Susceptibility of *Aphelinus certus* (Hymenoptera: Aphelinidae) to Neonicotinoid Seed Treatments used for Soybean Pest Management

Author(s): Andrew J. Frewin, Arthur W. Schaafsma, and Rebecca H. Hallett
Published By: Entomological Society of America
Susceptibility of *Aphelinus certus* (Hymenoptera: Aphelinidae) to Neonicotinoid Seed Treatments Used for Soybean Pest Management

ANDREW J. FREWIN,1,2,3 ARTHUR W. SCHAAFSMA,4 AND REBECCA H. HALLETT1

J. Econ. Entomol. 107(4): 1450–1457 (2014); DOI: http://dx.doi.org/10.1603/EC13523

ABSTRACT Soybean aphid is an economic pest of soybean in North America. Currently, management of soybean aphid is achieved through the use of foliar- and seed-applied insecticides. However, natural enemies play an important role in regulating soybean aphid populations, and may be adversely affected by insecticides. The effects of imidacloprid and thiamethoxam seed treatments on the soybean aphid parasitoid, *Aphelinus certus* Yasnosh, were examined using a tritrophic bioassay. *A. certus* was able to parasitize soybean aphids feeding on imidacloprid- and thiamethoxam-treated plants 5 and 6 wk after planting, respectively. However, up to 10 wk after planting, overall parasitism rates were reduced by 69–88% compared with the control. Therefore, neonicotinoid seed treatments may reduce the effectiveness of *A. certus* as a natural enemy of soybean aphid in seed-treated crops.

KEY WORDS *Aphelinus*, soybean aphid, neonicotinoid, tritrophic interaction, sublethal effect

Soybean aphid (*Aphis glycines* Matsumura) was first observed in North America in 2000 (Hunt et al. 2003, Ragsdale et al. 2004), and has since become a serious economic pest of soybean (*Glycine max* L.; Ragsdale et al. 2007). Soybean aphid feeding can reduce soybean yields (Beckendorf et al. 2008, Catangui et al. 2009), and therefore foliar- and seed-applied insecticides are often used for soybean aphid management (Ontario Ministry of Agriculture Food and Rural Affairs [OMAFRA] 2009). However, natural enemies play an important role in regulating soybean aphid populations (Fox et al. 2004, Rutledge et al. 2004, Costamagna and Landis 2006, Costamagna et al. 2007, Costamagna et al. 2008, Noma and Brewer 2008, Hallett et al. 2013). The susceptibility of soybean aphid natural enemies to insecticides used in soybean production should be assessed so that insecticides with minimal nontarget effects can be preferentially used to both preserve natural enemies and prevent soybean aphid resurgence.

The soybean aphid natural enemy community in North America consists of numerous generalist predators (Costamagna et al. 2007), but relatively few abundant parasitoids (Nielsen and Hajek 2005, Ohnesorg et al. 2009, Brewer and Noma 2010). Native to Asia, the soybean aphid parasitoid *Aphelinus certus* Yasnosh, has become naturalized in Ontario (Frewin et al. 2010) and in the eastern United States (Heimpel et al. 2010). This parasitoid is relatively common in Ontario, and is believed to contribute to soybean aphid suppression and control (Frewin et al. 2010, Hallett et al. 2013).

At the time this research was conducted, two neonicotinoid seed treatments, imidacloprid and thiamethoxam, were registered for use on soybeans in North America. Thiamethoxam is currently registered for use on soybean in Ontario and the United States, whereas imidacloprid is only registered in the United States (North Central Soybean Research Program [NCSRP] 2009, OMAFRA 2009). In response to pest pressure primarily from bean leaf beetle (*Cerotoma trifurcata* (Forster)), but also from seed corn maggot (*Delia platura* (Meigen)), wireworms (Elateridae: various species), and soybean aphid, seed-applied insecticides are recommended for soybean production to provide residual protection against these pests at the beginning of the growing season (OMAFRA 2012). However, soybean aphid outbreaks in North America typically occur after the insecticidal activity of these seed treatments has diminished, and insecticide seed treatments have been purported to have little value for managing soybean aphid (Johnson et al. 2009). Because of the widespread use of insecticidal seed treatments in soybean production, their effects on soybean aphid natural enemies should be examined. If natural enemies are more susceptible to insecticides than is the soybean aphid, use of seed treatments may negatively impact soybean aphid population regulation by natural enemies. Alternatively, if soybean aphid natural enemies are less susceptible to these insecticides, seed treatments and natural enemies may have a complimentary effect on aphid control.

natural enemies may be directly exposed to seed-applied insecticides through consumption of contam-
inated prey or prey waste products (i.e., honeydew). Insecticides can have lethal or sublethal effects on natural enemies, including reduced longevity, fecundity, and impaired feeding or searching behavior (Desneux et al. 2007), which may reduce the ability of natural enemies to limit pest populations (Stark et al. 2004, 2007).

This study employed a tritrophic experimental system to examine the effects of imidacloprid- and thiamethoxam-treated soybeans on soybean aphid and its associated parasitoid A. certus. The purpose of this research was to determine the compatibility of neonicotinoid seed treatments with A. certus, by exposing the parasitoids to aphids feeding on neonicotinoid-treated soybean plants of different ages.

Materials and Methods

Soybean Plants. For insect colonies, soybean plants ‘Colby’ (Hyland Seeds, Thompsons Limited, Blenheim, ON, Canada) were grown in a commercial potting mix (Pro-Mix BX/Mycorise, Premier Horticulture Inc., Quakertown, PA) in standard plastic greenhouse pots 12.5 cm in diameter. Before planting, seeds were soaked in Cell-Tech Soybean Bradyrhizobium japonicum inoculant (EMD Crop Bioscience Canada, Belgrave, ON, Canada). Plants were watered as necessary and fertilized once weekly with Plant-Prod 20-20-20 All Purpose Fertilizer (Plant Products Co. Ltd., Brampton, ON, Canada). Plants were maintained in a greenhouse on the University of Guelph campus under summer conditions (18–32°C) with ambient light.

Insect Rearing. Soybean aphid colonies were established from individual stem mothers collected on soybean from several locations in southern Ontario in the fall of 2007, and maintained on soybean plants in a growth room at 27 ± 2°C, 65–75% relative humidity (RH), and a photoperiod of 16:8 (L:D) h.

A colony of A. certus was established from individuals collected in southern Ontario during the fall of 2007, for details on collection information and specimen identification refer to Frewin et al. (2010). These colonies were maintained in a growth room at 25 ± 2°C, 65–75% RH, and a photoperiod of 16:8 (L:D) h. Parasitoids were reared on soybean aphid on soybean for three generations before use in any experiment. Aphid mummies were collected daily from colonies, placed individually in 1.5-ml centrifuge tubes and supplied with a droplet of undiluted honey. Aphid mummies were monitored daily for wasp emergence. To obtain mated females for experimentation, a naive female and a male wasp <24 h old were paired and introduced into a 1.5-ml centrifuge tube until mating was observed. Pairs failing to mate within 10 min were discarded. Mated pairs were left for at least 1 h before being used in experiments, at which time the male was removed. All matings occurred between 9:00 and 11:00 a.m. EST.

Insecticidal Seed Treatments. Soybean seeds for the greenhouse experiments were treated with either Cruiser 5FS (47.6% a.i. thiamethoxam, Syngenta Crop Protection, Guelph, ON, Canada) or Gaucho (480 g/liter a.i. imidaclorpid, Bayer Crop Science, Guelph, ON, Canada) at rates of 83 and 260 ml per 100 kg of seed, respectively. Two kilograms of soybean seeds were placed in a heavy clear plastic bag and treated with equal parts formulated seed treatment and distilled water. The bag was inflated and tied shut and then agitated by hand for 15 min. Treated seeds were left to dry on a tray in a fume hood for 3 d before planting.

Experimental Design. Forty seeds of each insecticide treatment and untreated seeds (control) were planted individually in 12.5-cm-diameter pots on the first day of the experiment, and maintained as described above with the following modifications. The pots were placed in a completely randomized design on greenhouse benches and rerandomized weekly with enough pots for five replicates for each treatment. To prevent any transfer of insecticide between plants via drainage water, pots were placed directly on the mesh surface of greenhouse benches, allowing uninterrupted drainage after each watering. Plants were watered three times weekly and fertilized weekly.

Starting 4 wk after planting, five pots (replicates) from each treatment were selected randomly from the bench for the bioassay each week for 7 wk, for a total of 35 plants per treatment. Similar to the methods of Magalhaes et al. (2008, 2009), excised plant material was used for this bioassay. The youngest fully expanded trifoliate from each plant was removed, and two leaflets (chosen randomly) from each trifoliate were placed individually in paired 10-cm petri dishes lined with damp cotton batting. Leaflets were placed adaxial side down on the cotton batting and 25 fourth-instar soybean aphids were transferred onto each. Twenty-four hours after introduction of the aphids, a mated female A. certus was placed in one of the paired leaflet dishes from each plant for each treatment. Aphids in the other paired leaflet dish were not exposed to a parasitoid to determine life table parameters in the presence and absence of parasitism. Petri dishes were then sealed with Parafilm and maintained in a growth chamber at 25 ± 2°C, 65–75% RH, and a photoperiod of 16:8 (L:D) h. The parasitoid was removed after 24 h. Petri dishes were monitored daily for 8 d and the number of alive, dead, juvenile, and parasitized aphids was recorded. Dead aphids, juvenile aphids, and A. certus mummies were removed daily after observations were complete.

Statistical Analysis. The proportion of initial aphids parasitized after 8 d was subjected to analysis of variance (ANOVA) using PROC MIXED in SAS v9.1 (SAS Institute 2004). Variance was partitioned into the fixed effects of treatment, plant age (in weeks), treatment by plant age, number of aphids at 0 h, and number of aphids surviving 24 h; and the random effect, replica. For all analyses, F-tests were used to test for significant effects, and mean estimates were compared using Tukey’s procedure. For all analyses, the assumptions of the variance analysis were verified by plotting the residuals by predicted and by all fixed effects. The mean of residuals was equal to zero, and the Shapiro–Wilk test confirmed that the residuals were normally distributed.
A Type 1 error rate $\alpha = 0.05$ was used for all statistical tests.

To determine the effect of insecticide and parasitoid presence on the soybean aphid, two life table parameters were calculated. Intrinsic rate of increase ($r$) was calculated using formula [1] (Wyatt and White 1977):

$$r = 0.74 (\log_e M_d)/d$$  \[1\]

where $M_d$ is the number of progeny produced in 24 h and $d$ is the prereproductive time in days. Prereproductive time at 25°C was determined to be 4.9 d (McCornack et al. 2004). The net reproductive rate ($R_0$) was calculated using formula [2] (Wyatt and White 1977):

$$R_0 = \sum l_i m_i$$  \[2\]

where $l_i$ is the age-specific survivorship, and $m_i$ is the age-specific fecundity. Life table parameters were calculated for each replication within each treatment. $R_0$ and $r$ data were subjected to an ANOVA using PROC MIXED in SAS v9.1. Variance was partitioned into the fixed effects treatment, plant age, parasitoid presence, treatment by plant age, parasitoid presence by treatment, parasitoid presence by plant age, and the random effect, replication. When significant, linear and lack-of-fit regression partitions were included for the effect of plant age for each treatment.

Results

The effect of insecticide on proportion of aphids parasitized (parasitism rate) was significant, whereas plant age, plant age by treatment, the initial number of aphids, and the number of aphids surviving for 24 h were not (insecticide: $F = 26.75$; $df = 2.82$; $P < 0.0001$; plant age: $F = 0.86$; $df = 6.82$; $P = 0.53$; plant age by treatment: $F = 0.47$; $df = 12.82$; $P = 0.92$; initial aphid number: $F = 2.27$; $df = 1.82$; $P = 0.13$; number aphids alive after 24 h: $F = 1.09$; $df = 1.82$; $P = 0.30$). The number of parasitized aphids was highest in the control and not significantly different between the two insecticide treatments (Fig. 1). The first mummies in the thiamethoxam and imidacloprid treatments were found during weeks 5 and 6 of the study, respectively (Fig. 2); however, because of the low number of parasitized aphids in the treatments, no further analysis of these data were conducted. Emergence rates of A. certus from parasitized aphids were 97.1 ± 6.5, 84.7 ± 31.9, and 77.1 ± 34.5%, for the control, imidacloprid, and thiamethoxam treatments, respectively.

The effects of treatment ($F = 22.89$; $df = 2,127$; $P < 0.0001$), plant age ($F = 5.35$; $df = 4.127$; $P = 0.0005$), treatment by plant age interaction ($F = 2.12$; $df = 8.127$; $P = 0.0386$), and parasitoid presence ($F = 18.29$; $df = 1.127$; $P < 0.0001$) on the $R_0$ of soybean aphid were all significant, whereas the effects of treatment by parasitoid ($F = 1.19$; $df = 2.127$; $P = 0.3081$), and parasitoid by plant age interactions ($F = 1.07$; $df = 4.127$; $P = 0.3723$) were not.

The linear regression partition for the effect of plant age on $R_0$ in the imidacloprid treatment was also significant ($F = 20.62$; $df = 1.42$; $P < 0.0001$), whereas the lack-of-fit partition was not ($F = 1.74$; $df = 3.42$; $P = 0.17$). The linear regression partitions for the effect of plant age on $R_0$ in the control and thiamethoxam treatments were not significant (control: $F = 0.16$; $df = 1.42$; $P = 0.68$; thiamethoxam: $F = 3.63$; $df = 1.42$; $P = 0.06$).

The effects of treatment ($F = 19.91$; $df = 2,127$; $P = 0.0001$), plant age by treatment ($F = 2.20$; $df = 8.127$; $P = 0.0316$), and parasitoid presence ($F = 4.25$; $df = 1.127$; $P = 0.0414$) on the $r$ of soybean aphid were significant, but the effects of plant age ($F = 1.25$; $df = 4.127$; $P = 0.17$), treatment by parasitoid ($F = 1.74$; $df = 3.42$; $P = 0.2940$), and parasitoid by plant age interactions were not ($F = 0.01$; $df = 4.127$; $P = 0.9945$).
The linear regression partition for the effect of plant age on $r$ in the imidacloprid treatment was significant ($F = 13.17; \text{df} = 1.42; P < 0.05$), whereas the lack-of-fit partition was not ($F = 0.91; \text{df} = 3.42; P = 0.44$). The linear regression partitions for the effect of plant age on $r$ in the control and thiamethoxam treatments were not significant (control: $F = 0.37; \text{df} = 1.42; P = 0.54$; thiamethoxam: $F = 0.01; \text{df} = 1.42; P = 0.99$).

In the absence of a parasitoid, the $R_0$ of soybean aphids in the imidacloprid treatment increased linearly with plant age (Fig. 3). However, there was no change in $R_0$ over time in the control and thiamethoxam treatments. The $R_0$ of the soybean aphid was numerically lower in both insecticide treatments compared with the control at all plant ages. Similarly, in the absence of a parasitoid, the $r$ of soybean aphids in the imidacloprid treatment increased linearly with plant age (Fig. 4), but there was no change in $r$ in the control and thiamethoxam treatments. The $r$ of the soybean aphid was numerically lower in both insecticide treatments compared with the control at all plant ages.

The highest $R_0$ of soybean aphid occurred in aphids not exposed to parasitoids or to insecticide seed treatment (Table 1). $R_0$ values of soybean aphids in all treatments were numerically lower with parasitoid exposure than without; however, a significant difference between parasitoid exposure treatments was only found in the control treatment. The $R_0$ of soybean aphid in the parasitoid control treatment was significantly greater than that of soybean aphids in the parasitoid thiamethoxam treatment. For parasitoid exposed treatments, $R_0$ was lowest in soybean aphids in the thiamethoxam treatment. The $r$ of the soybean aphid was highest in the no-parasitoid control treatment and was significantly different from all other treatments, except the parasitoid control treatment.

**Discussion**

This study demonstrated that $A. certus$ can successfully parasitize soybean aphids feeding on excised leaves from plants grown from neonicotinoid treated seeds. However, although parasitoids were able to complete development on these aphids, the parasitism rate was reduced by 69–88%. There are three likely explanations for the reduction in parasitism observed in insecticide treatments. First, the reduction in parasitism could be a result of the functional response of $A. certus$. $A. certus$ exhibits a type II functional response to the soybean aphid (Frewin et al. 2010), thus a reduction in the number of available aphids would reduce the parasitism rate. However, this is not a likely explanation for our results, as the initial number of aphids available to the parasitoid and the numbers of aphids alive when the parasitoid was removed from the experimental arena were not significant effects in our model. Second, exposure to insecticide residues within the host aphid may have caused mortality of immature parasitoids, or parasitism may have increased the likelihood of aphid mortality in the presence of insecticide residues. Third, $A. certus$ may avoid parasitizing insecticide-poisoned aphids. *Aphelinus* parasitoids are known to use internal, rather than extracuticular, cues to assess host suitability, and are capable of detecting con- and hetero-specific parasitism and fungal infection within potential hosts (Bai and Mackauer 1991, Mesquita et al. 1997, Mesquita and Lacey 2001). It is possible that these parasitoids use stress- or immune-related aphid-produced chemicals to assess host quality. If true, *Aphelinus* parasitoids should be able to reject a host that is stressed or injured for a variety of reasons, including insecticide-poisoning. If this is the case, then these two control mechanisms (i.e., parasitoids and insecticide seed treatments) may be complementary, as the parasitoid
selects only the healthiest hosts within the insecticide-exposed populations, therefore, increasing overall soybean aphid mortality.

Analyses of life table parameters showed that imidacloprid and thiamethoxam both had lethal and sub-lethal effects on soybean aphid. Across all treatments, soybean aphids exposed to contaminated plant material suffered a reduction in $r$ and $R_0$, a finding consistent with other work (Magalhaes et al. 2008, 2009). In addition to insecticide effects, parasitism numerically reduced $R_0$ and $r$ of the soybean aphid in all treatments. However, no additive effect of parasitism and
Fig. 4. The effect of plant age on the $r$ of the soybean aphid reared on excised soybean leaves from untreated plants or plants grown from seed treated with imidacloprid or thiamethoxam, in the absence of parasitoids. Within a treatment means with different letters are significantly different (Tukey’s HSD, $\alpha = 0.05$), $n = 35$ for each treatment.
insecticide in reducing life table parameters of the soybean aphid was evident. The lack of effect is likely because of the low parasitism rate found in the insecticide treatments. If the reduction in parasitism is due to insecticide-related mortality of A. certus larvae, then as the insecticide titer in the plant diminishes with plant age, an additive effect of parasitism and insecticide may become apparent.

The \( r \) for soybean aphid not exposed to parasitoids nor to insecticides was 0.37 per day, similar to values reported elsewhere (Magalhaes et al. 2008). However, estimates of \( r \) for soybean aphid in controlled environment chambers are higher than our estimate, for example 0.474 per day at 25°C (McCornack et al. 2004) and 0.415 per day at 22°C (Myers et al. 2005). Variability in \( r \) may be because of differences in test conditions, including temperature, humidity, light, and enclosure size. In addition, aphids in the current study originated from Ontario, whereas those used by McCornack et al. (2004) and Myers et al. (2005) originated from Nebraska, Minnesota, Indiana, and Wisconsin. Although the population genetic structure of soybean aphid in North America indicates a single population, populations from Michigan and Ontario are more similar to each other genetically than to populations in the southern and midwestern United States (Michel et al. 2009).

The \( r \) and \( R_0 \) of soybean aphids increased in a linear fashion in the imidacloprid treatment as plants aged. This result is likely due to aphid exposure to decreasing concentrations of imidacloprid in the leaves as plants aged. However, there was no change in these life history parameters in the thiamethoxam treatments, indicating that thiamethoxam has a longer residual activity than imidacloprid at the rates tested. More important is the fact that, while the \( R_0 \) of aphids increased as the plants aged in the imidacloprid treatment, there was no corresponding increase in parasitism rate. Thus, A. certus appears to be less tolerant than the soybean aphid to imidacloprid, and imidacloprid seed-treatments could therefore hinder the ability of A. certus to limit soybean aphid population growth.

An increasing parasitoid population early in the season is considered extremely important to the success of biological control in field crops, as natural enemies often impact pest populations most dramatically at low densities before serious outbreaks occur (Barbosa 1998). Imidacloprid and thiamethoxam dramatically reduced the parasitism rate of A. certus, which could well impair the ability of A. certus to control soybean aphid. Furthermore, it is unknown if these insecticides will have sublethal effects on A. certus emerging from contaminated hosts, which could further impair their ability to control aphid infestations.

Natural enemies provide important biological control services and can limit soybean aphid population growth (Hallett et al. 2013). Imidacloprid and thiamethoxam seed treatments reduced biological control services provided by A. certus for at least 10 wk after planting under greenhouse conditions. Similar results are expected in the field in terms of the dose–response of the parasitoid and aphid to the insecticide titer in treated plants; however, it is unclear how field conditions may affect the parasitoid titer in plants over time compared with greenhouse grown plants. Regardless, our results indicate that A. certus is more susceptible to imidacloprid and thiamethoxam than is the soybean aphid. Thus, it is important to weigh the need for seed treatments for early season soybean aphid control against the risk they pose to important natural enemies, especially in regions, such as Ontario, where A. certus is the dominant soybean aphid parasitoid and vegetative stage infestations of soybean aphid are rare (Hallett et al. 2013). Furthermore, the utility of seed treatments for soybean aphid management are questionable given that aphid infestations typically occur after the insecticidal activity of these chemicals has diminished (Johnson et al. 2009). Further studies are needed to determine whether and when the life table parameters of soybean aphid and the parasitism rates of A. certus return to normal levels on plants grown from insecticide-treated seed under field conditions.

### Acknowledgments

We thank K. Eddington, for assistance with insect colony and plant maintenance and data collection; M.K. Sears for providing feedback on experimental design; and Syngenta Crop Protection Canada and Bayer CropScience Canada for supplying insecticides. This research was supported by grants to R.H.H. and A.W.S. from the Agriculture and Agri-Food Canada Pesticide Risk Reduction Program and the Ontario Ministry of Agriculture, Food and Rural Affairs–University of Guelph Sustainable Production Program.

### References Cited


