

Risk and efficacy in biological control: an evaluation of the aphid parasitoid  
*Aphelinus certus* in North America

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## **Dedication**

To my parents, Ruth E. Frary Kaser and Steve W. Kaser

## Abstract

Invasive species are causing increasing harm to native biodiversity, ecosystems, agriculture, and other natural resources. Classical biological control is a powerful and cost-effective strategy for long-term invasive species management. However, while importation of biological control agents has many potential benefits, it also entails risks, such as harm to non-target native species. Therefore, candidate biological control agents are studied prior to release to predict their safety. Little is known, however, about how traits affecting the safety of biological control agents may also impact their efficacy in terms of reducing invasive pest populations. In this dissertation, I investigate the interacting causes and consequences of risk and efficacy in biological control. I approach this investigation first from a theoretical standpoint, including a literature review and a mathematical modeling framework. I then investigate the aphid parasitoid *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) which attacks the invasive soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). In the early 2000s, *Aphe. certus* was evaluated as a potential classical biological control agent of the soybean aphid, but it was precluded from release due to concerns over ecological safety. However, the parasitoid was accidentally introduced anyway, and thus provides an interesting case study to evaluate the causes of and consequences of biological control risk and efficacy.

Chapter 1 of this dissertation provides a literature review and synthesis of the potential impacts of natural enemy-mediated indirect effects on both risk and efficacy in biological control. Polyphagous natural enemies cause various indirect interactions between their prey/host populations. These indirect interactions may be reciprocally

negative (i.e. apparent competition), but can be any combination of positive, negative, or neutral. I focus on parasitoids to illustrate the importance of natural enemy-mediated indirect interactions in biological control risk-benefit assessment.

In Chapter 2, I utilize a mathematical modeling framework to investigate direct and indirect interactions between a generalized biological control agent, and its target and non-target hosts. I use Nicholson-Bailey form difference equations to simulate a one-parasitoid two-host system, and I evaluate conditions under which biological control safety and efficacy interact. Apparent competition can have important benefits for increasing biological control efficacy, even at low levels of non-target impact. However, under conditions of parasitoid egg limitation, high attack rates on resistant non-target hosts can dramatically decrease biological control efficacy while concurrently increasing non-target risk. These findings are discussed in the context of biological control agent pre-release risk-benefit assessment. The code for an interactive application of this model is provided as supplementary material for Chapter 2, and is presented in the Appendix.

In Chapter 3, I investigate the potential for perennial biofuel plantings to enhance biological control of the soybean aphid by parasitoids. Cultivated biofuels provide an important source of renewable energy, and may provide additional ecosystem services, such as enhanced natural enemy communities and increased biological control of pests in neighboring crops. I conducted a large-scale randomized experiment to test for effects of biofuel plantings on biological control of the soybean aphid in surrounding soybean fields. There was no significant effect of any biofuel treatment on the parasitoid community or on soybean aphid density compared to controls. However, the experiment

coincidentally captured the early colonization stages of the introduced aphid parasitoid *A. certus* in Minnesota. *Aphelinus certus* is increasing in density in Minnesota soybean fields, and this increase corresponds with a decrease of resident Aphidiinae parasitoids over a three-year period.

In Chapter 4, I present an evaluation of the host range of *A. certus*. I exposed *A. certus* to 17 species of aphid hosts in no-choice tests. *Aphelinus certus* attacked most species presented to it, but mummification rates and adult emergence were highly variable. I mapped host use data onto an aphid phylogeny to see if host phylogeny predicted host use. *Aphelinus certus* mummification significantly clustered with host relatedness ( $P=0.043$ ). Several native aphid species are shown to be at risk of attack by *A. certus*. Additionally, several pest aphid species present in North America may also be subjected to biological control by *A. certus*.

In Chapter 5, I evaluate the biological control impact of *A. certus* on the soybean aphid. The observed increase of *A. certus* prevalence in North American cultivated soybeans does not necessarily indicate that the parasitoid is substantially contributing to soybean aphid biological control. I conducted an exclusion-cage experiment designed to isolate the impact of parasitoids compared to other resident natural enemies of the soybean aphid. I found that *A. certus* greatly outnumbered all other soybean aphid parasitoids, and that it significantly reduced soybean aphid populations over a time span of less than two weeks compared to controls ( $P<0.0001$ ). Moreover, parasitoids alone resulted in aphid densities that were statistically equivalent to the combined effect of predators and parasitoids ( $P=0.95$ ). These results indicate that *A. certus* is an important



new component of the soybean aphid natural enemy community in North America.

*Aphelinus certus* provides an informative example of a biological control agent that is effective but also risky in terms of non-target impact to native species.

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## CHAPTER 1

### HIDDEN RISKS AND BENEFITS OF NATURAL ENEMY-MEDIATED INDIRECT EFFECTS

#### *Chapter Abstract*

Polyphagous natural enemies can mediate a variety of indirect interactions between resource populations. Such indirect interactions are often reciprocally negative (i.e. apparent competition), but the sign of effects between resource populations can be any combination of positive (+), negative (-), or neutral (0). In this article we focus on parasitoids to illustrate the importance of natural enemy-mediated indirect interactions in predicting risk and efficacy in biological control. We review recent findings to illustrate how an improved understanding of parasitoid behavioral ecology may increase model accuracy.

#### *1.1 Introduction*

In the 1925 publication ‘Elements of Physical Biology’, Alfred J. Lotka noted “a singularly interesting conclusion”: when a second resource population was added to a consumer-resource model, the first resource population could be driven to extinction (Lotka 1925). Such an interaction could occur if, for example, the second resource increases the carrying capacity of the consumer such that it completely consumes the first resource, the preferred prey. This result was due to what is now referred to as “apparent

competition” – i.e. a reciprocally negative indirect interaction between resource populations mediated by a shared consumer (Fig 1) (Holt 1977). Since Lotka’s early theoretical work, it has become clear that apparent competition is important in structuring many ecological communities (Jeffries and Lawton 1984; Wootton 1994; Holt and Lawton 1994; van Veen et al. 2006; Borer et al. 2007). However, natural enemy-mediated indirect interactions between resource populations (hereafter referred to as ‘enemy-mediated interactions’) are not always reciprocally negative (-,-) (Chailleux et al. 2014); they may result in apparent parasitism (-,+), (Hoogendoorn and Heimpel 2002), amensalism (0,-) (Bonsall and Hassell 1998), commensalism (0,+) (Nouhuys and Kraft 2011), or mutualism (+,+) (Frago and Godfray 2013). In this article we focus on parasitoids to review some of the latest research on enemy-mediated interactions. We argue that a community framework incorporating enemy-mediated interactions would aid ecological management (Memmott 2009), and these interactions have particular importance for both safety and efficacy in biological control (Holt and Hochberg 2001; Kaser and Heimpel 2015).

## *1.2 Background*

Some important characteristics and complexities of enemy-mediated interactions are illustrated by the now classic study of Settle and Wilson (Settle and Wilson 1990). The invasive variegated leafhopper *Erythroneura variabilis* (Hemiptera: Cicadellidae) was found to displace regionally populations of the native *E. elegantula* in California. This displacement was not caused by resource competition between leafhoppers, which

was roughly symmetrical both intra- and interspecifically. Rather, displacement occurred as a result of asymmetrical apparent competition mediated by the shared egg parasitoid *Anagrus epos* (Hymenoptera: Mymaridae) (Settle and Wilson 1990). The *A. epos*-mediated invasion of *E. variabilis* occurred in two different phases: 1) In the first phase, *A. epos* reduced *E. elegantula* densities, thereby alleviating resource competition between leafhoppers and allowing initial establishment of *E. variabilis*. 2) After local establishment of *E. variabilis*, the density of *A. epos* increased, causing increased parasitism of leafhoppers. Because *A. epos* attacked *E. elegantula* at a higher rate than *E. variabilis*, *E. elegantula* was displaced (Settle and Wilson 1990).

The leafhopper - *Anagrus* study demonstrates the importance of enemy-mediated effects for determining community structure. Additionally, by linking these experimental observations with theory, we can highlight three points important to the study of enemy-mediated interactions. First, it should be noted that the negative enemy-mediated effect of *E. variabilis* on *E. elegantula* is an effect on population density; that is, an increase in the population density of *E. variabilis* indirectly causes a decrease in the density of *E. elegantula*. However, the sign of interaction between populations [positive (+), negative (-), or neutral (0)] need not necessarily be measured in terms of population density. Interaction effects are also commonly measured in terms of population growth rate, individual fitness, and theoretically can be measured for any population level phenomenon (Abrams 1987). When interpreting and synthesizing experimental findings and theoretical developments on indirect effects, it is important to be clear about how the sign of interaction is measured (Abrams 2001). The same point can be made for how the

indirect effect is mediated by the natural enemy – i.e. is the effect due to an increase in density of the natural enemy, or due to some other factor? Furthermore, it is important to differentiate between density-mediated and trait-mediated indirect effects (Desneux et al. 2009). Density-mediated indirect effects are exemplified in the apparent competition model introduced by Lotka (Lotka 1925) and refined by Holt (Holt 1977), where the population density of the natural enemy is increased by one resource population thereby allowing for increased consumption of a different resource population. Apparent competition in the *E. variabilis* invasion is density mediated. Trait-mediated indirect effects, on the other hand, involve behavioral changes in one or more of the interacting populations. An example of this is in the avoidance behavior of the polyphagous parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in the presence of the intraguild predator *Coccinella septempunctata* (Coleoptera: Coccinellidae). This behavioral change results in an indirect mutualism between prey/host aphids *Acyrtosiphon pisum* and *Sitobion avenae* when both natural enemies are present (Frago and Godfray 2013).

A second feature of enemy-mediated interactions illustrated by the *A. epos* – leafhopper system is that the signs and strengths of the interaction may vary in space and time (Settle and Wilson 1990). The first phase of the *E. variabilis* invasion is perhaps better described as apparent amensalism (-,0) rather than apparent competition. There is an indirect negative effect of *E. elegantula* on *E. variabilis*, but little or no effect of *E. variabilis* on *E. elegantula*. Until *E. variabilis* reaches greater than 50% of the total leafhopper population, its contribution to the parasitoid population is very small. Thus, the density of *A. epos* is largely determined by the resident *E. elegantula* population

during the first phase. It is only later, in the second phase of invasion, when *E. variabilis* has increased the parasitoid's equilibrium population size that a reciprocally negative interaction (i.e. apparent competition) is observed.

The asymmetry of the apparent competition in the *E. elegantula*–*E. variabilis* system leads to a third point worth illustrating. Theory suggests that coexistence of resource populations experiencing apparent competition requires that  $r_i > a_i P$  (i.e. the intrinsic rate of increase,  $r$ , of resource population  $i$  must be greater than the attack rate,  $a$ , by the shared consumer on resource  $i$ , multiplied by the average density of the shared consumer,  $P$ ) (Holt 1977; Holt and Lawton 1994). Therefore, changes to intrinsic growth rates (e.g. via reduced resource competition between leafhopper species), changes in the density of the shared consumer (e.g. via arrival of a new resource population), or changes in attack rate (via functional response or other factors influencing host preference) could tip the balance between coexistence and exclusion. By increasing the density of *A. epos*, the ensuing *E. elegantula* mortality overwhelmed its intrinsic rate of increase. However, because the attack rate of *A. epos* on the invasive *E. variabilis* was sufficiently small, *E. variabilis* could persist.

The attack rate,  $a$ , represents many aspects of parasitoid searching behavior and host use. Host use is multifaceted (Desneux et al. 2009) with search efficiency, host suitability, and the relative contribution of egg and time limitation all known to influence attack rate (Getz and Mills 1996; Heimpel et al. 1998; Heimpel et al. 2003; Dieckhoff and Heimpel 2010; Dieckhoff et al. 2014). Moreover, host preference factors may be dynamic and host suitability can be mediated by the abiotic environment (Frewin et al.

2014) as well the internal microbiome of the host (McLean and Godfray 2015). In most interspecific interactions, the effect of density of one species on the growth rate of a second species is likely to be a nonlinear process (Abrams 2001). A key to developing better predictive models is to identify the form of functions describing these interactions (Abrams 2001). Recent breakthroughs in our understanding of the factors that shape enemy-mediated interactions promise to make predictions more accurate. In the remainder of this article we review recent research on enemy-mediated interactions in classical biological control. We focus on parasitoids, which represent about 75% of the classical biological control introductions against arthropod pests (Mills 2000), and we highlight the advantages of a community framework incorporating enemy-mediated interactions for evaluating biological control outcomes.

### *1.3 Case studies in biological control*

#### 1.3.1 Natural enemy-mediated effects and risk

Classical biological control involves the intentional importation and establishment of non-native natural enemy populations to control target non-native pests. Proponents of classical biological control point to its low cost-benefit ratio, broad spatial scale of control, and limited adverse environmental effects, while critics emphasize a history of non-target impacts on native species (Louda et al. 2003a; Messing and Wright 2006; Barratt et al. 2010; Hinz et al. 2014). Since the 1990s, attention to ecological risk has resulted in regulatory limits on the kinds of natural enemies that can be released in classical biological control programs (Van Driesche and Reardon 2004; Messing and

Wright 2006; Barratt et al. 2010). Biological control practitioners now focus on agents with narrow host ranges (i.e. agents that only attack one or a few phylogenetically related species), and this practice limits the likelihood of adverse impacts to non-target species (Fowler et al. 2000). However, if quarantine experiments on host use are not evaluated with the species they will encounter in their introduced habitat in a community context, researchers may miss risks due to indirect interactions between classical biological control agents and other resident species (Fowler et al. 2000). For example, the host-specific weed biological control agent *Mesoclanis polana* (Diptera: Tephritidae) was released in Australia against the target plant, the bitou bush *Chrysanthemoides monilifera* ssp. *rotundata*. Although *M. polana* has not effectively controlled the Bitou Bush, it has become well established and reaches high population densities where its target weed is present. *Mesoclanis polana* is a specialist herbivore and does not cause non-target impacts via direct consumptive effects on other plants. However, it is attacked by multiple generalist natural enemies in the introduced range, including at least seven species of parasitoids that attack related native herbivores (Carvalho et al. 2008). As a result, an enemy-mediated indirect interaction occurs between the biological control agent (*M. polana*) and several species of native herbivores (Fig 2) (Carvalho et al. 2008). Depending on the strength of this interaction over time, there is a risk that these native species may be driven extinct. Additionally, some native parasitoids that do not parasitize *M. polana* are negatively associated with *M. polana* abundance, likely due to increased exploitation competition with generalist natural enemies (Fig 2) (Carvalho et al. 2008). Recalling the theoretical rule for coexistence of apparent competitors ( $r_i > a_i P$ )

(Holt 1977; Holt and Lawton 1994), these findings highlight the importance of efficacy in the practice of classical biological control, because without a reduction in the pest density, a specialist biological control agent can maintain a large population which becomes a resource for higher trophic levels capable of multiple adverse indirect effects. The *M. polana* system suggest that biological control agents that become established in the area of introduction but which are ineffective at reducing the density of the target species, may remain at high densities and cause adverse indirect impacts via resource enrichment (Carvalho et al. 2008).

Given the discussion above, the decision whether to release a classical biological control agent should consider not only the likelihood of harm but also the likelihood of effective control of the target pest (McEvoy and Coombs 1999). Looking at releases of predators and parasitoids targeting arthropods prior to 1990, Greathead and Greathead (1992) estimated that “satisfactory control” of the target pest occurred in only 517 out of 4769 releases (~11%), and about three times that many (1445) became established in the introduced range. It should be noted, however, that in recent years major methodological and regulatory changes have occurred in the practice of classical biological control, so the criteria used by Greathead and Greathead (1992) may no longer be a sufficient reference to estimate recent successes. Nevertheless, efforts to increase efficacy in classical biological control are needed. Some authors have suggested that an overemphasis on risk may preclude effective biological control agents from release (Hinz et al. 2014). If efforts to increase safety by releasing specialist natural enemies reduces the rate at which



established biological control agents effectively control the target species, we may be replacing risks at one trophic level for risks at another.

### 1.3.2 Biological control efficacy and enemy-mediated effects

Establishment of classical biological control agents and effective pest suppression can also be inhibited by intraguild predation and attack from higher trophic levels (Fig 2). Hyperparasitoids can suppress populations of primary parasitoids, thereby interfering with effective herbivore population control (Chacón et al. 2008; Paynter et al. 2010; Schooler et al. 2011; Frago et al. 2012). In the Glanville fritillary butterfly system in Finland, two primary parasitoids, *Cotesia melitaerum* and *C. glomerata* (Hymenoptera: Braconidae), were experimentally shown to engage in apparent competition mediated by the wingless hyperparasitoid *Gelis agilis* (Hymenoptera: Ichneumonidae) (van Nouhuys and Hanski 2000). As the authors point out, however, this study represents an experimental combination of species which is unlikely to be found in nature; *C. glomerata* is a parasitoid of host species that do not naturally occur in the study area containing the Glanville fritillary butterfly and *C. melitaerum* habitat patches.

Generally speaking, the forms and frequency of occurrence of indirect effects mediated by hyperparasitoids are unclear. A field experiment to look for evidence of apparent competition between *Aphidius ervi* and *A. rhopalosiphi* (Hymenoptera: Braconidae) mediated by a community of generalist hymenopteran hyperparasitoids (families Megaspilidae, Pteromalidae, and Encyrtidae) found only weak support for indirect interactions (Morris et al. 2001). In addition to the obvious technical difficulties

of simultaneously manipulating four trophic levels, the generation time of hyperparasitoids is typically longer than that of their hosts, which may make enemy-mediated interactions particularly difficult to assess, especially if the effects predicted at equilibrium occur with time lags of several years (Holt and Lawton 1994). Better estimates of the functional relationships between the changes in density of one resource population and per capita growth rates are needed if we are to better match theoretical predictions with empirical findings (Abrams 2001). Research in to the potentially dynamic nature of host preference and parasitoid functional response would aid in this endeavor.

In another hyperparasitoid study, Acebes and Messing (2013) investigated two established classical biological control agents in Hawaii, the parasitoids *Binodoxys communis* and *A. colemani* (Hymenoptera: Braconidae), both introduced against the melon aphid *Aphis gossypii* (Hemiptera: Aphididae). Field observations indicated that *A. colemani* maintains higher population density (Acebes and Messing 2013). Both primary parasitoids are attacked by the hyperparasitoid *Syrphophagus aphidovorius* (Hymenoptera: Encyrtidae), and the authors hypothesized that asymmetrical apparent competition via differential hyperparasitism may explain this difference in primary parasitoid population density. However, *A. colemani* appears to be the more suitable host for *S. aphidovorius*, and in a host choice experiment, more hyperparasitoids emerged from *A. colemani* than *B. communis* (Acebes and Messing 2013). Recalling the *Anagrus* – leafhopper system, the more susceptible host was displaced by the less susceptible host (Settle and Wilson 1990). To explain this apparent discrepancy between theory and data,

the authors proposed that the earlier introduction of *A. colemani* to Hawaii, and/or local metapopulation factors like differential immigration and emigration could be important. We suggest an additional factor; in the presence of egg limitation and high attack rates on both hosts, asymmetric host resistance can generate apparent parasitism favoring the less resistant host (i.e. *A. colemani*) (Hoogendoorn and Heimpel 2002; Heimpel et al. 2003; Kaser and Heimpel 2015). This unintuitive result occurs because the more resistant host (i.e. *B. communis*) serves as an egg sink, thereby reducing parasitoid equilibrium density and thus increasing the equilibrium density of the less resistant host. In many systems, parasitoids attack at high rates hosts that are suboptimal for development (Heimpel et al. 2003; Desneux et al. 2009). In the case of *A. epos*, the difference in host susceptibility was due to differences in how the two leafhoppers oviposited in the host-plant; *E. variabilis* inserted its eggs more deeply in the plant tissue, providing some protection from attack (Settle and Wilson 1990). That is, the asymmetry in host mortality occurred because of an asymmetry in attack rate by *A. epos* not because of asymmetry in the innate resistance of the host. However, in the *S. aphidovor* – primary parasitoid system, the difference in host susceptibility between *A. colemani* and *B. communis* appears to be due to the fact that *B. communis* is a suboptimal host resulting in poor hyperparasitoid development. Supporting this, *S. aphidovor* produced a more male biased sex ratio when developing in *B. communis* (Acebes and Messing 2013). It would be interesting to evaluate if *S. aphidovor* becomes egg limited in Hawaii, and if hyperparasitism of partially resistant *B. communis* results in an egg sink effect.

Differential indirect effects of egg versus time limitation in primary parasitoids and in hyperparasitoids has emerged as a potentially important factor in determining the outcome of enemy-mediated interactions (Getz and Mills 1996; Hoogendoorn and Heimpel 2002; Heimpel et al. 2003; Kaser and Heimpel 2015). Kaser and Heimpel (2015) used a simple difference equation model with one parasitoid and two hosts to demonstrate the effects of parasitoid searching efficiency, host suitability, and egg versus time limitation on the safety and efficacy in biological control. Apparent competition can maximize biological control of the target species at intermediate levels of non-target impact, but in egg limited parasitoids, non-target attack can result in apparent parasitism that reduces biological control efficacy (Kaser and Heimpel 2015).

### 1.3.3 Exploiting enemy-mediated effects

Parasite- and parasitoid-mediated indirect interactions are ubiquitous components of biotic resistance to invasion in natural systems (Dunn et al. 2012). In a literature review, Kelly et al. 2009) found that native parasites and parasitoids attack 67% of non-native animals across a wide taxonomic range of hosts. Efforts to exploit polyphagous, native natural enemies are common in conservation biological control and in banker planting systems (Frank 2010; McClure and Frank 2015; Jaworski et al. 2015). Use of “lying-in-wait” predators (Murdoch et al. 1985) in conservation biological control is a clear example of exploiting enemy-mediated interactions to control pest populations. Efforts to provide alternative host plants to increase attack on target hosts are common and have proved repeatedly to be a successful pest management strategy. For example,

strips of clover, alfalfa, ryegrass, and meadow fescue provide habitat for alternative aphid hosts of generalist aphid parasitoids (Langer and Hance 2004). The presence of these alternate hosts increased parasitism of pest aphids in adjacent wheat crops (Langer and Hance 2004). Enemy-mediated interactions have been shown to be more important than interspecific resource competition in structuring an insect herbivore community in wheat, sweet pea, and semi-natural habitats at an experimental farm in Belgium. In this case, stinging nettle aphids in the semi-natural habitat supported a large population of generalist parasitoids which could then attack pest species in adjacent crop habitat (Alhmedi et al. 2011).

As the above examples show, enemy-mediated interactions can clearly enhance biological control of target species in conservation biological control and banker planting systems. Historically, enemy-mediated interactions have been exploited in classical biological control as well. For example, in the 20<sup>th</sup> century, the parasitoids *Blepharipa pratensis* and *Compsilura concinnata* (Diptera: Tachinidae) were released in anticipation of the gypsy moth (Lepidoptera: Erebidae) (Boettner et al. 2000; Kimberling 2004). Both parasitoids were intentionally established on native North American saturniid moths in hopes that they would create greater biotic resistance in northeastern North American forests in case the gypsy moth was to arrive. In retrospect of course, this strategy failed to control gypsy moth, which is still a persistent forest pest. In fact, gypsy moth in North America represents perhaps one of the more dismal examples of classical biological control of arthropods gone awry; over 50 species of biological control agents have been released in North America, of which none have been successful at controlling the moth

(Kimberling 2004). Of course, the practice of establishing a non-native biological control agent on native hosts prior to the arrival of the target pest would not be acceptable today due to concerns of ecological harm. Indeed, *C. concinnata* has caused serious declines of native saturniids (Boettner et al. 2000). Interestingly, however, *C. concinnata* is attributed with suppressing another pest, the brown-tail moth (Lepidoptera: Lymantriidae) (Elkinton et al. 2006; Elkinton and Boettner 2012). In the early 20<sup>th</sup> century, the brown-tail moth was considered one of the most dangerous invasive forest pests in North America (Elkinton and Boettner 2012). Currently, brown-tail moth escapes parasitism by *C. concinnata* only in a few coastal areas of low plant diversity where alternate early season lepidopteran hosts (e.g. gypsy moth) are scarce, suggesting that these hosts are critical to successful biological control by the parasitoid (Elkinton et al. 2006).

#### *1.4 Conclusions and future directions*

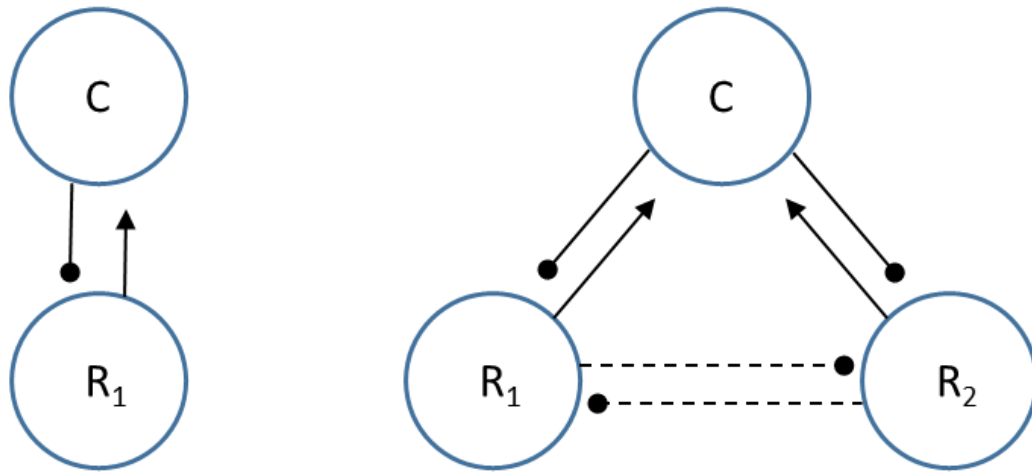
What factors cause *C. concinnata* to suppress the brown-tail moth, but not the gypsy moth? On the surface, we might expect both pest species to suffer equally from apparent competition, but as stated earlier, interspecific interactions are typically nonlinear and asymmetrical (Abrams 2001). A community framework for evaluating applied ecological questions such as biological control has several advantages (Holt and Lawton 1994; Shea and Chesson 2002; Memmott 2009; Tack et al. 2011). Unfortunately, the signs (positive, negative, or neutral) and strengths of enemy-mediated interactions are infrequently measured, and population-level outcomes remain difficult to predict in the field. To increase predictive capacity, improved understanding of functional relationships

between population density of resource and consumer populations as well as birth and death parameters are needed (Kaiser and Heimpel 2016). A fruitful direction for future research in parasitoid biological control is in measuring distinct components of host range, such as the rate parasitoid host encounter and oviposition, and host suitability for parasitoid development (Heimpel et al. 2003; Desneux et al. 2009). In the simple rule  $r_i > a_i P$  (Holt 1977; Holt and Lawton 1994),  $a$  represents many aspects of host search and development; determining the form and important elements of the attack function will improve predictive power.

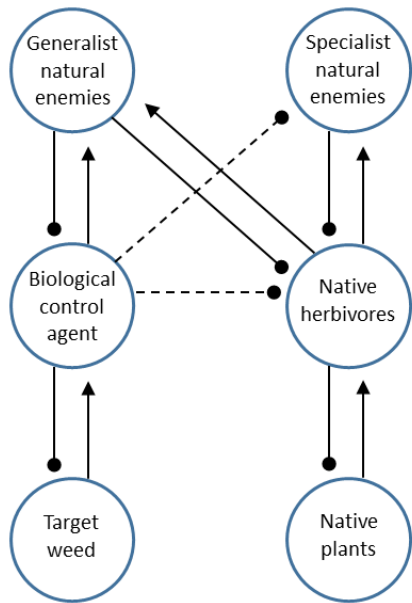
Another emerging factor seen as critical to biological control success is the differential impact of landscape complexity on biological control services. Increased landscape complexity (i.e. more non-crop areas and greater diversity of habitat types) has been shown to increase biological control of agricultural pests (Gardiner et al. 2009; Tscharrntke et al. 2012). The ‘intermediate landscape complexity hypothesis’ suggests that attempts to increase local (e.g. on-farm) complexity to increase biological control services will work best in landscapes of intermediate complexity (Tscharrntke et al. 2012; Jonsson et al. 2015). However, generalist and specialist natural enemies respond asymmetrically to habitat complexity. Generalists move at higher rates between agricultural habitat and unmanaged lands, and this difference is driven by the availability of alternative hosts/prey (Frost et al. 2015). Interestingly, some evidence suggests that hyperparasitism may be more strongly influenced by landscape complexity than is primary parasitism (Rand et al. 2012), likely because most hyperparasitoids have a broader host range than their primary parasitoid hosts. However, empirical support

demonstrating the trend of hyperparasitoids being more generalist than primary parasitoids is limited at best (Brodeur 2000). Host plants of target and non-target arthropod species can also influence the strength of enemy-mediated interactions (van Veen 2015). High patch connectivity (i.e. the frequency of individual movement between habitat patches) may be increased due to plant invasions (Bezemer et al. 2014). Increased connectivity may in turn increase variability of both herbivore and parasitoid densities, and increase local subpopulation extinction rates - with higher extinction rates for parasitoids than for their herbivore hosts (Cronin and Haynes 2004). Support is building for the hypothesis that parasitoids typically experience a more fragmented habitat than their hosts (Cronin and Haynes 2004), and for the notion that the release of biological control agents should consider how the habitat matrix experienced by the agent affects its population stability (Bezemer et al. 2014). Improved understanding of how enemy-mediated interactions interact with landscape complexity and variable biodiversity is needed.





**Figure 1.** Levins' diagrams (Levins 1974) showing a single consumer-single resource model (left), and a single consumer-two resource model with apparent competition occurring between resource populations. Solid lines represent direct interactions, and dashed lines represent indirect interactions; arrows represent positive effects on density, and circles represent negative effects on density.



**Figure 2.** A partial Levins' diagram (Levins 1974) representing a theoretical community similar to the weed biological control agent *Mesoclanis polana* and interacting species (Carvalho et al. 2008). Solid lines represent direct interactions, and dashed lines represent indirect interactions; arrows represent positive effects, and circles represent negative effects. Both indirect effects indicated are caused by the biological control agent increasing the density of the generalist natural enemies (via resource enrichment); the generalists directly consume native herbivores and engage in exploitation competition with the specialist natural enemies.

## CHAPTER 2

### LINKING RISK AND EFFICACY IN BIOLOGICAL CONTROL HOST-PARASITOID MODELS

#### *Chapter Abstract*

Exotic invasive arthropods present an increasing threat to native species, ecosystem function, crop production, forests, and other natural resources. Importation ('classical') biological control can be a cost-effective tactic for long-term pest management that reduces insecticide use. However, while importation of biological control agents has great potential benefits, it also entails risks to non-target native species. Therefore, candidate biological control agents are studied prior to release to predict safety. Little is known, however, about how traits affecting the safety of biological control agents may impact their efficacy in terms of reducing pest populations. We use a difference equation model to simulate a one-parasitoid two-host system and evaluate conditions under which biological control safety and efficacy interact. We vary the search efficiency and resistance to parasitism of both host species and interpret the results from the standpoint that one host is a target pest and the other a non-target species. We find that apparent competition can have important benefits for increasing biological control efficacy, even at low levels of non-target impact. However, under conditions of parasitoid egg limitation, high attack rates on resistant non-target hosts can dramatically decrease biological control efficacy while concurrently increasing non-target risk. These

findings are discussed in the context of biological control agent pre-release risk-benefit assessment.

## *2.1 Introduction*

Classical biological control is an important strategy for controlling the effects of invasive species over broad spatial scales while potentially minimizing risk of adverse consequences (Thomas and Willis 1998; Messing and Wright 2006). There are examples where classical biological control agents have caused harm to native biota (Louda et al. 2003b), but there are also many cases of biological control agents safely and effectively controlling pest populations (Hoddle 2004; Van Driesche et al. 2010; Clercq et al. 2011). Debate over how best to identify and value harms versus benefits in ecological risk assessment is an ongoing conversation in society (Nelson et al. 2009). One role of scientists is to provide stakeholders with the most precise and accurate predictions possible about potential ecological effects of management decisions (e.g. likelihood and magnitude of pest control, and likelihood and magnitude of impact to non-target populations) (Bigler and Köelliker-Ott 2006). Improved decision-making tools that allow managers to better predict both safety and efficacy of candidate biological control agents are needed (Thomas and Reid 2007).

No biological control agent release is without risk, and agents should be selected by simultaneously considering both risks and potential benefits (McEvoy and Coombs 1999; Heimpel et al. 2004; Kriticos et al. 2009). Minimizing harms while maximizing benefits is not straightforward, however, if organismal traits that increase biological

control efficacy are the same traits that increase the risk of harm to non-target species (Ehler 2000). For example, predators and parasitoids with generalist feeding habits may pose higher risk to non-target organisms than do specialists (Simberloff and Stiling 1996); however, a broader host range might also facilitate establishment of biological control agents, influence stability of the agent-target interaction, or increase suppression of target pests (Murdoch et al. 1985; Symondson et al. 2002). As an illustration of this, a number of studies have shown that the presence of alternative prey or host species can enhance biological control through apparent competition (Murdoch et al. 1985; Langer and Hance 2004; Chailleux et al. 2014). Apparent competition can also be detrimental to native species, however (Noonburg and Byers 2005; Borer et al. 2007). For example, rapid population decline of the native green leafhopper, *Erythroneura elegantula*, following the introduction of the variegated leafhopper, *E. variabilis*, in central California, U.S.A. was likely the result of apparent competition mediated by the shared parasitoid *Anagrus epos*, rather than due to direct competition for resources between hosts (Settle and Wilson 1990).

There is not necessarily a trade-off between risk and efficacy, however, and many highly effective biological control agents may be low-risk to non-target organisms. For example, specialists may have a faster numerical response to pest outbreaks and they have a lower likelihood of engaging in intraguild predation than do generalists (Snyder and Ives 2001). A specialist biological control agent may be both safe to non-target species and highly effective at controlling the target pest (Symondson et al. 2002; Kimberling 2004). The renowned *Rodolia cardinalis*, a specialist predator of cottony

cushion scale, *Icerya purchasi*, has been a classical biological control success in many parts of the world where it has been introduced (Caltagirone and Doult 1989). In the Galapagos Islands, where *R. cardinalis* is unable to complete its life cycle on any of the local fauna besides the invasive *I. purchasi* (Causton et al. 2004), it has successfully established and is reducing *I. purchasi* by 60 to 98% (Hoddle et al. 2013).

Laboratory host specificity testing, which estimates the physiological host range of a biological control agent (i.e. the number of species that the agent could complete development on if attacked), provides perhaps the most important evidence used to predict post-release host range (i.e. the ecological host range within the region of importation) (Pemberton 2000; Van Driesche and Reardon 2004; J.C. van Lenteren et al. 2006; Gilbert and Webb 2007). The physiological host range can include species that are not preferred by the biological control agent but that, when attacked, are suitable for the agent to complete development. The ecological host range is the suite of suitable hosts that are actually attacked in the field; thus, the ecological host range may often be narrower than the physiological host range (e.g. Morehead and Feener 2000; Haye et al. 2005). However, insects that are completely unsuitable for agent development are consistently attacked in some cases (Heimpel et al. 2003; Desneux et al. 2009). Laboratory and field studies can supplement host range information and improve predictions about the ecological host range upon introduction (e.g. Wyckhuys et al. 2008; Wyckhuys et al. 2009; Desneux et al. 2009); however, without an appropriate model for host-parasitoid interaction, these studies tell us little about the likely magnitude of impact

on non-target field populations. Moreover, host range testing tells us little about how non-target species may influence target pest suppression.

Cases where a biological control agent attacks both a target and a non-target species may result in apparent competition (Holt and Lawton 1994), but indirect interactions between host populations may also be +/- (apparent parasitism) (Hoogendoorn and Heimpel 2002), +/+ (apparent mutualism) (Abrams and Matsuda 1996), or any other combination of positive, negative, or neutral (Fig 3). To understand the complexity of outcomes of these potential indirect interactions, and to develop hypotheses for empirical testing, we examined a mathematical model describing two hosts and a shared parasitoid. Previous studies have utilized simple mathematical models to make qualitative predictions about transient risk (Lynch et al. 2002), and risk at equilibrium (Holt and Hochberg 2001) of biological control agents. Building on this work, we suggest that a model for interpreting pre-release data should evaluate both direct and indirect interactions between the biological control agent and resource populations so that the potential interplay between both harm and benefit can be assessed.

The objective of this paper is to model and evaluate conditions under which the population of a non-target host would affect a target host population indirectly via a shared parasitoid, and also to examine what conditions are associated with impacts to non-target hosts upon release of a parasitoid. We pay particular attention to model representations of physiological host range versus ecological host range and the different effects these components may have on biological control efficacy and non-target impact. We evaluate these parameters within the context of pre-release risk-benefit screening.

## 2.2 The Model

We ran simulations using a discrete-time Nicholson-Bailey form consumer-resource model described by Heimpel et al. (2003) (Equation set 1).

$$\begin{aligned} H_{1,t+1} &= e^{r_1(1-\frac{H_{1,t}}{K_1})} H_{1,t} (1-s_1(1-f[E_{1,t}])) \\ H_{2,t+1} &= e^{r_2(1-\frac{H_{2,t}}{K_2})} H_{2,t} (1-s_2(1-f[E_{2,t}])) \\ P_{t+1} &= H_{1,t}s_1(1-f[E_{1,t}]) + H_{2,t}s_2(1-f[E_{2,t}]) \end{aligned} \quad [1]$$

where  $f[E_{i,t}]$  is the escape function for the host species,  $H1$  and  $H2$ , which are attacked by a parasitoid,  $P$ . Host species  $i$  reproduces at the intrinsic rate of increase,  $r_i$ , adjusted by a density-dependent intraspecific competition term, wherein  $K_i$  is the carrying capacity of each host. The hosts do not directly compete interspecifically, and this absence of interspecific competition between host populations describes a common situation in arthropod biological control programs, where one host is a crop pest and the non-target host feeds on a non-crop plant. Host suitability,  $s_i$ , is the proportion of attacked hosts that die and become parasitoids in the next generation. A suitability value of 1.0 represents a host with no physiological resistance to the parasitoid; a suitability value equal to 0.0 describes a host with complete resistance to the parasitoid. Suitability could, for example, represent the outcome of an all-or-nothing encapsulation response of the host (i.e. the proportion of immature parasitoids that escape encapsulation) (Godfray and Hassell 1993). The proportion of hosts that are attacked but do not die (i.e.  $1-s_i(1-f(E_{i,t}))$ ), survive to the next generation and are able to reproduce.



The escape function (Equation set 2) assumes a Type II functional response of the parasitoid. Aggregation of the parasitoid to the host follows a negative binomial distribution (as  $k \rightarrow 0$  host encounters by the parasitoid become increasingly aggregated; as  $k \rightarrow \infty$  parasitoid encounter becomes random). The maximum fecundity of the parasitoid,  $\beta$ , represents the maximum egg load of a solitary parasitoid (Getz and Mills 1996; Heimpel 2000; Heimpel et al. 2003). The parasitoid is egg-limited when parasitoid search efficiency,  $a_i$ , approaches infinity; it becomes search-limited as  $\beta$  approaches infinity. In other words, the degree of egg limitation increases as  $\beta$  decreases.

$$f(E_{t_i}) = \left(1 + \frac{E_{t_i}}{k}\right)^{-k} \quad [2]$$

$$E_{t_i} = \frac{a_i \beta P_i}{\beta + a_i H_i + a_2 H_2}$$

This model is useful for exploring the interaction between non-target risk and biological control efficacy for several reasons. First, it was designed to investigate apparent competition in a parasitoid-host system, and allows us to examine both direct effects of parasitoids on host populations, as well as indirect effects between host populations. Additionally, the model separates out three important parameters that investigators may estimate in the laboratory for a candidate biological control agent prior to release (i.e.  $s_i$ ,  $a_i$ , and  $\beta$ ). No-choice host range tests, used to estimate the physiological host range of a biological control agent, often generate data on the proportion of attacked hosts that are able to complete development, corresponding to  $s_i$  (Van Driesche and

Reardon 2004; Desneux et al. 2009; Hopper et al. 2013). The ecological host range is not only influenced by  $s_i$  (i.e. host suitability) however, but also by parasitoid attack rate on the hosts. Search efficiency,  $a_i$ , is a key parameter in determining attack rate, and thus the ecological host range of the parasitoid. In the model, search efficiency interacts with egg load, which is constrained by  $\beta$ , to determine whether the parasitoid is egg- or time-limited (Heimpel and Rosenheim 1998; Heimpel et al. 1998). Egg limitation is expected to be important when host density is high (i.e. host encounter rate is high), and time-limitation is expected to be important when host densities are low, and these factors affect attack rate, per capita mortality, and allow for various forms of indirect interaction between hosts, depending on parameter combinations (Roitberg 2000; Heimpel et al. 2003).

We designate the target host as  $H1$  and the non-target host as  $H2$ . To explore model implications on risk-benefit analysis in biological control, we examined model output while varying the parameters  $s_2$  and  $a_2$ , and  $\beta$  across a gradient of values that lead to stable population equilibria (see Heimpel et al. (2003) for a discussion of stability criteria, including a sensitivity analysis involving the aggregation parameter  $k$ , and see Getz and Mills (1996) for a broader discussion of the stability constraints for the negative binomial escape function). First, we varied  $s_2$  (non-target suitability) across a gradient from 0.0 to 1.0 (see Heimpel et al. (2003) for some empirically derived suitability values), in increments of 0.01, while maintaining all other parameters constant. We repeated this process for different degrees of egg-limitation (i.e. for  $\beta=500, 50, 10$ , and 5). For each  $s_2$  value, we ran a simulation for 1000 generations to estimate the

equilibrium densities of hosts and the parasitoid ( $H_i^*$  and  $P^*$  respectively). Consumer-resource oscillations ceased or became negligible after ~100-300 generations for the parameter values evaluated (Fig 4 and Fig 1S). For each simulation we calculated the  $q$ -value ( $q=H^*/K$ , where  $H^*$  is host equilibrium density; Beddington et al. 1978). The  $q$ -value is the proportional reduction in host density below the carrying capacity of each host, and is a measure of the degree of biological control effect (for  $H1$ ) or non-target impact (for  $H2$ ) achieved by the parasitoid. To examine the impact of parasitoid search efficiency on biological control risk and benefit, we varied  $a_2$  across a gradient from 0.00 to 0.05, in increments of 0.0005, while keeping all parameters constant, and, again, this is repeated for parasitoids with different degrees of egg-limitation. While the range of  $a_2$  presented here is narrow compared to search values found in the literature (e.g. Hassell et al. 1976), the results are qualitatively the same when higher  $a$  values are used; however, at lower values, the resulting figures are easier to interpret because axes are constrained within a narrower range (see Figs 1S-3S to explore higher  $a_i$  values). We kept the parameters  $a_1 = 0.05$  and  $s_1 = 1.0$  constant at the high end of values because in classical biological control we expect the target host to be more suitable and attacked at a high rate than the non-target. Moreover, the results generated by varying attack and suitability of the host would be symmetrical to findings from varying these parameters for the non-target host. Finally, we re-evaluated the impact of variable  $s_2$  and  $a_2$  under the conditions of a more slowly reproducing non-target host – i.e. reduced intrinsic growth rate of the non-target ( $r_2 = 0.025$  instead of 0.05), reduced non-target carrying capacity ( $K_2 = 50$  instead of 100), and a combined reduction of both  $r_2$  and  $K_2$  (0.0125 and 50, respectively)

All simulations were run in the R version 3.1.0 (R Core Team 2015) and a full sensitivity analysis is presented as an interactive R-based application in the Supplementary Materials.

### 2.3 Model Results

Model simulations result in dampening oscillations of the parasitoid, target, and non-target host through time under a broad range of parameter values (Fig 4 and Fig 1S).

#### 2.3.1 Effects of non-target host suitability

We first examine the effect of non-target ( $H_2$ ) suitability ( $s_2$ ) on biological control efficacy and non-target impact for a time-limited parasitoid ( $r_i = 0.25$ ;  $\beta = 500$ ;  $a_i = 0.05$ ;  $K_i = 100$ ;  $k = 0.75$ ) (Fig 5a). We find that biological control can be greatly improved by the presence of the non-target host with the greatest suppression of the target host occurring at intermediate values of  $s_2$ . This result is due to the effect of the non-target host on parasitoid equilibrium density. At very low values of  $s_2$ , little parasitoid reproduction occurs on the non-target host, and parasitoid equilibrium is roughly the same as when there is no non-target host present. As  $s_2$  increases toward intermediate values, parasitoid equilibrium increases, resulting in greater suppression of the target host. The lowest target host density corresponds with high parasitoid equilibrium density. Under these conditions, parasitoid reproduction is largely influenced by attack and development in the moderately suitable non-target host which has a higher equilibrium density than the target host. As  $s_2$  increases beyond intermediate values, the parasitoid

exploits the non-target host more efficiently, reducing the non-target host equilibrium which in turn reduces the parasitoid equilibrium, resulting in reduced suppression of the target host. For a time-limited parasitoid, for all  $s_2 > 0.0$ , apparent competition occurs between hosts; we see lower densities of target and non-target hosts alike than we would see if only one host population were present (Fig 5a, 1S, and 2S), and this is true for all values of non-target suitability. Unsurprisingly, the non-target host is suppressed the most at the highest levels of  $s_2$ . Across this range of  $s_2$  for a time-limited parasitoid, the presence of the non-target host generated a minimum target  $q$ -value of 0.003, over 81 times lower than in absence of the non-target host (i.e.  $q$ -value of 0.25). The non-target value was reduced to a minimum  $q$ -value of 0.13.

Next, we examine effects of  $s_2$  for increasing levels of egg limitation for the parasitoid (i.e.  $\beta = 50, 10, \text{ and } 5$ ) (Fig 5b-d). Again, we examine the  $q$ -value and parasitoid equilibrium while varying non-target host suitability for an egg-limited parasitoid. The resulting patterns are more complex with highly egg-limited parasitoids than that observed for time-limited parasitoids. For low  $s_2$  values, we see an indirect +/- interaction between host populations, with the target host benefiting from high non-target host densities, and the non-target host declining with increasing target host densities. This “apparent parasitism” occurs because the non-target host population serves as an egg sink which does not produce many new parasitoids due to low suitability values (Heimpel et al., 2003). At higher suitability values, however, the interaction between hosts returns to one of apparent competition. Both the maximal egg-sink effect (i.e. the highest level of “apparent parasitism”) observed and the range of  $s_2$  values for which any loss of target

control occurs decrease with increasing  $\beta$ . Unlike under conditions of time-limitation, however, under egg limitation the highest parasitoid equilibrium values were not symmetrically associated with highest target host suppression. Search efficiency remains high for these egg-limited parasitoids, and they therefore still encounter hosts at a high rate, but without eggs they are not able to oviposit into them. While a large non-target host population drives the parasitoid population to higher densities, parasitoid egg limitation and the egg-sink effect caused by the marginally suitable non-target hosts make the overall risk of parasitism for the target host lower. Across this range of  $s_2$  for an egg-limited parasitoid of  $\beta = 5$ , the presence of the non-target host increased the  $q$ -value of the target host to a maximum of 0.58, and decreased it to a minimum of 0.11, compared to a  $q$ -value of 0.32 in absence of the non-target. The non-target  $q$ -value reached a minimum of 0.17 across this range of  $s_2$  values. Three dimensional representations of parasitoid equilibrium, and  $q$ -values of the target and non-target for variable  $s_2$  and  $\beta$  are available in the supplementary materials (Fig 6a-c).

### 2.3.2 Effects of parasitoid searching efficiency

In this section we examine the impact of varying search efficiency of a time-limited parasitoid on the non-target ( $a_2$ ) from 0.00 to 0.05 (Fig 7a). For a time-limited parasitoid, varying parasitoid search on the non-target has similar effects to varying  $s_2$ . Intermediate values of  $a_2$  drive the parasitoid equilibrium density to its highest value. As we found with varying  $s_2$  for a time-limited parasitoid, as  $a_2$  increases beyond intermediate values, the parasitoid exploits the non-target more efficiently. Again, this

reduces the non-target host equilibrium density which results in a decline in parasitoid equilibrium density and an increased target host equilibrium density (though the target is never higher than when  $a_2 = 0.0$ ). In our simulation, apparent competition occurs between host populations for all stable values of  $a_2 > 0.00$ . Across this range of  $a_2$ , the presence of the non-target host decreased the target  $q$ -value to a minimum of 0.002, compared to 0.25 in absence of the non-target host. The non-target  $q$ -value reached a minimum of 0.13 across this range. Also, as we found with variable  $s_2$  under conditions of time-limitation, the highest parasitoid equilibrium value is associated with the highest target suppression.

We then examined the effects of variable  $a_2$  for different degrees of parasitoid egg-limitation. The results of varying  $a_2$  for an egg-limited parasitoid looked qualitatively similar to a time-limited parasitoid; however, parasitoid equilibrium was lower for the egg-limited parasitoid, and  $q$ -values were higher (Fig. 7b-d). Under no values of  $a_2$  do we see apparent parasitism when  $s_1 = 1.0$ . No eggs are “wasted” when  $s_1 = 1$ , unlike conditions where the non-target is a poorly suitable host. Across range of  $a_2$  that we used, the presence of the non-target host decreased the target  $q$ -value to a minimum of 0.06, compared to 0.32 in the absence of the non-target host. The non-target  $q$ -value reached a minimum of 0.17 across this range. It is interesting to note that optimizing  $a_2$  values for maximal target suppression at a given  $s_2$  value leads to stronger target host suppression than optimizing  $s_2$  values for a given  $a_2$  value. The magnitude of this difference was greater for egg-limited parasitoids (target  $q$ -values of 0.06 versus 0.11 when optimizing  $a_2$  and  $s_2$ , respectively) than for time-limited parasitoids (0.002 versus 0.003). Overall, time-limited parasitoids suppress the target population more than egg-limited parasitoids

do for all combinations of  $s_2$  and  $a_2$ , and consequently egg-limited parasitoids result in higher parasitoid equilibrium values (Figs 5 and 7). Three dimensional representations of parasitoid equilibrium, and  $q$ -values of the target and non-target for variable  $a_2$  and  $\beta$  are available in the supplementary materials (Fig 8a-c).

### 2.3.3 Effects of host demography

Finally, we evaluated the effects of search efficiency of the non-target host ( $a_2$ ) and non-target host suitability ( $s_2$ ) on suppression of the non-target host population when the non-target population has reduced demographic parameters relative to the target population (Fig 9). A reduction in  $K_2$  has the general effect of decreasing the non-target equilibrium density, which in turn reduces the parasitoid equilibrium, and thus increases the target equilibrium. There is little effect from varying  $K$  on the non-target  $q$ -value, however. This can be seen by comparing figures 9a and 9b (low  $K_2$ ) with figures 6b and 8b (high  $K_2$ ). Reduced carrying capacity of the non-target also results in a slight increase in the target  $q$ -values (figures not shown). A reduction in  $r_2$  also has the effect of lowering parasitoid and non-target equilibria, while increasing target equilibrium. There is also a slight increase in the target's  $q$ -value at lower values of  $r_2$ ; the non-target  $q$ -value, however was reduced substantially, however, and this allows for non-target extinctions to occur. This can be seen by comparing figures 10c and 10d (low  $r_2$ ) with figures 6b and 8b (high  $r_2$ ). Reducing both  $r_2$  and  $K_2$  had roughly similar results to reducing  $r_2$  alone under a wide range of parameter values. This can be seen by comparing figures 10e and 10f (low  $K_2$  and low  $r_2$ ) with figures 6b and 8b.



## 2.4 Discussion

We found that under many combinations of parameter values for both time- and egg-limited parasitoids the presence of an alternative host can increase biological control impact on the target pest, and in our simulations, high levels of biological control occurred at intermediate non-target impact. However, under egg limitation, a non-target host of low suitability that is attacked by the agent at a high enough rate may result in decreased control of the target host. This egg sink effect can occur even when the average proportion of unused eggs per parasitoid is fairly high (Fig 9; Fig 3S). Egg-limitation is expected to occur when host densities are high (Heimpel and Rosenheim 1998; Mangel and Heimpel 1998; Heimpel et al. 2003; Dieckhoff et al. 2014), which may be common for biological control agents of outbreak pest populations. Not surprisingly, the  $q$ -value of the non-target population declines monotonically with either increasing suitability or increasing search efficiency. That is, while increased polyphagy, as measured by suitability and search efficiency on the non-target population, can increase or decrease biological control of the target population, it consistently increases suppression of the non-target population.

Suppression of the non-target species can be greater for hosts with low intrinsic growth rate. Low carrying capacity ( $K$ ) of the non-target host, however, has less effect on the degree of non-target impact. This is because  $K$  has a diminishing effect on growth rate the farther away equilibrium is from  $K$ . At low  $q$ -values,  $K$  has little impact on the growth rate of the population, and before accounting for parasitism, growth approaches  $e^r$ . We

might expect a non-target population to have lower  $K$  or  $r$ . In our model, decreased  $r$  is of greater importance for overall non-target impact, and extinction occurred under conditions of low  $r_2$  when combined with high  $a_2$  and  $s_2$ . Generally speaking, as discussed in Heimpel et al. (2003), three-species coexistence is possible in this model when parasitoid fecundity is low or when aggregation of the parasitoid to the hosts is high. Similar to findings of Lynch et al. (2003), we find that a highly suitable and highly attacked non-target species will be at greater risk of extinction if it has a low intrinsic growth rate.

There is strong evidence that indirect effects between species mediated by natural enemies are important in structuring ecological communities (van Veen et al. 2006). Decision-making tools that explicitly address how such indirect interactions may influence both risk and potential benefit of biological control agents could improve our ability to choose suitable agents for release. Apparent competition can increase biological control efficacy across a broad range of parameter values associated with the physiological and ecological host range of a natural enemy. In our models, the highest benefit from apparent competition occurred at intermediate levels of non-target impact, but the degree of impact and the corresponding density of biological control agents at equilibrium varied, depending on which trait limited host range (search efficiency or host suitability) and whether the biological control agent was time- or egg-limited (Figs 4-6).

Extreme generalist biological control agents have caused what are widely considered to be unacceptable levels of non-target harm while generating little biological control benefit (e.g. *Bufo marinus* in Australia (Shine 2010; CSIRO 2011)). Non-target

harm has in some cases reached the level of extinction (e.g. Pacific island tree snails in the family Partulidae attacked by the introduced predatory snail *Euglandina rosea* (Coote and Loève 2003)). Other generalist biological control agents have caused both harms and benefits. The parasitoid fly *Compsilura concinnata* clearly illustrates the adverse and beneficial consequences that can come about from the introduction of a generalist biological control agent. This parasitoid was introduced multiple times in the 20<sup>th</sup> century against 13 species of lepidopteran pests and is likely responsible for the declines of native saturniid moths in northeastern North America (Boettner et al. 2000). However, it is also likely responsible for suppressing the invasion of the brown-tail moth (Elkinton et al. 2006; Elkinton and Boettner 2012). The brown-tail moth is a polyphagous defoliator of native trees, and can also cause severe allergic reactions in humans from its urticating hairs (Blair 1979; Elkinton and Boettner 2012). *Compsilura concinnata* was also released against the gypsy moth, but it is unclear if it has had significant biological control impact on this target (Williams et al. 1993; Elkinton and Boettner 2012). Indeed, gypsy moth remains a serious pest problem in North America (Elkinton and Liebhold 1990). Interestingly, however, *C. concinnata* causes higher parasitism of gypsy moth than specialist parasitoids do in areas with low gypsy moth densities. This has been attributed to the availability of alternative saturniid hosts (Elkinton and Liebhold 1990) – i.e. apparent competition. We do not argue here that the release of *C. concinnata* was an optimal decision. However, we highlight that non-target populations do interact with biological control agents, as our models suggest, and decisions to release agents should be based on all available evidence that may predict both efficacy and safety (Bigler and

Köelliker-Ott 2006; Thomas and Reid 2007). One way of quantitatively comparing non-target impact and pest suppression is as a ratio of q-values (i.e. q-value of the target divided by q-value of the non-target). A low ratio would indicate a combination of high pest suppression and low non-target impact.

Unfortunately, only a small portion of classical biological control projects have monitored host range post-release (~1.5% based on a database survey) (Hoddle 2004); therefore, further work is needed to empirically evaluate how frequently non-target species increase or decrease biological control efficacy. Moreover, further work is needed to determine how various mechanisms of host range limitation influence risk in the field. Host use is often determined by ability of the parasitoid to find the host's habitat, find the host, accept and oviposit in the host, and by the physiological suitability of the host for parasitoid development (Godfray 1994; Hopper 2001). In our model, all but parasitoid development within the host is represented by the search efficiency parameter  $a_i$ . Additionally, our model assumes both phenological and spatial overlap of the target and non-target species with the biological control agent. The determinants of host range limitation, whether due to search efficiency or physiological resistance can have especially important implications under conditions of egg limitation. For egg-limited parasitoids, a minimally suitable non-target host can act as an egg sink (Heimpel et al., 2003), which results in the non-target population indirectly increasing the target population. Plastic host use is common among parasitoids, and an individual parasitoid may be time-limited or egg-limited at different stages of its adult life and in different environmental contexts (Heimpel et al. 1998).

When a parasitoid is egg-limited it may become more “choosy” and reject hosts of low-suitability (i.e. dynamic host use). Dynamic host use has been shown for parasitoids on different life stages that vary in suitability within a single host species (Minkenbergh et al. 1992; Heimpel and Rosenheim 1998; Papaj 2000). If an egg-limited biological control agent rejects hosts of low suitability, the apparent parasitism found in our model would be weakened. That is, if search efficiency declines as suitability declines, the egg-sink phenomenon could be avoided. However, to our knowledge, in the only published experimental tests of dynamic host use for different host species (i.e. dynamic host range) with variable suitability, the three parasitoid species tested were not plastic in their response based on egg load (Dieckhoff 2011; Hopper et al. 2013). However, host availability and abiotic factors (e.g. barometric pressure (Roitberg et al. 1993)), as well as parasitoid experience and age have been shown to influence utilization of host species of differing suitability (Dieckhoff 2011; Jenner et al. 2014), as well as the process of egg maturation itself, which may be particularly important in synovigenic species (Dieckhoff et al. 2014). Further work is required to determine whether our prediction of a +/- interaction is common for egg-limited biological control agents in the field; although it is known that suitability among parasitized host of a given parasitoid can vary widely (see Table 1 in Heimpel et al., (2003)), including in field conditions (Hoogendoorn and Heimpel 2002; Heimpel and Casas 2008).

It is important to differentiate between risk and observed non-target impact. Ecological risk is a measure of the likelihood of an adverse effect occurring due to a stressor (EPA 1998). Non-target impact is the actual effect observed on the non-target

population, which we measured as a  $q$ -value. Holt and Hochberg (2001) suggested that the highest risk to non-target species may correspond with the highest parasitoid equilibrium density which, in our model, never corresponded to the highest impact on the non-target host population. High parasitoid equilibrium density may also increase risk to non-target species if the agent serves as a resource population for generalist predators and hyperparasitoids (Holt and Hochberg 2001; Carvalheiro et al. 2008), or other indirect interactions (Pearson and Callaway 2006). In our model, the highest parasitoid equilibrium values were achieved with intermediate non-target host suitability and with high search efficiency. Importantly, we assumed both the target and the non-target population had similar carrying capacity. This might not be the case if the non-target is a native species feeding on a native plant and has lower  $K$ . However, if another pest species (with high carrying capacity) serves as an alternate host with intermediate suitability, this may drive the biological control agent population to very high densities (Fig 1S).

While there is little debate that specialist natural enemies tend to be safer than generalist biological control agents, it is not clear, from a theoretical perspective, that specialists will also be the most effective at pest control. Specialists may have faster numerical response to pest outbreaks, making them more effective at pest control (Murdoch et al. 1985; Snyder and Ives 2001). This conclusion rests on the classical assumption that the “jack-of-all-trades is master-of-none” (Futuyma and Moreno 1988). Evidence for this hypothesis is conflicting across taxa, and for parasitoids in particular it is unclear whether it holds (Kibota and Courtney 1991; Straub et al. 2011; Asplen et al. 2012). A better understanding of what factors underlie host use would improve our ability

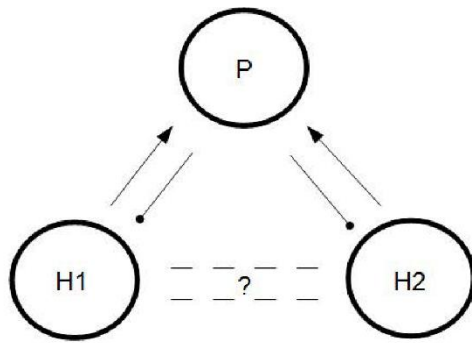
to predict when the trade-off hypothesis would be true (Asplen et al. 2012). In a meta-analysis of entomophagous biological control agents established between 1900 and 1981, Kimberling (2004) showed that monophagous (1 genus of hosts) or oligophagous (1-2 genera of hosts) species had higher likelihood of success than polyphagous (>2 genera of hosts) biological control agents. However, a single genus may contain many potential non-target species which are variably suitable for a biological control agent and experience variable search efficiency (e.g. Desneux et al. 2009).

One criticism of simple mathematical modeling of population interactions is that the models often fail to show predictive power when tested empirically (Simberloff 1982). However, in any empirical pre-release test of safety and efficacy, there is an *implicit* model in all measurements (and see Caswell 1988). Host suitability is measured in the laboratory and informs an implicit model that is assumed to provide some ecologically predictive information. By incorporating this information into a mathematical model we can begin to *explicitly* develop an appropriate framework within which to view laboratory data. For parasitoid biological control agents, we may need to choose between a species that feeds on two hosts (the target and a non-target) and a species that feeds only on the target host. Our model suggests that depending on how specificity is conferred and whether the parasitoid is likely to be egg- or time-limited, the “best” parasitoid may not always be the parasitoid with the narrowest host range. Evaluation of mathematical models, such as we present here, is an important method of hypothesis generation; however, we do not suggest that the parameter combinations we identified as leading to coexistence to indicate thresholds to be used assessment of risk.

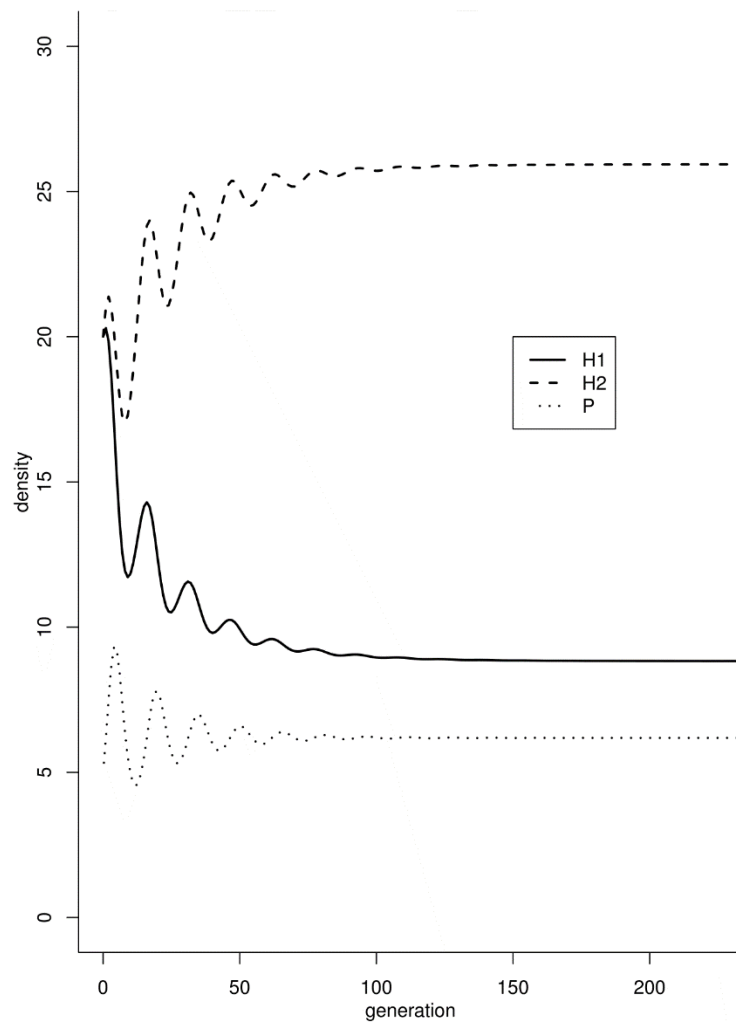
Ultimately, a broad range of ecological knowledge, both empirical and theoretical, should be brought to bear on the problem of risk-benefit analysis in biological control, including the potential for post-release rapid evolution (Van Klinken and Edwards 2002; Brodeur 2012; McEvoy et al. 2012) and effects due to a changing climate (Lu et al. 2014).

Considering the ubiquity of indirect interactions in nature, and the prevalence of third trophic level influences on population dynamics in herbivore communities (van Veen et al. 2006), models which explicitly incorporate these interactions can improve both the safety and efficacy of biological control in the future.

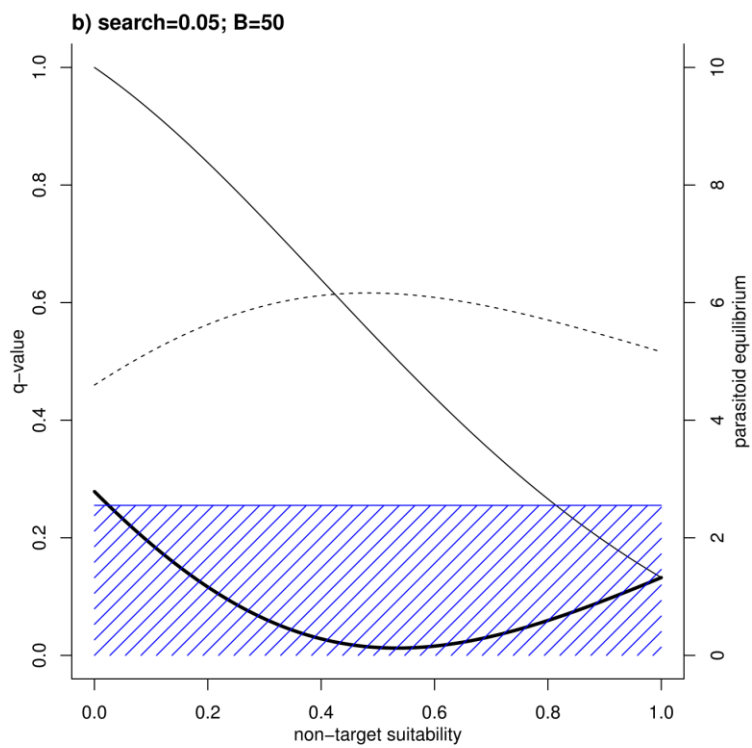
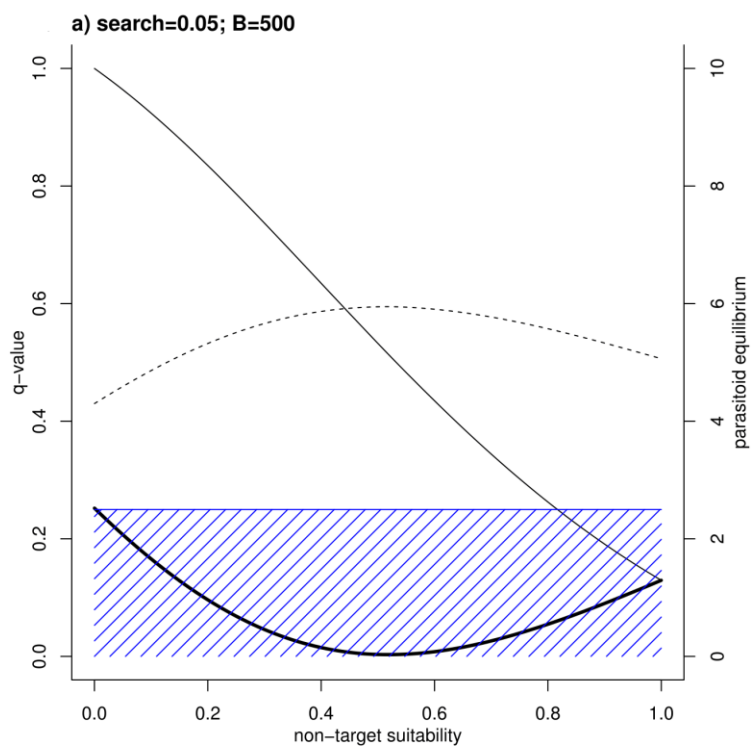


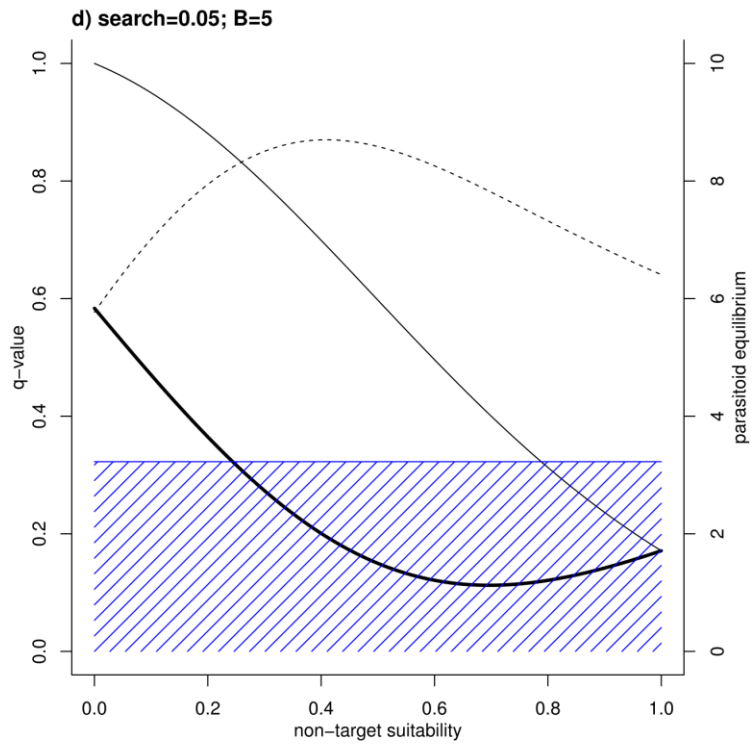
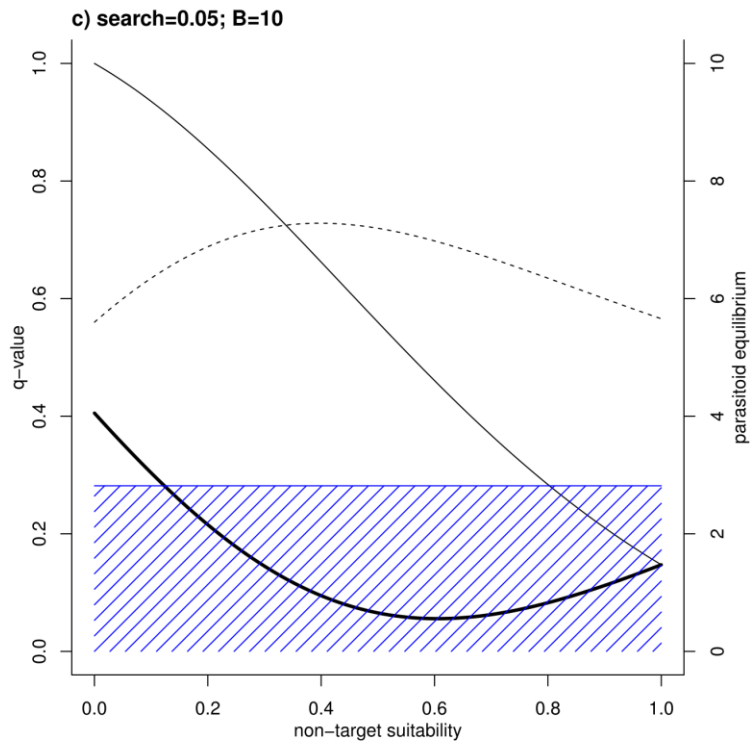


**Figure 3.** Levins' diagram (Levins 1974) depicting two hosts (*H1* and *H2*) and potential indirect interactions mediated by a shared parasitoid (*P*). Arrows represent positive interactions, and clubs represent negative interactions; solid lines represent direct interactions, and dashed lines represent indirect interactions.



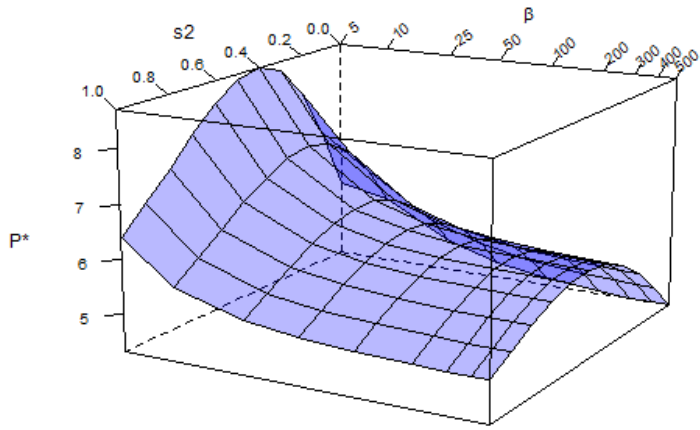
**Figure 4.** The typical model output shown here is for a simulation with parameters  $a_1=0.05$ ,  $a_2=0.045$ ,  $s_1=1.0$ ,  $s_2=0.9$ ,  $r_{1,2}=0.25$ ,  $K_{1,2}=100$ ,  $k=0.75$ , and initial starting densities are  $P=5$ ,  $H1=20$ , and  $H2=20$ . Equilibrium values were analyzed for different parameter combinations for the parasitoid (dotted line), target (solid line), and non-target (dashed line) populations.



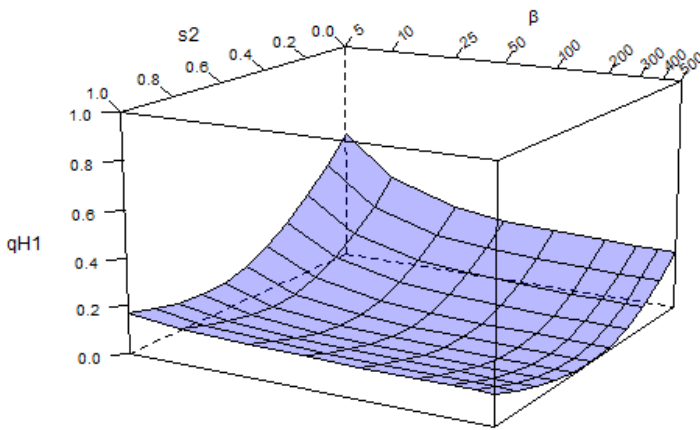


**Figure 5.** Host population suppression changes with variable levels of suitability of the non-target host. Population suppression is expressed as  $q = H_i^*/K$  along the left axis. The thick solid line is the target host; the thin solid line is the non-target host. The hatched region indicates the area of  $q$ -values below that attained by the target pest when the non-target population is set to zero density (i.e. improved levels of biological control). The dashed line is the estimated parasitoid equilibrium density measured along the right axis ( $K_i = 100$ ,  $r_i = 0.25$ ,  $a_i = 0.05$ ,  $s_I = 1.0$ ) for **a)**  $\beta = 500$ , **b)**  $\beta = 50$ , **c)**  $\beta = 10$ , and **d)**  $\beta = 5$ .

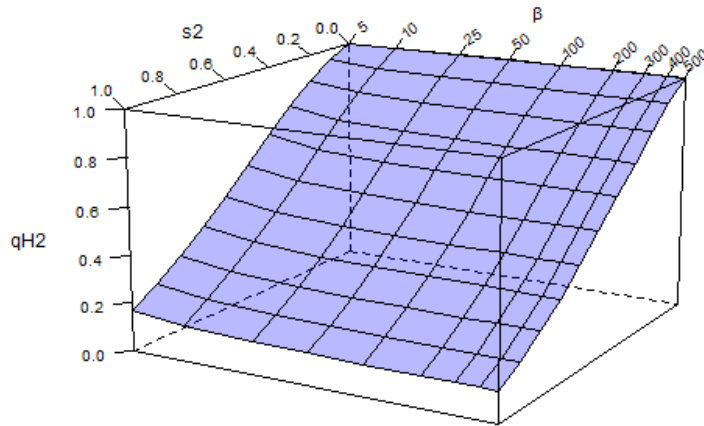
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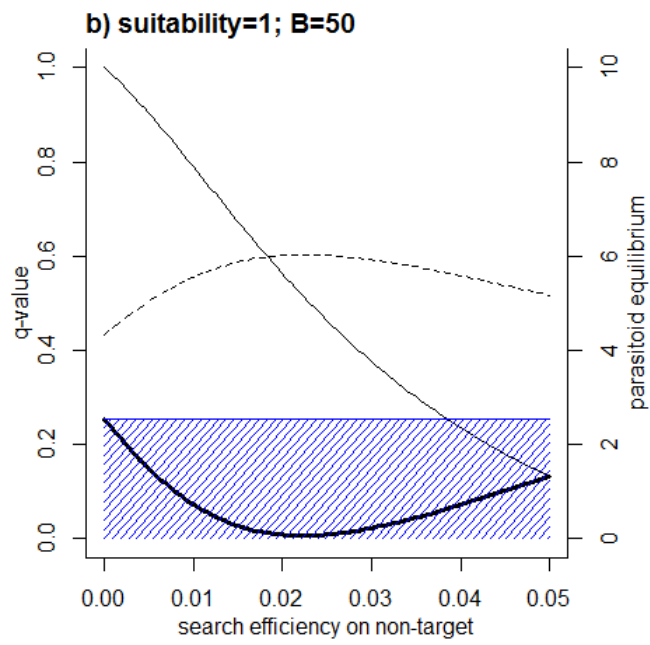
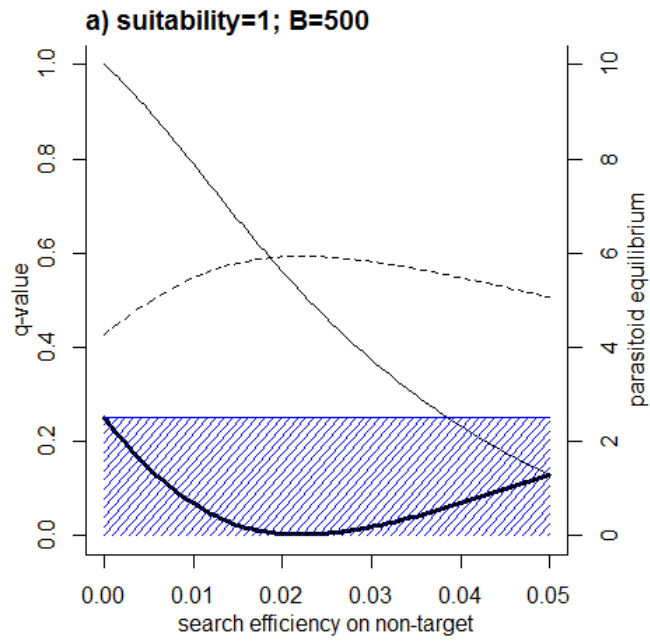
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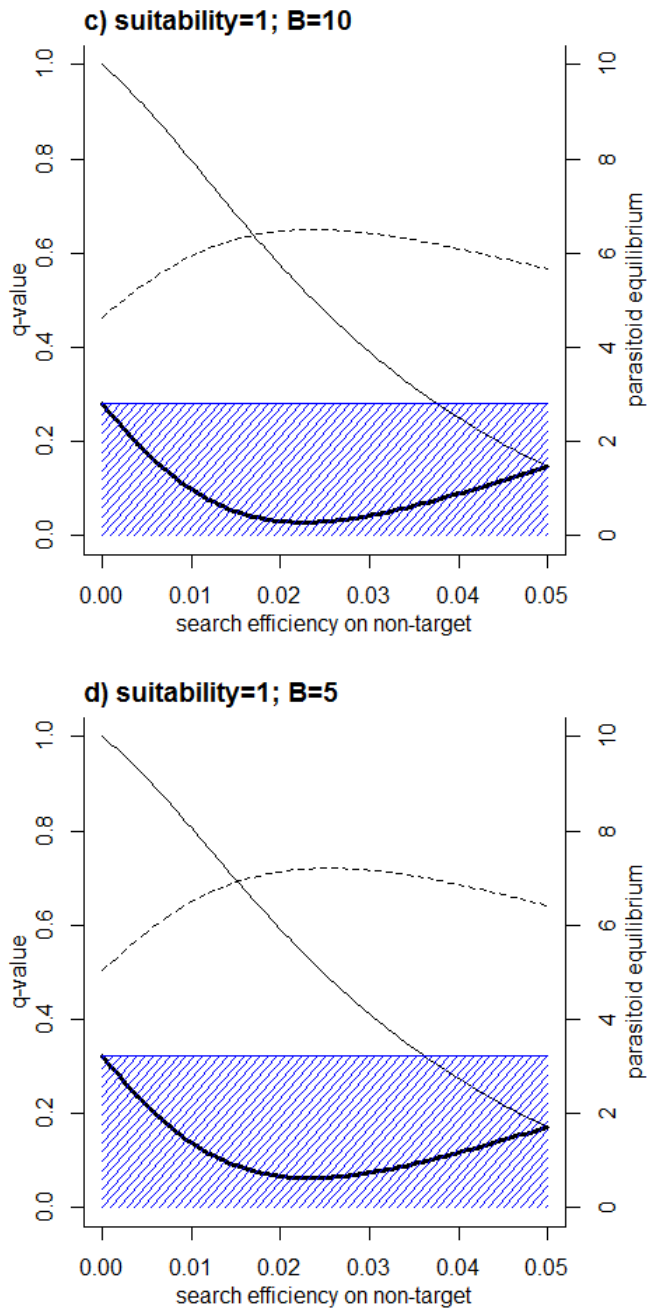
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**Figure 6.** Parasitoid equilibrium densities (**a**), and suppression at equilibrium, expressed as q-values, for the target host (**b**), and non-target host (**c**) populations appear on the z-axis for variable non-target host suitability (s2) and parasitoid egg load ( $\beta$ ) ( $K_i = 100$ ,  $r_i = 0.25$ ,  $a_i = 0.05$ ,  $s_I = 1.0$ ).

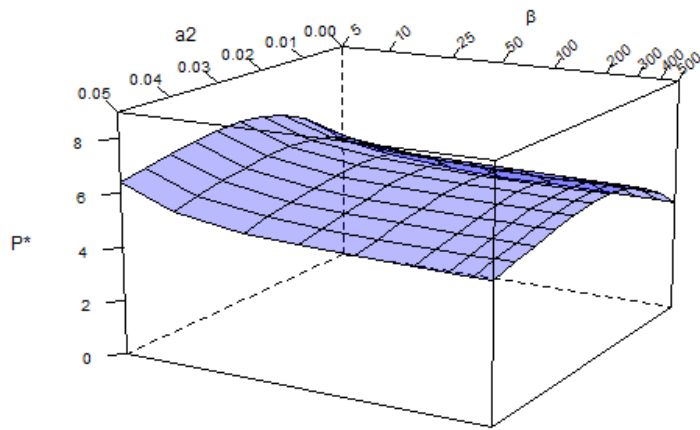




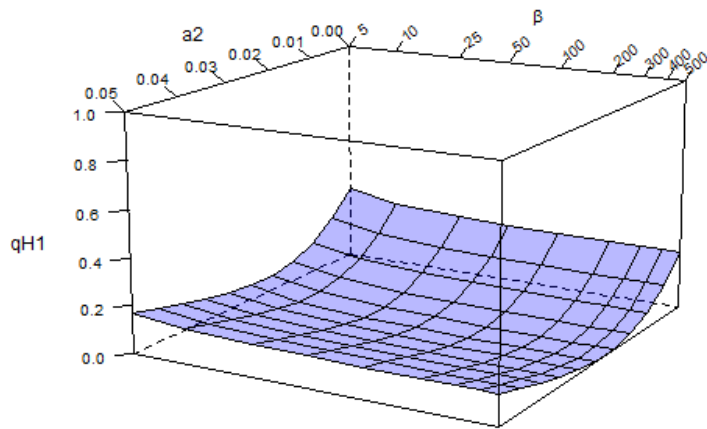


**Figure 7.** Host population suppression with variable levels of parasitoid search efficiency on the non-target host ( $a_2$ ), expressed as  $q$ -values along the left axis for the target host (thick solid line), and non-target host (thin solid line). The hatched region indicates the area of  $q$ -values below that attained by the target pest when the non-target population is set to zero density. The thin dashed line is the estimated parasitoid equilibrium density measured along the right axis ( $K_i = 100$ ,  $r_i = 0.25$ ,  $s_i = 1.0$ ,  $a_I = 0.05$ ) for **a)**  $\beta = 500$ , **b)**  $\beta = 50$ , **c)**  $\beta = 10$ , and **d)**  $\beta = 5$ .

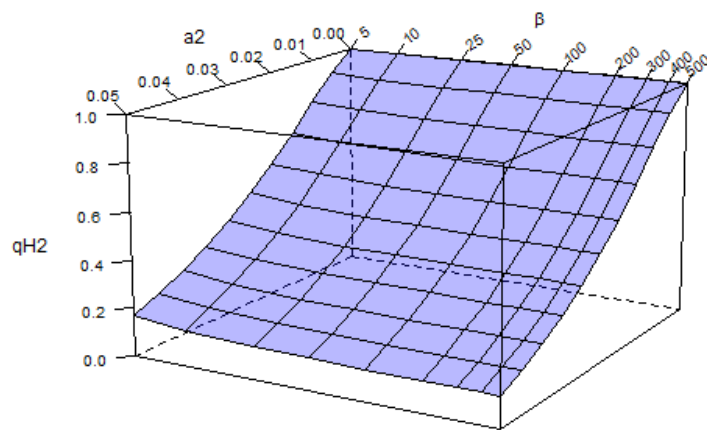
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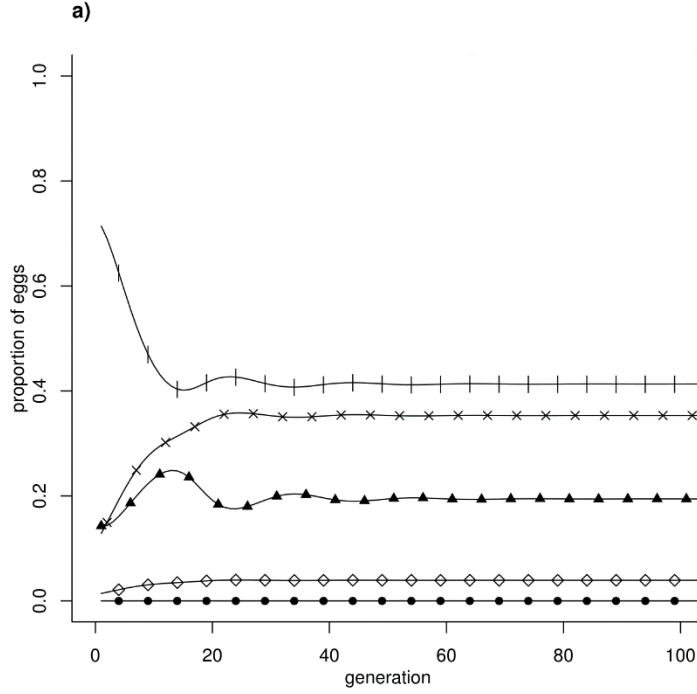
**b)**



**c)**

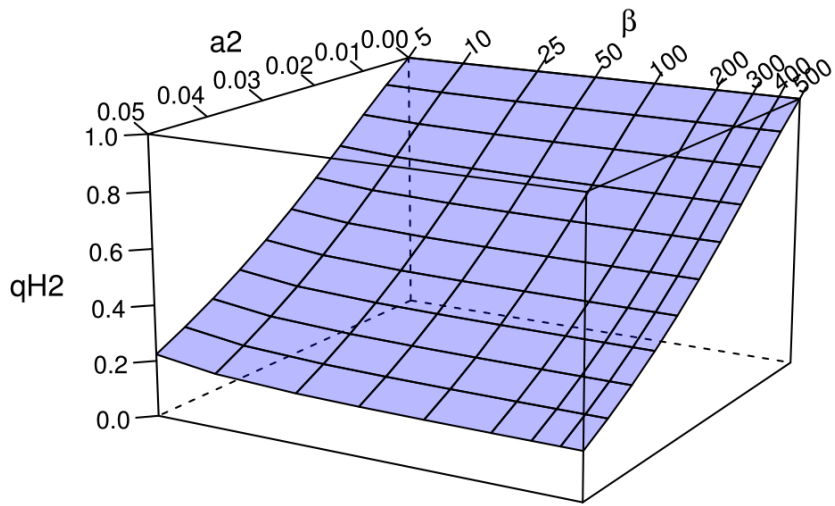


**Figure 8.** Parasitoid equilibrium densities (**a**), and suppression at equilibrium, expressed as  $q$ -values, for the target host (**b**), and non-target host (**c**) populations appear on the  $z$ -axis for variable search efficiency on the non-target ( $s_2$ ) and parasitoid egg load ( $\beta$ ) ( $K_i = 100$ ,  $r_i = 0.25$ ,  $s_i = 1.0$ ,  $a_I = 0.05$ ).

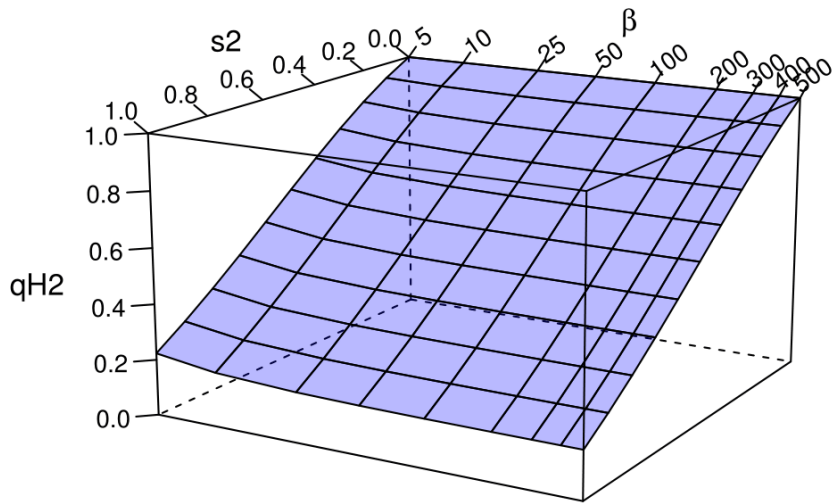


**Figure 9.** The proportion of eggs that successfully develop in the target (solid triangles) and in the non-target (open diamonds) hosts,  $(s_i E_i H_i)/(P\beta)$ ; the proportion of eggs that do not successfully develop to adults in the target (solid circle) and in the non-target ( $\times$  marks),  $((1-s_i)E_i H_i)/(P\beta)$ ; and the proportion of eggs that are not laid (| marks),  $1 - (s_i E_i H_i)/(P\beta) - ((1-s_i)E_i H_i)/(P\beta)$ , with  $K_i = 100$ ,  $a_i = 0.05$ ,  $s_1 = 1.0$ ,  $s_2 = 0.1$ ,  $r_i = 0.25$ , and  $k = 0.75$  for an egg-limited parasitoid ( $\beta = 5$ ).

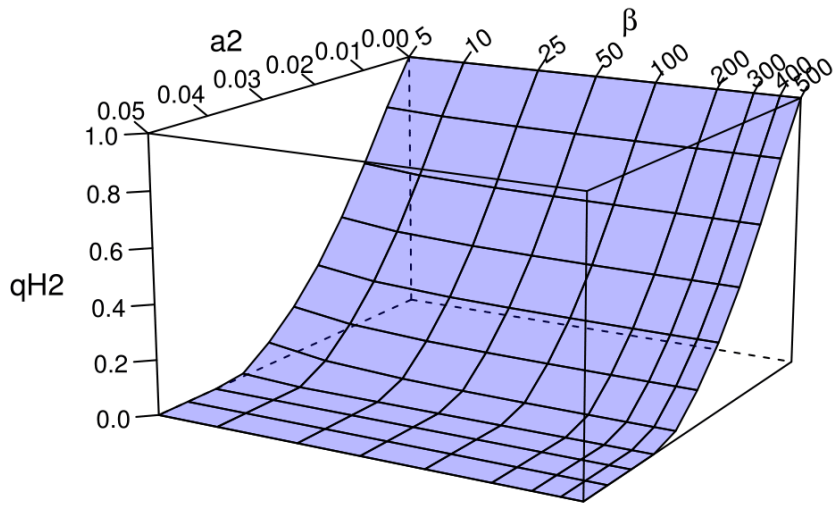
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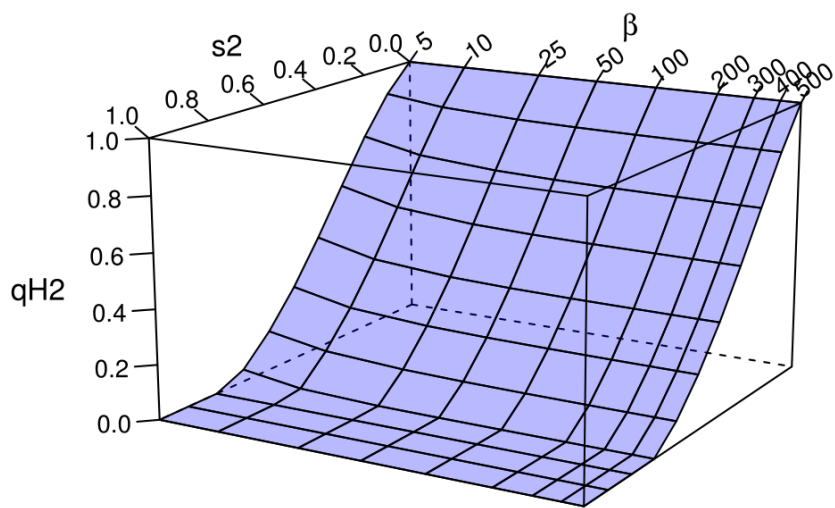
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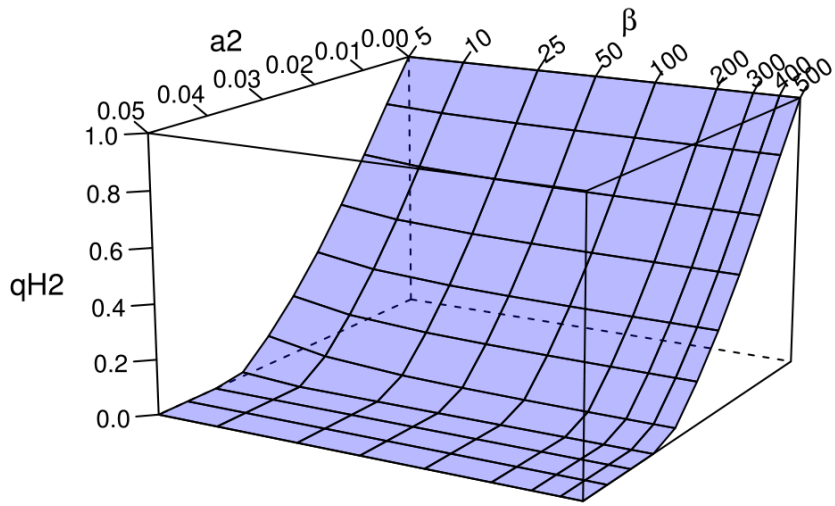
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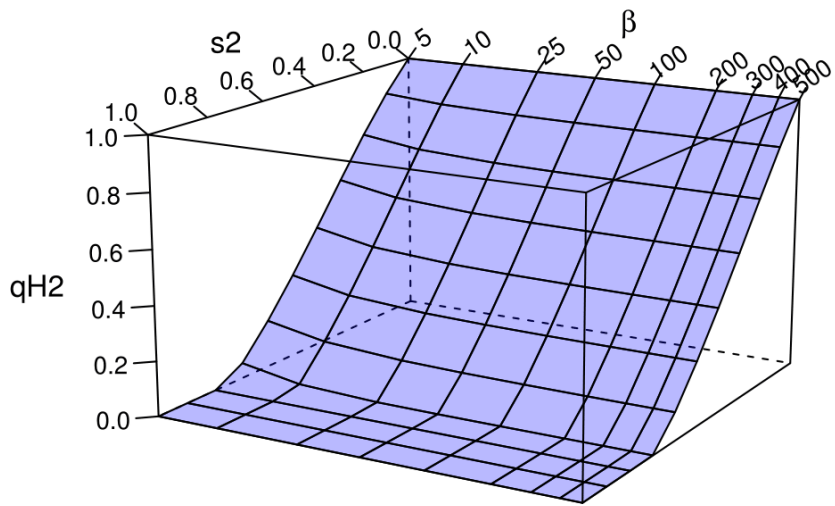
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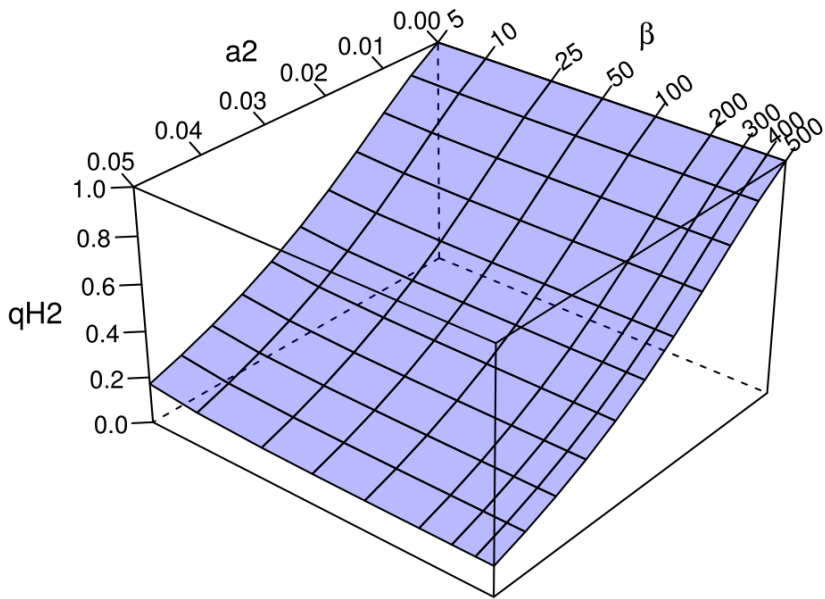
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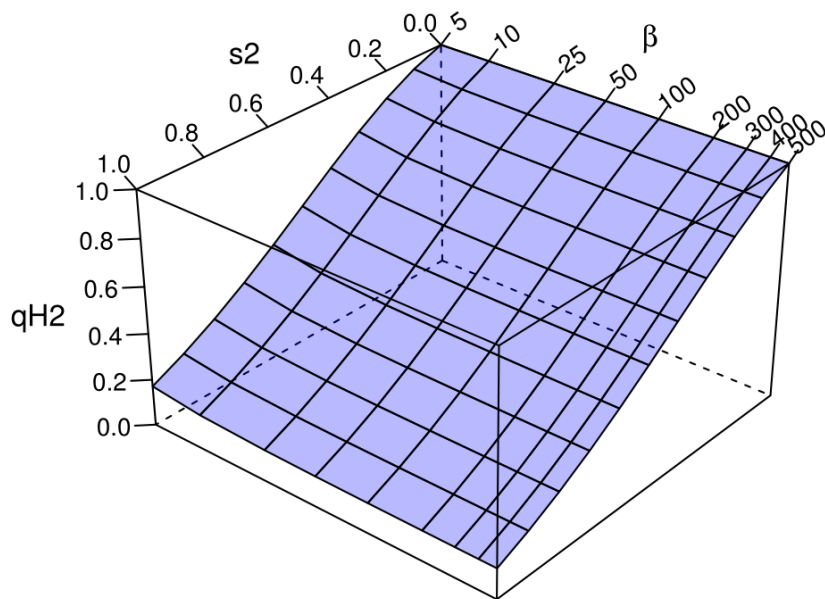
f)



g)



h)



**Figure 10.** The effects of variable  $s_2$ ,  $a_2$ , and  $\beta$  on the  $q$ -value of the non-target species ( $H_2$ ) under different demographic conditions of the non-target relative to the target: low  $K_2$  (a and b), low  $r_2$  (c and d), both low  $r_2$  and low  $K_2$  (e and f), and high  $r_2$  and low  $K_2$  (g and h).



## CHAPTER 3

### PERENNIAL BIOFUEL PLANTINGS AND THE APHID PARASITOID COMMUNITY IN ADJACENT SOYBEAN FIELDS

#### *Chapter Abstract*

Cultivated biofuels provide an important source of renewable energy, and may provide additional ecosystem services, such as enhanced natural enemy communities and increased biological control of pests in neighboring crops. We conducted a large-scale randomized experiment to test for effects of biofuel plantings on biological control of a focal pest, the soybean aphid, in surrounding soybean fields. Four biofuel treatments were replicated four times each by installing 15 m by 20 m plantings within 10-acre soybean fields, with one treatment per field. Biofuel treatments included 1) prairie polyculture, 2) willow biofuel cultivar, 3) “alley” strip (i.e. a combination of prairie polyculture and willow cultivar), and 4) soybean control plots. There was no significant effect of any treatment on the parasitoid community or on soybean aphid density compared to the control. However, the experiment coincidentally captured the early colonization stages of an introduced aphid parasitoid, *Aphelinus certus*, in Minnesota. *Aphelinus certus* is becoming one of the most important natural enemies of the soybean aphid. Moreover, observational data from this study suggest that *A. certus* may be competing directly or indirectly with native parasitoids. The potential impact of *A. certus* on other resident aphid parasitoids and hyperparasitoids are discussed.

### *3.1 Introduction*

Supplementation and replacement of fossil fuels with renewable energy sources, like biofuels, is an important approach to reduce global greenhouse gas emissions and improve energy security (Dale et al. 2014; RED 2016; US EPA 2016). However, increased biofuel production can involve difficult tradeoffs, including conflicts with food security and protection of natural ecosystems (Tilman 1982; Searchinger et al. 2008; Plevin et al. 2010). Changes in land use to produce more biofuels may result in higher food and feed prices as agricultural land is converted into biofuel crop production. Some models predict this will cause increased clearing of forests, grasslands, and other natural areas as more land is cultivated to offset lost food and feed production, driving net increased atmospheric carbon emissions decades into the future (Fargione et al. 2008; Searchinger et al. 2008; but see Dale et al. 2011). Bioenergy sources that do not compete with food and feed crops and that enhance ecosystem services should, therefore, be prioritized for development (Fargione et al. 2008; Tilman et al. 2009; Dale et al. 2014). Low-input high-diversity perennial biofuels present an important opportunity to generate renewable energy from non-food crops (Tilman et al. 2006; Tilman et al. 2009). Additionally, woody perennials like willow can perform well when fertilized and irrigated with municipal or industrial waste streams, and thus may also play an important role in sustainable biofuel production (Powelson et al. 2005; Groom et al. 2008). If production is optimally integrated into the landscape, biofuel cropping systems could decrease reliance on fossil fuels, reduce greenhouse gases, increase biodiversity, and provide a suite of ecosystem services like enhanced beneficial insect communities

(Tilman et al. 2006; Kline et al. 2009; Tilman et al. 2009; Haddad et al. 2009; Dale et al. 2011; Eckberg 2015).

One particularly enticing strategy would be to integrate production of perennial biofuels within traditional annual cropping systems like corn-soybean rotations (Malézieux et al. 2009; Dale et al. 2011). Biofuel plantings have the potential to increase biological control services -- for example, by providing alternative food sources and overwintering refuge for natural enemy communities. Perennial switchgrass monocultures and prairie polycultures grown for biofuels have been shown to increase within-field biological control of herbivores compared to corn fields (Werling et al. 2014). Increased plant biodiversity in polyculture plantings not only increases overall plant biomass production and belowground growth - good news from the standpoints of biofuel production and carbon sequestration - but it also increases biodiversity at higher trophic levels, which can result in increased biological control of pest species (Root 1973; Tilman et al. 2006; Tilman et al. 2012). Haddad *et al.* (2009) found a 3-fold increase in the ratio of predators and parasitoids relative to their herbivorous prey when comparing 16-species polyculture plantings to monocultures. At the landscape level, increased habitat complexity has been shown to increase biological control services in several different annual cropping systems (Rusch et al. 2016), and natural enemy movement between fields and habitat types can occur at biologically and economically meaningful levels (Thies and Tschardtke 1999; Werling et al. 2012). Spillover of biological control services from biofuel crops into annual crops could be an important added benefit for

growers, which would increase the economic incentive for planting biofuels on field edges, or on degraded and low productivity land on farms.

How to best to improve biological control services in annual crops through integration of perennial biofuel plantings on the landscape is a key knowledge gap (Dale et al. 2011; Werling et al. 2014). Here we present an experiment using the soybean agroecosystem as a model to evaluate how perennial polycultures and willow biofuel cultivars influence biological control of a focal pest, the soybean aphid. Elements of this study have been published or are in preparation for publication elsewhere (see Plečaš et al. *in prep* for a report on biological control by generalist predators and the overall effects of treatments on soybean aphid density; see Chapter 3 in Eckberg (2015) for a report on biofuel theoretical ethanol yields and associated natural enemy communities; see Plečaš et al. *in prep* for a report on the contribution of floral resources for enhancing biological control; and see Peterson et al. *in prep*. for a report on the impact of extra-floral nectar in sunflowers). We focus here on aphid parasitoid and hyperparasitoid communities as they relate to soybean aphid biological control.

The soybean aphid, *Aphis glycines*, is the most important insect pest in North American soybeans (Ragsdale et al. 2011). The objective of this experiment was to determine if biofuel plantings could enhance aphid parasitoid populations in surrounding soybean fields and contribute toward biological control of the soybean aphid. Natural enemies can dramatically reduce field populations of the soybean aphid in North America (Costamagna et al. 2008) and biological control of the soybean aphid is credited with the equivalent of a 200 million kg reduction in CO<sub>2</sub> emissions (Heimpel et al. 2013). Yet,

soybean aphid biological control can likely be enhanced further, as indicated by the wide variability in biological control depending on landscape context (Landis et al. 2008). Increased proportion of non-corn habitat in the landscape increases biological control of the soybean aphid (Landis et al. 2008; Gardiner et al. 2009), which points to the potential for habitat manipulation to enhance natural enemy communities. Additionally, parasitoids as a functional group have until recently been largely absent from the community of natural enemies attacking soybean aphid in North American commercial soybean fields (Costamagna et al. 2008; Noma et al. 2010). This stands in contrast to Asia, the native range of the soybean aphid, where it is a less serious crop pest and where its parasitoids are diverse and abundant (Heimpel et al. 2004; Wu et al. 2004).

Importation of specialist parasitoids (i.e. classical biological control) has been utilized as a strategy to improve biological control of the soybean aphid in North America (Heimpel et al. 2004; Chacón et al. 2008; Heimpel et al. 2010; Chacón and Heimpel 2010; Ragsdale et al. 2011; Asplen et al. 2011). Two classical biological control agents targeting the soybean aphid have been released in the United States to date: 1) *Binodoxys communis* (Hymenoptera: Braconidae), released from 2007 through 2009; and 2) *Aphelinus glycinis* (Hymenoptera: Aphelinidae), released in 2013 through 2016. A third parasitoid species intended to attack the soybean aphid, *Aphe. atriplicis* (previously misidentified as *Aphe. albipodus*; KR Hopper *pers. comm.*), was redistributed in 2002 to Minnesota from Wyoming where it was originally imported from Asia in the 1990s to control *Diuraphis noxia* (Heimpel et al. 2004). None of these three species (*B. communis*, *Aphe. glycinis*, or *Aphe. atriplicis*) have yet been confirmed to be established in the

release area. In contrast to these intentionally released parasitoids, a generalist aphid parasitoid, *Aphe. certus*, which was inadvertently introduced into North America, is becoming widely established. *Aphelinus certus* was first reported in eastern North America in Pennsylvania in 2005, and it has since spread through much of the soybean growing region of North America (Frewin et al. 2010; Ragsdale et al. 2011; Wulff et al. 2013; Lagos-Kutz and Voegtlin 2016). The most common parasitoids found in soybean fields of the north central United States are the native *Lysiphlebus testaceipes*, and the non-native *Aphe. asychis*, but they typically persist at low densities in soybean fields (Costamagna and Landis 2006; Kaiser et al. 2007; Noma and Brewer 2008; Noma et al. 2010).

### 3.2 Materials & Methods

#### 3.2.1 Study site

At the University of Minnesota Agricultural Experiment Station in Rosemount, MN, USA, 16 ten-acre soybean fields were planted a minimum of 0.5 km apart (76 cm wide rows of cultivars Pioneer 91M51 at 375,600 seeds ha<sup>-1</sup> in 2012 and 2013, and Pioneer 91Y70 at 370,658 seeds ha<sup>-1</sup> in 2014). Each field was assigned to one of four blocks, and within each block treatments were randomly assigned to each field. There were four treatments: 1) perennial polyculture mix; 2) willow biofuel cultivar; 3) an alley configuration of polyculture mix with two strips of willow planted on either side (for justification of alley configuration - see Strickland et al. 2015); and 4) a control plot consisting of soybeans.

### 3.2.2 Sampling

In 2012, 2013, and 2014, we recorded soybean aphid and parasitoid density per plant approximately weekly along each side of the treatment plots at “near” (2 meter) and “far” (17 meter) distances from the treatment plots. Sample locations were selected by using a random number generator to select locations along transects parallel to each side of the treatment plots at both near and far distances. At each randomly selected location, we non-destructively inspected a whole soybean plant and counted the total number of apterous and alate soybean aphids, parasitoid ‘mummies’ (*Aphelinus* spp. and Aphidiinae), and soybean trifoliates per plant. We collected subsamples of parasitoid mummies in gel capsules which were brought back to the lab so that the adults could emerge and be identified to species. We began sampling each growing season at the time of soybean unifoliolate expansion and continued to sample approximately weekly through plant senescence. In the beginning of each season, along each transect, four plants were selected for inspection. As the season progressed and aphid population grew, the number of plants inspected per transect was reduced to two, and then one plant along each transect per date, due to time constraints associated with counting increasing aphid densities. Combined, we surveyed 11,548 soybean plants.

Biofuel plots were inspected approximately weekly throughout the soybean growing season (May through September) for colonies of aphids in concert with visual inspections for predators during 2012 and 2013 (Plečaš *in prep.*). In each biofuel plot, weekly visual inspections consisted of two 0.25 m<sup>2</sup> quadrats, in the center and 1 meter

from the eastern edge of the plot. Within each quadrat, the entire plant was inspected for aphids and mummies. When colonies were discovered in polyculture, all aphids were counted. When colonies were discovered in willow, all aphids were counted on 10 infested stems. In 2014, aphid arrival was recorded, but densities were not estimated on a weekly basis (Plečaš *in prep*).

### 3.2.3 Aphid suitability

The most abundant aphids observed in the biofuel plots were giant willow aphids, *Tuberolachnus salignus*. In August 2013, field collected *T. salignus* were brought to the lab and presented to *Aphe. certus* from a colony maintained on soybean aphids. Ten mixed *T. salignus* of mixed instars were placed on a willow leaf in a petri dish. One mated female *Aphe. certus* was placed in the petri dish and observed under a dissecting scope for 5 minute. This was repeated 7 times. Parasitoids were monitored for signs of host recognition (antennation or ovipositor probing). See Chapter 4 for host range testing of additional species.

### 3.2.4 Insect Identification

Insects were identified based on morphological characteristics, and using the dichotomous keys presented in Hayat (1983), Pike et al. (1997), Hopper et al. (2012), and by comparison to specimens of known identity, with subsamples of *Aphelinus* spp. confirmed by KR Hopper.



### 3.2.5 Statistical analysis

All statistical analyses were conducted in R. version 3.2.3 (R Core Team 2015). Spatial and temporal non-independence of data were taken into account in two different ways: 1) by calculating cumulative insect days estimated from the average response for each plot at each date; 2) by utilizing crossed design generalized linear mixed effects models (details below).

#### Cumulative insect days

To account for spatial pseudoreplication in sampling, densities per plant of response variables (i.e. aphids and parasitoids) were averaged for each plot at each sampling date. Means were analyzed in two ways. First we estimated cumulative aphid (or parasitoid) days (CAD or CPD), following the methods of Hanafi et al. (1989), using the formula

$$CID = \sum_{i=1}^k \frac{n_i + n_{i+1}}{2} \Delta t$$

Where CID is the cumulative insect days (either CAD or CPD),  $n_i$  is the density of insects on sample date  $i$ , and  $\Delta t$  is the number of days between sampling date  $i$  and  $i+1$ . Analysis of variance (ANOVA) was conducted on the CAD and log-transformed CPD values (+0.1 for the Aphidiinae to account for zeros) against the year as a factor. Pairwise comparisons were conducted with TukeyHSD. Cumulative parasitism rate was calculated as  $CPD/(CPD+CAD)$ , and significance was determined with generalized linear model (gamma distribution) of the rate responses against the factor of year of observation.

Subsample species-level data was pooled for each plot and each year, and relationships between CID and hyperparasitism were assessed using logistic regression.

Hyperparasitism of Aphidiinae and of *Aphelinus* spp. were treated separately as binary response variables, with the full model of Hyperparasitism~Year + Cumulative Aphid Days + Cumulative Aphidiinae days + Cumulative Aphelinus days + Treatment, model selection was conducted using stepwise comparisons of AIC values, and model fit was assessed with Chi-square analysis of deviance.

#### Generalized linear mixed effects

While estimation CAD and CPD accounted for temporal pseudoreplication of our sampling design, this method loses information by averaging sample locations and summing counts through time. To investigate responses through time, we used a generalized mixed effects models (Package glmmADMB v. 0.8.3.3) with a negative binomial distribution (Zuur et al. 2009; Bolker et al. 2009; Skaug et al. 2016). Aphid density per plant, distance from biofuel planting, and the interaction of treatment and distance were fixed effects in the full model. Plot ID, date, and the interaction of date and plot ID were random effects on the intercept. Model comparisons were made using likelihood ratio tests (anova.glmmadmb), comparing the full model to a model without the variable of interest. Tukey corrected pairwise comparisons were conducted using the R package lsmeans (Lenth 2016). Yearly changes in cumulative Aphidiinae and *Aphelinus* days were indicative of potential competitive interactions. To investigate this

potential interaction, we added the mummy density as an explanatory variable and compared it to the full model using to the full model using a likelihood ratio test.

### 3.3 Results

#### 3.3.1 Within soybeans

The first soybean aphids of each season were observed on June 21, July 1, and June 26, in 2012, 2013, and 2014, respectively (Fig 11). The first *Aphelinus* spp. mummies of each growing season were recorded on July 12, July 7, and July 22 in 2012, 2013, and 2014, respectively (Fig 12), and the first Aphidiinae mummies of the season were observed on June 28, July 22, and July 29, in 2012, 2013, and 2014, respectively. Mean peak aphid densities occurred on August 23, August 28, and August 13 in 2012, 2013, and 2014, respectively. Cumulative aphid days increased significantly each year of the study ( $P < 0.01$  for each Tukey corrected pairwise comparison) (Fig 11). Mean peak *Aphelinus* spp. density occurred on September 2, August 28, and August 25 in 2012, 2013, and 2014, respectively. Similar to cumulative aphid days, cumulative *Aphelinus* days increased significantly each year of the study ( $P < 0.05$  for each Tukey corrected pairwise comparison) (Fig 12). Unlike *Aphelinus*, there were no significant differences between years for cumulative Aphidiinae days ( $P = 0.101$ ) (Fig 13). Cumulative parasitism rate (i.e. mummification rate) for *Aphelinus* spp. increased from 2012 to 2013, but was unchanged between 2013 and 2014 (Fig 14). In contrast, Aphidiinae parasitism decreased significantly each year of the study ( $P < 0.0001$ ) (Fig 14). There was a strong positive linear correlation between log cumulative *Aphelinus* days (+1) and log cumulative aphid

days (+1) ( $P < 0.0001$ ), with an  $R^2$  of 0.90 (Fig 15). Likewise, there was a significant correlation between log cumulative Aphidiinae days (+1) and log cumulative aphid days (+1) ( $P = 0.007$ ), but the  $R^2$  was lower ( $= 0.15$ ) (Fig 15). Mean peak Aphidiinae density was observed on September 2, August 28, and August 13 in 2012, 2013, and 2014, respectively.

There was significant positive effect on *Aphelinus* density from the fixed effect of aphid density ( $P < 0.0001$ ). There was also a significant effect on *Aphelinus* density of the interaction between treatment and distance from the treatment plot ( $P = 0.0007$ ); however, after correction for multiple comparisons, there were no significant pairwise interactions. There was no significant correlation between *Aphelinus* density and the main fixed effect of treatment ( $P \sim 1$ ), distance from biofuel planting ( $P \sim 1$ ), or year ( $P = 0.30$ ). Adding Aphidiinae mummy density as a fixed effect significantly improved model fit ( $P < 0.0001$ ), and it had positive linear correlation with the response. The mixed effects model with Aphidiinae mummies for the response indicated that the fixed effect of aphid density was significant ( $P < 0.0001$ ). There was no significant effect of treatment ( $P \sim 1$ ), distance from biofuel planting ( $P \sim 1$ ), the interaction of distance and treatment ( $P = 0.45$ ), or year ( $P = 0.10$ ). Adding *Aphelinus* mummy density as a fixed effect improved model fit ( $P < 0.0001$ ), and it had a positive correlation with the response Aphidiinae density. There was no significant effect of treatment on aphid density in any year of the study ( $P = 0.22$ ). Analysis of variance of cumulative insect days by treatment for each year of the study agreed with our findings from the generalized mixed models; there was no significant effect of treatment for each year of the study ( $P = 0.73$ ).

We collected a total of 1415 mummies throughout the experiment; however, only ~48% successfully emerged as adults, while the rest either died in the mummy stage or had already emerged prior to collection (Table 1). The vast majority of *Aphelinus* species collected were the introduced parasitoid *Aphelinus certus* (99.5%). The majority of Aphidiinae mummies collected were *Lysiphlebus testaceipes* (92.6%). Hyperparasitoids included Pteromalidae (73%), Figitidae (19%), and Encyrtidae (8%). Aphidiinae were hyperparasitized at a rate of 17.4, 25.5, and 2.2% in 2012, 2013, and 2014, respectively. *Aphelinus* spp. were hyperparasitized at a rate of 10.4, 11.4, and 3.2%, in 2012, 2013, and 2014, respectively. Pooling data from all years, Aphidiinae were hyperparasitized significantly more than were *Aphelinus* spp. (P=0.004).

Pooled for all years, the model which best described *Aphelinus* hyperparasitism (Table 2), included only cumulative *Aphelinus* days, which was significantly negatively correlated with hyperparasitism rate (P=0.003). The model with best described Aphidiinae hyperparasitism included year (significantly lower hyperparasitism in 2014 (P<0.05) Tukey corrected pairwise comparisons), a positive correlation with cumulative aphid days (P=0.04), and a negative correlation with cumulative *Aphelinus* days (P=0.04).

### 3.3.2 Aphids in biofuel plantings

Very few aphids were found in the biofuel plantings during the course of this experiment, other than the giant willow aphid (*T. salignus*), which was found exclusively in willow plantings and the willow portion of the alley treatment. No mummies were

observed in the *T. salignus* colonies. In 2012, *T. salignus* was observed in two of the willow plots (colonies averaged 34.6 aphids per infested stem). In 2013, *T. salignus* was observed in all willow plots and two of the alley plots, averaging 68 aphids per infested stem. In 2014, *T. salignus* was found in all willow and alley plots. A total of twenty-three *Uroleucon rudbeckiae* were observed on black-eyed Susan (*Rudbeckia hirta*) in the prairie polyculture in 2012, and nine *U. rudbeckiae* were observed in 2013, but no mummies were observed in any year of the study. No other aphids were observed in the treatment plots.

*Aphelinus certus* did not demonstrate signs of host recognition of the giant willow aphid. See chapter 4 for extended host range tests including *Uroleucon* spp., which *Aphe. certus* did not successfully develop within.

### 3.4 Discussion

We experimentally manipulated perennial polyculture, willow biofuel, and an alley configuration of both willow and polyculture plantings within a conventional soybean agroecosystem to study the effects of these biofuels on soybean aphid biological control by parasitoids. Biofuel treatment plots did not have a significant effect on overall parasitoid, hyperparasitoid, or aphid host densities compared to control soybeans plots. To our knowledge this is the first study to examine the effects of biofuel plantings on parasitoid communities in adjacent crops. Additionally, this study is the first to document the parasitoid *Aphelinus certus* attacking the soybean aphid in the state of Minnesota. We describe here the initial stages of colonization of *Aphe. certus* and present observations of

potential displacement of native Aphidiinae parasitoids. Interestingly, *Aphe. certus* had been evaluated in quarantine as a potential classical biological control agent of the soybean aphid (Heimpel et al. 2010), but it was precluded from release due to a wide host breadth indicated in the first phase of laboratory safety testing. It is unclear how *Aphe. certus* arrived in North America. In Minnesota, where the present study was conducted, *Aphe. certus* was first documented in August of 2011 at the University of Minnesota Agricultural Experiment Station in Rosemount (collected by MC Kaiser; identified by KR Hopper).

The intermediate landscape complexity hypothesis suggests that on-farm manipulations of diversity will have greatest impacts in landscapes of intermediate complexity, but lower impact in landscape of either high or low complexity (Tschamtko et al. 2012), and empirical evidence bears this out (Jonsson et al. 2015). In this study we found no evidence of soybean aphid parasitoids benefiting from the biofuel treatment plots. It is possible that the experiment here described was not conducted within a landscape of sufficient complexity to cause significant changes in parasitoid or aphid communities. Yet, this study is not the first example of prairie plantings that have failed to increase biological control of the soybean aphid (Cox et al. 2014). Further research is needed to determine the appropriate scale and type of landscape manipulation to optimize biological control in North American soybean agroecosystems. Biological control by parasitoids can be enhanced by the provision of alternative resources in habitats adjacent to a focal crop (Langer and Hance 2004; Alhmedi et al. 2011). Parasitoid abundance can be increased if the parasitoids build up their populations on alternate hosts in adjacent

habitat and spillover to attack the crop pest (i.e. apparent competition) (Holt and Lawton 1994; Langer and Hance 2004; Alhmedi et al. 2011). For example, the abundance and early season parasitism rate of *Anagrus* parasitoids on the western grape leafhopper, *Erythroneura elegantula* Osborn, were shown to be increased in vineyards downwind of prune trees which harbor prune leafhopper eggs, *Edwardsiana prunicola* (Edwards) (Corbett et al. 1996; Murphy et al. 1996; Corbett and Rosenheim 1996; Murphy et al. 1998). Parasitoid populations can also be enhanced through increased survivorship or reproductive capacity by provisions from non-host resources such as nectar from flowers in adjacent habitat (Tylianakis et al. 2004; Heimpel and Jervis 2005). Untilled habitat provided by perennial crops may also enhance beneficial parasitoid populations (Roger-Estrade et al. 2010; Kantar et al. 2016). Periodically disturbed crops like alfalfa allow aphid parasitoids to persist in hosts within the field and suppress pests when new outbreaks begin (Rauwald and Ives 2001).

Very low aphid abundance was observed in the polyculture plantings, and these were of *Uroleucon* spp. on black-eyed Susan (Plećaš et al. *in prep.*), which *Aphe. certus*, the most common aphid parasitoid observed in this study, does not develop in (see Chapter 4). The high diversity and abundance of predators in polyculture plantings (Plećaš et al. *in prep.*) may have reduced alternative aphid hosts from reaching high enough densities to significantly increase parasitoid populations in adjacent soybean fields. Aphid densities in diverse polyculture or prairie systems are often relatively low (Favret and Voegtlin 2001; Wyckhuys et al. 2009), and limited dispersal of some species may have made colonization of our fragmented experimental plots rare (Muller and



Wagenius 2016). Resource competition with generalist predators in biofuel plantings may decrease the likelihood that parasitoids are able to generate a biologically or economically significant spillover effect into adjacent soybean fields. Generalist predators may also have contributed to intraguild predation within biofuel plantings which could decrease parasitoid densities further (Brodeur and Rosenheim 2000; Chacón and Heimpel 2010). In willow plots, giant willow aphid was occasionally observed at high densities; however, we found no parasitoid mummies on these aphids, and field-collected giant willow aphids were not attacked by *Aphe. certus* when brought to the lab.

While biofuel plantings did not appear to enhance parasitoid populations in soybean fields when compared to control plots, we observed relatively high parasitoid densities overall, which stands in contrast to previous findings from the region (Heimpel et al. 2004; Costamagna and Landis 2006; Noma et al. 2010). In this study, we observed the initial stages of colonization of the accidentally introduced aphid parasitoids *Aphe. certus* in Minnesota. *Aphelinus certus* increased each year of the study and is now a ubiquitous presence in soybean fields throughout the state (Kaser unpublished data). *Aphelinus certus* appears to track soybean aphids across a range of host densities (Fig 15). Frewin et al. (2010) showed that *Aphe. certus* was able to complete development across a temperature range of 15.3 to 30.2 °C (the full range of temperatures they tested), and optimal growth occurred at 29.5 and 30.0 °C for the egg-pupa and pupa-adult growth stages, respectively. In the lab, *Aphe. certus* reaches parasitism levels of about 4.7% on V3 soybean plants with 250 soybean aphids over the course of 24 hours (Frewin *et al.*,

2010). *Aphelinus certus* shows a type II functional response on soybean aphids on V1 soybean plants up to a density of 96 aphids per plant (Frewin et al. 2010).

A criticism of utilizing generalist natural enemies in biological control is that they might not be able to respond quickly to target pest outbreaks with a sufficient numerical increase (Symondson et al. 2002; Snyder and Ives 2003). However, *Aphe. certus* maintained similarly high parasitism levels in 2013 and 2014, even though aphid densities were 228% higher in 2014. This contrasts with the native *L. testaceipes*, which also has a broad host range (Biondi et al. unpublished data), and which has been recorded attacking the soybean aphid since the early stages of the pest's invasion into North America (Noma and Brewer 2008; Noma et al. 2010). The parasitism rate of Aphidiinae, 92.6% of which were *L. testaceipes*, declined with increasing host density (Fig 14), and aphid density was a weaker predictor of Aphidiinae density ( $R^2 = 0.15$ ) compared to *Aphelinus* ( $R^2 = 0.90$ ) (Fig 15).

Below, we explore below three non-mutually exclusive hypotheses to explain these differing patterns in parasitoid densities: 1) The newly arrived *Aphelinus certus* are outcompeting resident Aphidiinae spp. for resources; 2) *Aphelinus certus* is competing indirectly asymmetrically with Aphidiinae spp. via shared hyperparasitoids; and 3) Minnesota soybean aphids are not the main host driving Aphidiinae densities in Minnesota soybean fields while they are for *A. certus*.

#### 3.4.1 Resource competition

While not statistically significant, the overall density of Aphidiinae spp. trended downward in soybean fields over the three years of this study, even though host resources increased. Corresponding with this downward trend, *Aphelinus* increased dramatically. Viewing cumulative aphid days for an entire season, the trend across years is that Aphidiinae density is negatively associated with *Aphelinus* density, which is consistent with resource competition. However, after controlling for aphid density and year, *Aphelinus* was positively associated with Aphidiinae density. This might have occurred because some unmeasured variable in soybean fields benefited both parasitoid groups similarly. Additionally, the cumulative parasitism rate of both species remained fairly low (<2%), and so resources were not particularly scarce when looking at each season as a whole. However, resource competition may be occurring outside of soybean fields - for example, in wheat. Both *L. testaceipes* and *Aphe. certus* are known to attack several species of grain aphids (see Chapter 4). Grain aphids typically occur earlier in the season than do soybean aphids, so competitive effects in wheat may only be observable between years in soybean fields rather than within a single season.

#### 3.4.2 Apparent competition via hyperparasitoids

Hyperparasitoids are thought to be more polyphagous than primary parasitoids, and differential spillover of generalists versus specialists may increase the likelihood that hyperparasitoids cross habitat boundaries (Rand et al. 2012; Frost et al. 2015). Aphidiinae were hyperparasitized at a great rate than *Aphelinus* spp., and this asymmetrical hyperparasitism rate is consistent with apparent competition displacing Aphidiinae (Holt

1977; Settle and Wilson 1990; Kaser and Ode 2016). Asymmetrical apparent competition might be particularly detrimental to Aphidiinae considering the very high densities of the introduced *Aphe. certus*. However, hyperparasitism of Aphidiinae did not increase with increasing *Aphelinus* density, either within a season or across years; therefore, we think it is unlikely that hyperparasitoids are the cause of differential attack of the soybean aphid by its primary parasitoids.

### 3.4.3 Alternative drivers of *L. testaceipes*

The low predictive capacity of soybean aphid density to explain aphidiine density may simply indicate that ecological processes within soybean fields are not the main factors determining aphidiine populations in the study region. For example, while *L. testaceipes* is known to regularly parasitize aphids during the summer in cold temperate regions, it is unknown if it can successfully survive cold winters or if it instead recolonizes these regions each summer through long distance migration. In Washington, in the western United States at a similar latitude but with much milder winters than the study region, *L. testaceipes* is known to remain active at least from April through November (Pike et al. 1996). Experiments conducted in the United Kingdom, where *L. testaceipes* was intentionally introduced, suggests the parasitoid remains mobile at temperatures as low as 0°C, but it enters a “chill coma” at temperatures of around -8°C (Hughes et al. 2010). Moreover, at least for the U.K. strains of *L. testaceipes*, they do not appear to enter diapause (Hughes et al. 2011). These findings suggest that *L. testaceipes* does not diapause within the study region. If this is this case, local populations may be

determined more by rates of seasonal immigration rather than by local top-down or bottom-up processes. However, Stary et al. (2004) suggested that due to the wide distribution of *L. testaceipes* throughout much of North America and in northern Korean peninsula, we should hold some skepticism for the hypothesis that it cannot enter diapause or otherwise overwinter at very low temperatures.

From this study and others, it appears that patches of perennial polyculture and willow plantings do not appear to enhance biological control of soybean aphids in adjacent soybean fields (Cox *et al.*, 2014; Plečáš *et al.* *In prep.*). However, it is well documented that habitat complexity often leads to increased biological control of aphid pests (Landis *et al.* 2008; Gardiner *et al.* 2009; Rusch *et al.* 2016). Adjacent to field habitat manipulations may need to occur in a landscapes of intermediate complexity in order to observe a difference in biological control impact (Tscharrntke *et al.* 2012), but what represents “intermediate” is not easily known *a priori*. *Aphelinus certus* is an important new natural enemy of the soybean aphid in North America, and it may be resulting in changes to the broader community of parasitoids and hyperparasitoids in soybean fields. Further work is needed to evaluate the biological control impact of *Aphe. certus*, and its impact on other resident natural enemies.

**Table 1.** Summary of mummy subsamples collected from each plot for parasitoid identification.

Year	Mummies collected	Aphelinus total	Aphidiinae total	Empty	Unemerged	Emerged Aphelinus	Emerged Aphidiinae	Hyperparasitoids
2012	309	193	116	68	161	48	23	9
2013	722	467	255	80	294	193	106	49
2014	384	265	119	39	167	127	46	5
<b>Totals:</b>	1415	925	490	187	622	368	175	63
Percent:	100%*	65.4%*	34.6%*	13.2%*	44.0%*	39.9%**	35.7%***	10.4%****

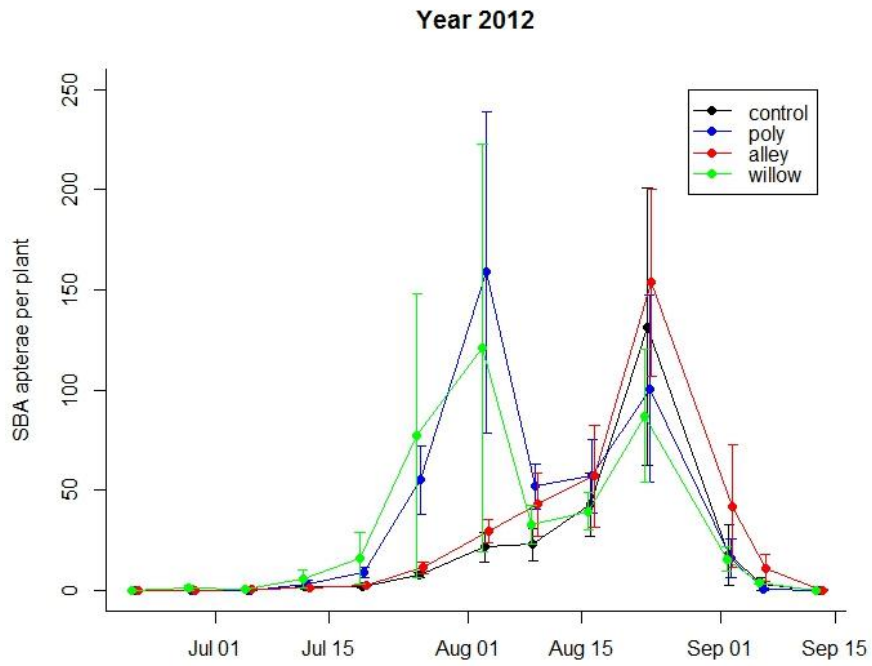
\*Percentage of total mummies; \*\*percentage of total *Aphelinus* mummies, excluding empty mummies; \*\*\*percentage of total Aphidiine mummies, excluding empty mummies; \*\*\*\*percentage of total emerged mummies (i.e. hyperparasitism rate).

**Table 2.** Model selection for logistic regression model simplification of hyperparasitism

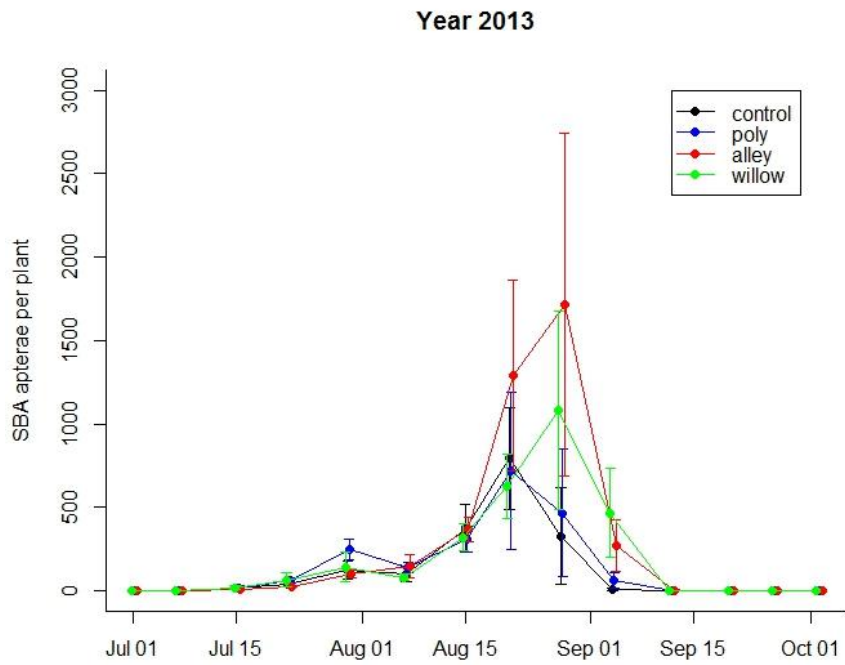
<b>Model</b>	<b>AIC value</b>
<u><i>Aphelinus</i> hyperparasitism</u>	
~CBD	209.6
~Year+CBD	210.28
~Year+CAD+CBD	211.9
~Year+CAD+CBD+Treat	213.84
~Year+CAD+CTD+CBD+Treat	215.8
<u>Aphidiinae hyperparasitism</u>	
~Year+CAD+CBD	169.96
~Year+CAD+CTD+CBD	171.8
~Year+CAD+CTD+CBD+Treat	173.78

AIC values are presented for the stepwise model selection, including the full model and reduced models down to the best model. All other models (which had higher AIC values) are not included. Explanatory variables include year of study (Year), cumulative aphid days (CAD), cumulative Aphidiinae days (CTD), cumulative *Aphelinus* days (CBD), and treatment (Treat).

**A)**

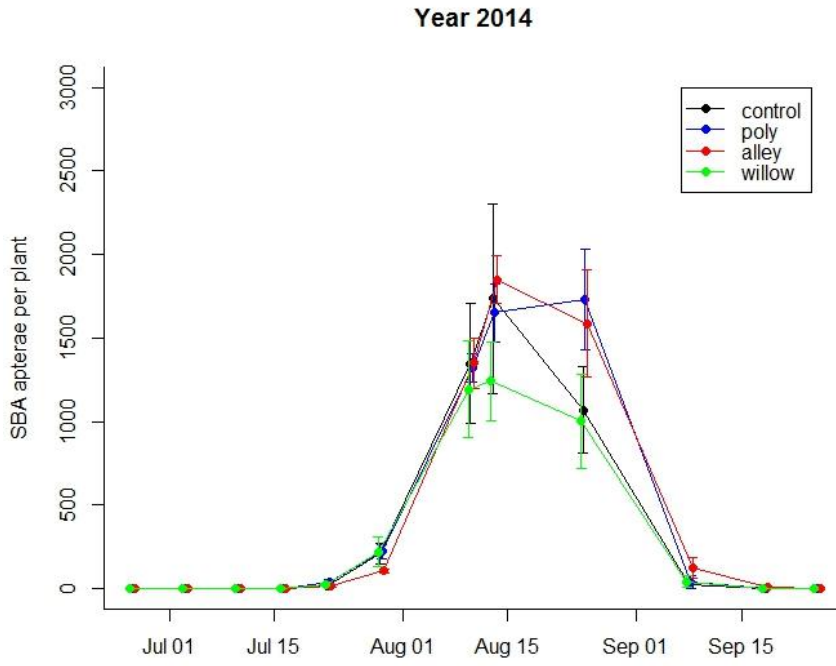


**B)**

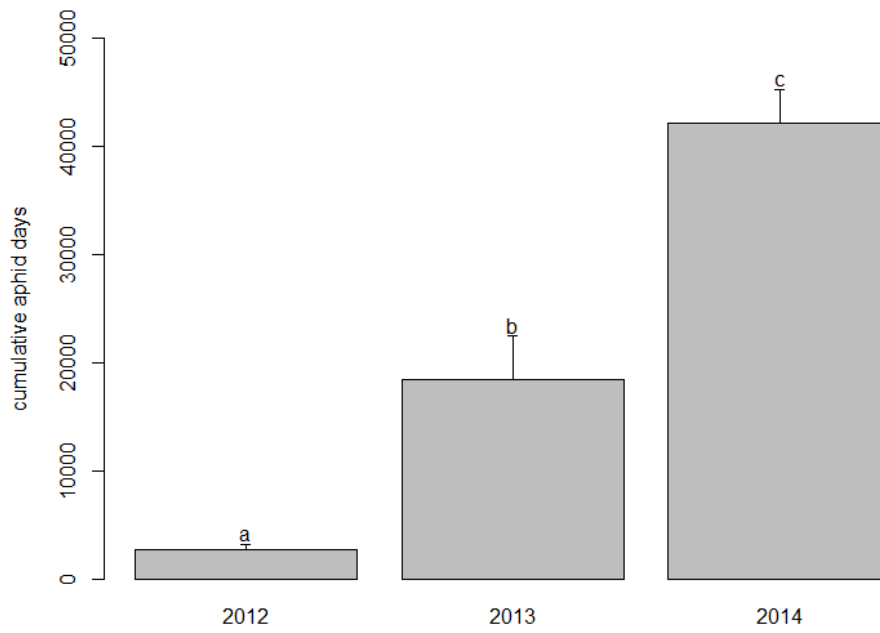




C)

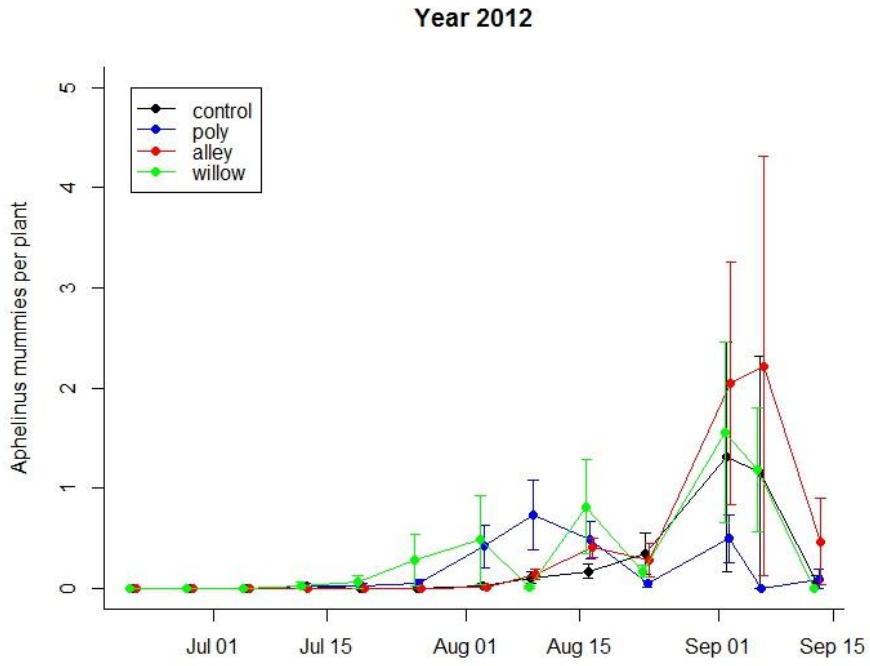


D)

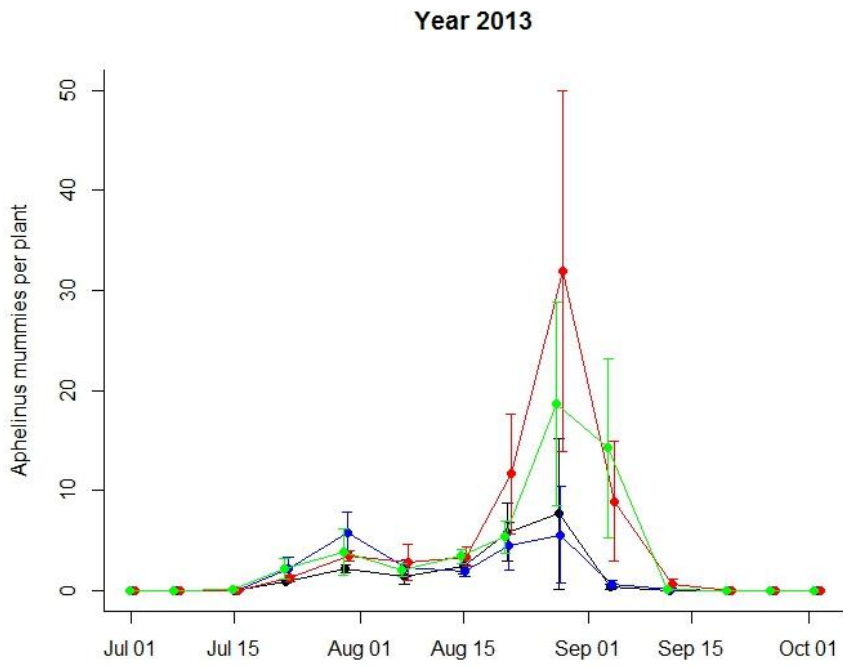


**Figure 11.** Mean density of apterous soybean aphids per plant for each treatment through time (+/- SE bars) for **A)** 2012, **B)** 2013, and **C)** 2014. Means were calculated by first averaging among samples at each plot then averaging among treatments. Standard error was calculated using the means at each plot (i.e. n=4 for each treatment). **D)** Cumulative aphid days (+SE bars) for each year of the study (P<0.01 for all TukeyHSD pairwise comparisons).

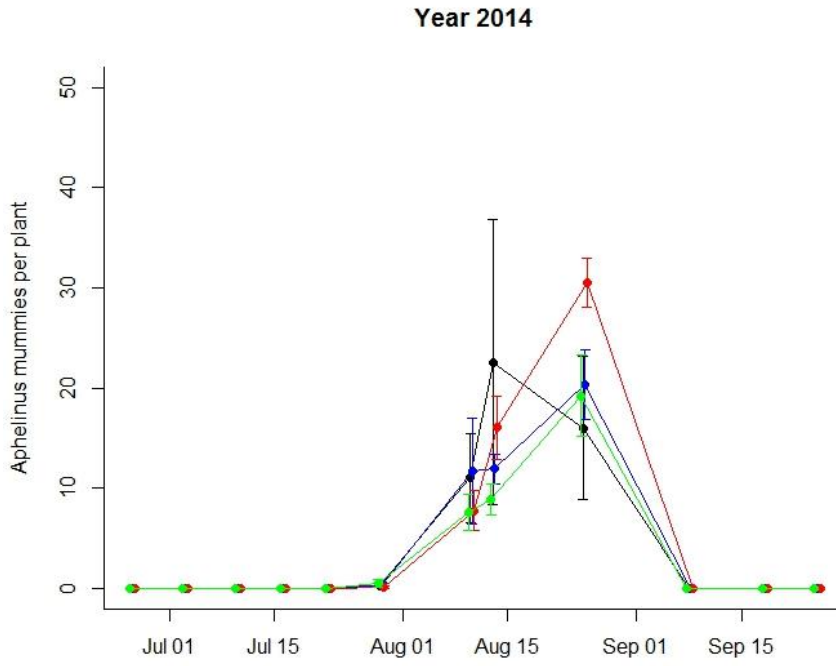
**A)**



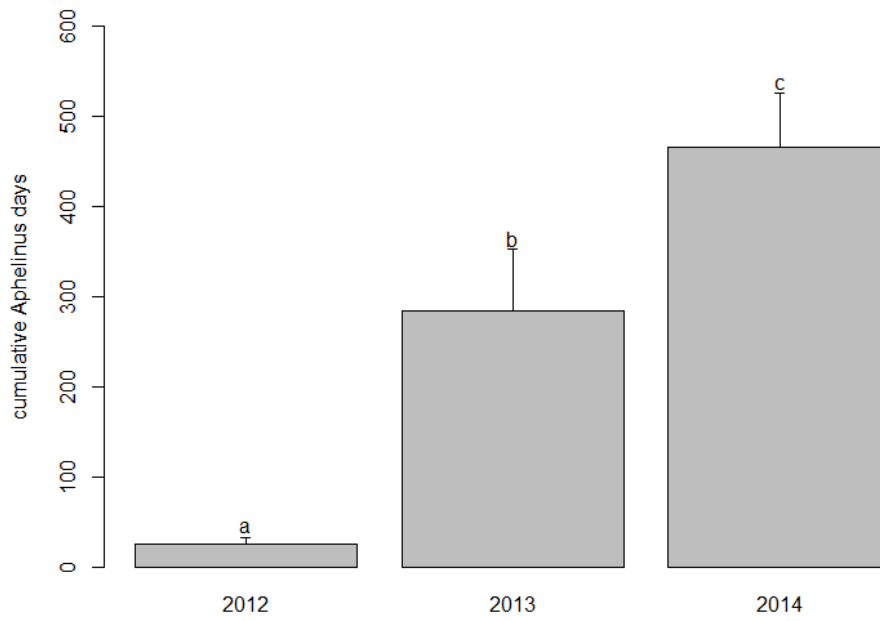
**B)**



C)

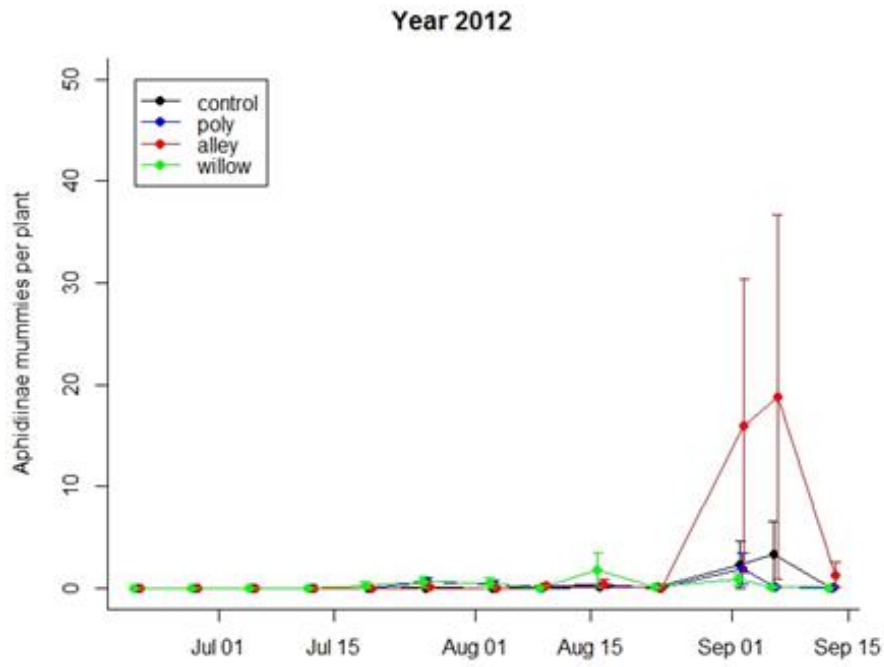


D)

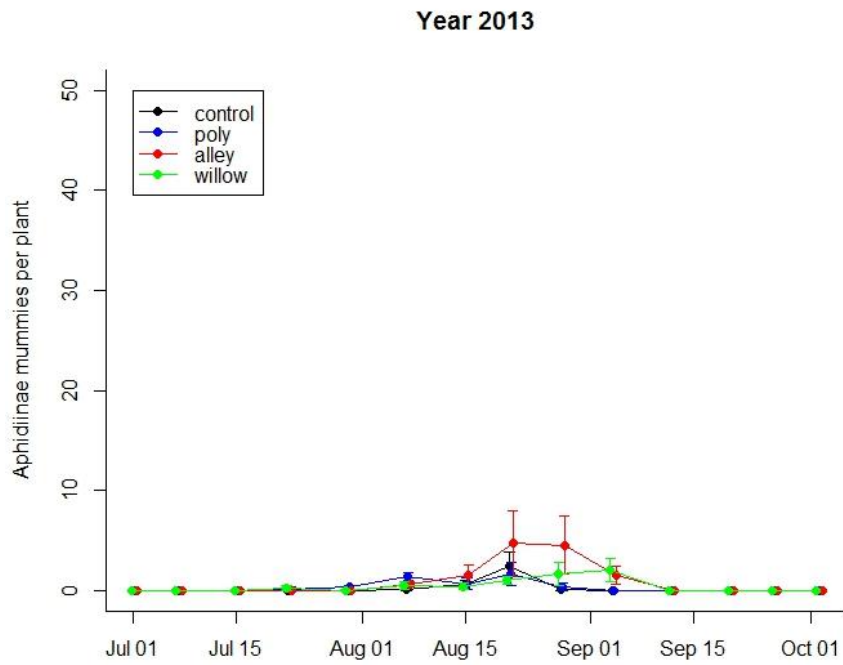


**Figure 12.** Mean density of *Aphelinus* spp. mummies per plant for each treatment through time (+/- SE bars) for A) 2012, B) 2013, and C) 2014. Means were calculated by first averaging among samples at each plot then averaging among treatments. Standard error was estimated using the means at each plot (i.e. n=4 for each treatment). D) Cumulative parasitoid days (+SE bars) for each year of the study (P<0.05 for each Tukey corrected pairwise comparison).

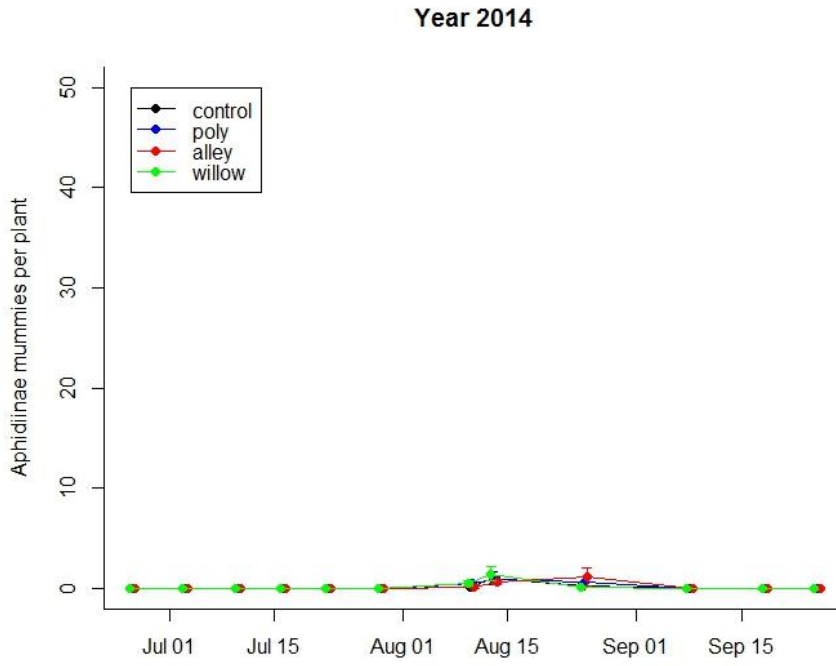
A)



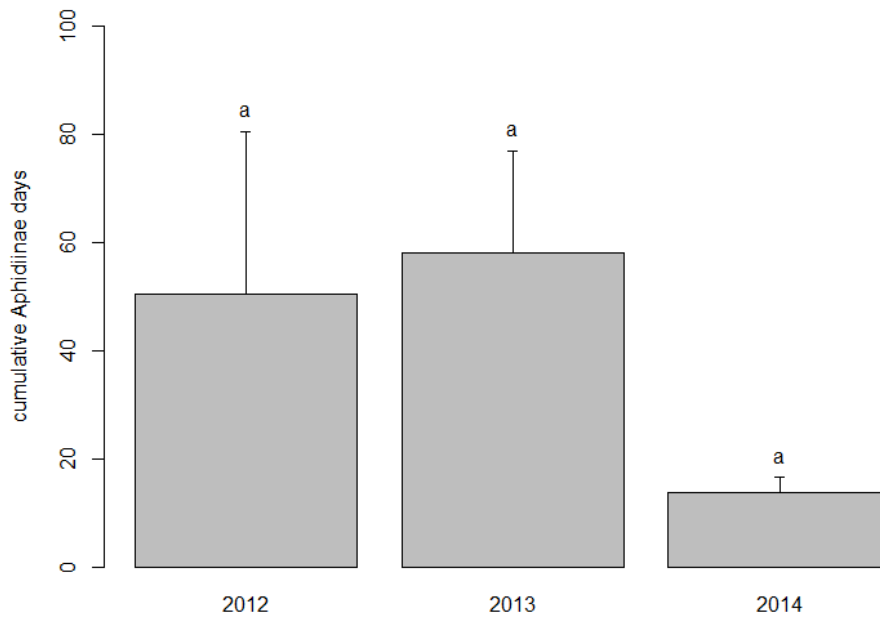
B)



C)



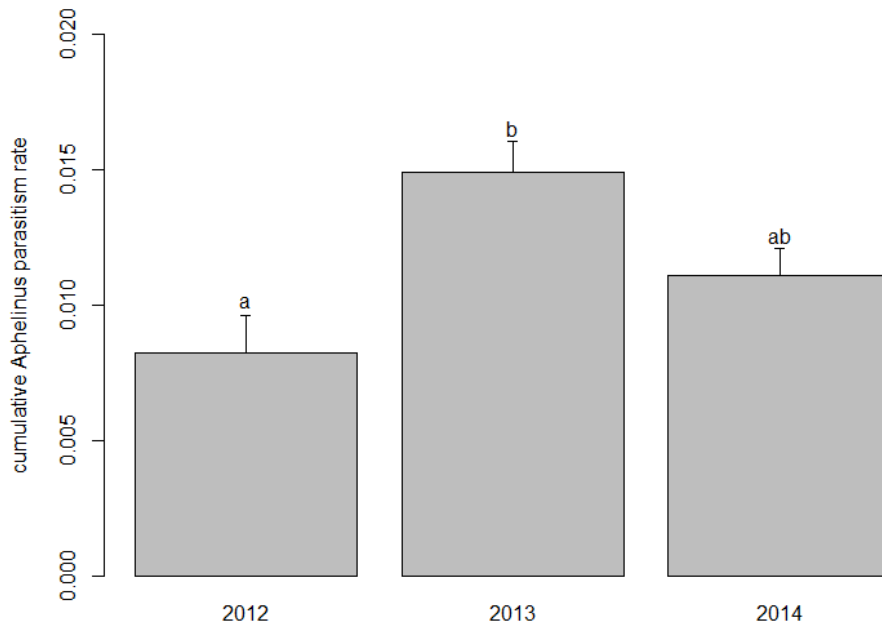
D)



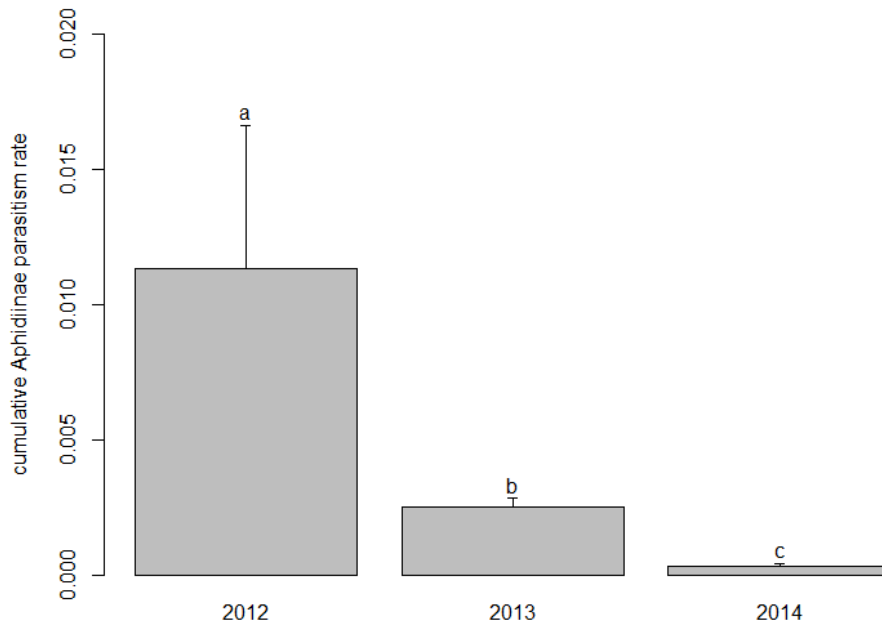
**Figure 13.** Mean density of Aphidiinae spp. mummies per plant for each treatment through time (+/- SE bars) for A) 2012, B) 2013, and C) 2014. Means were calculated by first averaging among samples at each plot then averaging among treatments. Standard error was estimated using the means at each plot (i.e. n=4 for each treatment). D) Cumulative parasitoid days (+SE bars) for each year of the study (ANOVA: P=0.101).



**A)**

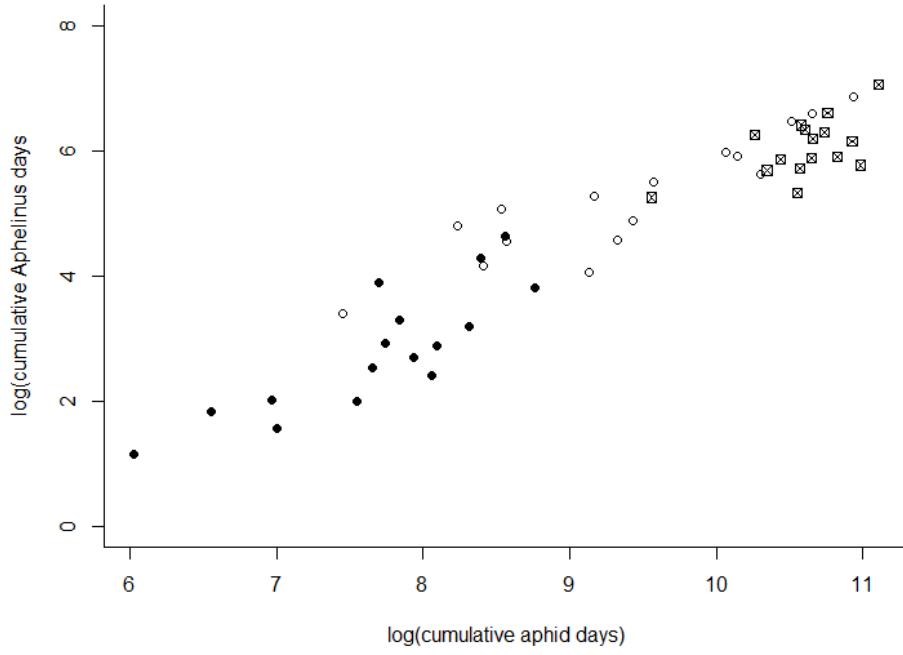


**B)**

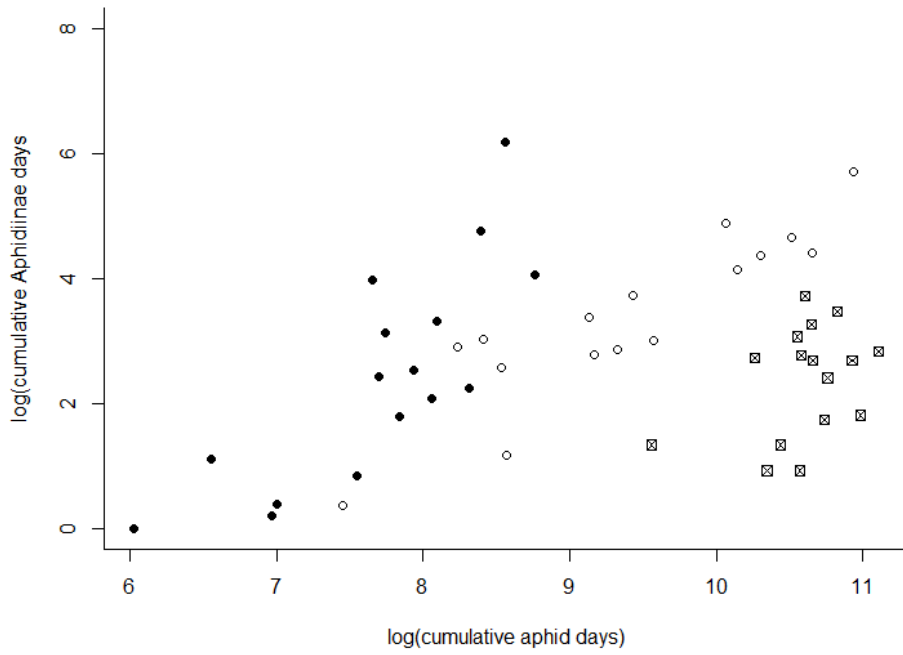


**Figure 14.** Cumulative mummification rate (CPD/(CPD+CAD)) for **A)** *Aphelinus* spp., and **B)** Aphidiinae (+/- SE) (significance at  $\alpha=0.05$  for Tukey corrected multiple comparisons).

**A)**



**B)**



**Figure 15.** Log (cumulative aphid days + 1) by **A)** log (cumulative *Aphelinus* days +1) and **B)** log cumulative Aphidiinae days +1) for each plot for the entire year. Filled circles are for 2012, empty circles are for 2013, and crossed squares are for 2014.

## CHAPTER 4

### PHYLOGENETIC CLUSTERING OF HOST USE BY THE APHID PARASITOID

#### *APHELINUS CERTUS*

##### *Chapter Abstract*

The aphid parasitoid *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) was inadvertently introduced into North America and has spread widely throughout the soybean growing regions of the United States and Canada. *Aphelinus certus* is known to attack and develop in a few aphid species besides the soybean aphid; but a more complete picture of the parasitoid's host range is needed to evaluate the risks and potential benefits posed by its introduction. We exposed *A. certus* to 17 species of hosts across two aphid subfamilies (three tribes) in no-choice tests. *Aphelinus certus* attacked most species presented to it, but mummification rate and adult emergence was highly variable. We mapped host use data onto aphid and aphid host-plant trees to see if host phylogeny predicted host use. *Aphelinus certus* mummification significantly clustered with aphid host relatedness ( $P=0.043$ ), but other host use traits did not. Aphid host-plant phylogeny did not predict any parasitoid host use traits measured. Several native species are at risk of attack by *A. certus*. Additionally, several pest aphid species present in North America may now be subjected to biological control by *A. certus*.

##### *4.1 Introduction*

*Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) is a koinobiont endoparasitoid of the soybean aphid, *Aphis glycines* Matsamura (Hemiptera: Aphididae). Both *A. certus* and the soybean aphid (SBA) were inadvertently introduced into North

America (Heimpel et al. 2010). The first record of SBA in North America was made in 2000 in the upper Midwestern United States (Wisconsin), and it rapidly spread through at least 30 U.S. states and three Canadian provinces (Ragsdale et al. 2011). SBA is considered the most economically important insect pest in North American soybeans (Ragsdale et al. 2011). The first record of *A. certus* was made in 2005 in the Eastern U.S. state of Pennsylvania, where it was found attacking SBA in soybean fields (Heimpel et al. 2010). *Aphelinus certus* has since spread to at least seven additional U.S. states (Illinois, Indiana, Kentucky, Minnesota, Missouri, Ohio, and Wisconsin) (Wulff et al. 2013; Lagos-Kutz and Voegtlin 2016; Chapter 3), and two Canadian provinces (Ontario and Quebec) (Frewin et al. 2010; Brodeur 2013). *Aphelinus certus* is quickly becoming the most common and abundant parasitoid attacking SBA throughout much of its adventive range (Frewin et al. 2010; Brodeur 2013; Hallett et al. 2014; Chapter 3).

Until the arrival of *A. certus*, parasitoids attacking the SBA in North America were generally in low abundance compared to its native range in Asia (Heimpel et al. 2004; Costamagna et al. 2008; Noma et al. 2010). This relatively low parasitoid abundance was a principal argument for the development of a classical biological control program targeting SBA (Heimpel et al. 2004); however, no intentionally released agents have yet been confirmed to be established (Gariépy et al. 2015). The establishment of *A. certus* across a broad geographic range in North America thus stands in contrast. Why has *A. certus* successfully colonized North America, while other species have not? One hypothesis is that the host breadth of *A. certus* allows it to parasitize alternative aphid hosts when SBA is scarce, thus freeing it from Allee effects and allowing it to maintain a viable population year-round (Heimpel and Asplen 2011; Asplen et al. 2011). Studies

conducted on *A. certus* at the United States Department of Agriculture's Beneficial Insect Laboratory prior to its inadvertent introduction indicated that *A. certus* is at least somewhat polyphagous (KR Hopper unpublished data). These initial studies were conducted on seven species in the aphid subfamily Aphidiinae, and all species tested were not native to North America. Interestingly, these findings precluded *A. certus* from intentional release as a classical biological control agent targeting the soybean aphid (KR Hopper pers. comm.). Now that *A. certus* has become established on its own, however, more data are required to predict ecological risks and benefits of *A. certus* in North America. Biological control agents with high levels of host specificity pose lower risk to non-target native species (Simberloff and Stiling 1996), and these were among the arguments supporting the release of the soybean aphid specialists *B. communis* (Wyckhuys and Heimpel 2007; Chacón et al. 2008; Wyckhuys et al. 2009; Desneux et al. 2009) and *A. glycinis* (USDA-APHIS 2012). While some ecological theory supports the idea that the safest classical biological control agent (i.e. the specialist) should also be the most effective and controlling the target pest (Snyder and Ives 2001; Symondson et al. 2002; Kimberling 2004), parasitoids with a broader host range may be able to take advantage of alternative hosts and thus enhance biological control efficacy (Murdoch et al. 1985; Settle and Wilson 1990; Symondson et al. 2002).

The objectives of this study were to 1) examine the ability of *A. certus* to successfully develop on a broad taxonomic range of aphid hosts, including species that are both native and non-native to North America; and 2) to test for a phylogenetic signal of host range. From the standpoint of ecological risk, a better understanding of the host breadth of *A. certus* will allow researchers to determine which native species are at risk of

attack. From the standpoint of biological control efficacy, information about *A. certus* host specificity will provide clues as to how landscape complexity and on-farm management may increase biological control of the soybean aphid or other pest species. For example, information about alternative host-use could inform development of banker planting systems (Frank 2010), or predict how crop diversity may impact pest management (Murphy et al. 1996; Corbett and Rosenheim 1996; Liu et al. 2016). While it is not feasible to test all aphid species which might be exposed to *A. certus* in the field, testing for a phylogenetic signal of host range allows us to make inferences about which untested species may be at risk of attack (Wapshere 1974; McEvoy 1996; van Lenteren et al. 2006; Desneux et al. 2012).

## 4.2 Materials & Methods

### 4.2.1 Aphid and parasitoid colonies

All insects used in this study were kept in colonies in growth chambers at 25 °C, 16:8 h (light:dark) photoperiod, and approximately 65% relative humidity. The 17 aphid species used, aphid host plants, and replication for each species are presented in Table 1. *Aphis gossypii*, which is among the hosts known to be attacked by *A. certus* (KR Hopper unpublished), was reared on two different hosts plants, *Gossypium hirsutum* (cotton) and *Asclepias syriaca* (common milkweed). Common milkweed is known to have high concentrations of anti-herbivore defensive chemicals like inducible cardenolides, which may be sequestered by herbivorous insects for defense against natural enemies (Desneux et al. 2009; Mohl et al. 2016). We included swamp milkweed in addition to cotton so we could compare potential interactions between aphid host-plant and *A. certus*.



The *A. certus* colony used in this study was started from field-collected mummies in August 2011, collected from the University of Minnesota's Rosemount Agricultural Experiment Station and the University of Minnesota Saint Paul campus. These collected mummies were the first records of *A. certus* in Minnesota (identified by KR Hopper, USDA ARS). The parasitoid colony was reared on SBA.

#### 4.2.2 No-choice assays

For each no-choice assay, plants with similar leaf surface area for each host-plant species were potted in plastic pots and covered in a cylindrical plastic cover. The covers were 11 cm in diameter and 21 cm tall, with several 3 cm wide mesh covered holes cut in the sides and on the top. The plants were grown in potting soil which was sealed covered with white plaster of Paris. The plaster of Paris served the dual roles of managing fungus gnats, and allowed to more easily detect parasitoid mummies that fell off the plants during the course of the assays. For each replicate, a plant was inoculated with 50 aphids of a given species and the aphids were allowed to settle for 2-4 hours. One adult virgin female parasitoid, less than 24-hours old, was allowed to mate within a gel capsule with one adult virgin male parasitoid that was also less than 24-hours old. All pairs were observed until copulation behaviors began (i.e. the male mounted the female without being quickly shaken off), and each pair was allowed to continue mating for at least 30 minutes and no more than 4 hours. If copulation behavior was not initiated within 20 minutes, the pair was discarded. Each successfully mated pair was placed into the covered pots after aphid settling time. After 24 hours, both wasps were aspirated out of the cage. If the female could not be found or was dead, that replicate was excluded from

analysis. Plants were inspected daily for 21 days, and all mummies were removed and placed into individual 0.6 mL microcentrifuge tubes. Total mummy counts, emergence rate, and adult offspring sex ratio were recorded.

#### 4.2.3 Statistical analysis

##### Host use data

Total mummy and viable offspring production were analyzed separately using a negative binomial generalized linear model, and goodness of fit was estimated with a likelihood ratio test (R v. 3.3.0, MASS package (Venables and Ripley 2002; R Core Team 2015)). We performed pairwise comparisons using a permutation based t-test from the RVAideMemoire package (Hervé 2016), with 50,000 permutations and the FDR correction (Benjamini and Hochberg 1995). Effects of hosts on the arcsin-square-root transformed sex ratio (proportion males) data were investigated with analysis of variance. Effects of host on arcsin-square-root transformed emergence rate was performed using analysis of variance, and pairwise comparisons were performed using permutation based t-tests. Correlations between mummy production and adult offspring emergence rate (arcsin-square-root transformed) were investigated with simple linear regression.

##### Phylogenetic analysis

We used the analysis of traits procedure in Phylocom (v. 4.2) to test for a phylogenetic signal of total mummy production and total adult offspring production on each aphid host species (Webb et al. 2008). The aphid phylogeny was constructed in Mesquite (v. 3.10, build 765), and the topology was determined from published records

(Ortiz-Rivas and Martínez-Torres 2010; Papatziropoulos et al. 2013). We generated a host plant phylogeny using branch relationships from Desneux et al. (2012) and the Angiosperm Phylogeny Group (2003). Using Phylocom, the average trait value for each host tested for phylogenetic signal using permutation-based methods. For traits associated with the host plant phylogeny we averaged across all trait values across all aphid hosts that developed on a given plant. We performed 100,000 randomizations of trait values across the tips of the phylogeny. For each randomization the standard deviation of the traits across each node descended from the basal node was calculated and averaged across the tree, and this statistic is called divergence,  $D$  (Webb et al. 2008). Higher values of  $D$  indicate lower clustering of traits (i.e. less phylogenetic signal). Significant clustering at  $\alpha = 0.05$  occurs when 5% or fewer of the randomizations result in  $D$  values less than the observed value (Webb et al. 2008; Desneux et al. 2012).

#### 4.3 Results

*Aphelinus certus* successfully produced adult offspring from 14 out of the 17 aphid species tested. Mean mummies produced across all successfully attacked species was 5.17 +/- 0.49 SEM, and mean adult offspring production was 3.60 +/- 0.37 SEM. The highest average number of mummies per host was produced on *Aphi. oestlundii* (8.90 mummies +/- 3.37 SEM), and highest average number of adult offspring was produced on SBA (6.50 adults +/- 1.57 SEM). There were significant differences in both mummy production ( $P < 0.0001$ ) (Fig 16) and adult offspring production ( $P < 0.0001$ ) (Fig 17), but there were no differences in the sex ratio of the adult offspring ( $P = 0.2$ ). Mummy emergence ranged from 100% for *D. noxia* (which only had 2 mummies out of 10

replicates), to a low of 21% for *Aphi. nerii* (which had 33 mummies from 10 replicates). To evaluate the correlation between preference and performance, we analyzed the relationship between the proportion of adults emerged from mummies (performance) and the total number of mummies produced (preference). There was no significant linear correlation between the proportion of adults that emerged from mummies and the total number of mummies produced for each host ( $P=0.54$ ) (Fig 18). However, there was a significant effect of host species on *A. certus* emergence rate ( $P=0.007$ ), with the emergence rate on *Aphi. nerii* significantly lower than on the soybean aphid, *Aphi. fabae*, or *R. maidis* (Fig 19). There was no FDR corrected significant difference between *Aphi. gossypii* strains on cotton versus milkweed for total mummies ( $P=0.98$ ), emerged adults ( $P=0.59$ ), emergence rate ( $P=0.83$ ).

There was significant clustering of mummies by aphid host phylogeny ( $P=0.043$ ), but not of emerged adult offspring ( $P=0.18$ ), and this is likely due to the *Uroleucon/Diuraphis* clade (Fig 20). There was no phylogenetic signal of insect hosts on emergence rate ( $P=0.17$ ) or sex ratio ( $P=0.17$ ). There was significant phylogenetic signal of host plant for mummies ( $P=0.59$ ), emerged adults ( $P=0.71$ ), emergence rate ( $P=0.23$ ), or sex ratio ( $P=0.41$ ) (Fig 21).

#### 4.4 Discussion

We here demonstrate that *A. certus* is a polyphagous aphid parasitoid that attacks both North American native species as well as several invasive aphid species across the subfamily Aphidinae (Fig 20; Table 3). No mummies formed on the native species *M. asclepiadis* (Eriosomatinae: Pemphigini), or either of the *Uroleucon* spp. (Aphidinae:

Macrosiphini) tested. On average, the highest mean adult offspring produced were from SBA, but eight other species produced statistically similar mean numbers of adults (Fig 17). This study suggests that *A. certus* poses a risk to non-target native aphids, such as *Aphi. monardae*, *Aphi. oestlundii*, and *Aphi. asclepiadis*, but further research is needed to estimate the likely exposure and effects of *A. certus* to these aphid species

The host-use data presented here estimate the physiological (or fundamental) host range of *A. certus*, which may be greater than the ecological host range (i.e. the species that are actually attacked in the field) (Morehead and Feener 2000; Haye et al. 2005). Similar to our findings, in host range testing of the classical biological control agent *Binodoxys communis* (Hymenoptera: Braconidae), *Aphi. monardae* was also found to be attacked (Desneux et al. 2009). However, due to ant tending and the observation that *Aphi. monardae* tends to feed with in the bracts of its host flower (*Monarda fistulosa*), it was thought that *Aphi. monardae* would not likely fall within the ecological host range of *B. communis* (Wyckhuys and Heimpel 2007; Wyckhuys et al. 2009). *Aphelinus certus* adults were reared from several field-collected mummies occurring in colonies of *Aphi. monardae* on the host plant *M. fistulosa* adjacent to a soybean field near the University of Minnesota Saint Paul campus (Kaser *pers. obs.*), and it may be that *A. certus* is not as easily detected by ants as *B. communis* is (Rasekh et al. 2010), or that its small size allows it to maneuver within flower bracts more readily than the larger Aphidiinae. However, Brewer et al. (1998) found, when comparing *Diaeretiella rapae* (Hymenoptera: Braconidae) and *Aphelinus albipodus* (Hymenoptera: Aphelinidae), which both attack *D. rapae* within curled and uncurled barley leaves, that the larger parasitoid (*Dia. rapae*) was the more effective biological control agent on curled leaves. The authors conclude

that parasitoid size may be a misleading characteristic in predicting its ability to reach hosts in protected areas of the plant (Brewer et al. 1998). In fact, *Dia. rapae* was more effective overall on curled leaves compared to uncurled leaves, possibly due to differences in host densities. However, in contrast, *Dia. rapae* attacking *D. noxia* on slender wheatgrass (*Elymus trachycaulum*) parasitized hosts at a lower rate on curled leaves versus uncurled leaves (Reed et al. 1992; Brewer and Elliott 2004). It is not clear if *A. certus* can distinguish between olfactory cues between infested and uninfested host-plant complexes (Wyckhuys and Heimpel 2007), or if the observed attack on *A. monardae* was strongly influenced by its proximity to soybean fields. *Aphis oestlundii* is another native species which may be particularly at risk to attack by *A. certus*, as it had the highest average mummy production of all host tested (Fig 16). Further research is needed to evaluate the ecological host range of *A. certus* on the native aphid species shown in this study to fall within its physiological host range.

*Aphelinus certus* represents an example where classical biological control researchers and regulatory protocol succeeded in precluding a potentially dangerous biological control agent from being released. Unfortunately, *A. certus* found its way into the continent through some unknown and inadvertent pathway, and it may now cause harm to native species. Importantly, however, the same factors influencing ecological risk may also be resulting in increased biological control efficacy on SBA and other pest aphids through apparent competition (Holt 1977; Kaser and Ode 2016). We show that *A. certus* can successfully attack several North American pest species. Adults have been reared from mummies collected from mixed field colonies of *S. avenae* and *R. padi* in wheat (Kaser pers. obs.), and both of these aphid species are here shown to be readily

attacked by *A. certus*. The subtribe Rhopalosiphina includes both *R. padi* and *R. maidis* (Papasotiropoulos et al. 2013), which *A. certus* can attack and develop on. The aphid *Melanaphis sacchari*, the sugarcane aphid, is another member of the Rhopalosiphina, and this species has recently emerged as a highly damaging invasive pest of sorghum in the southern and southwestern U.S., and in Mexico (Villanueva et al. 2014; Rodriguez-del-Bosque and Teran 2015). *Aphelinus certus* overlaps with at least some of the *M. sacchari* summer range (Kentucky) (Wulff et al. 2013), and so it may become an important natural enemy of sugarcane aphid.

The enemy release hypothesis proposes that species become invasive by escaping the coevolved natural enemies of their native range, and this has been cited as an explanation for the success of classical biological control (Keane and Crawley 2002; Colautti et al. 2004; Liu and Stiling 2006). There have been several parasitoids imported and released against SBA (Wu et al. 2004; Chacón and Heimpel 2010; Garipey et al. 2015), but none have yet been confirmed to be established in North America. For example, *B. communis*, a specialist koinobiont endoparasitoid that attacks SBA, underwent repeated area-wide releases beginning in 2007 (Chacón and Heimpel 2010). Hypotheses explaining its failure to establish include the loss of diapause ability while in quarantine (Garipey et al. 2015), intraguild predation (Chacón and Heimpel 2010), the inability to track its host to overwinter (Asplen et al. 2011), or Allee effects (Heimpel and Asplen 2011). It may be that a broader suite of potential hosts available to *A. certus* has allowed it to overcome fitness disadvantages faced by specialists.

While we did not in this study directly measure attack rate (preference), nor closely measure mortality at early parasitoid developmental stages (performance), we can

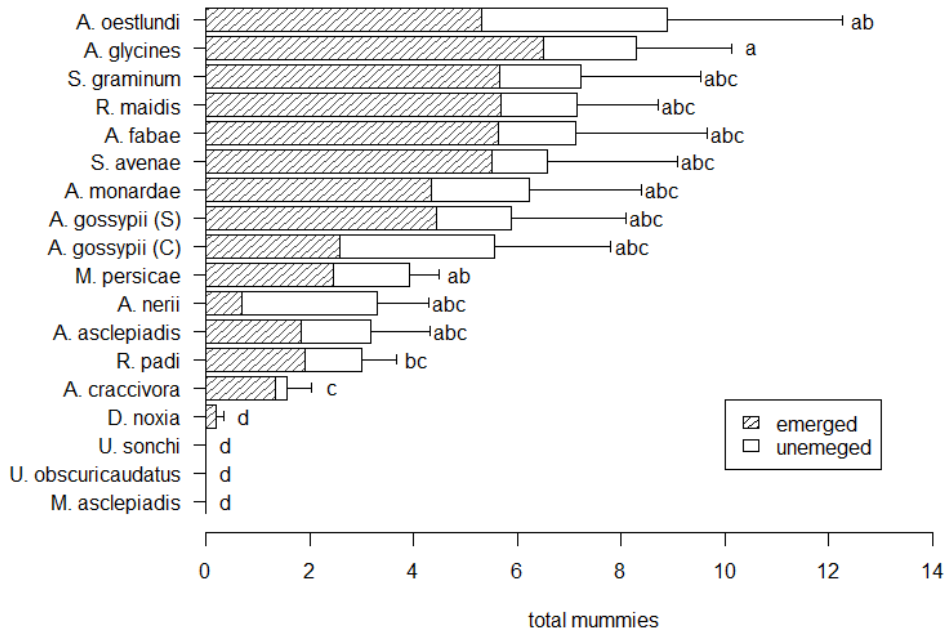
infer some clue as to the preference-performance relationship of *A. certus* on different hosts. For many parasitoid species, the rate of attack is different from the rate of mummification or adult emergence (e.g. Desneux et al. 2009). This could be caused by various factors, including host nutritional quality (Godfray 1994), host innate defenses (Godfray and Hassell 1993; Carton and Nappi 2000), host-plant mediated indirect effects (Ode 2006), and endosymbionts (Oliver et al. 2010; Asplen et al. 2014; McLean and Godfray 2015). While preference and performance are typically positively correlated there is often a great deal of additional variation (Jaenike and Holt 1991; Berenbaum and Feeny 2008; Craig and Itami 2008; Desneux et al. 2009). For example, *B. communis* attacks *Aphi. nerii* at a high rate, but rarely successfully develops on these hosts (Desneux et al. 2009), and we found similar results for *A. certus* attacking *Aphi. nerii*. By treating mummification rate as a rough proxy for parasitism rate, and the proportion of emerged adults over total mummies as a proxy for host suitability, we found no preference performance relationship (Fig 18). Hosts of low suitability that are oviposited in can serve as an egg sink, which can have important population level consequences in egg limited parasitoids (Hoogendoorn and Heimpel 2002; Heimpel et al. 2003; Kaser and Heimpel 2015). In *B. communis*, there is a phylogenetic signal of aphid host for pupal survival (Desneux et al. 2012), yet we found signal for emergence rate of *A. certus*. Further research is needed to evaluate the relationship between host preference – including host feeding – and performance for *Aphelinus certus*.



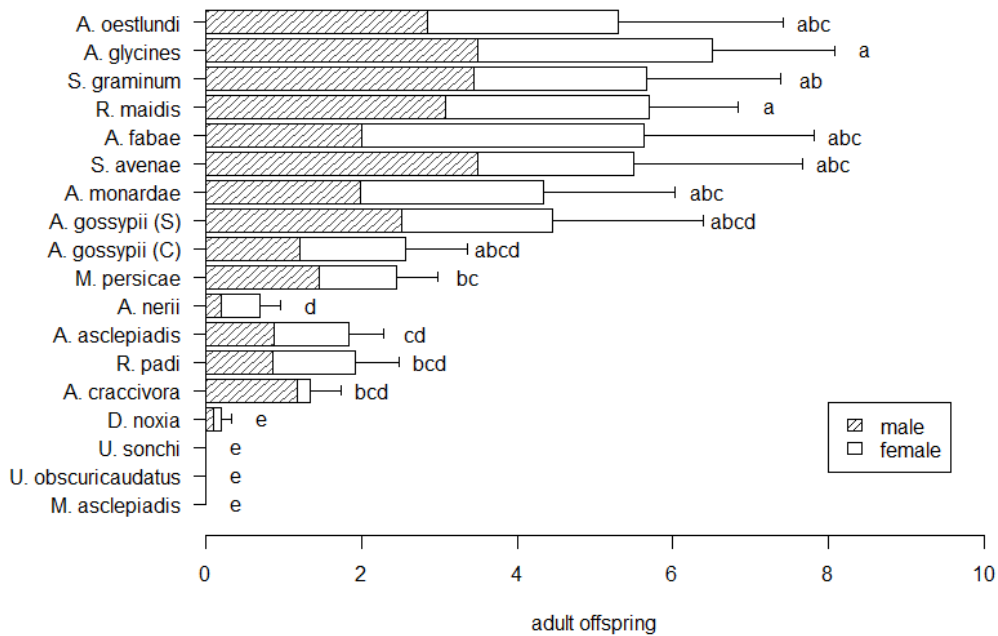
**Table 3.** Aphid host species, tribe and subfamily, associated host plant used in the study, and number of replicates (n).

Aphid species	Aphid Subfamily: Tribe	Host plant	n
<i>Aphis asclepiadis</i> F.	Aphidinae: Aphidini	<i>Asclepias syriaca</i> L.; common milkweed	12
<i>Aphis craccivora</i> Koch; cowpea aphid	Aphidinae: Aphidini	<i>Vicia faba</i> L.; fava bean	9
<i>Aphis fabae</i> Scopoli; bean aphid	Aphidinae: Aphidini	<i>Rumex altissimus</i> Alph. Wood; pale dock	8
<i>Aphis glycines</i> Matsumura	Aphidinae: Aphidini	<i>Glycine max</i> (L.) Merr.; soybean	10
<i>Aphis gossypii</i> Glover	Aphidinae: Aphidini	<i>Asclepias syriaca</i> L.; common milkweed	9
<i>Aphis gossypii</i> Glover	Aphidinae: Aphidini	<i>Gossypium hirsutum</i> L.; cotton	7
<i>Aphis monardae</i> Oestlund	Aphidinae: Aphidini	<i>Monarda fistulosa</i> L.; wild bergamot	9
<i>Aphis nerii</i> Boyer de Fonscolombe; oleander aphid	Aphidinae: Aphidini	<i>Asclepias incarnata</i> L.; swamp milkweed	10
<i>Aphis oestlundii</i> Gillette	Aphidinae: Aphidini	<i>Oenothera biennis</i> L.; common evening primrose	10
<i>Diuraphis noxia</i> (Kurdjumov); Russian wheat aphid	Aphidinae: Macrosiphini	<i>Hordeum vulgare</i> L.; common barley	10
<i>Myzocallis asclepiadis</i> (Monell)	Eriosomatinae: Pemphigini	<i>Asclepias syriaca</i> L.; common milkweed	12
<i>Myzus persicae</i> (Sulzer); green peach aphid	Aphidinae: Macrosiphini	<i>Rumex altissimus</i> Alph. Wood; pale dock	11
<i>Rhopalosiphum maidis</i> (Fitch); corn leaf aphid	Aphidinae: Aphidini	<i>Hordeum vulgare</i> L.; common barley	13
<i>Rhopalosiphum padi</i> (L.); bird cherry-oat aphid	Aphidinae: Aphidini	<i>Hordeum vulgare</i> L.; common barley	12
<i>Schizaphis graminum</i> (Rondani); greenbug	Aphidinae: Aphidini	<i>Hordeum vulgare</i> L.; common barley	9
<i>Sitobion avenae</i> (Fabricius); English grain aphid	Aphidinae: Macrosiphini	<i>Hordeum vulgare</i> L.; common barley	12
<i>Uroleucon obscuricaudatus</i> (Olive)	Aphidinae: Macrosiphini	<i>Heliopsis helianthoides</i> (L.) Sweet; smooth oxeye	10
<i>Uroleucon sonchi</i> (L.)	Aphidinae: Macrosiphini	<i>Sonchus oleraceus</i> L.; common sowthistle	7

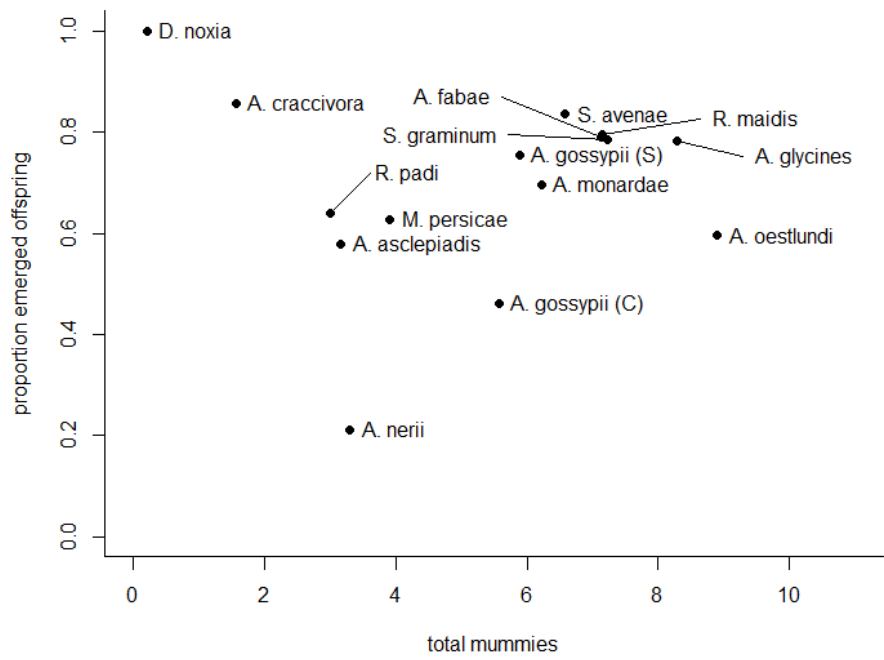
Entomological Society of America approved common names of aphids are included when available, as well common names host plant species USDA PLANTS database (ESA; USDA); higher taxonomy from Ortiz-Rivas & Martínez-Torres (2010) and Nováková et al. (2013).



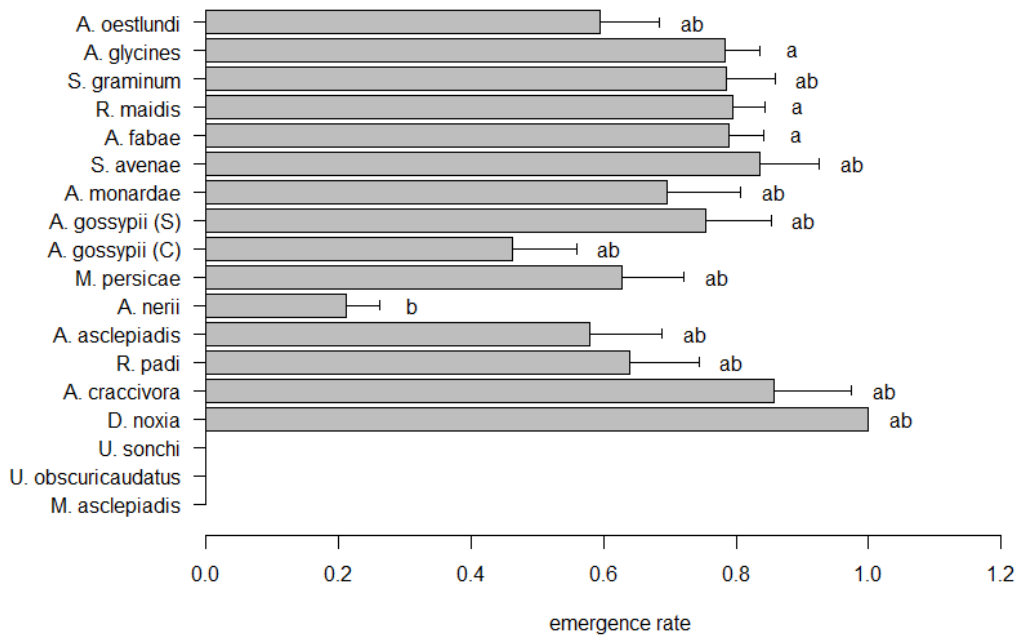
**Figure 16.** Stacked bar chart indicating mummification of host species by *A. certus*. Hashed areas indicate the mean number of mummies for which adult offspring successfully emerged; open areas indicate the mean remaining unemerged mummies. Standard error bars were calculated for total mean mummies (emerged + unemerged), and letters indicate significant difference for pairwise comparisons (FDR corrected  $\alpha=0.05$ ).



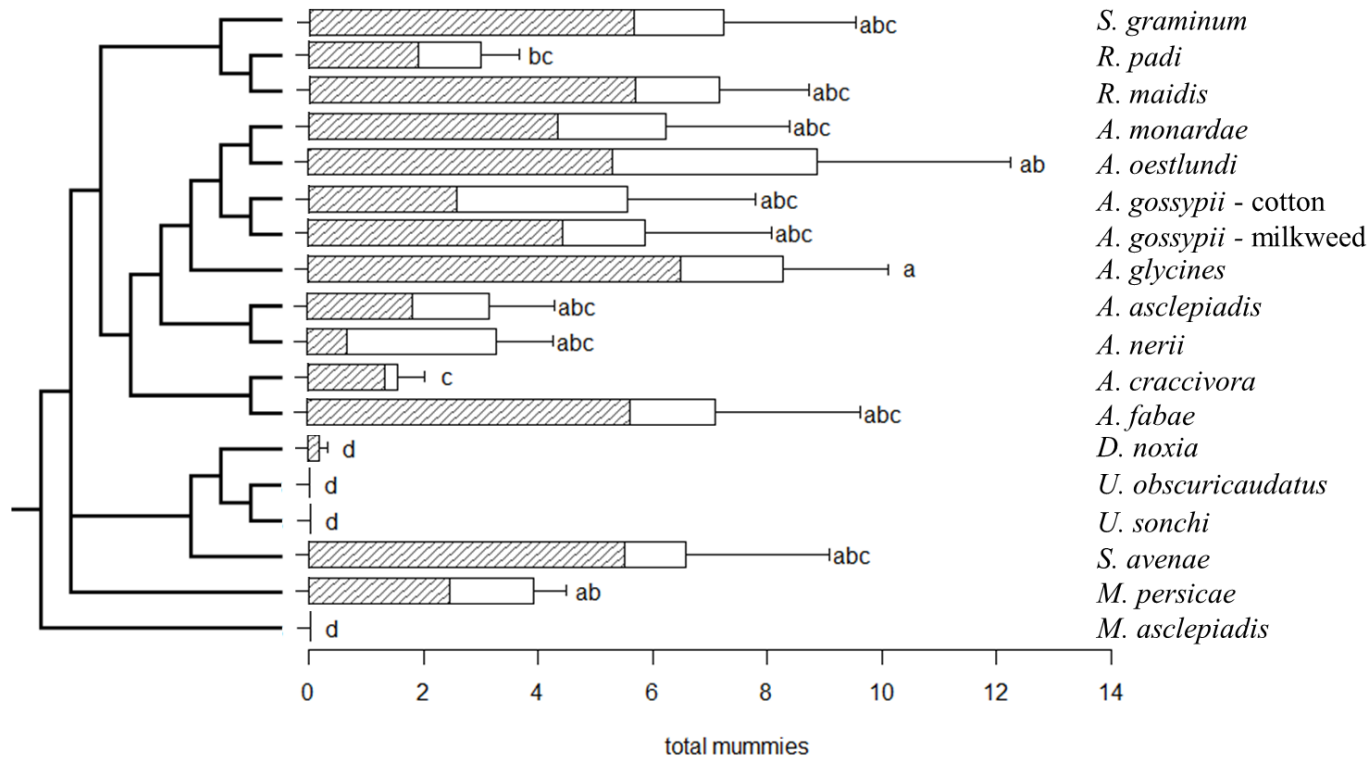
**Figure 17.** Stacked bar chart indicating emerged adult *A. certus* offspring from each host species. Hashed areas indicate the mean number of male offspring; open areas indicate the mean female offspring. Standard error bars were calculated for total adult offspring, and letters indicate significant differences for pairwise comparisons (FDR corrected  $\alpha=0.05$ ). For comparison with Fig 1, hosts are ordered by total mummy density (emerged + unemerged). For host *A. gossypii*, (C) indicates the aphid strain reared on cotton, and (S) indicates the aphid strain reared on swamp milkweed.



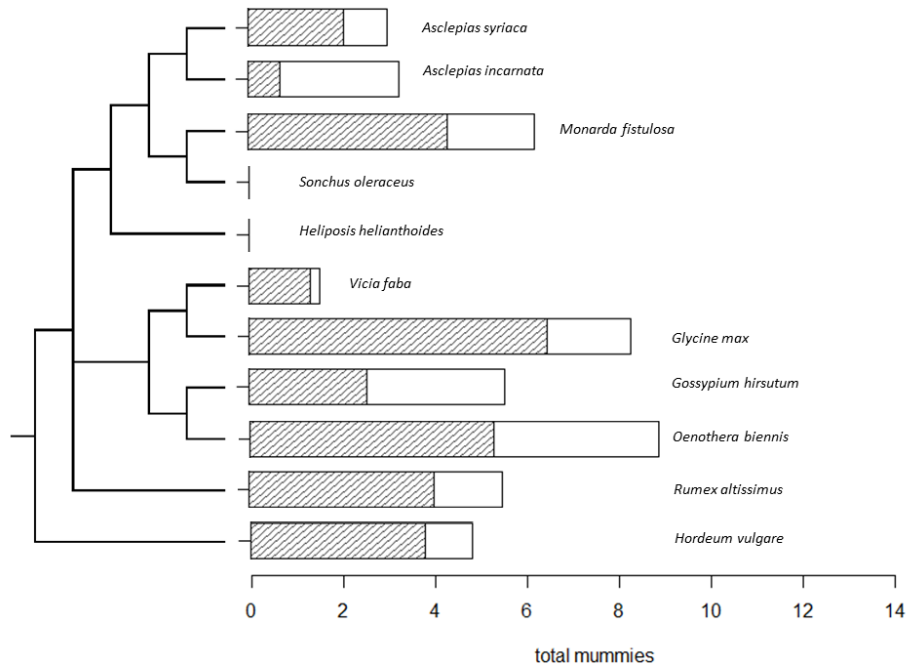
**Figure 18.** The proportion of emerged adult offspring plotted against the total number of mummies formed for each host.



**Figure 19.** Bar chart indicating mean mummy emergence rate for each host (+/- SEM). Letters indicate significant differences for pairwise comparisons (FDR corrected  $\alpha=0.05$ ) on the arcsin-square-root transformed rates. For comparison with Fig 1, hosts are ordered by total mummy density (emerged + unemerged). For host *A. gossypii*, (C) indicates the aphid strain reared on cotton, and (S) indicates the aphid strain reared on swamp milkweed.



**Figure 20.** Mummy density corresponding to aphid phylogeny with the hashed area representing offspring that emerged as adults and open area indicating mummies that died unemerged. Letter labels indicate pairwise separations from permutation test (FDR corrected  $\alpha=0.05$ ).



**Figure 21.** Average mummy density (hashed bar indicates emerged adults, and open bar indicates unemerged mummies) corresponding to the host plant phylogeny for the aphid hosts used in this study. For cases in which multiple aphid species were reared on the same plants species, trait values are averaged.

## CHAPTER 5

### IMPACT OF THE PARASITOID *APHELINUS CERTUS* ON SOYBEAN APHID POPULATIONS

#### *Chapter Abstract*

*Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) is an accidentally introduced parasitoid of the invasive soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in North America, and it has become one of the most common natural enemies of soybean aphids in its adventive range. It is unclear, however, if increased prevalence of *A. certus* has resulted in increased biological control. We conducted an exclusion-cage experiment designed to isolate the impact of parasitoids compared to other resident natural enemies of the soybean aphid. We found that *A. certus* greatly outnumbered all other soybean aphid parasitoids attacking the soybean aphid, and that it significantly reduced soybean aphid populations over a time span of less than two weeks compared to controls. Moreover, parasitoids alone resulted in aphid densities that were statistically equivalent to the combined effects of predators and parasitoids.

*Aphelinus certus* is an important new component of the soybean aphid natural enemy community in North America. Further research is needed to evaluate its role in integrated pest management and to update economic thresholds for the soybean aphid.

#### *5.1 Introduction*



The soybean aphid was first reported in North America in 2000, and it remains the most economically important insect pest in North American soybeans (Ragsdale et al. 2011; Heimpel et al. 2013). Soybean production value in the United States was estimated at over \$34.5 billion in 2015, and was planted over 33.4 million hectares (<http://usda.nass.gov>). Yield loss due to soybean aphid infestation can reach as high as 40%, increasing management costs by an average of \$16-\$33 per hectare (Ragsdale et al. 2007; Ragsdale et al. 2011). Insecticide use in soybeans increased 130-fold since the arrival of the soybean aphid, where conventional growers principally rely on foliar applied pyrethroids and organophosphates for soybean aphid management (Ragsdale et al. 2011). Soybean aphid management efforts are estimated to cause 10.6 kg of additional CO<sub>2</sub> equivalent greenhouse gas emissions per hectare (Heimpel et al. 2013). Without the contribution of natural enemies to reduce soybean aphid populations, insecticide use would increase, and emissions from soybean aphid management would be about 200 million kg higher in the U.S. alone (Heimpel et al. 2013).

Biological control is a critical component of effective soybean aphid integrated pest management (Ragsdale et al. 2007). A suite of natural enemies is known to attack the soybean aphid in North America (Rutledge et al. 2004; Ragsdale et al. 2011), and the most effective biological control is thought to be caused by generalist predators, particularly coccinellids like *Harmonia axyridis* (Rutledge et al. 2004; Mignault et al. 2006). Early studies of soybean aphid natural enemies in North America found several groups of parasitoids, both native and non-native, which attack the soybean aphid (Noma and Brewer 2008); but as a functional group, parasitoids have been only minor players in North American soybean aphid biological control (Heimpel et al. 2010; Ragsdale et al.

2011). As an exception to this conclusion, one potentially effective parasitoid species, *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae), has been increasing in prevalence since 2005 when it was first recorded in North America (Heimpel et al. 2010; Frewin et al. 2010; Ragsdale et al. 2011; Brodeur 2013; Chapter 3). *Aphelinus certus* is native to Asia (Heraty et al. 2007). Prior to its inadvertent introduction, it had been considered by researchers as a possible classical biological control agent to target the soybean aphid. However, quarantine host-specificity studies showed a wide host breadth within aphids and thus potential high risk to native aphid species (Hopper unpublished). As a result, no petition for release of *A. certus* in North America was filed, and we know of no intentional introduction. Nevertheless, *A. certus* was found in North America in 2005, presumably the result of an unintentional introduction, and it is now the dominant parasitoid attacking the soybean aphid throughout much of its adventive range (Frewin 2010; Heimpel et al. 2010; Brodeur 2013; Wulff et al. 2013; Lagos-Kutz and Voegtlin 2016; Chapter 3).

While *A. certus* is continuing to increase abundance in cultivated soybean fields (Chapter 3), reaching relatively high parasitism rates (Frewin et al. 2010), it remains to be shown experimentally that *A. certus* field populations can effectively control the soybean aphid. Local population densities of any pest species are determined by the cumulative effects of birth, death, immigration, and emigration (Thomas and Kunin 1999). These effects are influenced by a combination of top-down and bottom-up processes, and natural enemies are not always the driving force regulating populations (Hunter and Price 1992). Indeed, neither high abundance of natural enemies nor high rates of parasitism necessarily result in decreased population density. For example, *Orius insidiosus*

(Hemiptera: Anthocoridae) is thought to be an important predator of the soybean aphid (Rutledge and O'Neil 2005), but *O. insidiosus* has a preference for soybean thrips and may not consume soybean aphids when thrips densities are high (Desneux and O'Neil 2008). Alternatively, if intraspecific competition of the pest species is high, increased parasitism may simply be offset by reduced competition, without resulting in a net decrease in pest population size (Ortega et al. 2012). In a study conducted at the Kellogg Biological Station Long Term Ecological Research site, Costamagna and Landis (2006) found that soybean aphid is largely regulated by top-down processes, but their study was conducted prior to the arrival of *A. certus* in the region. To determine whether field populations of *A. certus* are causing increased control of the soybean aphid, we must differentiate between background aphid birth and death rates, while controlling for migration. Exclusion cage experiments are designed for this task, and have been utilized successfully in the past with soybean aphids to characterize the total effect of the community of natural enemies attacking the soybean aphid (Fox et al. 2004; Fox et al. 2005; Costamagna and Landis 2006; Miao et al. 2007; Gardiner et al. 2009). The objectives of the current study were to isolate the effects of parasitoids from that of other natural enemies and to determine if ambient densities *A. certus* alone are capable of reducing field populations of the soybean aphid.

## 5.2 Materials & Methods

### 5.2.1 Study site and experimental design

This study was conducted in a 0.7 hectare soybean field at the University of Minnesota Saint Paul Agricultural Experiment Station during July and August, 2015. The

field was planted with a mixture of cultivars MN0303SP and MN0209SP, neither of which have known soybean aphid resistance. Timing of the experiment was chosen based on aphid densities, so that aphid infestations were high enough to measure an effect of treatments, but early enough in the season to avoid density induced alate production and the summer alate migration phase (Hodgson et al. 2005). By conducting the experiment during this time window, we minimized the effects of immigration and emigration on soybean aphid density, so that treatments were largely a measure of birth and death factors. There were five treatment cages: 1) a fine mesh cage (240 micron holes) that was intended to exclude all natural enemies; 2) a predator exclusion mesh cage (1 mm holes) that would only let in very small natural enemies, which are principally parasitoids; 3) an open cage that would allow all natural enemies to enter; 4) a sham total exclusion cage that was open at the top and bottom; 5) a predator exclusion sham cage (Liu et al. 2004). The sham cages were intended to simulate the microclimate of the predator and total exclusion cages, but to allow all natural enemies to enter similarly to the open cages. We in this way control for the possibility that differences between treatments are due to microclimate rather than the amount and types of natural enemies entering the cages. We replicated each treatment 16 times, within 16 blocks. The cages in each block were placed ~1 meter apart from each other and treatment location was randomized within block, and blocks were spaced regularly throughout the field. As a further measure to monitor possible microclimate effects, we placed temperature loggers within all cages of three randomly selected blocks to directly compare temperatures between treatments.

Eight blocks of the experiment were initiated on July 27, 2015, and the other eight began on July 30, 2015. Twenty-four hours prior to initiating the treatments, 10 plants

within five meters of each block were haphazardly selected, and all alate and apterous soybean aphids were counted, in addition to all soybean aphid natural enemies (Table 1). The median aphid density of the 10 plants surround each block was used to determine the aphid inoculation level for each treatment cage within that block. This was done to avoid simulating an outbreak population or inducing aggregations of natural enemies (Schellhorn and Andow 2005) that might differ from ambient densities in the surrounding field. For each treatment, a single soybean plant was selected and all plants within contract distance were removed so that aphids could not easily migrate by walking between plants. All insects were removed from the selected plants prior to inoculation with soybean aphids from a laboratory colony. All treatments were covered with an additional fine mesh (240 microns) for 48 hours after inoculation to allow aphids to settle without exposure to natural enemies. After 48 hours the fine mesh was removed from all cages, aphid densities were recounted, and treatments were allowed to run for 12 days. After 12 days all aphids and natural enemies were counted on each plant, and parasitoid mummies were placed in gel capsules for emergence and identification to species. Each plant was then removed and placed in a sealed paper bag and brought back to the laboratory. After approximately five days, the bags were opened, and all mummies that had formed were counted and placed into individual gel capsules for emergence and identification to species. This was done to gain a more accurate estimate of parasitism rate, as *A. certus* about 6 days to reach the mummy stage at 25 °C (Frewin et al. 2010), and not all parasitized aphids observed in the field would have yet mummified.

### 5.2.2 Statistical analysis

Aphid densities among treatments were compared with a negative binomial generalized linear model (Venables and Ripley 2002). Aphid growth rates among treatment was compared with a generalized linear model with a log-link Gaussian distribution. Parasitism rates among treatments were compared using a generalized linear model with a gamma family distribution. Pairwise comparisons were conducted with Tukey corrections for significance. Standard deviations and means from each temperature logger were calculated, and these values were compared between treatment with analysis of variance.

### 5.3 Results

Both *Aphelinus* spp. and Aphidiinae mummies were recorded on plants surrounding each block in the 10-plant survey prior to establishing treatment cages. Multiple predator species were observed, including *Aphidoletes aphidomyza* larvae, Coccinellidae adults, larvae, and egg clusters, lacewing larvae and eggs, *Orius insidiosus* adults and nymphs, and Syrphidae larvae (Table 1). Alate aphid densities per plant were low prior to caging plants, averaging 0.8 (+/- 0.15 SEM) alates per plant. The average inoculation density for treatment cages was 54.44 (+/- 4.73 SEM) apterae per plant. At the end of the 12-day treatment period, there was some contamination of parasitoids observed in the fine mesh exclusion cages. In all cases where the fine mesh exclusion cage had greater than 0.10 parasitism rate (i.e. mummies/(aphids+mummies), the entire block in which it occurred was removed from the analyses. In total, five blocks were removed; the following analyses are on the 11 remaining blocks.

Aphid densities increased in all treatments from the beginning of the experiment to the end, indicating that aphid growth was positive during the course of the experiments, regardless of treatment. After controlling for block ( $P=0.34$ ), there was a highly significant effect of treatment on aphid density ( $P<0.0001$ ) (Fig 22). Aphid densities in the sham cages did not significantly differ from the open cages ( $P=0.91$  and  $P=0.81$  for the exclusion sham and parasitoid-only sham, respectively). The fine mesh exclusion cage was the only treatment where average aphid density exceeded the economic threshold of 250 aphids/plant (Ragsdale et al. 2007) (Fig 22). Aphid growth rates were also significantly different between treatments ( $P<0.0001$ ) after controlling for block ( $P<0.0001$ ) (Fig 23). Aphid growth was highest in the exclusion cage and significantly different from all other treatments ( $P<0.005$  for all pairwise comparisons to exclusion cages). Aphid growth in the open, sham, and parasitoid only cages were not significantly different from each other (Fig 23).

Aphidiine mummy densities were low, totaling only 12 for all treatments, and none of these mummies were successfully reared to adult stage. *Aphelinus* spp. totaled 1,354 mummies across all treatments. A total of 798 *Aphelinus* spp. mummies were collected, of which 587 adult parasitoids or hyperparasitoids emerged. *Aphelinus certus* represented 98.7% of emerged primary parasitoids. The remaining were identified as *Aphelinus asychis* (1.0%), or remained unidentified *Aphelinus* spp. (<1.0%). The hyperparasitism rate was 3.7% and included *Asaphes* spp. (Hymenoptera: Pteromalidae) and *Alloxysta* spp. (Hymenoptera: Figitidae). There was a significant effect of treatment on the *Aphelinus* parasitism rate ( $P<0.0001$ ) after controlling for block ( $P=0.004$ ). While there was some contamination of parasitoids into the exclusion cages, parasitism rate was

lowest in the exclusion cages ( $P < 0.005$  for all pairwise comparisons to exclusion cages) (Fig 24). Predators observed on treatment plants at the conclusion of the experiment included *Aphidoletes aphidomyza*, Coccinellidae, lacewings, and *O. insidiosus*. There were low levels of contamination into exclusion cages, with a mean of 2.1 ( $\pm 0.53$  SEM) predators in the parasitoid cage, and 2.45 ( $\pm 0.97$  SEM) predators in the exclusion cage. These contaminants were mostly *Orius insidiosus* which may have crawled into the cages as nymphs or emerged from eggs that were not seen when cleaning plants. Other than *O. insidiosus*, there were only a mean of 0.54 ( $\pm 0.21$  SEM) predators in the parasitoid cages, and 0.45 ( $\pm 0.16$  SEM) predators in the total exclusion cage.

To investigate the potential impact of migration on aphid densities, we estimated alates in each treatment. Overall soybean aphid alate densities averaged only 2.54 ( $\pm 0.51$  SEM) per treatment, or about 1.17% of total aphids (apterae+alates). There was a significant difference between treatments ( $P < 0.0001$ ) and block ( $P = 0.08$ ), with alate density highest on the open cages and lowest in the total exclusion cages (Fig 25). There were no significant differences between the sham cages and the open treatment for total aphid density, aphid growth rate, parasitism rate, or alate density. The mean temperature recorded by the temperature loggers was 24.23°C (standard deviation: 7.78). There was no significant difference in mean temperature between treatment ( $P = 0.45$ ). There was no significant difference in standard deviation of temperature between treatment ( $P = 0.30$ ).

#### 5.4 Discussion



This is the first study to our knowledge to experimentally demonstrate that the parasitoid *Aphelinus certus* can significantly reduce the growth rate of the invasive soybean aphid in North America. Over the course of only 12 days, restricting exposure to ambient parasitoid densities resulted in a 3.2-fold increase in aphid densities. Moreover, parasitoids resulted in similar aphid control when compared to treatments that allowed access to both predators and parasitoids combined, suggesting that even in the absence of predators, *A. certus* remains an effective biological control agent that is likely contributing to reduced insecticide use and associated adverse environmental impacts throughout the soybean growing region of North America (Desneux et al. 2007; Heimpel et al. 2013; Ragsdale et al. 2011). Parasitoids were able to keep aphids below the economic threshold of 250 aphids per plant (Ragsdale et al. 2007). This experiment demonstrates that *A. certus* is an important species in the soybean aphid natural enemy community in North American.

While exclusion cage experiments have been utilized successfully in multiple agriculture contexts to evaluate the effects of biological control (e.g. Rusch et al. 2016 and references therein), they have been occasionally criticized for not properly addressing insect immigration and emigration which could influence birth rates and population size between treatments (Kindlmann et al. 2015). Alate densities were low throughout this experiment, and significantly lower on the exclusion cages than any other treatment (Fig 25). While alates occurring on exclusion and parasitoid-only cages would represent potential emigrants, alates occurring on sham and open cages could represent either immigrants or emigrants. Therefore, our estimates of biological control effects are likely conservative, because we likely excluded immigrants from entering the total exclusion

cage which would have added aphid density, yet there were very few emigrants that we trapped. The most conservative comparison between treatments would be for the open cage (which had the highest mean density of alates) versus the total exclusion cage (which had the fewest alates) (Fig 25). Another criticism of exclusion cage experiments is that there may be microclimate effects that could bias birth and death rates between treatments (Kindlmann et al. 2015). However, our sham cages were intended to capture any potential cage effects on microclimate while allowing similar access to natural enemies as the open treatment. There were no significant differences between sham cages and open cages in aphid densities; therefore, cage microclimate did not likely differentially effect aphid birth and death rates between treatments. Moreover, the temperature logger data, a direct measurement of cage effects on microclimate, indicated that there were no significant differences in temperature between any of the treatments. Therefore, effects of treatments, other than differential exclusion of natural enemies, were not likely to have influenced aphid birth and death rates, and it is thus reasonable to infer that differences between treatments were principally due to differences in biological control.

We found significantly lower aphid densities with exposure to natural enemies over a 12-day period. The generalist predators observed in this study – in particular Coccinellidae – are known to consume soybean aphids quickly, especially when they aggregate in areas of high aphid density (Koch 2003; Rutledge and O’Neil 2005; Costamagna and Landis 2006; Donaldson et al. 2007; Koch and Galvan 2007). However, the generation time of *A. certus* is typically longer than 12 days (Frewin et al. 2010; Kaser pers. obs.), and, therefore, might not be expected to reduced soybean aphid

densities over that time period (Kindlmann and Dixon 1999). However, *Aphelinus* spp. in general are known to kill hosts via host feeding to support synovigenic egg development (Jervis and Kidd 1986; Heimpel and Collier 1996). Moreover, aphids parasitized by koinobiont parasitoids often have reduced fecundity prior to mummification (Kaiser and Heimpel 2016), and we have observed approximately halved reproductive rates of soybean aphids parasitized by *A. certus* compared to controls (Pitel et al. unpublished data).

*Aphelinus certus* has a broad host range (Chapter 4), so populations may be enhanced through banker cropping systems (Frank 2010; Frost et al. 2015). *Aphelinus certus* has been documented attacking other pest aphids such as *Sitobion avenae* and *Rhopalosiphum padi* in wheat, as well as native aphids such as *Aphis monardae* on *Monarda fistulosa* (Chapter 3; Kaser pers. obs.). Future work is needed to see if *A. certus* is important in the biological control of other pest aphids within its host range, and to determine the harm it may pose to native aphid species (Chapter 3). Another area of future research aimed to increase biological control by *A. certus* is of hyperparasitoids. Hyperparasitism was low in this study, but has been observed to be higher in other contexts (Chapter 3). Hyperparasitism may reduce biological control efficacy if it disrupts *A. certus* populations (Schooler et al. 2011).

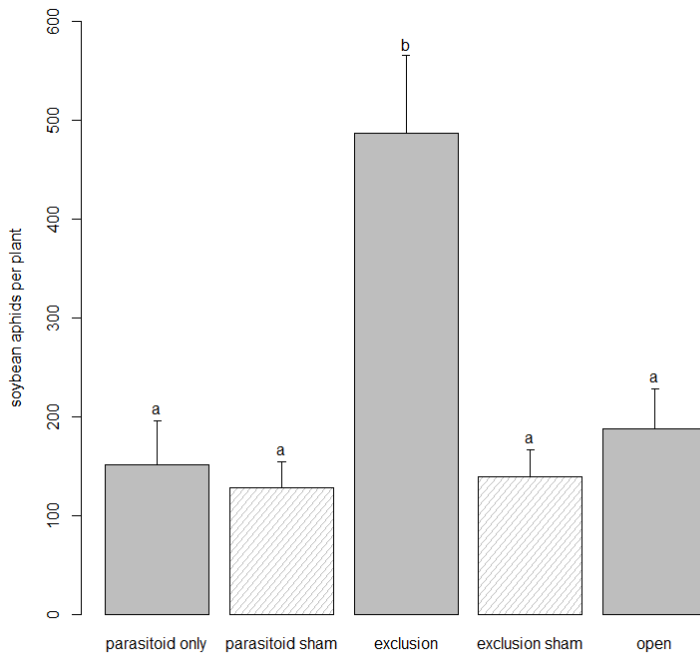
*Aphelinus certus* is an important and effective component of the natural enemy community controlling the soybean aphid in North America. This community has changed significantly since economic thresholds were initially established for the soybean aphid (Ragsdale et al. 2007), and therefore the threshold of 250 aphids per plant may need to be updated. This study emphasizes the need to increase conservation

biological control efforts and reduce use of foliar insecticides that are harmful to *A. certus*, like dimethoate and  $\lambda$ -cyhalothrin (Frewin et al. 2012), or prophylactic use of neonicotinoid seed treatments (Frewin et al. 2014). Since 2005, insecticidal seed treatments, largely neonicotinoids, have increased dramatically in soybeans (Douglas and Tooker 2015). The socioeconomic causes of this increase in soybeans is complex, and not only due to soybean aphid control efforts, for which the efficacy of seed treatments is debated (Bahlai et al. 2015; Douglas and Tooker 2015). Frewin et al. (2014) demonstrated that neonicotinoid seed treatments can be harmful to *A. certus* attacking soybean aphids on treated plants. Therefore, the overuse of seed treatments should be avoided to increase biological control and reduce harm to beneficial insects. For farmers who are utilizing economic thresholds, however, *A. certus* is likely already resulting in decreases in insecticide use throughout the soybean growing region of North America.

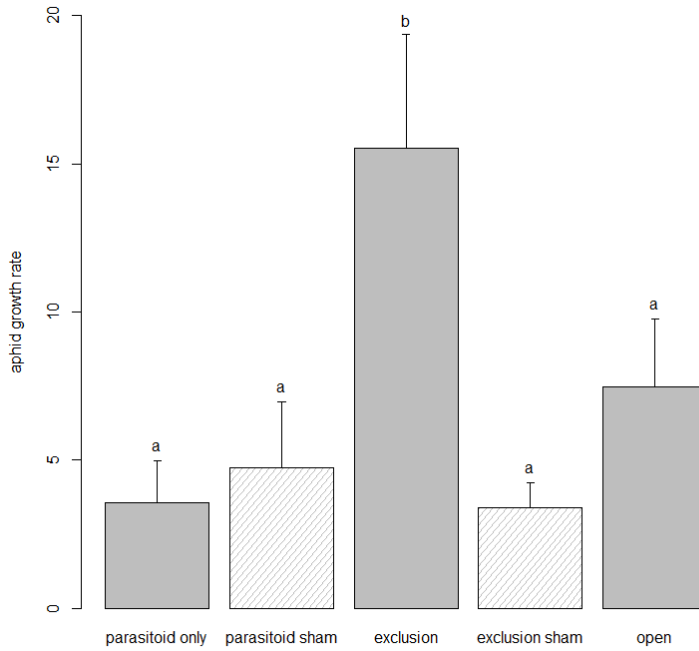
**Table 4.** Summary of natural enemies recorded at the beginning and end of the experiment, and median aphid densities selected.

Block	Median: Aphids		Mean: Predators				Mean: Parasitoids <sup>5</sup>	
	Apterae	Alates	<i>Aphidoletes aphidomyza</i> <sup>1</sup>	Coccinellidae <sup>2</sup>	Lacewings <sup>3</sup>	Syrphidae <sup>4</sup>	Aphidiinae	<i>Aphelinus</i> spp.
1	32	0.5	0.1	0.3	0	0	0.1	0.2
2	34	1	0	0.2	0.1	0.3	0	0.3
3	30.5	0	0.1	0.1	0.2	0.2	0	0.6
4	40	0	0	0.4	0	0.1	0	0.8
5	29.5	0	0	0	0.2	0.1	0	0.4
6	21.5	0	0	0.1	0.1	0.1	0	0.3
7	27	0.5	0.1	0	0.1	0	0	0.1
8	40.5	0	0	0.1	0.2	0	0.1	0.7
9	84	1	0.6	0	0	0	0	1.4
10	53	0	0	0	0	0	0	2
11	45	1	0	0.6	0	0	0	0.4
12	76	1	0	0.4	0	0.2	0	0.4
13	52	0	0	0	0.2	0.2	0	0
14	107	1	0	0.4	0.2	0	0	1.2
15	94	1	0.6	0.4	0	0	0	2.2
16	37	1	0	0	0.2	0	0	0.8

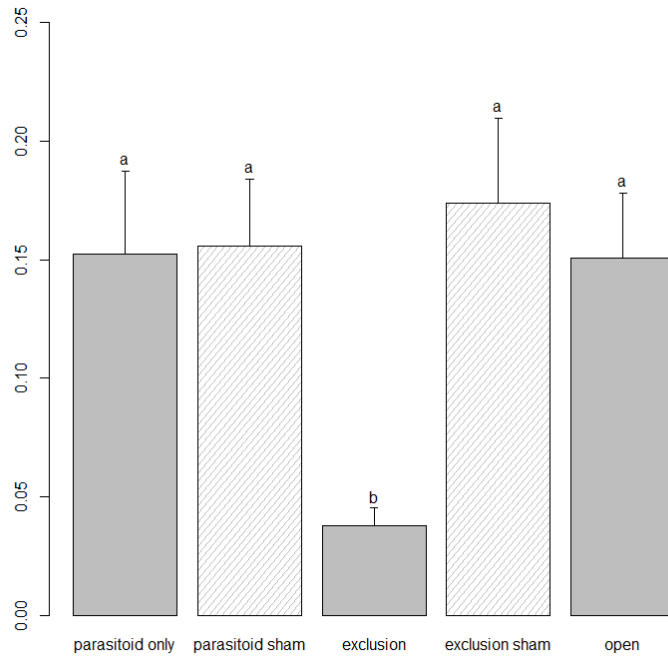
Results from ten-plant survey of aphids and natural enemies conducted prior to caging plants. Median aphid densities were used to determine aphid inoculation densities for the plants in each block. <sup>1</sup>Larvae only. <sup>2</sup>Includes adults, larvae, and egg clusters <sup>3</sup>Includes larvae and eggs. <sup>4</sup>Larvae only. <sup>5</sup>Includes unemerged mummies only.



**Figure 22.** Figure of mean aphid density by treatment with pairwise comparisons, with standard error bars.

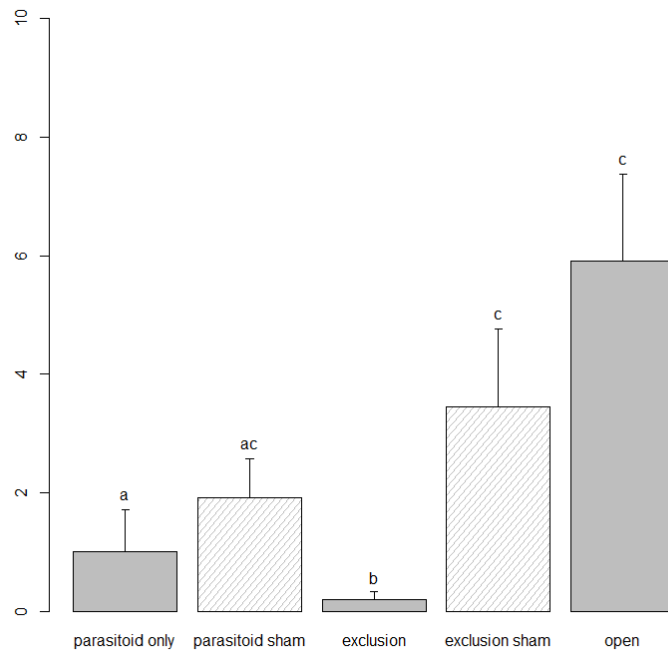


**Figure 23.** Aphid growth rate by treatment with pairwise comparisons (means and SE from untransformed data). Growth rate was calculated by dividing aphid inoculation density by the final observed density.



**Figure 24.** Parasitism by treatment with pairwise comparisons, with standard error bars.





**Figure 25.** Figure of alate density by treatment with pairwise comparisons with standard error bars.

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## APPENDIX

### *A.1 Supplementary materials for Chapter 2*

The following provide code and instructions to generate the interactive figures supplementary to Chapter 1. The code is written in the R programming language (R Core Team 2015) and utilizes the web application platform provided in the R package ‘shiny’ (R Studio, Inc. 2014). I first present the general instructions for displaying the supplementary figures, followed by the code for two files that are needed, ui.R and server.R.

#### A1.1 General Instructions

Supplementary materials were developed using the R package “shiny” (R Studio, Inc. 2014) and allow for interactive modulation of all model parameters presented in Chapter 1. To view these supplementary materials, R (R Core Team 2015) must be installed on your operating system, as well as a web browser (e.g. Chrome, Firefox). With the R software open, the package ‘shiny’ must be installed and loaded. The code presented in A1.2 and A1.3 must then be loaded. The following step-by-step instructions were written for Windows users but should work in most operating systems.

- 1) If an updated version of R is not installed on the computer, it can be acquired free here: <http://www.r-project.org/>
- 2) Download and install the free add-on package “shiny.” This can be completed by first opening RGui and clicking on Packages, then Install package(s)... If a pop-up window “CRAN mirror” appears, select the appropriate mirror location and

click OK. In the pop-up window “Packages” scroll down and select the package “shiny” and click OK. The package “shiny” will install.

- 3) Create a new folder called “name of folder”.
- 4) Copy the code presented in A1.2 below and paste it into a text editor. Save the file as ui.R, and move this file to the folder created in step 3).
- 5) Copy the code presented in A1.3 below and paste it into a text editor. Save the file as server.R, and move this file to the folder created in step 3). This folder should now contain two files, server.R and ui.R.
- 6) In R, navigate to the directory which contains “name of folder.” To do this in the standard RGui, click on File, then Change dir... In the Browse screen, navigate to the appropriate directory and click OK. Do not navigate into the directory “name of folder” but stay one directory above.
- 7) In R, type the following command and hit return

```
>library(shiny)
```

- 7) Next, type

```
>runApp("name of folder")
```

A web browser will open and display the supplementary materials, with figures on the right hand side of the screen and interactive bars on the left hand side. The interactive bars can be adjusted to automatically update model output. Figure captions are provided in A1.4.

#### A1.2 Code for file ui.R

```
#Copy and paste the following code into a text editor,  
#and save as 'ui.R'.
```

```
#Supplementary materials file for JM Kaser & GE Heimpel 2015,  
#Linking risk and efficacy in biological control host-parasitoid models  
#Previously published with Biological Control
```

```
library(shiny)
```

```
fluidPage(  
  titlePanel("Linking risk and efficacy in biological control host-  
  parasitoid models",  
  windowTitle="Kaser&Heimpel"),  
  sidebarLayout(  
    sidebarPanel(  
      sliderInput("t","Time (in  
      generations)",min=1,max=1000,value=300,step=1),  
      sliderInput("Pinit","Initial density of parasitoids",  
      min=0,max=100,step=1,value=5),  
      sliderInput("H1init",  
      "Initial host density of target",  
      min=0,max=200,value=20,step=1),  
      sliderInput("H2init",  
      "Initial host density of non target",  
      min=0,max=200,value=20,step=1),  
      sliderInput("a1","Search efficiency on target, a1",  
      min=0,max=2.0,value=0.05,step=0.01,  
      round=F),  
      sliderInput("a2",  
      "Search efficiency on non-target, a2",  
      min=0,max=2.0,value=0.05,step=0.01,  
      round=F),  
      sliderInput("s1","Suitability of target, s1",  
      min=0,max=1.0,value=1.0,step=0.01,round=F),  
      sliderInput("s2","Suitability of non-target, s2",  
      min=0,max=1.0,value=0.1,step=0.01,round=F),  
      sliderInput("B","Egg-limitation, Beta", min=0,  
      max=500, value=5, step=1),  
      sliderInput("r1",  
      "Intrinsic growth rate of target, r1",  
      min=0,max=3,value=0.25,step=0.01,  
      round=F),  
      sliderInput("r2",  
      "Intrinsic growth rate of non-target, r2",  
      min=0,max=3,value=0.25,step=0.01,  
      round=F),  
      sliderInput("K1", "Carrying capacity of target, K1",  
      min=0,max=1000,value=100,step=1),  
      sliderInput("K2",  
      "Carrying capacity of non-target, K2",  
      min=0,max=1000,value=100,step=1),  
      sliderInput("k","Aggregation parameter, k",  
      min=0,max=20,value=0.75,step=0.01,round=F)
```

```

    ),
    mainPanel(
      plotOutput("density"),plotOutput("qvalue"),
      plotOutput("eggplace"),plotOutput("egg.per.P")
    ))

```

### A1.3 Code for file server.R

#Copy and paste the following code into a text editor,  
#and save as 'server.R'.

#Supplementary materials file for JM Kaser & GE Heimpel,  
#Linking risk and efficacy in biological control host-parasitoid models  
#requires the R package 'shiny'

```
library(shiny)
```

```
shinyServer(function(input, output){
  #Figure 1S
  output$density=renderPlot({

    #number of time steps
    t=input$t

    #initial pop density for host 1 (H1), host 2 (H2) and
    #parasitoid (P)
    H1init=input$H1init
    H2init=input$H2init
    Pinit=input$Pinit

    #attack rates of P on H1 (a1) and H2 (a2)
    a1=input$a1
    a2=input$a2

    #susceptibility of hosts to parasitoid
    s1=input$s1
    s2=input$s2

    #maximum parasitoid fecundity per time step (i.e. egg load)
    B=input$B

    #intrinsic growth rate of H1 (r1) and H2 (r2)
    r1=input$r1
    r2=input$r2

    #carrying capacity of H1 (K1) and H2 (K2)
    K1=input$K1

```



```

K2=input$K2

#aggregation parameter; parasitoid aggregation to
#host density (0=random/no aggregation; infinity=completely
#aggregating to highest host density)

k=input$k

#####
H1=NULL
H2=NULL
P=NULL

gH1=NULL
gH2=NULL

fepsilon1=NULL
fepsilon2=NULL

epsilon1=NULL
epsilon2=NULL

H1[1]=H1init
H2[1]=H2init
P[1]=Pinit

#here's the model - written by JM Kaser using equations
#from Heimpel et al. (2003) Ecology Letters
for(i in 1:t) {

    epsilon1[i]=(a1*B*P[i])/(B+a1*H1[i]+a2*H2[i])
    epsilon2[i]=(a2*B*P[i])/(B+a1*H1[i]+a2*H2[i])

    fepsilon1[i]=(1+epsilon1[i]/k)^-k
    fepsilon2[i]=(1+epsilon2[i]/k)^-k

    gH1[i]=exp(r1*(1-H1[i]/K1))
    gH2[i]=exp(r2*(1-H2[i]/K2))

    H1[i+1]=H1[i]*gH1[i]*(1-s1*(1-fepsilon1[i]))
    H2[i+1]=H2[i]*gH2[i]*(1-s2*(1-fepsilon2[i]))
    P[i+1]=s1*H1[i]*(1-fepsilon1[i])+
        s2*H2[i]*(1-fepsilon2[i])

}

xindex=0:t

plot(xindex,H1,type="l",col="black",lwd=2,xlab="",
     ylab="",ylim=c(0,max(c(K1,K2))),
     main="Figure 1S. Population density plot",adj=0)
mtext("generation",side=1,line=2)

```

```

mtext("density",side=2,line=2)
lines(xindex,H2,lwd=2,lty=2)
lines(xindex,P,lwd=2,lty=3)
legend(t-.1*t,max(c(K1,K2))-
      .01*max(c(K1,K2)),lwd=c(2,2,2),lty=c(1,2,3),
      legend=c("H1","H2","P"))
})

#Figure 2S
output$qvalue=renderPlot({

#number of time steps
t=input$t

#initial pop density for host 1 (H1), host 2 (H2) and
#parasitoid (P)
H1init=input$H1init
H2init=input$H2init
Pinit=input$Pinit

#attack rates of P on H1 (a1) and H2 (a2)
a1=input$a1
a2=input$a2

#susceptibility of hosts to parasitoid
s1=input$s1
s2=input$s2

#maximum parasitoid fecundity per time step (i.e. egg load)
B=input$B

#intrinsic growth rate of H1 (r1) and H2 (r2)
r1=input$r1
r2=input$r2

#carrying capacity of H1 (K1) and H2 (K2)
K1=input$K1
K2=input$K2

#aggregation parameter; parasitoid aggregation to host
#density (0=random/no aggregation;
#infinity=completely aggregating to highest host density)
k=input$k

#####
H1=NULL
H2=NULL
P=NULL

gH1=NULL
gH2=NULL

```

```

fepsilon1=NULL
fepsilon2=NULL

epsilon1=NULL
epsilon2=NULL

H1[1]=H1init
H2[1]=H2init
P[1]=Pinit

#here's the model - written by JM Kaser using equations
#from Heimpel et al. (2003)
#Ecology Letters

for(i in 1:t) {

    epsilon1[i]=(a1*B*P[i]) / (B+a1*H1[i]+a2*H2[i])
    epsilon2[i]=(a2*B*P[i]) / (B+a1*H1[i]+a2*H2[i])

    fepsilon1[i]=(1+epsilon1[i]/k)^-k
    fepsilon2[i]=(1+epsilon2[i]/k)^-k

    gH1[i]=exp(r1*(1-H1[i]/K1))
    gH2[i]=exp(r2*(1-H2[i]/K2))

    H1[i+1]=H1[i]*gH1[i]*(1-s1*(1-fepsilon1[i]))
    H2[i+1]=H2[i]*gH2[i]*(1-s2*(1-fepsilon2[i]))
    P[i+1]=s1*H1[i]*(1-fepsilon1[i])+s2*H2[i]*
        (1-fepsilon2[i])

}

xindex=0:t

plot(xindex,H1/K1,ylim=c(0,1),xlab="generation",ylab="",
     lwd=2, type="l",
     main="Figure 2S. q-value (=H*/K) plot",adj=0)
lines(xindex,H2/K2,lty=2,lwd=2)
mtext("generation",side=1,line=2)
mtext("q-value",side=2,line=2)
legend(t-.1*t,.95,lwd=c(2,2),lty=c(1,2),
       legend=c("H1 q-value",
               "H2 q-value"))
})

#Figure 3S
output$eggplace=renderPlot({

    #number of time steps
    t=input$t

    #initial pop density for host 1 (H1), host 2 (H2) and
    #parasitoid (P)

```

```

H1init=input$H1init
H2init=input$H2init
Pinit=input$Pinit

#attack rates of P on H1 (a1) and H2 (a2)
a1=input$a1
a2=input$a2

#susceptibility of hosts to parasitoid
s1=input$s1
s2=input$s2

#maximum parasitoid fecundity per time step (i.e. egg load)
B=input$B

#intrinsic growth rate of H1 (r1) and H2 (r2)
r1=input$r1
r2=input$r2

#carrying capacity of H1 (K1) and H2 (K2)
K1=input$K1
K2=input$K2

#aggregation parameter; parasitoid aggregation to host
#density (0=random/no aggregation;
#infinity=completely aggregating to highest host density)
k=input$k

#####
H1=NULL
H2=NULL
P=NULL

gH1=NULL
gH2=NULL

fepsilon1=NULL
fepsilon2=NULL

epsilon1=NULL
epsilon2=NULL

LaidLiveH1=NULL
LaidLiveH2=NULL
LaidDeadH1=NULL
LaidDeadH2=NULL
Unlaid=NULL

H1[1]=H1init
H2[1]=H2init
P[1]=Pinit

#here's the model - written by JM Kaser using equations
#from Heimpel et al. (2003)

```

```

#Ecology Letters
for(i in 1:t) {

    epsilon1[i]=(a1*B*P[i])/(B+a1*H1[i]+a2*H2[i])
    epsilon2[i]=(a2*B*P[i])/(B+a1*H1[i]+a2*H2[i])

    fepsilon1[i]=(1+epsilon1[i]/k)^-k
    fepsilon2[i]=(1+epsilon2[i]/k)^-k

    gH1[i]=exp(r1*(1-H1[i]/K1))
    gH2[i]=exp(r2*(1-H2[i]/K2))

    H1[i+1]=H1[i]*gH1[i]*(1-s1*(1-fepsilon1[i]))
    H2[i+1]=H2[i]*gH2[i]*(1-s2*(1-fepsilon2[i]))
    P[i+1]=s1*H1[i]*(1-fepsilon1[i])+s2*H2[i]*(1-
        fepsilon2[i])

    LaidLiveH1[i]=(s1*epsilon1[i]*H1[i])/(P[i]*B)
    LaidLiveH2[i]=(s2*epsilon2[i]*H2[i])/(P[i]*B)
    LaidDeadH1[i]=((1-s1)*epsilon1[i]*H1[i])/(P[i]*B)
    LaidDeadH2[i]=((1-s2)*epsilon2[i]*H2[i])/(P[i]*B)
    Unlaid[i]=1-sum(LaidLiveH1[i],LaidLiveH2[i],
        LaidDeadH1[i],LaidDeadH2[i])

}

xindex=0:(t-1)

plot(xindex,LaidLiveH1,type="l",col="black",
     ylim=c(0,1),xlab="",lwd=1,xlim=c(0,t),
     main="Figure 3S. Egg placement plot",adj=0,ylab="")
mtext("proportion of eggs laid",side=2,line=2)
mtext("generation",side=1,line=2)
points(xindex[seq(1,t-1,5)],
       LaidLiveH1[seq(1,t-1,5)],pch=17)
lines(xindex,LaidLiveH2,col="black",lwd=1,lty=1)
points(xindex[seq(4,t-1,5)],LaidLiveH2[seq(4,t-1,5)],pch=5)
lines(xindex,LaidDeadH1,col="black",lwd=1,lty=1)
points(xindex[seq(3,t-1,5)],
       LaidDeadH1[seq(3,t-1,5)],pch=16)
lines(xindex,LaidDeadH2,col="black",lwd=1,lty=1)
points(xindex[seq(2,t-1,5)],LaidDeadH2[seq(2,t-1,5)],pch=4)
lines(xindex,Unlaid,col="black",lwd=1,lty=1)
points(xindex[seq(3,t-1,5)],Unlaid[seq(3,t-1,5)],pch=124)

legend(t-0.15*t, 0.95,
       col=c("black","black","black","black","black"),
       lty=c(1,1,1,1,1),
       lwd=c(1,1,1,1,1),
       legend=c("H1 live","H2 live","H1 dead",
               "H2 dead","Unlaid eggs"),
       pch=c(17,5,16,4,124))

})

```

```

#Figure 4S
output$egg.per.P=renderPlot({

  #number of time steps
  t=input$t

  #initial pop density for host 1 (H1), host 2 (H2) and
  #parasitoid (P)
  H1init=input$H1init
  H2init=input$H2init
  Pinit=input$Pinit

  #attack rates of P on H1 (a1) and H2 (a2)
  a1=input$a1
  a2=input$a2

  #susceptibility of hosts to parasitoid
  s1=input$s1
  s2=input$s2

  #maximum parasitoid fecundity per time step (i.e. egg load)
  B=input$B

  #intrinsic growth rate of H1 (r1) and H2 (r2)
  r1=input$r1
  r2=input$r2

  #carrying capacity of H1 (K1) and H2 (K2)
  K1=input$K1
  K2=input$K2

  #aggregation parameter; parasitoid aggregation to host
  #density (0=random/no aggregation;
  #infinity=completely aggregating to highest host density)
  k=input$k

  #####
  H1=NULL
  H2=NULL
  P=NULL

  gH1=NULL
  gH2=NULL

  fepsilon1=NULL
  fepsilon2=NULL

  epsilon1=NULL
  epsilon2=NULL

  H1[1]=H1init
  H2[1]=H2init
  P[1]=Pinit

```

```

LaidH1.per.P=NULL
LaidH2.per.P=NULL
Laid.total.per.P=NULL

#here's the model - written by JM Kaser using equations
#from Heimpel et al. (2003)
#Ecology Letters
for(i in 1:t) {

    epsilon1[i]=(a1*B*P[i])/(B+a1*H1[i]+a2*H2[i])
    epsilon2[i]=(a2*B*P[i])/(B+a1*H1[i]+a2*H2[i])

    fepsilon1[i]=(1+epsilon1[i]/k)^-k
    fepsilon2[i]=(1+epsilon2[i]/k)^-k

    gH1[i]=exp(r1*(1-H1[i]/K1))
    gH2[i]=exp(r2*(1-H2[i]/K2))

    H1[i+1]=H1[i]*gH1[i]*(1-s1*(1-fepsilon1[i]))
    H2[i+1]=H2[i]*gH2[i]*(1-s2*(1-fepsilon2[i]))
    P[i+1]=s1*H1[i]*(1-fepsilon1[i])+s2*H2[i]*(1-
        fepsilon2[i])

    LaidH1.per.P[i]=(s1*epsilon1[i]*H1[i])/(P[i])+ ((1-
        s1)*epsilon1[i]*H1[i])/(P[i])
    LaidH2.per.P[i]=(s2*epsilon2[i]*H2[i])/(P[i])+ ((1-s
        2)*epsilon2[i]*H2[i])/(P[i])
    Laid.total.per.P[i]=LaidH1.per.P[i]+LaidH2.per.P[i]

}

xindex=0:(t-1)

plot(xindex,LaidH1.per.P,type="l",ylab="",col="black",
     ylim=c(0,max(Laid.total.per.P)),
     xlab="",adj=0,lwd=1,xlim=c(0,t),
     main="Figure 4S. Eggs laid per parasitoid")
mtext("generation",side=1,line=2)
mtext("eggs laid per parasitoid",side=2,line=2)
points(xindex[seq(1,t-1,5)],
       LaidH1.per.P[seq(1,t-1,5)],pch=17)
lines(xindex,LaidH2.per.P,col="black",lwd=1,lty=1)
points(xindex[seq(4,t-1,5)],
       LaidH2.per.P[seq(4,t-1,5)],pch=5)
lines(xindex,Laid.total.per.P,col="black",lwd=1,lty=1)
points(xindex[seq(3,t-1,5)],
       Laid.total.per.P[seq(3,t-1,5)],pch=16)

legend(t-0.15*t,max(Laid.total.per.P)*.9,
       col=c("black","black","black"), lty=c(1,1,1),
       lwd=c(1,1,1),legend=c("in H1","in H2",
       "in all hosts"),pch=c(17,5,16))

```

})

})

#### A1.4 Supplementary figure captions for Chapter 1

**Figure 1S.** Population density of the parasitoid (dotted line), the target ( $H_1$ ) (solid line), and non-target ( $H_2$ ) (dashed line).

**Figure 2S.** The  $q$ -values ( $=H_i^*/K$ ) for the target ( $H_1$ ) (solid line) and non-target ( $H_2$ ) (dashed line).

**Figure 3S.** The proportion of eggs that successfully develop in the target host ( $H_1$ ) (solid triangles) and in the non-target ( $H_2$ ) (open diamonds),  $(s_i E_i H_i)/(P\beta)$ ; the proportion of eggs that do not successfully develop to adults in the target (solid circle) and in the non-target ( $\times$  marks),  $((1-s_i)E_i H_i)/(P\beta)$ ; and the proportion of eggs that are not laid ( $|$  marks),  $1-(s_i E_i H_i)/(P\beta)-((1-s_i)E_i H_i)/(P\beta)$ .

**Figure 4S.** The number of eggs laid per parasitoid that successfully develop in the target host ( $H_1$ ) (solid triangles) and in the non-target host ( $H_2$ ) (open diamonds), or any host  $(s_i E_i H_i)/(P\beta)$ .