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Author(s): Andrew J. Frewin, Yingen Xue, John A. Welsman, A. Bruce Broadbent, Arthur W. Schaafsma, and Rebecca H. Hallett

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# Development and Parasitism by *Aphelinus certus* (Hymenoptera: Aphelinidae), a Parasitoid of *Aphis glycines* (Hemiptera: Aphididae)

ANDREW J. FREWIN,<sup>1</sup> YINGEN XUE,<sup>1</sup> JOHN A. WELSMAN,<sup>2</sup> A. BRUCE BROADBENT,<sup>3</sup>  
ARTHUR W. SCHAAFSMA,<sup>2</sup> AND REBECCA H. HALLETT<sup>1,4</sup>

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**ABSTRACT** Since its introduction in 2000, the soybean aphid (*Aphis glycines* Matsumura) has been a serious pest of soybean in North America. Currently, insecticide application is the only recommended control method. However, a number of natural enemies have the potential to regulate soybean aphid populations. In 2007, *Aphelinus certus* Yasnosh, a soybean aphid parasitoid native to Asia, was found in commercial soybean fields in Ontario. This is the first record of this species in North America. To evaluate the potential biological control services provided by *A. certus* for soybean aphid management, temperature-dependent developmental parameters and functional response to soybean aphid were determined. *A. certus* is capable of completing its development between temperatures of 15.3 and 30.2°C. The lower thresholds of development for the egg-mummy and mummy-adult life stages were determined to be 9.1 and 11.6°C, respectively. The lethal temperature of development for the egg-mummy and mummy-adult life stages were 29.5 and 31.0°C, respectively. In this temperature range, *A. certus* did not exhibit temperature-dependent mortality; however, parasitism rate increased with temperature. *A. certus* exhibited a type II functional response to the soybean aphid.

**KEY WORDS** temperature-dependent development, functional response, biological control

Soybean aphid (*Aphis glycines* Matsumura) is an economically important pest of soybean (*Glycine max* L.) in North America (McCornack et al. 2004). Since its arrival in 2000, the soybean aphid has spread throughout the entire soybean growing region (DiFonzo 2009). Outbreaks of soybean aphid can dramatically reduce yields and seed quality (Diaz-Montano et al. 2007, Beckendorf et al. 2008). Currently, growers rely exclusively on insecticides for soybean aphid management (Baute 2007, NCSRP 2009). However, insecticide use can have negative effects on nontarget organisms, farm workers, and the environment and can result in the development of resistance within the target pest population (Devonshire and Moores 1982).

A potential cost-effective tool for integrated pest management (IPM) of soybean aphid is biological control by natural enemies. In its native range, soybean aphid populations are regulated by a natural enemy guild consisting of various predators and parasitoids (Miao et al. 2007). In the summer of 2006, a large number of soybean aphids parasitized by an unidentified *Aphelinus* spp. were found in Chatham-Kent, Ontario (Welsman 2007). Evaluation of life his-

tory traits of this parasitoid, such as its functional response, and temperature-dependent developmental rate, are important to predict its potential role in the management of soybean aphid.

Knowledge of the temperature-dependent development rate of an insect allows the estimation of seasonal occurrence, developmental time, and the number of generations per year (Bernal and Gonzalez 1993a). This information can be used in an IPM program to make informed management decisions to minimize contact between the natural enemy and pesticides and to reduce pesticide use when economically significant natural enemy populations are present.

The functional response describes the relationship between the number of prey consumed per predator and prey density (Solomon 1949). This is a key element to assess the impact of a natural enemy on a pest population (Hassell 1978, Houck and Strauss 1985, Tully et al. 2005). Detailed functional response data allows the incorporation of natural enemies into dynamic action thresholds for the soybean aphid (Zhang and Swinton 2009).

The objectives of this study were to determine the identity and range of the identified *Aphelinus* spp. in Ontario, Canada, to determine parameters related to the parasitoid's temperature-related developmental rate and its functional response to soybean aphid, and to determine its potential as a naturally occurring biological control agent for soybean aphid.

<sup>1</sup> School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

<sup>2</sup> Department of Plant Agriculture, Ridgetown Campus, University of Guelph, Ridgetown, Ontario, Canada N0P 2C0.

<sup>3</sup> Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario, Canada N5V 4T3.

<sup>4</sup> Corresponding author, e-mail: rhallett@uoguelph.ca.

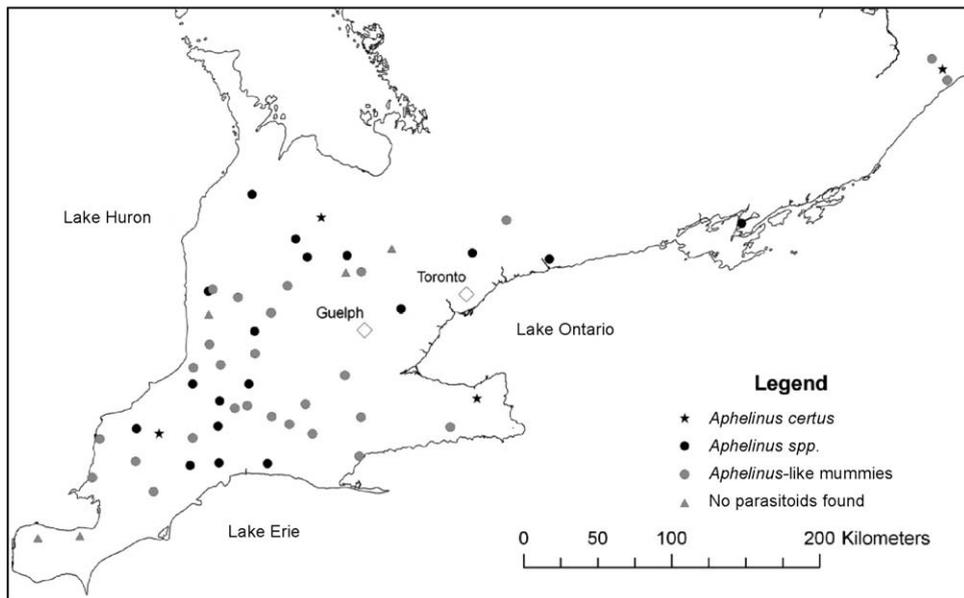


Fig. 1. Location of 54 commercial soybean fields surveyed for soybean aphid parasitoids during July–August 2007. *A. glycines* were found at all sites.

### Materials and Methods

**Survey.** In July and August 2007, a survey of soybean aphid parasitoids was conducted in Ontario, Canada. Soybeans infested with soybean aphid were collected from 54 locations across the province (Fig. 1). At least 10 soybean plants were sampled at each location, and the presence of black *Aphelinus*-like aphid mummies was recorded. Soybean trifoliates with aphids and aphid mummies from 22 sites were held in growth chambers maintained at  $25 \pm 1^\circ\text{C}$ , 16:8-h light:dark photoperiod, and 70% RH for 2 wk. All wasps emerging from black *Aphelinus*-like aphid mummies were collected. Individuals from four sampling locations were subsequently identified to species by Dr. Keith R. Hopper, USDA Beneficial Insect Introduction Research Unit, Newark, DE, using morphological characters.

**Rearing Methods.** Parasitoids emerging from the field-collected soybean aphids were used to establish a single laboratory colony. The colony was provided with soybean plants infested with soybean aphids once weekly and maintained at  $24 \pm 2^\circ\text{C}$ , 65–75% RH, and a 16:8-h light:dark photoperiod. Individuals from the laboratory colony were also sent to Dr. Hopper for species identification. This parasitoid was later identified as *Aphelinus certus* (see below) and is referred to as such hereafter.

**Developmental Rate.** All experiments were conducted in controlled environment chambers held at mean temperatures of 15.3, 18.3, 20.6, 25.3, 26.6, or  $30.2 \pm 0.3^\circ\text{C}$ , 70% RH, and 16:8-h light:dark photoperiod. Temperature was monitored using a StowAway XTI (Onset Computer, Pocasset, MA) temperature logger and a Fisher Traceable Digital Thermometer (Fisher, Suwanee, GA). Soybean leaves were col-

lected from 3- to 5-wk-old plants not previously exposed to soybean aphid. Each leaf was placed adaxial side down on damp cotton batting in an arena consisting of a 10-cm petri dish bottom. Twenty-five third- or fourth-instar soybean aphids were transferred onto each leaf. The aphids were allowed to settle for 4 h, after which one mated naïve female *A. certus* between 24 and 48 h old was introduced into the dish. Petri dishes were sealed with Parafilm and assigned randomly to a controlled environment chamber.

To obtain mated female *A. certus*, aphid mummies were collected from colonies and placed individually in 0.5-ml Eppendorf tubes and supplied with a droplet of honey. Once emerged, all parasitoids were sorted by sex. Females were mated before experimentation by introducing a male into their Eppendorf tube and visually observing them until mating had occurred.

The experiment had a randomized complete block design (RCBD), where blocks were replicates, with replicates separated by 2 d. Ten replications of the experiment were conducted at all temperatures with the exception of five replicates at  $30.2^\circ\text{C}$ ; the same environment chamber was used for each temperature replication. Female parasitoids were removed 24 h after they were introduced to the arenas; no mortality occurred over this period. Aphid mummies were removed daily from the leaves, placed in a 0.5-ml Eppendorf tube, and returned to their respective controlled environment chambers. The total number of mummies per dish (mummification rate, used as an estimate for parasitism rate), days to mummification (an estimate of parasitoid pupation), days to emergence, and sex of adult wasps were assessed.

Statistical analyses of developmental data were conducted using SAS v 9.1 (SAS Institute, Cary, NC). To

ensure data conformed to the assumptions of analysis of variance (ANOVA) and regression, the residuals were examined for homoscedasticity, independence, and randomness using plots of residuals by predicted values and by model effects. Normality was tested using the Shapiro-Wilk test. Lund's test of studentized residuals was performed to identify outliers in the data (Bowley 1999). All tests were conducted at a significance level of 0.05.

Developmental rate data were subjected to ANOVA using the MIXED procedure. Variance was partitioned into the random effect block, and the fixed effects temperature, sex of wasp, and the temperature by sex interaction. Linear and lack-of-fit regression partitions were also included for temperature and the temperature by sex interaction where significant. The lack-of-fit partition was included to confirm the existence of a higher-order relationship between development and temperature within the temperature range tested. Developmental rate data were fit to a nonlinear temperature dependent growth model (equation 1),

$$R(T) = \begin{cases} 0 & \text{for } T \leq T_0 \\ \alpha T(T - T_0) \sqrt{T_L - T} & \text{for } T_0 \leq T \leq T_L \\ 0 & \text{for } T \geq T_L \end{cases} \quad [1]$$

where developmental rate ( $R$ ) is a function of temperature ( $T$ ) (Briere et al. 1999). Developmental rate ( $R$ ) was calculated as the reciprocal of development time.  $T_L$  is the lethal temperature or upper threshold of development,  $T_0$  is the lower threshold of development, and  $\alpha$  is a constant (Briere et al. 1999). Parameters were estimated by nonlinear iterative regression using the Marquardt method (SAS Institute 2004), with a convergence criteria of 0.00001. Briere's model was chosen for this study because it provides estimates of upper and lower thresholds of development with the fewest terms in the equation (Roy et al. 2003). Optimal growth rate,  $T_{opt}$ , was calculated using equation 2 (Briere et al. 1999):

$$T_{opt} = \frac{4T_L + 3T_0 + \sqrt{16T_L^2 + 9T_0^2 - 16T_0T_L}}{10} \quad [2]$$

Following the method described by Campbell et al. (1974), the linear component of the developmental data, obtained with removal of the two highest temperatures, was subjected to ANOVA using the GLM procedure. Variance was partitioned into the fixed effect temperature and the linear, quadratic, and lack-of-fit regression partitions. This regression was used to estimate the degree-day ( $^{\circ}D$ ) requirements for each life stage and an additional estimate of the lower threshold of development ( $LT_0$ ) (Campbell et al. 1974). The linear estimate of  $LT_0$  is defined as the  $x$ -intercept of the regression of developmental rate on temperature, and  $^{\circ}D$  is the reciprocal of the slope.

Developmental rate data were subjected to ANOVA using the MIXED procedure. Variance was partitioned into the random effect block, and the fixed effects temperature, sex of wasp, and the temperature by sex interaction.

The effect of temperature on pupal mortality, parasitism rate, and  $F_1$  sex ratio was examined by ANOVA using the MIXED procedure. Variance was partitioned into the random effect block and the fixed effect temperature. Linear, quadratic, and lack-of-fit regression partitions were also included for temperature. Oviposition rate was calculated as proportion of parasitized aphids per petri dish. Parasitism rate data were subjected to an arcsine square-root transformation to better meet the assumptions of ANOVA.

**Functional Response.** Soybean plants at the V1 stage with two fully expanded unifoliate leaves were cut above the cotyledon. Stems of these plants were inserted through the lids of bottles containing 20 ml of water. Third-instar soybean aphids were transferred to the plants at densities of 4, 8, 16, 32, 64, and 96 per plant. The individual plants were placed in clear plastic containers (10 cm diameter by 8 cm height; Shortreed Paper, Guelph, Ontario, Canada). Mesh covered holes provided ventilation. A single mated naïve female (as described above) of *A. certus* between 24 and 48 h old was introduced into each container. Containers were placed in a controlled environment chamber at 26°C with a 16:8-h light:dark photoperiod. The temperature was monitored as above. After 24 h, the wasp was removed (no mortality of females occurred over this period), and after 7 d, the number of mummies was counted. An RCBD was used, blocked by replicate, with replicates separated by at least 1 d. Each aphid density was replicated 12 times.

**Parasitism of *A. certus* on Soybean Plants.** Soybean plants at the V3 stage with three unfolded leaflets were used to examine parasitism at a density of 250 aphids per plant, which is the economic threshold (Ragsdale et al. 2007). To get 250 mixed stage soybean aphids per plant, infested soybean leaves with ~400 aphids were transferred to the V3 soybean plant. After 24 h, excess aphids were removed or additional aphids introduced using a paint brush to obtain the desired density. An RCBD was used, blocked by replicate, with 14 replicates, each separated by at least 1 d. Individual plants were held within a 9 by 30-cm plastic cage with a mesh top to provide ventilation. A single mated naïve female (as described above) of *A. certus* between 24 and 48 h old was introduced into each cage, and cages were held in a controlled environment chamber, as above. After 24 h, the wasp was removed (no mortality occurred over this period), and after 7 d the number of mummies was counted.

Statistical analyses of functional response and parasitism data were conducted using SPSS software (SPSS 2006). Functional responses were examined using two-stage analysis (Juliano 2001). A cubic logistic regression (equation 3) between proportion of aphids parasitized and initial host density was first performed to determine the shape of the functional response:

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)} \quad [3]$$

where  $N_a$  is the number of hosts parasitized,  $N_0$  is the initial host density, and  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the intercept, linear, quadratic, and cubic coefficients, respectively (Juliano 2001). If the linear coefficient,  $P_1$ , is significantly negative, the parasitoid is displaying a type II functional response; if positive, there is a type III functional response (Juliano 2001). If a cubic equation yields a nonsignificant cubic coefficient, it is desirable to reduce the model by eliminating the cubic term from equation 4 and to retest the other parameters (Juliano 2001).

Because the logistic regression analysis indicated that our data fit type II (see Results), further analyses were restricted to the type II functional response. Holling's disc equation (equation 4) (Holling 1959):

$$N_a = \frac{aN_0}{1 + aT_hN_0} \quad [4]$$

and the random predator equation (equation 5) (Royama 1971, Rogers 1972):

$$N_a = N_0[1 - \exp(aT_hN_a - a)] \quad [5]$$

were used to model the relationship between the number of host parasitized ( $N_a$ ) and initial host density ( $N_0$ ), where  $a$  is the instantaneous searching rate and  $T_h$  is the handling time per host item. A nonlinear regression procedure based on the Levenberg-Marquardt method (SPSS 2006) was performed to estimate the parameters  $a$  and  $T_h$ . The starting values of  $a$  and  $T_h$  required by the nonlinear regression procedure were found by a linear regression of  $1/N_a$  against  $1/N_0$ . The resultant  $y$ -intercept is the initial estimate of  $T_h$  and the reciprocal of the regression coefficient (slope) is an estimate of  $a$  (Livdahl and Stiven 1983, Watson et al. 2000). These initial estimates were refined by nonlinear regression. Data did not fit the model if the asymptotic 95% confidence intervals of  $a$  and  $T_h$  estimates included zero (i.e., the estimates did not differ significantly from zero) (Watson et al. 2000). If the data fit both models, the best model was selected based on the value of the coefficient of determination ( $R^2$ ).

**Results**

**Survey.** Soybean aphids were present at all sites surveyed, and black *Aphelinus*-like aphid mummies were observed at 49 of 54 sites (Fig. 1). At 22 of these sites, wasps reared from aphids were identified as belonging to the genus *Aphelinus* Dalman (Hymenoptera: Aphelinidae). Individuals from four sites and the laboratory colony were identified as *A. certus* Yasnosh 1963 (K. Hopper, personal communication).

**Development Rate.** *Aphelinus certus* completed development at all temperatures tested. The development rate increased over the entire temperature range tested for both life stages (Fig. 2; Table 1). Both temperature and sex had a significant effect on development rate for the egg-mummy and mummy-adult life stages (Table 2). Males developed more slowly than females during both life stages at the three highest temperatures (Table 1). However, a significant tem-

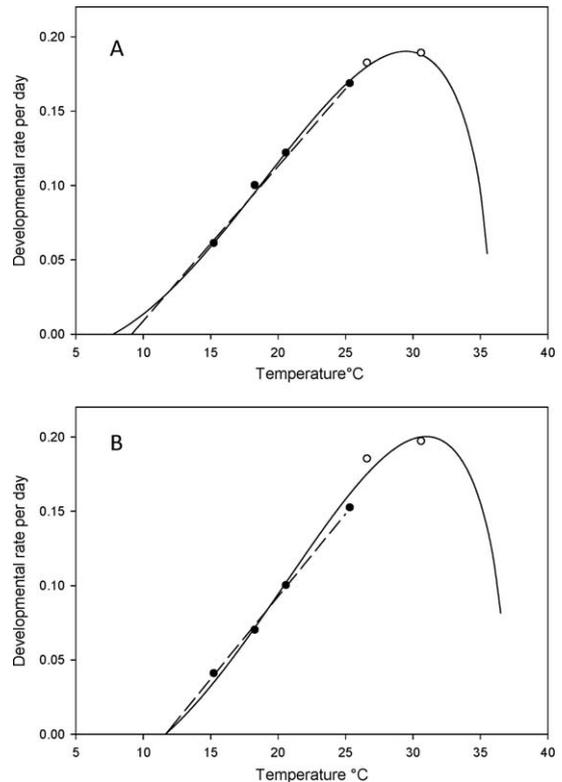


Fig. 2. Mean temperature-dependent development rate of *A. certus* from (A) egg to mummy and (B) mummy to adult. Physiological growth curve, solid line; linear regression, dotted line. Only solid circles were used in linear regression.

perature by sex interaction was only found for the mummy-adult life stage (Table 2). The linear and lack-of-fit regression partitions for the effect of temperature on egg-mummy and mummy-adult development rate were significant. The linear and lack-of-fit regression partitions for the temperature by sex interaction were omitted for the egg-mummy life stage because the term was not significant. The linear regression partition of the temperature by sex interaction for the mummy-adult development rate was significant (Table 2).

**Nonlinear Growth Model.** The nonlinear growth model provided an excellent fit for both the egg-pupae and pupae-adult developmental data sets (see  $r^2$ ; Table 3). Optimal growth rates for egg-mummy and mummy-adult life stages were achieved at 29.5 and 31.0°C, respectively (Fig. 2).  $T_0$  was higher for the mummy-adult life stage than for the egg-mummy, but  $T_L$  was similar for both life stages (Table 3).

**Linear Growth Model.** The linear, quadratic, and lack-of-fit regression partitions for growth rate were significant. Therefore, to obtain the linear component of the developmental curve (Fig. 2), developmental data collected at 26.6 and 30.2°C were removed from the analysis, after which only the linear regression partition remained significant. The regression pro-

**Table 1.** Mean ± SE development times and rates for egg to mummy and mummy to adult lifestages of the parasitoid *A. certus* developing on *A. glycines* at six constant temperatures

Temperature (°C)	Pooled (males + females) <sup>a</sup>			Females <sup>b</sup>			Males <sup>b</sup>		
	N	d	Rate (d <sup>-1</sup> )	N	d	Rate (d <sup>-1</sup> )	N	d	Rate (d <sup>-1</sup> )
<b>Egg-mummy</b>									
15.3	57	16.4	0.061 ± 0.0030E	32	16.1	0.062 ± 0.0039a	25	16.7	0.060 ± 0.0044a
18.3	30	10	0.100 ± 0.0044D	21	9.7	0.103 ± 0.0049a	9	10.4	0.096 ± 0.0074a
20.6	89	8.3	0.121 ± 0.0024C	46	8.1	0.124 ± 0.0033a	41	8.4	0.119 ± 0.0035a
25.4	154	6	0.168 ± 0.0018B	81	5.6	0.178 ± 0.0025a	70	6.3	0.159 ± 0.0027b
26.6	174	5.5	0.182 ± 0.0018A	109	5.3	0.189 ± 0.0021a	63	5.7	0.175 ± 0.0028b
30.6	40	5.3	0.188 ± 0.0036A	16	5	0.200 ± 0.0056a	24	5.7	0.176 ± 0.0045b
<b>Mummy-adult</b>									
15.3	72	24.4	0.041 ± 0.0021F	32	24.4	0.041 ± 0.0028a	25	24.4	0.041 ± 0.0031a
18.3	40	14.3	0.070 ± 0.0032E	21	14.1	0.071 ± 0.0035a	9	14.5	0.069 ± 0.0052a
20.6	98	10	0.100 ± 0.0017D	46	10	0.100 ± 0.0023a	41	10	0.100 ± 0.0025a
25.4	178	6.6	0.152 ± 0.0013C	81	6.4	0.157 ± 0.0018a	70	6.8	0.147 ± 0.0019b
26.6	203	5.4	0.185 ± 0.0013B	109	5.2	0.191 ± 0.0015a	63	5.6	0.179 ± 0.0020b
30.6	50	5.1	0.196 ± 0.0026A	16	4.9	0.204 ± 0.0039a	24	5.3	0.187 ± 0.0033b

<sup>a</sup> Means within the pooled column for a given life stage followed by different capital letters are significantly different ( $P < 0.05$ ) according to Tukey's procedure.

<sup>b</sup> Means within a row for a given life stage followed by different lowercase letters are significantly different ( $P < 0.05$ ) according to Tukey's procedure.

vided  $LT_0$  and  $^{\circ}D$  estimates of  $9.1 \pm 0.26$ ,  $96 \pm 2.2$  and  $11.6 \pm 0.14$ ,  $90 \pm 1.4$  for egg-mummy and mummy-adult, respectively.

**Mortality, Sex Ratio, and Parasitism.** Temperature had no effect on pupal mortality (mean 12%;  $F = 1.59$ ,  $df = 5,39$ ,  $P > 0.18$ ), or  $F_1$  sex ratio (1:0.75 female:male) ( $F = 1.74$ ,  $df = 5,508$ ,  $P > 0.12$ ). Temperature had a significant effect on parasitism rate, with parasitism increasing with temperature (Table 4; Fig. 3). The linear regression partition for the effect of temperature on parasitism rate was significant (Fig. 3).

**Functional Response.** Cubic response parameters were not significant, so the model was reduced to a

quadratic equation and retested. The linear coefficient,  $P_L$ , in the reduced logistic model was  $-0.0668 \pm 0.0106$  ( $\pm SE$ ,  $P = 0.008$ ), which indicates that *A. certus* had a type II functional response to soybean aphid. The asymptotic 95% confidence intervals of the estimates of  $a$  and  $T_h$  from nonlinear regression indicate that the data fit the Holling's disc equation but not the random predator equation (Table 5).

A type II functional response of *A. certus* to soybean aphid is shown in Fig. 4. The model predicts a theoretical maximum parasitism of 22 aphids per female adult per day, which occurs when the host densities exceed 2,000 aphids per arena. However, if the limitation of the leaves to support aphids is taken into account, the maximum parasitism rate should be  $\approx 20$  aphids per female adult per day at a density of 200 aphids per arena (i.e., maximum capacity of two unifoliate leaves).

**Parasitism of *A. certus* on Soybean Plants.** The average parasitism of soybean aphid by *A. certus* was  $11.83 \pm 0.63$  on V3 plants at a density of 250 aphids per plant, which is less than both the theoretical maximum parasitism rate and the adjusted maximum rate based on limitations of the arena.

**Discussion**

This study provides the first record of *A. certus* in Ontario. The presence of *A. certus* was confirmed at four sites on the periphery of the sampling range. However, because of the presence of unidentified

**Table 2.** Variance analysis of the effect of temperature and sex on the development rate of *A. certus*, from egg to mummy and from mummy to adult, developing within *A. glycines* at six constant temperatures

	Estimate	SE	Z value	Pr > Z
<b>Egg-mummy</b>				
Block	0			
Residual	0.000496	0.000031	16.2	<0.0001
<b>Effect</b>				
	Num df	Den df	F value	Pr > F
Sex	1	502	24.18	<0.0001
Temperature	5	502	342.74	<0.0001
Linear	(1)	502	1701.23	<0.0001
Lack of fit	(4)	502	19.84	<0.0001
Temperature × sex	5	504	2.06	0.069
<b>Mummy-adult</b>				
Block	1.44E-06	3.98E-06	0.36	0.3586
Residual	0.000243	0.000015	15.88	<0.0001
<b>Effect</b>				
	Num df	Den df	F value	Pr > F
Sex	1	502	16.64	<0.0001
Temperature	5	502	1024.55	<0.0001
Linear	(1)	502	5143.94	<0.0001
Lack of fit	(4)	502	53.86	<0.0001
Temperature × sex	5	502	3.37	0.0053
Linear	(1)	502	35.84	<0.0001
Lack of fit	(4)	502	0.48	0.7489

**Table 3.** Parameter estimates from nonlinear iterative regression of developmental data for *A. certus* developing within *A. glycines* at six constant temperatures

Life stage	$a$ ( $\times 10^{-4}$ )	$T_0$	$T_L$	$r^2$
Egg-mummy	$1.19 \pm 0.063$	$7.8 \pm 0.59$	$35.7 \pm 0.48$	0.99
Mummy-adult	$1.37 \pm 0.060$	$11.6 \pm 0.31$	$36.9 \pm 0.53$	0.99

$a$ , constant;  $T_0$ , lower threshold of development;  $T_L$ , lethal temperature or upper threshold of development.

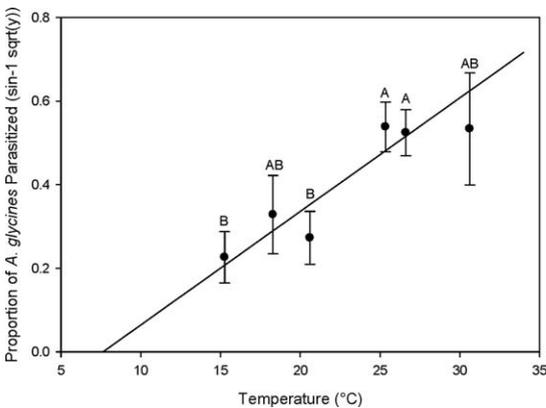
**Table 4.** Variance analysis of the effect of temperature on parasitism rate of *A. certus*, i.e., the proportion of available *A. glycines* parasitized by *A. certus* in a 24-h period, at six constant temperatures

Cov. Param.	Estimate	SE	Z value	Pr > Z
Block	0.02623	0.01568	1.67	0.0473
Residual	0.05513	0.01218	4.53	<0.0001
Effect	Num df	Den df	F value	Pr > F
Temperature	5	43	5.63	0.0004
Linear	(1)	43	24.76	<0.0001
Quadratic	(1)	43	0.00	0.9738
Lack of fit	(3)	43	1.14	0.343

*Aphelinus* spp. and black *Aphelinus*-like aphid mummies in other samples, it is reasonable to assume *A. certus* occurs throughout the sampling range. Further work will be needed to confirm the range and distribution of *A. certus* in North America.

*Aphelinus certus* is native to Asia, first collected and described from unidentified aphids in the former USSR (Yasnosh 1963). More recently it has been found parasitizing soybean aphid in China, Korea, and Japan (Heraty et al. 2007). It is unclear when *A. certus* was introduced into North America, but it was likely present in Ontario before 2006 when an unidentified *Aphelinus* spp. was found parasitizing soybean aphid in Chatham-Kent (Welsman 2007).

Because of the sudden appearance and extent of its range, it is likely that *A. certus* is capable of long range dispersal, either as an adult or as a larva within an alate host. Considering that *Aphelinus* spp. are poor fliers (Viggiani 1984, DeFarias and Hopper 1997), it is unlikely that they would travel long distances as adults. Some aphid parasitoids have the capacity to be transported long distances as an egg or larva within an alate host during aphid dispersal events (Feng et al. 2007, Huang et al. 2008). Considering soybean aphids are strong fliers (Zhang et al. 2008), it is possible that *A. certus* was distributed in this way. To confirm this suggestion, the parasitism status of migratory aphids should be assessed.



**Fig. 3.** Mean proportion ( $\pm$ SE) of 25 *A. glycines* parasitized by *A. certus* at six constant temperatures and linear regression of the proportion of parasitized *A. glycines* on temperature (solid line).

The development pattern of *A. certus* is also similar to other *Aphelinus* spp. Typically an increase in development rate is seen up to temperatures of  $\approx 30^\circ\text{C}$  (Asante and Danthanarayana 1992, Bernal and Gonzalez 1993b, Bernal and Gonzalez 1996, Prinsloo and du Plessis 2000, Rohne 2002). The lower threshold of development is also consistent with other *Aphelinus* spp., because a range of 7–10°C is often reported (Asante and Danthanarayana 1992, Bernal and Gonzalez 1993b, Bernal and Gonzalez 1996, Prinsloo and du Plessis 2000). The high lower threshold of development and the ability to develop successfully at temperatures of  $\approx 30^\circ\text{C}$  indicate that *Aphelinus* spp. are tolerant of high temperatures. This is in contrast to Aphidiinae parasitoids that typically show signs of physiological stress at temperatures  $>26^\circ\text{C}$ . For example, *Aphidius matricariae* was not able to complete development at 29.4°C and *Diaeretiella rapae* showed a reduction in developmental rate at 29.4°C compared with 25°C (Bernal and Gonzalez 1993b). However, in the same experiment, two *Aphelinus* species increased development rate over the entire temperature range tested (Bernal and Gonzalez 1993b). The lower threshold of development of Aphidiinae aphid parasitoids is often much lower than that of *Aphelinus* spp. and values  $<7^\circ\text{C}$  are often reported (Bernal and Gonzalez 1993b, Sigsgaard 2000).

Sex also had a significant effect on development rate. In general, male Aphelinidae develop faster than females (Viggiani 1984), but the opposite is true for *A. certus*. In this experiment, female *A. certus* developed more quickly than males during both life stages at all temperatures tested. However, the difference was only significant at the three highest temperatures. This is consistent with other work on *Aphelinus albipodus* and *A. spiraeocolae* (Tang and Yokomi 1995, Bernal and Gonzalez 1996). In this study, host aphid instar was not fixed. Therefore, it is possible that the differences in development time were a result of differences in host quality and parasitoid behavior. Parasitoids, including *Aphelinus* spp., often deposit female eggs into larger hosts (Honek et al. 1998). Generally, larger hosts support faster development in parasitoids (Rohne 2002). In this study, female eggs may have been preferentially deposited into the larger fourth-instar aphids and third-instar aphids used for males, resulting in faster development of females. Additionally, the rate of development for females increased with temperature at a greater rate than that of males, which could be caused by underlying physiological differences between the sexes or an artifact of host size.

Consistent with previous work, temperature had no effect on pupal mortality (Bernal and Gonzalez 1996). However, Aphidiinae aphid parasitoids (Bernal and Gonzalez 1993b, Sigsgaard 2000) and other endoparasitoids (Krugner et al. 2007) often exhibit temperature-dependent larval and pupal mortality. As temperatures approach  $T_L$ , pupal mortality would be expected to increase; however, the temperatures tested here were well within the tolerable range for *A. certus*. The effect of exposure to low temperature is less clear. *Aphelinus* spp. in temperate climates exhibit

**Table 5.** Estimates of the instantaneous searching rate ( $a$ ) and handling time ( $T_h$ ) of *A. certus* parasitizing *A. glycines* at  $26 \pm 1^\circ\text{C}$  in a 24-h prey exposure period, with asymptotic 95% confidence intervals using the Holling's disc and random predator models

Equation	$r^2$	$a$ (95% CI)	$T_h$ (95% CI)
Holling's disc	0.6503	0.9789 (0.6277–1.3301)	0.0448 (0.0366–0.0529)
Random predator	0.3803	0.1077 (0.0021–0.2133)	–0.0865 (–0.2259 to 0.0529)

various diapause regimens (Bernal et al. 2001, Tatsumi and Takada 2006), and it is likely that *A. certus* is capable of entering diapause at similar temperatures.

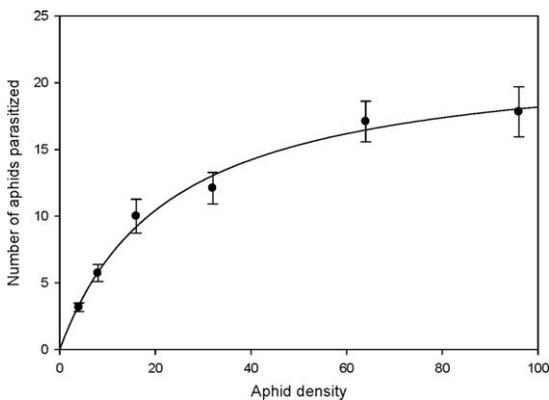
The lower threshold of development of the soybean aphid is  $8.6^\circ\text{C}$  (McCornack et al. 2004),  $3^\circ\text{C}$  lower than that of *A. certus* pupae. Thus, the soybean aphid will be able to complete development earlier in the season than *A. certus*. However, throughout Ontario, high populations of soybean aphid normally do not occur until July, at which point average temperatures are well above  $11.6^\circ\text{C}$ , and thus, differences in development parameters may not affect the biological control potential of *A. certus*. It is not yet known how *A. certus* overwinter. Other *Aphelinus* spp. overwinter as last-instar larvae (Bernal et al. 2001, Tatsumi and Takada 2005), within mummies (Prinsloo and du Plessis 2000), or as free living adults (Hamilton 1973). Overwintering strategy affects the ability of the parasitoid to develop early in the season. More work is needed to resolve how *A. certus* overwinters and evaluate its potential as an early season biological control agent of soybean aphid.

The optimal temperatures of development for the egg-mummy and mummy-adult life stages of *A. certus* are both greater than the optimal temperature for development of the soybean aphid (i.e.,  $27.8^\circ\text{C}$ ) (McCornack et al. 2004), suggesting that *A. certus* is more tolerant than soybean aphid of high temperatures. The population doubling time, intrinsic rate of increase, and gross fecundity of the soybean aphid have been shown to be lower at  $30^\circ\text{C}$  than at  $25^\circ\text{C}$  (McCornack et al. 2004). Considering that *A. certus* does not experience temperature-dependent mortality and parasitism rate increased with temperature, biological control of soybean aphid by *A. certus* may be enhanced by warm weather. However, it should be noted that pre-

pupal mortality was not measured in this experiment, and it is possible that mortality during this life stage could vary in relation to temperature. Furthermore, mummification rate was used as a proxy for parasitism rate; therefore, it is possible that the increase in parasitism rate across the temperature gradient is simply a reflection of increased survival of parasitoid larvae (pre-mummy) within the aphid host rather than an increase in egg deposition.

*Aphelinus certus* displayed a type II functional response to soybean aphid. The type II functional response indicates that the number of prey parasitized increases with prey density, whereas the rate of parasitism (estimated by mummification rate) decreases with prey density. The random predator equation has been considered more appropriate than Holling's disc equation in studies where prey are not replaced and trial duration is relatively long, which leads to a significant reduction in prey over time (Houck and Strauss 1985). The random predator equation assumes a random search pattern, whereas the disc equation assumes a systematic search pattern. Therefore, our results indicate that searching behavior of *A. certus* is not random but systematic. Similarly, the type II functional response for *Aphidius colemani* and *A. matricariae* to the cotton aphid is best described using Holling's disc equation rather than the random predator equation (Zamani et al. 2006).

The maximum number of soybean aphids that *A. certus* can parasitize in 24 h is lower than other aphid parasitoids commonly used as biological control agents. For example, *D. rapae* can theoretically parasitize a maximum of 23.5–59.5 aphids per day (Bernal et al. 1994, Fathipour et al. 2006), whereas *Binodoxys communis* can parasitize 15.7 aphids in 4 h (Wyckhuys et al. 2008). However, the functional response and related values (i.e., theoretical maximum parasitism) can change over time (Bernal et al. 1994), and thus a functional response determined from a parasitoid's first encounter with a host cannot be extrapolated over its entire life. Jervis et al. (2008) identified four age-specific realized fecundity patterns in parasitoids, which may be useful to predict the change in functional response over time. Aphidiinae braconids exhibit a type II age-specific realized fecundity pattern, characterized by increasing egg deposition over the first few days of adult life followed by a rapid decline (Jervis et al. 2008). This pattern is similar to that found for *D. rapae*, which at high host densities exhausted its egg supply, resulting in reduced parasitism the following day (Bernal et al. 1994). Aphelinidae display type III or IV fecundity patterns, characterized by a prolonged low egg deposition rate (Jervis et al. 2008). Therefore, even if daily egg deposition rates differ, this



**Fig. 4.** Functional response ( $\pm\text{SE}$ ) of *A. certus* to *A. glycines* in a 24-h prey exposure period at  $26 \pm 1^\circ\text{C}$ .

may not reflect lifetime fecundity. As a result, direct comparisons between parasitoids are only meaningful if they have the same age-specific fecundity pattern. Future work should attempt to determine the maximum lifetime fecundity of *A. certus* and determine whether it exhibits a type III or IV age-specific fecundity pattern.

Because of the extent of its range and its relationship with soybean aphid, *A. certus* seems to be a valuable asset for soybean aphid management in North America. There are few members of this guild in North American soybean ecosystems (Kaiser et al. 2007). The extent of its geographic range and its impact on soybean aphid populations under field conditions still need to be determined. The data collected here are a valuable first step in determining the utility of *A. certus* as a biological control agent and its importance as a component of an IPM program for soybean aphid.

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