Population ecology of *Aphelinus certus*, an adventive parasitoid of soybean aphid in North America, with implications for biological control

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Abstract

Parasitoids are excellent model systems in addressing fundamental aspects of biology and ecology while offering a high degree of economic and ecological value in regulating the population densities of their host species in both natural and agroecological settings. The single most important arthropod pest of soybean is the soybean aphid (*Aphis glycines*). Although not recommended for release due to its broad host range, the parasitoid *Aphelinus certus* has since been accidentally introduced into North America and has been hypothesized to be a key natural enemy of soybean aphid. However, the overall impact of *A. certus* in the biological control of soybean aphid is uncertain.

The chapters in this thesis are united by broader theories and concepts addressing the role of *A. certus* in biological control. Chapter 1 presents a coupled-equations matrix population model parameterized by a series of laboratory bioassays outlining the life history of soybean aphid and *A. certus*; analysis of the model suggested that a parasitism rate of 0.21 d^{-1}, which would be equivalent to at least 3.4% mummies, is capable of maintaining soybean aphid below economically damaging levels in 31.0% of simulations. Chapter 2 further explores the effects of host density on parasitoid lifespan and reproduction as well as establishes a quantitative wing wear index for estimating the age of field-collected parasitoids. Moving from theoretical to in-field efficacy, Chapter 3 evaluates the impact of *A. certus* on soybean aphid in soybean fields across central and western Minnesota (United States) from 2017–2019; contrary to the predictions of the model in Chapter 1, the results of this field experiment did not find a strong effect of *A. certus* in reducing the population growth rates of soybean aphid. Finally, Chapter 4 evaluates the prevalence of parasitism by *A. certus* on the native aphid species *Aphis* *asclepiadis* and *Aphis monardae* in prairie ecosystems; these results suggest that *A. certus* readily colonizes or is already established in natural habitats and poses a potential threat to endemic aphid populations. Lastly, I offer conclusions by more broadly discussing this work within the context of population dynamics, ecology, and biological control.

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Preface

Parasitoids are a homoplastic group of insects with a rich and fascinating natural history in which free-living adults produce parasitic offspring that slowly kill their hosts in order to complete development (Godfray 1994). Not only are parasitoids excellent model systems to address fundamental aspects of biology and ecology, but they offer a large amount of economic and ecological value in regulating the population densities of their host species in natural and agroecological settings (Godfray and Shimada 1999, Murdoch et al. 2003, Heimpel and Mills 2017). My interest in parasitoid wasps began after meeting Bradleigh Vinson and learning about his research on the effects of an ichneumonid venom that contains a symbiotic, domesticated virus (Stoltz and Vinson 1979, Vinson 1980, Burke and Strand 2012) as well as the complementary roles of specialized cells called "teratocytes" in the development of larval endoparasitoids (Vinson 1970, Strand 2014). Since this initial exposure to parasitoid wasps, I have found myself particularly intrigued by the influence of parasitoid life history and ecology on population-level processes, demography, and interactions with other species.

Modern population ecology arose largely from early studies in human demography, biometry, and general and comparative physiology (Park 1946). The field of demography was pioneered by John Graunt, an "opulent haberdashery merchant" lacking a formal education, due to his interest in studying the composition of his customer base using the records from different parish churches throughout London (Harper 2018). While Graunt's success in extracting some basic population patterns (e.g. plague mortality) earned him a recommendation from King Charles II, he lost his business in the Great Fire of London and died bankrupt in 1674 (Harper 2018). Similarly, the earliest host–parasitoid models also arose out of an economic need—in this case to apply the use of parasitoids to control pest insects (Godfray and Shimada 1999). The earliest model of host–parasitoid population dynamics was proposed by William R. Thompson in 1924 with later improvements by the Australian entomologist Alexander John Nicholson and physicist Victor Bailey in 1935 (see Godfray and Shimada 1999, Hassell 2000). Interestingly, in 1939, in part criticizing the work of Nicholson and Bailey but also a reflection of his changing beliefs regarding the utility of models, Thompson wrote that "mathematical theories, though a natural and useful development… can [not] be considered as an adequate representation of events in nature" and that "observation and experiment are still the only safe basis for practical operations in… biology." We might therefore consider mathematical models as a "language" rather than a standalone science (Watt 1962), since models function to test, improve, and apply our understanding of theoretical relationships akin to how observations and experiments elucidate empirical problems, each with their own set of benefits and limitations (Caswell 1988).

A brief introduction to soybean: Plant, aphid, and parasitoids

Soybean was domesticated in northeastern China around 3100 years ago and was first brough to North America in 1765 by Samuel Bowen, who was a seaman with the East India Company (Hymowitz and Shurtleff 2005). Production of soybean in the United States rose sharply during World War II due to demand for oils and other products; following the war, soybean meal began to be used widely as livestock feed (Shurtleff and Aoyagi 2004). Today, soybean is one of the most widely cultivated crops in the United States (2016: 83.7 million planted acres valued at $38 billion), and 44–49% of American soybean is exported to counties including China, Mexico, Japan, and the European Union (USDA ERS 2019). The primary component of soybean is meal, which is largely used for animal feed but also as a protein alternative in food products and soy milk; the other component is oil, which is used for foods, biofuels, paints, plastics, and cleaners. In North America, soybean is attacked by a variety of diseases and pests, including soybean rust, pod and stem blight, *Fusarium* root rot, and arthropods such as leaf feeders and defoliators (leafhoppers, Japanese beetles, and spider mites), pod feeders, stem feeders (including soybean gall midge, an emerging pest species), and root and other below-ground feeders (University of Minnesota Extension 2020).

The single most important arthropod pest of soybean is the soybean aphid (*Aphis glycines*), which was first detected in North America in 2000 (Ragsdale et al. 2011). Soybean aphid has a heteroecious holocyclic life cycle, reproducing asexually on soybean throughout the summer before switching to its primary host plant, common buckthorn (*Rhamnus cathartica*), for sexual reproduction and overwintering (Ragsdale et al. 2004). Populations of soybean aphids are capable of rapidly expanding and readily moving between fields (McCornack et al. 2004, Ragsdale et al. 2007, 2011). Soybean aphids may result in yield losses as high as 40% (Ragsdale et al. 2007) due to reductions in plant height, pod size, seed production, and oil content (see Ragsdale et al. 2011).

To combat soybean aphid, foliar insecticides (predominately pyrethroids and organophosphates) are applied to 16% of planted acres (USDA NASS 2019) and preventative neonicotinoid seed treatments are used in 34–44% of soybean acreage (Douglas and Tooker 2015). Resident natural enemies—mostly coccinellids—offer some protection against soybean aphid in North America, but damaging outbreaks still occur (Ragsdale et al. 2011, Heimpel et al. 2013). The importation and release of nonnative parasitoid species (*Aphelinus atriplicis*, *Binodoxys communis*, *Aphelinus glycinis*, and *Aphelinus rhamni*) have been attempted unsuccessfully due to biotic interference, intraguild predation, and challenges related to overwintering and dispersal (Chacón et al. 2008, Chacón et al. 2010, Asplen et al. 2011, Gariepy et al. 2015, Heimpel and Asplen 2011, Asplen et al. 2016).

Although not recommended for release due to its broad host range, the parasitoid *Aphelinus certus* was accidentally introduced into North America in or before 2005 and has since spread throughout the north central United States and southeastern Canada (Ragsdale et al. 2011, Frewin et al. 2010, Heimpel et al. 2010, Kaser 2016, Hopper et al. 2017). Recent work on *A. certus* in Saint Paul, Minnesota, suggests that this parasitoid may be able to maintain soybean aphid populations below the economic threshold of 250 aphids per plant (Kaser and Heimpel 2018), although a different study in Québec, Canada, found that *A. certus* only decreased peak aphid population densities by 1–7%, possibly due to low early-season parasitism rates (Leblanc and Brodeur 2018). Thus, the overall impact of *A. certus* in the biological control of soybean aphid remains uncertain.

The work herein

As I note throughout the body of this dissertation, the design and outcome of empirical experiments testing applied problems have often motivated my development and usage of models (and vice versa). The purpose, content, and conclusions for each chapter are united by broader theories and concepts: in the most general sense, Chapters 1 and 2 largely focus on the theoretical aspects of *A. certus* in terms of its potential as a biological control agent, whereas Chapters 3 and 4 investigate the real and actual effects of *A. certus* in both agricultural and natural ecosystems.

At the start, Chapter 1 evaluates the extent to which *A. certus* might suppress soybean aphid below damaging levels, investigates the dynamics of interacting stage-structured populations (including the effects of parasitoid lifespan and post-parasitism reproduction), and serves to generate hypotheses related to the potential economic and ecological effects of *A. certus* in biological control. Building on this, Chapter 2 tests the effects of host density on parasitoid lifespan and fecundity and then incorporates these variables into the model developed in Chapter 1 in order to investigate the population-level and dynamical consequences of host-density-dependent parasitoid lifespan. The final portion of Chapter 2 attempts to bridge the gap between laboratory- and field-based studies by establishing a quantitative method for measuring the age of parasitoids in natural settings. At this point, the dissertation moves entirely into the field with Chapter 3, which functions to identify the actual impact of *A. certus* (as well as the entire natural enemy community) using a series of field experiments based on Kaser and Heimpel (2018) with the purpose of informing management practices throughout the region. Finally, Chapter 4 switches the focus from agricultural to natural habitats as it assesses the prevalence of parasitism by *A. certus* on native prairie aphids, generates hypotheses of invasion into prairies, and discusses these finding within the context of laboratory-conducted host-range testing and retrospective risk–benefit analyses in biological control.

Chapter 1 A matrix model describing host–parasitoid population dynamics: The case of *Aphelinus certus* and soybean aphid

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Synopsis

Integrating elements from life tables into population models within a matrix framework has been an underutilized method of describing host–parasitoid population dynamics. This type of modeling is useful in describing demographically structured populations and in identifying points in the host developmental timeline susceptible to parasitic attack. We apply this approach to investigate the effect of parasitism by the Asian parasitoid *Aphelinus certus* on its host, the soybean aphid (*Aphis glycines*). We present a matrix population model with coupled equations that are analogous to a Nicholson–Bailey model. To parameterize the model, we conducted several bioassays outlining host and parasitoid life history and supplemented these studies with data obtained from the literature. Analysis of the model suggests that, at a parasitism rate of 0.21 d^{-1}, *A. certus* is capable of maintaining aphid densities below economically damaging levels in 31.0% of simulations. Several parameters—parasitoid lifespan, colonization timeline, host developmental stage, and mean daily temperature—were also shown to markedly influence the overall dynamics of the system. These results suggest that *A. certus* might provide a valuable service in agroecosystems by suppressing soybean aphid populations at relatively low levels of parasitism. Our results also support the use of *A. certus* within a dynamic action threshold framework in order to maximize the value of biological control in pest management programs.

Introduction

The ways in which demography, life history, interspecific interactions, and the biotic or abiotic characteristics of a habitat affect the dynamics of consumer–resource interactions may be investigated through simple experiments and ecological models (Murdoch et al. 2003). Host–parasitoid systems are ideal for these studies not only because of their straightforward structure, but because of their application in the biological control of insect pests (Hassell 2000, Heimpel and Mills 2017). Thus, population modeling has often been used to quantify the importance of parasitoids as natural enemies within a broad range of ecological and evolutionary processes (Hochberg and Ives 2000). Other approaches, such as life table analyses, also provide valuable insight into the effect of mortality imposed by parasitoids and other natural enemies on host populations (Ryan 1990, Bellows et al. 1992).

Matrix population models are well adapted to studying structured populations (Leslie 1945, Birch 1948, Lefkovitch 1965, Caswell 2001), although they have seldom been used to describe aspects of host–parasitoid systems. Yet, these life table-based models have been successfully used in a variety of systems to address heterogeneity in populations and in identifying vulnerable aspects of the life history of a species, making them useful not only in theory, but in evaluating the impact of biological control agents in practice as well (Stark et al. 2004, Davis et al. 2006, Evans et al. 2012, Meisner et al. 2014, Heimpel and Mills 2017, Rand et al. 2017, Rand et al. 2020). For example, Lin and Ives (2003) constructed a size-classified matrix model for soybean aphid (*Aphis glycines*) and the parasitoid wasp *Aphidius colemani*, showing that parasitoid preference for larger individuals tended to have the greatest impact on host population growth, and Mills

(2005) utilized a stage-structured matrix to identify the developmental stages of the codling moth (*Cydia pomonella*) most susceptible to increased parasitism pressure in a competitive environment.

We present a coupled stage-classified matrix model for a host–parasitoid system. Our method of coupling two species follows that of the more-familiar Nicholson–Bailey equations, $N_{t+1} = \lambda N_t e^{-aP_t}$ and $P_{t+1} = N_t(1 - e^{-aP_t})$, in which *N* and P are the host and parasitoid population densities, λ is the natural rate of increase for the host, and e^{-aP_t} is the escape function (Nicholson and Bailey 1935). We parameterized the matrix model for the soybean aphid–*Aphelinus certus* system through a series of developmental and behavioral bioassays as well as with data from the literature. The purpose of this model—which may be adapted to describe other host–parasitoid systems—is to (1) evaluate the extent to which *A. certus* might suppress soybean aphid populations below damaging levels, (2) generate hypotheses related to the potential economic and environmental effects of *A. certus* in biological control of soybean aphid, and (3) investigate the dynamics of interacting stage-structured populations.

Materials and Methods

Study system

Soybean aphid (*Aphis glycines* Matsumura; Hemiptera: Sternorrhyncha: Aphididae) is an important pest of soybean (*Glycine max* (L.) Merrill; Fabaceae) in North America, and often requires treatment with broad-spectrum insecticides that pose risk to an array of non-target organisms (Ragsdale et al. 2011, Tilmon et al. 2011). The practice of biological control reduces risk to beneficial species (such as pollinators and natural enemies) as it complements or acts as an alternative to insecticide use (Heimpel and Mills 2017, Heimpel et al. 2004). In North America, the biological control services provided by resident enemies offer some protection against soybean aphid (and reduce its overall environmental impact), but damaging outbreaks still occur (Ragsdale et al. 2011, Heimpel et al. 2013). The importation and release of exotic specialized parasitoids from the native range of soybean aphid have been attempted unsuccessfully, and various hypotheses for this lack of establishment have been proposed, such as biotic interference (Chacón et al. 2008), intraguild predation (Chacón et al. 2010), and challenges related to overwintering (Asplen et al. 2011, Gariepy et al. 2015) and dispersal (Heimpel and Asplen 2011, Asplen et al. 2016).

The Asian parasitoid *Aphelinus certus* Yasnosh (Hymenoptera: Chalcidoidea: Aphelinidae) was evaluated for importation and release against soybean aphid. However, *A. certus* was determined to be an unsuitable classical biological control agent because it parasitized a broad range of aphid species during tests in quarantine (Kaser 2016, Hopper et al. 2017). In or before 2005, *A. certus* was accidentally introduced into North America—possibly during a secondary invasion of soybean aphid—and has since spread throughout the north central United States and southeastern Canada (Frewin et al. 2010, Heimpel et al. 2010, Ragsdale et al. 2011). Recent work on *A. certus* in Saint Paul, Minnesota, suggests that this parasitoid may be able to maintain soybean aphid populations below the economic threshold of 250 aphids per plant (the pest density at which management practices should be applied) (Kaser and Heimpel 2018), although a different study in the Montérégie area of Québec, Canada, found that *A. certus* only decreased peak aphid population densities (and cumulative aphid-days) by 1–7%, possibly due to low rates of parasitism early in the season (Leblanc and Brodeur 2018). Thus, the overall impact of *A. certus* as a biological control agent of soybean aphid remains uncertain.

The matrix model

Following Caswell (2001), a host population vector **n** (the abundance of each developmental stage in the host population) is projected to $t + 1$ (projection interval = 1 d) using a transition and fertility matrix **A** and the probability of escaping parasitism **H**, as well as with a temperature-scaling matrix $\mathbf{C}_{\mathrm{SBA}}$. Similarly, the parasitoid population vector **p** is projected with the transition and fertility matrix **W** and modified by a temperature-scaling matrix $\mathbf{C}_{\mathrm{Ac}}$. The model also includes a carrying capacity K for the total host population N following Allen (1989) and Jensen (1995, 1997, 2000), and this formulation includes the identity matrix **I** such that $\mathbf{HAC}_{\mathrm{SBA}} - \mathbf{I}$ is analogous to the rate of increase. Thus, the model takes the form

$$\mathbf{n}_{t+1} = \mathbf{n}_t + \frac{K - N_t}{K}(\mathbf{HAC}_{\mathbf{SBA}} - \mathbf{I})\mathbf{n}_t$$

$$\mathbf{p}_{t+1} = \mathbf{WC}_{\mathbf{Ac}}\mathbf{p}_t \qquad (1)$$

The matrices **A** and **W** represent the proportion of individuals in stage j (columns) surviving or transitioning to stage i (rows) from time t to $t + 1$. For the host, the survival probabilities (P_i), the transition probabilities (G_i), and the fertilities (F_i) are reduced by parasitism (g_i). Equation (2) details the host transition and fertility matrix **A** as well as the probabilities of escaping parasitism expressed in **H**

$$\mathbf{AH} = \begin{bmatrix} P_1 & 0 & 0 & 0 & F_5 \\ G_1 & P_2 & 0 & 0 & 0 \\ 0 & G_2 & P_3 & 0 & 0 \\ 0 & 0 & G_3 & P_4 & 0 \\ 0 & 0 & 0 & G_4 & P_5 \end{bmatrix} \begin{bmatrix} g_1 & 0 & 0 & 0 & g_5 \\ g_1 & g_2 & 0 & 0 & 0 \\ 0 & g & g_3 & 0 & 0 \\ 0 & 0 & g_3 & g_4 & 0 \\ 0 & 0 & 0 & g_4 & g_5 \end{bmatrix} \tag{2}$$

in which P_i, G_i, and F_i were calculated assuming a postbreeding census birth-pulse, so that the probability of observing an individual of a specific developmental stage is a function of the sampling period. Following Caswell (2001), $P_i = l(i)/l(i-1)$, $G_i = l(i)/l(i-1)$, and $F_5 = P_i m_i$; l_i is the number or proportion of individuals surviving from $i - 1$ to i, m_i is per capita reproduction, and g_i is the proportion of hosts escaping parasitism. g_i was based on a type II functional response for parasitoids attacking hosts that was previously applied to *A. certus* by Frewin et al. (2010) and takes the form

$$g_i = \exp\left(\frac{-a_i \alpha P_3 p_{3♀}}{1 + a_i \alpha T_h N}\right)\left(\frac{\sum_{i=1}^{5} n_i}{N}\right) \tag{3}$$

in which a_i is the fraction of all attacks on host stage i, α is the instantaneous search rate of the parasitoid, and T_h is the handling time. While $\sum_{i=1}^{5} n_i$ represents the total number of unparasitized hosts (the scalar sum of the host population vector **n**), N represents the entire host population, including both the unparasitized ($\sum_{i=1}^{5} n_i$) and parasitized, but still-living, hosts (the element p_1 in the parasitoid population vector **p**). Because only female parasitoids exert parasitism pressure on the host population, the element p_3 from the population vector **p** is multiplied by the proportion of adult parasitoids that are female, and is represented in Equation 3 as $p_{3♀}$. As only unparasitized individuals may be parasitized, the escape function is multiplied by the relative number of available hosts, $\sum_{i=1}^{5} n_i/N$. Note that the stage-specific probability of escaping parasitism g_i was referred to as "p_i" by Lin and Ives (2003); the symbol for this variable was changed here for clarity as entries in our parasitoid population vector p are referred to as p_i in conventional matrix notation.

The transition and fertility matrix for the parasitoid (a combined egg and larval stage, mummy/pupal stage, and adult) is

$$\mathbf{W} = \begin{bmatrix} P_1 & 0 & F_3 \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{bmatrix} \tag{4}$$

in which P_i and G_i are calculated as before, with the exception of P_3, which incorporates host-density-dependent survival of adult parasitoids modeled using the Verhulst model of logistic growth (Miksanek JR & Heimpel GE, in revision). Here, $P_3 = [l(3)/l(3-1)]\theta_1/[1+\theta_2 e^{\theta_3 N_{\text{total}}}]$, in which l is the proportion of parasitoids surviving as before, θ_1 is the maximum mean adult parasitoid lifespan, and θ_2 and θ_3 are shape and growth rate parameters. The fertility of adult parasitoids is $F_3 = (1-g_i)n_i/p_3$. Finally, the parasitoid survival and transition matrix **W** was additively decomposed to reflect the effects of the host carrying capacity on parasitoid eggs and larvae (as there is an equal probability of the carrying capacity affecting either parasitized or unparasitized hosts), such that the second line of Equation (1) becomes $\mathbf{p}_{t+1} = \begin{bmatrix} p_1 \\ 0 \\ 0 \end{bmatrix}_t + (\mathbf{WC_{Ac}} - \boldsymbol{I}) \begin{bmatrix} p_1 \\ 0 \\ 0 \end{bmatrix}_t +$ $\mathbf{WC_{Ac}} \begin{bmatrix} 0 \\ p_2 \\ p_3 \end{bmatrix}_t .$

Offspring produced by parasitized hosts were added to the element n_1 (number of 1st stadium hosts) in the population vector $\mathbf{n}_{t+1}$ as $\sum_{i=i}^{5} p_1 J_i \frac{n_i}{N}$. Here, post-parasitism reproduction is accounted for by multiplying the stage-specific per capita reproduction of parasitized hosts (J_i) and the proportional stage structure n_i/N with the number of still-living parasitized hosts (p_1). This formulation approximates the stage structure of the parasitized host population by equating it to that of the unparasitized population.

A temperature-scaling matrix was implemented for the host ($\mathbf{C}_{\mathrm{SBA}}$) and parasitoid ($\mathbf{C}_{\mathrm{Ac}}$) in order to adjust population growth rates for temperatures outside of those used in laboratory assays. The temperature-scaling matrices take the form

$$\mathbf{C}_{\mathrm{SBA}} = \begin{bmatrix} \mathrm{c}_{P_1} & 0 & 0 & 0 & \mathrm{c}_5{}^4 \\ \mathrm{c}_1 & \mathrm{c}_{P_2} & 0 & 0 & 0 \\ 0 & \mathrm{c}_2 & \mathrm{c}_{P_3} & 0 & 0 \\ 0 & 0 & \mathrm{c}_3 & \mathrm{c}_{P_4} & 0 \\ 0 & 0 & 0 & \mathrm{c}_4 & \mathrm{c}_5 \end{bmatrix} \tag{5}$$

$$\mathbf{C}_{\mathrm{Ac}} = \begin{bmatrix} \mathrm{c}_{P_1} & 0 & \mathrm{c}_3{}^4 \\ \mathrm{c}_1 & \mathrm{c}_{P_2} & 0 \\ 0 & \mathrm{c}_2 & \mathrm{c}_3 \end{bmatrix}$$

in which c_i represents a scaling function for the rate of increase and c_{P_i} is $[1 - (1 - P_i)\mathrm{c}_i]/P_i$. Thus, the term c_i adjusts sampling probabilities based on the temperature at which laboratory-conducted assays were performed as $\lambda(T)/\lambda(T_0)$, in which T_0 = 25 °C. As $T \rightarrow T_{\max}$ (the upper temperature threshold for development), individuals have a decreasing probability of being resampled from t to $t + 1$ ($c_{P_i}P_i < P_i$) and an increasing probability of being sampled in the subsequent developmental stage ($c_iG_i > G_i$), with $c_{P_i}P_i + c_iG_i = P_i + G_i$. Fertilities ($F_5$ for the host and F_3 for the parasitoid) exhibit the same trend exponentially, with c_i^4 providing the best fit for the matrix approximation of the native function. Our formulation of $\mathbf{C}_{\mathrm{SBA}}$ and $\mathbf{C}_{\mathrm{Ac}}$ was necessary so that the population growth rate can follow temperature-dependent changes in juvenile development and adult survival and fertility. Direct application of the scaling function, e.g. $\mathbf{p}_{t+1} = \lambda(T)/\lambda(T_0)\mathbf{W}\mathbf{p}_t$, would yield the correct rate of population growth but only by adding or removing individuals from the population in a biologically unrealistic manner; while our formulation is an approximation, it holds from 5–30° C, which spans the normal range of average historical daily temperatures during the modeling period.

Temperature-dependent development was added for the host following McCornack et al. (2004) and for the parasitoid following Frewin et al. (2010). The McCornack et al. (2004) model is a modified Logan model (Logan et al. 1976) that expresses the intrinsic rate of growth (r) as a function of temperature, and incorporates the upper development threshold ($T_{\max}$, the maximum lethal temperature), the range of thermal breakdown (Δ), and a constant (ρ) so that $r(T) = e^{\rho T} - e^{[\rho T_{\max} - (T_{\max} - T)/\Delta]}$. (For reference, the intrinsic rate of growth was related to the natural rate of increase λ using the approximation $\lambda = e^r$; see Gotelli 2008.) The model used by Frewin et al. (2010) was based on an earlier model by Briere et al. (1999) (also based on Logan et al. 1976) and estimates the intrinsic rate of growth (r) given an upper temperature threshold ($T_{\max}$), a lower temperature threshold (T_0), and a constant (a) so that $r(T) = aT(T - T_0)\sqrt{T_{\max} - T}$. These modified Logan models build on improvements made by Lactin et al. (1995) and are advantageous in that they decrease the number of necessary parameters while maximizing their biological relevance; Shi et al. (2011) has since proposed a similar model based on physiological mechanisms (enzyme kinetics), but the McCornack et al. (2004) and Frewin et al. (2010) formulations were selected because they were parameterized for the host and parasitoid species used in the present study.

Bioassays

Aphid development

Soybean aphids were observed to determine the amount of time required to reach maturity. Reproducing adult aphids from a mixed-aged colony raised at 25 ± 2° C, 16:8 L:D, were transferred to the underside of an excised soybean leaflet. After 1.5 hr, 1st-stadium nymphs (n = 31) were transferred with a fine brush to the underside of a fresh excised leaflet from a V1–V2 soybean plant. Leaflets were positioned vertically with the stem placed in 3 cm^3 of moist, fine sand at the bottom of a 6 dram plastic vial that was ventilated by puncturing sixteen pinholes through the cap. Individual aphids were identified to developmental stage at 12 hr intervals until reaching reproductive maturity. Although nymphs and adults are visually similar, developmental stage can be distinguished by unique differences in antennal segmentation and caudal morphology; antennal segmentation increases from four (1st stadium) to five (2nd stadium) to six (3rd stadium and higher), and the caudum characteristically increases in size before tapering into an elongated teardrop shape at adulthood (Hodgson et al. 2005). Additionally, 4th-stadium nymphs often exhibit the eyespots of well-developed embryos that may be seen through their integument (JRM, personal observation). The presence of exuviae and analysis of exuvial antennal segmentation was also used to confirm stage transitions. The entire assay was conducted in a growth chamber at constant 25 ± 2° C, 16:8 L:D.

Parasitoid development

Parasitoids were evaluated for their capacity to complete development on each of five apterous stages of soybean aphid (1st–4th stadia and adult). Mummies—the darkened exoskeletal remains of recently killed aphids that contain late larval parasitoids or pupae—were collected from laboratory colonies of *A. certus* maintained at 23 ± 2° C, 16:8 L:D (first established in August 2011 with field-collected mummies from Saint Paul and Rosemount, Minnesota). Mummies were stored individually in 0.6 mL plastic microcentrifuge tubes supplied with a droplet of honey water (approx. 50 vol%). Each newly emerged female parasitoid (< 24 hr old, n = 59) was paired with a newly emerged male and observed for copulatory behavior; after copulation, the male was discarded and the female left overnight. Each female was randomly assigned a treatment (one of the five host stages), and twenty soybean aphids of representative size and quality for that stage were transferred from a mixed-aged laboratory colony to the underside of a soybean leaflet placed in a plastic vial (as previously described). The aphids were allowed to settle for ten minutes, after which time the parasitoid was introduced into the tube and left to interact with the aphids for 24 hr. The parasitoid was then removed and the aphids were allowed to continue development. Aphids were checked daily for the formation of mummies, which were individually collected in 0.6 mL microcentrifuge tubes and observed at 3–12 hr intervals for the emergence of adult parasitoids. Hind tibia length was measured for a subsample (n = 194) of the emerged offspring as a proxy for size and fitness. The assay was conducted in a growth chamber at 25 ± 2° C, 16:8 L:D. An ANOVA was used to compare the main effects of host stage, sex, and parental identity on parasitoid developmental time as well as hind tibia length. Tukey's *post hoc* was used to separate means for multiple comparisons using the *agricolae* package and base R version 3.4.4 (The R Foundation for Statistical Computing, 2017), which was also used for all subsequent analyses. Differences in parasitoid sex ratio in response to different host stages were determined using a binomial generalized linear model with a χ^2 test and Tukey's pairwise comparisons via the glht() function in the *multcomp* package; the response variable (proportion male) was weighted based on brood size.

Host-stage preference

To determine host-stage preference (defined as the deviation in the proportion of host stages attacked by a single female *A. certus* from random chance when all stages present are of equal abundance), *A. certus* mummies were collected and mated as before. At the start of the assay, a single female (n = 73) was allowed to exit the microcentrifuge tube onto the underside of a leaflet containing three each of the 1st–4th stadia and apterous adult soybean aphids in a plastic vial (as previously described). Only aphids of visually similar quality and of representative size for their stage were used in the assay. Each parasitoid was allowed to interact with aphids for two hours at 25 ± 2° C, which provides ample time to locate and parasitize approximately one host (JRM, personal observation). Immediately after parasitoid exposure, aphids were stored in 70% ethanol and later dissected to recover parasitoid eggs. Host-stage preference was determined using the Friedman rank-sum test (in the base R package *stats*), which follows a χ^2 distribution. Parasitoids that did not oviposit during the assay were excluded from the analysis.

Post-parasitism reproduction

Aphids were assessed for their reproductive capacity following parasitism. A single 3rd, 4th, or adult stadium aphid was collected from the laboratory colony and transferred to the underside of a V1 soybean leaflet, which was situated in a plastic vial as previously described. 1st and 2nd stadium aphids were not included because preliminary testing revealed that these stages do not reproduce prior to mummification. A total of 105 vials were assembled, fifteen for each unparasitized (control) 3rd, 4th, and adult stadium soybean aphid and twenty for each parasitized 3rd, 4th, and adult stadium aphid. Adult *A. certus* (n = 60) were aspirated from a two-week old laboratory colony and placed individually into the appropriate vials. (In the colony, individual wasps had the opportunity to mate, acquire host handling experience, and feed on honeydew or host hemolymph, thus they were considered to be reproductively, behaviorally, and nutritionally prepared for the bioassay.) Each parasitoid was allowed to interact with its aphid for 24 hr, after which time the parasitoid was removed. Aphids were observed daily for 8 d for the production of offspring, and nymphs were removed with each observation. The assay was performed in a growth chamber held at 25 ± 2° C, 16:8 L:D.

Reproduction of parasitized and unparasitized adult hosts was analyzed with a cumulative link mixed effects model (CLMM). This approach consists of a multivariate analysis of variance with a logit link function that assesses ordinal response variables while accounting for random factors. The daily number of offspring was the response variable; treatment (parasitized or control), initial host stage (3rd, 4th, or adult stadium), and number of days since exposure (1–7, a discrete variable) were fixed effects; and individual aphid was included as a random factor to account for repeated measures. An interaction term between treatment and day was included to account for any time-dependent effects of parasitism (i.e. delayed impact on host reproduction). Pairwise comparisons were determined using a *post hoc* test of least square means with a Bonferroni correction. An ANOVA was performed to compare the number of days between molts for parasitized and unparasitized aphids. Aphids that died within the parasitoid exposure period (e.g. due to host feeding or overstinging) were excluded from the analysis, and aphids in the parasitism treatment that did not mummify by the end of the seven-day study period were excluded as well. The CLMM was analyzed using the *ordinal* package in R, with ten quadrature points used for Gauss-Hermite likelihood approximation, and *emmeans* was used for pairwise comparisons of least square means.

Model analysis

Population dynamics

A 90 d period was simulated given a randomly selected initial number of individuals ranging from 0.3–1.82 hosts and 0.15–4.08 parasitoid mummies per plant. These values represent the range of early-season host and parasitoid densities sampled at four sites surveyed during 2017: Hitterdal, MN (47.0° N, 96.2° E), Starbuck, MN (45.6° N, 95.7° E), Appleton, MN (45.3° N, 95.9° E), and Pipestone, MN (44.0° N, 95.9° E) (United States). In order to reflect natural conditions, the initial stage structure for the host was juvenile-biased as colonizing soybean aphid alatae deposit a few offspring per plant without themselves settling (Ragsdale et al. 2004); the initial parasitoid population was similarly biased towards younger stages. Colonization timeline followed field observations: aphids were introduced on June 22nd and parasitoids were introduced 20 d later. Simulations were conducted in R and replicated 10^4 times.

Parasitism and host suppression

Accurate comparisons of field data and ecological models require clear differentiation of the various methods of measuring parasitism in a host population. *A. certus* and other aphid parasitoids are surveyed during their late larval and pupal stages because, at this point, their host has died, leaving behind a mummy (the darkened exoskeletal remains), which are easily sampled in field settings and identifiable to subfamily or genus (Powell 1982). However, the relative abundance of mummies—referred to as *mummy fraction*—is not synonymous with other measures of parasitism. To clarify this terminology, we use *parasitism rate* to denote a temporal unit of measurement expressing an absolute or proportional change in the individuals succumbing to parasitism over time (Post van der Burg et al. 2009). In contrast, *percent* (or *proportion*) *parasitism* is a unitless measure that compares a subset of hosts (the parasitized) to the larger population at some specific point in time; percent parasitism is thus the *result* of parasitism rate interacting with other competing rates (birth/death, immigration, dispersal, etc.), following van Driesche (1983). Operating under these definitions and following the format of the matrix model, we define *parasitism rate* as $\sum_{i=1}^{5}[(1-g_i)n_i/N]$, *percent parasitism* as $p_1/N \times 100\%$, and *mummy fraction* as $p_2/(N + p_2)$.

Sensitivity analyses

The influences of adult parasitoid lifespan, date of parasitoid colonization, host-stage preference, and mean daily temperature were evaluated in ecologically plausible parameter space. The effects of these parameters on host population densities were calculated as a percent difference in maximum host population density with or without the parasitoid present (“peak pest reduction”). The sensitivity analysis for adult parasitoid lifespan (uncoupled from host density) evaluated a mean adult parasitoid survival period of 2–26 d. Parasitoid colonization was evaluated from 2–32 d after host establishment. For host-stage preference, a total of 21 graded preferences were assessed, which ranged from a strong preference for early-stage juveniles ($a_{1\text{–}5} = \{0.50, 0.35, 0.10, 0.05, 0.00\}$), to no overall preference ($a_i = 0.20$), to a strong preference for adults ($a_{1\text{–}5} = \{0.00, 0.05, 0.10, 0.35, 0.50\}$). The effects of average daily temperature were assessed over a range of ± 3° C compared to publicly available historical records from the station GHCND:USC00215204 located at (44.4706° N, 95.7908° E) in Marshall, MN. With the exception of the manipulated parameter, all parameters were the same as previously described and simulated using median starting densities for the host and parasitoid populations.

Results

Laboratory assays

Aphid development

All aphids successfully reached reproductive maturity within seven days. All adults began reproducing within 24 hours of their final molt, and most produced their first offspring within 12 hours; thus, a significant non-reproductive adult stage (referred to as S5 by Lin and Ives [2003]) was not noted in our study.

Parasitoid development

Host stage affected the amount of time required for *A. certus* to complete development, both in terms of the time until host mummification ($F_{4,\,455} = 17.23$, $p < 0.001$) and time until adult parasitoid emergence ($F_{4,\,455} = 18.87$, $p < 0.001$) (Table 1.1: *Mean time to mummy* and *Mean time to emerge*). Both parasitoid sexes developed more slowly on 1st stadium hosts compared to adult hosts (Table 1.1: *Total development time*). Males developed more rapidly than females ($F_{1,\,455} = 17.38$, $p < 0.001$) (Table 1: *Total development time*). The amount of time from mummification to emergence did not differ significantly between sexes ($F_{1,\,455} = 0.06$, $p = 0.807$) (Table 1.1: *Mean time to emerge*). There was an effect of experimental block on developmental rate (time to mummification: $F_{54,\,455} = 6.52$, $p < 0.001$; time to emergence: $F_{54,\,455} = 4.89$, $p < 0.001$) and size ($F_{52,\,136} = 1.86$, $p = 0.002$), indicating similarities among offspring of the same parental parasitoid. Offspring reared on adult hosts were smaller than those developing on other stages ($F_{4,136} = 8.37$, $p < 0.001$), and males tended to be smaller than females ($F_{1,\,136} = 12.66$, $p < 0.001$) (Figure 1.1). A female-biased sex ratio was produced on most host stages; the proportion male was 0.37 ± 0.05, 0.48 ± 0.04, 0.52 ± 0.04, 0.27 ± 0.06, 0.38 ± 0.06 (mean ± SEM) on 1st, 2nd, 3rd, and 4th stadia and adult hosts, respectively. Sex ratio varied with host stage (χ^2 [deviance] = 21.23, $p < 0.001$, n = 49), with parasitoids ovipositing on 4th stadium hosts producing a lower proportion of males compared to those provided with 2nd and 3rd stadium hosts.

Host-stage preference

Parasitoid eggs recovered from dissected aphids were typically located within the anterior abdomen or posterior thorax of the host. Host-stage preference for *A. certus* was 0.21, 0.23, 0.21, 0.17, and 0.19 for 1st–4th stadia and apterous adults respectively, but did not demonstrate a significant deviation in oviposition from random (Friedman test, $F_R = 0.640$, $p = 0.958$).

Post-parasitism reproduction

Parasitism by *A. certus* negatively affected soybean aphid reproduction (CLMM, likelihood ratio $\chi^2_{1,440} = 89.29$, $p < 0.001$) and varied by day ($\chi^2_{6,440} = 33.91$, $p < 0.001$) and with the host stage parasitized ($\chi^2_{2,440} = 45.84$, $p < 0.001$). Additionally, there was an interaction between treatment and day ($\chi^2_{6,440} = 195.44$, $p < 0.001$), indicating that the effect of parasitism on host reproduction changed over time (parasitism-induced changes in fertility did not begin until after the third day). The difference in reproduction between parasitized and control aphids was not statistically significant until four days after parasitism, at which time parasitized aphids were rendered infertile (Figure 1.2). Parasitism did not influence the amount of time between soybean aphid molts (3rd stadium to 4th stadium: $F_{1,20} = 1.34$, $p = 0.261$; 4th stadium to adult: $F_{1,38} = 0.229$, $p = 0.635$).

Matrix model parameterization

Results from the bioassays were supplemented with data from peer-reviewed literature to parameterize the matrix model. Survival and transition probabilities for soybean aphid nymphs were obtained from the aphid development data, and fertility of parasitized aphids was taken from the post-parasitism reproduction assay. Pooled data from the parasitoid development assay were used determine survival and transition probabilities for immature parasitoids because, even though *A. certus* developed more slowly on 1st stadia hosts, this difference was relatively small. Parasitoid sex ratio data were obtained from the parasitoid development assay, and adult parasitoid survival was calculated using unpublished data (Miksanek JR & Heimpel GE, in revision). Because *A. certus* did not exhibit a significant host-stage preference, the null hypothesis $a_i = 0.2$ was used in the model. The remaining parameters in the model were obtained from the literature, and all parameters used in the model are summarized in Table 1.2.

Model analysis

Population dynamics

Soybean aphid densities peaked just before day 45 of the simulation, which corresponds to the last week of July (Figure 1.3a). In 9.9% of simulations including *A. certus*, soybean aphid densities were below the economic threshold (ET) of 250 aphids per plant (the density at which pest management practices should be applied), and in 31.0% did not exceed the economic injury level (EIL) of 674 aphids per plant (the density at which yield loss exceeds management costs) (Figure 1.3b). Densities in simulations not including *A. certus* reached the carrying capacity of 6000 aphids per plant, and there was a 74.2 ± 0.2% decrease in peak aphid abundance in the presence of *A. certus*.

Parasitism and host suppression

Because the model was stage-structured for both the host and the parasitoid, parasitism rate at any time throughout the season can be equated with mummy fraction and percent parasitism. The parasitism rate associated with no host population growth ($\lambda = 1$, or the apex of peak aphid density for each of the 10000 simulations) was 0.208 ± 0.012 d^{-1} (mean ± SD), which equates to 11.3 ± 3.7% parasitism or 3.4 ± 1.4% mummies (Figure 1.4). Regardless of the method of measuring parasitism, parasitism increased with host density before decreasing as the host population declined; however, time-dependent measures of parasitism (percent parasitism and mummy fraction) exhibited a notable lag in comparison with parasitism rate (Figure 1.4).

Sensitivity analyses

Biological control efficacy of *A. certus* was greatest for long-lived parasitoids that colonized fields early and attacked hosts without a strong preference for either younger or older host stages. Longer-lived adult parasitoids had a higher impact on the aphid population, and the slope of this relationship was greatest when parasitoid longevity was exceeded 10 days (Figure 1.5a). Delaying the date of parasitoid introduction reduced the effect of *A. certus* such that, for parasitoids colonizing fields more than a month after the arrival of soybean aphid, their effect was nearly zero (Figure 1.5b). An increase in parasitoid preference from younger to older hosts produced a concave response in peak pest reduction, indicating that parasitoids attacking all host stages indiscriminately have the greatest effect on aphid population dynamics (Figure 1.5c). Additionally, lower temperatures were more conducive to host suppression (Figure 1.5d). Finally, post-parasitism reproduction had a minor effect on peak population reduction; exclusion of this term from the model increased peak population reduction by 0.08% (no figure).

Discussion

The developmental bioassays revealed a host with a high capacity for growth and a parasitoid that exhibits optimal growth on intermediate host stages but without a significant preference for any individual stage. The fully parameterized matrix model predicted that *Aphelinus certus* reduces soybean aphid populations below the economic threshold in 9.9% of simulations and below the economic injury level in 31.0% of simulations. Host suppression was predicted at a parasitism rate of 0.21 d^{-1}, which corresponds with 3.4% of the aphid population being visibly mummified; notably, because parasitism was dynamic, relatively low parasitism rates early in the season could still be associated with low peak host densities. Assuming that the 9.9% modeled reduction in fields exceeding threshold due to parasitism by *A. certus* is scalable, then *A. certus* might reduce insecticide applications by 1.8 million acres annually, saving an average of $2.43/ha in management costs and contributing to a commensurate reduction in greenhouse gas emissions (Ragsdale et al. 2007, Landis et al. 2008, USDA 2015, Heimpel et al. 2013). Our assessment of *A. certus* supports the conclusions of Hallett et al. (2014) in calculating the value of this parasitoid for implementation in a dynamic action threshold, which would adjust the traditional economic treatment threshold for a pest based on the relative abundance of its natural enemies (Zhang and Swinton 2009).

Coupled host–parasitoid dynamics

The matrix model described increasing soybean aphid densities that peaked in late July. This pattern is characteristic of soybean aphid population dynamics in North America (Ragsdale et al. 2004, Bahlai et al. 2010). In its native range in Asia, soybean aphid exhibits the same early/mid-season peak, although densities are considerably lower overall and midsummer migrations are of decreased importance (Liu et al. 2004, Wu et al. 2004). In our model, this unimodal pattern of soybean aphid abundance was driven by a single natural enemy, *A. certus*. Soybean aphid is limited by a suite of natural enemies in its native range (Wang et al. 1962, Wu et al. 2004), and, in North America, *Harmonia axyridis*, *Coccinella septempunctata*, *Orius insidiosus* (debatably), and *A. certus* have been identified as important predators in certain landscapes (Rutledge et al. 2004, Rutledge and O'Neil 2005, Costamagna and Landis 2006, Desneux et al. 2006, Brosius et al. 2007, Donaldson et al. 2007, Hallet et al. 2014, Kaser and Heimpel 2018).

Our model suggests that *A. certus* is capable of suppressing soybean aphid at a parasitism rate of 0.21 d^{-1} (i.e. parasitizing 21% of the total host population per day). This value is consistent with the 20–30% total daily parasitism range required for soybean aphid population suppression previously determined by Lin and Ives (2003), but was relatively low in comparison to the field-estimated 42% parasitism rate proposed by Kaser and Heimpel (2018). This discrepancy may be due to different methods of analysis. The matrix model was analyzed using a non-equilibrium approach, and, as a result, our model was able to show that parasitism rate fluctuates dynamically in response to aphid population densities, in which high mid-season parasitism rates followed low early-season parasitism and contributed to an overall increase in percent parasitism over time.

This time difference between increasing parasitism rates associated with host suppression and percent parasitism suggests that it may be difficult to identify the impact of *A. certus* in field settings until the pest population is already in decline.

Insights into host–parasitoid dynamics

The sensitivity analyses demonstrated that adult parasitoid lifespan, date of parasitoid colonization, host-stage preference, mean daily temperature, and post-parasitism reproduction all affect peak host densities to some degree, but the ways in which host-stage preference and post-parasitism reproduction influenced the system merit further discussion. Interestingly, we did not find host suppression to be at a maximum when parasitoids preferentially attacked the oldest host stages and we did not identify post-parasitism reproduction as a mechanism of notably increasing peak population densities, both of which are contrary to the results of Lin and Ives (2003).

Host-stage preference

The parasitoid *A. certus* did not show a significant preference for any individual host developmental stage. Although many *Aphelinus* species readily accept all host stages, there is broad variability in host-stage preference (Gerling et al. 1990, Mueller et al. 1992, Tang et al. 1996, Bayram and Bayhan 2016). While Lin and Ives (2003) showed that preference for older host stages produces the lowest equilibrium host densities, we found that the relationship between host-stage preference and peak host densities produced a different result in our nonequilibrium analysis. When preference for younger hosts became less pronounced, peak aphid densities began decreasing, which is consistent with parasitoids removing hosts of a higher reproductive value (Lin and Ives 2003). However, as preference for older individuals continued increasing, peak aphid densities began to rise again. In our model, the initial aphid population consists predominately of immature host stages, which mimics the conditions imposed by colonizing alate aphids at the beginning of the season (Ragsdale et al. 2004); thus, the adult hosts are much scarcer, so parasitoid preference for late-stage hosts suggests that parasitism rate will be low until the relative abundance of their preferred stage eventually increases as the host population approaches its stable stage structure.

Post-parasitism reproduction

Soybean aphid reproduction was decreased 72 hr after parasitism by *A. certus*, and soybean aphids were reproductively dead the following day. Compared to parasitism by the aphidiine *Aphidius colemani* (Lin and Ives 2003), soybean aphids parasitized by *A. certus* reproduce a full day longer, but compared to parasitism by the aphidiine *Binodoxys communis* (Kaiser and Heimpel 2016), post-parasitism reproduction was similar. Aphid parasitoids decrease host reproduction when they compete with developing host embryos for nutritional resources (Colinet et al. 2005), which indirectly leads to embryonic degeneration via starvation (Polaszek 1986). Additionally, parasitoids influence the fertility of their hosts by venomous castration (Digilio et al. 2000) or by directly feeding on embryos (Polaszek 1986). In response, parasitized aphids may allocate additional resources to any surviving embryos (Kaiser and Heimpel 2016). Lin and Ives (2003) showed that continued reproduction by parasitized aphids during the early stages of parasitoid development produces a partially compensatory effect that leads to higher population growth rates compared with non-reproducing parasitized hosts, and this compensation may be high enough that parasitoids attacking adult aphids—especially older adults—do not affect the maximum growth rate (r_m) or doubling time of their host populations (Campbell and Mackauer 1975, Tang and Yokomi 1996). However, our model did not indicate a strong effect of post-parasitism reproduction on peak soybean aphid densities. Instead, our analysis supports the hypothesis that total lifetime reproduction of aphids has little impact on population growth rates and that the reproductive output during early adulthood contributes disproportionately to population growth (e.g. van Steenis and El-Khawass 1995 and references therein). As a result, preference for the oldest host stages allows for high survival for mid- to late-stage immature hosts, which then mature and begin reproducing before succumbing to parasitism themselves.

An alternative modeling approach: Decreasing *r*

A different approach to modeling herbivorous pest species of annual crops—aphids in particular—involves a linear decline in the intrinsic rate of growth, *r*, due to bottom-up effects of decreasing plant quality as a result of plant phenology; this approach is termed the *decreasing* r *model* (Williams et al. 1999, Costamagna et al. 2007). The decreasing *r* model produces a distinctive bell-shaped population curve defined as $N_t = N_0 e^{r_{\max} t(1-0.5at)}$, in which *N* is aphid density, $r_{\max}$ is the maximum rate of population growth, *t* is time, and *a* is the rate of decline for *r*. Decreasing *r* was field-validated for soybean aphid by Costamagna et al. (2007) and applied to the host–parasitoid system of soybean aphid and *A. certus* by Leblanc and Brodeur (2018). Both studies reported a high degree of success using this bottom-up model to describe population dynamics in the field even though soybean aphid dynamics have been previously linked to the strong top-down effect of predation (e.g. Brosius et al. 2007).

Decreasing *r* may be incorporated into a matrix model as $\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t \lambda^{-at}$, in which **n** is the aphid population vector, **A** is the transition and fertility matrix, and λ is the dominant eigenvalue of the matrix **A** representing the natural rate of population increase. Analysis of the matrix model (as described in *Materials and Methods: The matrix model*) with the addition of decreasing *r* (in which $a = 0.0247$ per Costamagna et al. 2007) predicts that peak soybean aphid densities are reduced by 49.6 ± 0.2% in the presence of *A. certus* and, even in the absence of *A. certus*, do not exceed the economic injury level, suggesting that soybean aphid might be unlikely to be considered an economically damaging species in any scenario. Regardless, the biggest challenge to the decreasing *r* model in general is that it oversimplifies aphid population dynamics by imposing a season-long effect of plant phenology that confounds density-dependent effects of the aphid on host plant quality with density- and time-dependent changes in aphid behavior and physiology such as emigration, mid-summer migration, or a parthenogenic shift and migration to the primary host plant (Lamb and MacKay 2017, Ríos Martínez and Costamagna 2018). Additionally, host population dynamics can be affected by hyperparasitoids and other higher-order natural enemies if, for example, they trigger avoidance behaviors in primary parasitoids or signal hosts of a reduced risk of parasitism, leading to increased reproduction (reviewed by Frago 2016). The overall course of soybean aphid colonization and growth throughout the season is also influenced by landscape-level resource availability, such as proximity to buckthorn or agricultural intensification (Gardiner et al. 2009, Noma et al. 2010, Hallet et al. 2014, Liere et al. 2015, Whitney et al. 2016).

Final remarks

Our study highlighted the value of including host stage-specific parameters as well as parasitoid lifespan and colonization timeline in host–parasitoid population models. We also showed a negligible effect of post-parasitism reproduction on peak host densities, and that relatively low parasitism rates early in the season could maintain peak host densities below the economic injury level during the mid-season. Although there have been successes applying real-time monitoring protocols to assess the influence of natural enemies on pest population dynamics and adjust the economic thresholds accordingly (e.g. Hoffmann et al. 1990), such programs may face challenges related to development and implementation and are not currently recommended for management of soybean aphid in the United States (Koch et al. 2016).

Table 1.1 Development time for *Aphelinus certus* on the various stages of soybean aphid separated by parasitoid sex with two-factor comparisons

	Host-stage	Mean time to mummy (days ± SE) [a]		Mean time to emerge (days ± SE)[a]		Total development time (days ± SE) [a]		n
female	1st stadium	6.18 ± .05	a	7.00 ± .07	a	13.18 ± .07	a	72
	2nd stadium	6.00 ± .05	abc	6.43 ± .08	d	12.43 ± .07	b	67
	3rd stadium	6.02 ± .04	abc	6.60 ± .07	bcd	12.61 ± .09	b	57
	4th stadium	6.08 ± .07	ab	6.47 ± .09	cd	12.55 ± .07	b	66
	adult	5.75 ± .07	de	6.76 ± .07	abc	12.51 ± .09	b	51
male	1st stadium	6.07 ± .06	abc	6.93 ± .06	ab	13.00 ± .06	a	42
	2nd stadium	5.84 ± .07	cde	6.49 ± .09	cd	12.33 ± .07	b	61
	3rd stadium	5.93 ± .05	bcd	6.46 ± .08	cd	12.39 ± .09	b	56
	4th stadium	5.85 ± .11	bcde	6.65 ± .15	abcd	12.50 ± .07	b	20
	adult	5.57 ± .14	e	6.74 ± .13	abcd	12.30 ± .08	b	23
	Pooled:	5.97 ± .02		6.64 ± .03		12.61 ± .03	*Total:*	515

[a] Shared letters indicate no significant difference.

Table 1.2 Values and sources of parameters used in the matrix model

	Parameter	Symbol(s)	Value(s) (excluding units)	Source(s)
soybean aphid	juvenile survival probabilities	P_1, P_2, P_3, P_4	0.500, 0.143, 0.311, 0.205	bioassay
	adult survival probability	P_5	0.86	Lin and Ives 2003
	transition probabilities	G_1, G_2, G_3, G_4	0.484, 0.857, 0.689, 0.795[a]	bioassay
	per capita reproduction	F_5	2.56	bioassay, Lin and Ives 2003
	post-parasitism reproduction	J_1, J_2, J_3, J_4, J_5	0, 0, 0.563, 1.521, 1.471	bioassay
	temperature-curve	$\rho, T_{\max}, \Delta$	34.9, 7.1, 0.14	McCornack et al. 2004
	carrying capacity	K	6000	Catangui et al. 2009
Aphelinus certus	egg+larval survival probability[b]	P_1	0.832	bioassay
	pupal survival probability	P_2	0.869	bioassay
	adult survival probability	P_3	0.932	Miksanek & Heimpel (in revision)
	transition probabilities	G_1, G_2	0.168, 0.131	bioassay
	sex ratio	–	0.412	bioassay
	host-stage preference	a_i	0.2	bioassay
	functional response	α, T_h	0.979, 0.045	Frewin et al. 2010
	temperature: egg to mummy	$a, T_0, T_{\max}$	1.19×10^{-4}, 7.8, 35.7	Frewin et al. 2010
	temperature: mummy to adult	$a, T_0, T_{\max}$	1.37×10^{-4}, 11.6, 36.9	Frewin et al. 2010
	host-density-dependent survival	$\theta_1, \theta_2, \theta_3,$	18.6, 13.5, −0.562	Miksanek & Heimpel (in revision)

[a] There is also a 0.016 probability of sampling a first stadium as a third stadium 24 hours later that was included in the model.

[b] Probability of being resampled as an egg or larva at time $t + 1$ assumes no mortality during this period because egg and larva survival were not measured during the assay.

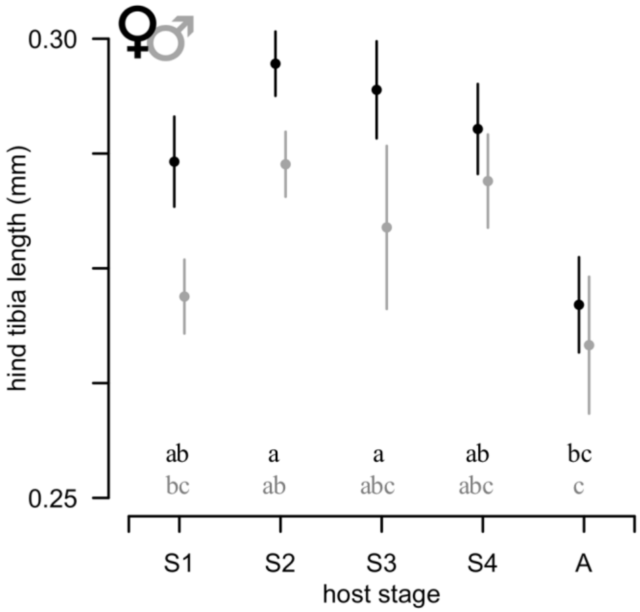

Figure 1.1 Hind tibia length as a function of host developmental stage

Black: female *A. certus*; gray: male. S1: 1st stadium aphid, S2: 2nd stadium, S3: 3rd stadium, S4: 4th stadium, A: adult. Mean ± SE; shared letters indicate no significant difference.

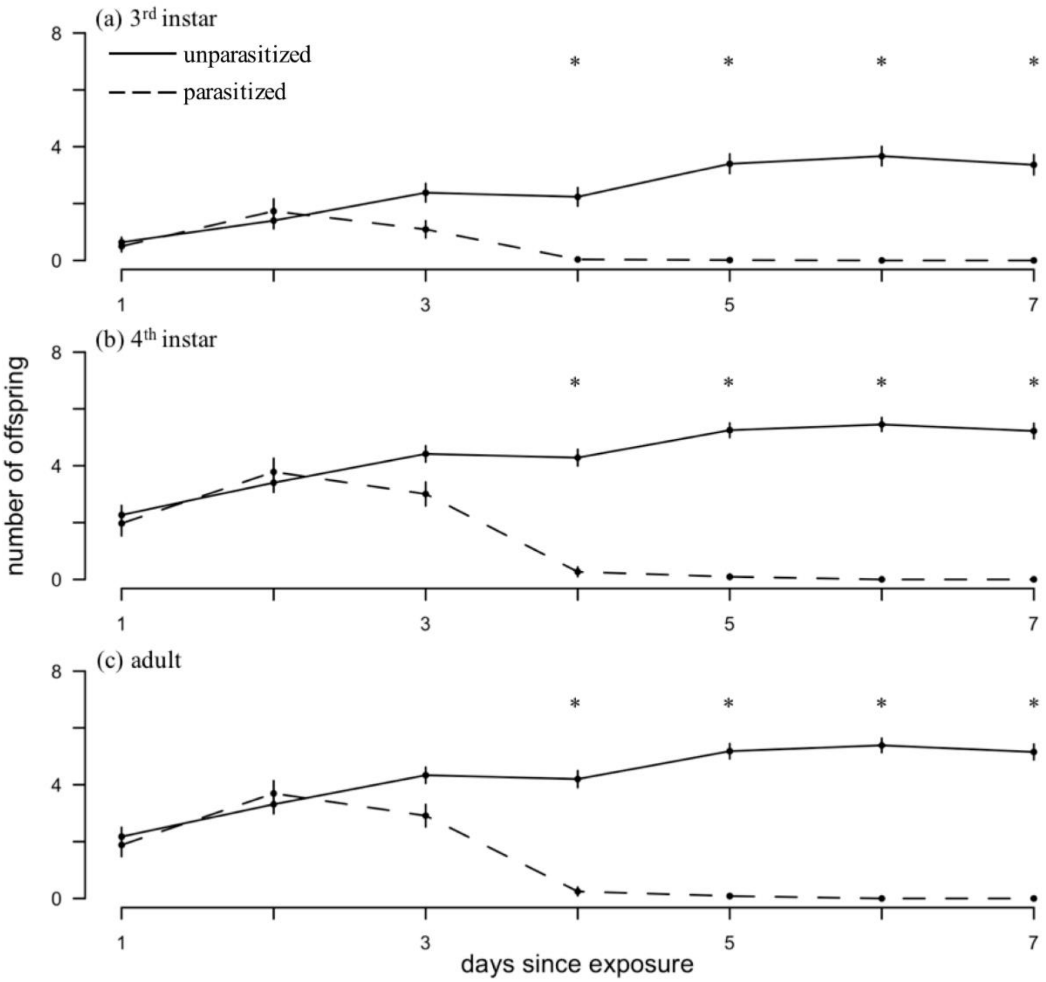

Figure 1.2 Daily reproduction of parasitized and unparasitized soybean aphids

(a) 3rd stadium, (b) 4th stadium, and (c) adult. Points plot least square means ± SE. Solid lines: unparasitized (control) aphids; dashed lines: parasitized aphids. Asterisks indicate significant differences between control and parasitized aphid reproduction on each day following parasitoid exposure.

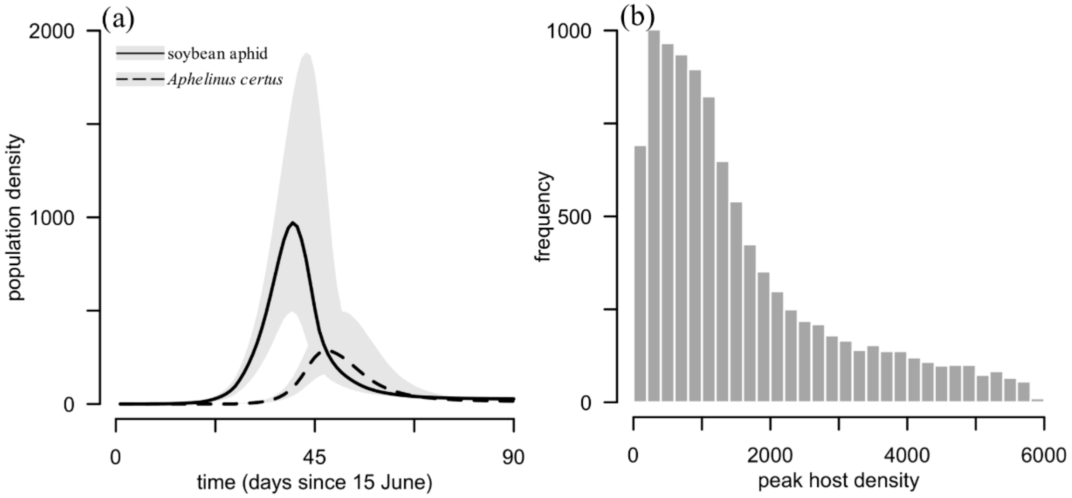

Figure 1.3 Population dynamics predicted by the matrix model

(a) Population dynamics of soybean aphid and *A. certus*. Black lines represent median densities with the interquartile (Q1–Q3) range shaded in gray. Solid line: soybean aphid (all living aphids); Dashed line: *Aphelinus certus* (all stages). (b) Histogram of peak aphid densities simulated from the model.

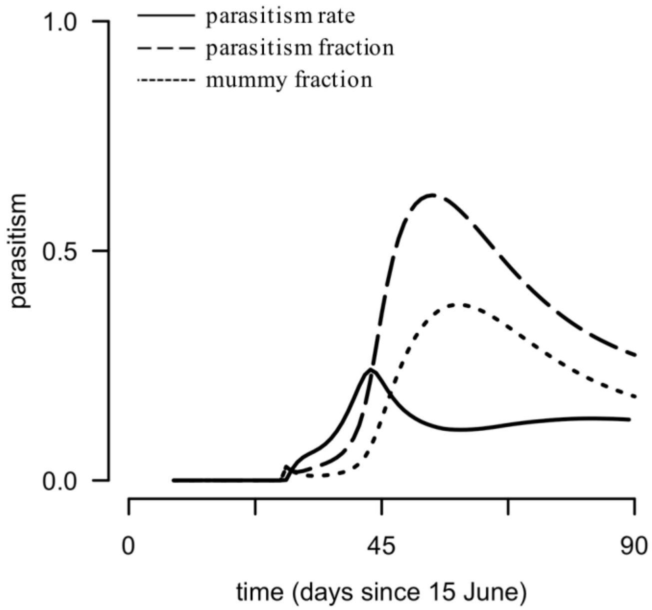

Figure 1.4 Comparing measures of parasitism in model simulations

Solid line: parasitism rate (d^{-1}); dashed line: parasitism fraction (×100 = percent parasitism [%]); dotted line: mummy fraction.

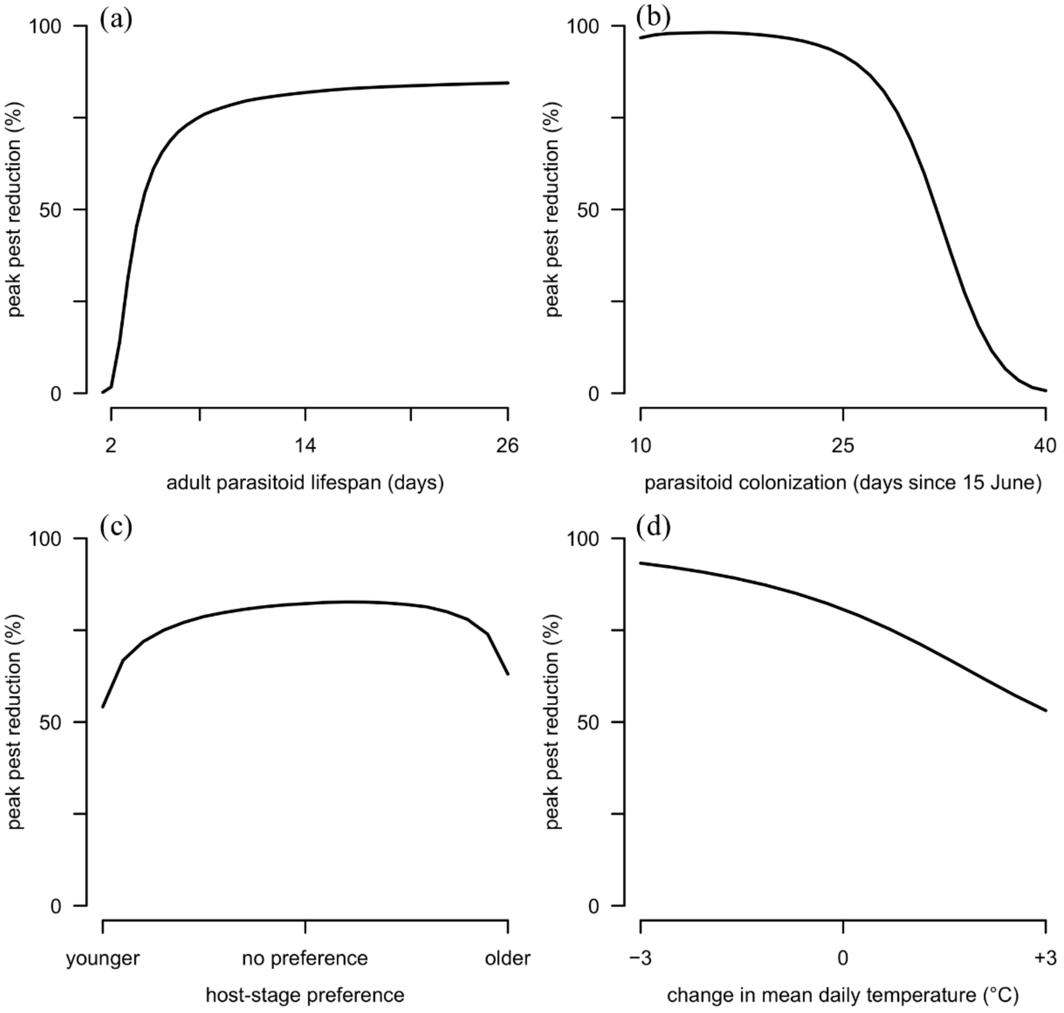

Figure 1.5 Sensitivity analyses

The effect of the parasitoid is shown as a percent reduction in peak host densities (solid line) as a function of (a) adult parasitoid lifespan, (b) the date of parasitoid colonization, (c) host-stage preference, and (d) mean daily temperature.

Chapter 2 Density-dependent lifespan and estimation of life expectancy for a parasitoid with implications for population dynamics

This chapter has been submitted for publication and is currently undergoing revisions.

Synopsis

Parasitoid lifespan is influenced by nutrient availability, thus the lifespan of parasitoids that rely on their hosts for nutritional resources should vary with host density. We assessed the survival and reproduction of one such species, *Aphelinus certus*—a parasitoid of the soybean aphid, *Aphis glycines*—over a range of host densities using a laboratory assay. We found a positive, asymptotic relationship between host density and the lifespan and fecundity of *A. certus* that was supported by a traditional survivorship analysis as well as a logistic model. Parasitoids from this assay were also used to develop a wing wear index relating setae damage to parasitoid age. This index was used to estimate the life expectancy of field-collected parasitoids, which was shorter than the life expectancy of laboratory-reared female parasitoids. Finally, host-density-dependent parasitoid lifespan was incorporated into a coupled-equations matrix population model that revealed that decreasing the degree of host density dependence leads to higher equilibrium host densities and changes in the quality of equilibrium (e.g. from stable limit cycles to a stable point before the host escapes control). These results detail the relatively unstudied phenomenon of host-density-dependent parasitoid lifespan and suggest that differences between laboratory- and field-determined parasitoid life expectancy have important implications for population dynamics and the biological control of insects.

Introduction

Density-dependent consumption rates are critically important in the population-level outcomes of consumer–resource interactions (May et al. 1981, Stiling 1987, Murdoch et al. 2003). Parasitoids are key natural enemies of insects and are often studied using population models to test hypotheses related to population regulation, stability, and other fundamental and applied aspects of the ecology of populations and communities (Hassell 2000, Heimpel and Mills 2017). In addition to the well-studied density-dependent patterns of prey acquisition that provides predators with the resources required for survival and reproduction (e.g. Holling 1959, Royama 1971), host density can similarly influence the survival and fecundity of parasitoids that rely primarily on their hosts for nutrients (Sahragard et al. 1991, Mahmoudi et al. 2010).

Many species of adult parasitoids indirectly obtain nutritional resources from their host, such as through the consumption of the honeydew excreted by soft-bodied, plant-feeding homopterans (Wäckers et al. 2008, Tena et al. 2018). Parasitoids may also directly feed on host hemolymph through a process termed “host feeding,” which often kills the host (Jervis and Kidd 1986, Heimpel and Collier 1996, Heimpel et al. 1997). If available in the ecosystem, floral resources such as nectar, pollen, and other plant tissues may utilized by foraging parasitoids as well (reviewed by Jervis et al. 1993). However, for parasitoid species that rely solely on their hosts for nutrients (either via host feeding or by consuming honeydew), survival—and, by extension, lifetime reproduction—is hypothesized to reach its maximum when hosts are most abundant relative to parasitoid density. While the functional role of host feeding is typically linked to the fecundity of synovigenic parasitoids (the proteins obtained are used to mature eggs), host feeding contributes to longevity in a number of species as well (Heimpel and Collier 1996, Casas et al. 2005). From a dynamical perspective, host feeding can reduce equilibrium host densities and contribute to stability in coupled host–parasitoid interactions (Kidd and Jervis 1989, 1991, Briggs et al. 1995). Overall, host-density-dependent effects could play a particularly important role in parasitoid population dynamics, such as reducing the impact of the parasitoid population during periods of low host availability or increasing the value of biological control services when hosts are abundant (Siekmann et al. 2001).

Although host–parasitoid population dynamics are linked to parasitoid lifespan, the lifespan of parasitoids in the field is generally unknown. However, a variety of methods have been used to investigate parasitoid lifespan in field settings. Lifespan has been indirectly assessed for *Aphytis* parasitoids (Hymenoptera: Aphelinidae) by calculating encounter and predation rates from direct observations (Heimpel et al. 1997) and for the parasitoid *Binodoxys communis* (Hym.: Braconidae) by measuring sugar profiles to determine starvation probabilities (Dieckhoff et al. 2014). Another approach has been to develop a laboratory-parameterized wing wear index that correlates physical damage to wing setae with parasitoid age (Heimpel et al. 1996, Lee and Heimpel 2008). Such an index was initially developed for *Aphytis aonidiae* (Hym.: Aphelinidae) to confirm that older parasitoids break more wing setae than younger parasitoids (Heimpel et al. 1996). This index was later adapted to provide a rough estimate of lifespan for *Diadegma insulare* (Hym.: Ichneumonidae), and, as a result, found the parasitoid to be relatively "short-lived" in the field (Lee and Heimpel 2008). Wing wear indices have also been used for other groups of insects, including tseste flies and mosquitoes (reviewed by Hayes and Wall 1999), wild bees (Michener et al. 1955), and lepidopterans (Watt et al.

1977). Thus, further development of wing wear indices for parasitoid wasps might allow for more detailed insights into the lifespan and demography of field-collected specimens (Lee et al. 2006).

The purpose of this paper is to (1) determine the effects, if any, of host density on parasitoid lifespan and fecundity; (2) formulate a method of estimating the age of field-collected parasitoids in order to facilitate comparisons between laboratory- and field-based studies; and (3) investigate the dynamical consequences of parasitoid lifespan on the dynamics of the system. To accomplish this, we studied the parasitoid *Aphelinus certus* (Hym.: Aphelinidae), an adventive natural enemy of soybean aphid (*Aphis glycines*; Hemiptera: Aphididae) in North America. Like other species of *Aphelinus,* adult *A. certus* obtains nutrients by feeding on honeydew and through host feeding, the latter of which is destructive and non-concurrent with parasitism (Cate et al. 1974, Bai and MacKauer 1990, Röhne 2002, Wu and Heimpel 2007, Hopper et al. 2013, Yashima and Murai 2013). We used a laboratory bioassay to test the hypothesis that adult parasitoid lifespan is negatively affected by decreasing host density. The results of this assay were incorporated into a model of the lifetime reproduction of parasitoids and used to construct a wing wear index that quantitatively relates parasitoid age to wing damage accrued over time, allowing for estimation of the age structure and adult lifespan of field-collected parasitoids. Lastly, we used a matrix population model adapted from Miksanek and Heimpel (2019) [also presented in Chapter 1] to evaluate the effects of host density dependence on host–parasitoid population dynamics.

Methods

Laboratory experiment

A laboratory-conducted bioassay was used to determine the lifespan and fecundity of adult female parasitoids over a range of host densities. Laboratory colonies of soybean aphid were maintained at 25 ± 2° C, 16:8 L:D, and approximately 65% relative humidity. Parasitoid colonies were reared on soybean aphid and maintained at 22 ± 2° C, 16:8 L:D, and 65% R.H.; colonies were founded in August 2011 from field-collected soybean aphid mummies (the dead, blackened aphid carcasses containing maturing parasitoids) originating from Saint Paul and Rosemount, Minnesota (United States). Prior to the bioassay, parasitoid mummies were collected individually from the laboratory colonies and placed into 0.6 mL microcentrifuge tubes supplied with a droplet of honey-water (approx. 50 vol%). A newly emerged (< 24 hours in age) *A. certus* female was paired with a newly emerged male and observed for successful copulation behavior, after which the male was discarded. The female parasitoid was then transferred onto a single V1–V3 soybean leaflet supplied with 0, 0.5, 1, 10, or 50 1st– 4th instar aphids in approximately equal proportions from the mixed-age laboratory colony (the 0.5 aphid treatment consisted of one aphid provided on alternating days; see below). Each leaflet was placed into a 6 dram plastic vial (ventilated with pinholes in the lid) with its petiole submerged in about 3 cm^3 of fine, wet sand. The aphid densities chosen for this assay represent low (0–1 aphids per day), medium (10), and high (50) host densities; the use of multiple low density treatments was intended to identify the minimum host availability required for survival of *A. certus*, and the highest density treatment assured that *A. certus* parasitized a number of hosts consistent with the theorized asymptote of its functional response curve of 22 soybean aphids per day at constant 26° C (Frewin et al. 2010). These treatments (which limit parasitoids to host-based resources) are realistic because soybean is a nutrient-poor environment in which the flowers produce nectar for only a short period during the field season (Lee 2004). Additionally, the impact of water is not considered in this study as it is present as condensation on the interior of the experimental arenas where it may be imbibed by parasitoids (JRM, personal observation); in the field, water may be present in the soil or as dew or precipitation on the plant.

Parasitoids were aspirated into a new vial containing a fresh leaflet and aphids every 24 hours until death; the 0.5 aphid treatment consisted of one aphid provided on alternating days, beginning with the first day. The aphids in the former vial were visually inspected to determine the number of deaths over the previous 24-hour period and the contents of the vial were examined daily for mummification of aphids. Mummies were placed individually into microcentrifuge tubes and allowed an additional 10 days for adults to emerge, at which time the sex of each parasitoid was determined. All five treatments were each replicated 10 times and run concurrently; in addition, three control treatments containing 1, 10, or 50 aphids in the absence of a parasitoid were each replicated 10 times to determine baseline host mortality over a 24-hour period, which can be compared to the number of deaths in the experimental treatments to estimate the effects of parasitoid host feeding. The experiment was performed in a growth chamber at constant 25 ± 2° C, 16:8 L:D, and approximately 65% R.H. Two parasitoids from the 10 hosts per day and two from the 50 hosts per day treatments were lost during transfer, so those data were censored when conducting the analyses.

Lifespan and life expectancy

Kaplan–Meir survivorship curves were compared using the log-rank test in the R package *survival* with pairwise differences determined using a Bonferroni correction with *survminer*; in this case, host density was treated as a discrete variable to be consistent with similar analyses in previous studies (e.g. Sahragard et al. 1991, Mahmoudi et al. 2010, Xu et al. 2012), but was treated as a continuous variable in later analyses. These and all subsequent models and analyses were conducted in R version 3.4.4 (The R Foundation for Statistical Computing).

The relationship between parasitoid lifespan and host density was also modeled using a formula for logistic growth:

$$y = \frac{\theta_1}{1 + \theta_2 e^{\theta_3 N}}$$

(1)

where y is parasitoid lifespan and N is host density. θ_1 is the upper asymptote, which represents the maximum mean lifespan of an adult parasitoid. $\theta_1/(\theta_1 + \theta_2)$ is parasitoid lifespan when hosts are absent, and θ_3 determines host fast lifespan reaches is maximum. This function is ecologically intuitive in that when resources are high, parasitoid lifespan reaches its maximum (upper asymptote), but when resources are low, nutrient stores are mobilized as the parasitoid starves (lifespan decreases with a declining slope). The *critical survival threshold*, or the average host density at which adult parasitoid lifespan reaches 95% of its maximum value, was defined as $0.95 \cdot \theta_1$. Values for constants in the logistic model were obtained using the R packages *stats* and *car* by nonlinear least squares, with linear transformation and stepwise addition of parameters used to estimate starting values for θ_2 and θ_3; the starting value for θ_1 was estimated as the maximum age of the longest-lived parasitoid from the experiment. Model predictions were obtained using *propagate* for first- and second-order Taylor series expansion.

Life expectancy, e_x, was calculated following Southwood (1978):

$$e_x = \frac{T_x}{l_x} = \frac{\sum_x^w \left(\frac{l_x + l_{x+1}}{2}\right)}{l_x}$$

(2)

in which T_x represents the total number of animal age units from age x to death w and l_x is the number of individuals surviving at the beginning of age x.

Lifetime reproduction model

Lifetime reproductive success was described using a nonlinear model that incorporates parasitoid satiation at high host densities and an exponential decrease in fecundity with age that is more prominent at high host densities, drawing from Enkegaard (1993; also see Mahmoudi et al. 2010). The lifetime reproduction model relates daily fecundity m to parasitoid age x and host density N so that

$$m = \frac{(\varphi + \alpha N)e^{-(\delta + \varepsilon N)x}}{1 + (\varphi + \alpha N)\beta^{-1}e^{-(\varepsilon N)x}}$$

(3)

and φ, α, δ, ε, and β are parameters. α expresses the instantaneous search rate, β is the maximum egg maturation rate, δ represents an age-related change in daily fecundity, ε refers to the strength of the interaction between parasitoid age and host density, and $-\varphi/\alpha$ represents the lowest number of hosts (zero). Lifetime reproduction was obtained from the model as $\int_0^{e_0} m(x)dx$ for any given host density N, in which e_0 is the life expectancy at emergence; the theoretical maximum number of offspring is $\int_0^{\infty} m(x)dx$. Values for constants in the model were obtained using the R packages *stats* and *car* by nonlinear least squares. Nonlinear model predictions and confidence intervals were obtained using *propagate* for Monte Carlo simulations for error propagation.

Wing wear index and age determination of field-collected parasitoids

Parasitoids that died over the course of the bioassay were used to generate a wing wear index by quantifying damage to forewing fringe setae over time at different host densities. This index takes the form of a linear regression model with the log-transformed number of broken setae as a function of parasitoid age and host density. To parameterize the index, *A. certus* that died at a known age in the bioassay were dry mounted on microscope slides and analyzed for wing damage using standard lightfield microscopy at 400x total magnification. Wing damage was scored as the number of broken or missing setae lining the outer edge of each forewing, beginning at the submarginal vein and terminating basally (Figure 2.1); both wings were scored and the mean number of broken setae per wing was determined.

The wing wear index was then used to estimate the age of field-collected parasitoids. Adult *A. certus*, which are present in overlapping generations in the field

(JRM, personal observation), were collected from a conventional soybean farm in Hitterdal, Minnesota (United States) (46.958, −96.222), from 14–21 August 2018. Adult parasitoids were collected by beating 5–10 soybean plants (in the late reproductive stage) into a tray of water from 30 haphazardly selected areas within approximately two hectares of the field. While in the field, adult parasitoids were transferred from the water (where they were held on the surface by tension) into 95% ethanol using a fine brush. Specimens were later dry mounted on microscope slides in the laboratory and wing damage was assessed as previously described. The mean, median, and maximum age (and Wald 95% confidence interval) of field-collected parasitoids were determined by calculating the inverse estimate of the regression model (wing wear index) using the R package *investr*, and life expectancy was calculated as in Equation 2 using a time-specific (vertical) life table (Southwood 1978, Henderson and Southwood 2016).

Dynamical consequences of host-density-dependent parasitoid lifespan

The effects of host-density-dependent parasitoid lifespan on equilibrium host density and parasitism rate were investigated using a stage-structured matrix population model described by Miksanek and Heimpel (2019) [also presented in Chapter 1]. This model is analogous to a Nicholson–Bailey host–parasitoid model with a type II functional response for the parasitoid; the model also includes the developmental rate, fecundity, and lifespan for both species as well as host-stage preference and post-parasitism reproduction. Our analysis of host density dependence follows the sensitivity analyses of Miksanek and Heimpel (2019), with the exception that we excluded the temperature-scaling matrices and expanded the projection interval to 1500 d in order to assess the influence of host density dependence under conditions of equilibrium (days 1000–1500 in the simulation), which allows us to interpret our sensitivity analyses within a more generalizable context rather than one that is restricted to the specific seasonality of the soybean aphid system. The sensitivity analysis of host-density-dependent parasitoid lifespan (P_3 in Miksanek and Heimpel 2019) was performed by applying a inverse linear multiplier that we refer to as $1/z$ such that for $1/z = 1$, parasitoid lifespan is density dependent, but as $1/z \rightarrow 0$, parasitoid lifespan becomes independent of host density.

Results

Lifespan and life expectancy

Parasitoid survival increased significantly with host density (log-rank test of median survival: $\chi^2 = 63.2$, df = 4, $p < 0.001$, n = 51). Parasitoids provided with host densities of 0 and 0.5 hosts per day had the poorest survival, and although 1 host per day significantly improved survival, both 10 and 50 hosts per day yielded the greatest survival (Figure 2.2a); this pattern was also observed in the logistic model of host density and mean lifespan (Figure 2.2b). In the logistic model, maximum mean lifespan of adult parasitoids (θ_1) was 18.6 ± 1.3 days, and estimates for the remaining parameters (which were all statistically significant) are listed in Table 2.1. The critical survival threshold (95% survivorship) was calculated to be 9.9 hosts per day. Life expectancies at emergence for adult female *A. certus* at host densities of 0, 0.5, 1, 10, and 50 hosts per day were 1.9, 2.2, 3.8, 18.6, and 19.7 days, respectively.

Host utilization and lifetime reproduction

The relative number of hosts used for parasitism as opposed to host feeding was similar for parasitoids supplied with 10 and 50 hosts per day but was highest for parasitoids supplied with 1 host per day (Figures 2.3a, 2.3b, and 2.3c). The sex ratio of parasitoid offspring was initially female-biased at all densities but became strongly male-biased later in life for parasitoids given 10 and 50 hosts per day (Figures 2.3b and 2.3c). Parasitoids provided with 50 hosts per day ceased to produce female offspring after reaching an age of 17 days (Figure 2.3c).

Over the course of the experiment, 4092 mummies were produced; of these, live parasitoids emerged from 3615 (88.34%). At host densities of 0.5, 1, 10, and 50 hosts per day, 0.3 ± 0.0 (mean ± SE), 1.7 ± 0.0, 104.1 ± 0.4, and 339.4 ± 1.6 hosts were parasitized during the lifetime of the parasitoid, respectively; an additional 1.0 ± 0.0, 1.1 ± 0.1, 11.9 ± 2.0, and 46.8 ± 5.2 hosts died from host feeding. Control (baseline) mortality was 0.0 ± 0.0, 0.0 ± 0.0, and 0.2 ± 0.4 for host densities of 1, 10, and 50 hosts per day. Parasitism rate (daily fecundity) decreased with parasitoid age, and this trend was most prevalent at the highest host density of 50 hosts per day (Table 2.1; Figure 2.4).

Wing wear index and age determination of field-collected parasitoids

Wing damage increased significantly with age ($F_{1,37} = 217.9$, $p < 0.001$; $R^2 = 0.855$; Table 1; Figures 2.5 and 2.6). Analysis of a similar model including the effects of both parasitoid age and host density also showed that wing damage increased with age ($F_{1,35} = 228.5$, $p < 0.001$) but equivocally with host density ($F_{1,35} = 3.797$, $p = 0.059$; interaction: $F_{1,35} = 0.002$, $p = 0.964$).

A total of 208 parasitoids were collected in the field (141 females and 67 males) and analyzed for wing damage (Figure 2.7). The estimated mean age of adult parasitoids (with 95% CI) was 17.1 ± 7.9 days for females and 6.0 ± 7.8 days for males. The median age (with 95% CI) was 4.4 ± 7.9 days for females and 0.0 ± 7.9 days for males, and the life expectancy at emergence was 14.6 days for females and 6.7 days for males. The female with the most broken setae had an estimated age of 38.3 ± 8.9 (maximum with 95% CI) days, and the male with the most broken setae had an estimated age of 23.6 ± 8.1 days (minimum with 95% CI).

Dynamical consequences of host density dependence on parasitoid lifespan

Decreasing the degree of host density dependence decreased parasitoid life expectancy at equilibrium (Figure 2.8a). The host density at equilibrium generally increased with decreasing density dependence (Figure 2.8b). Leading up to equilibrium, the population dynamics initially changed from stable limit cycles (Figure 2.9a) to a single point equilibrium (Figure 2.9b) to stabilizing cycles (Figure 2.9c) before reaching complete density independence (Figure 2.9d). The proportion of parasitized hosts fluctuated when the equilibrium state was periodic, but as host density dependence decreased and the state of equilibrium changed to a single point, the proportion of parasitized hosts remained relatively constant before falling to zero as the host population escaped control when parasitoid longevity neared complete independence (Figure 2.8c).

Discussion

We found a positive, asymptotic relationship between host density and parasitoid lifespan for the aphid parasitoid *Aphelinus certus*, a pattern that was supported by both the traditional survivorship analysis as well as the logistic model for parasitoid lifespan. Additionally, a laboratory-parameterized wing wear index was used to estimate the age of *A. certus* as a function of damaged wing fringe setae; newly emerged, field-collected female parasitoids had an estimated life expectancy of 14.6 days, which appeared to be shorter than the life expectancy of laboratory-reared parasitoids provided with a sufficient number of hosts per day to reach their maximum lifespan of 19.7 days. Finally, a sensitivity analysis of host-density-dependent parasitoid lifespan in a coupled-equations population model revealed that increasing the degree of host density dependence generally resulted in lower equilibrium host densities.

There are few published studies investigating the relationship between host density and parasitoid survival. In one of these, Sahragard et al. (1991) found that parasitoid lifespan and fecundity increased with host density for *Dicondylus indianus* (Hymenoptera: Dryinidae), a parasitoid of the rice brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). The results reported by Sahragard et al. (1991) were consistent with those of our study; however, while *D. indianus* engages in host feeding, it is unclear if it also consumes honeydew produced by *N. lugens*. In a similar study on the non-host-feeding species *Trioxys angelicae* (Hym.: Braconidae) attacking the black bean aphid, *Aphis fabae* (Hem.: Aphididae), Mahmoudi et al. (2010) did not find a relationship between parasitoid lifespan and host density. In that experiment, however, parasitoids were offered honey as a source of sugar. In those conditions, an effect of host density would not be expected under the hypothesis that host-derived nutrients determine density dependence because sugar water usually supports parasitoid lifespan better than honeydew (Wackers et al. 2008). The contrast between that and the results reported here and by Sahragard et al. (1991) is thus consistent with the hypothesis that host-associated nutrition is a key component mediating the effect of host density on parasitoid lifespan.

We fit a logistic model to the relationship between host density and parasitoid lifespan. Parasitoids reared below this threshold exhibited a marked decrease in lifespan. A logistic model of parasitoid lifespan added a fundamental dimension of physiological realism: the slope of the function decreases when hosts are plentiful (parasitoid lifespan reaches its maximum) but also when hosts are scarce because starving parasitoids catabolize stored sugars and carbohydrates (Ellers 1996, Eijs et al. 1998, Olson et al. 2000) or resorb eggs (Jervis and Kidd 1986, Collier 1995, Heimpel et al. 1997, Rivero-Lynch and Godfray 1997) before eventually dying. Although our model was fit with relatively few data points, it was consistent with the results of the traditional survivorship analysis (lifespan was at its maximum at host densities of 10 and 50 hosts per day, decreased at a density of 1 host per day, and was lowest at densities of 0 and 0.5 hosts per day). Future studies should evaluate parasitoid lifespan at additional host densities to better test the suitability of the logistic model and to investigate how parasitoids respond to extremely high host densities that might be detrimental to lifespan or fecundity (e.g. Líznarová and Pekár 2013).

Although the age distribution of field-collected parasitoids suggests that some individuals do not reach their theoretical maximum lifespan, the life expectancy of field-collected parasitoids was only moderately reduced compared to that of laboratory-reared parasitoids. Overall, the risks of mortality are typically higher in natural settings than in the laboratory due to extrinsic factors such as predation and weather conditions (Heimpel et al. 1997b, Weisser et al. 1997). Also, parasitoids were more likely to parasitize hosts than to feed at high densities, consistent with the results of Bai and MacKauer (1990) for *Aphelinus asychis*. It is important to note that estimates provided by wing wear indices may differ from the field to the laboratory, so a field-parameterized index as opposed to a laboratory-based index may be of more value in providing quantitative estimates of age (Lee et al. 2006).

At the population level, increasing parasitoid lifespan decreases peak host population densities (Miksanek and Heimpel 2019), but the degree of host-density-dependence also plays an important role in host–parasitoid population dynamics since parasitoid life expectancy is higher during periods of high host abundance. Reducing the influence of host density dependence on parasitoid lifespan generally raised equilibrium host densities as shorter-lived parasitoids exerted a smaller effect on the growing host population. When parasitoid lifespan was at its maximum, an initial reduction in host-density-dependence changed the quality of equilibrium from unstable to stable and reduced the maximum equilibrium host density. These patterns parallel the effects of host feeding on host–parasitoid population dynamics, which also reduces equilibrium host densities and contributes to stability (Kidd and Jervis 1989, 1991, Briggs et al. 1995).

The population dynamics identified here are relevant to parasitoids that obtain nutritional resources from their host—as through host feeding or the consumption of honeydew—and in habitats with low floral resources (e.g. agricultural settings). A number of population models have been constructed to elucidate the effects of host feeding on host–parasitoid population dynamics (Kidd and Jervis 1989, 1991, Briggs et al. 1995, Křiven and Sirot 1997), but, to our knowledge, ours is the first to identify the effects of density dependence on parasitoid lifespan within the context of population dynamics. Although not a host-feeding model *per se* (the model simplifies host feeding in that it is nondestructive; in a more complex model we might expect to find additional interactions as parasitoids choose to utilize hosts as food [predation] or for reproduction [parasitism]), our findings should be relevant for any interaction in which host-feeding enhances parasitoid lifespan (Heimpel and Collier 1996, Giron et al. 2002, 2004). And, in practical application, density-dependent effects promote stability in interspecies interactions and contribute to the biological control of pests (Heimpel and Mills 2017).

It is important to continue the development of methods such as the wing wear index in order to be able to relate the results of laboratory studies to natural systems, because ecological and statistical models parameterized primarily with laboratory data may otherwise be of limited utility in predicting, describing, or evaluating host–parasitoid interactions as they occur in nature. In practice, a wing wear index may aid in identifying the impact of different environmental variables on parasitoid lifespan and in understanding patterns in population demographics, contributing to the monitoring and management of natural enemies in a variety of ecosystems.

Table 2.1 Parameter estimates for the logistic model of host-density-dependent parasitoid lifespan, lifetime reproduction model, and wing wear index

	Parameter	Estimate (± SE)	*t*-value	Total df	*p*-value
Lifespan (logistic)	θ_1	18.6 ± 1.3	13.96	43	<0.001
	θ_2	13.5 ± 6.6	2.05		0.047
	θ_3	−0.56 ± 0.23	−2.40		0.021
Lifetime reproduction	φ	−0.26 ± 0.61	−0.42	410	0.671
	α	0.74 ± 0.08	9.00		<0.001
	δ	−0.17 ± 0.02	−7.15		<0.001
	ε	−0.00 ± 0.00	−2.54		0.011
	β^{-1}	0.01 ± 0.00	3.38		<0.001
Wing wear index	intercept	0.12 ± 0.08	1.63	37	0.111
	slope	0.09 ± 0.01	14.76		<0.001

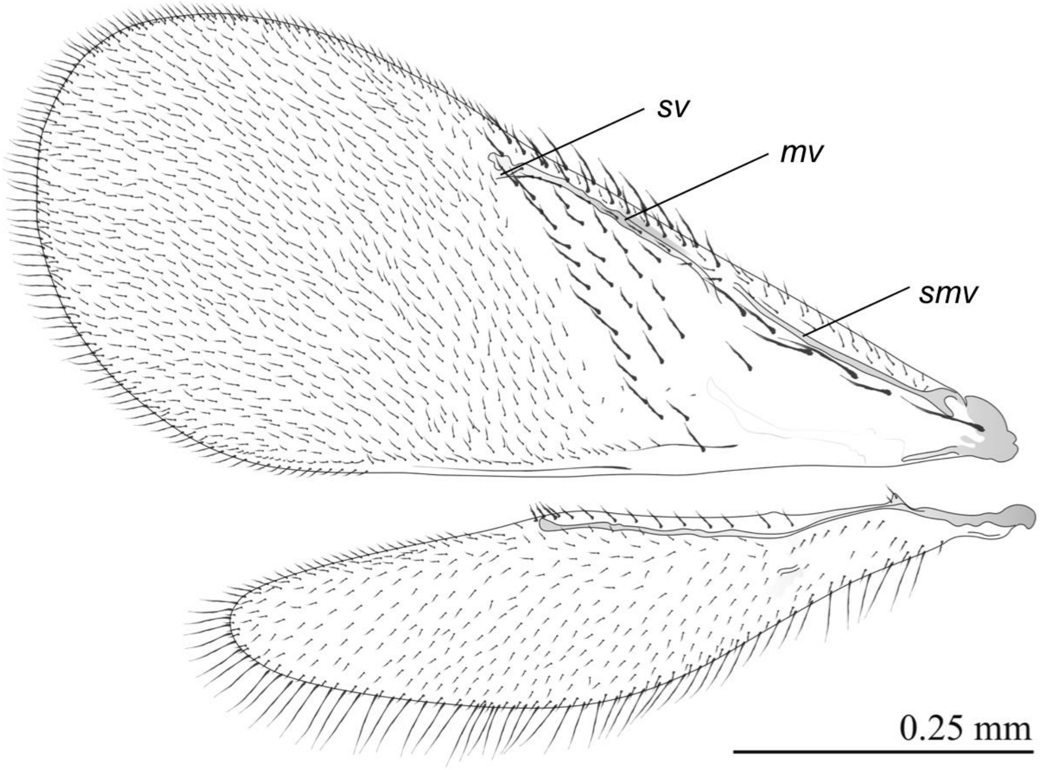

Figure 2.1 Left fore- and hindwing of female *Aphelinus certus*, dorsal view

sv: stigmal vein; *mv*: marginal vein; *smv*: submarginal vein. Illustration: JRM.

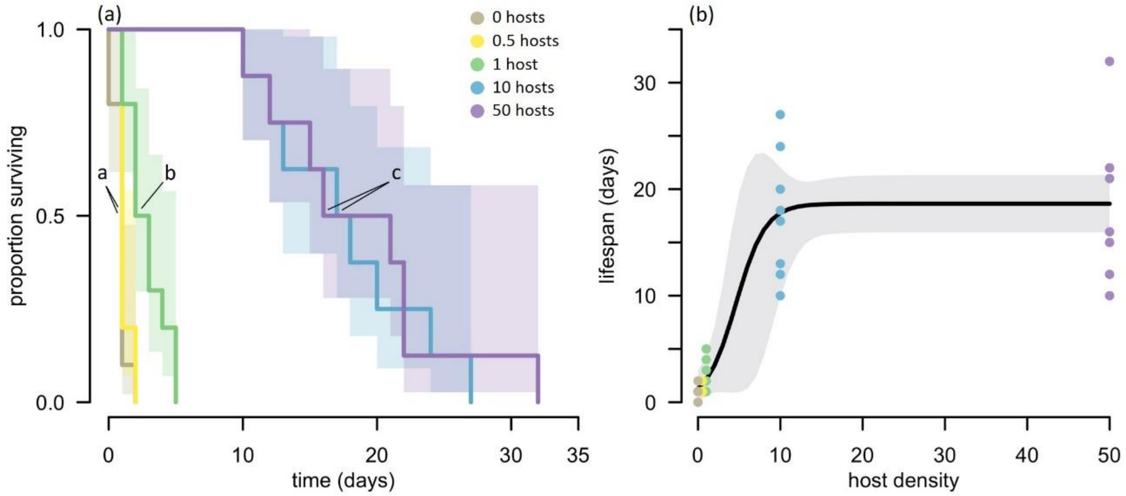

Figure 2.2 Survivorship of adult *A. certus* at different host densities

(a) Traditional survivorship curves. Shared letters indicate no significant differences among groups. Shaded regions represent 90% CI. (b) Host-density-dependent parasitoid lifespan (± SEM) fitted with the logistic model with 95% CI.

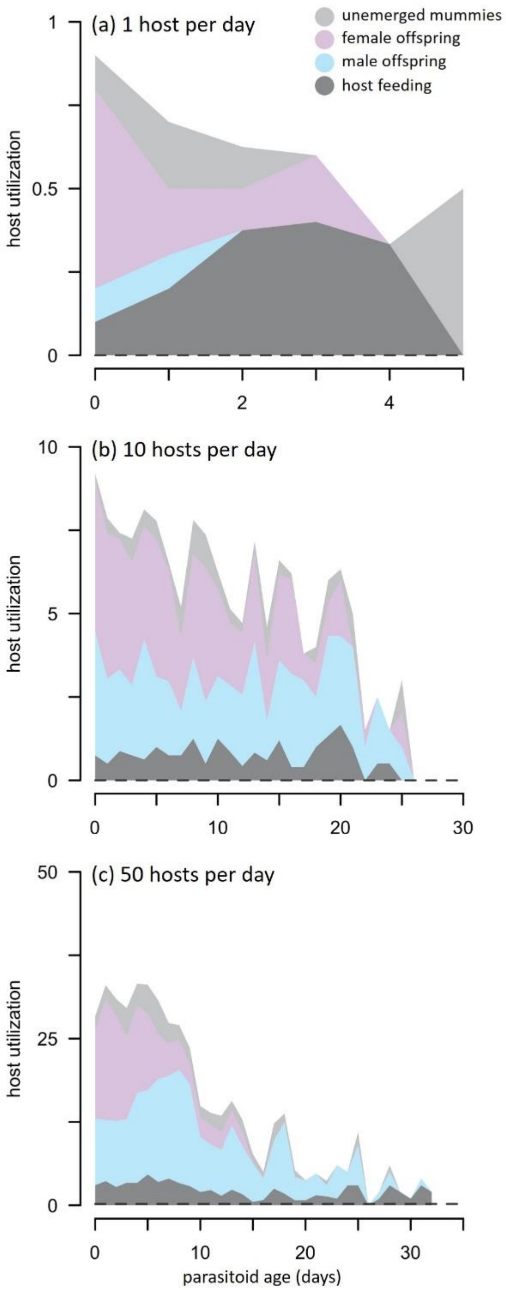

Figure 2.3 Lifetime utilization of host aphids by female *A. certus*

Dark gray: proportion of hosts killed by host feeding. Light gray, purple, and blue (together): proportion of hosts killed by parasitism. Light gray: unemerged parasitoid mummies. Purple: emerged females. Blue: emerged males. The dashed line indicates baseline host mortality. Densities of 0 and 0.5 hosts per day are not shown.

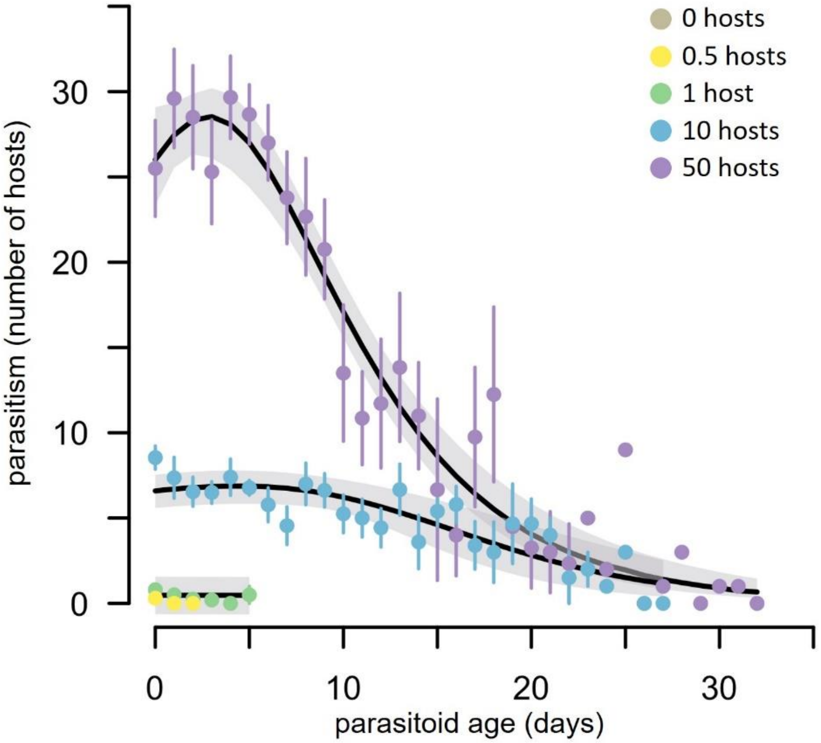

Figure 2.4 Lifetime reproduction of *A. certus*

Color-coded points represent means ± SE for each host density in the experiment. Black lines with 95% CI depict the fitted lifetime reproduction model. For graphical clarity, model predictions are only shown for host densities of 1, 10, and 50 hosts per day.

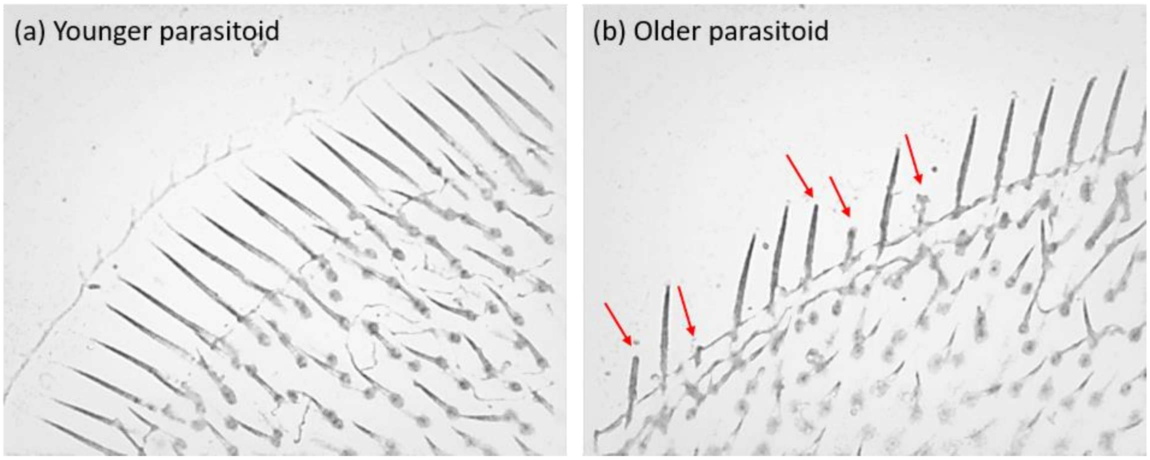

Figure 2.5 Grayscale micrograph of setae on the apical edge of the forewing

Images enhanced for increased visibility of transparent structures. (a) No setae damage on a relatively young parasitoid. (b) Broken setae on an older parasitoid. Arrows indicate broken setae. Images: JRM.

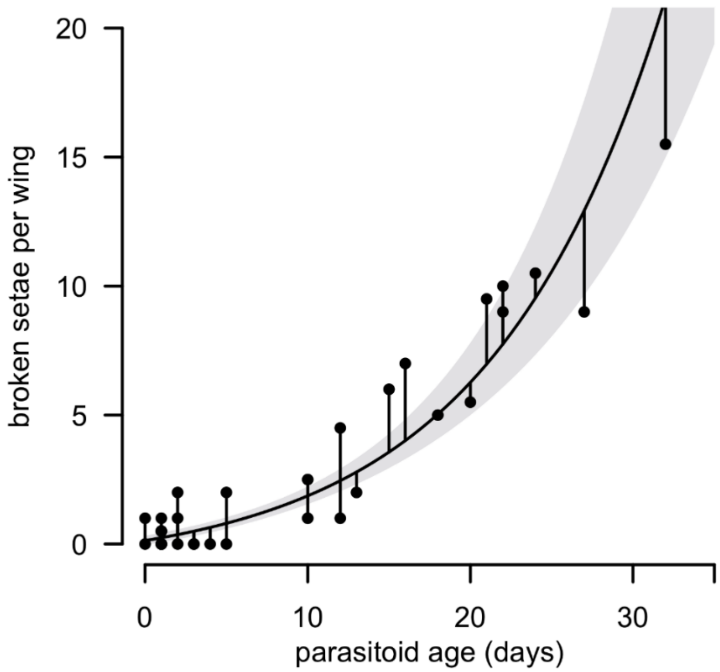

Figure 2.6 Wing wear index for female *A. certus*

Observed values with residuals; solid line depicts fitted model with 95% CI.

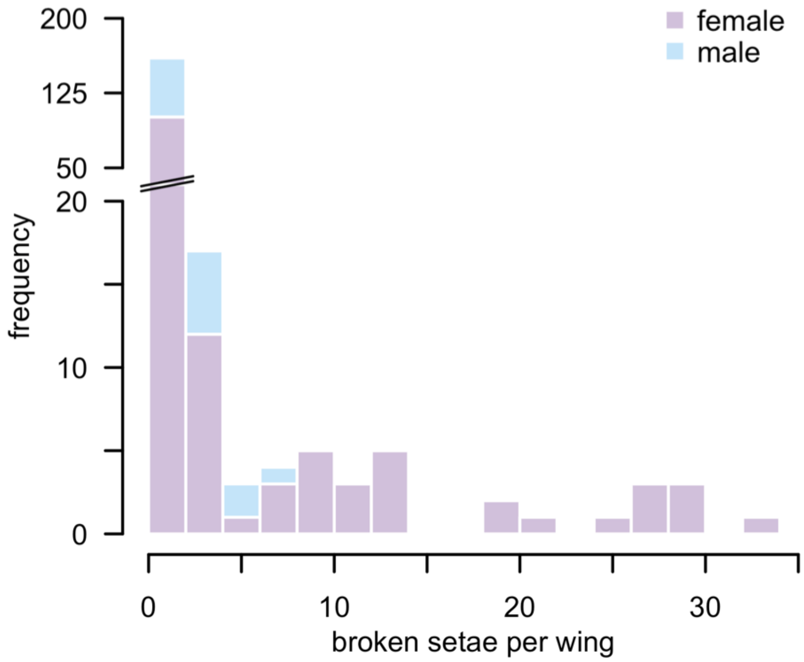

Figure 2.7 Stacked histogram of setae damage from male and female field-collected *A. certus*

Note the change in scale following the break in the *y*-axis.

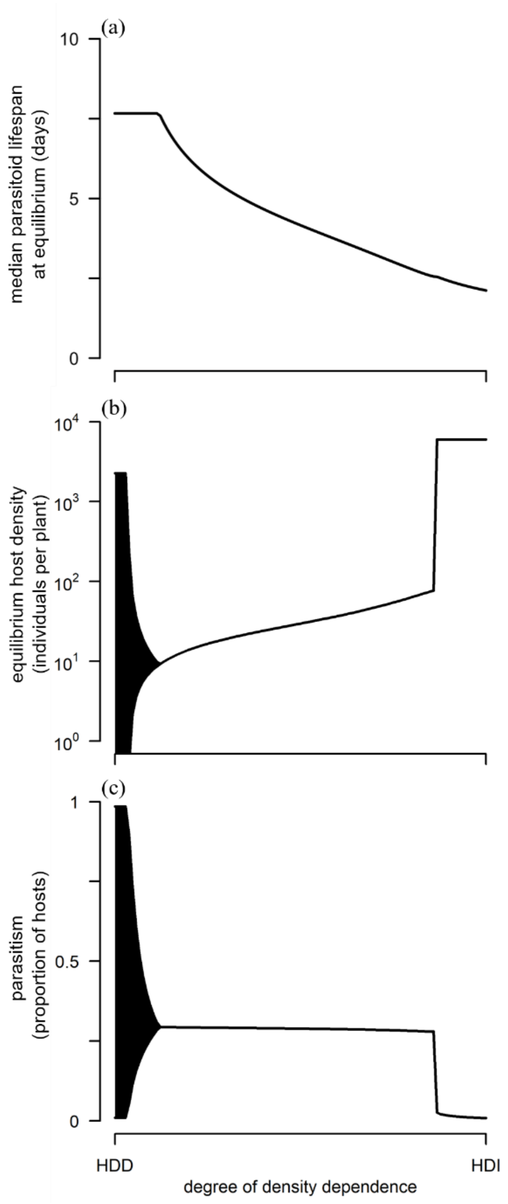

Figure 2.8 Equilibrium dynamics as a function of the degree of host-density-dependent parasitoid lifespan

(a) Median lifespan of parasitoids at equilibrium; (b) host density at equilibrium; and (c) proportion of hosts parasitized at equilibrium. The *x*-axis represents the degree that parasitoid lifespan is dependent on host density; the minimum value of *x* represents complete host density dependence (HDD) on parasitoid lifespan, and increasing values decouple lifespan and host density towards density independence (HDI).

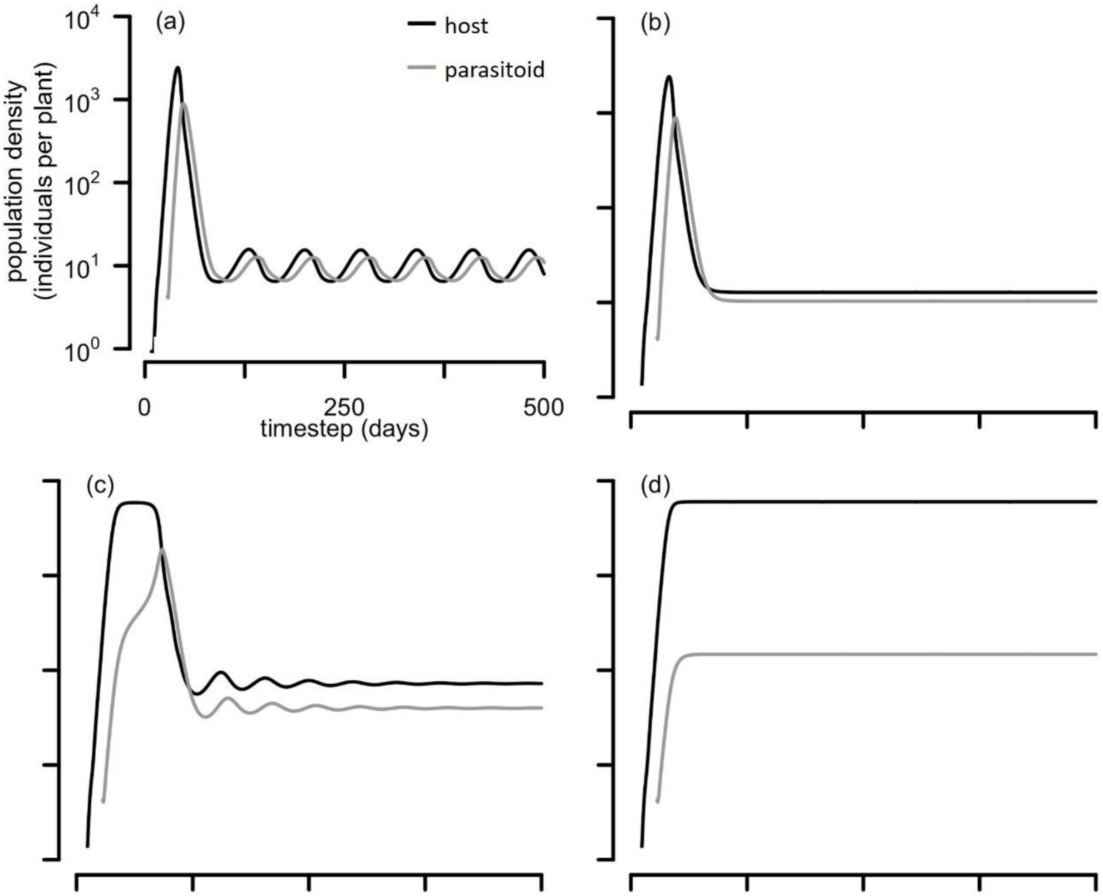

Figure 2.9 Simulation timeseries for different levels of host-density-dependent parasitoid lifespan

Black: host density (individuals per plant); gray: parasitoid density. (a) Host-density-dependent lifespan (*z* = 10); (b) moderate decoupling of host-density-dependent lifespan (*z* = 20); (c) high decoupling of host-density-dependent lifespan (*z* = 90); (d) approximate density independence (*z* = 100). Axis labels and numbering are identical for each plot and were omitted for b–d for graphical clarity.

Chapter 3 A field-based assessment of the parasitoid *Aphelinus certus* as a biological control agent of soybean aphid in North America

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Synopsis

Damaging outbreaks of soybean aphid continue to occur in North America despite the valuable biological control services provided by resident natural enemies. The parasitoid *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) has recently established in North America and has been hypothesized to be a key component of the resident natural enemy community, but there have been few studies evaluating the efficacy of this parasitoid in suppressing soybean aphid populations. We used an exclusion cage study to quantify the effect of *A. certus* on soybean aphid population growth at four sites spanning western and east-central Minnesota from 2017–2019. There were minimal differences in soybean aphid population growth between experimental treatments that excluded natural enemies and control treatments, suggesting that parasitism of soybean aphid by *A. certus* did not have a strong impact on soybean aphid during this study. Because, for example, *A. certus* larvae can reduce host reproduction prior to mortality (resulting in underestimates of effects in short-term studies), our results reflect the challenges of using exclusion cages to assess the effect of individual natural enemy species, especially those with complex life cycles.

Introduction

Soybean aphid (*Aphis glycines* Matsumura; Hemiptera: Aphididae) is a highly damaging pest of soybean (*Glycine max* (L.) Merrill; Fabaceae) that is native to Asia and invasive in North America. Populations of soybean aphids are capable of rapidly expanding and readily moving between fields (McCornack et al. 2004, Ragsdale et al. 2007, 2011). In its native range, soybean aphid is largely controlled by natural enemies (Liu et al. 2004, Wu et al. 2004, Miao et al. 2007), whereas in North America, biological control has been less effective albeit valuable (Heimpel et al. 2004, Ragsdale et al. 2011). For example, Costamagna et al. (2007) found that large generalist predators (predominately coccinellid beetles) resulted in an average 176-fold decrease in soybean aphid densities compared to control treatments over the course of a six-week field experiment in 2004, consistent with a previous study finding that predators provide top-down control of soybean aphid (Costamagna and Landis 2006). Similarly, Gardiner et al. (2009), Bannerman et al. (2018) and Samaranayake and Costamagna (2019) have demonstrated that resident natural enemies decrease soybean aphid population densities.

All of these studies incorporated exclusion cages to quantify the effect of the natural enemy community on pest growth, which can then be compared directly to the economic threshold (ET) of 250 aphids per plant (below which insecticides should not be applied; Ragsdale et al. 2007) to determine the realized benefit of resident enemies. Overall, the biological control services provided by natural enemies of soybean aphid in North America has been estimated at \$33 ha^{-1} in 2006 and \$25 ha^{-1} in 2007 through decreasing costs associated with insecticide applications and a reduction in yield loss (Landis et al. 2008). Additionally, the services provided by natural enemies of soybean aphid indirectly reduce environmental emissions by an estimated 200 million kg of CO_2-equivalent greenhouse gases annually (Heimpel et al. 2013).

Parasitoid hymenopterans have been shown to be one of the most important natural enemies of many aphid pests, including cereal aphids (Schmidt et al. 2003), pea aphids (Snyder and Ives 2003), and wooly apple aphids (Gontijo et al. 2015). Since the previous studies of the resident natural enemies of soybean aphid were conducted, a novel enemy has established and spread throughout the soybean growing region: *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae). *Aphelinus certus* is a solitary koinobiont endoparasitoid native to Asia that attacks a broad range of aphid species in a laboratory setting, and was first detected in North America around 2005 (Frewin et al. 2010, Heimpel et al. 2010, Kaser 2016, Hopper et al. 2017). As with other species of *Aphelinus*, *A. certus* obtains nutrients during as an adult by consuming aphid honeydew and through destructive host feeding (Cate et al. 1974, Bai and MacKauer 1990, Röhne 2002, Wu and Heimpel 2007, Yashima and Murai 2013, Hopper et al. 2013). *Aphelinus certus* is believed to have been accidentally introduced, possibly coinciding with a secondary invasion of soybean aphid (Ragsdale et al. 2011). Following its introduction, *A. certus* spread throughout the central United States and has exhibited steadily increasing rates of parasitism (Heimpel et al. 2010, Kaser 2016). A field experiment conducted during July and August of 2015 by Kaser and Heimpel (2018) found *A. certus* to provide a 3.2-fold decrease in soybean aphid abundance over 12 days. However, there may be geographical or bioclimatic limitations to the success of *A. certus* as a biological control agent, since a 2012–2013 study in Québec, Canada, by Leblanc and Brodeur (2018) did not find *A. certus* to notably affect soybean aphid population dynamics.

The purpose of this study is to better understand the role of *A. certus* in the soybean agroecosystem by replicating the study by Kaser and Heimpel (2018) across Minnesota to better inform management practices in the region. Because arthropod abundance and diversity vary over time and space, we might expect more variation in the present study compared to the case-specific results of the previous study. By utilizing exclusion cages with differing mesh sizes, we aim to determine the specific impact of *A. certus* on soybean aphid populations by itself as well as within the context of the other species comprising the natural enemy community.

Methods

This study was performed at two privately owned conventional farms and at two university-owned research sites from 2017–2019. The private farms were located at 47.0° N, 96.2° E in Hitterdal, MN, USA, and at 45.6° N, 95.7° E in Glenwood, MN, and the university-owned plots were located at 44.7° N, 93.1° E in Rosemount, MN, and at 44.1° N, 93.5° E in Waseca, MN. The dates of the study roughly corresponded with the time period leading up to peak aphid population densities during the soybean growing season. No insecticides were used prior to or during the study.

Exclusion cages were used to quantify the effects of the natural enemy community on soybean aphid population growth over time, following Kaser and Heimpel (2018). There were three treatments constructed using a 38 x 38 x 100 cm wire frame embedded 15 cm below ground surrounding a single soybean plant: (1) an *open* cage, which consisted of the bare frame only; (2) a *predator exclusion* cage, which was constructed by draping a fine mosquito mesh (1 mm^2 holes) around the wire frame; and

(3) a *total exclusion* cage consisting of a very fine mesh (about 0.1 mm^2 holes). The purpose of the open cage was to determine the effect of the entire natural enemy community on soybean aphid populations. The predator exclusion cage functioned to quantify the effect of *A. certus* alone on soybean aphid as the size of the mesh prevents most other natural enemies from accessing the aphids. The mesh of the total exclusion cage was small enough to prevent all natural enemies from entering so that the maximum growth rate of aphids in enemy-free space could be ascertained. Sham cages, which consisted of the very fine total exclusion mesh (0.1 mm2 holes) with an approximately 20-cm cut through the mesh on two of the four sides of the cage that facilitates the movement of insects into and out of the cage, served to control for changes in microclimate in the total exclusion cage. Shame cages were set up at Hitterdal and Glenwood in 2017; comparisons between sham and treatment cages are reported in Appendix A. Kaser and Heimpel (2018) previously demonstrated that the type of cloth mesh used as the predator exclusion material in this study does not significantly affect microclimate. Parasitoids were free to enter and exit the open cages and predator exclusion cages. Total exclusion cages were excluded from analysis if they were contaminated by parasitoids (percent mummies exceeded ½ of one percent) (Appendix B). The omission of contaminated total exclusion cage replicates is necessary because the presence of parasitoids can depress aphid population growth and reduce the accuracy of the treatment in estimating aphid growth in an enemy-free space (see Kaser and Heimpel 2018). In addition, open treatments were excluded if immigrating alatae were detected or if severe weather damaged exposed soybean plants (Appendix B). Omitting open cage

replicates with immigrating alatae is justifiable because reproduction by winged migrants can compensate for the effects of predation (Costamagna et al. 2013).

Prior to the study, 4–8 sections (blocks) spaced about 10 meters apart within each field were surveyed for soybean aphids (5–10 plants per block), and the median density of aphids in each block was calculated. Median densities were used because means can be skewed towards higher aphid densities, potentially simulating an outbreak or causing aggregation of natural enemies (see Kaser and Heimpel 2018). Plants (roughly 60–80 cm in height) to be used in the experiment were cleaned of aphids and natural enemies by removing them individually by hand. In 2017, the median number of non-parasitized soybean aphids of mixed age for each block was introduced into each cage on infested plant material from a laboratory colony reared at 25 ± 2° C, 16:8 L:D, and approximately 65% relative humidity. The cage frames were then covered with a total exclusion mesh and left for 24 hours for the aphids to transfer to the plant of their own accord, after which time the mesh was removed and the aphids were counted to determine colonization success and the initial aphid density for each cage. In 2018 and 2019, the median number of aphids for each block was transferred directly from the laboratory-reared colony to the soybean plant using a fine brush, which ensured consistent colonization across replicates (Miksanek JR, personal observation). A total of 3–9 cages were set up in each block and randomly assigned to an experimental treatment (all with equal replication within block), for a total of 24 cages in Hitterdal, 24 cages in Glenwood, 36 cages in Rosemount, 24 cages in Waseca in 2018, and 27 cages in Waseca in 2019. The experiment was then left to run for 12 days. This relatively short period of time functions to prevent the development of alatae at high densities and allows for only one generation of *A. certus*

(Frewin et al. 2010, Kaser and Heimpel 2018). The total numbers of aphids and parasitoid mummies were assessed for each cage at the end of the study, and mummies (the darkened exoskeletal remnants of dead aphids that contain late-stage parasitoid larvae and pupae) were placed in small groups of up to 10 individuals into 0.6 mL plastic microcentrifuge tubes for rearing (in the laboratory at 23 ± 2° C, 16:8 L:D, and approximately 65% relative humidity) and identification of primary parasitoids (to species) and hyperparasitoids (to genus).

All statistical analyses were performed in R version 3.6.1 (The R Foundation for Statistical Computing 2019). Aphid population growth was calculated using the natural rate of increase, $\lambda = N_{t+1}/N_t$ for the aphid density (N) in each cage at the beginning (t) and end ($t + 1$) of the experiment. To report daily values of population growth, λ was related to the intrinsic rate of growth r using the approximation $\lambda = e^r$; r (which has the units of days^{-1}) was divided by the experiment length (in days) and then converted back to λ (see Gotelli 2008). Differences in daily aphid population growth among treatments were assessed using a multiple regression model with treatment, site-year, and block as factors, and an interaction term for treatment and site-year. Multiple comparisons were conducted *post hoc* using sequential/step-down Bonferroni-corrected p-values (the Holm–Bonferroni method) within the LSD.test() function in the R package *agricolae*. Changes in daily aphid population growth as a function of the level of parasitism at the end of the experiment were assessed using a multiple regression model with percent mummies (calculated as the number of mummies divided by the total number of aphids plus mummies—see Miksanek and Heimpel 2019), and site-year as main factors and an interaction term for percent mummies and site-year to account for local differences in parasitoid populations. Pearson's product-moment coefficient was used to test the relationship between population growth and percent mummies individually for each site-year as well as the relationship between *Aphelinus* density and hyperparasitism rate. Data were pooled across blocks for this analysis, and only treatments with parasitoids present (open and predator exclusion cages) were used.

Results

Median field densities at the start of the experiments ranged from 6–302 soybean aphids per plant (Table 3.1). At the end of the experiments, parasitism in the predator exclusion and open treatments ranged from 0.59–1.71% and hyperparasitism ranged from 0.0–31.0% of *Aphelinus* mummies (Table 3.2). The aphidiine parasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae), a native generalist that also attacks soybean aphid, was only recovered at Hitterdal. Although hyperparasitism of *Aphelinus* at this site was relatively high, no hyperparasitoids were reared from *L. testaceipes*. There was no significant correlation between *Aphelinus* density and the rate of hyperparasitism (n = 23, Pearson's $r = 0.094$, $p = 0.669$).

Daily aphid population growth did not vary significantly among treatments ($F_{2, 103} = 0.329$, $p = 0.721$), but did vary among site-year ($F_{4, 103} = 105.7$, $p < 0.001$) and block ($F_{25, 103} = 2.74$, $p < 0.001$) (Figure 3.2); the overall model had a multiple r^2 value of 0.884. Additionally, there was a marginally significant interaction between site-year and treatment ($F_{6, 103} = 2.07$, $p = 0.069$). The marginal significance of the interaction term indicates that treatment potentially affected aphid population growth differently among site-years. Indeed, population growth rates were lower in open cages compared to the predator exclusion treatment in Waseca during 2018 (Figure 3.2).

In the regression model incorporating daily aphid population growth as a function of parasitism (percent mummies), site-year, and their interaction, there was not a significant effect of parasitism on aphid population growth ($F_{1, 68} = 0.236$, $p = 0.629$). However, aphid population growth varied significantly across site-year ($F_{4, 68} = 48.2$, $p < 0.001$), and the interaction between site-year and parasitism was significant as well ($F_{4, 68} = 3.32$, $p = 0.0167$), indicating that there was a negative relationship between parasitism and aphid population growth. Individual analyses of site-years identified a significant negative correlation at Hitterdal in 2017 and Waseca in 2018 (Figure 3.3; Table 3.3).

Discussion

Although *Aphelinus certus* did not significantly affect daily soybean aphid population growth rates over the course of this study spanning multiple site-years, an increase in parasitism (measured as percent mummies) was correlated with a reduction in aphid population growth rates in two of five replications of the experiment. Overall, our results suggest that, in the context of exclusion cage studies, the effects of natural enemies on soybean aphid are small and variable.

Our evaluation of the biological control efficacy of *A. certus* was most consistent with the results of a previous field study conducted by Leblanc and Brodeur (2018) finding that *A. certus* depresses peak aphid population densities by 1–7%. Our results and those of Leblanc and Brodeur (2018) differ from the findings of Kaser and Heimpel (2018), who found that *A. certus* alone effectively suppressed soybean aphid populations below economically damaging levels during a single 12-day experiment conducted during a similar period of time (late July/early August) in Saint Paul, MN. Altogether, these studies suggest that *A. certus* may be of limited value as a biological control agent under certain conditions but that it is capable of control in others. It may be that other important population- or community-level factors affect the efficacy or reliability of *A. certus* in biological control, such as early-season parasitism (Leblanc and Brodeur 2018) or interactions with higher-order predators and hyperparasitoids (Schooler et al. 2011, Frago 2016).

High hyperparasitism has been shown to negatively impact populations of primary parasitoids and disrupt biological control services (Rosenheim et al. 1995, Schooler et al. 2011). For the primary parasitoids of aphids in North America, hyperparasitoids in the genera *Asaphes* (Hymenoptera: Pteromalidae), *Dendrocerus* (Hym.: Megaspilidae), and *Syrphophagus* (Hym.: Encyrtidae) attack the mummy stage, and hyperparasitoids in the genus *Alloxysta* (Hym.: Figitidae) attack parasitoid larvae but delay their own development until after mummification (reviewed by Sullivan 1987). In mathematical models, while the addition of hyperparasitoids increases food web complexity and overall stability, it also raises equilibrium host density (Sullivan 1987). Our observations of 31% hyperparasitism at the Hitterdal site suggest a high capacity for hyperparasitism to affect the biological control services provided by *A. certus*, especially if hyperparasitoids are capable of colonizing agroecosystems early in the season. Furthermore, the fact that we did not find a negative correlation between *Aphelinus* density and hyperparasitism rate at our Hitterdal site (the only site with an appreciable number of hyperparasitoids) suggests that hyperparasitoids, when present, have the capability of negatively impacting *A. certus* even at low densities. However, we were not able to formally evaluate the population-level impacts of hyperparasitism as the length of the experiment only allowed for one generation of *A. certus*. This may have resulted in an underestimation of the impact of hyperparasitoid species, especially those that attack the mummy stage.

Low parasitism rates likely caused the low efficacy of *A. certus* in our study. Miksanek and Heimpel (2019) used a coupled-equations matrix population model to predict that a mummification level of at least 3.4% is required for *A. certus* to suppress soybean aphid population growth, and that *A. certus* would maintain soybean aphid populations below damaging levels in approximately 10% of simulations under a realistic colonization scenario [see Chapter 1]. This level of parasitism was not met in our study, and Leblanc and Brodeur (2018) reported similarly low levels of parasitism (mummification rates less than 10%) leading up to peak aphid densities in field surveys in Québec, Canada. However, much higher levels of parasitism (about 15% mummies) were observed by Kaser and Heimpel (2018), who found that *A. certus* was able to maintain soybean aphid below damaging levels in Minnesota during the 12-day period of their experiment.

But what might be driving these low rates of parasitism? Leblanc and Brodeur (2018) suggest that timing could be key. One mechanism for this would be a delayed date of parasitoid colonization, which drastically decreases the effect of *A. certus* on peak aphid densities if parasitoids arrive more than one month after the start of the season (Miksanek and Heimpel 2019). Other mechanisms include hyperparasitism (as previously discussed), high overwintering mortality (reviewed by Ramsden et al. 2015), and low aphid densities that serve as a refuge from parasitism due to inverse density dependence (Hassell 2000, Donaldson et al. 2007). For *A. certus*, overwintering mortality is increased at higher latitudes and significantly reduces the number of individuals available to colonize soybean fields in the spring (Stenoien C, Welch K, and Heimpel GE, unpublished), and low aphid densities reduce the amount of nutritional resources (i.e. honeydew and host feeding) available to adult parasitoids, resulting in a decreased life expectancy (Miksanek JR and Heimpel GE, in revision) [presented in Chapter 2]. However, as the aphid densities in our experiment appeared to exceed the minimum number of hosts required for adult parasitoid survival—approximately ten hosts per day for 95% survivorship (J. R. Miksanek and G. E. Heimpel, in revision), host-density-dependent parasitoid lifespan should not have been a limiting factor. Regardless, low aphid densities could still pose a challenge for colonizing populations of *A. certus* earlier in the season through either reduced recruitment, increased starvation, or allocation of fewer resources towards reproduction.

Limitations of exclusion cage studies

It is important to acknowledge that the present study may have underestimated the efficacy of *A. certus*. Exclusion cages have long been criticized for altering a number of microclimatic variables including soil moisture, wind speed, and solar radiation (Perillo et al. 2015). Additionally, exclusion cages may artificially elevate host populations due to limited dispersal (Kindlmann et al. 2015). We propose another limitation of exclusion cage studies: poor synchronization of host–parasitoid dynamics between caged and field populations. Field experiments that have been conducted during entire seasons have identified a definite lag between peak soybean aphid densities and parasitoid/predator response (Costamagna et al. 2007, Bannerman et al. 2018), which suggests that short-term field exclusion cage studies may not be sufficient to detect the effects of natural enemies on host populations. This may be because (1) short-term cage studies conducted during the middle of the season are not able to control for the effects of early-season predation or parasitism; and (2) because exclusion cages do not faithfully recreate field conditions (e.g. exclusion of previously-parasitized, still-living aphids when inoculating cages at the start of the experiment). The inclusion of previously parasitized, still-living aphids could be important because these individuals exhibit lower reproductive rates or infertility (Lin and Ives 2003, Miksanek and Heimpel 2019). A compromising approach may be to conduct exclusion cage experiments at multiple points during the season to allow for better detection of the long-term effects of natural enemies (see Mohl et al. 2016, Bannerman et al. 2018) and to incorporate a subpopulation of parasitized hosts into the experimental design.

Conclusions

The efficacy of *A. certus* as a biological control agent observed during our experiments appears to have been limited by its low rates of parasitism, although the present study did not definitively negate the potential value of this parasitoid in the soybean aphid system. We suggest that further field research is warranted on this interaction due to the variability of these results and those of other published works (Kaser & Heimpel 2018, LeBlanc & Brodeur 2018). Colonization timeline, overwintering mortality, low host densities acting as a refuge for aphids, and hyperparasitism may all be important in diminishing the influence of *A. certus*. If factors that increase parasitism can be identified or promoted, *A. certus* could significantly reduce the necessity of insecticide treatments used to manage soybean aphid and support the use of a dynamic action threshold (Hallett et al. 2014, Miksanek and Heimpel 2019).

Table 3.1 Median number of soybean aphids by block that were used to inoculate soybean plants with laboratory-reared aphids at the start of the experiment

Block	2017		2018		2019
	Hitterdal (15–27 Jul)	Glenwood (24 Jul–5 Aug)	Rosemount (27 Jul–8 Aug)	Waseca (17–29 Aug)	Waseca (8–20 Aug)
1	62.5	8	68	65	6
2	142	11.5	64	115	22
3	53.5	15.5	84	100	21
4	190	34.5	73	105	9
5	157.5	24	64	–	–
6	302.5	21.5	67	–	–
7	123	20.5	–	–	–
8	145	14.5	–	–	–

Table 3.2 Soybean aphid parasitism and prevalence of primary and secondary parasitoid species at the end of the experiment

Site-year	*Aphelinus* parasitism* (%)	Mummies collected (total)		*Aphelinus* emergence (% reared to adulthood)	*Aphelinus* sex ratio (% female)	Hyper-parasitism (% emerged *Aphelinus*)	Hyperparasitism (total number of hyperparasitoids collected)			
		Aphelinus	*Lysiphlebus*				*Alloxysta* (Figitidae)	*Asaphes* (Pteromalidae)	*Dendrocerus* (Megaspilidae)	*Syrphophagus* (Encyrtidae)
Hitterdal-17	1.71	930	127	51.0	39.1	31.0	142	4	1	0
Glenwood-17	1.22	156	0	23.7	58.8	5.4	0	1	0	1
Rosemount-18	0.59	108	0	47.2	34.7	3.9	1	0	0	1
Waseca-18	1.05	3	0	33.3	100	0.0	0	0	0	0
Waseca-19	1.20	12	0	83.3	30.0	0.0	0	0	0	0

*percent mummies (calculated from the predator and total exclusion cages pooled)

Table 3.3 Individual correlations between aphid population growth and parasitism for each site-year

Site-year	Sample size (*n*)	Pearson’s *r*	*p*-value
Hitterdal-2017	15	−0.667	0.002
Glenwood-2017	16	0.063	0.799
Rosemount-2018	10	0.247	0.293
Waseca-2018	16	−0.513	0.015
Waseca-2019	12	−0.027	0.900

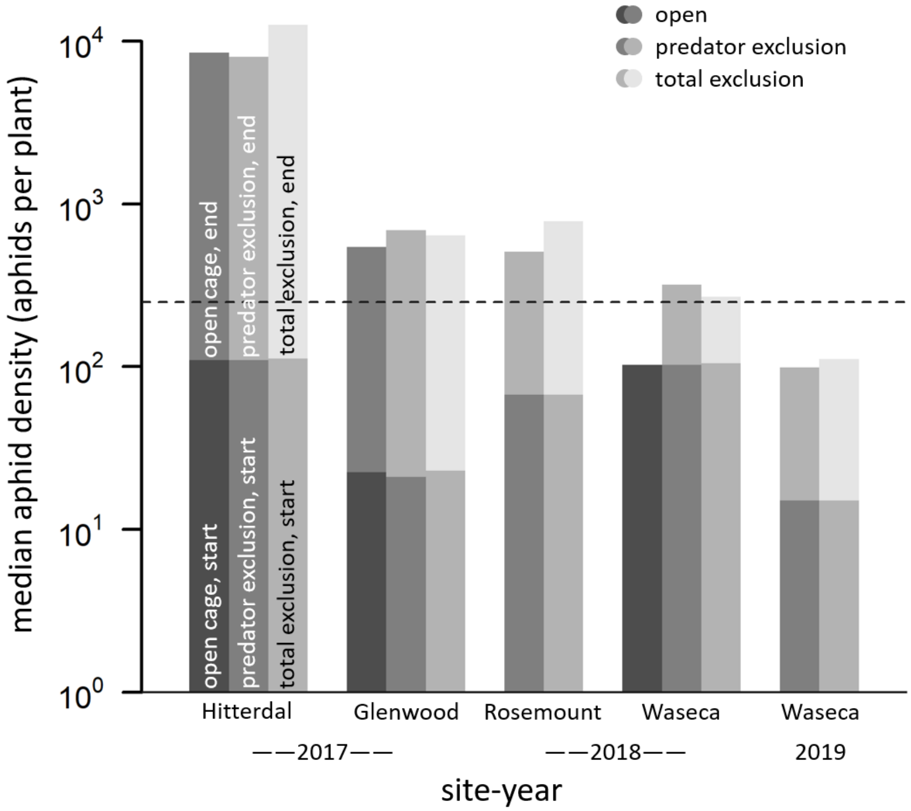

Figure 3.1 Median soybean aphid population densities by site and treatment at the beginning and end of the experiment

The dashed horizontal line indicates the economic threshold of 250 aphids per plant. Note that the open cage treatments at Rosemount in 2018 and at Waseca in 2019 were omitted due to alate immigration and severe weather damage, respectively (refer to Appendix B).

n = 104

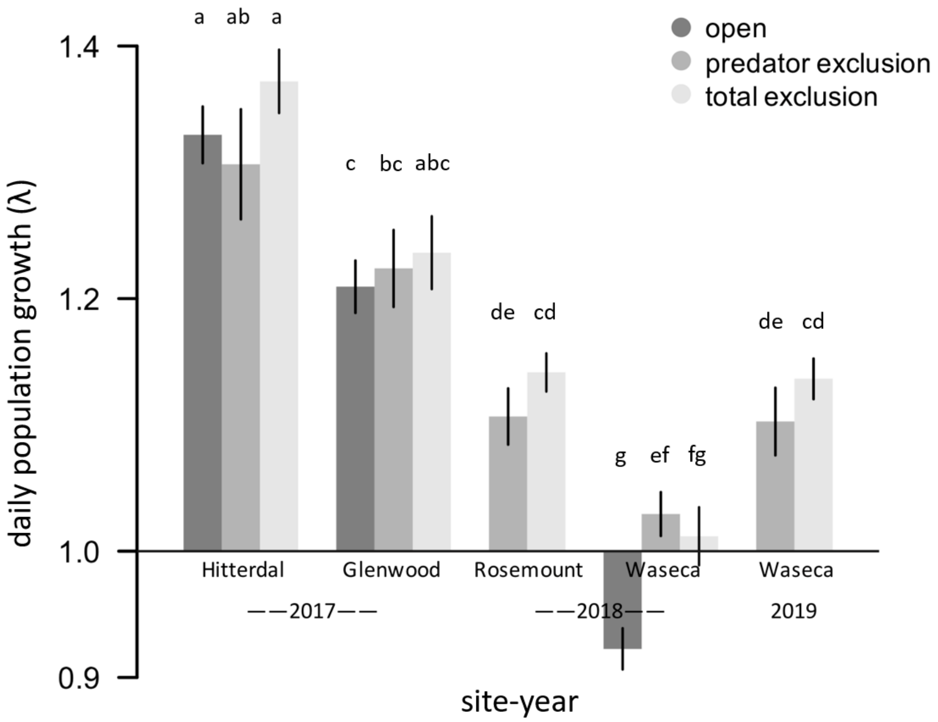

Figure 3.2 Proportional aphid population growth by site and treatment

Bars indicate SEM. Columns not sharing letters are significantly different. Note that the open cage treatments at Rosemount in 2018 and at Waseca in 2019 were omitted due to alate immigration and severe weather damage, respectively (see Appendix B). n = 104

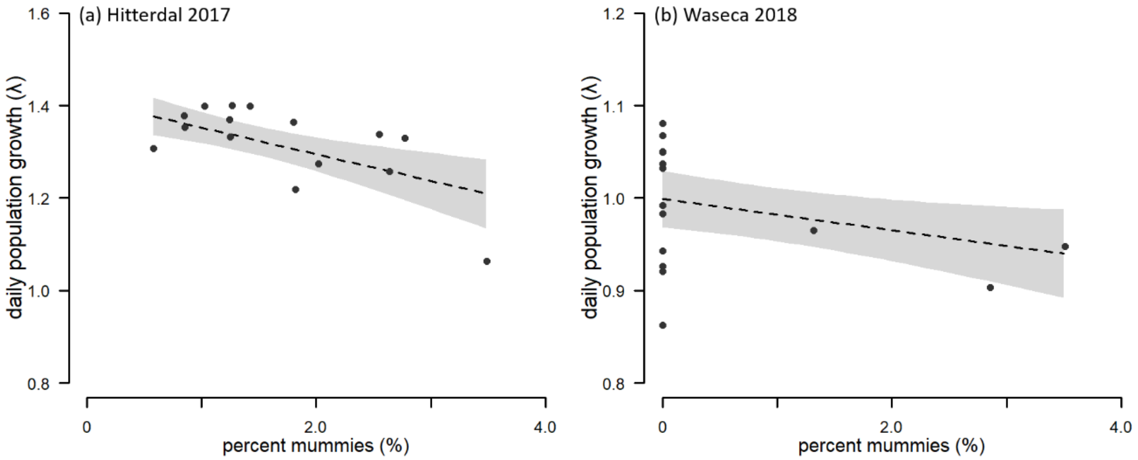

Figure 3.3 Proportional aphid population growth rate as a function of parasitism (percent mummies) in open cages and predator exclusion cages

(a) Hitterdal in 2017 (n = 15) and (b) Waseca in 2018 (n = 16). Site-years with nonsignificant slopes are not shown (see Table 3.1). Dotted lines: prediction from linear regression model with 95% CI.

Chapter 4 Prevalence of *Aphelinus certus*, an accidentally introduced parasitoid of soybean aphid, on native prairie aphids

Synopsis

North American prairies are highly threatened ecosystems that have seen the loss of many iconic animal species in recent decades. Prairie aphids have a wide variety of ecological roles, including their well-known mutualisms with ants that are sometimes regarded as “keystone interactions.” *Aphelinus certus*, a recently introduced parasitoid of the soybean aphid *Aphis glycines*, has been found to parasitize native aphid species in the laboratory, but its effects on aphids in natural ecosystems are unknown. To address this, we conducted field surveys to assess the prevalence of parasitism by *A. certus* on prairie aphids. We found that *A. certus* parasitizes two native aphid species, *Aphis asclepiadis* and *Aphis monardae*, at levels exceeding those of *A. certus* on soybean aphid. The colonization timelines of *A. certus* in prairies and in agroecosystems were roughly parallel, suggesting that *A. certus* readily colonizes or is established in both systems. A broader survey identified *Aphelinus* parasitism at 33% of sites with either *Aphis asclepiadis* or *Aphis monardae* present, and that the relative amount of cropland in the landscape did not predict the presence of *A. certus* in natural areas. Although the overall impact of *A. certus* on native prairie aphids is unknown, it appears likely that *A. certus* poses a threat. A better understanding of the impact of *A. certus* in prairies will contribute to our understanding of the spread of invasive species, and how *A. certus*—as well as other invasive species—might be a beneficial resident enemy in certain ecosystems, whereas in others it may be considered a damaging invader.

Introduction

Facing landscape fragmentation, habitat degradation and loss, invasive species, pesticide drift, and a myriad of other challenges, the prairies of North America are one of the most threatened ecosystems on the planet (Weaver 1954, Leach and Givnish 1996, Robertson et al. 1997, Goldsborough and Crumpton 1998, Pergams and Nyberg 2001, Cully et al. 2003, Bauer 2012). Populations of iconic animals, such as bison, prairie dogs, black-footed ferrets, and grassland birds, have been decimated or nearly lost (reviewed by Samson and Knopf 1994). Insects, too, are vulnerable, with grass skippers (e.g. the Dakota skipper *Hesperia dacotae* and the Poweshiek skipperling *Oarisma poweshiek*) and native bees (e.g. the rusty-patched bumble bee *Bombus affinis*) receiving mainstream publicity as their populations have drastically declined in recent years (Cameron et al. 2011, Swengel and Swengel 2015). These losses in the biodiversity of prairies negatively affect functional species interactions, community composition and trait diversity, nutrient cycling, and trophic dynamics (Coppock et al. 1983, Fisher 1998, Stoner and Joern 2004, Flynn et al. 2011).

Native prairie aphids—although rarely the focus of ecological studies or conservation efforts—are participants in a variety of interspecific interactions with wide-ranging impacts. Perhaps the most recognizable of these interactions is the mutualism between aphids and the ants that tend to them, in which aphids produce high-quality, sugar-rich honeydew in exchange for protection from enemies and a reduction in disease transmission (e.g. *Pandora neoaphidis*) (Stradler and Dixon 2005, Nielsen et al. 2010). Of general importance, ants influence the composition of soil by transforming organic matter (e.g. via aphid tending) by increasing drainage and aeration, consequently affecting the abundance and distribution of plants and other species (reviewed by Nemec 2014). More specifically, aphid–ant mutualisms might be considered “keystone interactions” in terrestrial ecosystems because aphid-tending ants act as natural enemies of other, more harmful herbivores and exert a disproportionally large effect on the structure of the entire arthropod community (Eubanks and Styrsky 2006).

In 2005, the generalist parasitoid *Aphelinus certus* was found parasitizing the soybean aphid (*Aphis glycines*) in North America (Frewin et al. 2010, Heimpel et al. 2010). Prior to its invasion, *A. certus* was evaluated for use in classical biological control but was not approved for release as it attacked a broad range of aphid species in laboratory experiments (Kaser 2016, Hopper et al. 2017). Many of the aphids attacked by *A. certus* participate in aphid–ant mutualisms and are members of native floral communities, including *Asclepias* (milkweed), *Monarda fistulosa* (wild bergamot or beebalm), and *Andropogon geradi* (big bluestem) (Blackman and Eastop 2006, Wyckhuys et al. 2009, Keller 2016). Since its invasion, *A. certus* has quickly spread throughout the North Central United States and exhibits steadily increasing rates of parasitism on soybean aphid (Heimpel et al. 2010, Kaser 2016), which increases the need to investigate the potential exposure of native aphid species.

The purpose of this study is to assess the prevalence of parasitism by *A. certus* on native prairie aphids and to generate hypotheses of parasitoid invasion in natural habitats. To accomplish this, we monitored the population dynamics of aphids and their parasitoids on *Monarda fistulosa* and *Asclepias* spp. (in prairies) and on *Glycine max* (soybean) (in agricultural fields) across western Minnesota. We also conducted a series of presence/absence surveys in prairies, grasslands, savannas, and parklands throughout the midwestern United States (centering on Minnesota). This work is an important first step in documenting the exposure of an invasive parasitoid to native prairie aphids, and may also be applied to validate previously published results of laboratory-conducted host-range testing and contribute to a retrospective quantitative risk–benefit analysis of *A. certus* as an introduced biological control agent.

Methods

Population surveys were conducted in 2017 to outline the colonization timeline of aphids and their parasitoids in natural and agricultural habitats. *Monarda fistulosa* and *Asclepias* spp. (*syriaca*, *speciosa*, *incarnata*, or *sullivantii*) were non-destructively sampled to measure the species composition and abundance of aphids and their associated parasitoids at four prairies: Prairie Coteau (44.122, −96.151), Chippewa Prairie (Unit 1 [45.115, −95.984]), Glacial Lakes State Park (Douglas A. Bean Memorial Tract [45.539, −95.485]), and Felton Prairie (Assiniboia Skipper Unit [47.101, −96.410]). Each prairie was paired with a conventional soybean field located within a 25-kilometer radius. Sampling occurred within a roughly 0.2 km^2 area within the interior of each site, with 50–200 plants of each species sampled per site per visit at approximately two- or three-week intervals. Prairie sampling was conducted at least 0.8 km from the nearest agricultural field to minimize spillover effects. Parasitism was measured as *percent mummies* (following Miksanek and Heimpel 2019), which is a fractional measure of the number of mummified aphids—the dark, sclerotized husks of dead aphids containing late-instar parasitoid larvae, pupae, or pre-emerged adults—over the total number of aphids, living and dead.

A statewide presence/absence survey was conducted during late July and early August of 2019 to determine the prevalence of *A. certus* parasitism on *Aphis asclepiadis* and *Aphis monardae* in natural habitats. For this, *Asclepias* and *Monarda fistulosa* were non-destructively sampled as previously described to detect the presence or absence of aphids and their associated parasitoids at 40 potentially suitable natural areas (prairies, grasslands, savannas, and parklands) evenly distributed throughout Minnesota, Wisconsin, and North Dakota (Supplement 1). The results of the survey were mapped using an Albers equal area conic projection (EPSG:102003) in QGIS 3.8.2. State, country, lake, and river boundaries were obtained from the United States Geological Survey (2004, 2006) and Minnesota county boundaries from the Minnesota Department of Natural Resources (2013). Land use data were obtained from the National Land Cover Database (MRLC Consortium 2016) and processed in QGIS to obtain the relative amount of cropland within radii of 1 and 5 km around each site, and a logistic (binomial) regression was used to test for correlations between the presence/absence of *A. certus* and the proportion of cropland in the surrounding landscape. Unless otherwise stated, all analyses were performed in base R version 3.6.0 (The R Foundation for Statistical Computing 2019).

Results

In the 2017 surveys, *Aphis asclepiadis* was recorded on *Asclepias* spp. at all four sites, and *Aphis monardae* was recorded on *M. fistulosa* at Glacial Lakes State Park and Chippewa Prairie (it was not present at Felton Prairie or Prairie Coteau because of the absence its host plant). *Aphis glycines* was present on *G. max* at all four sites. Mummies formed by an *Aphelinus* parasitoid were observed on *Aphis monardae* at Glacial Lakes State Park and Chippewa Prairie (Figure 4.1a) and on *Aphis asclepiadis* at Felton Prairie, Glacial Lakes State Park, and Prairie Coteau (Figure 4.1b). We identified the parasitoids that emerged from these mummies as *Aphelinus certus* based on morphological characters described by Yasnosh (1963); however, species-level identification could not be confirmed on a later date due to issues related to specimen age (Keith Hopper, personal communication).

Parasitism of *Aphis asclepiadis* by *A. certus* ranged from 0.33–8.3% mummies (Table 4.1). Parasitism of *Aphis monardae* by *A. certus* peaked at 11.8% mummies (Table 4.1), and *Aphelinus* mummies were often found in high abundance inside of flower heads (Figure 4.1a). Parasitism of *Aphis glycines* in paired agricultural fields ranged from 1.8–12.6% mummies (Table 4.1). Where present, parasitism by *A. certus* was first recorded in mid-July, a colonization timeline that paralleled that of *A. certus* in soybean fields (Figure 4.2).

In the 2019 statewide survey, *A. certus* was found parasitizing native aphid species at 33% of sites with either *Aphis monardae* or *Aphis asclepiadis* present (Figure 4.3). Parasitism of *Aphis monardae* was recorded at Glacial Lakes State Park, Carlos Avery State WMA, Chippewa Prairie, St. Croix Savanna, Iron Horse Prairie, and Mound Prairie (Figure 4.3a; Appendix C), and parasitism of *Aphis asclepiadis* was recorded at Felton Prairie and Glacial Lakes State Park (Figure 4.3b). Additionally, a second *Aphelinus* species (clearly a different morphospecies than *A. certus*, but species-level identification was not possible; Keith Hopper, personal communication) was recovered from Rice Lake. The presence or absence of *A. certus* was not correlated with the relative amount of cropland in the surrounding landscape within a 1 km radius (AOV: $p = 0.975$; McFadden's $R^2 = 0.000$) or a 5 km radius (AOV: $p = 0.222$; McFadden's $R^2 = 0.037$).

Discussion

The accidentally introduced parasitoid *Aphelinus certus*, once considered for classical (importation) biological control of soybean aphid, was found parasitizing native aphid species in prairie ecosystems. The concurrent colonization of natural habitats that were geographically isolated from agroecosystems (consistent with the temporal overlap reported by Wyckhuys et al. 2009) and the lack of correlation between the presence of *Aphelinus* and the proportion of cropland in the landscape were not indicative of spillover effects, indicating that *A. certus* has either permanently invaded prairie ecosystems or readily colonizes prairies early in the season. The overall impact of this parasitoid on prairie aphid communities is yet to be determined. Although there are three native *Aphelinus* species that have been reported parasitizing aphids in the genus *Aphis* in the United States—*Aphelinus marlatti, A. nigritis, and A. semiflavus*—there are no historical records of parasitism on *Aphis monardae* or *Aphis asclepiadis* despite thorough surveys in the Midwestern United States (Ohnesorg 2008, Wyckhuys et al. 2009, Kaser 2016, USDA 2012, Martens and Johnson 2018); thus, to the best of our knowledge, our findings of *Aphelinus* parasitism on *Aphis asclepiadis* and *Aphis monardae* constitute a novel ecological interaction in the region.

Observed levels of parasitism on *Aphis asclepiadis* and *Aphis monardae* (8–10% mummies) exceeded the theoretical threshold for zero population growth (3.4% mummies for *Aphis glycines* [Miksanek and Heimpel 2019]) and are higher than historical levels of parasitism by braconid parasitoids in the Midwest (4% mummies [Ohnesorg 2008]). Thus, it appears likely that *A. certus* will pose a threat to these species of prairie aphids, and these negative effects may be compounded by the fact that the population growth rates of prairie aphids tend to be much lower than those of soybean aphid in agricultural settings (e.g. McCornack et al. 2004, Züst and Agrawal 2016). The relatively low levels of parasitism on soybean aphid (approximately 2.5% mummies) were consistent with the low/variable effect *A. certus* is understood to have on soybean aphid (Kaser and Heimpel 2018, Leblanc and Brodeur 2018, Miksanek and Heimpel 2020 [Chapter 3]). Physical refuges may offer some protection against parasitism (Wyckhuys et al. 2007). For example, parasitism by the nonnative aphidiine *Binodoxys communis* is reduced for *Aphis monardae* when the aphids feed within the flowerheads of *Monarda fistulosa* (Wyckhuys et al. 2007). However, during the period of this study, *Aphelinus certus* was found parasitizing *Aphis monardae* deep within flowerheads, which may have been due to its smaller size or differences in foraging behaviors. Similarly, the presence of ants may serve as a refuge for aphids from parasitoids (Wyckhuys et al. 2007) and merits further study.

Enrichment of the hyperparasitoid guild and disruption of aphid–ant mutualisms are also of concern with the advent of *A. certus*. The addition of *A. certus* to the community may increase hyperparasitism by *Alloxysta* (Figitidae), *Asaphes* (Pteromalidae), *Dendrocerous* (Megaspilidae), and *Syrphophagus* (Encyrtidae) (Kaser 2016, Kaser and Heimpel 2018; Dregni J and Heimpel GE, unpublished data), resulting in apparent competition with other species of primary parasitoids such as the endemic aphidiine *Lysiphlebus testaceipes* (see Heimpel et al. 2004). However, as many prairie aphids are tended by ants, it is possible these effects may be mitigated; for example, ants attending *Aphis fabae* reduce hyperparasitism as they are not aggressive towards *Lysiphlebus cardui* but do attack *Trioxys angelicae* (Völkl 1992). It is also possible that the presence of ants could be indirectly beneficial for *A. certus* in that (1) ants would protect *A. certus* from predation during the early developmental stages and (2) *A. certus* provided with higher quality honeydew manufactured in the presence of ants (and often at a cost to the population growth rate of aphids—see Stradler and Dixon 1998, Yao 2014) may have longer lifespans (Tena et al. 2018; Miksanek and Heimpel, in revision). On the other hand, aphid–ant interactions may be negatively affected by *A. certus* if the size of the aphid colony is reduced below what tending ants require; even so, because ants involved in aphid mutualisms are expected to interact with more than one species of aphid, the loss of any one aphid species may not disrupt the keystone interaction between aphids and ants in prairies ecosystems (Stradler and Dixon 2005).

As *A. certus* was once considered for importation as a natural enemy of soybean aphid, our findings qualitatively support the ultimate decision not to pursue the release of *A. certus* as a biological control agent. Although host specificity is often associated with effectiveness in biological control (see Snyder and Ives 2003, Kaser & Heimpel 2015), it may come at a cost of reduced virulence, host coevolution, or inability of the agent to persist in an ecosystem in the absence of the primary host (Brodeur 2012). Depending on the severity of the pest, the potential benefits of biological control must be weighed against the ecological risks of an introduced enemy, as well as the effects of continuing current management practices (or "doing nothing") or developing and implementing other new technologies (Heimpel et al. 2004, Heimpel and Mills 2017, Heimpel and Cock

2018). But if the risk of exposure of a generalist to non-target hosts is minimal or if the realized host range of an enemy is more limited than the physiological host range, these types of factors may be incorporated into risk-benefit models (Wright et al. 2005, Kaser and Heimpel 2015)—something that is referred to as a “new era” in biological control (see Heimpel and Cock 2018, Wyckhuys et al. 2018)—although the broader ecological impacts of introduced arthropods under modern risk assessment practices are complex and poorly understood (Hajek et al. 2016).

In addition to novel challenges posed by *Aphelinus*, prairie aphids face threats stemming from land management strategies, other invasive species, and climate change. Prescribed fires that exclude unburned refuges or with an insufficient interval between burns significantly reduces aphid abundance and destroys overwintering eggs deposited in aboveground plant tissue (Harper et al. 2000). The multicolored Asian (harlequin) ladybeetle *Harmonia axyridis* has been recorded attacking native aphid species in grasslands (reviewed by Roy et al. 2016), and the oleander aphid (*Aphis nerii*), a cosmopolitan species, directly competes with *Aphis asclepiadis* (Mooney et al. 2008, Wong et al. 2017). Although there is little consensus whether a warmer climate might ultimately benefit (increased reproductive rates, range expansion) or harm (disrupted trophic interactions, habitat loss) aphid populations (see Kindlemann et al. 2010), a warming climate may disrupt aphid–ant mutualisms by inducing a phenological mismatch or by reducing the benefits for tending ants (decreased sugar concentration in honeydew) thus decreasing the vigor with which ants protect aphids (Barton and Ives 2014, Mooney et al. 2019).

While our understanding of prairie aphid communities is far from complete, native aphid species could be rarer or present in lower densities that they were historically due to the invasion of *A. certus*. Going forward, it will be important to continue population surveys to determine the magnitude and frequency of parasitism by *A. certus* as well as its overall effects on the population dynamics of native prairie aphids, plants, hyperparasitoids, and other natural enemies. It will also be useful to evaluate the potential for movement of *A. certus* between agricultural and natural habitats, or if parasitism of non-target aphid hosts is an important component of the natural history of *A. certus* in North America (and a critical aspect of its life cycle as a resident biological control agent of soybean aphid). Finally, an interdisciplinary approach is needed in order to better understand the trade-offs of *A. certus* in reducing the use of insecticides in managing soybean aphid as well as the landscape-level indirect benefits of reducing pesticide drift for at-risk arthropod species, such as the Dakota skipper and other prairie lepidopterans.

Table 4.1 Number of aphids at peak percent parasitism for each site in 2017

Aphid/plant species	Site	Date	Aphids per plant (mean ± SD)	Parasitism[*] (%)
Aphis asclepiadis on *Asclepias* spp.	Felton Prairie	5 Aug	26.1 ± 72.1	8.3
	Glacial Lakes	24 Jul	9.2 ± 30.2	0.33
	Chippewa Prairie	14 Aug	13.4 ± 37.9	0
	Prairie Coteau	14 Aug	1.1 ± 5.4	0.93
Aphis monardae on *M. fistulosa*	Felton Prairie	–	–	–
	Glacial Lakes	24 Jul	0.34 ± 1.9	0
	Chippewa Prairie	14 Aug	4.6 ± 9.9	11.8
	Prairie Coteau	–	–	–
Aphis glycines on *G. max*	Felton Prairie	27 Jul	166.7 ± 140.0	2.4
	Glacial Lakes	14 Jul	3.6 ± 5.9	12.6
	Chippewa Prairie	14 Aug	29.8 ± 22.1	1.8
	Prairie Coteau	13 Jul	10.3 ± 47.7	2.7

[*] percent mummies

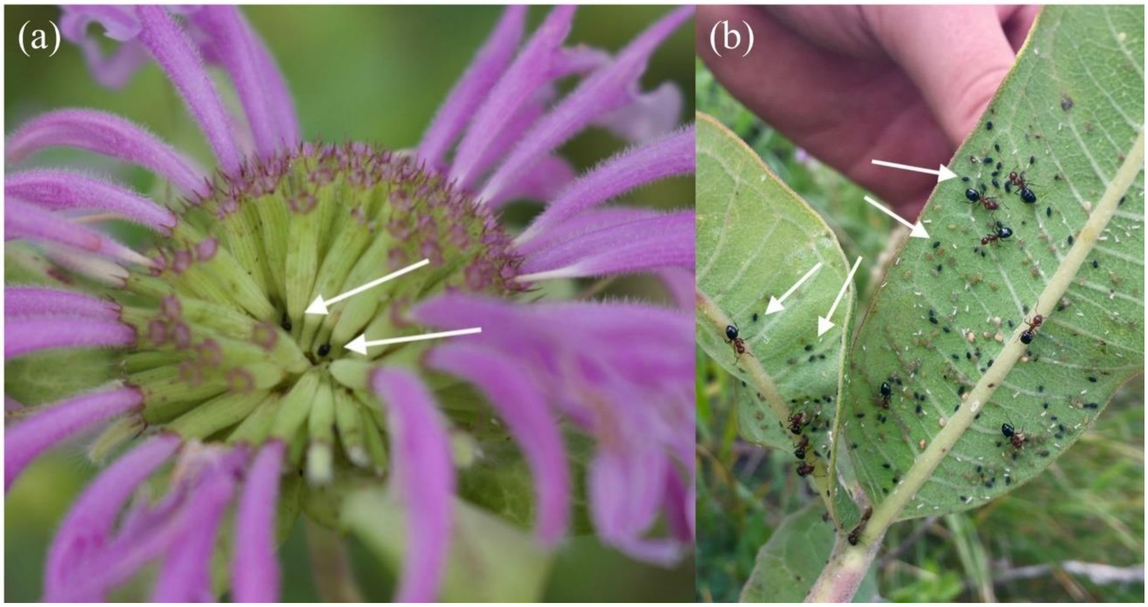

Figure 4.1 *Aphelinus* mummies in prairies

Arrows indicate *Aphelinus* mummies on (a) *Aphis monardae* at Chippewa Prairie and (b) *Aphis asclepiadis* at Felton Prairie. Photos: JRM.

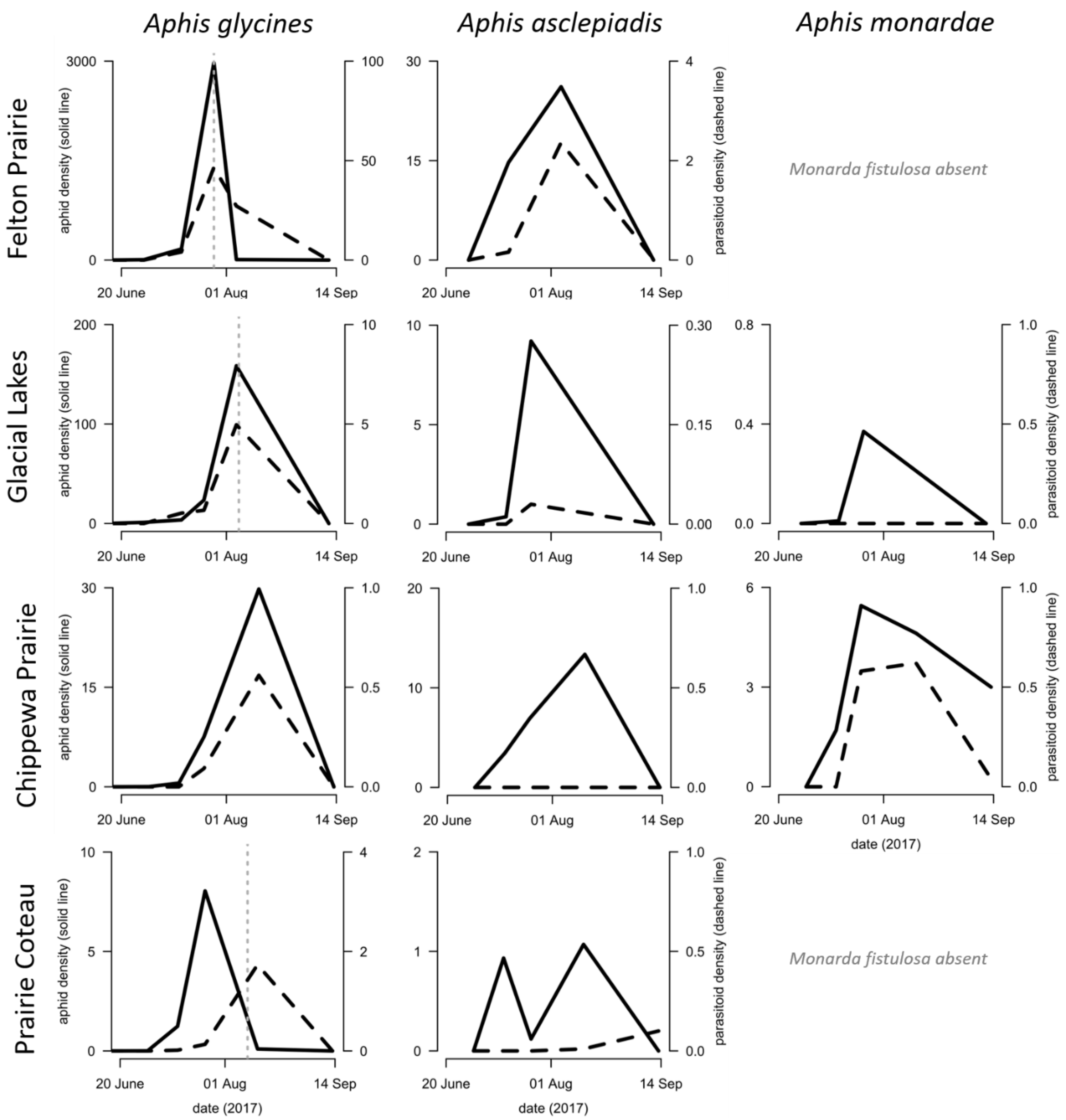

Figure 4.2 Population dynamics of *A. certus* and its hosts by site and aphid species

Solid lines (left *y*-axes): *Aphis glycines*, *Aphis asclepiadis*, and *Aphis monardae*; dashed lines (right *y*-axes): *A certus*. Dotted gray vertical lines indicate insecticide spray dates in soybean fields.

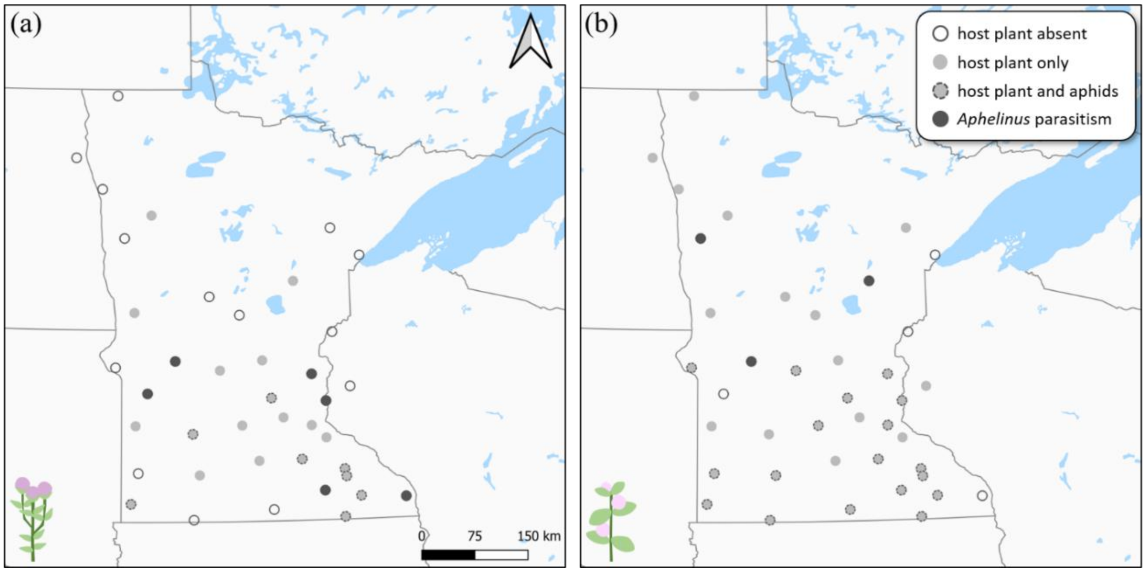

Figure 4.3 Presence/absence data for host plants, endemic aphid species, and *A. certus* in July and August of 2019

(a) *M. fistulosa* and (b) *Asclepias* communities.

Final thoughts and conclusions

The theoretical work presented in the first half of this thesis suggested that *Aphelinus certus* may be capable of suppressing soybean aphid in approximately 10% of cases, and certain aspects of parasitoid life history (i.e. lifespan and fecundity) acted in a host-density-dependent manner to affect the overall dynamics of the system. The fieldwork detailed in the latter half of this thesis revealed that *A. certus* has a small but variable impact in the biological control of soybean aphid across Minnesota and that endemic aphid species in native habitats are readily attacked by this parasitoid.

As reported in Chapter 1, *A. certus* exhibited optimal growth on intermediate host stages and lacked a preference for any individual host stage. The parameterized model predicted that *A. certus* reduces soybean aphid densities below economic threshold in 9.9% of simulations, and host suppression was predicted at a parasitism rate of 0.21 d^{-1} (equivalent to 3.4% of the aphids being mummified). Because parasitism was dynamic, relatively low parasitism rates early in the season could still be associated with low peak host densities. Scaling this estimated 9.9% reduction in cases exceeding economic threshold, then *A. certus* might reduce insecticide applications by 1.8 million acres annually, saving an average of $2.43/ha in management costs along with any commensurate environmental benefits of reduced insecticide use (Ragsdale et al. 2007, Landis et al. 2008, Heimpel et al. 2013, USDA 2015).

Expanding on the matrix model from Chapter 1, the data presented in Chapter 2 outlined a positive, asymptotic relationship between host density and parasitoid lifespan supported by both a traditional survivorship analysis as well as the logistic model. A sensitivity analysis of host-density-dependent parasitoid lifespan incorporated into the model introduced in Chapter 1 revealed that increasing the degree of host density dependence generally resulted in lower equilibrium host densities. Thus, for parasitoid species that rely on their host for nutrients, biological control may be less effective during periods of low host abundance, such as during the early season, after spraying insecticides, or late in the season. Lastly, a laboratory-parameterized wing wear index was used to estimate the age of *A. certus* as a function of damaged wing fringe setae. In the field, newly emerged female parasitoids had an estimated life expectancy of 14.6 days, which was not significantly shorter than the life expectancy of laboratory-reared parasitoids provided with a sufficient number of hosts. The wing wear index offers a promising quantitative approach for estimating parasitoid age and may be used to monitor cohorts in the field, conduct life table analyses, and identify the impact of environmental variables on lifespan and demography.

The field-based assessment of *A. certus* reported in Chapter 3 demonstrated that this parasitoid did not significantly affect daily soybean aphid population growth rates over the course of the experimental study replicated over multiple site-years. However, an increase in parasitism (measured as percent mummies) was correlated with a reduction in aphid population growth rates in two of five replications of the experiment. Altogether, these results suggest that, in the context of exclusion cage studies, the effects of natural enemies on soybean aphid are small and variable. It appears likely that low parasitism rates caused the low efficacy of *A. certus* in the study. From the model presented in Chapter 1, at least 3.4% mummies were required to suppress soybean aphid population growth, a value that was mot observed during the experimental period (however, in 2015, Kaser and Heimpel [2018] reported 15%). Timing could be key—delayed parasitoid colonization results in higher peak host densities (Leblanc and Brodeur 2018, Miksanek and Heimpel 2019); likewise, high hyperparasitism, overwintering mortality, and low-density refuges/inverse density dependence may also be contributing factors (Hassell 2000, Donaldson et al. 2007, Ramsden et al. 2015).

Despite a relatively low effect on soybean aphid, Chapter 4 suggested that *A. certus* may be having a negative impact on native aphid species. The concurrent colonization of natural habitats that were geographically isolated from agroecosystems and the lack of correlation between the presence of *Aphelinus* and the proportion of cropland in the landscape were not consistent with spillover effects, indicating that *A. certus* has either permanently invaded prairie ecosystems or readily colonizes prairies early in the season. While the overall impact of this parasitoid on prairie aphid communities is yet to be determined, the observed levels of parasitism on *Aphis asclepiadis* and *Aphis monardae* (8–10% mummies) exceeded the theoretical threshold for zero population growth (3.4% mummies for *Aphis glycines*) and are higher than historical levels of parasitism (4% mummies) by braconid parasitoids in the Midwest. Thus, it appears likely that *A. certus* will pose a threat to these species of prairie aphids.

Balancing risks and benefits in biological control

Depending on the severity of the pest in question, the potential benefits of biological control must be weighed against the risk of the introduced enemy as well as the effects of continuing current management practices or implementing new techniques (Heimpel et al. 2004, Heimpel and Mills 2017, Heimpel and Cock 2018). In soybean, the management of soybean aphid tends to rely on broad spectrum foliar insecticides including pyrethroids and organophosphates, but these insecticides have been shown to drift into nontarget habitats and negatively affect endemic arthropod populations including the Dakota skipper (*Hesperia dacotae*) and Poweshiek skipperling (*Oarisma poweshiek*) (Runquist and Heimpel 2017, Belitz et al. 2020). While these two butterfly species were historically common in the prairies of the upper Midwest, the past century of habitat fragmentation and degradation has contributed to a steady decline (Delphey et al. 2017, Belitz et al. 2020); however, local extinctions have been occurring at an accelerated rate since 2004 in correlation with insecticide applications targeting soybean aphid (Runquist and Heimpel 2017, Belitz et al. 2020). Other soybean aphid management techniques, such as the use of plant lines containing naturally occurring resistance genes (e.g. *Rag1* or *Rag2*), have yet to be widely adopted despite extensive research (reviewed by Hesler et al. 2013).

Analysis of the population model in Chapter 1 suggested that *A. certus* may be a key natural enemy of soybean aphid under certain conditions, indirectly decreasing insecticide applications and benefitting these endangered prairie lepidopterans as well as other species of conservation interest. Contrary to the predictions of the model, *A. certus* was not found to be effective in controlling soybean aphid under the field conditions described in Chapter 3. Furthermore, the evidence presented in Chapter 4 indicates that *A. certus* is already established in (or readily colonizes) natural habitats and attacks *Aphis asclepiadis*, a specialist of milkweed (*Asclepias* spp.), and *Aphis monardae*, a specialist of beebalm (*Monarda fistulosa*). While some degree of risk to native aphid species may be acceptable in a risk-benefit analysis, there is a paucity of field-based evidence showing that *A. certus* reduces soybean aphid population densities thus, per our current understanding of the system, it would appear that the benefits of *A. certus* do not outweigh the risks.

Future research

Tritrophic models of *A. certus*, soybean aphid, and the soybean plant could provide valuable insight into the dynamics of the pest and the influence of biological control. As I discuss in Chapter 1, the *decreasing* r *model*, which postulates a linear decline in the intrinsic rate of growth of the aphid due to bottom-up effects of plant quality (Williams et al. 1999, Costamagna et al. 2007), while not a true tritrophic model, suggests that this approach may be useful in describing the dynamics of soybean aphid. Likewise, models that incorporate landscape-level factors (e.g. proximity to buckthorn, agricultural intensification) may also be useful as these variables affect soybean aphid colonization and growth (Gardiner et al. 2009, Noma et al. 2010, Hallet et al. 2014, Liere et al. 2015, Whitney et al. 2016).

Future studies should further evaluate parasitoid lifespan at additional host densities to better test the suitability of the logistic model presented in Chapter 2 and to investigate how parasitoids respond to extremely high host densities that might be detrimental to lifespan or fecundity (e.g. Líznarová and Pekár 2013). It is also important to continue developing methods such as wing wear indices in order to be able to relate the results of laboratory studies to natural systems, because ecological and statistical models parameterized primarily with laboratory data may otherwise be of limited utility in predicting, describing, or evaluating host–parasitoid interactions as they occur in nature.

While the in-field efficacy of *A. certus* as a biological control agent appears to have been limited by its low rates of parasitism, the series of experiments in Chapter 3 do not necessarily negate the potential value of this parasitoid in the soybean aphid system. Additional field-based research is warranted due to the variability of these results and those of other published works (Kaser & Heimpel 2018, LeBlanc & Brodeur 2018). Factors including colonization timeline, overwintering mortality, low host densities acting as a refuge for aphids, and hyperparasitism may all contribute to diminishing the influence of *A. certus*. Most importantly, the mechanisms driving low early season parasitism should be thoroughly investigated as it is appearing more and more likely the reason for the low efficacy of *A. certus* in multiple cases.

Even though Chapter 4 took the critical first steps in determining the prevalence of parasitism by *A. certus* for native aphid species, it will be important to continue surveys of these aphid hosts to detail the population-level effects of parasitism. It could also be useful to refine the sampling methodology to investigate whether there is movement of *A. certus* between agricultural and natural habitats or if parasitism of non-target aphid hosts is a vital component of the natural history of *A. certus* in North America. More broadly, future studies should continue to focus on incorporating indirect effects, such as the potential for *A. certus* to reduce insecticide use that in turn protects species of conservation interest, into the decision-making process for classical biological control agents.

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Appendix A

The very fine mesh that was used in the sham and total exclusion cages appears to have had a negligible effect on in-cage temperature at all sites (Figure A.1). This mesh increased thermal accumulation (calculated as the area under the time–temperature curve) by about 3–4%, which allowed for approximately one additional soybean aphid generation. Due to the small sample size of eight temperature loggers distributed across four treatments at two sites, no statistical analysis was possible. However, there was no difference in aphid population growth rates between total exclusion and sham exclusion cages (Welch two sample *t*-test; $t = -0.265$, df = 26.311, $p = 0.793$).

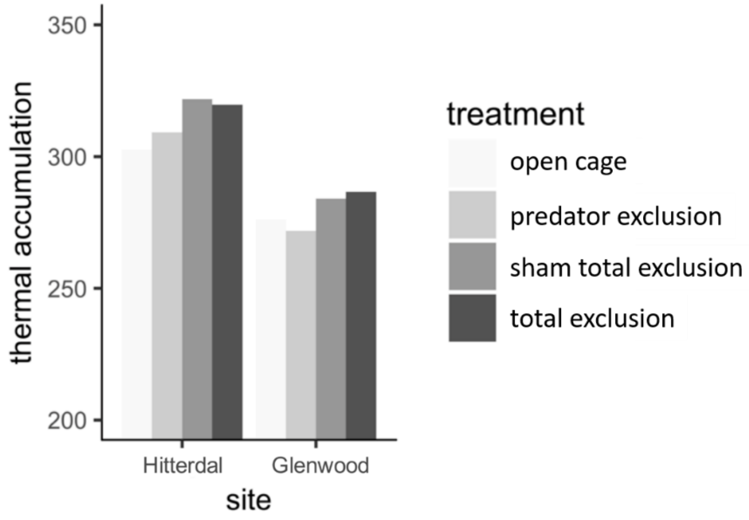

Figure A.1 Thermal accumulation (°C × days) in open, predator exclusion, total exclusion, and sham exclusion treatments

Appendix B

Replicates excluded from statistical analyses due to contamination (> 0.5% mummies in total exclusion cages) are listed in Table B.1. All open treatments were excluded from Rosemount in 2018 due to immigration of soybean aphid alatae into the field (migrating aphids did not colonize caged plants). All open treatments were excluded from Waseca in 2019 due to severe wind and hail damage to exposed plants (caged plants were not affected).

Table B.1 Replicates excluded from analysis

Year	Site	Block	Treatment
2017	Hitterdal	1	total exclusion
		2	total exclusion
		2	predator exclusion
		3	total exclusion
		6	total exclusion
	Glenwood	3	total exclusion
		4	total exclusion
		5	total exclusion
		6	total exclusion
		8	total exclusion
2018	Rosemount	2	total exclusion
		3	total exclusion
		3	predator exclusion
		5	predator exclusion
	Waseca	1	total exclusion
		3	total exclusion

Appendix C

Survey dates, location (latitude and longitude), and a description of the habitat type for each of the forty sampled sites are provided in Table C.1.

Table C.1 Site information

date (2019)	site	latitude	longitude	habitat type
17 July	St. Croix Savanna	45.007	-92.786	dry sand-gravel oak savanna
	Cylon WMA	45.181	-92.345	sedge meadow
	Carlos Avery	45.351	-93.030	oak savanna; grassland
18 July	Rice Lake Savanna	45.538	-93.915	oak savanna; sedge meadow
	Roscoe Prairie	45.417	-94.680	southern mesic prairie
	Schaefer Prairie	44.718	-94.295	northern tallgrass; mesic prairie
	Seminary Fen	44.809	-93.555	seepage meadow
	River Terrace Prairie	44.542	-92.800	dry sand-gravel prairie
21 July	Oronoco Prairie	44.139	-92.490	dry bedrock bluff prairie
	Iron Horse Prairie	43.876	-92.850	southern mesic prairie
	Pin Oak Prairie	43.795	-92.216	sedge meadow; dry barrens prairie
22 July	Walnut Lake	43.649	-93.759	prairie wetland; upland/lowland forest
	Kasota Prairie	44.268	-94.002	southern mesic prairie
	Rock Ridge Prairie	44.095	-95.061	southern mesic prairie
	Sangl WMA	43.531	-95.170	–
	Touch the Sky Prairie	43.733	-96.278	southern mesic prairie
	Prairie Coteau	44.122	-96.151	dry hill prairie
23 July	Cedar Rock	44.616	-95.178	wet prairie; wet seepage prairie
	East Canby Reserve	44.719	-96.200	–
	Chippewa Prairie	45.129	-95.986	southern mesic prairie
	Clinton Prairie	45.458	-96.564	wet prairie; southern mesic prairie
	Glacial Lakes	45.539	-95.485	dry sand-gravel prairie
	Ottertail Prairie	46.154	-96.225	northern mesic prairie
	Felton Prairie Complex	47.101	-96.410	northern wet prairie
	Malmberg Prairie	47.726	-96.824	northern mesic/wet prairie
24 July	Prairie Chicken/Ed Bry	48.124	-97.319	–
	Wallace C. Dayton	48.920	-96.547	aspen parkland
	Santee Prairie	47.393	-95.912	northern mesic/brush prairie
	Dower Lake	46.355	-94.857	lowland grassland
	Ripley Esker	46.116	-94.313	oak savanna
27 July	River Bend Prairie	44.277	-93.242	–
	Lake Louise	43.536	-92.509	southern mesic/wet prairie
	Mound Prairie	43.764	-91.429	southern dry bedrock bluff prairie
29 July	Vermillion Highlands	44.703	-93.055	oak savanna
31 July	Rice Lake	46.537	-93.317	grassland
	Sax-Zim Bog	47.197	-92.606	–

	Hartley Park	46.837	-92.085	sedge meadow
	Crex Sand Prairie	45.878	-92.636	sand prairie
8 August	Lake Rebecca	45.058	-93.762	sedge meadow
	Olmsted Rec Area	44.046	-92.464	–