THE EFFECTS OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' ON COCCINELLID PREDATION OF THE POTATO PSYLLID AND INTRAGUILD INTERACTIONS BETWEEN PSYLLIDS AND APHIDS ON TOMATO

A Thesis

by

SASHA KAY

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Chair of Committee,	Cecilia Tamborindeguy
Committee Members,	Raul F. Medina
	Elizabeth Pierson
	Carlos E. Bográn
Head of Department,	David Ragsdale

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ABSTRACT

‘*Candidatus* Liberibacter solanacearum’ (Lso), a pathogen of many solanaceous crops, and its insect vector, *Bactericera cockerelli*, are increasing their geographic range in North America, Central America, and abroad. Understanding the mechanisms by which their range is expanding will be key to both pest and disease management. This study aimed to determine if Lso could protect its insect vector from predation by *Hippodamia convergens* by examining beetle preference for infected tomato and/or insect volatile combinations in a 2-choice olfactometer, and the consumption of uninfected and Lso-infected psyllids in a no-choice feeding bioassay. Additionally, a previously unreported aphid facilitation interaction between the psyllid and *Myzus persicae* on tomato was examined to determine if either plant infection by Lso or the presence of the psyllid could promote aphid facilitation. Beetles significantly preferred the odors of uninfected tomato to those of infected tomato, but their preference was insignificant when both plants were infested with psyllids. Beetle consumption of psyllids did not vary significantly according to their infection status. Lso may confer slight indirect protection to its insect vector through modification of their shared host plant. The presence of the psyllid, rather than plant infection by Lso, was determined to be responsible for aphid facilitation on tomato. Aphid populations persisted for 30 days or longer on plants with psyllids. Aphid populations increased on psyllid-infested plants upon which the majority of psyllids were young nymphs, and that had previously been infested with aphids.

DEDICATION

ΤΗΙ ΚΑΛΛΙΣΤΗΙ

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CHAPTER I
INTRODUCTION

'*Candidatus* Liberibacter solanacearum' (Lso) is a phloem-limited, α-proteobacterium. The Lso bacterium cannot be cultured *in vitro*, and is identifiable only by DNA sequencing. It has four currently known haplotypes (Nelson et al. 2011; Nelson et al. 2013), two of which, A and B, infect solanaceous plants (Liefting et al. 2009a; Liefting et al. 2009b), causing tomato vein-greening (Brown et al. 2010), and zebra chip disease in potatoes (Secor et al. 2009). Zebra chip disease results in reduced vigor (Munyaneza et al. 2007), reduced yield (Munyaneza et al. 2008; Butler et al. 2011a; Buchman et al. 2012), and eventual death of the potato plant (Henne et al. 2012). The bacterium also causes glucose and sucrose to build up in potato tubers (Buchman et al. 2012). These sugars are believed to cause brown streaking (loosely resembling zebra stripes) when tubers are fried that gives the disease its name (Buchman et al. 2012). While there are no known adverse health effects from human consumption of infected potatoes, their bitter taste and unsightly appearance make them unsuitable for the production of potato chips and, more recently, French fries (Crosslin et al. 2012b). Symptomatic crops are rejected by producers in both the U.S. and abroad (Crosslin et al. 2010). This has led to millions of dollars in potato crop losses (Munyaneza et al. 2008).

In North America, Central America, and New Zealand, Lso is transmitted by its insect vector, the potato or tomato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae) (Munyaneza et al. 2007). This insect is native to North America. It is highly mobile, known to undertake migrations northward during the summer in southern latitudes (Liu et al. 2006). It is a highly efficient vector – one infected adult may transmit the pathogen to a potato plant in as little as six hours (Buchman et al. 2011b).

Both Lso and *B. cockerelli* are increasingly being found in areas where they had rarely been observed (Liu et al. 2006), or were never previously reported (Liefting et al. 2009a; Munyaneza et al. 2009a; Munyaneza et al. 2009b; Brown et al. 2010; French-Monar et al. 2010; Rehman et al. 2010; Crosslin et al. 2012a; Crosslin et al. 2012b;

Murphy et al. 2013; Nelson et al. 2013; Aguilar et al. 2013a; Bextine et al. 2013a; Aguilar et al. 2013b; Bextine et al. 2013b). Currently, Lso haplotypes A and B are spreading across North America (Wen et al. 2009; Brown et al. 2010; French-Monar et al. 2010; Crosslin et al. 2012a; Crosslin et al. 2012b; Murphy et al. 2013); Central America (Gudmestad and Secor 2007; Munyaneza et al. 2009a; Munyaneza et al. 2009b; Rehman et al. 2010; Ling et al. 2011; Aguilar et al. 2013a; Bextine et al. 2013a; Munyaneza et al. 2013a; Aguilar et al. 2013b; Bextine et al. 2013b; Munyaneza et al. 2013b; Munyaneza et al. 2013c); and New Zealand (Liefting et al. 2009a). (Lso haplotypes C and D have also recently been found in carrots and celery in Spain, Finland, Norway, Sweden, and the Canary Islands (Alfaro-Fernandez et al. 2012a; Alfaro-Fernandez et al. 2012b; Nelson et al. 2013), where two novel vectors, *Bactericera trigonica* and *Trioza apicalis* (Hemiptera: Triozidae), have been implicated in their spread (Munyaneza 2010; Nissinen et al. 2012; Alfaro-Fernandez et al. 2012b; Nelson et al. 2013).) The spread of Lso haplotypes A and B in different parts of the world is partly due to human-mediated transport of infected plants and vectors – as in New Zealand – but climate change or a mutually beneficial association between pathogen and vector may also be contributing factors. As global temperatures increase, southern latitudes in North America may become inhospitable for *B. cockerelli* and the pathogen, while conditions further north become more favorable (Munyaneza et al. 2012a; Murphy et al. 2013). In some instances, plant pathogens have also been known to facilitate insect adaptations to new environments (Ebbert and Nault 1994), or reduce the fitness of natural enemies (Christiansen-Weniger et al. 1998; Hodge and Powell 2008; Calvo and Fereres 2011).

Currently, insecticides provide the only substantial means of controlling *B. cockerelli* (Lacey et al. 2009; Lacey et al. 2011; Butler et al. 2011a; Munyaneza 2012; Butler et al. 2012b). However, concerns over the development of insecticide resistance and the negative impacts of broad-spectrum insecticide use on non-target, beneficial organisms, which may control secondary pest outbreaks, have led to investigations of the utility of natural enemies (Lacey et al. 2009; Lacey et al. 2011; Butler and Trumble 2012a),

coating plants with a particle film (Peng et al. 2011), and the development of resistant cultivars (Munyaneza et al. 2011a; Butler et al. 2011b; Anderson et al. 2013; Wuriyanghan and Falk 2013) for controlling both the vector and the pathogen. Yet the effects of harboring Lso by *B. cockerelli* on the psyllid's natural enemies have not been studied. Understanding the effects of the interaction between psyllids and Lso on the psyllid's natural enemies is important for integrated pest management. If the association between the psyllid and the pathogen decreases psyllid susceptibility to natural enemies, thereby making biological control less efficacious, pest management strategies must take this into account when designing a program for psyllid control.

Psyllids, like other hemipteran insects, are known to harbor both primary (obligate) and secondary (facultative) microbial symbionts (Nachappa et al. 2011; Hail et al. 2012). Primary symbionts have undergone long-term symbioses with their hosts, resulting in mutual co-evolution, and can no longer survive outside of their hosts. In the case of hemipterans, primary symbionts (called endosymbionts) are housed in specialized cells of the insects called bacteriocytes, and they are passed from mother to offspring only via transovarial transmission. Lso is considered a secondary symbiont of *B. cockerelli* since it is not always present in the insect (although Lso may not be able to disseminate without the vector), and it is not yet known where the bacterium is localized inside the insect when it is present. While Lso can be transovarially transmitted, psyllids may also acquire it by feeding on an infected host plant (Hansen et al. 2008). In the Tamborindeguy lab, uninfected and Lso-infected *B. cockerelli* colonies have been maintained for several years. The symbionts of psyllids in these colonies are identified through DNA sequencing. These colonies are valuable research tools for understanding the effects of different symbiont associations on psyllid performance, plant infection, and interspecific interactions.

Insect symbionts are known to alter, and often enhance, the fitness of their hosts in numerous ways. Some benefits conferred by symbionts to their hemipteran hosts include: supplemental nutrition (Hansen and Moran 2011), parasite resistance (Hansen et al. 2007; Oliver et al. 2009), pathogen resistance (Scarborough et al. 2005; Haine 2008),

pesticide resistance (Kikuchi et al. 2012), and the ability to tolerate adverse environmental conditions (Ebbert and Nault 1994; Burke et al. 2010) *Hamiltonia defensa*, a secondary symbiont of the pea aphid, has been shown to increase parasitoid resistance at the cost of fecundity (Gwynn et al. 2005). Although Lso-infected psyllids were found to be less fecund than uninfected psyllids on tomato (Nachappa et al. 2012), it remains to be determined if infected psyllids experience less predation or parasitism risk than uninfected psyllids. Plant infection by Lso can also alter plant volatile production (Davis et al. 2012), which could affect how plants are perceived by natural enemies that use volatile cues to locate their insect prey.

There has been little research on the impact of natural enemies on *B. cockerelli* populations (Al-Jabar 1999; Lacey et al. 2009; Lacey et al. 2011; Butler and Trumble 2012a), but several promising candidates for natural enemy research have emerged. One of these is the convergent lady beetle, *Hippodamia convergens*. This insect is native to and found throughout North America. Although it primarily feeds upon aphids, it also feeds on *B. cockerelli* (Hoffmann and Frodsham 1993), and is often found in association with *B. cockerelli* in field crops (Butler and Trumble 2012a). *Hippodamia convergens* was recently identified as a "key" natural enemy of *B. cockerelli* after laboratory feeding assays showed that it consumed significantly high numbers of all psyllid life stages (Butler and Trumble 2012a). This insect is also known to use olfactory cues to locate prey (Hamilton et al. 1999; Acar et al. 2001). All of these attributes make *H. convergens* a model predator for use in determining the effect of Lso on the natural enemies of *B. cockerelli*.

Although knowledge of psyllid interactions with natural enemies is scarce, virtually nothing is known concerning interactions between *B. cockerelli* and other phytophagous hemipterans. However, such interactions may contribute to the spread of both Lso and its vector throughout the world. Interactions between phytophagous hemipterans are known to be common in nature (Denno et al. 1995), and may occur between different species (interspecific), or among members of the same species (intraspecific). The outcomes of interspecific interactions are often asymmetric – one species disproportionately feels the

effects (Denno et al. 1995; Kaplan and Denno 2007). Facilitation is an outcome that benefits one participant while the other participant either benefits or is unaffected (Stachowicz 2001), unlike competition, in which both participants are disadvantaged, or commensalism, where neither participant incurs a benefit or detriment resulting from the interaction. Competitive interactions between phytophagous hemipterans can enhance invasion by non-native species (McClure 1981; Settle and Wilson 1990). Facilitative interactions between phytophagous hemipterans can result in increased fitness of one or both participants (Denno et al. 1995; Dugravot et al. 2007; Brunissen et al. 2009) and increased species diversity (Kaplan and Denno 2007). Since phytophagous hemipterans are also the pre-eminent vectors of disease-causing agents of plants, their interactions could have multi-layered consequences, including increased herbivory and spread of vector-borne diseases.

In the spring of 2012, large numbers of aphids (species unknown) were found on potatoes in large potato fields in Weslaco, TX, where psyllids are endemic (Levy, pers. comm.). Psyllids and whiteflies were co-occurring in potato that same year in Weslaco, also during the spring (Villanueva and Esparza-Diaz 2012). *Bactericera cockerelli* was found to co-occur on bittersweet nightshade, *Solanum dulcamara*, with the potato aphid, *Macrosiphum euphorbiae*, and foxglove or glasshouse potato aphid, *Aulacorthum solani*, in Idaho (Goolsby, pers. comm.). Although it is clear that interactions between psyllids and other vascular-feeding hemipterans are occurring in natural and agricultural systems, so far, the nature of these interactions (competition, facilitation, or commensalism) has not been characterized.

An interesting interaction has been observed between *Myzus persicae*, the green peach aphid, and *B. cockerelli* on tomato in the Tamborindeguy lab. Accidental infestations of the psyllid colonies by dispersing aphids have occurred occasionally when the aphid and psyllids colonies were maintained together on the same shelf. The *Myzus* clone in the Tamborindeguy lab cannot normally survive on tomato, yet large aphid populations lasting several generations were sometimes observed on tomato plants where psyllids were also present, in both uninfected and infected psyllid colonies. It

seems that the psyllid may facilitate the aphid's survival on this host. However, a review of the literature revealed no published studies of interactions between aphids and psyllids.

The objectives of this work were 1) to determine if Lso could protect its insect vector from predation by *Hippodamia convergens*, and 2) to investigate the interaction between green peach aphids, Lso, and psyllids to determine whether the presence of psyllids, plant infection with Lso, or both are necessary for green peach aphids to survive on tomato.

CHAPTER II

IMPACT OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' ON PREDATION OF THE POTATO PSYLLID BY CONVERGENT LADY BEETLE

Introduction

Plants produce volatile organic compounds, or VOCs, to communicate with each other and with organisms that aid in their reproduction, such as pollinators (Das et al. 2013). Herbivory can cause plants to produce specific VOCs, called herbivore-induced plant volatiles or HIPV (Heil and Karban 2010). Such volatiles may signal other herbivores to the presence of a suitable host, and natural enemies to the presence of prey (Dicke and Baldwin 2010; Heil and Karban 2010). Insect parasitoids have been shown to rely on volatile cues to locate their hosts, and there is evidence to suggest that insect predators may do the same (Hamilton et al. 1999; Sengonca and Kranz 2001; Kessler and Baldwin 2002; Moayeri et al. 2006; Gencer et al. 2009). For example, the coccinellid *Stethorus gilvifrons* is attracted to HIPV induced by two species of spider mite prey, *Tetranychus urticae* and *Pononychus ulmi,* and also to prey odors alone (Gencer et al. 2009). In a two-choice (y-tube) olfactometer bioassay, the beetle's attraction to odor sources varied in response to plant and mite species (Gencer et al. 2009). Another coccinellid, *Hippodamia convergens*, was attracted to odors of *Myzus persicae* as well as HIPV from aphid-infested radish leaves in two separate eight-arm airflow olfactometer studies (Hamilton et al. 1999; Acar et al. 2001). The predatory mirid, *Macrolophus caliginosus,* was also found to be attracted to the HIPV of *T. urticae*-infested pepper plants in a y-tube olfactometer study (Moayeri et al. 2006).

Vector-borne plant pathogens may also alter plant volatile production in ways that make infective plants attractive to insect vectors, and thus facilitate their spread (Davis et al. 2012). *Myzus persicae,* a competent vector of numerous plant viruses, is attracted to the odors of potato plants infected with potato leafroll virus (PLRV) (Eigenbrode et al. 2002). Newly emerged female *Cacopsylla picta* are attracted by the odor of apple trees

infected with '*Candidatus* Phytoplasma mali', which is transmitted by *C. picta* (Mayer et al. 2008). Similarly, *B. cockerelli*, the vector of Lso, are initially attracted to and settles upon potato plants previous fed upon by Lso-infected psyllids, but are later repelled, preferentially settling on plants fed upon by uninfected psyllids and potentially transmitting the pathogen to new hosts (Davis et al. 2012). It remains to be determined whether olfactory cues are responsible for this change in behavior by *B. cockerelli* (Davis et al. 2012). However, the volatile profile of a potato plant fed upon by infected psyllids was found to be distinctly different from that of a potato plant fed upon by uninfected psyllids: plants fed upon by infected psyllids emitted significantly more α-caryophyllene (Davis et al. 2012).

Both HIPV and plant pathogen-induced VOCs can have the effect of increased herbivory of the producing plants (Cardoza et al. 2003; Mayer et al. 2008; Dicke and Baldwin 2010).Yet little is known about the effect of plant pathogen-induced VOCs on the behavior of natural enemies. There have been two studies of the effects of fungal plant pathogen-induced VOCs on parasitoid foraging behavior (Cardoza et al. 2003; Rostas et al. 2006). The volatiles induced by white mold (*Sclerotium rolfsii*) on peanut plants were found to be attractive to *Cotesia marginiventris* (Cardoza et al. 2003), but there was no effect of volatiles induced by *Setosphaeria turcica*-infected maize on either *C. marginiventris* or *Microplitis rufiventris* (Rostas et al. 2006). Neither of these fungi is borne by a specific vector. Although several vector-borne plant pathogens (all luteoviruses) have been shown to reduce the fitness of one parasitoid, *Aphidius ervi*¸ developing inside three species of infected aphid hosts (Christiansen-Weniger et al. 1998; Hodge and Powell 2008; Calvo and Fereres 2011), it is not known whether virus-induced volatiles from infected insect prey or their host plants would influence the foraging behavior by any natural enemy. Additionally, no previous studies have addressed the effect of bacterial plant pathogen-induced VOCs on the behavior of any natural enemy.

Understanding the effects of plant pathogens on the behavior of natural enemies is important for biological control. If the presence of a plant pathogen reduces the efficacy

of natural enemies, the success of a biological control program is jeopardized. Lso may protect its insect vector indirectly if it changes the volatile profile of the host plant so that it no longer produces the volatiles associated with herbivory, thus masking the presence of *B. cockerelli*. Lso may also directly protect its vector if harboring the pathogen changes the palatability of the insect to predators.

The objectives of this study were first to elucidate the preference of *Hippodamia convergens* for Lso-induced plant volatiles, and second, to quantify the actual predation rates on uninfected vs. infected psyllids. The first objective was to determine whether plant infection would influence foraging behavior by the beetle. The second objective was to determine if actual psyllid consumption by the beetle varied if the psyllids were infected or not.

Materials and Methods

Olfactometer Bioassay

Tomato (cv. Moneymaker) seeds (Thompson & Morgan) were planted in 20x10" plastic flats (TRF-1020-OPEN, 710211C) each lined with thirty-two 2.15x2.18" inserts (TRI-804, 715320C) filled with professional growing media (Metro-Mix 300, Sun Gro Horticulture, SKU553001), and sprinkled with a slow-release fertilizer (Osmocote Plant Food, Scotts, 273260). No additional fertilizer was supplied. Plants were grown at room temperature on metal shelving (Style Selections, 0071034) underneath forty-eight inch fluorescent shop lights (Utilitech NXU-6000, 0245536) with plant and aquarium bulbs (GE F40T12). Plants were watered twice per week, using reverse osmosis water. After at least two true leaves were present, the plants were thinned to one seedling per insert. When plants were at least three weeks old, they were individually transplanted into 3.5" square pots (SVD-350 (3x6), 700026C), using the same media. All pots, inserts, and flats were manufactured by T.O. Plastics, Inc.

The tomato plants were divided into two groups (16 plants per group) in 14" x 24" rectangular mesh cages (BioQuip 1466B). Each plant was labeled with a unique number.

Each group was assigned to one of two treatments: uninfected and infected. One leaflet on each plant in the infected group was infested with two 3^{rd}-4^{th} instar psyllid nymphs from an infected colony. Petioles of infested leaflets in the infected groups were wrapped loosely with white yarn (Peaches & Cream, worsted 4-ply, color 01005, 262001), in order to mark the leaflets to keep track of the insects' locations. One leaflet of each plant in the uninfected groups was also loosely wrapped with yarn to control for effects of the yarn. Both insects and yarn were left in place for a one-week inoculation access period (IAP). During this period, the plants in the infected groups were checked daily, and missing or dead insects were replaced. At the end of the period, the yarn and insects were removed from all plants.

Three weeks after the end of the IAP (the minimum latency required to first detect Lso in potato or tomato (Levy et al. 2011)), leaf samples from the terminals of all plants in both groups were collected. Most of the leaf lamina was cut away from the midvein using a straight razor, and the midveins were stored in 1.5ml microtubes (Axygen 311-08-051) at -20°C. Genomic DNA was extracted from the leaf tissue using a slightly-modified Arabidopsis quick genomic DNA prep for PCR (Meyerowitz, CALTECH) (Table 2.1). Unlike the original protocol, the solution is vortexed in step 2 following the addition of SDS; the 65°C incubation in step 3 is increased from 5 to 10 minutes; solution is inverted in step 9 following the addition of NaOAc; the -20°C incubation in step 10 is increased from 10 minutes to at least 2 hours; and the amount of re-suspension needed for PCR applications is reduced from 2 to 1 µl.

Table 2.1. Arabidopsis quick genomic DNA prep for PCR (modified)

Step	Procedure
1	In fume hood, add 500 µl extraction buffer (5 ml of 1M Tris pH 8; 5 ml of 0.5M eDTA pH 8; 5 ml of 5M NaCl; 34.7 µl of 14.4M beta ME; 35 ml distilled H_2O) to tissue sample in microtube and homogenize using a pestle.
2	Add 35 µl 20% SDS. Vortex briefly.
3	Incubate on 65° C heat block for 10 minutes.
4	Add 130 µl 5M CH_3CO_2K.
5	Incubate 5 minutes on ice.
6	Centrifuge at 15,000g for 10 minutes.
7	Transfer supernatant to new microtube.
8	Add 640 µl isopropanol.
9	Add 60 µl 3M NaOAc. Invert briefly.
10	Incubate at -20° C for at least 2 hours.
11	Centrifuge at 15,000g for 10 minutes. Discard supernatant.
12	Wash with 70% EtOH (add EtOH and invert 3 times). Add about as much EtOH as supernatant in step 7.
13	Centrifuge at 15,000g for 5 minutes. Discard EtOH. Vacuum dry.
14	Re-suspend pellet in 40 µl molecular grade H_2O.
15	Add RNAse (20µg/ml) to eliminate RNA that could interfere with PCR.
16	Use 1 µl of re-suspension for PCR applications.

Before testing plant DNA for infection, DNA quality and quantity was determined by gel electrophoresis and spectrophotometry, respectively, in order to ensure that the DNA was not degraded, and that enough DNA was present in the samples to detect infection. The infection status of each plant was determined by PCR using primers that amplified the elongation factor (EF1) gene as an internal control, and primers that amplified the 16S rDNA gene of Lso to test for infection. Forward and reverse primer sequences are listed in Table 2.2, below.

Table 2.2. Diagnostic primers used to amplify genes of interest

Gene	Forward Primer	Reverse Primer
EF1	5'-AGATGGTCAGACCCGTGAAC-3'	5'-GTCAAACCAGTAGGGCCAAA-3'
16S rDNA	5'-ATGCAAGTCGAGCGCTTATT-3'	5'-CGAGCGCTTATTTTTAATAGGAGC-3'

Both EF1 and Lso PCR assays were performed under the following conditions: 94°C for 2 minutes, followed by 40 cycles of 94°C for 30 seconds (denaturation), 60°C for 30 seconds (annealing), and 72°C for 1.5 minutes (extension), plus a final extension at 72°C for 10 minutes. The PCR products were resolved on a 1% agarose gel stained with ethidium bromide, and visualized using a Foto/Analyst Investigator photographic system with one of two software packages: Image J 1.34s or PC Image 10.41 (Fotodyne, Inc.)

Hippodamia convergens pupae were supplied by Dr. Steven Arthurs of the University of Florida (2725 Binion Rd., Apopka, FL 32703-8504). Upon arrival, the pupae were housed in groups of ten in 100 mm x 15 mm Petri dishes (VWR 25384-302), which were positioned upside down, with 9 cm filter paper (VWR 55411-050) lining their lids. This filter paper was moistened with distilled water twice daily to facilitate adult emergence.

Upon emergence, adults were kept in Petri dishes described above. Filter paper liners were changed once per week. Two milliliters of filtered water were placed on the filter paper once per day for hydration. Organic raisins, soaked in filtered water to plump them up, were cut in half, placed on small pieces of Parafilm ("M" laboratory film, Pechiney Plastic Packaging), and given daily to supply additional hydration and carbohydrates. Lady beetle diet (Bio-Control Honeydew, 144230, Carolina Biological Supply Co.) was also prepared according to package instructions, dried, ground into small flakes, and 4 mg of diet were given once per week. Frozen *Macrosiphum euphorbiae* or *Myzus persicae* were given once per week, when available.

To ensure that beetles used in the olfactometer experiment were familiar with both psyllids and tomato, beetles underwent a two-day priming period followed by a one-day starvation period prior to testing, as per Gencer et al (2009). During the priming period, groups of up to ten individuals were placed in 150 mm x 15 mm Petri dishes (Fisher Scientific, 8-757-14), each containing one excised tomato leaf of the cultivar described above, its petiole inserted through a Parafilm membrane into a water-filled 1.5 ml microtube to provide hydration to the leaf. Each leaf was also infested with twenty 3rd-5th instar psyllid nymphs from an uninfected colony, in order to feed the beetles. A

filtered water-soaked cotton ball provided hydration for the beetles. Twenty-four hours later, the leaves were removed, re-infested with nymphs, and returned to the dishes. After forty-eight hours, leaves were removed, and beetles were given only water via the cotton balls for twenty-four hours. Throughout priming and starvation periods, Petri dishes were held at room temperature on a bench top under indirect fluorescent light.

During the starvation period, plants requiring psyllids for the experimental treatments (Table 2.3) were infested with thirty 3^{rd}-4^{th} instar psyllid nymphs. In most cases, only infected plants received infected nymphs, but due to a shortage of uninfected insects in 2012, infected nymphs from an infected colony were placed on uninfected plants.

Table 2.3. Treatments for the olfactometer experiment

TREATMENT	DESCRIPTION
Tomato	Control plants
Tomato + Psyllids	Plants infested with thirty psyllid nymphs (3^{rd}-4^{th} instar) one day prior to experiments
Infected Tomato	Plants previously inoculated with Lso by allowing two infected psyllid nymphs (3^{rd}–4^{th} instar) a one-week inoculation access period (IAP) on three week-old tomato plants. Plants were used in experiments following a three-week incubation period after IAP
Infected Tomato + Psyllids	Plants previously inoculated with Lso as described above, with thirty psyllid nymphs (3^{rd}-4^{th} instar) added one day prior to the experiments

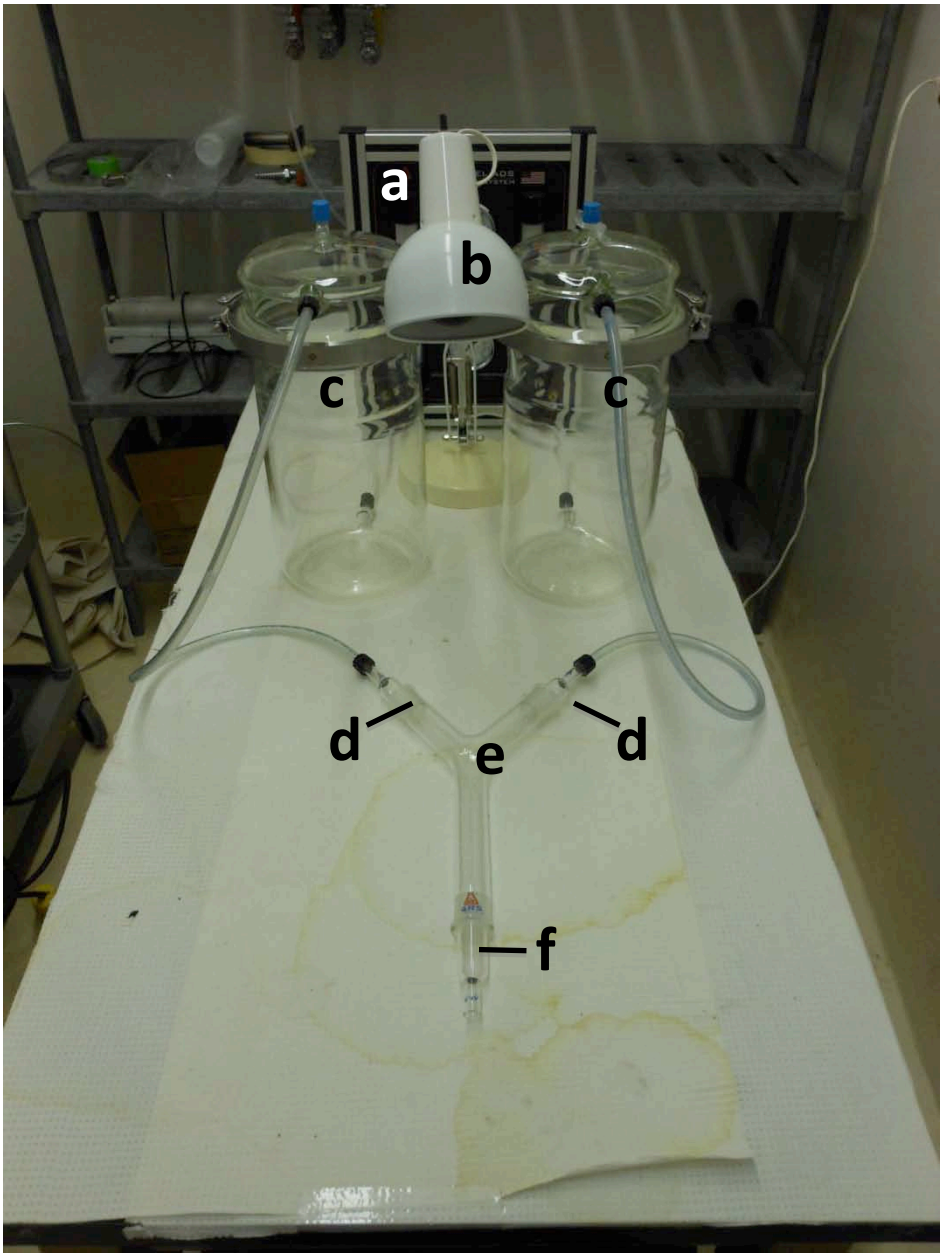
The experiment was conducted in a windowless, non-climate controlled room of the Biological Control Building at Texas A&M University. Experiments were only conducted during periods with stable barometric pressure, without inclement weather, as preliminary experiments revealed that precipitation and cold fronts could significantly impact the beetles' behavior, as has been noted for other insects (Pellegrino et al. 2013). Figure 2.1 (below) shows the olfactometer setup. The building's air supply was connected to a two-choice arena olfactometer air delivery system (ADS-2PFM1C

Economy 2-Chamber). Tubing connected the ADS to the bases of two 14" tall, 6" diameter volatile collection chambers with 1-port lids, which contained the volatile sources (i.e. the treatment plants) being tested. Additional tubing attached to the lids of the collection chambers connected to the two external odor source adapters (OLFM-XO-2425M, male ground- glass joint) of a y-tube olfactometer (24 mm OLFM-YT-2425, standard-taper female ground-glass joints (size 24/25)) with an insect inlet adapter (OLFM-IN-2425M, male ground-glass joint). With the exception of the tubing, all components listed in the above paragraph were manufactured by Analytical Research Systems, Inc. of Gainesville, Florida.

Plants in the treatment combinations being tested (Table 2.4) were randomly assigned to each volatile collection chamber. In three cases, the plants used for the experiment proved to be too large for the volatile collection chambers, which caused stems or leaflets to break upon insertion. If this happened to only one of a pair of plants, the undamaged plant in the pair was deliberately damaged in a similar manner to preserve symmetry.

Once plants were in place, the entire apparatus was flushed with air for ten minutes before starting tests. Airflow was approximately 1.3 liters per minute (LPM) (maximum); inlet and outlet pressure were adjusted to 20 PSI using the ADS's pressure adjust dial. The fluorescent light on the ceiling was turned off for the duration of the tests. A desk lamp with a 17 watt red incandescent light bulb was positioned between the two volatile collection chambers to provide illumination for the researcher (but not the beetles) during the tests.

Figure 2.1. Olfactometer setup



a = ADS; b = desk lamp with 17 watt red light bulb; c = volatile collection chamber; d = external odor source adapter; e = y tube olfactometer; f = insect inlet adapter

Table 2.4. Treatment combinations for the olfactometer experiment

Combination	Description
1	Tomato v. Infected Tomato
2	Tomato v. Tomato+Psyllids
3	Tomato+Psyllids v. Infected Tomato+Psyllids
4	Infected Tomato v. Infected Tomato+Psyllids

Individual beetles were induced to crawl into the insect inlet adapter, which was then connected to the entrance arm of the y-tube. The y-tube lay flat on the table during the tests, and was rotated between tests. Beetles were monitored for up to five minutes using a stopwatch, or until they had crawled at least as far as the inner joint of either choice arm. Those that did not crawl this distance in the time allotted were marked "no-choice" and excluded from analysis.

On the initial assumption that females were "choosier" than males (as per Gencer et al. 2009), females were used exclusively for 35 out of 55 test subjects in treatment combination 2. These females were collected *in copula* and the males were removed. Later, both sexes were tested; beetles were killed by freezing after the tests, and sexed under a light microscope by examining the distal margin of the 5th abdominal sternite (McCornack et al. 2007).

The total number of beetles choosing one odor source per test was compared using the X^2 test of independence. The X^2 value and the degrees of freedom were used to compute the probability for each comparison using the Chi-Square Calculator (www.fourmilab.ch/rpkp/experiments/analysis/chiCalc.html, August 5th, 2013, John Walker).

No-Choice Feeding Bioassay

Excised tomato leaves were prepared and housed in the manner described above for priming beetles during the olfactometer bioassay, except that only ten psyllid nymphs were used to infest each leaf. There were two treatments: uninfected psyllids and infected psyllids. Only uninfected tomato leaves were used for this experiment, in order

to focus on the effect of psyllid infection on beetle behavior. Following infestation, the Petri dishes were sealed with Parafilm for an eight-hour acclimation period (Brown et al. 1999). Afterwards, the dishes were opened and the number of surviving nymphs was recorded in order to have an accurate count of live nymphs at the start of the experiment.

One adult *H. convergens* (male or female) was added to all but one of the dishes in each treatment before dishes were resealed and housed at room temperature on a bench top under indirect fluorescent light for twelve hours. (It was determined that the 24-hour period suggested by Brown et al. (1999) resulted in close to 100% psyllid mortality in both treatments.) Dishes without beetles served as references for psyllid survival in this environment during the experimental period. After twelve hours, the dishes were unsealed once more, and the number of surviving psyllid nymphs, plus any natural mortality, in each dish was recorded. There were twenty-four replicates of each treatment.

The percent mortality of psyllid nymphs caused by the beetles (i.e. the number of psyllids that were no longer present), minus natural mortality (uneaten, dead psyllids), was compared using the Wilcoxon rank sums test using JMP Pro 10.

Results

Olfactometer Bioassay

Most of the beetles tested (77%) made choices in all treatment combinations (Table 2.5). Of those beetles that made choices, 77% were females. However, more females (n = 72) than males (n = 29) were tested. This was partly due to a female bias in the beetle population that was detected after the conclusion of the experiment when beetles were sexed, and partly due to the deliberate usage of only female beetles for 35 out of 55 choice tests for treatment combination 2. Of the 23 beetles that failed to make choices, 43.5% were males and 56.5% were females.

Table 2.5. Number of beetles tested, total number making choices, and percentages of males and females making choices for each treatment combination

Combination[1]	Treatments	Beetles Tested	Choices (%)	% Male	% Female
1	Tomato	13	10 (76.9%)	40	60
	Infected Tomato				
2	Tomato	55	42 (76.4%)	9.5	90.5
	Tomato+Psyllids				
3	Tomato+Psyllids	17	13 (76.5%)	23	77
	Infected Tomato+Psyllids				
4	Infected Tomato	16	13 (81.2%)	53.8	46.2
	Infected Tomato+Psyllids				

[1]Refer to Table 2.4

Chi square analyses revealed no significant differences in beetle preference for treatment combinations 2 – 4. But for treatment combination number 1, significantly more beetles preferred the odors of the uninfected plant to those of the infected plant (Table 2.6).

Table 2.6. Statistics for the olfactometer experiment

Combination	Treatments	Choice	X^2	df	Prob>X^2
1	Tomato	8	9	1	0.0026*
	Infected Tomato	2			
2	Tomato	17	1.524	1	0.2170
	Tomato+Psyllids	25			
3	Tomato+Psyllids	9	1.923	1	0.1655
	Infected Tomato+Psyllids	4			
4	Infected Tomato	7	0.077	1	0.7815
	Infected Tomato+Psyllids	6			

No-Choice Feeding Bioassay

In dishes without beetles, the mean percent mortality of psyllid nymphs was 0% for both treatments. In dishes with beetles, mean percent mortality for uninfected and infected psyllid treatments was not significantly different (Table 2.7). Each beetle consumed 5 psyllids on average in a 12-hour period.

Table 2.7. Statistics for dishes with beetles in the no-choice feeding bioassay

Treatment	N Beetle	Mean % Psyllid Mortality	X^2	df	Prob>X^2
Uninfected Psyllid	24	47.7	0.0315	1	0.8590
Infected Psyllid	24	47.6			

Discussion

Induced changes to the volatile profile of its host plant by a vector-borne plant pathogen could have an effect on a predator that uses volatile cues to locate prey. The results of the olfactometer experiment indicated that Lso infection of tomato plants could significantly affect the foraging behavior of *H. convergens*. Beetles significantly preferred the odors of the uninfected tomato plant to those of the infected plant (Table 2.6). This is evidence that Lso infection changes volatile production by tomato plants.

It appears unlikely that *H. convergens* would be repelled by Lso-induced plant volatiles. Das et al. (2013) found that many HIPV are also induced by plant pathogens. Although some HIPVs are repellant to herbivores (Kessler and Baldwin 2002), the literature suggests that natural enemies are often attracted to HIPV even while herbivores are repelled (Kessler and Baldwin 2002; Dicke and Baldwin 2010).

Lso plant infection may be disrupting volatile cues that *H. convergens* normally uses for orientation, perhaps by changing them in such a way as to make them unrecognizable to the predator. Unlike Rostas et al. (2006), who found that maize plants infected with white mold emitted 47% fewer volatiles than uninfected maize plants, Davis et al. (2012) reported that potato plants fed upon by Lso-infected psyllids did not reduce the number of volatiles produced by infected potato plants as compared with plants fed upon by uninfected psyllids. Furthermore, potato plants fed upon by Lso-infected psyllids emitted significantly more α-caryophyllene. However, Davis et al. (2012) collected volatiles from potatoes that were being actively fed upon by psyllids, making it difficult to separate HIPV and Lso-induced volatiles. In this olfactometer bioassay, infected tomato plants in the treatments without psyllids had not been fed upon by psyllids for three weeks before being used. The persistence of psyllid HIPV is unknown in tomato, but HIPV is expected to have dissipated almost completely, if not entirely, by the time the experiment began. Therefore, the possibility that Lso masks volatile cues by reducing their emissions cannot presently be ruled out.

However, psyllid HIPV also appears to negate the effect of Lso-induced volatiles on beetle foraging behavior. Upon comparing both uninfected and infected plants with psyllids, the difference in beetle preference for both odor sources was not significant, although more beetles chose the odors of the uninfected plant over those of the infected plant (Table 2.6). Beetles also did not significantly prefer the odors of plants with psyllids over those of plants without psyllids, regardless of plant infection status (Table 2.6). This indicates that Lso plant infection may not confer indirect protection to its insect vector. However, the effects of plant infection on psyllid settling behavior vary over time (Davis et al. 2012), and effects on natural enemy foraging behavior may do the same. This study examined the effects of plant infection three weeks after the end of the IAP. It would be interesting to see the effects of more recent plant infection on both the volatile profile of the tomato plant and the behavior of the beetle.

To the author's knowledge, this is the first study of the effects of plant pathogen-induced VOCs on the foraging behavior of a predatory insect and the first study of the

effects of bacterial plant pathogen-induced VOCs on the foraging behavior of any natural enemy. Two previous studies have examined the effects of fungal plant pathogen-induced VOCs on the foraging behavior of parasitoids (Cardoza et al. 2003; Rostas et al. 2006). Using a wind tunnel, Cardoza et al. (2003) found that *Cotesia marginiventris* preferred the odors of peanut plants infected with *Sclerotium rolfsii* to the odors of healthy peanut plants. Because the beet armyworm, *Spodoptera exigua*, preferentially oviposits on mold-infected peanuts, the authors hypothesized that the parasitoid has learned to associate the odor of the mold-infected plants with potential host locations (Cardoza et al. 2003). By contrast, Rostas et al. (2006) found that *C. marginiventris* and *Microplitis rufiventris* displayed no preference between odors of uninfected and *Setosphaeria turcica*-infected maize seedlings in a six-arm olfactometer. However, *S. littoralis*, the caterpillar host used in their experiment, also was not positively associated with infected maize (Rostas et al. 2006). Unlike Lso, neither fungal pathogen used in these experiments is borne exclusively by an insect vector. Assuming an obligate pathogen-vector association, pathogen-induced VOCs that are attractive to natural enemies of the pathogen vectors should be selected against, from an evolutionary standpoint. But where there is no obligate association between pathogen and vector, parasitoids would not be expected to become attracted to pathogen-induced VOCs unless there was a positive association between such volatiles and locations of potential hosts.

Percent psyllid mortality rates in the no-choice feeding bioassay did not vary significantly across treatments (Table 2.7); that is, the beetles seemed equally inclined to consume infected or uninfected psyllids. This indicates that Lso does not confer direct protection to its insect vector as a consequence of harboring Lso inside its body, as has been observed with aphids challenged by parasitoids while aphids were harboring the bacterial symbiont *Hamiltonia defensa* or luteoviruses (Christiansen-Weniger et al. 1998; Gwynn et al. 2005; Hodge and Powell 2008; Calvo and Fereres 2011). However, a choice experiment between uninfected and Lso-infected psyllids may reveal differences in psyllid predation by *H. convergens*. Perhaps through the use of a fluorescent marker or dye, distinct psyllids treatments could be easily differentiated on the surface of a leaf

to allow such a test. However, care must be taken to ensure that the marking method does not adversely affect the fitness of psyllids (Warner and Bierzychudek 2009).

These studies provide evidence that the presence of Lso will not adversely affect biological control of *B. cockerelli* by *H. convergens*. Additional studies should be conducted to determine the effects of Lso plant or psyllid infection on the behavior of other natural enemies of *B. cockerelli*. Volatiles could be collected from uninfected and Lso-infected tomato at different time points and analyzed using a GC/MS (Davis et al. 2012). Then, further olfactometer experiments could be used to determine if *H. convergens*, or other natural enemies, are attracted to individual volatiles or volatile blends of interest (Hamilton et al. 1999). A choice experiment between uninfected and Lso-infected insects may reveal differences in psyllid predation by various natural enemies.

CHAPTER III
INTRAGUILD INTERACTION BETWEEN THE POTATO PSYLLID AND GREEN PEACH APHID ON TOMATO

Introduction

Facilitation between members of the same species or different species can play an important role in shaping ecological communities, but the importance of facilitation has often been ignored in ecological studies (Stachowicz 2001). Competitive interactions are by far the most commonly described in the literature, and this is true of most descriptions of interactions between phytophagous hemipterans (Denno et al. 1995; Kaplan and Denno 2007). Certainly hemipteran herbivores do compete, especially if they depend upon the same resources (Denno et al. 1995). However, habitat modifications by one herbivore can potentially promote competition or facilitation between herbivores (Stachowicz 2001). Outcomes may vary according to plant growth stage (Gianoli 2000), plant genotype (Moran and Whitham 1990), and plant species (Xu et al. 2011).

Herbivory depletes plant nutrients and may also induce plant defenses, thereby decreasing plant suitability as a host (Denno et al. 1995; Denno et al. 2000; Ohgushi 2005; Dugravot et al. 2007), but it may also attenuate host plant defenses (Ohgushi 2005; Dugravot et al. 2007), or increase the nutritional quality of the host plant (Forrest 1971; Brunissen et al. 2009). Both scenarios can potentially benefit conspecific or heterospecific herbivores feeding on these plants. Multiple herbivores may also work together to increase the availability of nutrients (Wise et al. 2006), or create a protected environment for their offspring (Luft et al. 2001). Beneficial interactions may be dependent on an optimal herbivore density – depending on plant resource availability, there can be both too few and too many herbivores present for a beneficial outcome (Chongrattanameteekul et al. 1991; Luft et al. 2001; Wise et al. 2006).

Since most plant pathogens are transmitted by hemipteran vectors, they may also influence the outcomes of hemipteran interactions. Plant pathogens can induce or reduce plant defenses, just like herbivory. Reductions in plant defenses due to plant pathogens have been known to benefit their insect vectors (Belliure et al. 2005). For example, *Myzus persicae* develops faster and is more fecund on potato plants infected with the potato leafroll virus, which is transmitted by this aphid (Eigenbrode et al. 2002). Being able to develop faster and produce more progeny may lend a competitive edge in an interspecific interaction with another herbivore. Yet vector-borne plant pathogens may sometimes benefit non-vector herbivores. For example, the survival of juvenile *Tetranychus urticae* was found to be enhanced on pepper plants infected with tomato spotted wilt virus (TSWV), which is transmitted by the thrips *Frankliniella occidentalis* (Belliure et al. 2010). This type of facilitation may increase the levels of herbivory on infected plants, and could potentially lead to the vector dispersing more often to locate new hosts, which would in turn increase the spread of plant disease.

The Tamborindeguy lab maintains colonies of both *B. cockerelli* and *Myzus persicae*, the green peach aphid. Although *M. persicae* is a highly polyphagous species, exhibiting a high degree of phenotypic plasticity (Agarwala 2007), the clone of *Myzus* in the Tamborindeguy lab has repeatedly been shown to languish on tomato cv. Moneymaker, typically dying off after a few days. However, some aphid populations were observed to survive, and even thrive, for months on tomato plants in both uninfected and Lso-infected psyllid colonies before inexplicably going extinct.

Such was the case in the spring of 2012, when an accidental infestation of an infected psyllid colony led to a veritable outbreak of aphids on tomato. Indeed, at its peak, the aphids appeared more numerous than the psyllids. Many of the aphids had developed into winged forms, or alates, which, for *M. persicae,* are usually induced by reductions in host plant quality (Müller et al. 2001). Several months following the initial infestation, the aphids died off in the colony. The original tomato plants in the colony at the time of the outbreak had also died, leading one to speculate that either the psyllids or

Lso may first have to "condition" the plants for a certain amount of time before they become suitable for aphids.

Both Lso and its insect vector can attenuate tomato plant defenses (Casteel et al. 2012), which may benefit the vector, and possibly other phloem-feeding herbivores. The objective of this work was to determine whether the presence of psyllids, plant infection with Lso, or both would allow aphids to survive on an otherwise unsuitable host. Several questions were addressed: 1) Are aphids becoming adapted to tomato? 2) Could plant infection with Lso promote aphid facilitation? 3) Could the presence of psyllids promote aphid facilitation? 4) Do aphids that complete their development on tomato suffer fitness consequences?

Materials and Methods

All tomato plants (cv. Moneymaker) and bell pepper plants (cv. Calwonder) used in this work were grown in the manner described for tomatoes in Chapter 2. Wild tobacco (*Nicotiana benthamiana*) was planted using the same media as tomato and bell pepper, but in 4.5" round pots (Kord Regal Standard Pots, Meyers Industries, Inc., CN-STD 0450). Tobacco seeds were sprinkled on the surface of moistened media, and covered with a fine layer of media. Soil was kept perpetually moist prior to and following germination by watering three times per week.

Aphid Adaptation Experiment

The purpose of this experiment was to determine if *Myzus persicae* in the psyllid colony had become adapted to tomato.

Ten 42-day old tomato plants were divided into two groups of six and four in two 14x24" mesh rectangular cages (BioQuip 1466B). Each plant was labeled with a unique number. In case transmission of Lso was not 100% efficient, the larger group of six was infected with Lso by placing two 3^{rd}-4^{th} instar nymphs from an infected colony on one leaflet of each plant, and allowing them to feed on the plants for a 1-week IAP. During this period, the plants in the infected group were checked daily, and missing or dead

psyllids were replaced. At the end of the period, the psyllids were removed from all plants.

Leaf tissue samples from plant terminals were collected from all plants and stored at -20°C. Genomic DNA was extracted from the samples using the Arabidopsis quick genomic DNA prep for PCR (Meyerowitz, CALTECH) detailed in Chapter 2, and standard PCR (procedure described in Chapter 2) determined that all un-infested plants tested negative, and all psyllid-infested plants tested positive for Lso.

Three weeks after the end of the IAP, eight plants (four uninfected, four infected) were individually caged in 12" mesh cube cages (BioQuip 1466A) and randomly assigned to one of two shelves (Style Selections, 0071034) under forty-eight inch shop lights (Utilitech NXU-6000, 0245536) with plant and aquarium fluorescent light bulbs (GE, F40T12). Five aphids (4^{th} instars and adults) from an aphid-infested, infected psyllid colony were collected and placed on each plant. The caged plants were monitored every other day, and the numbers of surviving aphid adults and nymphs were recorded.

Fitness Bioassay

The purpose of this experiment was to determine if the interaction between psyllid feeding and plant infection with Lso could promote aphid facilitation on tomato.

Four week-old tomato plants were infected with Lso using psyllid nymphs in the manner described above. Two weeks after the end of IAP, 20-30 adult *Myzus persicae* were isolated on a clean bell pepper plant in a 12" cube cage (described above) and allowed to nymphoposit for 24 hours, or until at least 75 1^{st} instar nymphs were present on the pepper plant. When a sufficient number of nymphs were present, the adults were removed.

Table 3.1 below lists the treatments used in the experiment. Eighteen days after the end of IAP, twelve plants (three replicates per treatment) were individually housed in the mesh cube cages described above, and were randomly assigned to one of three shelves (Style Selections, 0071034).

Table 3.1. Fitness bioassay treatments

Treatment	Description
Uninfected	Plants without psyllids
Infected	
Uninfected+Psyllids	Plants infested with ten 3rd-4th instar psyllid nymphs three days before the start of experiments
Infected+Psyllids	

Three weeks after the end of IAP, five adult aphids from the group isolated one week before were placed on each plant. Plants were monitored daily for a 1-week period, and the numbers of adult aphids, aphid nymphs, and psyllid nymphs were recorded. If psyllids died or went missing, they were replaced.

At the conclusion of the experiment, terminal leaf samples were collected from all plants, and their genomic DNA was extracted and tested for Lso. If plants in the infected treatments tested negative, a second PCR was performed using the first PCR product as a template. If plants still tested negative even after performing PCR on the PCR product, data from those plants were excluded from analysis.

The mean numbers of aphid adults and nymphs per treatment per day were compared using the Kruskal-Wallis rank sums test in JMP 9.

Life History Bioassay

The purpose of this experiment was to determine if plant infection with Lso alone could facilitate aphid survival on tomato.

Flats of tomato plants (ranging in age from 42-46 days old) were divided into groups of between 9-15 plants and placed in 14” x 24” mesh rectangular cages (BioQuip 1466B). Groups were randomly assigned to one of two treatments: uninfected and infected. One leaflet on each plant in the infected group was infested with two 3rd-4th instar psyllid nymphs from the infected “W2” or “C2” colonies. Leaflets of all plants were wrapped loosely with yarn as described above in Chapter 2 during the one-week IAP.

At least three weeks after the end of the IAP, leaf tissue samples were collected from all plant terminals, and stored at -20°C. Genomic DNA was extracted and tested in the manners described above. When plants were conclusively determined to be uninfected or infected, they were then used in experiments. The number of plants per treatment was equal to the number of infected plants available for this experiment.

First-instar *Myzus persicae* nymphs were collected from bell pepper and confined to one leaf per plant in all treatments using 1" diameter foam clip cages (BioQuip 1458). Nymphs were monitored every other day, and the number of surviving nymphs and their instars were recorded until death of the nymphs. Exuvia was retrieved from the clip cages using a fine paintbrush in order to ascertain molts, although molt data were not analyzed.

Survival data were analyzed using the Kaplan Meier log rank test in the Survival Program in StatsToDo (http://www.statstodo.com/Survival_Pgm.php, July 9th, 2013, Allen Chang).

Psyllid Life Stages Experiment

The purpose of this experiment was to determine if a particular psyllid life stage could facilitate aphid survival on tomato.

Twenty 43 day-old tomato plants were individually caged in the 12" cube cages described above. Five caged plants were assigned each of four shelves underneath lighting conditions described above. Beginning on March 15, 2013, every week for four weeks, one group of five plants was infested with 20 uninfected adult psyllids (10 male, 10 female) from an uninfected colony. The psyllids were left on the plants for one week to10 days, or until approximately 50 eggs had been laid per plant. Then, psyllid adults were removed with an aspirator (BioQuip 1135A). This was done in order to obtain psyllid populations of definite life stages, as described in Table 3.2, below.

Table 3.2. Treatments for the psyllid life stages experiment

Treatment	Description
1	Adult psyllids
2	4^{th}-5^{th} instar nymphs
3	2^{nd}-3^{rd} instar nymphs
4	1^{st} instar nymphs and eggs

Twelve days after the last group of plants was infested (on April 5^{th}), twenty *M. persicae* (4 adults, and 4 of each of the 4 nymphal instars) were placed on a leaf of each plant. Aphids were typically placed on a leaf with psyllid nymphs present, if available. Following aphid infestation, plants were monitored every three days for a fifteen-day period, and the numbers of aphids were recorded.

The mean numbers of aphids per treatment were analyzed using the Kruskal-Wallis rank sums test in JMP Pro 10.

Psyllid Infestation Duration Experiment

The purpose of this experiment was to determine if the length of a psyllid infestation affected aphid facilitation on tomato.

Following the conclusion of the psyllid life stages experiment, the decision was made to reuse the plants from this experiment to examine the effect of psyllid infestation duration on aphid survival. Since cohorts of same-age psyllids were initially present in those plants at the end of the psyllids life stages experiment, psyllids populations still remained synchronized, but there were significantly more psyllids present per plant. Table 3.3 below lists the treatments in the experiment.

Table 3.3. Treatments for the psyllid infestation duration experiment

Treatment	Infestation Date (2013)	Length of Infestation (Days)
1	March 15th	55
2	March 22nd	48
3	March 29th	41
4	April 5th	34

Two plants, one in treatment 3 and one in treatment 4, still had small aphid populations left over from the psyllid life stages experiment, and were not re-infested with aphids. The remaining 18 plants were re-infested with the same number and life stages of aphids as in the psyllid life stages experiment. Aphids were placed on leaves that were previously infested with aphids. All plants were monitored every 3rd day for a 15-day period. The mean numbers of aphids and nymphs per treatment were analyzed in the manner described above for the psyllid life stages experiment.

Plants were also evaluated 30 days after the start of this experiment, and the number of plants with aphids, as well as the numbers of adults and nymphs on each, was recorded.

Plant Age Experiment

The purpose of this experiment was to determine if plant age was a determining factor in aphid facilitation on tomato.

Three Lso-negative tomato plants that were the same age as those used in both the psyllid life stages and psyllid infestation duration experiments, but which had never been exposed to psyllids, were individually housed in 12” cube cages described above, on the same shelves and lighting conditions as plants in the aforementioned experiments. Each was infested twice with aphids, as in the aforementioned experiments, and the numbers of aphid adults and nymphs were recorded every three days for 15-day periods. Matched pairs analysis of the mean numbers of aphids in each infestation was performed using JMP Pro 10.

Fitness Comparisons of Aphids from Three Host Plants

The purpose of this experiment was to determine if aphids that complete their development on tomato exhibit reduced fitness as compared to aphids that complete their development on favorable hosts.

In aphid-infested psyllids colonies, alate aphids were abundant, which usually indicates poor nutrition for *M. persicae*. However, only four out of seventeen aphids developed into alates on plants in the psyllid life stages/infestation duration experiments. In order to determine if aphids experienced reduced fitness on tomato as compared to favorable hosts, comparisons were made between adult weight and fecundity of *M. persicae* from tomato plants used in the psyllid life stages/infestation duration experiments, and from two hosts known to be suitable for the aphid: bell pepper and wild tobacco.

Adult aphids (alatae and apterae) were collected from wild tobacco (n=12), bell pepper (n=5), and tomato plants (n=12). Aphids were placed in 1.5ml microtubes embedded in ice to make them less active, and taken to the Biological Control Building, where they were individually weighed using a microbalance (Mettler-Toledo UMX2). Individual aphids were handled using a fine paintbrush. Following weighing, 10 individuals from tomato, 10 from wild tobacco, and 4 from bell pepper were individually isolated on excised wild tobacco leaves in Petri dishes (prepared as described for tomato leaves in Chapter 2). Dishes were sealed with Parafilm for 24 hours, after which the number of nymphs present in each dish was recorded.

Aphid weight and 24-hour fecundity data were analyzed using the Kruskal-Wallis rank sums test in JMP Pro 10. If significant differences were discovered, Wilcoxon rank sums tests were performed to compare pairs of means.

Results

Aphid Adaptation Experiment

There were no significant differences in the numbers of adults or nymphs across treatments. Adult aphids taken from the "C2" psyllid colony tomato and placed on tomato without psyllids died within three days, irrespective of treatment (Table 3.4). Most aphid nymphs from the "C2" colony were dead by day five (Table 3.4).

Table 3.4. Statistics for the aphid adaptation experiment

Day	Treatment	Aphid Stage	Mean Aphid Number	X^2	df	Prob>X^2
1	Adult	Uninfected	2.25	0.0897	1	07645
		Infected	2.5			
	Nymph	Uninfected	3.25	0.3590	1	0.5491
		Infected	2.0			
3	Adult	Uninfected	0	0.0000	1	1.0000
		Infected	0			
	Nymph	Uninfected	1.75	0.3684	1	0.5439
		Infected	1.0			
5	Adult	Uninfected	0	0.0000	1	1.0000
		Infected	0			
	Nymph	Uninfected	0.75	1.0000	1	0.3173
		Infected	0			
7	Adult	Uninfected	0	0.0000	1	1.0000
		Infected	0			
	Nymph	Uninfected	0.75	1.0000	1	0.3173
		Infected	0			

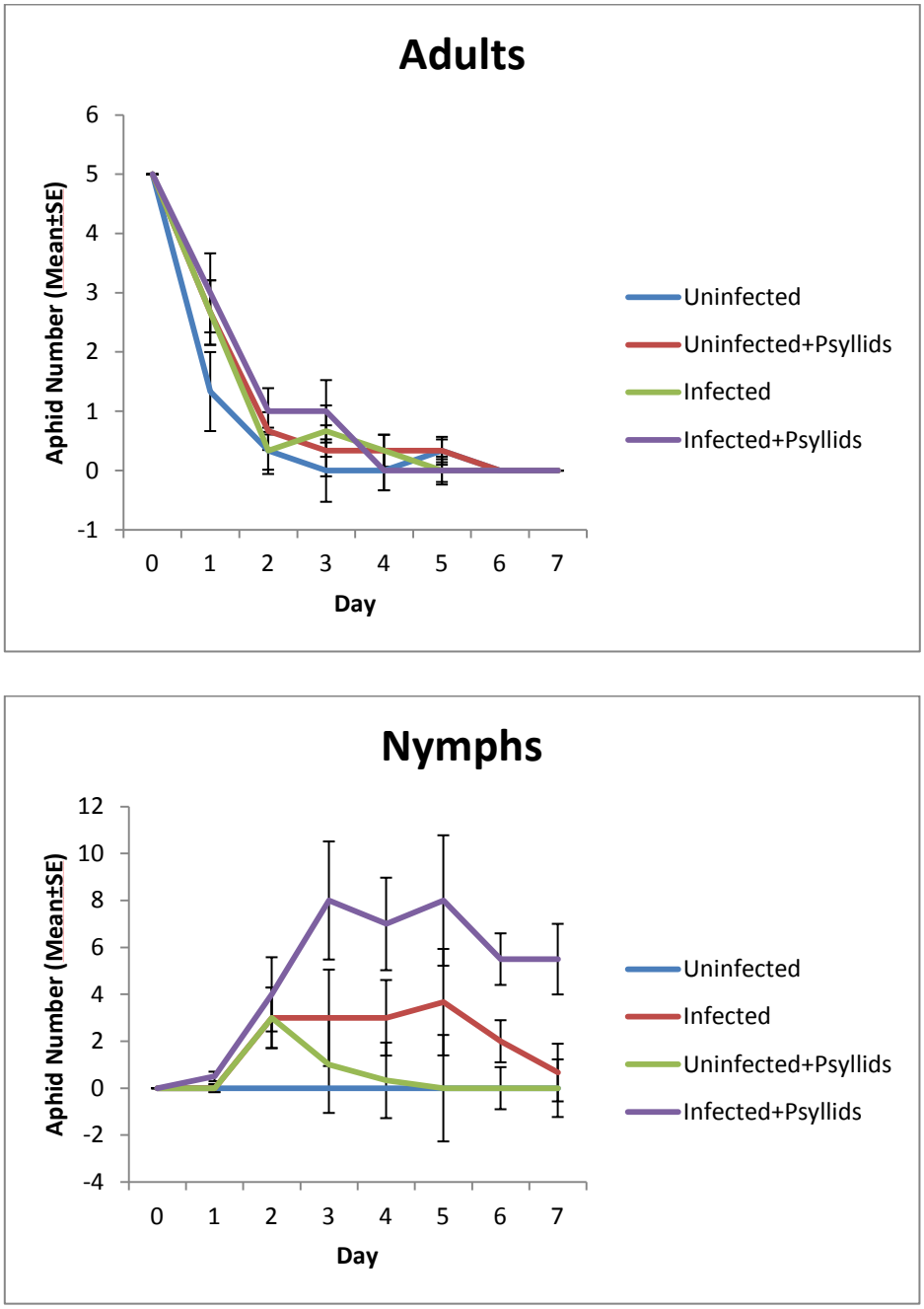
Fitness Bioassay

Mean numbers of adult aphids did not vary significantly across treatments; most were dead by day 4 (Figure 3.1 Adults). Mean numbers of nymphs per treatment were significantly different on days 5 and 6 (Table 3.5, Figure 3.1 Nymphs).

Table 3.5. Statistics for the fitness bioassay

Day	Aphid Stage	Treatment	Mean	X^2	df	Prob>X^2
1	Adult	Uninfected	1.3333	3.7914	3	0.2849
		Infected	2.6667			
		Uninfected +Psyllids	2.6667			
		Infected +Psyllids	3.0000			
	Nymph	Uninfected	0	4.0000	3	0.2615
		Infected	0			
		Uninfected +Psyllids	0			
		Infected +Psyllids	0.5000			
2	Adult	Uninfected	0.3333	4.9833	3	0.1730
		Infected	0.3333			
		Uninfected +Psyllids	0.6667			
		Infected +Psyllids	1.0000			
	Nymph	Uninfected	0	3.8815	3	0.2745
		Infected	0			
		Uninfected +Psyllids	3.0000			
		Infected +Psyllids	4.0000			
3	Adult	Uninfected	0	3.0476	3	0.3843
		Infected	0.6667			
		Uninfected +Psyllids	0.3333			
		Infected +Psyllids	1.0000			
	Nymph	Uninfected	0	6.0955	3	0.1071
		Infected	3.0000			
		Uninfected +Psyllids	1.0000			
		Infected +Psyllids	8.0000			
4	Adult	Uninfected	0	1.5000	3	0.6823
		Infected	0.3333			
		Uninfected +Psyllids	0.3333			
		Infected +Psyllids	0			
	Nymph	Uninfected	0	7.3442	3	0.0617
		Infected	3.0000			
		Uninfected +Psyllids	0.3333			
		Infected +Psyllids	7.0000			
5	Adult	Uninfected	0.3333	2.3333	3	0.5062
		Infected	0			
		Uninfected +Psyllids	0.3333			
		Infected +Psyllids	0			
	Nymph	Uninfected	0	8.2292	3	0.0415*
		Infected	3.6667			
		Uninfected +Psyllids	0			
		Infected +Psyllids	8.0000			
6	Adult	Uninfected	0	0.0000	3	1.0000
		Infected	0			
		Uninfected +Psyllids	0			
		Infected +Psyllids	0			
	Nymph	Uninfected	0	8.4635	3	0.0373*
		Infected	2.0000			
		Uninfected +Psyllids	0			
		Infected +Psyllids	5.5000			
7	Adult	Uninfected	0	0.0000	3	1.0000
		Infected	0			
		Uninfected +Psyllids	0			
		Infected +Psyllids	0			
	Nymph	Uninfected	0	6.5625	3	0.0872
		Infected	0.6667			
		Uninfected +Psyllids	0			
		Infected +Psyllids	5.5000			

Figure 3.1. Mean numbers of aphids per treatment per day in the fitness bioassay



Life History Bioassay

There was no significant difference in aphid survival across treatments (Table 3.6, Figure 3.3). In many instances, nymphs escaped from the clip cages. Some were recovered and returned to the cages, but 4 and 5 individuals from the uninfected and infected treatments, respectively, were never found. Nymphs that remained in the cages stopped molting at the 2nd or 3rd instar, and most survived no more than 6 days. The mean survival time in days was the same for both treatments (Table 3.6).

Table 3.6. Statistics for the life history bioassay

Treatment	Mean Aphid Survival (Days)	X^2	df	Prob>X^2
Uninfected	5.09	0.2474	1	0.6189
Infected	5.09			

Psyllid Life Stages Experiment

There were no significant differences in mean numbers of aphids per day across treatments (Table 3.7). At the end of the experiment, three plants – one each in treatments 1, 2, and 4 – still had small aphid populations. The plant from treatment 4 supported a population of aphids from the 17th of April to early July, when the plant died.

Table 3.7. Kruskal-Wallis analysis of mean numbers of aphids per treatment in the psyllid life stages experiment

Day	Treatment	Mean Aphids	X^2	df	Prob>X^2
3	Adults	2.2	7.5386	3	0.0566
	4^{th}-5^{th} Instar	1.2			
	2^{nd}-3^{rd} Instar	3.8			
	1^{st} Instar + Eggs	4.0			
6	Adults	0.8	4.2233	3	0.2383
	4^{th}-5^{th} Instar	1.0			
	2^{nd}-3^{rd} Instar	1.4			
	1^{st} Instar + Eggs	2.2			
9	Adults	0.8	1.2062	3	0.7515
	4^{th}-5^{th} Instar	1.4			
	2^{nd}-3^{rd} Instar	1.0			
	1^{st} Instar + Eggs	1.4			
12	Adults	0.2	4.6698	3	0.1976
	4^{th}-5^{th} Instar	0.6			
	2^{nd}-3^{rd} Instar	0			
	1^{st} Instar + Eggs	1.2			
15	Adults	0.2	1.1385	3	0.7678
	4^{th}-5^{th} Instar	0.4			
	2^{nd}-3^{rd} Instar	0			
	1^{st} Instar + Eggs	0.8			

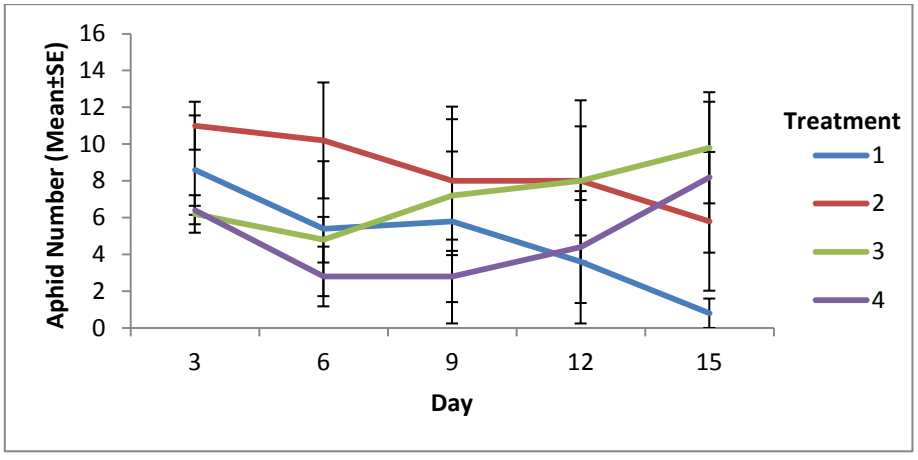
Psyllid Infestation Duration Experiment

There were no significant differences in the mean numbers of aphids per day across treatments (Table 3.8). Aphid populations declined in treatments 1 and 2, but were increasing on plants in treatments 3 and 4 – the 41-day and 35-day infestation treatments, respectively, by the end of the experiment (Figure 3.2). At the 30-day evaluation after the start of this experiment, aphids were present on five plants, three in treatment 3 and two in treatment 4.

Table 3.8. Statistics for the psyllid infestation duration experiment

Day	Treatment	Mean Aphid Number	X^2	df	Prob>X^2
3	March 15	8.6	6.6705	3	0.0832
	March 22	11.0			
	March 29	6.2			
	April 5	6.4			
6	March 15	5.4	4.8102	3	0.1862
	March 22	10.2			
	March 29	4.8			
	April 5	2.8			
9	March 15	5.8	3.3878	3	0.3356
	March 22	8.0			
	March 29	7.2			
	April 5	2.8			
12	March 15	3.6	3.4360	3	0.3292
	March 22	8.0			
	March 29	8.0			
	April 5	4.4			
15	March 15	0.8	6.0464	3	0.1094
	March 22	5.8			
	March 29	9.8			
	April 5	8.2			

Figure 3.2. Mean numbers of aphids per treatment per day in the psyllid infestation duration experiment



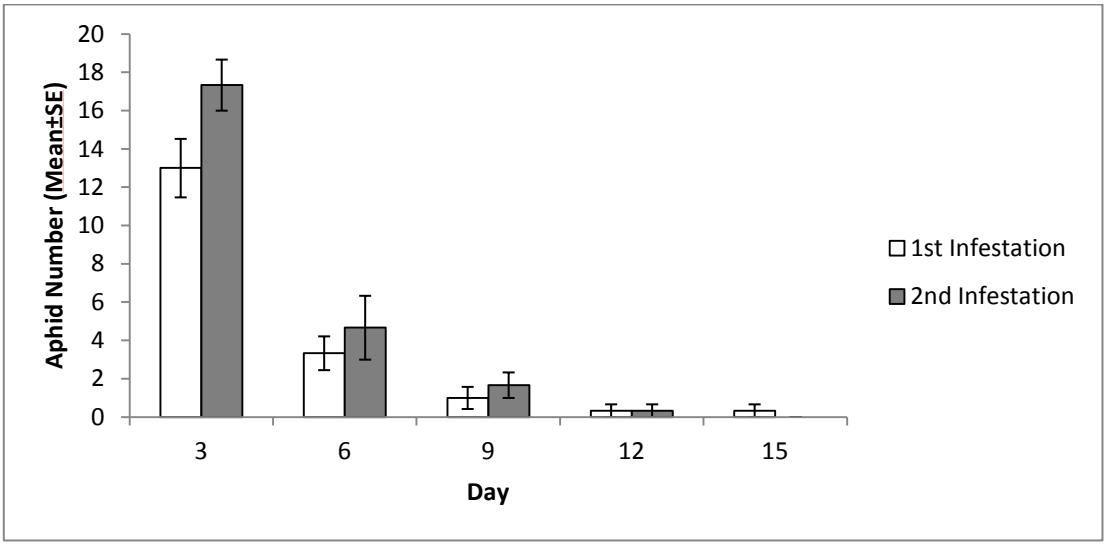
Plant Age Experiment

In both infestations, aphid populations were initially high, but declined rapidly (Figure 3.3). The mean number of aphids per day in the second infestation was significantly higher than in the first infestation on day 3 (Table 3.9).

Table 3.9. Comparison of 1st and 2nd aphid infestations in the plant age experiment

Day	Infestation	Mean Aphids	X^2	df	Prob>X^2
3	1	13.0000	3.9706	1	0.0463*
	2	17.3333			
6	1	3.3333	0.4839	1	0.4867
	2	4.6667			
9	1	1.0000	0.4839	1	0.4867
	2	1.6667			
12	1	0.3333	0.0000	1	1.0000
	2	0.3333			
15	1	0.3333	1.0000	1	0.3173
	2	0			

Figure 3.3. Mean numbers of aphids per day in the plant age experiment



Fitness Comparisons of Aphids from Three Host Plants

Mean weights of adult aphids from tomato and pepper were not significantly different, but both were significantly less than the mean weight of adult aphids from wild tobacco (Table 3.10). Mean 24-hour fecundity was not significantly different across plant hosts (Table 3.11). The numbers of nymphs produced in 24 hours ranged from 0–4, with most aphids producing zero nymphs. Mortality during the 24-hour period among aphids from tomato and wild tobacco was high: mean percent mortality for aphids from bell pepper, tomato, and wild tobacco was 25, 50, and 60%, respectively.

Table 3.10. Mean weights of adult aphids from three host plants

Host Plant	Mean Adult Weight* (mg)	X^2	df	Prob>X^2
Wild Tobacco	0.3928**a**	7.7759	2	0.0205*
Tomato	0.2994**b**			
Bell Pepper	0.2608**b**			

*Means not connected by the same letter are significantly different

Table 3.11. Mean 24-hour fecundity of aphids from three host plants

Host Plant	Mean Nymphs Per Aphid In 24 Hours	X^2	df	Prob>X^2
Wild Tobacco	0.7	0.7946	2	0.6721
Tomato	1.0			
Bell Pepper	1.0			

Discussion

From the various experiments, it can be said that the presence of psyllids contributes to the aphid facilitation on tomato, and appears to be crucial for aphid survival. Although no significant differences in mean numbers of aphids per treatment per day were detected in the psyllid life stages experiment, the mean number of aphids in treatment 4, which had the youngest psyllids nymphs, was consistently higher than the mean numbers of aphids in all other treatments, which had older nymphs or adults (Table 3.7). This indicates that the presence of the youngest psyllid nymphs is the most important for aphid facilitation. Indeed, one plant in treatment 4 supported a population of aphids for approximately 80 days. This amounts to at least eight generations of aphids. Aphid populations also increased on plants with populations of young nymphs (Figure 3.2), and

persisted for 30 days or longer only on plants with populations of young nymphs in the psyllids infestation duration experiment.

The role of Lso is more puzzling, for although mean numbers of aphid nymphs were higher on infected plants in the fitness bioassay (Figure 3.1), in the life history bioassay, there was no significant difference in survival across infected and uninfected plants (Table 3.6). The life history bioassay was performed with one aphid per plant, while multiple aphids were used on each plant in the fitness bioassay. Possibly, having multiple aphids feeding at the same time could have an effect apart from that of individual aphids feeding. Meadow spittlebugs, for example, often share spittle masses and collectively are able to overcome barriers to xylem feeding more easily than they could individually (Wise et al. 2006). But in the plant age experiment, having 20 aphids on each plant did not prevent their populations from going extinct (Figure 3.3). Lso has been shown to attenuate plant defenses (Casteel et al. 2012), which may benefit the aphid. However, aphid infestations were occurring on both uninfected and Lso-infected psyllid colony tomato. This, coupled with the fact that there were relatively few replicates of the fitness bioassay as compared with the life history bioassay, is another indication that psyllid presence is the determining factor in aphid survival on tomato rather than Lso.

How psyllids are able to facilitate aphid survival on tomato is an open question, but likely answers are that they are knocking down some defensive pathway (Casteel et al. 2012), or freeing up nutrients that are otherwise unavailable to aphids, or both. Increased nutritional quality of the tomato plant is indicated by the fact that few aphids from tomato in the psyllid life stages/infestation duration experiments developed into alates. For *Myzus persicae*, reductions in host plant quality are principally responsible for alate production (Müller et al. 2001).

The mean weights of adult aphids from tomato plants were not significantly different from those from bell pepper (Table 3.10), the host plant from which the aphids placed on tomato originated. In addition, 24-hour fecundity was not significantly different for aphids reared on tomato, bell pepper, or wild tobacco (Table 3.11). The presence of

psyllids makes it possible for aphids to perform as well on tomato with psyllids as they would on favorable hosts without psyllids.

The duration of the psyllid infestation of a tomato plant appeared to influence the likelihood of aphid facilitation. However, the length of psyllid infestation was confounded by the psyllid life stages, plant age, and previous infestation by conspecific aphids. Due to their development rate, the majority of psyllids present in each treatment of the psyllid infestation duration experiment was still in a particular life stage. Psyllid ages in treatments 1-4 of this experiment largely conformed to the treatments 1-4 in the psyllids life stages bioassay (Table 3.2), although adults were present in all treatments of this experiment. The time between the first and second aphid infestations of the tomato plants was 19 days, and the suitability of a host plant changes over time. Particular growth stages can make plants more or less suitable to herbivores (Gianoli 2000). Finally, aphids appeared to benefit from previous infestation by conspecifics, which has been noted before for *M. persicae* on other hosts (Sauge et al. 2002; Sauge et al. 2006). More work must be done to determine if the length of a psyllid infestation has an effect apart from psyllid age, plant age, or previous infestation by conspecifics.

Another factor that may be important for aphid facilitation is psyllid density. At the 30-day evaluation after the conclusion of the psyllid infestation duration experiment, the numbers of adult psyllids were relatively low (between 56-155 individuals) on plants that still supported aphids, compared with many that did not (often over 400 individuals). The numbers of psyllid nymphs on leaves shared by aphids were also relatively low in most cases. Other researchers have noted that beneficial interactions between phytophagous hemipterans may depend on low-to-moderate densities of both parties (Luft et al. 2001; Wise et al. 2006). When members of one species become too numerous, competition replaces facilitation. This seems to be the case with aphids and psyllids. There may be an optimum density of psyllids required for aphid facilitation. Future experiments in this system should attempt to quantify the effects of psyllid density on aphid survival.

Interestingly, in these experiments with aphids and psyllids, the psyllids appeared to be superior competitors, while the aphids seemed to out-compete the psyllids in the psyllids colonies with sheer numbers. Based on the fitness comparisons between aphids from favorable and unfavorable hosts, the psyllid colony tomato infestations are not likely the result of one-time introductions of small numbers of aphids. *Myzus persicae* is a highly mobile aphid that disperses at relatively low densities compared to some other aphids (Vehrs et al. 1992). The best explanation for the high population densities of aphids in the psyllids colonies is that aphids are infiltrating the colonies multiple times, amounting to higher overall numbers of aphids. Upon their arrival, if the colony tomato plants are properly conditioned for aphids – if they currently have populations of very young psyllid nymphs, have previously been infested by aphids, and the overall psyllid population is low – the aphids that infiltrate the colonies can survive and increase. However, once those tomato plants decline and are replaced with newer tomato plants, the aphids are unable to effectively colonize the newer plants and thus perish.

CHAPTER IV
SUMMARY

Tomato plant infection with Lso can affect the foraging behavior of *Hippodamia convergens*. In a 2-choice olfactometer bioassay, beetles significantly preferred the odors of uninfected plants to those of infected plants. However, there was no significant difference in odor preference when *B. cockerelli* were present on both uninfected and Lso-infected plants. Beetles did not significantly prefer odors of plants with psyllids to those of plants without psyllids, regardless of plant infection. Beetles did not consume significantly more uninfected versus Lso-infected psyllid nymphs in a no-choice feeding bioassay. Taken together, these data suggest that *B. cockerelli* receives neither direct nor indirect protection from *H. convergens* due to its association with Lso. However, more work needs to be done to determine if these findings are applicable to other natural enemies of *B. cockerelli*.

Myzus persicae is able to survive on tomato plants due to the presence of *B. cockerelli* and not plant infection by Lso. Plants with young psyllid nymphs supported higher mean numbers of aphids than plants with older nymphs or adults. In addition, aphid populations increased on plants whose psyllid populations were predominantly young nymphs. Aphid populations persisted for 30 days or longer on plants with predominantly young psyllid nymphs. However, the effect of the length of psyllids infestation on aphid performance was confounded by psyllid life stage, plant age, previous aphid infestation, and possibly psyllid density.

REFERENCES

Acar, E. B., J. C. Medina, M. L. Lee and G. M. Booth (2001). Olfactory behavior of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). The Canadian Entomologist **133**(03): 389-397.

Agarwala, B. (2007). Phenotypic plasticity in aphids (Homoptera: Insecta): Components of variation and causative factors. Current science **93**(3): 308-313.

Aguilar, E., V. G. Sengoda, B. Bextine, K. F. McCue and J. E. Munyaneza (2013a). First report of "*Candidatus* Liberibacter solanacearum" on tobacco in Honduras. Plant Disease. http://dx.doi.org/10.1094/PDIS-04-13-0453-PDN.

Aguilar, E., V. G. Sengoda, B. Bextine, K. F. McCue and J. E. Munyaneza (2013b). First report of "*Candidatus* Liberibacter solanacearum" on tomato in Honduras. Plant Disease. http://dx.doi.org/10.1094/PDIS-04-13-0354-PDN.

Al-Jabar, A. (1999). Integrated pest management of tomato/potato psyllid, *Paratrioza cockerelli* (Sulc) (Homoptera, Psyllidae) with emphasis on its importance in greenhouse grown tomatoes. PhD Dissertation, (Internet download). Colorado State University, Fort Collins, CO. p.89.

Alfaro-Fernandez, A., M. C. Cebrian, F. J. Villaescusa, A. H. de Mendoza, J. C. Ferrandiz, et al. (2012a). First report of '*Candidatus* Liberibacter solanacearum' in carrot in mainland Spain. Plant Disease **96**(4): 582-582.

Alfaro-Fernandez, A., F. Siverio, M. C. Cebrian, F. J. Villaescusa and M. I. Font (2012b). '*Candidatus* Liberibacter solanacearum' associated with *Bactericera trigonica*-affected carrots in the Canary Islands. Plant Disease **96**(4): 581-582.

Anderson, J. A. D., G. P. Walker, P. A. Alspach, M. Jeram and P. J. Wright (2013). Assessment of susceptibility to zebra chip and *Bactericera cockerelli* of selected potato cultivars under different insecticide regimes in New Zealand. American Journal of Potato Research **90**(1): 58-65.

Belliure, B., A. Janssen, P. C. Maris, D. Peters and M. W. Sabelis (2005). Herbivore arthropods benefit from vectoring plant viruses, Wiley-Blackwell. **8:** 70-79.

Belliure, B., M. W. Sabelis and A. Janssen (2010). Vector and virus induce plant responses that benefit a non-vector herbivore. Basic and Applied Ecology **11**(2): 162-169.

Bextine, B., E. Aguilar, A. Rueda, C. O., V. G. Sengoda, et al. (2013a). First report of "*Candidatus* Liberibacter solanacearum" on tomato in El Salvador. Plant Disease **97**(9): 1245-1245.

Bextine, B., A. Arp, E. Flores, E. Aguilar, L. Lastrea, et al. (2013b). First report of Zebra Chip and '*Candidatus* Liberibacter solanacearum' on potatoes in Nicaragua. Plant Disease **97**(8): 1109-1109.

Brown, A., M. Simmonds and W. Blaney (1999). Influence of species of host plants on the predation of thrips by *Neoseiulus cucumeris*, *Iphiseius degenerans* and *Orius laevigatus*. Entomologia Experimentalis Et Applicata **92**(3): 283-288.

Brown, J. K., M. Rehman, D. Rogan, R. R. Martin and A. M. Idris (2010). First report of "*Candidatus* Liberibacter psyllaurous" (synonym "*Ca*. L. solanacearum") associated with 'Tomato Vein-Greening' and 'Tomato Psyllid Yellows' diseases in commercial greenhouses in Arizona. Plant Disease **94**(3): 376-376.

Brunissen, L., A. Cherqui, Y. Pelletier, C. Vincent and P. Giordanengo (2009). Host-plant mediated interactions between two aphid species. Entomologia Experimentalis Et Applicata **132**(2): 209-209.

Buchman, J. L., T. W. Fisher, V. G. Sengoda and J. E. Munyaneza (2012). Zebra chip progression: from inoculation of potato plants with Liberibacter to development of disease symptoms in tubers. American Journal of Potato Research **89**(2): 159-168.

Buchman, J. L., V. G. Sengoda and J. E. Munyaneza (2011). Vector transmission efficiency of Liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. Journal of Economic Entomology **104**(5): 1486-1495.

Burke, G., O. Fiehn and N. Moran (2010). Effects of facultative symbionts and heat stress on the metabolome of pea aphids. Isme Journal **4**(2): 242-252.

Butler, C. D., F. J. Byrne, M. L. Keremane, R. F. Lee and J. T. Trumble (2011a). Effects of insecticides on behavior of adult *Bactericera cockerelli* (Hemiptera:

Triozidae) and transmission of "*Candidatus* Liberibacter psyllaurous". Journal of Economic Entomology **104**(2): 586-594.

Butler, C. D., B. Gonzalez, K. L. Manjunath, R. F. Lee, R. G. Novy, et al. (2011b). Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), to potato germplasm and transmission of *Candidatus* Liberibacter psyllaurous. Crop Protection **30**(9): 1233-1238.

Butler, C. D. and J. T. Trumble (2012a). Identification and impact of natural enemies of *Bactericera cockerelli* (Hemiptera: Triozidae) in Southern California. Journal of Economic Entomology **105**(5): 1509-1519.

Butler, C. D., G. P. Walker and J. T. Trumble (2012b). Feeding disruption of potato psyllid, *Bactericera cockerelli*, by imidacloprid as measured by electrical penetration graphs. Entomologia Experimentalis Et Applicata **142**(3): 247-257.

Calvo, D. and A. Fereres (2011). The performance of an aphid parasitoid is negatively affected by the presence of a circulative plant virus. Biocontrol **56**(5): 747-757.

Cardoza, Y. J., P. E. A. Teal and J. H. Tumlinson (2003). Effect of peanut plant fungal infection on oviposition preference by *Spodoptera exigua* and on host-searching behavior by *Cotesia marginiventris*. Environmental Entomology **32**(5): 970-976.

Casteel, C. L., A. K. Hansen, L. L. Walling and T. D. Paine (2012). Manipulation of plant defense responses by the tomato psyllid (*Bactericerca cockerelli*) and its associated endosymbiont *Candidatus* Liberibacter psyllaurous. Plos One **7**(4): e35191.

Chongrattanameteekul, W., J. E. Foster and J. E. Araya (1991). Biological interactions between the cereal aphids *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) (Hom., Aphididae) on wheat. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie **111**(3): 249-253.

Christiansen-Weniger, P., G. Powell and J. Hardie (1998). Plant virus and parasitoid interactions in a shared insect vector/host. Entomologia Experimentalis Et Applicata **86**(2): 205-213.

Crosslin, J. M., P. B. Hamm, J. E. Eggers, S. I. Rondon, V. G. Sengoda, et al. (2012a). First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Oregon and Washington State. Plant Disease **96**(3): 452-453.

Crosslin, J. M., J. E. Munyaneza, J. K. Brown and L. W. Liefting (2010). Potato zebra chip disease: a phytopathological tale. Online. Plant Health Progress doi: 10.1094/PHP-2010-0317-01-RV.

Crosslin, J. M., N. Olsen and P. Nolte (2012b). First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Idaho. Plant Disease **96**(3): 453-453.

Das, A., S.-H. Lee, T. Hyun, S.-W. Kim and J.-Y. Kim (2013). Plant volatiles as method of communication. Plant Biotechnology Reports **7**(1): 9-26.

Davis, T. S., D. R. Horton, J. E. Munyaneza and P. J. Landolt (2012). Experimental infection of plants with an herbivore-associated bacterial endosymbiont influences herbivore host selection behavior. Plos One **7**(11): e49330.

Denno, R. F., M. S. Mcclure and J. R. Ott (1995). Interspecific interactions in phytophagous insects: competition reexamined and resurrected. Annual Review of Entomology **40**: 297-331.

Denno, R. F., M. A. Peterson, C. Gratton, J. A. Cheng, G. A. Langellotto, et al. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. Ecology **81**(7): 1814-1827.

Dicke, M. and I. T. Baldwin (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. Trends in Plant Science **15**(3): 167-175.

Dugravot, S., L. Brunissen, E. Letocart, W. F. Tjallingii, C. Vincent, et al. (2007). Local and systemic responses induced by aphids in *Solanum tuberosum* plants. Entomologia Experimentalis Et Applicata **123**(3): 271-277.

Ebbert, M. A. and L. R. Nault (1994). Improved overwintering ability in *Dalbulus maidis* (Homoptera, Cicadellidae) vectors infected with *Spiroplasma kunkelii* (Mycoplasmatales, Spiroplasmataceae). Environmental Entomology **23**(3): 634-644.

Eigenbrode, S. D., H. J. Ding, P. Shiel and P. H. Berger (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera : Aphididae). Proceedings of the Royal Society B-Biological Sciences **269**(1490): 455-460.

Forrest, J. M. S. (1971). Growth of *Aphis fabae* as an indicator of nutritional advantage of galling to apple aphid *Dysaphis devecta*. Entomologia Experimentalis Et Applicata **14**(4): 477-483.

French-Monar, R. D., A. F. Patton, J. M. Douglas, J. A. Abad, G. Schuster, et al. (2010). First report of "*Candidatus* Liberibacter solanacearum" on field tomatoes in the United States. Plant Disease **94**(4): 481-481.

Gencer, N. S., N. A. Kumral, H. O. Sivritepe, M. Seidi, H. Susurluk, et al. (2009). Olfactory response of the ladybird beetle *Stethorus gilvifrons* to two preys and herbivore-induced plant volatiles. Phytoparasitica **37**(3): 217-224.

Gianoli, E. (2000). Competition in cereal aphids (Homoptera: Aphididae) on wheat plants. Environmental Entomology **29**(2): 213-219.

Gudmestad, N. C. and G. A. Secor (2007). Zebra chip: a new disease of potato. Nebr. Potato Eyes **19**(1): 1-4.

Gwynn, D. M., A. Callaghan, J. Gorham, K. F. A. Walters and M. D. E. Fellowes (2005). Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. Proceedings of the Royal Society B-Biological Sciences **272**(1574): 1803-1808.

Hail, D., S. E. Dowd and B. Bextine (2012). Identification and location of symbionts associated with potato psyllid (*Bactericera cockerelli*) lifestages. Environmental Entomology **41**(1): 98-107.

Haine, E. R. (2008). Symbiont-mediated protection. Proceedings of the Royal Society B-Biological Sciences **275**(1633): 353-361.

Hamilton, R. M., E. B. Dogan, G. B. Schaalje and G. M. Booth (1999). Olfactory response of the lady beetle *Hippodamia convergens* (Coleoptera: Coccinellidae) to prey related odors, including a scanning electron microscopy study of the antennal sensilla. Environmental Entomology **28**(5): 812-822.

Hansen, A. K., G. Jeong, T. D. Paine and R. Stouthamer (2007). Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. Applied and Environmental Microbiology **73**(23): 7531-7535.

Hansen, A. K. and N. A. Moran (2011). Aphid genome expression reveals host-symbiont cooperation in the production of amino acids. Proceedings of the National Academy of Sciences of the United States of America **108**(7): 2849-2854.

Hansen, A. K., J. T. Trumble, R. Stouthamer and T. D. Paine (2008). A new huanglongbing species, "*Candidatus* Liberibacter psyllaurous," found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Applied and Environmental Microbiology **74**(18): 5862-5865.

Heil, M. and R. Karban (2010). Explaining evolution of plant communication by airborne signals. Trends in Ecology & Evolution **25**(3): 137-144.

Henne, D. C., F. Workneh and C. M. Rush (2012). Spatial patterns and spread of potato zebra chip disease in the Texas Panhandle. Plant Disease **96**(7): 948-956.

Hodge, S. and G. Powell (2008). Complex interactions between a plant pathogen and insect parasitoid via the shared vector-host: consequences for host plant infection. Oecologia **157**(3): 387-397.

Hoffmann, M. P. and A. Frodsham (1993). Natural enemies of vegetable insect pests. Cornell University, Ithaca, NY (USA). p.63.

Kaplan, I. and R. F. Denno (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecology Letters **10**(10): 977-994.

Kessler, A. and I. T. Baldwin (2002). Plant responses to insect herbivory: The emerging molecular analysis. Annual Review of Plant Biology **53**: 299-328.

Kikuchi, Y., M. Hayatsu, T. Hosokawa, A. Nagayama, K. Tago, et al. (2012). Symbiont-mediated insecticide resistance. Proceedings of the National Academy of Sciences of the United States of America **109**(22): 8618-8622.

Lacey, L. A., F. de la Rosa and D. R. Horton (2009). Insecticidal activity of entomopathogenic fungi (Hypocreales) for potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae): Development of bioassay techniques, effect of fungal species and stage of the psyllid. Biocontrol Science and Technology **19**(9): 957-970.

Lacey, L. A., T. X. Liu, J. L. Buchman, J. E. Munyaneza, J. A. Goolsby, et al. (2011). Entomopathogenic fungi (Hypocreales) for control of potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) in an area endemic for zebra chip disease of potato. Biological Control **56**(3): 271-278.

Levy, J., A. Ravindran, D. Gross, C. Tamborindeguy and E. Pierson (2011). Translocation of '*Candidatus* Liberibacter solanacearum', the Zebra Chip pathogen, in potato and tomato. Phytopathology **101**(11): 1285-1291.

Liefting, L. W., P. W. Sutherland, L. I. Ward, K. L. Paice, B. S. Weir, et al. (2009a). A new '*Candidatus* Liberibacter' species associated with diseases of solanaceous crops. Plant Disease **93**(3): 208-214.

Liefting, L. W., B. S. Weir, S. R. Pennycook and G. R. G. Clover (2009b). '*Candidatus* Liberibacter solanacearum', associated with plants in the family Solanaceae. International Journal of Systematic and Evolutionary Microbiology **59**: 2274-2276.

Ling, K. S., H. Lin, M. L. L. Ivey, W. Zhang and S. A. Miller (2011). First report or '*Candidatus* Liberibacter solanacearum' naturally infecting tomatoes in the state of Mexico, Mexico. Plant Disease **95**(8): 1026-1027.

Liu, D., J. T. Trumble and R. Stouthamer (2006). Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera cockerelli*) into western North America. Entomologia Experimentalis Et Applicata **118**(3): 177-183.

Luft, P. A., T. D. Paine and R. A. Redak (2001). Limiting the potential for intraspecific competition: regulation of *Trioza eugeniae* oviposition on unexpanded leaf tissue. Ecological Entomology **26**(4): 395-403.

Mayer, C. J., A. Vilcinskas and J. Gross (2008). Pathogen-induced release of plant allomone manipulates vector insect behavior. Journal of Chemical Ecology **34**(12): 1518-1522.

McClure, M. S. (1981). Effects of voltinism, interspecific competition and parasitism on the population dynamics of the hemlock scales, *Fiorinia externa* and *Tsugaspidiotus tsugae* (Homoptera: Diaspididae). Ecological Entomology **6**(1): 47-54.

McCornack, B., R. Koch and D. Ragsdale (2007). A simple method for in-field sex determination of the multicolored Asian lady beetle *Harmonia axyridis*. 12pp. Journal of Insect Science **7**:10, available online: insectscience.org/7.10

Moayeri, H. S., A. Ashouri, H. Brødsgaard and A. Enkegaard (2006). Odour-mediated responses of a predatory mirid bug and its prey, the two-spotted spider mite. Experimental & Applied Acarology **40**(1): 27-36.

Moran, N. A. and T. G. Whitham (1990). Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. Ecology **71**(3): 1050-1058.

Müller, C. B., I. S. Williams and J. Hardie (2001). The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecological Entomology **26**(3): 330-340.

Munyaneza, J. E. (2010). Psyllids as vectors of emerging bacterial diseases of annual crops. Southwestern Entomologist **35**(3): 471-477.

Munyaneza, J. E. (2012). Zebra chip disease of potato: biology, epidemiology, and management. American Journal of Potato Research **89**(5): 329-350.

Munyaneza, J. E., J. L. Buchman, V. G. Sengoda, T. W. Fisher and C. C. Pearson (2011). Susceptibility of selected potato varieties to zebra chip potato disease. American Journal of Potato Research **88**(5): 435-440.

Munyaneza, J. E., J. L. Buchman, J. E. Upton, J. A. Goolsby, J. M. Crosslin, et al. (2008). Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtropical Plant Science **60**: 27-37.

Munyaneza, J. E., J. M. Crosslin and J. E. Upton (2007). Association of *Bactericera cockerelli* (Homoptera : Psyllidae) with "zebra chip," a new potato disease in southwestern United States and Mexico. Journal of Economic Entomology **100**(3): 656-663.

Munyaneza, J. E., V. G. Sengoda, E. Aguilar, B. Bextine and K. F. McCue (2013a). First report of "*Candidatus* Liberibacter solanacearum" associated with psyllid-infested tobacco in Nicaragua. Plant Disease **97**(9): 1244-1244.

Munyaneza, J. E., V. G. Sengoda, E. Aguilar, B. Bextine and K. F. McCue (2013b). First report of "*Candidatus* Liberibacter solanacearum" on pepper in Honduras. Plant Disease. http://dx.doi.org/10.1094/PDIS-06-13-0598-PDN.

Munyaneza, J. E., V. G. Sengoda, E. Aguilar, B. Bextine and K. F. McCue (2013c). First report of "*Candidatus* Liberibacter solanacearum" infecting eggplant in Honduras. Plant Disease. http://dx.doi.org/10.1094/PDIS-06-13-0641-PDN.

Munyaneza, J. E., V. G. Sengoda, J. L. Buchman and T. W. Fisher (2012). Effects of temperature on '*Candidatus* Liberibacter solanacearum' and zebra chip potato disease symptom development. Plant Disease **96**(1): 18-23.

Munyaneza, J. E., V. G. Sengoda, J. M. Crosslin, J. A. Garzon-Tiznado and O. G. Cardenas-Valenzuela (2009a). First report of "*Candidatus* Liberibacter solanacearum" in pepper plants in Mexico. Plant Disease **93**(10): 1076-1076.

Munyaneza, J. E., V. G. Sengoda, J. M. Crosslin, J. A. Garzon-Tiznado and O. G. Cardenas-Valenzuela (2009b). First report of "*Candidatus* Liberibacter solanacearum" in tomato plants in Mexico. Plant Disease **93**(10): 1076-1076.

Murphy, A. F., S. I. Rondon and A. S. Jensen (2013). First report of potato psyllids, *Bactericera cockerelli*, overwintering in the Pacific Northwest. American Journal of Potato Research **90**(3): 294-296.

Nachappa, P., J. Levy, E. Pierson and C. Tamborindeguy (2011). Diversity of endosymbionts in the potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), vector of zebra chip disease of potato. Current Microbiology **62**(5): 1510-1520.

Nachappa, P., A. A. Shapiro and C. Tamborindeguy (2012). Effect of '*Candidatus* Liberibacter solanacearum' on fitness of its insect vector, *Bactericera cockerelli* (Hemiptera: Triozidae), on tomato. Phytopathology **102**(1): 41-46.

Nelson, W. R., T. W. Fisher and J. E. Munyaneza (2011). Haplotypes of "Candidatus Liberibacter solanacearum" suggest long-standing separation. European Journal of Plant Pathology **130**(1): 5-12.

Nelson, W. R., V. G. Sengoda, A. O. Alfaro-Fernandez, M. I. Font, J. M. Crosslin, et al. (2013). A new haplotype of "*Candidatus* Liberibacter solanacearum" identified in the Mediterranean region. European Journal of Plant Pathology **135**(4): 633-639.

Nissinen, A. I., A. Lemmetty, J. M. Pihlava, L. Jauhiainen, J. E. Munyaneza, et al. (2012). Effects of carrot psyllid (*Trioza apicalis*) feeding on carrot yield and content of sugars and phenolic compounds. Annals of Applied Biology **161**(1): 68-80.

Ohgushi, T. (2005). Indirect interaction webs: Herbivore-induced effects through trait change in plants. Annual Review of Ecology Evolution and Systematics **36**: 81-105.

Oliver, K. M., P. H. Degnan, M. S. Hunter and N. A. Moran (2009). Bacteriophages encode factors required for protection in a symbiotic mutualism. Science **325**(5943): 992-994.

Pellegrino, A. C., M. F. G. V. Peñaflor, C. Nardi, W. Bezner-Kerr, C. G. Guglielmo, et al. (2013). Weather forecasting by insects: modified sexual behaviour in response to atmospheric pressure changes. Plos One **8**(10): e75004.

Peng, L. N., J. T. Trumble, J. E. Munyaneza and T. X. Liu (2011). Repellency of a kaolin particle film to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae), on tomato under laboratory and field conditions. Pest Management Science **67**(7): 815-824.

Rehman, M., J. C. Melgar, J. M. Rivera, A. M. Idris and J. K. Brown (2010). First report of "*Candidatus* Liberibacter psyllaurous" or "*Ca*. Liberibacter solanacearum" associated with severe foliar chlorosis, curling, and necrosis and tuber discoloration of potato plants in Honduras. Plant Disease **94**(3): 376-377.

Rostas, M., J. Ton, B. Mauch-Mani and T. C. J. Turlings (2006). Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naive parasitoids. Journal of Chemical Ecology **32**(9): 1897-1909.

Sauge, M. H., J. P. Lacroze, J. L. Poessel, T. Pascal and J. Kervella (2002). Induced resistance by *Myzus persicae* in the peach cultivar 'Rubira'. Entomologia Experimentalis Et Applicata **102**(1): 29-37.

Sauge, M. H., F. Mus, J. P. Lacroze, T. Pascal, J. Kervella, et al. (2006). Genotypic variation in induced resistance and induced susceptibility in the peach - *Myzus persicae* aphid system. Oikos **113**(2): 305-313.

Scarborough, C. L., J. Ferrari and H. C. J. Godfray (2005). Aphid protected from pathogen by endosymbiont. Science **310**(5755): 1781-1781.

Secor, G. A., V. V. Rivera, J. A. Abad, I. M. Lee, G. R. G. Clover, et al. (2009). Association of '*Candidatus* Liberibacter solanacearum' with zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Disease **93**(6): 574-583.

Sengonca, C. and J. Kranz (2001). A modified, four-armed olfactometer for determining olfactory reactions of beneficial arthropods. Anzeiger für Schädlingskunde = Journal of Pest Science **74**(5): 127-132.

Settle, W. H. and L. T. Wilson (1990). Invasion by the variegated leafhopper and biotic interactions: parasitism, competition, and apparent competition. Ecology **71**(4): 1461-1470.

Stachowicz, J. J. (2001). Mutualism, facilitation, and the structure of ecological communities. Bioscience **51**(3): 235-246.

Vehrs, S. L. C., 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 chrysanthemums. Journal of Economic Entomology **85**(3): 799-807.

Villanueva, R. T. and G. Esparza-Diaz (2012). Psyllids, whiteflies and environmental factors affecting potato trials in S. Texas: 2010-2012. In: Workneh F., Rashed A., Rush C.M., eds. Proceedings of the 12th Annual SCRI Zebra Chip Reporting Sesssion, October 30-November 2, 2012, San Antonio, TX, p.163.

Warner, K. A. and P. Bierzychudek (2009). Does marking with fluorescent powders affect the survival or development of larval *Vanessa cardui*? Entomologia Experimentalis Et Applicata **131**(3): 320-324.

Wen, A., I. Mallik, V. Alvarado, J. Pasche, X. Wang, et al. (2009). Incidence, distribution, and genetic variations of '*Candidatus* Liberibacter sp.' associated with zebra chip of potato in North America. Phytopathology **99**(6): S140-S140.

Wise, M. J., D. L. Kieffer and W. G. Abrahamson (2006). Costs and benefits of gregarious feeding in the meadow spittlebug, *Philaenus spumarius*. Ecological Entomology **31**(5): 548-555.

Wuriyanghan, H. and B. W. Falk (2013). RNA interference towards the potato psyllid, *Bactericera cockerelli*, is induced in plants infected with recombinant tobacco mosaic virus (TMV). Plos One **8**(6): e66050.

Xu, J., K. K. Lin and S. S. Liu (2011). Performance on different host plants of an alien and an indigenous *Bemisia tabaci* from China. Journal of Applied Entomology **135**(10): 771-779.

APPENDIX
INTRAGUILD INTERACTION BETWEEN THE POTATO PSYLLID AND TWO SPECIES OF APHIDS ON BELL PEPPER AND POTATO

Introduction

Predator-prey interactions, as well as interactions between herbivores, can be largely mediated by host plant quality. The outcomes of these interactions vary among herbivores, and among different host plant species being fed upon. For this reason, it was initially proposed that coccinellid predation of *B. cockerelli* and intraguild interactions among psyllids and two species of aphids (*Myzus persicae* and *Macrosiphum euphorbiae*) be studied on three hosts: potato, bell pepper, and tomato. Although this proved unfeasible, some data related to aphid-psyllid interactions was collected from potato and bell pepper that may be relevant to future studies.

Materials and Methods

Fitness Bioassay on Potato and Bell Pepper

Bell pepper (cv. Calwonder) was cultivated in the same manner as tomato in Chapter 2, but were transplanted into 3.5” round pots. Potato (cv. Atlantic) was grown from tuber propagules. Tubers were quartered using a steak knife, making sure that each quarter contained at least one eye, and planted in the same media used for tomato in 1-gallon round garden pots. Propagules were watered sparingly, once per week or less, to ensure the tubers did not rot. Stems were individually transplanted to 8.5” round garden pots when at least 3 true leaves had unfurled. Stems were watered once per week or less, depending on soil moisture.

The methodology closely follows that of the tomato fitness bioassay in Chapter 3. Three week-old bell pepper and potato plants were infected with Lso in the manner described in Chapter 2, using nymphs from either the infected “C2” or “P2” colonies.

Plant samples were collected and tested for Lso at the end of the experiment, or when an experimental plant began to decline, so there was no way of knowing beforehand if plants were infected or not. DNA extraction and PCR were performed as described in Chapter 2. Plants in the infected treatments that did not test positive for Lso even after performing PCR on the first PCR product were excluded from analysis. Two weeks after the end of IAP, a same-age cohort of *Myzus persicae* or *Macrosiphum euphorbiae* nymphs was obtained for use in the experiment by allowing adult apterae to nymphoposit on either bell pepper (*Myzus*) or potato (*Macrosiphum*) for a 24-hour period.

Eighteen days after the end of IAP, twelve bell pepper plants (three replicates per treatment) were individually housed in the mesh cube cages described in Chapter 3, and were randomly assigned to one of three shelves as described for tomato in Chapter 3. Potato plants were loosely encased in plastic cylinders. Each cylinder was made from four-to-six 8.5" x 11" transparency films (3M, PP2500) bound together with masking tape. Small holes were made using a push-pin to encircle the cylinders at one end, to provide ventilation. The tops of the cylinders were covered with thrips-proof mesh (BioQuip 7261A) held in place with four micro binder clips (Wal-Mart, WMCR-BC1-70). Due to their size, potatoes could only be placed on one tall shelf, where their positions were randomly assigned.

Treatments were the same as those described in Table 3.1 (Chapter 3). Plants requiring psyllids were infested with ten 3^{rd}-4^{th} instar nymphs 3 days prior to the experiment. Each plant was infested with five adult *Macrosiphum* (potato) or *Myzus* (bell pepper). Counts of the numbers of aphid adults and nymphs were made daily for a one-week period, and mean numbers of aphid adults and nymphs per treatment were analyzed using the Kruskal-Wallis rank sums test in JMP 9.0.

Aphid Life History Experiment on Bell Pepper and Potato

Treatments were the same as in Chapter 3: uninfected and infected. Unlike the fitness bioassay above, plants were sampled prior to performing this experiment, but

were not tested for Lso until after the experiment. Plants in the infected treatment that did not test positive for Lso even after performing PCR on the first PCR product were excluded from analysis. Each treatment group of plants was housed in a separate 14" x 24" rectangular mesh cage (BioQuip 1466B).

Adult *Macrosiphum euphorbiae* or *Myzus persicae* apterae were collected from potato or bell pepper and confined for 24 hours to one leaf per plant using 1" diameter foam clip cages (BioQuip 1458). Then, the adults and all but one 1st-instar nymph were removed from the cages. (The potato experiment differed in that focal nymphs were not confined using clip cages; instead, each potato plant was enshrouded with a sleeve cage tied around the stem of the plant with white yarn (Peaches & Cream, worsted 4-ply, color 01005, 262001) at one end, and tied closed above the top of the plant with more yarn to confine the aphids. This was done based on results of the pepper life history experiment, in which many nymphs escaped from the clip cages. The sleeve cages were much more effective at containing aphids, although it was more difficult to locate exuvia in these larger cages.)

The focal nymphs were monitored every other day, and the number of surviving aphids and their instars were recorded until death of the aphids. Exuvia was retrieved from the clip cages using a fine paintbrush in order to ascertain molts, although molt data were not analyzed.

The experiment on pepper took place in the same location as the fitness bioassay. The experiment on potato took place in two locations. In October 2012, a growth chamber (Percival) operating at a constant temperature of 23° C and a 16:8 L:D photoperiod, was used in order to prevent plant infestation by western flower thrips, *Frankliniella occidentalis*, which had become endemic. In February 2013, when thrips were less of a concern, the potato experiment was carried out on a bench top under fluorescent light.

Survival data were analyzed using the Kaplan Meier log rank test in the Survival Program in StatsToDo (http://www.statstodo.com/Survival_Pgm.php, September 8th, 2013, Allen Chang).

Results

Fitness Bioassay on Potato and Bell Pepper

PCR confirmed that three out of six bell pepper plants and three out of six potato plants used in the infected treatments of the pepper fitness experiment were, in fact, uninfected. Data from these plants were excluded from analysis.

On pepper, mean numbers of *Myzus persicae* adults and nymphs per day were not significantly different across treatments (Table A1).

Table A1. Mean numbers of *Myzus persicae* adults and nymphs per treatment per day in the fitness bioassay on bell pepper

Day	Aphid Stage	Treatment	Mean	X^2	df	Prob>X^2
1	Adult	Uninfected	3.3333	3.7078	3	0.2948
		Infected	6.0			
		Uninfected+Psyllids	5.0			
		Infected+Psyllids	4.0			
	Nymph	Uninfected	7.0	1.2520	3	0.7406
		Infected	15.0			
		Uninfected+Psyllids	13.6667			
		Infected +Psyllids	12.0			
2	Adult	Uninfected	5.0	7.0000	3	0.0719
		Infected	5.5			
		Uninfected+Psyllids	5.0			
		Infected +Psyllids	4.3333			
	Nymph	Uninfected	26.3333	4.5823	3	0.2051
		Infected	25.5			
		Uninfected+Psyllids	30.0			
		Infected +Psyllids	29.3333			
3	Adult	Uninfected	4.6667	3.3704	3	0.3380
		Infected	2.5			
		Uninfected+Psyllids	4.6667			
		Infected +Psyllids	4.3333			
	Nymph	Uninfected	35.3333	3.5556	3	0.3136
		Infected	22.5			
		Uninfected+Psyllids	48.6667			
		Infected+Psyllids	39.3333			
4	Adult	Uninfected	3.6667	5.0502	3	0.1682
		Infected	2.5			
		Uninfected+Psyllids	4.6667			
		Infected+Psyllids	4.0			
	Nymph	Uninfected	38.3333	4.4418	3	0.2175
		Infected	23.0			
		Uninfected+Psyllids	62.0			
		Infected+Psyllids	55.0			

Table A1. Continued

Day	Aphid Stage	Treatment	Mean	X^2	df	Prob>X^2
5	Adult	Uninfected	3.3333	6.5214	3	0.0888
		Infected	2.5			
		Uninfected+Psyllids	5.0			
		Infected+Psyllids	4.3333			
	Nymph	Uninfected	41.3333	6.3333	3	0.0965
		Infected	36.5			
		Uninfected+Psyllids	69.0			
		Infected+Psyllids	64.3333			
6	Adult	Uninfected	6.0	6.0325	3	0.1100
		Infected	9.0			
		Uninfected+Psyllids	10.3333			
		Infected+Psyllids	12.6667			
	Nymph	Uninfected	48.3333	4.8889	3	0.1801
		Infected	29.0			
		Uninfected+Psyllids	65.3333			
		Infected+Psyllids	60.0			
7	Adult	Uninfected	12.6667	3.2610	3	0.3531
		Infected	16.5			
		Uninfected+Psyllids	21.6667			
		Infected+Psyllids	22.6667			
	Nymph	Uninfected	38.6667	4.1111	3	0.2497
		Infected	25.0			
		Uninfected+Psyllids	50.6667			
		Infected+Psyllids	49.0			

On potato, mean numbers of adults and nymphs were not significantly different across treatments (Table A2). Some aphids were able to escape from one cage in the uninfected treatment due to the plant growing up through the mesh at the top of the cage. These aphids then managed to enter another cage in the infected+psyllids treatment one day later.

Table A2. Mean numbers of *Macrosiphum euphorbiae* adults and nymphs per treatment per day in the fitness bioassay on potato

Day	Aphid Stage	Treatment	Mean	X^2	df	Prob>X^2
1	Adult	Uninfected	3.3333	5.4720	3	0.1403
		Infected	3.0			
		Uninfected+Psyllids	3.6667			
		Infected+Psyllids	5.0			
	Nymph	Uninfected	1.6667	6.1572	3	0.1042
		Infected	7.0			
		Uninfected+Psyllids	2.6667			
		Infected+Psyllids	5.5			
2	Adult	Uninfected	3.6667	3.2389	3	0.3562
		Infected	4.0			
		Uninfected+Psyllids	4.0			
		Infected+Psyllids	5.0			
	Nymph	Uninfected	4.0	3.1484	3	0.3693
		Infected	18.0			
		Uninfected+Psyllids	12.0			
		Infected+Psyllids	11.0			
3	Adult	Uninfected	3.6667	0.9167	3	0.8214
		Infected	4.0			
		Uninfected+Psyllids	4.3333			
		Infected+Psyllids	6.0			
	Nymph	Uninfected	10.0	3.1373	3	0.3709
		Infected	24.0			
		Uninfected+Psyllids	18.0			
		Infected+Psyllids	22.0			
4	Adult	Uninfected	3.0	1.0101	3	0.7988
		Infected	3.0			
		Uninfected+Psyllids	4.0			
		Infected+Psyllids	5.5			
	Nymph	Uninfected	22.3333	0.5552	3	0.9066
		Infected	24.0			
		Uninfected+Psyllids	32.3333			
		Infected+Psyllids	29.0			
5	Adult	Uninfected	2.6667	2.1470	3	0.5425
		Infected	1.0			
		Uninfected+Psyllids	4.0			
		Infected+Psyllids	3.0			
	Nymph	Uninfected	20.6667	3.5128	3	0.3191
		Infected	30.0			
		Uninfected+Psyllids	38.0			
		Infected+Psyllids	67.0			
6	Adult	Uninfected	4.3333	0.9237	3	0.8197
		Infected	2.0			
		Uninfected+Psyllids	4.3333			
		Infected+Psyllids	2.5			
	Nymph	Uninfected	26.6667	2.5860	3	0.4600
		Infected	38.0			
		Uninfected+Psyllids	49.3333			
		Infected+Psyllids	52.5			
7	Adult	Uninfected	5.0	0.9769	3	0.8068
		Infected	3.0			
		Uninfected+Psyllids	5.0			
		Infected+Psyllids	4.5			
	Nymph	Uninfected	40.0	2.0199	3	0.5683
		Infected	44.0			
		Uninfected+Psyllids	63.6667			
		Infected+Psyllids	54.0			

Aphid Life History Experiment on Bell Pepper and Potato

There were no significant differences in aphid survival across treatments on either host (Table A3).

Table A3. Statistics for the life history bioassays on bell pepper and potato

Host	Aphid	Treatment	Mean Survival (Days)	X^2	df	Prob>X^2
Bell Pepper	*Myzus persicae*	Uninfected	8.50	0.8681	1	0.3515
		Infected	13.67			
Potato	*Macrosiphum euphorbiae*	Uninfected	25.91	1.1506	1	0.2834
		Infected	17.50			

Discussion

The fitness bioassays with *M. persicae* on bell pepper and *M. euphorbiae* on potato did not indicate that the presence of psyllids or plant infection with Lso facilitates survival of either aphid on either host (Tables A1&2). Some *M. euphorbiae* escaped from one cage and entered another during the potato experiment, which did not happen during the bell pepper experiment with *M. persicae*. Before it can be said that the presence of psyllids, alone or in conjunction with Lso plant infection, does not affect *M. euphorbiae*, sufficient barriers to aphid escape must be found.

The life history bioassay showed no significant differences in survival of either aphid on its respective host across treatments (Table A3). Western flower thrips colonized potato plants in October 2012, in spite of their being in a growth chamber, and the plant damage they caused may have affected the performance of the potato aphid.