.7 ± 1.5 (13) C	+ 5	P = 0.0376
Dwarf. snapdragon [d]	Budding (12.1 ± 0.26cm)	Flowering (15.1 ± 0.12cm)		
Top	64.0 ± 8.7 (49) a	29.8 ± 5.3 (36) A	– 13	$F_{2,10.3}$ =
Middle	18.0 ± 6.9 (14) b	32.8 ± 10.3 (40) A	+ 26	3.55
Bottom	48.8 ± 5.7 (37) a	19.8 ± 3.3 (24) A	– 13	P = 0.0671
Poinsettia	Vegetative (22.0 ± 0.56cm)	Flowering		
Top	12.0 ± 5.3 a (13)	–	–	–
Middle	31.8 ± 5.6 ab (33)	–	–	–
Bottom	64.0 ± 17.8 b (54)	–	–	–

[a] Means within plant species within growth stages followed by same letter are not significantly different (Tukey-Kramer test, alpha = 0.05).

[b] Mixed-model ANOVA on total aphids per stratum per plant, within species; data log (n+1) transformed before analysis; includes the random effect of plant as a repeated measure.

[c] Top stratum includes flowers and buds for plants in the reproductive stage.

[d] Dwarf snapdragons were the only plant species where flowers and buds were also present in the middle stratum.

have already attracted aphids to the top of the plants, resulting in little difference between the budding and flowering stages. Interestingly, our results show that aphids prefer bottom leaves of vegetative peppers, even though most plant tissue was present in the top stratum due to lateral branching there.

One caveat to the data presented in Table 6.2 is that overall aphid numbers are a mix of adults and their offspring. High numbers of aphids in a particular stratum may be biased by higher reproduction in that stratum. Since aphids were only present on plants for 1week, and thus few nymphs would have reached the adult stage by this time (see Jandricic et al. 2010), we also analyzed data from adult aphids only (Table 6.3). These data are a stronger indicator of *A. solani* distribution preferences based on plant canopy differences. As shown in Table 6.3, trends are extremely similar to those presented in Table 6.2, except for large snapdragons, which did not show a statistically different shift in aphid distribution upon flowering. Due to these similarities, data were not separated into adults and nymphs for other data analyses.

Looking at the frequency of adult aphids on the same patch (leaf, meristem, flower or bud) as a measure of initial dispersion, data indicate that adult aphids of this species tend not to aggregate together, despite starting at the same location (Figure 6.1). A single adult on a patch was most common, though it was observed to have up to 27 adults in one patch. Patches with >8 adults generally only occurred on scarlet sage, where the large racemes supported high numbers of aphids. Exceptions to this were a single case of a leaf containing 15 adults occurring on flowering sage, as well as a case of 18 adults on a poinsettia leaf.

Table 6.3. Influence of plant species and growth stage on within-plant distribution of adult *Aulacorthum solani* only.

Plant species and stratum	Avg. adult aphids/stratum/plant of indicated growth stage ± standard error [a] (percent of total population)		Percentage-point difference between stages	ANOVA: [b] Stage x stratum Interaction F-test
Pansy	Vegetative	Flowering		
Top	0.0 (0) a	7.3 ± 1.1 (80) A	+ 80	$F_{2,19.6} = 32.9$
Middle	1.5 ± 0.4 (19) b	1.0 ± 0.4 (11) B	– 8	$P < 0.0001$
Bottom	6.2 ± 3.2 (81) c	0.8 ± 0.4 (9) B	– 72	
Flowering sage	Vegetative	Flowering		
Top	0.0 (0) a	9.7 ± 2.7 (80) A	+ 80	$F_{2,12.4} = 48.9$
Middle	9.7 ± 3.2 (85) b	0.7 ± 0.4 (6) B	– 79	$P < 0.0001$
Bottom	1.7 ± 0.6 (15) c	1.7 ± 0.8 (14) B	– 1	
Scarlet sage	Budding	Flowering		
Top	6.2 ± 1.2 (42) a	55.5 ± 19.4 (95) A	+ 53	$F_{2,14.2} = 14.9$
Middle	4.2 ± 1.2 (28) a	1.2 ± 0.8 (2) B	– 26	$P = 0.0003$
Bottom	4.5 ± 2.0 (30) a	1.7 ± 1.5 (3) B	– 27	
Zinnia	Vegetative	Flowering		
Top	0.2 ± 0.2 (3) a	3.8 ± 1.3 (50) A	+ 47	$F_{2,19.9} = 10.4$
Middle	3.0 ± 0.9 (40) b	1.8 ± 0.7 (24) A	– 16	$P = 0.0008$
Bottom	4.2 ± 0.6 (57) b	2.0 ± 0.7 (26) A	– 31	
Peppers	Vegetative	Flowering		
Top	0.7 ± 0.4 (12) a	15.5 ± 2.1 (60) A	+ 48	$F_{2,18.6} = 11.2$
Middle	1.8 ± 0.5 (30) a	4.0 ± 0.8 (16) B	– 14	$P = 0.0007$
Bottom	3.5 ± 0.8 (58) a	6.3 ± 2.5 (24) AB	– 34	
Tall snapdragon	Vegetative	Flowering		
Top	0.3 ± 0.2 (6) a	2.0 ± 0.5 (21) A	+ 15	$F_{2,14} = 1.0$
Middle	0.3 ± 0.2 (6) a	1.2 ± 0.3 (13) A	+ 7	$P = 0.3918$
Bottom	4.2 ± 1.1 (88) b	6.2 ± 1.7 (66) B	– 22	
Petunia	Vegetative	Flowering		
Top	2.3 ± 1.2 (35) a	0.7 ± 0.2 (29) A	– 6	$F_{2,20.5} = 0.6$
Middle	3.0 ± 1.6 (45) a	1.5 ± 0.8 (63) A	+ 18	$P = 0.9401$
Bottom	1.3 ± 0.6 (20) a	0.2 ± 0.2 (8) A	– 12	
Dwarf snapdragon	Budding	Flowering		
Top	5.3 ± 1.7 (49) a	3.0 ± 0.3 a (45) A	– 4	$F_{2,9.4} = 10.2$
Middle	1.3 ± 0.8 (12) a	3.2 ± 0.9 a (49) A	+ 37	$P = 0.0044$
Bottom	4.2 ± 0.7 (39) a	0.4 ± 0.4 a (6) B	– 33	

Table 6.3 (Continued)

Plant species and stratum	Avg. adult aphids/stratum/plant of indicated growth stage ± standard error [a] (percent of total population)		Percentage-point difference between stages	ANOVA: [b] Stage x stratum Interaction F-test
	Vegetative	Flowering		
Poinsettia				
Top	1.2 ± 0.8 (7) a	–	–	
Middle	5.8 ± 3.1 (32) ab	–	–	–
Bottom	11.0 ± 2.9 (61) b	–	–	

[a] Means within plant species within growth stages followed by same letter are not significantly different (Tukey-Kramer test, alpha = 0.05). [b] Mixed-model ANOVA on the number of adult aphids per stratum per plant, within species; data log (n+1) transformed before analysis; includes plant as the random effect.

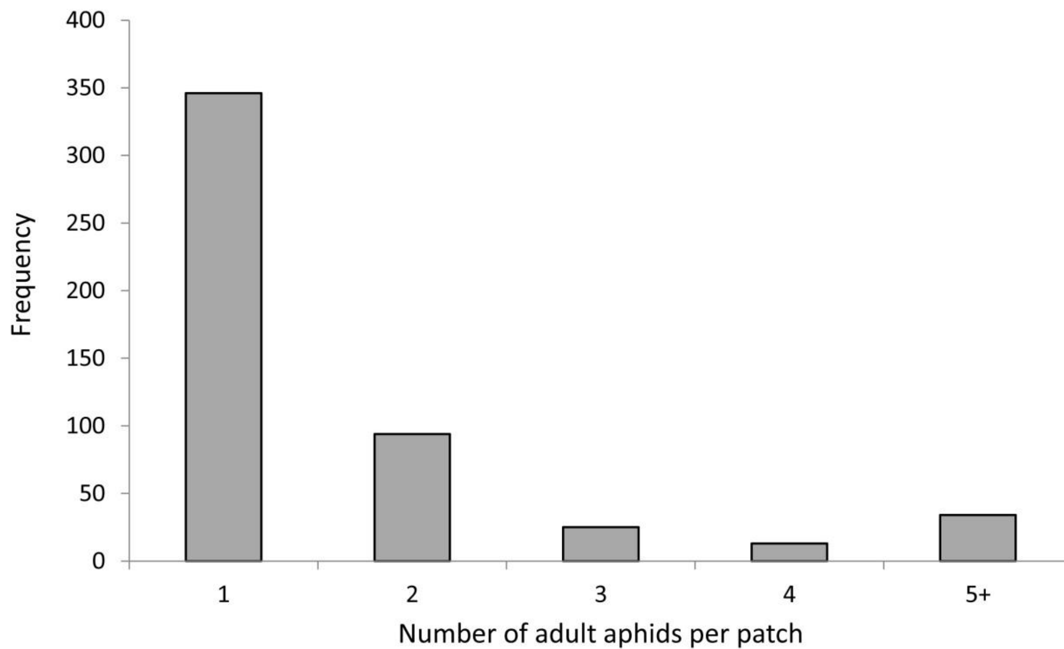


Figure 6.1. Frequencies of adult *Aulacorthum solani* occurring per patch (*i.e.* leaf, meristem, flower or bud) across 10 species/varieties of greenhouse crops.

Although adults of unknown age were used, as thus aphid numbers are not directly comparable between plant species, the much higher number of total aphids on flowering sage suggests the intrinsic rate of increase of *A. solani* is higher on this plant species (1187 aphids total in the flowering stage; 1139 when vegetative; 176-785 for all other plant species; n=6 for all plants). Thus, the presence of more adults after 1 week of reproduction on flowering sage likely contributed to greater clustering of adults on this species.

Results presented in Table 6.4 indicate the plant organ type on which *A. solani* preferred to feed within each plant species, regardless of stratum. Leaves were favored over meristems in most cases when plants were vegetative or budding. Exceptions to this were marigolds, flowering sage, and dwarf snapdragons. For the first two of these, most aphids were instead found on growing tips of small, under-developed lateral meristems (< 2cm) terminating in either the top (marigolds) or middle stratum (flowering sage). For dwarf snapdragons, aphids were found in almost equal numbers on buds as well as leaves. On buds, aphids were found feeding almost exclusively on the calyx and sepals, versus any petal tissue showing on buds that were starting to open. Flower buds were quite hirsute, although this did not seem to inhibit aphids from feeding on them. Pepperswere the only plants where aphids were sometimes observed feeding on the top (adaxial) side of leaves; generally this was observed more with adults and later instars. In no cases were aphids observed feeding on the fruits of pepper plants.

When plants were in the flowering stage, aphids were found predominately on open flowers for pansies, scarlet sage, and marigolds. More aphids were found on buds

Table 6.4 Influence of plant species and growth stage on feeding of *Aulacorthum solani* on different plant organs

Plant species and stratum	Average aphids/organ/plant of indicated growth stage ± standard error [a] (percent of total population)	
Pansy	Vegetative	Flowering
Flowers	–	48.2 ± 8.1 (52) A
Flower Buds	–	19.8 ± 4.6 (22) AB
Meristems	9.8 ± 3.2 (13) a	12.7 ± 3.1 (13) B
Leaves	65.8 ± 31.5 (87) b	12.5 ± 3.0 (13) B
Flowering Sage	Vegetative	Flowering
Flowers	–	8.5 ± 4.3 (9) AB
Flower Buds	–	6.5 ± 3.8 (7) A
Meristems	46.8 ± 7.0 (51) a	55.0 ± 14.7 (60) B
Leaves	44.5 ± 12.5 (49) a	21.8 ± 8.6 (24) AB
Scarlett Sage	Budding	Flowering
Flowers	–	171.3 ± 40.7 (87) A
Flower Buds	5.0 ± 2.4 (2) a	–
Meristems	22.2 ± 6.2 (12) b	6.8 ± 3.2 (3) B
Leaves	162.7 ± 16.5 (86) c	19.7 ± 5.2 (10) C
Zinnia	Budding	Flowering
Flowers	–	7.7 ± 2.7 (9) A
Flower Buds	–	1.3 ± 1.3 (1) B
Meristems	4.3 ± 3.4 (4) a	20.7 ± 6.1 (25) C
Leaves	106.0 ± 12.3 (96) b	52.5 ± 7.3 (65) C
Peppers	Vegetative	Flowering
Flowers	–	13.6 ± 5.8 (13) A
Flower Buds	–	7.0 ± 2.2 (6) A
Meristems	0.5 ± 0.5 (1) a	4.7 ± 1.2 (4) A
Leaves	63.2 ± 17.5 (99) b	98.3 ± 28.1 (77) B
Tall snapdragon	Vegetative	Flowering
Flowers	–	1.2 ± 1.2 (1) A
Flower Buds	–	1.2 ± 1.0 (1) A
Meristems	19.5 ± 3.5 (31) a	18.3 ± 3.1 (17) B
Leaves	46.7 ± 10.0 (69) b	98.8 ± 17.8 (81) C

Table 6.4 (Continued)

Plant species and stratum	Average aphids/organ/plant of indicated growth stage ± standard error [a] (percent of total population)	
Petunia	Vegetative	Flowering
Flowers	–	0 ± 0 (0) A
Flower Buds	–	0 ± 0 (0) A
Meristems	0 ± 0 (0) a	0 ± 0 (0) A
Leaves	29.3 ± 6.4 (100) b	15.8 ± 7.8 (100) B
Marigold	Budding	Flowering
Flowers	–	36.0 ± 6.0 (49) A
Flower Buds	11.2 ± 3.6 (22) a	6.2 ± 2.9 (8) B
Meristems	32.4 ± 6.5 (64) a	16.7 ± 3.3 (23) A
Leaves	7.0 ± 0.3 (14) a	15.0 ± 3.7 (20) AB
Dwarf Snapdragon	Budding	Flowering
Flowers	–	19.0 ± 4.8 (23) AB
Flower Buds	49.0 ± 6.0 (38) a	36.6 ± 7.3 (44) A
Meristems	34.8 ± 8.3 (26) a	8.4 ± 3.1 (10) B
Leaves	47.0 ± 6.2 (36) a	18.4 ± 1.9 (22) AB
Poinsettia	Vegetative	Flowering
Meristems	39.8 ± 6.5 (37) a	–
Leaves	68.0 ± 15.1 (63) a	–

[a] Means within plant species within growth stages followed by same letter are not significantly different (Tukey-Kramer test, alpha = 0.05; Mixed model ANOVA conducted on log (n+1) transformed data; the random effect of plant was the repeated measure.

vs. open flowers for dwarf snapdragons. Although more aphids were found in the top stratum of tall snapdragons when they were flowering compared to the vegetative stage, aphids were still predominately found on leaves. It is unclear whether flowers were less attractive, or if apterous aphids could simply not travel this far up the tallest plant in our study in the given time frame. Interestingly, for marigolds, most of the aphids were present on senescing flowers (45% of the total population), feeding primarily on the wilting petals (64% of all *A. solani* found on senescing marigold flowers). In cases where aphids were found on the calyx or sepals, this generally occurred when there were no petals left.

Despite being described as a “stem feeding” aphid, stem feeding was only observed in a few select cases. The predominate case was on flowering sage, where a relatively high proportion of the total aphids across all plants (31%) were found on the stems of young lateral shoots (thus, these were incorporated into the number of aphids found on meristems in Table 6.4), with some occasionally found on the central stem. Aphids feeding on the buds and flowers of flowering sage were almost exclusively found on flower stalks just below the racemes as well on stems between florets (vs. on the florets themselves). Similarly, although no aphids were found feeding on stems in the leaf canopy for scarlet sage, when it came to the racemes, roughly equal numbers of aphids were found on the stem of the raceme between or below the florets as were on the petals of the large, individual florets (46 % and 54 % of aphids found on racemes, respectively). For pansies, most aphids feeding on flowers were found on the actual petals, but aphids could also be found on the calyx and sepals, and the upper portion of the stem of the flower (all data considered “flowers” for Table 6.4). For all other plants

species (marigolds, zinnias, peppers, petunias, snapdragons), no aphids were recorded on stems.

DISCUSSION

Our study on a variety of ornamental plants commonly grown in greenhouses supports our hypothesis that *A. solani* prefer to colonize lower leaves of plants, and confirms previous anecdotal evidence of this behavior. Our study also reveals that this aphid pest moves up the plant canopy when plants are reproductive, though they do not necessarily colonize flowers themselves. Despite anecdotal descriptions of *A. solani* as "stem feeding", this was only observed on species of *Salvia*, where aphids were commonly found on stems of racemes. Additionally, this is the first record of any aphid reproducing on poinsettia, a plant species usually more susceptible to other phloem sucking insects such as whiteflies.

Previous research supports that aphids are often attracted to lower leaves of plants due to elevated concentrations of free amino acids here, resulting from leaf senescence (Taylor 1962). The aphid *Myzus persicae* Sulzer was shown to be more abundant and increase more rapidly on lower leaves of potato (Jansson and Smilowitz 1986); *A. solani* densities were also reported to be higher on lower leaves of this field crop as well (Robert 1979). In ornamental crops, however, reports suggests that *M. persicae* is more attracted to growing points of plants (Vehrs et al 1992; Bethke 2010; Jandricic et al. 2013), possibly due to the shunting of plant resources to new growth in these often fast-growing, highly fertilized crops. Interestingly, *A. solani* still prefers lower leaves of ornamentals. One possible reason for this difference may be the stylet

size between these two aphids: Gibson (1972) showed that *A. solani* has considerably longer mouthparts, and thus was able to feed on primary veins of mature leaves, while *M. persicae* was more commonly found on the leaf lamina (Lowe 1967; Gibson 1972). Thus, *A. solani* may be able to more effectively use the niche of lower leaves than other, smaller, aphid species, avoiding resource competition. Alternatively, *A. solani* may have evolved to feed on older, lower leaves as a response to plant defenses, both physical and chemical. New growth of plants can often contain a higher trichome density (*e.g.* Lucas and Brodeur 1999). Our results on petunia (as well as the fact that few aphids were recovered from tomato) suggest that *A. solani* does not succeed well on on leaves with heavy trichomes numbers. This may explain why *A. solani* are commonly found on bottom leaves of potato plants (Robert 1979), where trichome densities would be lower. Plant chemical defenses, including secondary metabolites, also tend to be present at higher concentrations in new, productive tissue than older tissue of terrestrial vascular plants (see McKey 1979; Raupp and Denno 1983 and others). Additionally, this study confirmed that *A. solani* chooses to colonize higher up in the plant canopy when the plant is in the reproductive stage. This may be because a greater proportion of food in the plant is being allocated to the formation of plant reproductive organs (Wyatt 1969; Guldemond et al. 1998), which may provide higher quality resources than senescing leaves at this point in the crop cycle. More in-depth experiments, such as life table studies of *A. solani* feeding on different plant strata, are needed to further study the effects of nutritional quality and plant defences on *A. solani* feeding site selection. Additionally, analyses of honeydew composition between different locations could be done to assess efficiency of resource use across strata.

On flowering scarlet sage, pansy and marigold, *A. solani* were found to noticeably colonize petal tissue. That this occurred over a range of distantly related plant species suggests this could be a fairly common occurrence. From a pest management standpoint, this could be cause for concern, as systemic insecticides commonly used for aphids do not translocate into petal tissue (G. Murphy, personal communication). Though aphids at this location could be easily covered by contact insecticides, flowers are very susceptible to pesticide phytotoxicity. Conversely, for plants in the vegetative stage, colonies would be hard to affect by direct contact sprays because of their distribution on lower leaves. Thus, if chemical control is to be used, systemic insecticides would be appropriate for plants in the vegetative stage, while a combination of systemic and direct contact sprays may be needed to fully eradicate aphids feeding on reproductive plants. However, extreme care by growers will need to be taken to avoid phytotoxic effects of chemical sprays on very sensitive flowers.

Given the ability of aphid populations to become resistant to numerous chemical classes of pesticides (Devonshire 1989), limiting pesticide applications is prudent with these pests, and biological control options for *A. solani* should be seriously considered. However, their within-plant canopy distributions also have impacts on biological control programs, including those incorporating the commercially available "generalist" aphid predator *Aphidoletes aphidimyza*. Given that *A. aphidimyza* considers aphid colonies on growing points of plants to be of higher quality as oviposition sites (Jandricic et al. 2013), aphid species that generally colonize this location (*e.g. Myzus persicae*) are disproportionally attacked, and subsequently receive more consistent control than *A. solani* (which occurs lower in the canopy) when both pest species are

present in the greenhouse (Jandricic et al. unpublished data). Meristematic tissue is also preferred over flowers as oviposition sites by this predator (Jandricic et al.; unpublished data), possibly due to the relatively transient existence of flowers. As *A. solani* were shown to heavily colonize mersitem tissue of flowering sage (though numbers here were not statistically different than for lower leaves), this suggests good control of *A. solani* using *A. aphidimyza* may be possible on this plant species. However, high colonization of new growth only occurred for one out of the ten plant species/varieties tested here, suggesting that *A. solani* may generally be less susceptible to control by *A. aphidimya* across a wide variety of crops than other greenhouse aphid pest species would be if these tended to feed on new growth. In light of this, it's possible that feeding site preferences for lower, mature leaves by *A. solani* may have evolved in direct response to predator avoidance. Other natural enemies besides *A. aphidimyza* might also not search effectively on leaves close to the soil surface or be less likely to deposit their offspring here; more research needs to be done with *A. solani* and other predators/parasitoids to corroborate this.

Pest management researchers have also noticed that, despite good parasitism rates in lab bioassays, effective control of *A. solani* is generally hard to achieve using the parasitic wasps (*e.g. Aphidius ervi*) (R. Buitenhuis, personal communication) Furthermore, *A. solani* engages in defensive dropping behavior upon parasitoid attack, with aphids dispersing to new plants (resulting in more widespread damage) after an attempted attack is made (Henry et al. 2010). Perhaps this behavior and the preference for lower leaves are coupled to result in a shorter fall increase survival rates. Studies comparing survival from falls from flower stalks vs. lower leaves would be needed to

confirm this, and biocontrol studies should be conducted on a plant species such as flowering sage to see how control rates would differ from a plant species where *A. solani* are more commonly found on lower leaves.

As discussed here, the preference of *A. solani* for bottom leaves of vegetative plants may have a evolved as in response to differing plant nutrition or plant defenses within strata, a method of avoiding resource competition or predation, or (as yet unmentioned) possibly even a mechanism for finding cooler temperatures at which this aphid develops better (Jandricic et al. 2010). Regardless of the biological reasons behind these distribution trends, the information provided in this study can serve as a resource for scouting for this important pest species. Particular emphasis should placed on scouting lower leaves of vegetative plants and top leaves/flowers of flowering plants. Furthermore, our results suggest that the outcomes observed by Jandricic et al. (unpublished data), indicating reduced control of *A. solani* compared to *M. persicae* by *A. aphidimyza* on pansies as a result of differing within-plant distributions, is potentially translatable for *A. solani* across a variety of greenhouse ornamental crops. Separate tests with *M. persicae* would need to be done to fully confirm its within-plant distribution across a range of ornamental crops. Moreover, the demonstrated preference of *A. solani* for hard-to-reach/lower “quality” lower leaves as their primary feeding site provides growers and biocontrol practitioners a reasonable hypothesis as for why some current control programs might be failing. Thus, these results detail important aspects of the biology and behavior of *A. solani* and provide needed research toward control tactics for this difficult-to-control pest.

REFERENCES

Bethke, J.A. 2010. Insects and mites. UC IPM Pest Management Guidelines: Floriculture and Ornamental Nurseries: UC ANR Publication 3392.

Devonshire, A.L. 1989. Resistance of aphids to insecticides. In A.K. Minks and P. Harrewijn (eds), *Aphids, Their Biology, Natural Enemies and Control*, Volume C, pp. 127-137. Elsevier Science Publishers BV, Amsterdam, The Netherlands.

Gibson, R. W. 1972. The distribution of aphids on potato leaves in relation to vein size. Entomol. Exp. Appl. 15: 213-233.

Hall, R. A., and H. D. Burgess. 1979. Control of aphids in glasshouses with the fungus, *Verticillium lecanii*. Ann. Appl. Biol. 93: 235-246.

Henry, L. M., J.A. Bannerman, D. R. Gillespie, and B.D. Roitberg. 2010. Predator identity and the nature and strength of food web interactions. J. Anim. Ecol. 79: 1164-1171.

Jandricic, S.E., S. P. Wraight, K.C. Bennett and J. P. Sanderson. 2010. Developmental times and life table statistics of the *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models. Environ. Entomol. 39(5): 1631-1642.

Jandricic, S.E., S. P. Wraight, K.C. Bennett and J. P. Sanderson. 2011. Correction for the article "Developmental times and life table statistics of the *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models". Environ. Entomol. 40(3): iv-vi.

Jandricic S.E., S.P. Wraight, D.R. Gillespie and J.P. Sanderson. 2013. Oviposition behavior of the biological control agent *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) in environments with multiple pest aphid species (Hemiptera: Aphididae). Biol. Contr. 65: 234-245.

Kim, D-H., G-H. Lee, J-W. Park, and C-Y. Hwang. 1991. Occurrence aspects and ecological characteristics of foxglove aphid *Aulacorthum solani* Kaltenbach Homoptera Aphididae in soybean. Res. Rept. RDA (Crop. Prot.) 33 : 28-32 (in Korean).

Lee, S.G., H.H. Kim, T.H. Kim, G-J. Park, K.H. Kim, and J.S Kim. 2008. Development model of the foxglove aphid, *Aulacorthum solani* (Kaltenbach) on lettuce. Kor. J. Appl. Entomol. 47: 359-364.

Littell, R.C, G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. (2006). SAS System for Mixed Models (second edition), Cary, NC: SAS Institute Inc.

Lowe, H. J. B. 1967. Interspecific differences in the biology of aphids (Homoptera: Aphididae) on leaves of *Vicia faba* II: growth and excretion. Entomol. Exp. Appl. 10: 413-420.

Lucas, E. and J. Brodeur. 1999. Oviposition site selection by the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). Environ. Entomol. 28(4): 622-627.

McKey, D. 1979. The distribution of secondary compounds within plants: In: Rosenthal, G.A. and Jansen D.H. (eds) Herbivores: their interactions with secondary plant metabolites. Academic Press, NY, pp. 55-133.

Raup, M.J. and R.F. Denno. 1983. Leaf age as a predictor of herbivore distribution and abundance. In: Denno, R.F. and McClure, M.S. (eds.) Variable plants and herbivores in natural and managed systems. Academic Press, NY, pp. 91-124.

Robert, Y. 1979. Recherches écologiques sur les pucerons *Aulacorthrum solani* Kltb., *Macrosiphum euphorbiae* Thomas et *Myzus persicae* Sulz. dans l'Ouest de la France. II. Evolution spatio-temporelle de leurs populations sur la pomme de terre. Ann. Zoo. Ecol. Anim. 11: 67-83 *(in French).*

Sanchez, J.A., F. Canovas, and A. Lacasa. 2007. Thresholds and management strategies for *Aulacorthum solani* (Hemiptera: Aphididae) in greenhouse pepper. J. Econ. Entomol. 100: 123-130.

SAS Institute Inc., 2011. SAS Software Release 9.3. Cary, N.C.: SAS Institute Inc.

van Driesche, R., S. Lyon, J.P. Sanderson, K.C. Bennett, E. J. Stanek III, and R. Zhang. 2008. Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker plants for control of aphids (Hemiptera: Aphididae) in greenhouse spring floral crops. Fla. Entomol. 91(4): 583-591.

Vehrs, S.L.C., G.P. Walker and M.P. Parella. 1992. Comparison of population growth rate and within-plant distribution between *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae) reared on potted chrysanthemums. J. Econ. Entomol. 85(3): 799-807.

Verider, K. 1999. Improvement of the biological control of the aphid *Aulacorthum solani* in glasshouses. Master’s thesis, Horticultural Science Program, Swedish University of Agricultural Sciences, Uppsala Sweden: 1999: 6.

Warton, D.I., and F.K.C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92(1): 3-10.

Wave, H. E., W.A. Shands and G.W. Simpson. 1965. Biology of the foxglove aphid in the Northeastern United States. US. Dep. Agr. Tech. Bull. 1338: 1-40.

CHAPTER 7

GENERAL SUMMARY & DISCUSSION

The overall objectives of these dissertation studies were to investigate a) how the biology and ecology of *Aulacorthum solani* (foxglove aphid) could impact control programs for this emerging pest, and b) the efficacy of various aphid biological control agents (the predator *Aphidoletes aphidimyza;* entomopathogenic fungi in the genera *Beauveria*, *Metarhizium* and *Isaria*) for foxglove aphid control when present in mixed aphid species environments, which is common in floriculture crops.

The studies herein of the developmental times and life table statistics of *A. solani* were the first comprehensive study conducted on a North American population, and provides information on responses in an ornamental crop. Our results support what has been reported anecdotally by floriculture growers in Canada and the Northeastern U.S.: that *A. solani* is a relatively "cool weather pest", having the highest intrinsic rates of increase (r_m) between 20-25°C (0.24 and 0.25, respectively), and a negative r_m at constant temperatures of 30°C. Our survey of *A. solani* within-plant distributions on 10 different species/varieties of ornamental crops supported anecdotal statements in the literature that *A. solani* often feeds on the lower leaves of plants, but can move up the plant to feed directly on flower petals once in flower. These studies can inform grower decisions regarding scouting practices and control of *A. solani* by increasing their knowledge of the temperature range/plant canopy strata in which this aphid is likely to be a problem.

Where a pest is located on a plant may have repercussions for control by a natural enemy. This was demonstrated in our studies of oviposition decisions by the aphidophagous predator *Aphidoletes aphidimyza* in the presence of the two main aphid pests in Northeastern U.S. greenhouses: *A. solani* and *Myzus persicae* (the green peach aphid). In tests where aphid feeding location was controlled (*i.e.* aphid nymphs were confined to middle leaves only, or colonies of aphids of all ages were confined to bottom leaves in a separate trial), only aphid density was a significant factor in the number of *A. aphidimyza* eggs received by a prey patch. In all cases, the aphid species within the patch was not significant. When *A. aphidimyza* was given a choice of 2 patch locations (either top vs. bottom leaves, or plant meristems vs. bottom leaves), *A. aphidimyza* deposited significantly more eggs on top leaves/meristems, with many prey patches on bottom leaves being ignored/rejected as oviposition sites. As in the first set of trials, the aphid species within the patch did not contribute to the outcome. When aphids were allowed to naturally distribute on plants (aphid location was uncontrolled), a significant species × location interaction was observed, with *M. persicae* aggregations on apical meristems receiving a disproportionally higher number of *A. aphidimiza* eggs compared to other colony locations. *A. solani* patches were mostly distributed among bottom leaves of plants, and these were ignored by the predator, even when they were high density colonies. *Aulacorthum solani* colonies present on meristems received the most *A. aphidimyza* eggs within this aphid species, supporting the significance of location as a main effect, although the oviposition rates were still less than on *M. persicae* for similar sized colonies at the same location. Although this suggests that there may be at least a weak species preference for *M. persicae* by *A. aphidimyza*, our

overall results suggest that within-plant location of aphid colonies supersedes aphid species for *A. aphidimyza* oviposition choices, with this predator perhaps perceiving aphid colonies present on younger plant tissue to be higher quality patches than those present on older tissue.

To assess the impact of patch quality on biological control outcomes using *A. aphdimyza* as the sole control agent for a multi-species aphid outbreak, 9 to 11-day trials were conducted in which *A. aphidimyza* were allowed to oviposit on aphid infested plants, with aphid populations naturally distributed within the plant, and larvae were allowed to eclose and prey on aphids until pupation. These were compared to plants for which no aphid management tactic was used. Trials were done on plants in several growth stages (vegetative, budding, or flowering) to determine if within-plant distributions of the two aphid species changed with crop growth stage, and if changes in aphid distribution affected the degree of control by *A. aphidimyza*. At the beginning of each experiment, *M. persicae* was found at the highest densities on plant meristems, although the highest densities shifted to flowers over time when plants were in the reproductive phase. The distribution of *A. solani*, however, differed with plant stage: most aphids were found on bottom leaves during the vegetative stage, with a greater percentage found on meristems/flower buds during the budding stage, to the highest proportion being on flowers in the reproductive stage. Fairly consistent and effective control of *M. persicae* (78-95%) with a single release of *A. aphidimyza* was observed, regardless of plant stage or time of year the trial was conducted. Given our previous experiments on oviposition preferences of *A. aphidimya*, this likely is a direct result of *M. persicae* occurring on plant meristems coincident with the highest oviposition of this

predator during these experiments. In contrast, control of *A. solani* by *A. aphidimyza* in the presence of *M. persicae* was variable, with 12-80% control achieved. The highest control rates occurred when plants were in the budding stage, which may be a result of a greater proportion of *A. solani* occurring on the meristem or on flower buds (close to the meristem). However, time of year and quality of the commercially-supplied natural enemy cannot entirely be ruled out as confounding factors in this variable control. Specifically, plant, and thus aphid quality, can be lower in the winter months if adequate supplemental light in commercial insect rearing facilities is not used, which in turn can result in smaller and possibly less fecund *A. aphidimyza*.

Given the disproportionate oviposition by *A. aphidimyza* on aphids colonizing new plant tissue compared to other plant locations, as well as its possible preference for *M. persicae* over *A. solani*, we suspected that apparent competition might be acting within this system. Specifically, the low *A. aphidimyza* attack rates on *A. solani*-infested plants may likely be a result of the combination of a preferred oviposition location and a preferred prey species offered by nearby *M. persicae*-infested plants. To test this, we presented populations of *A. aphdimyza* with 2 scenarios: greenhouses containing 24 aphid infested plants, half of which were *A. solani* and half of which were *M. persicae*, or greenhouses containing 24 *A. solani*-infested plants only. Both treatment types were compared to the same number of aphid-infested plants receiving no aphid management (controls). In the compartments where *A. solani* was presented simultaneously with *M. persicae*, results revealed similar rates of biological control of *M. persicae* as before (91%), but only 12% control of *A. solani*. In the compartments where *A. solani* was presented alone, however, control was 40%. An analysis of *A.*

aphidimyza egg/larval numbers and canopy locations suggests that this discrepancy in control is primarily attributable predator attack-rates at the whole-plant level. Specifically, in the compartments with *A. solani* alone, all aphid-infested plants showed some sign of attack (presence of larvae or consumed aphid carcasses) by the end of the experiment. However, 43% of *A. solani* plants in the compartments with the mixed-species aphid population showed no evidence of *A. aphidimyza* attack. This suggests that the presence of *M. persicae*-infested plants may have distracted *A. aphidimyza* from discovering all *A. solani*-infested plants. Confirming our hypothesis of apparent competition in this system, this experiment also demonstrates that, unlike other aphidophagous Diptera such as Syrphid flies, there appears to be no egg-deterrence by conspecific females which would steer *A. aphidimyza* females towards unfound *A. solani* plants once initial oviposition had taken place in colonies of *M. persicae*.

Along with arthropod natural enemies, entomopathogenic fungi are also available for aphid biological control. Although there are several commercially available products for "sucking insects" that include aphids on their product labels, these products are generally not considered sufficiently efficacious against aphids, possibly due to the host specificity of the isolate. Thus, one goal of this dissertation research was to determine if a more pathogenic fungal isolate against an aphid pest could be found, and if this isolate would be effective against multiple aphid species. Assuming that greater pathogencity should occur with isolates originally recovered from taxonomically-related species, the majority of the novel isolates selected from the USDA-ARS collection were originally from hosts in the family Aphididae or other insects from the suborder Sternorrhyncha (although isolates from other hosts were also

included). Primary screening tests using a Burgerjon spray tower and applied against 1st instar nymphs of *M. persicae* and the melon aphid, *Aphis gossypii* (another important aphid in the greenhouse aphid complex) narrowed results to 2 isolates which appeared to cause greater mortality than commercial isolates. Lethal concentration studies (LC_{50}) were then conducted against all 3 main aphid pests in greenhouses: *A. solani*, *M. persicae*, and *A. gossypii*, where activity of the novel isolates was compared with commercial strains. The overall results of these experiments indicated that, unfortunately, there is little difference in pathogenicity between novel and commercial strains of these fungi. Although *B. bassiana* 5493 (originally isolated from an aphid host) had the most consistent results against all 3 aphid species, spore counts of > 900 mm^2 at minimum were needed to result in 50% mortality of a nymphal aphid population. Compared to effective doses against other sucking insect species (*e.g.* LC_{50}s of 50-100 conidia/mm^2 for thrips and whiteflies), these doses are extremely high; thus entomopathogenic fungi for use against aphid pests of ornamentals seems untenable.

Recommendations to Growers Based on Results

Results from the study on development of *A. solani* at 6 different temperatures indicate that when temperatures approach constant 30°C, population growth of this pest is unlikely. Thus, in the summer months, growers could more confidently switch their aphid management program to focus on *M. persicae* and *A. gossypii*, which do better at warmer temperatures. However, during the Spring and Fall (at temperatures between 10-25°C), growers should be cognizant that *A. solani* may comprise part of their pest

aphid complex; thus, management strategies that are only successful for smaller-bodied aphids (such as the use of banker plants containing the parasitoid *Aphidius colemani*) will not control this pest. Growers should learn to identify the main aphid pests that arrive in their greenhouse in order to select the most appropriate control measures. In terms of scouting for *A. solani*, growers should also be certain to check the lowest leaves in the crop canopy, as high density colonies can accumulate here.

Unfortunately, the use of entomopathogenic fungi in the genera *Beauveria*, *Metarhizium* or *Isaria* (commercially available products or otherwise) do not currently seem to be a realistic control option for aphids in greenhouse crops, particularly ornamentals which are sold for their aesthetic value. At best, only 30% control of aphids is likely to be seen in applications in commercial greenhouse settings (S. Wraight, personal communication), which does not justify the cost of these products.

Given the consistent control of *M. periscae*–infested plants in our studies using a single release of *A. aphidimyza*, regardless of plant stage or the presence of alternative prey, the use of this natural enemy for curative control of *M. persicae* outbreaks may be recommended, as long as appropriate release rates are used and aphid infestations are not severe. In these studies, a release rate of 1 predator: 10 aphids was used. Note that the use of release rates based on pest density rather than square footage of growing area is recommended. This release rate was found to be generally successful, almost completely eliminating *M. persicae* in one experiment. However, additional releases of the predator (or perhaps a parasitoid) would likely be required to to ensure complete control. Given that remaining aphid populations are found on bottom leaves due to high levels of *A. aphidimyza* predation on aphid-infested meristems, there may be a

question if *A. aphidimyza* will be as effective at finding these remaining colonies. However, our *A. aphidimyza* oviposition experiment using aphid colonies on bottom-leaves only suggests this predator may utilize this location successfully to some degree if no other choice is present.

Unfortunately, recommendations for the use of *A. aphidimyza* for controlling *A. solani* are harder to outline. If *M. persicae* is present simultaneously at equal or higher numbers (which will likely always be the case, given the higher intrinsic rate of increase of *M. persicae* vs. *A. solani*), then our results strongly suggest that control of *A. solani* could suffer, even at high rates of midge release. Growers should be aware of this, and *A. solani*-infested plants should always be flagged and monitored carefully to confirm they are receiving *A. aphidimyza* eggs/larvae (large groupings of eggs can be seen with a hand lens, though single eggs can be hard to detect; larvae are more easily visible). Because ≥70% control of *A. solani* occurred in the presence of *M. persicae* in 2 out of 4 greenhouse experiments, both conducted in the late spring, this control measure has the potential to be efficacious, but depends strongly on the within-plant location of the *A. solani* infestation and, potentially, the strength/quality of the commercial batch of *A. aphidimyza* and its relation to time of year. Given that all the experiments in this dissertation were based on a single release, it's also possible that several releases over time may offer better control of *A. solani*. However, the same issues regarding repeated releases of *A. aphidimyza* apply here as discussed above with *M. persicae* (especially considering that the number of unfound, bottom leaf colonies remaining at the end of experiments was higher for *A. solani*). Longer term testing, preferably in larger greenhouses more closely resembling commercial operations, should be done with *A.*

solani infestations alone to determine the true potential of *A. aphidimyza* for controlling this pest before full recommendations can be made. The efficacy of prophylactic releases of *A. aphidimyza* for control of *A. solani* and other aphid pests should also be validated experimentally, given that our results generally support previous research suggesting this natural enemy lays few eggs in prey patches with < 10 aphids, potentially resulting in very-low initial aphid infestations being ignored. Lastly, the cost of potentially releasing greater numbers of *A. aphidimyza* than currently recommended (or more frequent releases) needs to be balanced with monetary benefits of *A. solani* control (*i.e.* marginal values) before growers will accept any recommendations.

Future Research Questions

This project primarily elucidated reasons behind failures of various biocontrol agents against mixed-aphid populations; determining effective release rates/combinations of natural enemies for aphid control was outside the scope of this project. Thus, future research should be done on the use of multiple types of biological control agents for control of *A. solani*: both in isolation and as part of a multi-species outbreak (as these two scenarios can have very different outcomes, as demonstrated by the research in this project). The use of parasitoids combined with *A. aphidimyza* should be explored, though the possible disturbance and defensive dropping of *A. solani* when attacked by parasitoids is a concern, as is the cost of *Aphidius ervi* (the main parasitoid for larger bodied-aphid species). However, *Aphelinus abdominalis*, though slow to build up populations in the greenhouse, has been shown to result in less disturbance of *A. solani*

colonies (D. Gillespie, personal communication), and is less expensive than *A. ervi*, and thus may be a good potential candidate for mixed-enemy releases. Additionally, though the cheaper *Aphidius colemani* is currently used for smaller-bodied aphid species, this is thought be partially a consequence of its commercial production on smaller aphids (G. Messelink, personal communication). Thus, the breeding of *A. colemani,* or even *A. aphidimyza*, using *A. solani* as the natal prey/host should also be explored to potentially enhance natural enemy specificity to this challenging pest species. The use of insect growth regulators tank-mixed with entomopathogenic fungi to increase their efficacy should also be investigated as a pesticide option with less risk towards arthropod natural enemies. Additionally, the formulation of commercial strains of *Beauveria* and *Metarhizium* as blastospores should be investigated, as these propagules germinate far more quickly than conidia, and may thus be more pathogenic against nymphal aphids.