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Research Article

Efficacy of afidopyropen against soybean aphid (Hemiptera: Aphididae) and toxicity to natural enemies

Running title: Afidopyropen for soybean aphid management

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ABSTRACT

BACKGROUND: Soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), remains the most significant soybean insect pest in the North Central Region of the United States. The sustainability of reliance on only a few insecticide groups for this pest is questionable. We evaluate afidopyropen, a novel pyropene insecticide (Group 9D), for efficacy against *A. glycines* in field and greenhouse experiments and toxicity to common natural enemies in laboratory experiments.

RESULTS: Across four site-years of field experiments and a greenhouse experiment, afidopyropen reduced *A. glycines* populations similar to commonly used broad spectrum (i.e., lambda-cyhalothrin (Group 3A) and chlorpyrifos (Group 1B)) insecticides and potential selective insecticides (i.e., sulfoxaflor (Group 4C) and flupyradifurone (Group 4D)). In the greenhouse, however, *A. glycines* mortality was delayed slightly for afidopyropen compared to the other insecticides. In laboratory experiments with natural enemies of *A. glycines*, afidopyropen was not toxic to adult or third instar *Hippodamia convergens* (Coleoptera: Coccinellidae) or adult *Orius insidiosus* (Hemiptera: Heteriptera), and was only moderately toxic to *Aphelinus certus* (Hymenoptera: Encyrtidae).

CONCLUSION: Afidopyropen is effective against *A. glycines* and relatively nontoxic to natural enemies, and appears to be an effective option for integrated pest management and insecticide resistance management programs for *A. glycines*.

Keywords: Integrated pest management; selective insecticide; *Aphis glycines*; *Aphelinus certus*; *Orius insidiosus*; *Hippodamia convergens*

1. INTRODUCTION

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the most significant insect pest of soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), production in North Central Region of the United States.[1,2] Infestation of soybean by *A. glycines* can decrease plant biomass, pod number, seed number, seed size, and concentration of oil in seed.[1,3] In addition, *A. glycines* can vector Soybean mosaic virus and Alfalfa mosaic virus,[4,5] and facilitate growth of saprophytic sooty mold fungi on aboveground plant parts.[6] Yield reductions in soybean of up to 40% have been attributed to this pest.[7]

Pest management recommendations for *A. glycines* in soybean rely primarily on scouting and threshold-based applications of broad-spectrum insecticides.[8,9] The economic threshold for *A. glycines* is 250 aphids per plant with 80% of plants infested, and populations increasing.[7] Foliar insecticides used for *A. glycines* management are generally from only three insecticide sub-groups: pyrethroids (Group 3A), organophosphates (Group 1B) and neonicotinoids (Group 4C),[10] with pyrethroids and organophosphates most commonly used.[8] This approach to management of *A. glycines* has proven effective;[11-13] however, reliance on relatively few broad-spectrum insecticides increases risks for ecological backlash through impacts to natural enemies and development of resistance in the pest.[14]

Aphis glycines is attacked by a diverse suite of natural enemies, including predators, parasitoids and pathogens.[1,15] Predators, such as Coccinellidae and Anthocoridae, contribute to suppression of *A. glycines*.[1,16] In addition, the parasitoid, *Aphelinus certus* Yasnosh

(Hymenoptera: Encyrtidae), is becoming a more prominent natural enemy of *A. glycines* in the North Central Region of the United States.[17-19] Insecticides commonly used for management of *A. glycines* have relatively broad-spectrum activity and are detrimental to predators and parasitoids.[20-23] In contrast, conservation biological control using selective insecticides (i.e., those with reduced risk to natural enemies) holds promise to better integrate biological and chemical controls. Selective insecticides, such as flonicamid (Group 29), pymetrozine (Group 9B), spirotetramat (Group 23), and sulfoxaflor (Group 4C), have been evaluated for efficacy against *A. glycines* and toxicity to natural enemies,[24-29] but currently lack registration for use in soybean in the United States. Flupyradifurone (Group 4D), however, has shown selectivity in other systems[30] and is registered for use in the United States. Selective insecticides could improve the integration of chemical and biological controls for *A. glycines* and improve sustainability of soybean production.

The potential for pests to develop resistance to insecticides is an additional concern. Insecticide resistance is a genetically based reduction in susceptibility of a pest to an insecticide.[31] Reliance on insecticides often results in pests developing resistance to those insecticides.[14] Resistance of *A. glycines* to pyrethroid insecticides was first documented in North America in 2015.[32] Laboratory bioassays showing resistance and reports of pyrethroids failing to control field infestations of *A. glycines* indicate a recurring problem (i.e., multiple consecutive years) covering a broad geography (i.e., Minnesota, Iowa, South Dakota, North Dakota and Manitoba).[10,32] Pyrethroid resistance in *A. glycines* threatens the current insecticide-based

management programs for this pest.[10] Additional insecticide modes of action are needed for more robust insecticide rotations to mitigate development of further resistance by *A. glycines*.

A novel pyropene insecticide, afidopyropen (Group 9D), which received registration from the United States Environmental Protection Agency (US EPA) for use in soybean in 2018, may prove an effective insecticide for management of *A. glycines* by offering an alternate insecticide group for resistance management as well as provide selectivity to conserve natural enemies. This insecticide is a semi-synthetic derivative of a fermentation product called pyripyropene, which is produced by the microbe, *Aspergillus fumigatus* Fresenius (Eurotiales: Trichocomaceae).[33-35] Afidopyropen acts on the vanilloid-type transient receptor potential (TRPV) channels of the chordotonal stretch receptor neurons of insects.[36,37] Chordotonal receptors serve a mechanosensory role in insects by detecting movement at articulations.[38] This insecticide targets sap-feeding Hemiptera, such as Aphididae,[39-41] Aleyrodidae,[41] and Liviidae,[42-44] by inhibiting feeding, which leads to starvation, desiccation and mortality.[33,39] In contrast, afidopyropen has shown little to no toxicity to species in other insect taxa, including Blattellia, Blattidae, Diptera, and Coleoptera.[39] To determine the potential role of afidopyropen in *A. glycines* management programs, we performed field and greenhouse experiments to evaluate the efficacy of this insecticide against *A. glycines* and laboratory experiments to evaluate its toxicity to some representative natural enemies, including *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), and *A. certus*.

2. MATERIALS AND METHODS

2.1. Field efficacy experiments with *A. glycines*

Field experiments were performed over 4 site-years in Minnesota and Iowa. In each site year, the experimental design was a randomized complete block with four replications of each treatment. In Minnesota, experiments were performed at the University of Minnesota Southwest Research and Outreach Center near Lamberton, Minnesota, in 2016 and 2017. Untreated (i.e., no insecticidal seed treatment) 'P22T69' (Pioneer, Johnston, IA, United States) soybean seed was sown 1 May 2016 and untreated 'AG2015' soybean seed (Asgrow Seed Company, Kalamazoo, MI, United States) was sown on 30 May 2017. For both site-years in Minnesota, seed was sown with 76.2-cm row spacing and plots were established that were four rows wide and 9.1 m long. In Iowa, experiments were performed at Iowa State University's Northeast Research Farm near Nashua, Iowa, and Northwest Research Farm near Sutherland, Iowa, in 2017. Untreated 'NK S24-K2' soybean seed (Syngenta, Basel, Switzerland) was sown with 76.2-cm row spacing in plots that were 6 rows wide and 15.2 or 13.4 m long on 14 and 30 May 2017 at the Northeast and Northwest Research Farms, respectively.

At the Southwest Research and Outreach Center, insecticides were applied on 9 August 2016 and 11 August 2017 (R4 soybean growth stage[45]) using TeeJet 8001XR nozzles (TeeJet Technologies, Springfield, IL United States) with 140.3 L/ha and 275.8 kPa. At the Northeast Research Farm, insecticides were applied on 18 August 2017 (R5 soybean growth stage) using TeeJet 11002 twinjet nozzles (TeeJet Technologies, Springfield, IL, United States) with 187.0 L/ha and 275.8 kPa. At the Northwest Research Farm, insecticides were applied on 18 August

2017 (R5 soybean growth stage) using TeeJet 8002 flat fan nozzles (TeeJet Technologies, Springfield, IL, United States) with 130.9 L/ha and 275.8 kPa. Treatments included afidopyropen, flupyradifurone, sulfoxafor, lambda-cyhalothrin, chlorpyrifos, and an untreated check (Table 1). These treatments, which represent a subset of treatments from larger insecticide efficacy trials, were selected to compare the efficacy of afidopyropen to commonly used broad-spectrum insecticides (i.e., lambda-cyhalothrin and chlorpyrifos) and to insecticides with a narrower spectrum (i.e., sulfoxaflor and flupyradifurone).

In each site-year, *A. glycines* were counted (nymphs and adults combined) on randomly-selected whole plants within each plot on weekly intervals through vegetative and reproductive growth stages in Iowa and from trial initiation through reproductive growth stages in Minnesota. In Minnesota, the number of plants inspected was 3 plants per plot prior to treatment and 5 plants per plot after treatment. In Iowa the number of plants inspected ranged from 3 to 20 plants per plot and was determined by soybean growth stage and by the proportion of plants infested.[46] Plots were harvested using small-plot combines on 11 October 2016 and 18 October 2017 at the Southwest Research and Outreach Center, and on 3 and 20 October 2017 at the Northeast Research Farm and Northwest Research Farm, respectively. Soybean grain yields were adjusted to 13% moisture.

2.2. Greenhouse efficacy experiment with *A. glycines*

A greenhouse experiment was performed at the University of Minnesota, St. Paul, Minnesota, in June 2018. Untreated (i.e., no insecticidal seed treatment) 'SD01-76R' soybean

seed were sown in potting soil (Sunshine MVP, Sun Gro Horticulture Products, Agawam, MA, United States) in small pots (10 × 10 × 10 cm) with two soybean plants per pot. Potted plants were maintained in an environmental growth chamber at approximately 25°C, 70% RH, and a photoperiod of 16:8 h (L:D). Soybean plants were watered twice per week. Plants were infested at the V1 soybean growth stage[45] with 20 mixed-age *A. glycines*. Five days (d) after infestation, the number of live *A. glycines* (nymphs and adults combined) were counted on each plant. On this same day, plants were sprayed using a motorized spray chamber[47] calibrated prior to each replication to deliver 233 L/ha of spray at 242 kPa with a single TeeJet 8002 flat fan nozzle (TeeJet Technologies, Springfield, IL United States). The experiment was performed as a randomized complete block design with 5 treatments and 10 replications with two soybean plants per replication, for a total of 20 soybean plants per treatment. Treatments included afidopyropen, flupyradifurone, sulfoxafor, lambda-cyhalothrin, and an untreated control (i.e., water) (Table 1). After application of insecticides, plants from each replicate were placed in separate cages (45 × 68 × 70 cm; L × W × H) in a greenhouse. The number of live *A. glycines* was recorded 1, 2, 5 and 7 d after application.

2.3. Laboratory experiments with natural enemies

Toxicity of insecticide residues to natural enemies was assessed in December 2017 to April 2018. For all bioassays with natural enemies, treatments included lambda-cyhalothrin, afidopyropen, and an untreated control (i.e., water). The interior surfaces of Petri dishes were treated using the motorized spray chamber and using methods described above. After

application, dishes were allowed to dry for 1.5 hours (h) prior to transfer of insects to the dishes. Predators were received from a commercial supplier (Arbico Organics, Oro Valley, AZ, United States).

Hippodamia convergens adults were shipped overnight and used within 24 h after arrival. Groups of five *H. convergens* were transferred to treated Petri dishes (100 × 15 mm). Dishes were placed inside a growth chamber at 25°C, 55–60% RH and a photoperiod of 16:8 h (L:D). The experiment was performed as a completely randomized design with 3 treatments with 5 independent replications. Each replication contained 2 petri dishes of 5 individuals for a total of 50 insects per treatment. After 24 h in the treated dishes, insects were transferred to clean Petri dishes and provided with water-moistened florist foam and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs *ad libitum* as food source. Food and water were replenished every 2 d. Mortality was checked daily and insects were rated as live (i.e., ability to walk and showing coordinated movement), moribund (i.e., inability to walk and other uncoordinated movement), or dead (i.e., no movement).

Hippodamia convergens larvae were reared from the population obtained for the previous bioassay with adults. Larvae were reared individually to the third instar in Petri dishes (60 × 15mm) on a diet of *E. kuehniella* eggs and water. Third instars were transferred individually to treated Petri dishes (60 × 15mm) and placed inside a growth chamber at 25°C, 55–60% RH and a photoperiod of 16:8 h (L:D) for 24 h. The experiment was performed as a completely randomized design with 3 treatments and 6 independent replications. Each replication consisted

of 5 individuals, totaling 30 individuals per treatment. After 24 h in treated dishes, the larvae were transferred to clean Petri dishes provisioned with water and *E. kuehniella* eggs *ad libitum* as food source. Food and water were replenished every 2 d. Mortality was recorded every 2 d in the same manner as the adult bioassay.

Orius insidiosus adults were shipped overnight and used within 2 h after arrival. Different routes of exposure were tested in this experiment, residual and ingestion. Groups of 5 individuals were transferred to treated Petri dishes (100 × 15 mm), with each dish containing 35 plastic beads (approximately 9 × 9 mm) and one whole organic green bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae). Beads and beans were treated using the motorized spray chamber following the methods described above and were allowed to dry for 1.5 h prior to use in experiments. Beads provided increased surface area to decrease cannibalism[28,29] and beans provided an additional food source for *O. insidiousus*. Each dish was then sealed around 50% of the perimeter with a laboratory film (Parafilm M®, Neenah, WI, United States) to prevent escape of the insects, while maintaining some ventilation. The experiment was performed as a completely randomized design with 3 treatments and 5 independent replications. Each replication contained 2 petri dishes of 5 individuals for a total of 50 insects per treatment. After 24 h, insects were transferred to untreated petri dishes and provisioned with water through water-moistened florist foam and *E. kuehniella* eggs *ad libitum* as food. Food and water were replenished every 2 d. Mortality was recorded for 1, 2, 3, 5 and 7 d after application, with survival assessed as previously described.

A colony of *A. certus* was initiated using field-collected individuals from Minnesota in 2012. Pots of aphid-infested soybean plants were placed in clear acrylic cages (30 × 35 × 39 cm) with 25 to 40 *A. certus* females added to each cage. From these cages, approximately 200 parasitized aphid mummies were collected 4 d prior to the experiment and transferred to micro-centrifuge tubes containing a small drop of a 1:1 mixture of honey and water, and held inside a growth chamber at 25°C, 55–60% RH and a photoperiod of 16:8 h (L:D) until adult emergence. One adult *A. certus* was transferred to each treated Petri dish (60 × 15mm) with a small drop of the 1:1 mixture of honey and water. Dishes with insects were placed inside a growth chamber at 25°C, 55–60% RH and a photoperiod of 16:8 h (L:D) for 24 h. The experiment was performed as a completely randomized design with 3 treatments and 6 independent replications. Each replication consisted of 5 individuals, totaling 30 individuals per treatment. After 24 h in treated dishes, *A. certus* were transferred to untreated Petri dishes and provisioned with a 1:1 honey and water mixture as food source. Mortality was recorded at 1, 2, 4 and 7 d after application and survival was assessed as previously described.

2.4. Analyses

Data were analyzed using R Development Core Team software (version 3.5.0).[48] For the field and greenhouse experiments, cumulative aphid days (CAD) were calculated according to Hanafi et al.[49] to compare exposure of soybean to *A. glycines* among treatments using mixed effect models with normal distributions using the function “lmer” from package “lme4”. CAD was calculated at the plot level for the field experiment and the plant level for the greenhouse

experiment. The units of replication for analyses for the field and greenhouse experiments were the plots and pots, respectively. Prior to all analyses of each data set, conformity of the data to assumptions of analyses were assessed via visual inspection of qqplot, histograms of residuals, and plots of the residuals against fitted value. In the final model for the field experiments, log-transformed CAD was described as a function of a fixed effect for treatment and a random effect for plot. To determine the best model for the greenhouse experiment, a likelihood-ratio-test was used to compare random effects (plant or plant nested in block) that should be included in the models. In the final model for the greenhouse experiment, log-transformed CAD was described as a function of a fixed effect for treatment and a random effect for plant nested in pot. Yield from field experiments was described as function of insecticides as a fixed effect and plot as random effect. For the above analyses, overall significance of treatment was calculated using a Type II chi-square test (ANOVA type II) using the function "Anova" from package "car". Mean separations among treatments were performed using Tukey HSD tests using package "lsmeans"[50] and package "MulticompView" with $\alpha = 0.05$.[51]

To compare survival of each natural enemy species or life stage after exposure to treatments, data were analyzed using a generalized linear mixed effect model with the function "glmer" from the package "lme4"[52] with a binomial response variable (i.e., dead or alive) and logit as the link-function. The units of replication for these analyses were as described in the methods for these experiments, and were not the individual insects. Survival at 7 d was described as a function of a fixed effect for treatment and a random effect for replication. Mean separations

among treatments were performed using Tukey HSD tests using package "lsmeans"[50] and package "MulticompView" with $\alpha = 0.05$.[51]

3. RESULTS

3.1. Field efficacy experiments with *A. glycines*

In Minnesota, experiment-wide mean (±SEM) *A. glycines* densities prior to application of insecticides were 264 ± 32 and 273 ± 27 aphids per plant at the Southwest Research and Outreach Center in 2016 and 2017, respectively (data not shown). Peak *A. glycines* densities in untreated plots reached 663 ± 234 and 289 ± 122 aphids per plant, respectively, on 22 August 2016 and 14 August 2017 (data not shown). Mean CAD at the final sample date in plots treated with insecticides did not differ from one another, but were lower than that of untreated in 2016 ($\chi^2 = 35.9$, df = 3, $P < 0.0001$) (Figure 1A) and 2017 ($\chi^2 = 13.0$, df = 3, $P = 0.005$) (Figure 1B).

In Iowa, experiment-wide mean *A. glycines* densities prior to application of insecticides were 9 ± 4 and 125 ± 17 aphids per plant at the Northeast Research Farm and Northwest Research Farm, respectively (data not shown). At the Northeast Research Farm and Northwest Research Farm, peak *A. glycines* densities in untreated plots reached 132 ± 39 and 650 ± 129 aphids per plant, respectively, on 11 September (data not shown). At the Northwest Research Farm, mean CAD at the final sample date in insecticide-treated plots did not differ from one another, but were lower than that of the untreated plots ($\chi^2 = 12.99$, df = 3, $P = 0.005$) (Figure 1C). At the Northeast Research Farm, mean CAD at the final sample date in plots treated with

afidopyropen, flupyradifurone, sulfoxaflor or chlorpyrifos did not differ from one another and were lower than that of untreated plots; however, plots treated with lambda-cyhalothrin did not differ from untreated plots or plots treated with the other insecticides ($\chi^2 = 35.91$, df =3, P < 0.0001) (Figure 1D).

In Minnesota, mean soybean yields at the Southwest Research and Outreach Center in 2016 were greater in the insecticide treated plots (3.77 ± 0.09 to 3.82 ± 0.05 metric ton per ha) compared to untreated plots (3.47 ± 0.05 metric ton per ha) ($\chi^2 = 20.6$, df = 3 P = 0.001). At the Southwest Research and Outreach Center in 2017, mean soybean yields ranged from 4.03 ± 0.13 to 4.17 ± 0.05 metric ton per ha, but did not differ among treatments ($\chi^2 = 0.9$, df = 3 P = 0.83). In Iowa, mean soybean yields ranged from 3.88 ± 0.13 to 4.11 ± 0.05 metric ton per ha at the Northeast Research Farm and from 3.86 ± 0.10 to 4.19 ± 3.50 metric ton per ha at the Northwest Research Farm, but yields did not differ among treatments at either location (Northeast Research Farm: $\chi^2 = 5.9$, df = 5, P = 0.30; Northwest Research Farm: $\chi^2 = 6.6$, df = 5, P = 0.25).

3.2. Greenhouse efficacy experiment with *A. glycines*

Prior to treatment, mean *A. glycines* densities were 66.1 ± 2.4 aphids per plant and peaked at 493 ± 60 *A. glycines* per plant for untreated plants at 7 d after application (data not shown). Mean CAD at the final sample date for insecticide treated plants was lower than that of the untreated plants ($\chi^2 = 1226.1$, df = 4, P < 0.0001) (Figure 2). Among the insecticide treatments, mean CAD was lowest on flyupyradifurone treated plants, intermediate on

sulfoxaflor and lambda-cyhalothrin treated plants, and highest on afidopyropen treated plants (Figure 2).

3.3. Laboratory experiments with natural enemies

Mean proportion survival of all natural enemies at the final sample date was significantly affected by treatment (*H. convergens* adults: $\chi^2 = 29.6$, df = 2, $P < 0.0001$; *H. convergens* larvae: $\chi^2 = 25.2$, df = 2, $P < 0.0001$; *O. insidiosus* adults: $\chi^2 = 63.5$, df = 2, $P < 0.0001$; *A. certus* adults: $\chi^2 = 46.7$, df = 2, $P < 0.0001$) (Figure 3A-D). For both life stages of *H. convergens* and adults of *O. insidiosus,* survival in dishes treated with afidopyropen did not differ from that of the untreated dishes, while survival in dishes treated with lambda-cyhalothrin was less than that in the other two treatments (Figure 3). For adult *A. certus*, survival in dishes treated with either insecticide was lower than that in the untreated dishes, but survival in dishes treated with afidopyropen was greater than in dishes treated with lambda-cyhalothrin (Figure 3). By the final sample date, all individuals were either dead or alive (i.e., no moribund individuals). However, prior to this, moribund individuals were observed particularly for *H. convergens* exposed to lambda-cyhalothrin (Figure 4C,F) and *A. certus* exposed to afidopyropen (Figure 4K). Over time, many of those moribund individuals recovered (i.e., change from moribund to live), but no dead individuals recovered (i.e., none changed from dead to live or moribund) (Figure 4).

4. DISCUSSION

Management of *A. glycines* continues to rely on threshold-based applications of foliar insecticides.[8,9] Due to development of resistance to pyrethroids by *A. glycines*[10,32] and continued regulatory threats to registration of organophosphates (i.e., chlorpyrifos)[53] and neonicotinoids,[54] there is a need for additional insecticide groups for continued management of this pest.[10] Afidopyropen is a novel pyropene insecticide (Group 9D) toxic to Aphididae[39-41] and currently the only member of this insecticide sub-group. The present study provides the first evaluation of the potential role of afidopyropen in management programs for *A. glycines*, the most significant insect pest of soybean in North America.[2]

Under field conditions, afidopyropen reduced exposure of soybean to *A. glycines* (i.e., cumulative aphid days, CAD) similar to commonly used broad spectrum insecticides (i.e., lambda-cyhalothrin and chlorpyrifos) and potential selective insecticides (i.e., sulfoxaflor and flupyradifurone) (Figure 1). At the Northeast Research Farm in Iowa, where efficacy of lambda-cyhalothrin was moderate (Figure 1D) and suggestive of the presence of pyrethroid-resistant *A. glycines*, afidopyropen and the other non-pyrethroid insecticides effectively suppressed *A. glycines*. Cross resistance between afidopyropen and other insecticides has not been documented.

Afidopyropen acts by quickly inhibiting feeding and disorienting aphids, which leads to mortality by starvation and desiccation.[33,39] In many experiments, including those described here, determination of insecticide efficacy is based on number of insects present and does not consider whether those insects are feeding. In the greenhouse, CAD for *A. glycines* on plants treated with afidopyropen increased slightly through day 5, but stopped increasing after day 5 (Figure 3).

While feeding cessation occurs quickly, time to mortality (and subsequent reduction in aphid densities) will fluctuate based on ambient conditions with the controlled environment of a greenhouse being conducive to a longer survival period than natural weather conditions in a soybean field.

Pymetrozine (Group 9B) and flonicamid (Group 29) also affect chordotonal receptors.[36,37] Pymetrozine and afidopyropen both activate the TRPV channels of the chordotonal receptors, though afidopyropen binds to these sites with greater affinity than pymetrozine.[36] Unlike afidopyropen and pymetrozine, flonicamid does not activate the TRPV channels and is assumed to act on a different target site on the chordotonal organs.[36] Leichter et al.[39] found afidopyropen to be 43- and 75-fold more toxic to *A. pisum* than flonicamid and pymetrozine, respectively, based on comparison of LC_{50} values for each insecticide. Both insecticides have been evaluated for efficacy against *A. glycines*.[24,25,28] Under field conditions, pymetrozine provided intermediate levels of *A. glycines* control and yield protection compared to lambda-cyhalothrin.[24] Under field and greenhouse conditions, flonicamid provided a level of *A. glycines* control similar to that of lambda-cyhalothrin.[25]

Considering the challenges posed by pyrethroid-resistant *A. glycines*, Koch et al.[10] indicated that management programs for *A. glycines* should move from nearly sole reliance on chemical control to integration of other non-chemical management tactics. Conservation biological control, through the use of selective insecticides, provides a means for better integration of chemical and biological controls for pests.[21] Kumar et al.[55] showed potential for

integrating afidopyropen and a predatory mite (*Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae)) for management of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Based on assessment of survival on the final sample dates in the laboratory experiments of the present study, afidopyropen was not toxic to *H. convergens* adults and larvae nor to *O. insidiosus* adults, and was only moderately toxic to *A. certus* when compared to lambda-cyholothrin (Figure 3). The relative lack of toxicity of afidopyropen to natural enemies is not surprising considering that this insecticide has shown little to no toxicity to a range of other taxa (i.e., Blattodea, Diptera, and Coleoptera).[39] Similarly, flonicamid was not toxic to *H. convergens* adults and showed no to moderate toxicity to *O. insidiosus* adults,[28] and was not toxic to *A. certus*.[26] Furthermore, soybean treated with pymetrozine in a field study had intermediate abundance of natural enemies of *A. glycines* compared to plots treated with lambda-cyhalothrin and an untreated control.[24] However, over the course of the experiments in the present study, a knock-down effect was observed for some insecticides, as indicated by moribund individuals later recovering (Figure 4). While moribund, such individuals could be considered 'functionally dead' because they are unlikely to contribute to biological control. The knock-down effect of lambda-cyhalothrin on *H. convergens* has been observed in other studies.[56] Further research could examine the knock-down effect of afidopyropen on *A. certus*.

5. CONCLUSIONS

Use of afidopyropen as an additional chemical tool for *A. glycines* management could contribute to improved sustainability of soybean production in the region. Afidopyropen and the potential selective insecticides (i.e., sulfoxaflor and flupyradifurone) provided levels of *A. glycines* control similar to commonly used broad-spectrum insecticides. The relative lack of toxicity of afidopyropen to natural enemies suggests this insecticide, along with other selective insecticides, could improve integration of chemical and biological controls for *A. glycines*. In addition, afidopyropen would provide an additional option for alternation of insecticide groups for insecticide resistance management, beyond the current reliance on and alternation among only three insecticide groups.

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Table 1. Rates (amount of product per hectare) of insecticides used in field and greenhouse efficacy experiments for *Aphis glycines* and toxicity experiments in laboratory experiments for natural enemies

Site-year	Afidopyropen[a]	Flupyradifurone[b]	Sulfoxaflor[c]	Lambda-cyhalothrin[d]	Chlorpyrifos[e]
Southwest Research and Outreach Center, MN, 2016	219.2 mL	--	52.5 g	116.9 mL	--
Southwest Research and Outreach Center, MN, 2017	219.2 mL	--	--	138.8 mL	1,169.2 mL
Northeast Research Farm, IA, 2017	219.2 mL	511.5 mL	70.1 g	138.8 mL	1,169.2 mL
Northwest Research Farm, IA, 2017	219.2 mL	511.5 mL	70.1 g	138.8 mL	1,169.2 mL
Greenhouse, MN	200.0 mL	1,023.1 mL	70.1 g	138.8 mL	--

Laboratory, MN	200.0 mL	--	--	116.9 mL	--

[a] Sefina (Group 9D), BASF, Ludwigshafen, Germany
[b] Sivanto Prime (Group 4D), Bayer Crop Sciences, Leverkusen, Germany
[c] Transform WG (Group 4C), Dow AgroSciences, Indianapolis, U.S.
[d] Warrior II with Zeon Technology (Group 3A), Syngenta, Basel, Switzerland
[e] Lorsban Advanced (Group 1B), Dow AgroSciences, Indianapolis, U.S.

Fig. 1. Mean cumulative aphid days (CAD) by date from field experiments examining efficacy of insecticides against *Aphis glycines* at the Southwest Research and Outreach Center, Lamberton, Minnesota in 2016 (A) and 2017 (B), and the Northwest Research Farm, Sutherland, Iowa (C) and the Northeast Research Farm, Nashua, Iowa (D) in 2017. Analyses were performed on CAD at the last sample date for each experiment. Different letters in superscripts after treatment names in legends indicate differences among mean CAD values within each experiment (Tukey HSD, $P > 0.05$).

Fig. 2. Mean cumulative aphid days (CAD) by date from a greenhouse experiment examining efficacy of insecticides against *Aphis glycines*. Analyses were performed on CAD at the last sample date. Different letters in superscripts after treatment names in the legend indicate differences among mean CAD values (Tukey HSD, $P > 0.05$).

Fig. 3. Mean survival of natural enemies (adult *Hippodamia convergens* [A]; third-instar *H. convergens* [B]; adult *Orius insidiosus* [C] and adult *Aphelinus certus* [D]) at 7 d after exposure from laboratory experiments examining toxicity of dried residues of insecticides. Different letters above bars indicate differences among mean survival values within each experiment (Tukey HSD, $P > 0.05$).

Fig. 4. Mean proportion of live (light gray), moribund (light gray with diagonal black lines) and dead (dark gray) natural enemies (adult *Hippodamia convergens* [A, B and C]; third-instar *H.*

convergens [D, E and F]; adult *Orius insidiosus* [G, H and I] and adult *Aphelinus certus* [J, K and L] from laboratory experiments examining toxicity of dried residues of insecticides (untreated [A, D, G and J]; afidopyropen [B, E, H and K] and lambda-cyhalothrin [C, F, I and L]).

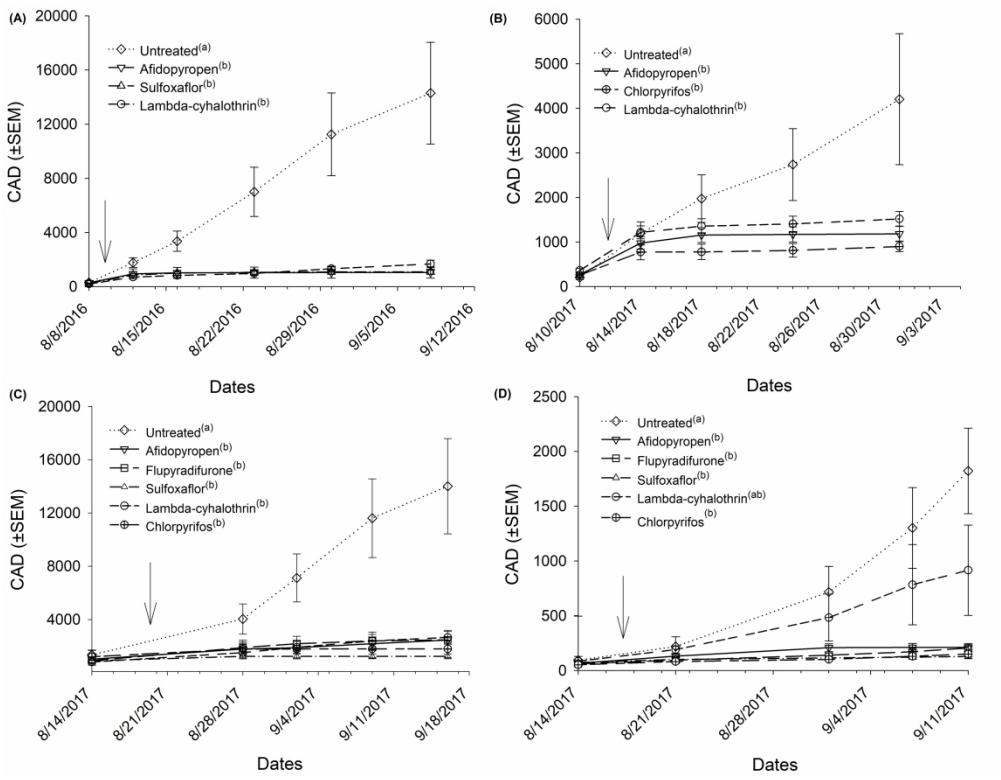

Fig. 1

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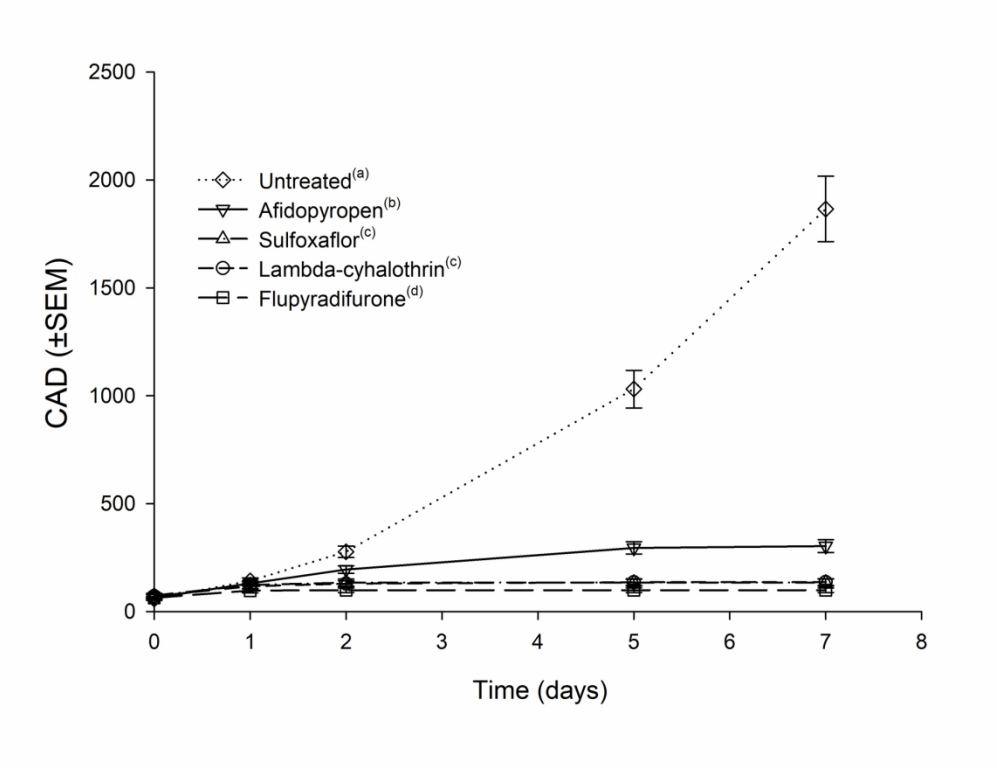

Fig. 2

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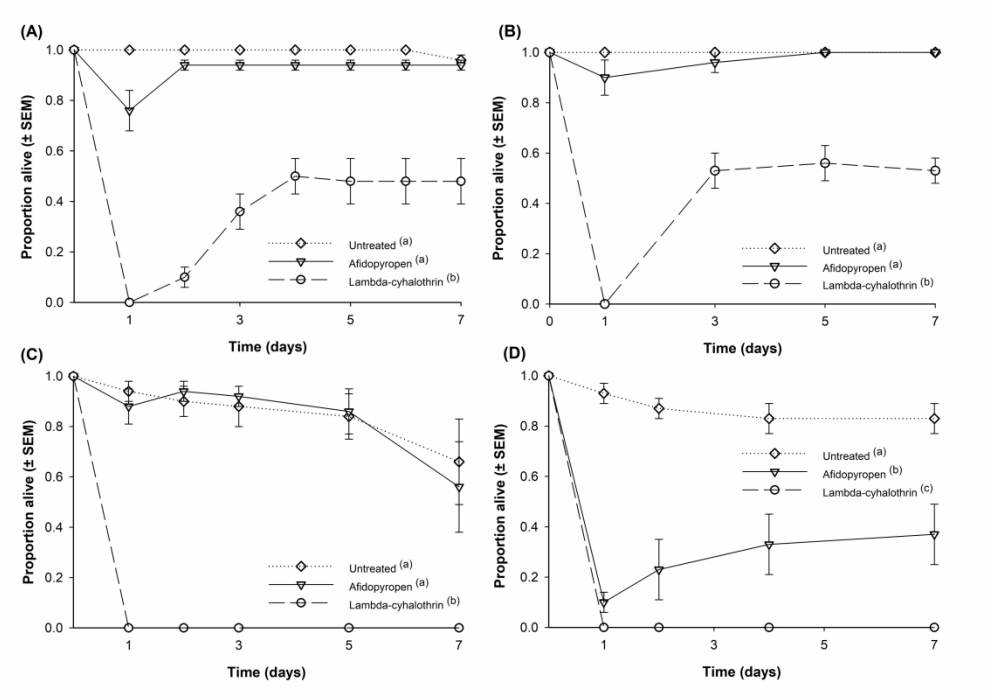

Fig. 3

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