



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

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## ABSTRACT

Damaging outbreaks of soybean aphid continue to occur in North America despite the valuable biological control services provided by resident natural enemies. The adventive 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 to 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 population growth 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 effects of individual natural enemy species, especially those with complex life cycles.

## 1. 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<sup>-1</sup> in 2006 and \$25 ha<sup>-1</sup> 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<sub>2</sub>-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 woolly apple aphids (Gontijo et al., 2015). Since the previous studies of the resident enemies of soybean aphid were conducted, a novel enemy has established and spread throughout the soybean growing region: *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae). *A. certus* is a solitary koinobiont endoparasitoid native to Asia that attacks a broad range of aphid species in laboratory studies 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*, adult *A. certus* obtain nutrients 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, Miksanek and Heimpel [in revision]). *A. certus* is believed to have

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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 our 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 Kaser and Heimpel (2018). 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.

## 2. Methods

This study was performed at two privately owned conventional farms and at two university-owned research plots from 2017 to 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. 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 × 38 × 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<sup>2</sup> holes) around the wire frame; and (3) a *total exclusion* cage consisting of a very fine mesh (about 0.1 mm<sup>2</sup> 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 mm<sup>2</sup> holes) with an approximately 20-cm cut through the mesh on two of the four sides of the cage facilitating the movement of insects into and out of the cage, served to control for changes in microclimate in the total exclusion cage. Sham 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 m apart within the interior of 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 using a fine brush. 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 h 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 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 (JRM, 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), 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* in order to minimize overestimation of enemy impacts due to restricting aphid emigration from exclusion cages (Frewin et al., 2010; Kaser and Heimpel, 2018). The total numbers of aphids 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,  $\lambda$  was related to the intrinsic rate of growth  $r$  using the approximation  $\lambda = e^r$ ;  $r$  (which has units of days<sup>-1</sup>) was divided by the experiment length (in days) and then converted back to  $\lambda$  for compatible comparisons of daily aphid population growth across site-time (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 by means of a least significant differences (LSD) test with false discovery rate (FDR)-corrected  $p$ -values. 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.

### 3. Results

Median field densities at the start of the experiments ranged from 6 to 302 soybean aphids per plant (Table 1). At the end of the experiments, the mean parasitism in the predator exclusion and open treatments ranged from 0.59 to 1.71% and hyperparasitism ranged from 0.0 to 31.0% of *Aphelinus* mummies in individual cages (Table 2, Fig. 1). *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae: Aphidiinae), a native generalist parasitoid that also attacks soybean aphid, was only recovered at Hitterdal, and 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 ( $n = 104$ ,  $F_{2, 103} = 0.329$ ,  $p = 0.721$ ), but did vary among site-year ( $F_{4, 103} = 105.7$ ,  $p < 0.001$ ) and block ( $F_{25, 103} = 2.74$ ,  $p < 0.001$ ) (Fig. 2); the overall model had an 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 significantly lower in open cages compared to either exclusion cage treatment in Waseca during 2018 (LSD test with FDR-corrected  $p$ -values; Fig. 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 ( $n = 69$ ,  $F_{1, 68} = 0.236$ ,  $p = 0.629$ ). However, aphid population growth varied significantly across site-years ( $F_{4, 68} = 48.2$ ,  $p < 0.001$ ), 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

**Table 1**

Median number of soybean aphids (number of individuals) by block that were used to inoculate clean soybean plants with laboratory-reared aphids the start of the experiment.

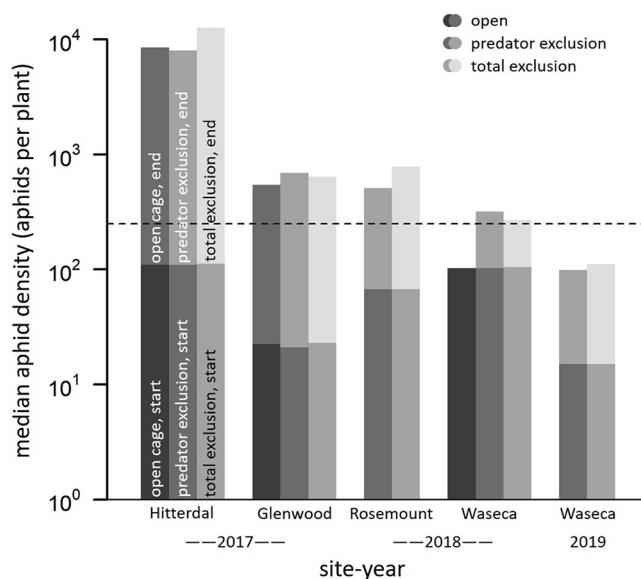
Block	2017		2018		2019
	Hitterdal (15–27 Jul)	Glenwood (24 Jul–5 Aug)	Rosemount (27 Jul–8 Aug)	Waseca (17–29 Aug)	Waseca (8–20 Aug)
1	62.5	8	68	65	6
2	142	11.5	64	115	22
3	53.5	15.5	84	100	21
4	190	34.5	73	105	9
5	157.5	24	64	–	–
6	302.5	21.5	67	–	–
7	123	20.5	–	–	–
8	145	14.5	–	–	–

**Table 2**

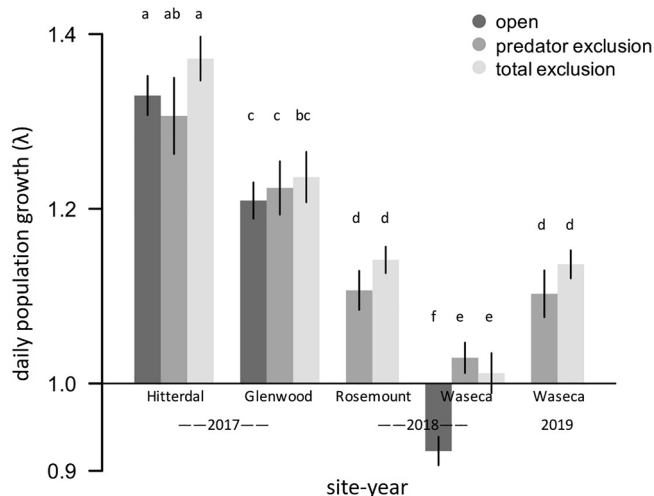
Soybean aphid parasitism and prevalence of primary and secondary parasitoid species at the end of the experiment.

Year	Site	<i>Aphelinus</i> parasitism (% mummies)*	Mummies collected (total)		<i>Aphelinus</i> emergence (% reared to adulthood)	<i>Aphelinus</i> sex ratio (% female)	Hyperparasitism (% emerged <i>Aphelinus</i> )	Hyperparasitism (total number of hyperparasitoids collected)			
			<i>Aphelinus</i>	<i>Lysiphlebus</i>				<i>Alloxysta</i> (Figitidae)	<i>Asaphes</i> (Pteromalidae)	<i>Dendrocerus</i> (Megaspilidae)	<i>Syrphophagus</i> (Encyrtidae)
2017	Hitterdal	1.71	930	127	51.0	39.1	31.0	142	4	1	0
	Glenwood	1.22	156	0	23.7	58.8	5.4	0	1	0	1
2018	Rosemount	0.59	108	0	47.2	34.7	3.9	1	0	0	1
	Waseca	1.05	3	0	33.3	100	0.0	0	0	0	0
2019	Waseca	1.20	12	0	83.3	30.0	0.0	0	0	0	0

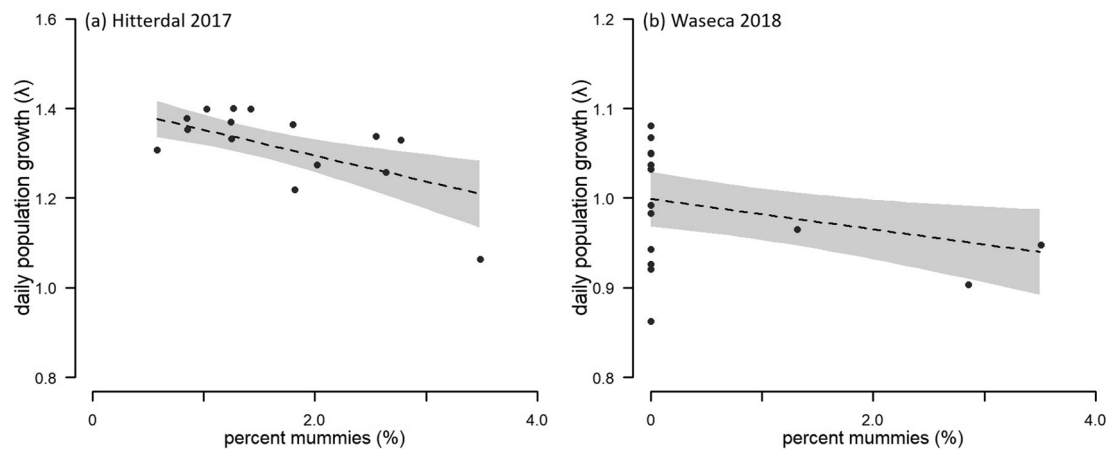
\*percent mummies = mummies ÷ (aphids + mummies) (calculated from the predator and total exclusion cages pooled).



**Fig. 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 late immigration and severe weather damage, respectively (refer to Appendix B).  $n = 104$ .



**Fig. 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 late immigration and severe weather damage, respectively (refer to Appendix B).  $n = 104$ .



**Fig. 3.** Proportional aphid population growth rate as a function of parasitism (percent mummies) in open cages and predator exclusion cages at (a) Hitterdal in 2017 ( $n = 15$ ) and (b) Waseca in 2018 ( $n = 16$ ). Site-years with nonsignificant slopes are not shown (see Table 3). Dotted lines: prediction from linear regression model with 95% CI.

**Table 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

significant negative correlation at Hitterdal in 2017 and Waseca in 2018 (Fig. 3; Table 3).

#### 4. Discussion

Although *Aphelinus certus* did not significantly affect daily soybean aphid population growth rates ( $\lambda$ ) 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), *Dendrocercus* (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 evaluate the population-level impacts of hyperparasitism as the length of the experiment only allowed for one generation of *A. certus* and 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. 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 (C. Steinoien, K. Welch and G. E. Heimpel, unpublished), and low aphid



densities reduce the amount of nutritional resources (i.e. from honeydew and host feeding) available to adult parasitoids, resulting in a decreased life expectancy (J. R. Miksanek and G. E. Heimpel, in revision). 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.

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 population densities 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.

## 5. Conclusions

The efficacy of *A. certus* as a biological control agent observed

## 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 (Fig. A.1). This mesh increased thermal accumulation (calculated as the area under the time–temperature curve) by about 3–4%, which allowed for

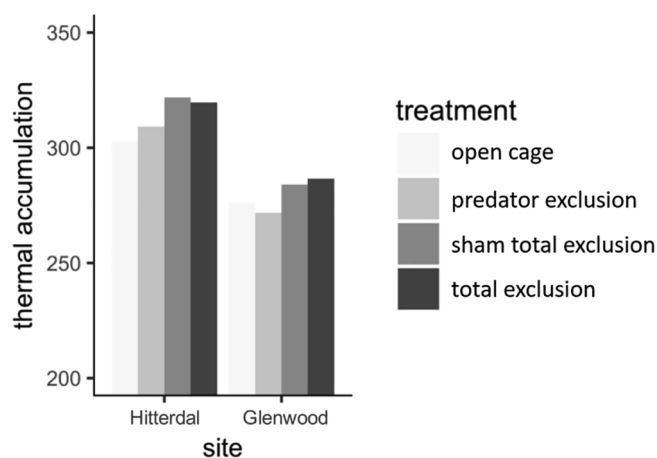


Fig. A1. Thermal accumulation appeared to be slightly greater in sham and total exclusion treatments compared to predator exclusion and open treatments.

during our experiments appears to have been limited by 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 and Heimpel, 2018; LeBlanc and 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).

## CRediT authorship contribution statement

**James Rudolph Miksanek:** Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **George E. Heimpel:** Methodology, Conceptualization, Funding acquisition, Writing - review & editing.

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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;  $t = -0.265$ ,  $df = 26.311$ ,  $p = 0.793$ ).

## 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 B1**  
Replicates excluded from analysis.

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

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