

# Incorporating natural enemy units into a dynamic action threshold for the soybean aphid, *Aphis glycines* (Homoptera: Aphididae)

Rebecca H Hallett,<sup>a\*</sup> Christine A Bahlai,<sup>a,b</sup> Yingen Xue<sup>a,c</sup> and Arthur W Schaafsma<sup>c</sup>

## Abstract

**BACKGROUND:** Recommended action thresholds for soybean aphid, *Aphis glycines*, do not adjust for natural enemy impact, although natural enemies contribute important biological control services. Because individual natural enemy species have varied impacts on pest population dynamics, incorporating the impact of a diverse predator guild into an action threshold can be cumbersome.

**RESULTS:** Field surveys identified an aphidophagous natural enemy complex dominated by *Orius insidiosus*, *Coccinella septempunctata*, *Harmonia axyridis* and *Aphelinus certus*. Functional responses of *O. insidiosus* were determined in the laboratory, while predation rates of all other natural enemies were obtained from the literature. Natural enemy impacts were normalized using natural enemy units (NEUs), where 1 NEU = 100 aphids consumed or parasitized. A dynamic action threshold (DAT) was developed by combining NEUs with an *A. glycines* population growth model. With the DAT, an insecticide application was only triggered if natural enemy numbers were insufficient to suppress pest populations. In field experiments, DAT provided equivalent yields to the conventional action threshold and reduced the average number of pesticide applications.

**CONCLUSION:** The DAT approach has the potential to reduce pesticide use, will help preserve natural enemy populations and can be applied to other pest systems with diverse natural enemy guilds.

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**Keywords:** biological control; natural enemies; natural enemy units; predation; parasitism

## 1 INTRODUCTION

Action thresholds are the cornerstone of integrated pest management.<sup>1</sup> It is recommended that management action be taken in agricultural systems when levels of damage or pest populations occur in excess of the action threshold in order to prevent damage from exceeding another, higher threshold, the economic injury level (EIL). The EIL occurs when economic cost associated with damage is incurred, i.e. when the point at which the cost of yield loss exceeds the cost of a given management action is reached. For insect pest populations exceeding conventional action thresholds, however, a number of factors may slow or even reverse their population growth, preventing economic injury from occurring. In pest species with diverse guilds of natural enemies, pest population suppression can be particularly effective.<sup>2</sup> However, incorporating natural enemies into management decisions and action thresholds presents a functional challenge. Members of the natural enemy guild may contribute unequally to pest suppression, and thus quantifying the impact of each species in real time becomes daunting and cumbersome, which limits the likelihood of widespread adoption. Thus, it is essential that action thresholds incorporating natural enemy populations strike a balance between ease of use and precision. The present paper sets out a method for developing and testing a dynamic action threshold (DAT) incorporating natural

enemies standardized in terms of natural enemy units (NEUs),<sup>3</sup> using a case study from Canadian field crops.

The soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae), has become a serious exotic pest of soybean, *Glycine max* (L.) Merrill, since its discovery in North America in 2000.<sup>4</sup> Natural enemies are important in regulating soybean aphid populations in Asia<sup>5–8</sup> and in North America.<sup>9–14</sup> At 25 °C and in the absence of natural enemies, soybean aphid populations can double in 1.5 days.<sup>15</sup> Soybean aphid density on plants in natural enemy exclusion cages in the field reached peak populations up to 12 times higher than on non-caged plants.<sup>16,17</sup>

Natural enemy surveys in soybean fields have identified the presence of members of Coccinellidae, Anthocoridae,

\* Correspondence to: RH Hallett, School of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada. E-mail: rhallett@uoguelph.ca

<sup>a</sup> School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

<sup>b</sup> Department of Entomology, Michigan State University, East Lansing, Michigan, USA

<sup>c</sup> Department of Plant Agriculture, Ridgetown Campus, University of Guelph, Ridgetown, Ontario, Canada

Chrysopidae, Syrphidae and Nabidae, with *Harmonia axyridis* (Pallas) and *Orius insidiosus* (Say) frequently identified as the key predators of *A. glycines* in the midwestern United States,<sup>11,17</sup> Michigan<sup>9,10</sup> and Quebec.<sup>18</sup> Only one prior natural enemy survey for *A. glycines* exists for Ontario.<sup>19</sup> Natural enemies vary in their ability to affect population growth of *A. glycines*, and their relative voracity (i.e. the number of aphids consumed or parasitized per day) is key to developing action thresholds that incorporate their impact. The voracity of the main coccinellid species and the parasitoid *Aphelinus certus* Yasnosh for *A. glycines*,<sup>20,21</sup> as well as of lacewings, syrphids and *Aphidoletes* on other aphid species, is known.<sup>14,22–25</sup> However, for *O. insidiosus* feeding on *A. glycines*, this information was only available for adult females,<sup>11,26</sup> and not for males or nymphs.

In North America, insecticides were used infrequently in soybean production before the occurrence of *A. glycines* as a pest.<sup>4</sup> Soybean producers now rely on foliar applications of pyrethroids and organophosphates for control of *A. glycines*. A single foliar insecticide application can provide cost-effective management of *A. glycines* when application is based on weekly scouting and use of an action threshold.<sup>27</sup> The use of neonicotinoid seed treatments has become widespread in soybean production; however, most studies suggest that seed treatments are not effective in preventing yield losses to *A. glycines*.<sup>27–30</sup>

In Ontario and through much of the American midwest, an action threshold of 250 soybean aphids plant<sup>-1</sup>, if populations are actively increasing in late vegetative to R4 soybeans, is recommended (henceforth referred to as the conventional action threshold, or CAT).<sup>31,32</sup> However, the CAT does not explicitly take natural enemy numbers into account. Several studies<sup>33–36</sup> have attempted to develop profit-maximizing or environmentally conservative action thresholds, but none can be used to make real-time management decisions, and none explicitly considers the impact of a diverse natural enemy guild on soybean aphid numbers.

Ragsdale *et al.*<sup>33</sup> developed an EIL and economic threshold for soybean aphid under field conditions in which biotic and abiotic factors were allowed to influence soybean aphid densities. The economic threshold (ET = 273 aphids plant<sup>-1</sup>) does not specify the dynamic influence of natural enemies in space and time on soybean aphid populations. Catangui *et al.*<sup>34</sup> developed a threshold with a dynamic EIL that took into account soybean growth stages and growing conditions and allowed input of the actual market value of soybean and control costs; however, it ignored the abundance of natural enemies and was criticized for being unrealistic to field conditions.<sup>37</sup>

Zhang and Swinton<sup>35</sup> were the first to incorporate both the effect of natural enemies on soybean aphid density and the non-target mortality effect of insecticides on natural enemy abundance in an ET. However, they did not account directly for the relative voracity of different natural enemies. Moreover, their profit-maximizing decision could only be achieved by an optimizing simulation approach, limiting its in-field use by producers. Recently, Zhang and Swinton<sup>36</sup> have refined their model to incorporate the voracity of coccinellids; however, the impacts of other important natural enemies, such as *O. insidiosus* and *A. certus*,<sup>20,38</sup> were ignored. In addition, their estimates of coccinellid voracities were very low compared with available data for the dominant coccinellid predators of soybean aphid.<sup>21</sup>

The goal of the present study was to develop a DAT for *A. glycines* that would prescribe an insecticide application only if natural enemy numbers were insufficient to keep aphid populations below the EIL. The authors had several objectives:

(i) to confirm which natural enemies were the main contributors to regulation of *A. glycines* populations in their region; (ii) to describe the functional response of *O. insidiosus*; (iii) to study relationships between populations of *A. glycines*, its key natural enemies and the insecticide regimes; (iv) to normalize the impact of various species of natural enemies on aphid populations; (v) to incorporate these results into a DAT; and (vi) to validate the DAT in commercial fields for one season as proof of concept.

## 2 EXPERIMENTAL METHODS

### 2.1 The natural enemy complex of *A. glycines*

To determine the composition of the foliar-based natural enemy community in Ontario, Canada, destructive plant sampling was undertaken weekly from V1 to R6 stage<sup>39</sup> at each site included in the population dynamics experiment (see below) in 2007 and 2008. These samples consisted of three (2007) or five (2008) whole plants, randomly selected and removed at the base from the ground in each plot, placed immediately into plastic bags and transported back to the lab for assessment. Whole-plant sampling yields greater diversity of natural enemies than other sampling methods.<sup>19</sup> Assessments consisted of identification and quantification of all predatory insects and all parasitized and healthy aphids on the plant samples.

### 2.2 Relationships between populations of *A. glycines*, its natural enemies and the insecticide regime

To examine the relationship between aphid density and natural enemy populations under different insecticide and action threshold regimes, experiments were conducted in six soybean fields in 2007 and 2008. In 2007, three experiments were established (one in Lambton County and two in Kent County). In 2008, three experiments were established (one in Lambton County and two in Kent County); however, because of very low populations (<1 aphid plant<sup>-1</sup> for most of the growing season) of *A. glycines*, 2008 data were used only for determining the composition of the natural enemy complex (see above), and not for examining interactions with natural enemy populations.

A factorial design was employed with four seed treatments and three foliar insecticide timings as main effects (i.e. 12 treatment combinations), replicated 3 times in randomized complete blocks. The four seed treatments levels were: (1) untreated; (2) fungicide alone (ApronMaxx RTA<sup>®</sup>, fludioxonil + metalaxyl-M, 0.025 + 0.038 g AI kg<sup>-1</sup> seed; Syngenta Crop Protection Canada, Guelph, ON); (3) thiamethoxam (Cruiser<sup>®</sup> 350FS, 0.5 g AI kg<sup>-1</sup> seed; Syngenta) plus fungicide (as above); (4) imidacloprid (Gaucho<sup>®</sup> 480 FS, 1.2 g AI kg<sup>-1</sup> seed; Bayer<sup>®</sup> CropScience, Guelph, ON) plus fungicide. The three foliar insecticide timing levels were: (1) untreated; (2) CAT, i.e.  $\lambda$ -cyhalothrin (Matador<sup>®</sup> 120EC, 10 g AI ha<sup>-1</sup>, Syngenta) applied at 250 aphids plant<sup>-1</sup>; (3) a high threshold, i.e.  $\lambda$ -cyhalothrin applied at 500 aphids plant<sup>-1</sup>. Individual plots were 3.7 m by 15 m. Foliar insecticide was applied with a high-clearance sprayer (Model 4730; John Deere, Moline, IL) with a 18.3 m boom divided into five 3.3 m sections and with duo TT11003 spray tips spaced at 50 cm on the boom, held at 50 cm above the plant canopy, travelling at 19.3 km h<sup>-1</sup> and delivering 187 L water ha<sup>-1</sup> at 483 kPa. Plant sampling was as described above. In 2007, yellow sticky cards (14 cm × 11.75 cm) were also placed in the center of each plot at plant canopy height. Sticky cards were replaced weekly and at the end of each week were taken back to the lab for assessment.

Very few natural enemies were observed early in the growing season, and these data did not follow a normal distribution, so only natural enemy data from samples taken once soybeans reached the R-stages were used for analyses. Three measures of natural enemy density were determined: natural enemy counts plant<sup>-1</sup>, NEUs plant<sup>-1</sup> (see below for calculation of the NEUs) and NEUs sticky card<sup>-1</sup>. Aikake's information criterion (AIC), a measure of statistical noise remaining in the data after the application of a model,<sup>40</sup> was generated by linear models (lm command) using R 2.10.1 (R Project for Statistical Computing, <http://www.r-project.org/>) and was used to determine which measure of natural enemy density was the best predictor of aphid density. Models were considered to be different from one another if their AIC values differed by more than two units, with the lower AIC value indicating the model with better performance.<sup>40</sup>

Total aphid density was determined as the sum of aphid-days (that is, the cumulative aphid population, given by  $\sum_{i=1}^N n_i \cdot 7$  days,

where  $n_i$  is the mean aphid density plant<sup>-1</sup> in a given plot each week, summed over  $N$  weeks) from the first sampling week until soybean leaves began to senesce at a given site. Data were subjected to ANOVA (using R 2.10.1), with seed treatment and foliar treatment used as fixed effects in the model to determine differences in yield, total aphid density and natural enemy density in response to these treatments. Site effects and interaction terms were also included in the model to account for variations between sites. A *post hoc* Tukey's HSD was applied to determine differences between treatments. A type I error rate of  $\alpha = 0.05$  was employed for all relevant statistical tests.

### 2.3 Functional response of *O. insidiosus* to *A. glycines*

To examine the functional response of nymphal and adult *O. insidiosus* to *A. glycines*, laboratory assays were conducted using *O. insidiosus* obtained from commercial suppliers (BioBest Biological Systems Canada, Brampton, ON, and MGS Horticultural Inc., Leamington, ON). In preparation for assays, *O. insidiosus* were reared in a clear plastic container (10 cm diameter  $\times$  8 cm height; Shortreed Paper Inc., Guelph, ON) with *A. glycines* on infested soybean leaves. Mesh-covered holes in the lid provided ventilation. The containers were kept in a controlled environment chamber at  $25 \pm 1$  °C with a 16:8 h L:D photoperiod.

One leaflet of a soybean trifoliolate leaf was placed, abaxial surface up, on wet cotton wool in a 90 mm petri dish. Third- or fourth-instar aphids were obtained from laboratory colonies<sup>21</sup> and transferred to leaflets at treatment densities of 2, 4, 8, 16, 32 or 64 leaflet<sup>-1</sup>. Individual fifth-instar or female or male adult *O. insidiosus* were starved for 24 h and then introduced singly into each petri dish. Fifth instars were differentiated from other instars by body size and presence of wing buds. Adult sexes were differentiated by presence of the ovipositor in females. Dishes were sealed with finely punctured Parafilm<sup>®</sup> M laboratory film (Pechiney Plastic Packaging, Menasha, WI), for ventilation, and kept under the same conditions as above. After 24 h, the number of prey consumed was recorded. There were ten replications of each aphid density treatment. Controls without predators for each aphid density were included to control for other sources of aphid mortality, and were replicated 3 times.

A two-stage analysis, as per Juliano,<sup>41</sup> indicated that the type II functional response fitted the present data best, so further analyses were restricted to the type II functional response. Holling's disc equation<sup>42</sup> and the random predator equation<sup>43</sup> were used to

model the relationship between the number of prey consumed and the initial prey density. These parameters were estimated by a non-linear regression process in SPSS (SPSS for Windows, v.15.0; SPSS Inc., Chicago, IL). If the asymptotic 95% confidence intervals (CIs) of instantaneous searching rate and handling time included zero (i.e. the estimates did not differ significantly from zero), the model did not fit the data.

## 2.4 Dynamic action threshold development

### 2.4.1 Natural enemy unit calculations for predominant natural enemies

To normalize the impact of varying species of natural enemies on *A. glycines*, NEUs<sup>3</sup> were used for quantifying biological control services; this is similar to previously developed strategies for quantifying total pest damage.<sup>44</sup> Here, 1 NEU is defined as the number of predators or parasitoids required to kill 100 pest insects in 24 h. The voracity (i.e. the number of aphids consumed or parasitized per day) of *C. septempunctata*, *H. axyridis* and *Aphelinus certus* on *A. glycines* was determined previously.<sup>20,21</sup> The voracities of Chrysopidae, Syrphidae and *Aphidoletes* were estimated from data for *Chrysoperla carnea* (Stephens) feeding on *Aphis gossypii* Glover<sup>23,24</sup> and for *Allograpta obliqua* (Say)<sup>22</sup> and *Aphidoletes aphidimyza* (Rondani)<sup>14,25</sup> feeding on various aphid species. *A. obliqua* and *C. carnea* were selected as representatives of the taxa Syrphidae and Chrysopidae, respectively, as they are the most common representatives of these taxa in Ontario.<sup>22,45</sup> *A. aphidimyza* was selected to represent *Aphidoletes* because it is a common predator in soybean fields in the midwestern United States.<sup>14</sup> Sex ratios of 2:1 female:male for *A. certus* and 3:2 for *O. insidiosus* were observed in the present colonies and were used to correct the voracities when calculating NEUs for these species.

### 2.4.2 Dynamic action threshold model

To develop the DAT, the impact of natural enemies on *A. glycines* populations was incorporated into a population growth model for *A. glycines* developed by Ragsdale *et al.*<sup>33</sup> The impact of total natural enemies was defined as

$$NEU_{\text{total}} = \sum_{i=1}^N n_i V_i \quad (1)$$

where  $N$  is the total number of natural enemy species,  $n_i$  is the total number of individuals of natural enemy species  $i$  observed on one plant and  $V_i$  is the average voracity of natural enemy species  $i$  divided by 100.

The underlying model for *A. glycines* population growth<sup>33</sup> is

$$N_t = N_0 e^{rt} \quad (2)$$

where  $N_t$  is the *A. glycines* population density plant<sup>-1</sup> at time  $t$ , in days,  $N_0$  is the initial aphid density and  $r$  is the population growth rate. As an external source of mortality (in the form of NEUs) was to be incorporated into the model, the field-derived population growth rate used in developing the ET<sup>33</sup> was replaced in equation (2) with the temperature-dependent intrinsic rate of increase. For *A. glycines*, the temperature-dependent intrinsic rate of increase,  $r$  (in day<sup>-1</sup>), was described by a two-part function developed

from data for intrinsic rate of increase at constant temperatures presented in Hirano *et al.*<sup>46</sup> and McCornack *et al.*<sup>15</sup>

$$r(T) = 0.021T - 0.047, \quad T < 25.7 - 0.009T^2 \\ + 0.445T - 5.000, \quad T \geq 25.7 \quad (3)$$

The *A. glycines* population at time  $t$  adjusted for natural enemy impact was therefore defined as

$$N_t \text{ (adjusted)} = \sum_{i=1}^t \frac{(N_{i+1} - 100NEU_i) + (N_i - 100NEU_i)}{2} \quad (4)$$

To calculate revised action thresholds, equation (4) was run over a 7 day period for different values of NEUs plant<sup>-1</sup>, using long-term average minimum and maximum temperatures (16.9 and 27.5 °C respectively) for July and August in southwestern Ontario. The long-term averages were calculated on the basis of average maximum and minimum temperatures for Windsor, London and Toronto, Ontario, in July and August (i.e. when soybean reproductive stages occur) between 1971 and 2007 (Environment Canada National Climate Data and Information Archive, [http://www.climate.weatheroffice.gc.ca/climateData/canada\\_e.html](http://www.climate.weatheroffice.gc.ca/climateData/canada_e.html)). A 7 day period was used because the ET provides a 7 day lead-time before populations should reach the EIL,<sup>33</sup> and the CAT is implemented with the recommendation that an action be taken within 7 days of an assessment indicating that the CAT has been reached (Baute T, private communication). According to equation (4), in the absence of natural enemies, a population of 250 aphids plant<sup>-1</sup> will increase to 4408 aphids within 7 days at the temperatures specified. Thus, for a given NEU value, the starting population of *A. glycines*, which can reach a density of 4408 aphids plant<sup>-1</sup> on day 7, was designated as the action threshold for that NEU value.

## 2.5 Field validation of the dynamic action threshold

To assess the efficacy of the DAT in making pest management decisions for *A. glycines*, field experiments were conducted at three locations, Ridgeway, Louisville and Seaforth, in southwestern Ontario in 2009. Experiments had a randomized complete block design with three replicates at each site and three treatments consisting of the CAT, the DAT (i.e. foliar insecticide application made according to *A. glycines* numbers and natural enemy counts) and an untreated control, where no insecticide was applied regardless of insect populations. Once aphid populations exceeded 100 aphids plant<sup>-1</sup>, whole-plant sampling (as described above) of aphid and natural enemy numbers at each site was conducted on 3–5 randomly selected plants per plot. The second sampling event occurred 3–4 days after the first in order to monitor aphid populations closely prior to reaching the CAT, but was extended to 7 days thereafter. Sampling dates and respective plant stages were as follows: Seaforth – 13, 17 and 24 August (R3-4, R4-5 and R6); Louisville – 14, 18, 24 and 31 August (R5, R5, R5 and R6); Ridgeway – 17, 20 and 27 August (R4-5, R5 and R6).

Insecticide applications of  $\lambda$ -cyhalothrin at the Canadian registered rate (10 g AI ha<sup>-1</sup>) for soybean were made to individual plots only during the R-stages of soybean when directed by the associated action threshold regime. Foliar insecticide treatments were applied as described for the 2007 experiments. Each plot was 12.2 m by 15.2 m in size, with the exception of plots at

the Seaforth location, which were 27.4 m by 15.2 m in order to accommodate larger spray equipment. The buffer zones at the four borders of the experiment location were 15.2 m wide. The central 6.1 m by 9.1 m area of each plot was harvested at crop maturity (early to mid-November), and soybean yields at 13% moisture were determined. ANOVA was used to determine differences among yields.

## 2.6 Operational dynamic action threshold demonstrations

To assess operational use of the DAT, on-farm demonstrations of the DAT were conducted in collaboration with growers and crop consultants on four commercial soybean fields, two in eastern Ontario and two in southwestern Ontario, in 2009. Each cooperating grower was visited at least twice during the season, i.e. at the beginning to provide training on the use of the DAT and at the end of the season to collect feedback. Cooperators were asked to conduct their usual field scouting practices for *A. glycines* and to count the number of aphids and natural enemies observed on ten plants per field. A hand-held disc calculator was provided to cooperators to simplify the DAT calculation process and to provide an in-field method of assessing the need for insecticide management of *A. glycines*. To simplify NEU calculations, the most frequently observed natural enemies were grouped into six categories (Coccinellidae, *Orius* spp., Chrysopidae, Syrphidae, *Aphidoletes* spp. and hymenopteran parasitoids), and representative NEUs were used for all members of each group. A table was provided to help determine the total NEUs for the field. With the disc calculator, users would then line up the NEU number with the mean number of aphids plant<sup>-1</sup>, and one of three possible recommendations would be indicated: no insecticide needed (i.e. DAT not reached), no insecticide needed now but scout again within 1 week (i.e. DAT exceeded by <10%) or an insecticide application is needed (DAT exceeded by  $\geq$ 10%). In order to provide a margin of safety before the EIL of 674 aphids plant<sup>-1</sup> was reached,<sup>33</sup> the disc calculator was designed to recommend an insecticide application if there were  $\geq$ 550 aphids plant<sup>-1</sup> regardless of NEUs. Similarly, an insecticide application was never recommended if there were  $\leq$ 250 aphids plant<sup>-1</sup>.

## 3 RESULTS

### 3.1 The natural enemy complex of *A. glycines*

Foliar natural enemies of *A. glycines* observed in 2007 and 2008 included predators, parasitoids (observed as aphid mummies) and pathogens. The most common predators were *O. insidiosus* and coccinellids (Table 1), with *C. septempunctata* and *H. axyridis* representing 77.7% of the coccinellids found. The characteristic black mummies of *A. certus* were the most frequently observed, suggesting that this species was the dominant parasitoid. Aphids exhibiting symptoms of infestation by pathogens were infrequently observed (<1%) and were typically seen only in soybeans with dense canopies at late R-growth stages. In 2007, a high aphid year, the natural enemy community was dominated by parasitoids, whereas in 2008, when aphids were less abundant, predators were more abundant than parasitoids.

### 3.2 Relationships between populations of *A. glycines*, its natural enemies and the insecticide regime

Aphid density was better predicted by calculated NEUs plant<sup>-1</sup> (AIC = 12 820;  $P < 0.0001$ ), as indicated by the lower AIC value,

**Table 1.** Percentage abundance of natural enemies of *A. glycines* on soybean plants from field experiments in southwestern Ontario, by site and year. The total counts for each category and site are given in parentheses

Natural enemies	2007				2008				Pooled
	Erie	Alvinston	Shetland	Total	Ridgetown	Dresden	Alvinston	Total	
<b>Predators</b>	(860)	(877)	(869)	(2606)	(406)	(74)	(100)	(580)	(3186)
<i>Harmonia axyridis</i>	1.9	1.1	1.7	1.6	0.5	0	2.0	0.7	1.4
<i>Coccinella septempunctata</i>	11.3	20.5	16.6	16.2	0	1.4	0	0.2	13.2
Other Coccinellidae <sup>a</sup>	2.3	8.1	4.8	5.1	0.3	0	1.0	0.3	4.2
<i>Orius insidiosus</i>	51.5	60.1	53.9	55.2	37.0	63.5	30.0	39.1	52.3
Chrysopidae	5.4	3.7	3.5	4.1	35.7	6.8	46.0	33.8	9.5
Syrphidae	10.0	0.7	7.1	5.9	25.4	21.6	21.0	24.1	9.2
<i>Aphidoletes aphidimyza</i>	0	0	0	0	0.5	4.1	0	0.9	0.3
Spiders	17.7	5.8	12.4	11.9	0.7	2.7	0	0.8	9.9
<b>Parasitoids</b>	(1152)	(3547)	(11 395)	(16 094)	(369)	(22)	(7)	(398)	(16 492)
<i>Aphelinus certus</i>	94.4	99.6	97.5	97.7	100	100	100	100	97.8
<i>Aphidius colemani</i>	5.6	0.4	2.5	2.3	0	0	0	0	2.2
<b>Total natural enemies</b>	(2012)	(4424)	(12 264)	(18 700)	(775)	(96)	(107)	(978)	(19 678)
Predators	42.7	19.8	7.1	13.9	52.4	77.1	93.5	59.3	83.81
Parasitoids	57.3	80.2	92.9	86.1	47.6	22.9	6.5	40.7	16.19

<sup>a</sup> Chiefly *Coleomegilla maculata* and *Propylea quatuordecimpunctata*.

than by total natural enemy individuals plant<sup>-1</sup> (AIC = 12 848;  $P < 0.0001$ ) or total NEUs sticky card<sup>-1</sup> (AIC = 13 455;  $P = 0.786$ ). Thus, NEUs plant<sup>-1</sup> was chosen as the best measure of natural enemy density in subsequent calculations.

Total aphid density differed by site, but there were no interactions between site and treatments, nor between foliar and seed treatments, so data were pooled for analyses. Total aphid density varied by both threshold (i.e. foliar) treatment ( $F = 8.4$ ;  $df = 2, 84$ ;  $P = 0.0004$ ) and seed treatment ( $F = 8.8$ ;  $df = 3, 84$ ;  $P < 0.0001$ ); aphid density was reduced in all plots receiving insecticide treatments, but both seed treatments and foliar application timings had similar total season aphid densities (Table 2). Similarly, NEUs plant<sup>-1</sup> was lower both in plots with insecticide-treated seed ( $F = 6.3$ ;  $df = 3, 947$ ;  $P = 0.0003$ ) and in those that received foliar insecticides ( $F = 4.8$ ;  $df = 2, 947$ ;  $P = 0.009$ ) than for the untreated controls (Table 2). No differences in yield were observed between seed treatments ( $F = 1.4$ ;  $df = 3, 98$ ;  $P = 0.247$ ) or between foliar treatments ( $F = 0.3$ ;  $df = 2, 98$ ;  $P = 0.745$ ) and untreated controls on data pooled between sites (Table 2); when sites were analyzed individually, one site had slightly higher yields in the two threshold treatments relative to the control ( $F = 9.7$ ;  $df = 2, 32$ ;  $P = 0.029$ ), but the two threshold treatments did not differ from each other by Tukey's HSD.

### 3.3 Functional response of *O. insidiosus* to *A. glycines*

Fifth instars ( $P_1 = -0.1708$ ;  $P = 0.0347$ ) and female adults ( $P_1 = -0.1043$ ;  $P = 0.0488$ ) exhibited a type II response<sup>41</sup> to soybean aphids. Male adults of *O. insidiosus* seldom killed aphids, so their predation rates of soybean aphid could not be subjected to statistical analysis and were assumed to be negligible. For females and fifth instars, Holling's disc model fitted the data well, but the random predator model did not, because the asymptotic 95% CIs of handling time estimates of fifth instars (from  $-0.6828$  to  $0.2070$ ) and female adults (from  $-3.3825$  to  $1.6426$ ) included zero. The functional responses predict theoretical maximum predation rates of 8 and 11 aphids day<sup>-1</sup> for fifth instars and female adults

**Table 2.** Total aphid density (in aphid-days), NEU density and yield for plots treated with seed and/or foliar insecticide treatments for soybean aphid according to different insecticide regimes in 2007. Values within a treatment grouping followed by the same letter do not significantly differ by Tukey's HSD test,  $\alpha = 0.05$

Treatment	Total aphid density (mean $\pm$ SEM)	NEUs plant <sup>-1</sup> (mean $\pm$ SEM)	Yield (kg m <sup>-2</sup> ) (mean $\pm$ SEM)
<b>Seed treatments</b>			
Untreated	9300 $\pm$ 1700a	6.5 $\pm$ 1.4a	0.295 $\pm$ 0.005a
Fungicide	10 000 $\pm$ 1700a	5.9 $\pm$ 1.2ab	0.290 $\pm$ 0.009a
Imidacloprid	4000 $\pm$ 800b	3.7 $\pm$ 0.8bc	0.290 $\pm$ 0.009a
Thiamethoxam	5300 $\pm$ 900b	2.9 $\pm$ 0.6c	0.313 $\pm$ 0.005a
<b>Foliar treatments</b>			
Untreated	9900 $\pm$ 1400a	6.3 $\pm$ 1.1a	0.299 $\pm$ 0.007a
CAT <sup>a</sup>	5000 $\pm$ 1100b	3.9 $\pm$ 0.9b	0.299 $\pm$ 0.005a
High threshold <sup>b</sup>	6500 $\pm$ 900b	4.1 $\pm$ 0.6b	0.305 $\pm$ 0.005a

<sup>a</sup> CAT: conventional action threshold, insecticide applied at 250 aphids plant<sup>-1</sup>.

<sup>b</sup> Insecticide applied at 500 aphids plant<sup>-1</sup>.

respectively, with maximum predation achieved at densities of approximately 250 aphids arena<sup>-1</sup> (Fig. 1).

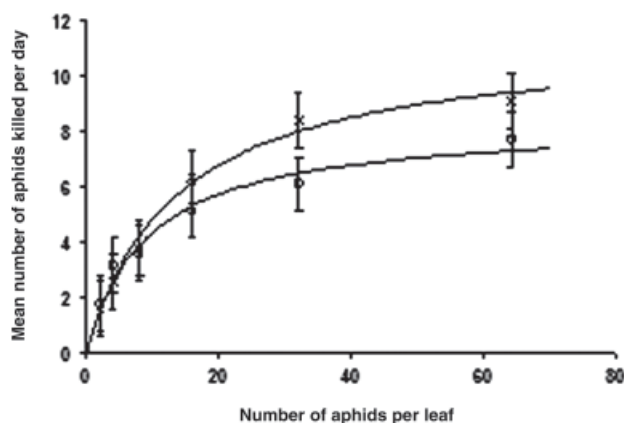
### 3.4 Dynamic action threshold development

#### 3.4.1 Natural enemy unit calculations for predominant natural enemies

Ladybeetles were the most voracious of all predators examined, followed by lacewings and *Aphidoletes aphidimyza* (Table 3). The most abundant predator, *O. insidiosus*, and the most abundant parasitoid, *A. certus*, are equivalent in terms of NEUs, with 12 individuals of either species being equivalent to one coccinellid, which is approximately equivalent to 1 NEU.

#### 3.4.2 Dynamic action threshold model

The starting densities of *A. glycines* that would result in a population of  $\sim 4408$  plant<sup>-1</sup> after 7 days were calculated for different NEU



**Figure 1.** Functional responses of fifth instar larvae (L) and adult females of *Orius insidiosus* to third- and fourth-instar *Aphis glycines*.

**Table 3.** Voracity and NEUs of natural enemies of *A. glycines* frequently observed in field experiments in southwestern Ontario, 2007 and 2008

Natural enemies	Number of aphids killed or parasitized in 24 h			NEUs
	Larva	Adult female	Adult male	
<b>Predators</b>				
<i>Harmonia axyridis</i>	107	114	62	0.94
<i>Coccinella septempunctata</i>	118	101	66	0.95
<i>Orius insidiosus</i>	8	11	—	0.08
Chrysopidae <sup>a</sup>	36	—	—	0.36
Syrphidae <sup>b</sup>	17	—	—	0.17
<i>Aphidoletes aphidimyza</i>	25	—	—	0.25
<b>Parasitoids</b>				
<i>Aphelinus certus</i>	—	12	—	0.08

<sup>a</sup> Based on *Chrysoperla carnea*.<sup>21,22</sup>

<sup>b</sup> Based on *Allograpta obliqua*.<sup>20</sup>

levels, using average maximum and minimum temperatures of 27.5 and 16.9 °C (Table 4). The resultant DAT values range from 250 aphids plant<sup>-1</sup> for 0 NEUs plant<sup>-1</sup> to 600 aphids plant<sup>-1</sup> for 1.5 NEUs plant<sup>-1</sup>.

### 3.5 Field validation of the dynamic action threshold

At the Ridgeway site, use of the DAT delayed the insecticide application by 3 days compared with the CAT (Fig. 2a). This delay was accompanied with natural enemy population growth (Fig. 2b). The natural enemy populations in DAT plots were significantly higher than in CAT plots for the duration of the experiment after insecticide was applied to the CAT plots (ANOVA:  $F = 11.17$ ;  $df = 2, 14$ ;  $P = 0.0013$ ). There was no significant difference between yields of DAT and CAT plots, but yields of both treatments were higher than the yield of the untreated control (ANOVA:  $F = 10.76$ ;  $df = 2, 6$ ;  $P = 0.0104$ ) (Table 5).

At the Louisville site, one insecticide application was made to CAT plots, but the DAT indicated that no spray was needed, so DAT plots received no insecticide application. Aphid populations declined in DAT plots to levels equivalent to the CAT at 2 weeks post-insecticide application (Fig. 2c). The natural enemy populations in DAT plots were numerically, but not statistically,

higher than in CAT plots after treatment (ANOVA:  $F = 0.13$ ;  $df = 2, 14$ ;  $P = 0.8766$ ) (Fig. 2d). There was no significant difference between yields of the CAT, DAT and control plots (ANOVA:  $F = 0.15$ ;  $df = 1, 3$ ;  $P = 0.7244$ ) (Table 5).

At the Seaforth site, both soybean aphid densities and natural enemy numbers were relatively high at the time of treatment of the CAT plots (Fig. 2e). One insecticide application was made to CAT plots, but the DAT indicated that no spray was needed, so DAT plots received no insecticide application. Aphid populations in DAT plots declined 1 week later, in spite of there being no insecticide application. The natural enemy populations in DAT plots were significantly higher than in CAT plots after treatment (ANOVA:  $F = 14.67$ ;  $df = 2, 6$ ;  $P = 0.0049$ ) (Fig. 2f). There was no apparent difference between yields of CAT and DAT plots, although no statistical analyses could be performed because seed from the three replicates for each treatment were inadvertently pooled at harvest (Table 5).

When pooled for all sites, DAT plots had significantly higher natural enemy populations than CAT plots after insecticide treatment (ANOVA:  $F = 6.15$ ;  $df = 2, 38$ ;  $P = 0.0049$ ) and no difference in yield compared with CAT plots (ANOVA:  $F = 13.70$ ;  $df = 2, 11$ ;  $P = 0.0010$ ), and insecticide application to DAT plots was only required at one of three sites.

### 3.6 Operational dynamic action threshold demonstrations

Cooperators in both eastern and southwestern Ontario found the DAT disc calculator to be a valuable decision-making tool for management of *A. glycines*. All cooperators indicated that, as a result of having access to and using the DAT disc calculator, they did not apply any insecticides on their fields or on those under their management (Bennett H, Buurma R, Lumley M and Simpson B, private communications). Without access to the disc calculator, all cooperators indicated that they likely would have sprayed those fields with insecticides; thus, a 100% reduction in insecticide use was achieved in those fields.

## 4 DISCUSSION AND CONCLUSIONS

### 4.1 The natural enemy complex of *A. glycines*

The aphidophagous natural enemy complex in Ontario soybean ecosystems is similar to that observed in other soybean-producing regions in North America, with the predator guild dominated by *O. insidiosus* and coccinellids. While at least five other parasitoid species have been recorded in soybean ecosystems elsewhere in North America,<sup>4</sup> in the present authors' region only two species were detected, *Aphelinus certus* and *Aphidius colemani*, with the former being dominant.

### 4.2 Functional response of *O. insidiosus* to *A. glycines*

As observed for *C. septempunctata* and *H. axyridis*,<sup>21</sup> the searching behavior of *O. insidiosus* is not random, but systematic, probably owing to the use of semiochemical cues.<sup>47</sup> The maximum predation rates of *O. insidiosus* observed were lower than those reported elsewhere,<sup>11,26</sup> likely because of the larger prey used in the present experiments, resulting in more rapid satiation of predators.

### 4.3 Relationships between populations of *A. glycines*, its natural enemies and the insecticide regime

The NEUs plant<sup>-1</sup> was the best predictor of aphid density, indicating that this measure is useful for quantifying the biological

**Table 4.** Estimates of *Aphis glycines* density (plant<sup>-1</sup>), based on different aphid starting densities and different NEU densities, as determined using the revised aphid population growth model [equation (4)], with maximum and minimum temperatures of 27.5 and 16.9 °C, respectively

Day	NEUs plant <sup>-1</sup>											
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5
0	250	273	297	320	343	367	390	413	437	460	483	600
1	377	399	422	445	467	490	512	535	558	580	602	716
2	568	588	611	632	653	676	697	718	741	762	782	891
3	855	874	896	915	934	956	975	994	1016	1035	1053	1154
4	1289	1304	1325	1341	1357	1378	1394	1409	1430	1446	1462	1551
5	1942	1953	1972	1983	1994	2013	2025	2036	2055	2066	2078	2149
6	2925	2930	2946	2950	2955	2971	2975	2980	2996	3000	3005	3051
7	4408	4402	4414	4408	4402	4414	4408	4402	4414	4408	4402	4408

control services of a natural enemy complex. Foliar applications of insecticides for *A. glycines* control cause mortality of both predators and parasitoids.<sup>3,48</sup> In foliar insecticide plots, reduced natural enemy numbers are likely a result of both direct mortality effects and reduced aphid densities, while in seed treatment plots, lower NEUs are more likely a result of reduced aphid densities alone. However, it is important to investigate the impact of all insecticides on natural enemies and, wherever possible, to minimize pesticide use in soybean systems in order to conserve biological control agents and maximize potential biological control services.

#### 4.4 Action thresholds for *A. glycines*

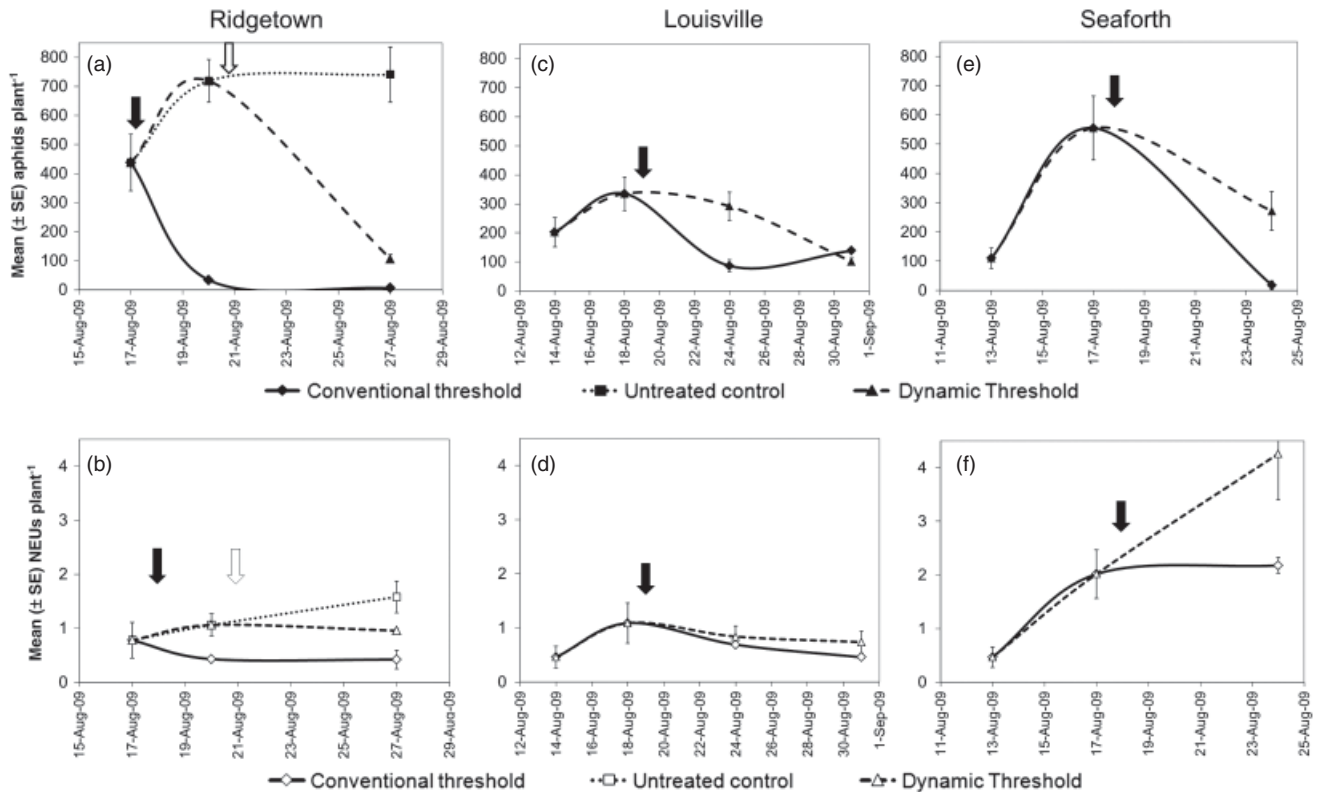
Results from the present field experiments suggest that the CAT is likely too conservative and results in overuse of insecticides. In 2007, insecticide use only conferred a yield advantage at one site, and no yield differences were observed between the CAT and high threshold treatments. Similarly, in 2009, use of the DAT resulted in equivalent yield protection compared with the CAT, but only prescribed an insecticide application at one site, whereas the CAT prescribed an insecticide application at all sites. This result is attributable, at least in part, to the effect of natural enemies on aphid population growth: the 250 aphids plant<sup>-1</sup> CAT assumes that aphid populations will continue to grow and that treatment should occur before the EIL of 674 aphids plant<sup>-1</sup> is reached.<sup>33</sup> When aphid populations peaked at the present sites in 2007, they did not exceed 513 aphids plant<sup>-1</sup> in untreated plots, indicating that the natural enemy complex was capable of suppressing aphid populations below the EIL, even when aphid populations exceeded the CAT. These data suggest that the economic and environmental costs associated with excess insecticide application when the CAT is used can be reduced by refining the action threshold to include the potential impact of natural enemies present in a given field, resulting in a more dynamic action threshold. Both delay and reduction in insecticide applications were also achieved with the use of a threshold incorporating multiple natural enemies for cotton aphid management,<sup>49</sup> but in that study natural enemies alone were unable to suppress aphid populations.

Assuming that the impact of a given predaceous species is broadly similar across the range of the soybean aphid in North America, and given that the natural enemy complex in Ontario is very similar to those in other soybean regions in North America, the present results will help to quantify the impact of these predators on soybean aphid populations outside the present study area. Similar procedures can be adopted to develop location-specific DATs for a given area on the basis of prevailing environmental

conditions. The DAT calculator could also be modified to include new biological control agents introduced from other locations or natural enemies that adopt *A. glycines* as a host, such as the braconid wasp *Binodoxys communis*<sup>50</sup> and the entomopathogenic fungus *Pandora neoaphidis*.<sup>51</sup> The DAT developed herein is suitable for use during the R-stages of soybean, and has not been evaluated for vegetative stages of soybean. Economic aphid infestations occurring before flowering of soybean are rare in Ontario, and a vegetative-stage action threshold for soybean aphids in this region has not yet been established (Baute T, private communication).

Quantitative models of interactions between predator and prey can be used to predict prey–predator dynamics.<sup>52</sup> DATs should be developed on the basis of functional response curves and predation capacities of key predators in conjunction with projections of aphid population growth under given environmental conditions. It should be noted that the projected density of 4408 aphids plant<sup>-1</sup> on day 7 (Table 4) is not intended as a realistic estimate of aphid populations in the field; this value is unrealistically high because it is based on the theoretical intrinsic rate of increase, which assumes no external mortality factors or other environmental resistance to population growth, and only mortality due to NEUs has been accounted for. Thus, the authors would not expect to observe 4408 aphids plant<sup>-1</sup> in the field, but have used this approach in order to couple the ET (based on field population growth rates) with voracity of natural enemies (based on laboratory experiments). This approach helps to avoid an unrealistically low economic threshold, which can result if environmental resistances which may prevent exponential growth of soybean aphid populations are not considered.<sup>33</sup>

Although laboratory estimates of predation and parasitism rates may be higher than field rates, the authors have incorporated a level of conservatism into the soybean aphid DAT that may help to offset this discrepancy, and may result in a realistic assessment of natural enemy impacts on aphid population growth: (i) the assumption of stable predator numbers over the upcoming 7 day period in the soybean aphid DAT somewhat underestimates the impact of coccinellids, as larval coccinellid numbers have been found to increase within 2–3 days of increasing aphid numbers in other systems;<sup>49</sup> (ii) counts of mummified aphids alone may underestimate parasitism rates, as newly parasitized aphids are not counted;<sup>53</sup> while other studies have utilized a correction factor to estimate total parasitism rates,<sup>54,55</sup> the present use of mummified aphids alone underestimates total parasitism, but more closely reflects the number of wasps that will emerge and parasitize additional aphids in the upcoming week. Finally, owing to their disruptive nature, in-field counts are likely to underestimate



**Figure 2.** *Aphis glycines* and natural enemy population dynamics in CAT (solid line), DAT (dashed line) and untreated control (dotted line) plots at Ridgetown (a and b), Louisville (c and d) and Seaforth (e and f), ON, in 2009. Arrows (●CAT, ◻DAT) indicate timing of insecticide applications. At Louisville and Seaforth, where no insecticides were applied to DAT plots, data for DAT and untreated control plots are pooled.

natural enemy populations, particularly where mobile predators, such as coccinellids and *O. insidiosus*, dominate. A model using NEUs to describe the dynamics between aphids and natural enemies corresponded very well to aphid and natural enemy populations under field conditions when it was assumed that the NEUs followed a type III functional response and that average voracity was approximately 75% of peak voracity.<sup>56</sup> The results of that study<sup>56</sup> suggested that, when aphids were abundant (i.e. at densities relevant to management), interspecific interactions within the natural enemy guild were minimal, and the effects of natural enemies were additive.

The DAT ranges from 250 to 600 aphids plant<sup>-1</sup> for NEU values of 0–1.5 NEUs plant<sup>-1</sup>. In contrast, the natural-enemy-adjusted economic threshold,<sup>35,36</sup> generates thresholds of 30–150 aphids plant<sup>-1</sup> for a natural enemy density of 1–2 lady beetles plant<sup>-1</sup>. The natural-enemy-adjusted economic threshold is considerably lower than the DAT owing to reliance on theoretical growth rates as well as unrealistically low estimates of lady beetle voracity. Although the ET for *A. glycines* (273 aphids plant<sup>-1</sup>)<sup>33</sup> was developed without explicitly taking natural enemies into account, it is identical to the DAT indicated with the presence of 0.1 NEUs plant<sup>-1</sup> (Table 4). The DAT may thus provide some insight into the background natural enemy populations present at sites used by Ragsdale *et al.*<sup>33</sup> in determining the ET.

DATs that incorporate fluctuating values of natural enemies, economic inputs, etc., have great potential to facilitate pest management decision-making and increase the efficiency of agricultural production. However, an overly complex decision-making process that incorporates too many components may hinder the adoptability and appeal of such a tool. Because of its

**Table 5.** Mean soybean yields obtained under different soybean aphid management regimes in field experiments conducted at three locations in southwestern Ontario, 2009. Values within a location followed by the same letter do not significantly differ by Tukey's HSD test,  $\alpha = 0.05$

Treatment	Soybean yield (mean ± SEM) (t ha <sup>-1</sup> ) (number of insecticide applications)		
	Ridgetown	Louisville	Seaforth <sup>c</sup>
CAT <sup>a</sup>	2.7 ± 0.1 a (1)	2.05 ± 0.004 a (1)	3.05 (1)
DAT <sup>b</sup>	2.5 ± 0.1 a (1)	— <sup>d</sup> (0)	— <sup>d</sup> (0)
Untreated control	2.3 ± 0.1 b (0)	2.04 ± 0.005 a (0)	3.00 (0)

<sup>a</sup> CAT: conventional action threshold, insecticide applied at 250 aphids plant<sup>-1</sup>.

<sup>b</sup> DAT: dynamic action threshold, insecticide applied according to aphids plant<sup>-1</sup> and NEUs.

<sup>c</sup> Owing to inadvertent pooling of samples from each replicate, yields could not be compared statistically and SE values could not be calculated.

<sup>d</sup> Where no insecticides were applied to DAT plots, data for DAT and untreated control plots are pooled and reported under 'Untreated control'.

simplicity, the DAT method readily lends itself to application in other cropping systems where diverse guilds of natural enemies are present. Although several other action thresholds incorporating impacts of natural enemies have been developed for aphids<sup>49,53</sup> and other crop pests,<sup>54,55</sup> the soybean aphid DAT is the only one to incorporate multiple natural enemies and relate natural enemy impacts explicitly to estimates of voracity. The balance of precision



and facility of the DAT will be important to its rate of adoption and compliance when evaluated against other management decision-making tools.

The DAT developed here for *A. glycines* provided equivalent control to the CAT and resulted in equivalent yields in the present experiments. With additional validation and widespread adoption, this DAT has the potential to reduce or eliminate insecticide applications on an operational scale, thereby reducing production costs for growers, reducing environmental and health risks associated with pesticide use and helping to conserve natural enemies. A smartphone application (Aphid Advisor, © RH Hallett; www.aphidapp.com) has been developed to replace the DAT disc calculator and facilitate use of the DAT for decision-making by soybean producers. Very low soybean aphid populations occurred in the several years following this study, preventing further in-field refinement, and thus the Aphid Advisor app incorporates the same margin of safety as used for the disc calculator, in order to protect growers from economic losses. Data collected through the use of this app will allow future refinements and validation of the underlying model.

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