Soybean aphid (Hemiptera: Aphididae) response to lambda-cyhalothrin varies with its virulence status to aphid-resistant soybean

Running title: Soybean aphid response to lambda-cyhalothrin

Ivair Valmorbida[a] *, Dionei S. Muraro[b], Erin W. Hodgson[a], and Matthew E. O'Neal[a] *

BACKGROUND: Soybean aphid, *Aphis glycines*, is an invasive insect in North America, considered one of the most important pests of soybean. Their management relies heavily on foliar insecticides, but there is growing effort to expand these tools to include aphid-resistant varieties. We explored if the LC_{50} and LC_{25} of lambda-cyhalothrin varied between virulent (Resistance to *Aphis glycines* (*Rag*) soybeans) and avirulent (susceptible to *Rag*-genes soybeans) populations of soybean aphid with a leaf-dip bioassay. We also investigated the response to the LC_{25} of lambda-cyhalothrin on adults (F0) and their progeny (F1) for both avirulent and virulent soybean aphid.

RESULTS: The LC_{50} of the virulent aphid population was significantly higher compared with the LC_{50} of the avirulent population. The LC_{25} significantly reduced fecundity of the F0

* Correspondence to: Ivair Valmorbida, E-mail: ivairvalmorbida@gmail.com; Matthew E. O'Neal, oneal@iastate.edu
[a] Department of Entomology, Iowa State University, Ames, IA 50011, United States.
[b] Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), São Paulo, Brazil.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ps.5661

generation of avirulent soybean aphid, but no significant effect was observed for virulent aphids. In addition, the LC_{25} significantly shortened the adult pre-oviposition period (APOP) and lengthened total pre-oviposition period (TPOP) of avirulent aphids, while the mean generation time (*T*) was significantly increased. For the virulent aphid, sublethal exposure significantly lengthened development time of first and third instars, TPOP, and adult longevity. In addition, all demographic parameters of virulent soybean aphid were significantly affected when they were exposed to the LC_{25} of lambda-cyhalothrin.

CONCLUSION: Our results demonstrate lambda-cyhalothrin is less toxic to virulent aphids and exposure to the LC_{25} can trigger hormesis which may have implications for the long-term management of this pest with this insecticide as well as with aphid-resistant varieties of soybean.

Keywords: Hormesis; pyrethroid; life table analysis; resistance; IPM

1 INTRODUCTION

Insect pests can be exposed to a plethora of chemicals, including defensive chemicals within the host plant[1] and insecticides used for their management.[2,3] Mechanisms used by insects to overcome plant defenses and chemical insecticides may be shared, which in turn may affect the susceptibility of insects to insecticides.[4,5,6] Indeed, decreased susceptibility to insecticides has been observed in several pest species due to the effect of plant allelochemicals.[6,7,8] Conversely, host plant resistance has also been reported to interfere with susceptibility of pest species to insecticides.[9,10,11]

Insects can be exposed to sublethal concentrations of insecticides in several situations within agro-ecosystems. Insecticide degradation by rainfall, temperature, and sunlight can lead to a reduction in concentration after initial application.[3] In addition, defective spraying equipment,

drift, and missaplication may also affect the final concentration of the insecticide that the target insect experiences.[12] Furthermore, if the pest colonizes a field after an insecticide is applied, there is a potential for sublethal exposure as the active ingredient degrades. A sublethal concentration of an insecticide can induce hormesis in insects, a biphasic-response phenomenon where a low-dose of an insecticide may have a stimulatory effect on population parameters, while a high-dose leads to inhibition.[2,13, 14,15] These sublethal effects can favor pest resurgence[2,15] and the development of insecticide resistance.[16]

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was first detected in the United States in 2000,[17,18] and rapidly spread across the major soybean production areas of the North Central United States.[18] This species has a complex life cycle [18] and uses soybean as a secondary host during the summer resulting in as many as 15 generations.[19] Soybean aphid feed on phloem while residing on soybean leaves and stems,[19] reducing pods per plant, seed per pod, individual seed weight, and consequently, seed yield.[20,21] If these populations are left unmanaged, soybean aphids can reduce yield by as much as a 40%.[22]

Evidence of hormesis in the form of stimulatory effects on life history traits resulting in increased longevity and fecundity have been observed in numerous arthropods species,[2,15] including the soybean aphid.[23,24] Soybean aphids were positively affected by sublethal exposure to imidacloprid (Group 4A)[23] and beta-cypermethrin (Group 3A).[24] Net reproduction rate was significantly higher in aphids exposed to sublethal concentrations of imidacloprid than those in the control treatment.[23] Similarly, net reproduction rate, intrinsic rate of increase, and finite rate of increase were significantly higher when soybean aphids were exposed to 0.005 $\mu g\ mL^{-1}$ of beta-cypermethrin compared to unexposed aphids.[24] During the first 15 years of soybean aphid occurrence in North America (NA), the most common active ingredient used has been lambda-

cyhalothrin (Group 3A).[18] To what extent lambda-cyhalothrin produces hormesis in soybean aphids found in NA is not known.

Despite passing through a genetic bottle-neck common for invasive species, there is evidence of genetic diversity with soybean aphid populations found in North America.[25,26,27] This diversity has implications for management, in the form of phenotypic variation to genes confereing resitance to soybean aphid, i.e., *Rag*-genes.[27] Populations avirulent to all *Rag*-genes are refered to as biotype 1.[28] To date, several distinct virulent biotypes have been identified in North America[29] and found throughout a multi-state region of the US.[28] The most virulent biotype, biotype 4, is capable of surviving on soybeans with one or more *Rag*-genes.[30]

The role of *Rag*-genes against soybean aphids are not fully understood. Soybean isolines containing *Rag1* and *Rag2* genes confer resistance to soybean aphids primarily through antibiosis, although antixenosis may also play a role against soybean aphids.[31] The stress caused by the feeding of the aphid on a *Rag* plant induces a higher expression of genes related to the activation of response mechanisms common in resistant plants.[32] The identity of products from these genes and subsequent mechanisms has not been identified. Enzymatic systems in a virulent soybean aphid have been suggested to account for their capacity to develop on Rag-containing varieties of soybeans.[33] Although there is overlap between the mechanisms confering pesticide resistance and resistance to plant toxins,[34] especially for generalists herbivores, it is unclear how robust such overlap exists for specialists herbivores like the soybean aphid.

We used two distinct biotypes of soybean aphid to determine if virulence affected the aphids' susceptibility to lambda-cyhalothrin. Furthermore, we explored how both populations responded to the LC_{25} of lambda-cyhalothrin, and if this induced hormesis. We first determined if the LC_{50} for lambda-cyhalothrin varied between virulent and avirulent soybean aphids. Once

we established this base-line susceptibility, we then tested if the biotypes responded differently to their respective LC_{25} for lambda-cyhalothrin. We predicted that a virulent biotype would be less susceptible to lambda-cyhalothrin and more likely to show evidence of hormesis than avirulent biotypes. We looked for evidence for hormesis in adults (F0) exposed to lambda-cyhalothrin as well as their offspring (F1).

2 MATERIALS AND METHODS

2.1 Insects, plants and insecticides

We used avirulent (biotype 1) and virulent (biotype 4) soybean aphids that came from colonies initially collected by colleagues at The Ohio State University. At Iowa State University, these colonies were maintained on their respective susceptible plants in growth chambers (25 ± 2°C, 50% RH and a photoperiod of 16:8 [L:D]) without exposure to insecticides. Avirulent soybean aphids (i.e., biotype 1) were reared on the soybean genotype LD14-8007, which does not contain *Rag*-genes. Virulent soybean aphids (i.e., biotype 4) were reared on soybean genotype LD14-8001 expressing both *Rag1* and *Rag2* genes (written as *Rag1+2* throughout). For the sublethal exposure bioassays, avirulent and virulent aphids were reared and tested on their respective plant genotypes that they were kept on while in these colonies. Technical grade of lambda-cyhalothrin (active ingredient 97.7%) was obtained from Control Solutions Inc. (Pasadena, USA).

2.2 Concentration-mortality response for virulent and avirulent soybean aphids

A leaf-dip bioassay[35] was used to assess the susceptibility of avirulent and virulent soybean aphids to a technical formulation of lambda-cyhalothrin. A stock solution of lambda-cyhalothrin was prepared in analytical acetone and diluted into seven to eight concentrations with distilled

water containing 0.05% (v/v) Triton X-100 (Alfa Aesar, Tewksbury, USA). A control treatment contained distilled water, 0.05% (v/v) Triton X-100, and 0.01% of acetone, equal to the concentration of acetone in the treatment with the highest concentration of lambda-cyhalothrin.

Soybean seeds were planted in plastic pots filled with a soil mixture (Sungro Horticulture Products, SS#1-F1P) and kept in a greenhouse (25 ± 5°C and a photoperiod of 16:8 [L:D]). Plants were watered three times per week, and after emergence, fertilized weekly with a water-soluble formulation (Peters Excel Multi-Purpose Fertilizer, 21-5-20 NPK). Disks (3.8-cm diameter) from first and second trifoliate leaves were cut with a hole punch (Fiskars, Helsinki, Finland) when plants reached the mid-vegetative stage (V4).[36] Leaf disks were manually submerged with gentle agitation in a treatment solution for 10 s and then allowed to air dry, abaxial side-up on a paper towel. Subsequently, leaf disks were placed with their abaxial surface downward onto 29.6 mm plastic souffle cups (Choice Paper Company, New York, USA) containing 1% w/v agar (Bacto^{TM} Agar, Becton, Dickinson and Company, Franklin Lakes, USA) prior to congealing. Each cup was filled with approximately 20 mm of agar, leaving 10 mm to the top of the cups. A drop of distilled water was added to the agar bed to increase leaf disk adherence.

We selected apterous, mixed age adult aphids from our colonies and transferred them to the bottom of Petri dishes containing filter paper moistened with distilled water. We randomly selected twenty, uninjured aphids from these Petri dishes, transferring them onto a leaf disk. Each cup was sealed with a close-fitting, ventilated lid. Cups were stored in a growth chamber (25 ± 2°C, 70% RH and 16:8 [L:D]). Assessment of mortality was performed after 24 and 48h and data from 48h post treatment was used to estimate the LC_{50}. Aphids unable to right themselves within 10 s after they were turned on their back were considered dead.[35,37] Each cup

contained 20 aphids and was considered an experimental unit, and each concentration of lambda-cyhalothrin and the control was replicated three times.

2.3 Effects of LC_{25} of lambda-cyhalothrin on F0 generation

The leaf-dip bioassay was used to determine the effects of lambda-cyhalothrin on both virulent and avirulent soybean aphids. We used data collected in the previous section to estimate the LC_{25} of lambda-cyhalothrin for each biotype. To determine the response of soybean aphids to this concentration, the same control treatment was used as described above, with adult aphids exposed to their respective LC_{25} of lambda-cyhalothrin prepared in acetone and diluted in distilled water containing 0.05% (v/v) Triton X-100. Aphid mortality was assessed at 48 h after exposure, and individual surviving aphids were gently transferred to an untreated leaflet kept in a Petri dish within a growth chamber (25 ± 2°C, 50% RH and 16:8 [L:D]). Each Petri dish contained a moistened circular filter paper at the bottom and a string of Parafilm (Fisher Scientific, Ottawa, Canada) was used to seal the Petri dishes preventing escape of aphids. Nymphs were recorded and removed daily until the death of the adult aphid. The soybean leaflet was replaced every 7 d, and filter paper was moistened when necessary. Each aphid was considered an experimental unit, and 100 adults were used for each treatment and biotype combination, for a total of 400 adult aphids.

2.4 Effects of LC_{25} of lambda-cyhalothrin on F1 generation

We used the same experimental protocol as described above to estimate the effect on the F1 generation of adult aphids exposed to the LC_{25} of lambda-cyhalothrin. The same control was used as described above, with adult aphids of virulent and avirulent biotypes exposed to their respective LC_{25} of lambda-cyhalothrin prepared in acetone and diluted in distilled water

containing 0.05% (v/v) Triton X-100. Twenty-four hours after the F0 generation was exposed to a treatment, the F1 nymphs were removed and only the adults (F0 generation) remained on the leaf disks. At 48 h post-treatment of the F0 generation, the 24 h old nymphs (F1) were transferred to untreated soybean leaflets and maintained individually in a Petri dish as described for the parental generation (F0). For the avirulent aphid, 100 nymphs were used for the control treatment and 61 for the LC_{25} treatment. For the virulent aphid, 100 nymphs were used for each treatment. The following parameters were assessed daily during the lifespan of the F1 generation: development time, number of surviving aphids at each life stage, nymphs per aphid, and longevity of adults. Exuviae were removed once detected and morphological characteristics of nymphs[38,39] were used to assess growth stage.

2.5 Data analysis

Concentration-mortality data was analyzed using a three-parameter log-logistic function of the 'drc' package in R[40] to estimate slope, LC_{50} and LC_{25} of lambda-cyhalothrin, and whether the LC_{50} of lambda-cyhalothrin differed between avirulent and virulent aphid populations. Individual aphid development time, survival rate, longevity, and daily fecundity of virulent and avirulent soybean aphids exposed to the LC_{25} of lambda-cyhalothrin and control treatments were analyzed following the age-stage, two-sex life table theory,[41,42] using TWOSEX-MSChart program.[43] Parameters such as age-stage specific survival rate (s_{xj}), probability a newly emerged nymph would survive to age x and stage j, (x is age in days and j is the stage), age-specific survival rate (l_x), age-specific fecundity (m_x), intrinsic rate of increase (r), net reproductive rate (R_0), finite rate of increase (λ), and mean generation time (T) were calculated according to Chi and Liu[41] and Chi[42]. Means and standard error of population parameters in the life table were estimated using a

bootstrap procedure,[44] with 100,000 replicates. Differences between control and treated aphids within life table parameters were analyzed using a paired bootstrap test at 5% significant level using TWOSEX-MSChart program.[43]

3 RESULTS

3.1 LC_{50} for virulent and avirulent soybean aphids

Susceptibility to lambda-cyhalothrin varied significantly between the two soybean aphid biotypes (Table 1). Lambda-cyhalothrin was less toxic to the virulent biotype than the avirulent biotype. Based on these data, we estimated an LC_{25} of 0.25 μg mL^{-1} and 0.53 μg mL^{-1} for avirulent and virulent biotypes, respectively. The corrected mortality for adult aphids exposed to the LC_{25} was 23.70% and 25.02% for avirulent and virulent aphids, respectively. Our estimate of the LC_{50} (0.40 ± 0.17 μg mL^{-1}) for the avirulent soybean aphid is similar to the LC_{50} (0.32–0.44 μg mL^{-1}) reported by Hanson[37] using leaf-dip bioassays to evaluate the susceptibility of biotype 1 to lambda-cyhalothrin. This comparison suggests that our estimate is within the range of what others have reported for avirulent soybean aphid populations.

3.2 Effects of LC_{25} of lambda-cyhalothrin on F0 generation

Regardless of virulence status, we did not observe a significant effect of lambda-cyhalothrin applied at the LC_{25} on the longevity of adults when compared to their respective controls. However, the LC_{25} of lambda-cyhalothrin had a variable effect on fecundity based on the virulence status of soybean aphid. The fecundity of avirulent aphids exposed to their LC_{25} was significant lower when compared with the control treatment (t = 3.045; d.f. = 198; P = 0.002), while the LC_{25} did not affect fecundity of virulent aphids (t = 0.1502; d.f. = 198; P = 0.8808) (Table 2).

3.3 Effects of LC_{25} of lambda-cyhalothrin on F1 generation of avirulent soybean aphid

Exposure to the LC_{25} of lambda-cyhalothrin had limited effects on biological and demographic parameters of avirulent soybean aphid when compared to the control treatment (Table 3). The developmental duration of 1st, 2nd, 3rd, and 4th instars (N1 through N4), oviposition period, adult longevity and fecundity were not significantly affected by the exposure to the LC_{25} of lambda-cyhalothrin. Conversely, the LC_{25} of lambda-cyhalothrin significantly shortened adult pre-oviposition period (APOP) and lengthened total pre-oviposition period (TPOP). There were no significant differences in the net reproductive rate (*Ro*), finite rate of increase (λ), intrinsic rate of increase (r) and gross reproduction rate (GRR) of avirulent soybean aphids exposed to LC_{25} when compared with the control treatment. However, the mean generation time (T) significantly increased when avirulent aphids were exposed to the LC_{25} of lambda-cyhalothrin.

Variability in developmental rates of individual avirulent soybean aphids, and overlap among stages were observed between those exposed to the LC_{25} and control treatments (Fig. 1). Nymphal development was delayed, as the peak of the fourth instar occurred at 5 d in the control treatment and 6 d in the LC_{25} treatment (Fig. 1). The maximum survival time was decreased in the LC_{25} treatment and a higher l_x was observed in the control group from age 2 to 12 d (Fig. 2). After 12 d, the l_x decreased, and a higher l_x was observed in the LC_{25} treatment from age 13 to 32 d. The age-specific maternity ($l_x m_x$) highest peaks occurred earlier in the control treatment (age 12) compared to the LC_{25} treatment (age 15) (Fig. 2). However, the fecundity peaks were higher for the LC_{25} treatment compared with control treatment (4 and 5.1 aphids/day, respectively). The age-stage life expectancy (e_{xj}) (Supporting Information, Fig. S1) demonstrates the time that an individual of age x and stage y is expected to live. In general, the life expectancy decreased as

age increased, and estimates of life expectancy were similar for both control and treated aphids. The age-stage reproductive values (v_{xj}) peaked earlier for the control treatment (12.12 at day 9; Supporting Information, Fig. S2) and was lower than the peak for aphids receiving the LC_{25} treatment (14.04 at day 10).

3.4 Effects of LC_{25} of lambda-cyhalothrin on F1 generation of virulent soybean aphid

Exposure to an LC_{25} of lambda-cyhalothrin significantly affected more biological and demographic parameters of the nymphs of virulent aphids than the avirulent aphids (Table 3). Development time of the first and third instars, TPOP, and adult longevity were significantly longer for virulent aphids exposed to lambda-cyhalothrin as compared to the control treatment. Net reproductive rate, mean generation time, and GRR were significantly lower for the treatment control. On the contrary, exposure to the LC_{25} significantly reduced finite rate of increase (λ) and intrinsic rate of increase (r). Overall, the effect of the LC_{25} on virulent aphids was more pronounced than in the avirulent aphid. For example, all the demographic parameters of the virulent aphid were significantly affected by the exposure to the LC_{25}, while for the avirulent aphid, only the mean generation time (T) was significantly affected.

Overlapping between stages showing variable developmental rates among individuals were observed in the control and LC_{25} treatments for the virulent soybean aphid (Fig. 1). Exposure to the LC_{25} delayed developmental time of the virulent aphid (Fig. 1). The virulent aphid exposed to the control treatment had a higher age-specific survival rate (l_x) at the beginning (age 3 and 4 d), which then decreased and was lower than that observed for aphids exposed to the LC_{25}, from ages six to 27 d (Fig 2). The LC_{25} did not affect the maximal survival time of the virulent aphids. The age-specific maternity ($l_x m_x$) peak occurred later for virulent

aphids exposed to the LC_{25} compared to the control treatment; however, the number of aphids per day was higher in the LC_{25} treatment (Fig. 2). The age-stage life expectancy (e_{xj}) (Supporting Information, Fig. S1) and the age-stage reproductive values (v_{xj}) (Supporting Information, Fig. S2) for the virulent aphid followed a similar pattern as observed for the avirulent soybean aphid. The age-stage reproductive peak occurred later and was higher for the LC_{25} treatment when compared with control treatment (Supporting Information, Fig. S2).

4 DISCUSSION

We observed differences between the two biotypes when exposed to two different concentrations of lambda-cyhalothrin, one representing a concentration more consistent with a lethal dose (LC_{50}) and a lower concentration (LC_{25}) that could represent a sublethal dose. This latter dose produced an interesting difference in the response of aphids consistent with hormesis in the F1 generation, at least for the virulent biotype. Difference in response by the two biotypes to lambda-cyhalothrin extended to effects on longevity and fecundity of the F0 generation when exposed to the LC_{25}. There were no significant differences on longevity and fecundity of virulent aphids exposed to the LC_{25} of lambda-cyhalothrin, whereas the fecundity of avirulent aphids were significantly reduced when exposed to the LC_{25} of lambda-cyhalothrin.

Based on life table analysis of the F1 generation, exposure to the LC_{25} of lambda-cyhalothrin had a stimulatory effect on several parameters for the virulent biotype but not the avirulent biotype. The F1 generation produced from virulent aphids exposed to the LC_{25} of lambda-cyhalothrin had greater adult longevity, longer oviposition period and produced more nymphs per female than those produced from a generation exposed to the control treatment. Furthermore, the LC_{25} exposure significantly increased net reproductive and gross reproductive

rates of virulent soybean aphid compared to the untreated control. This is in contrast to the avirulent aphids, whose net reproductive and GRR rates were numerically higher; but did not significantly differ from the control treatment. All of these differences suggest a stimulatory effect from the exposure of this concentration of lambda-cyhalothrin is limited to the virulent biotype. These results suggest that this virulent biotype experiences hormesis when exposed to its' LC_{25} of lambda-cyhalothrin.

Evidence of hormesis in the F1 generation of soybean aphid exposed to another pyrethroid insecticide has been revealed with lower concentrations. Soybean aphids exposed to the LC_{15} of beta-cypermethrin significantly decreased the intrinsic rate and finite rate of increase, while a lower concentration (nearly LC_5) increased these parameters.[24] The LC_5 of beta-cypermethrin significantly increased net reproduction rate, intrinsic rate of increase, and finite rate of increase for soybean aphids compared to the control treatment.[24] Although we did not evaluate the response of soybean aphids to concentration below the LC_{25} for lambda-cyhalothrin, it is likely concentrations below the LC_{25} for lambda-cyhalothrin could produce an even greater stimulatory effect, regardless of virulence status.

In summary, our results suggest that lambda-cyhalothrin was less toxic to the virulent biotype of the soybean aphid at varying concentrations than the avirulent biotype. This is the first evidence that virulence to *Rag*-genes affects the response to insecticides. Variation in susceptibility to insecticides has been demonstrated for sub-populations of other Hemipterans. For example, sub-populations of *Aphis gossypii* Glover,[45] *Bemisia tabaci* Gennadius,[10] and *Bactericera cockerelli* (Sulc)[9] that vary in their capacity to exploit different plant species, also had varying levels of insecticide susceptibility. Unlike these previous studies that include Hemipterans with a broad host-range, soybean aphid is specialist with a restricted host range

(e.g., soybean as a summer host), and the sub-populations we used were identified based on their response to a genetic difference in their host plant (i.e., presence/absence of *Rag*-genes).

Although the mechanism for virulence to *Rag*-genes is not known, a role for effector proteins secreted by the aphid into the host plant[46,47] and detoxification enzymes within the aphid have been suggested.[33] Variation in the form and amount of these effector proteins injected into the plant by feeding aphids, as well as variation in detoxification enzymes may contribute to the various biotype phenotypes observed in North America and Asia. For example, up-regulation of P450s, glutathione S-transferases (GSTs), carboxylesterases (COEs), and ABC transporters was observed when avirulent soybean aphids fed soybean containing *Rag 1*, suggesting a specific stress response to the xenobiotic compounds produced by *Rag 1* soybean variety.[33] These mechanisms are similar to those used by insects against synthetic insecticides[34,48], and could explain the difference in susceptibility of avirulent and virulent soybean aphid to lambda-cyhalothrin, assuming the virulent aphid also presents similar mechanisms that allow them to survive on *Rag1+2* plants. For example, similar mechanisms against plant secondary compounds were observed for *Bradysia odoriphaga* larva reared on garlic and humus, leading to a higher tolerance to insecticides (e.g., Phoxim and clothianidin) compared with other host plants.[11] Furthermore, *Helicoverpa armigera* (Hübner) larvae fed on a gossypol-diet demonstrated higher tolerance to deltamethrin, associated with an increase in P450 activity within the midgut.[7] Activity of EST and P450 were also associated with development of resistance to deltamethrin in *Spodoptera exigua* (Hübner) fed gossypol.[8]

To what extent the response of the F0 generation of soybean aphid to lambda-cyhalothrin is typical for other insecticides is not clear. Similar experiments suggest effects on longevity and fecundity for the F0 generation varies by aphid species and insecticide combination. For

example, the exposure of *Myzus persicae* (Sulzer) to the LC_{25} of flupyradifurone significantly reduced adult longevity and fecundity.[49] However, no differences in longevity and fecundity were observed for *A. gossypii* when exposed to theLC_{25} of flupyradifurone[50] and sulfoxaflor.[51] The longevity of *A. gossypii* was not affected by the LC_{10} and LC_{50} of nitenpyram, but fecundity was significantly reduced.[52]

Although we observed a difference in the LC_{50} between virulent and avirulent biotypes when exposed to lambda-cyhalothrin, this difference is likely not immediately important for management of the soybean aphid. However, the difference observed at a lower concentration (as defined by the LC_{25}) is important for soybean aphid management, and reinforces a rational application of insecticides within an IPM program.

Insecticide induced hormesis in agricultural pests can be a serious problem, because it can result in pest resurgence.[16] Sublethal exposure may also increase mutation frequencies, and if related to the target-site of insecticides, it might reduce the pest's susceptability to insecticides.[53] In addition, sublethal exposure to insecticides may directly increase the selection of resistant by stimulating the expression of advantageous phenotypes, and indirectly by providing conditions that may prime the insect pest to better tolerate stressful conditions (e.g., resistant host plants).[54] Interestingly, our data suggest that the virulent soybean aphid may have an advantage over avirulent aphid when exposed to low concentrations of lambda-cyhalothrin. This is disconcerting given the often prophylactic or calendar-based use of insecticides for managing the soybean aphid in North America.[55] This approach to insect pest a management may inadvertently favor the selection of virulent over avirulent soybean aphids within North America. Such selection pressure may limit the durability of aphid-resistant soybean varieties that are in development,[31,56]

and this relatively new technology also may suffer the consequences of unnecessary insecticide applications in soybean fields.

ACKNOWLEDGEMENTS

We would like to thank Aaron Eckley and Kelsey Shepherd for helping in data collection. This work was funded by soybean checkoff funds from the Iowa Soybean Association.

REFERENCES

1 Mithöfer A and Boland W, Plant defense against herbivores: chemical aspects. *Annu Rev Plant Biol* **63**:431-50 (2012).

2 Cutler GC, Insects, insecticides and hormesis: evidence and considerations for study. *Dose-Response* **11**:154-177 (2013).

3 França SM, Breda MO, Barbosa DR, Araujo AM and Guedes CA, The sublethal effects of insecticides in insects. In: Shields VDC, editor. Biological Control of Pest and Vector Insects. (2017).

4 Silva AX, Bacigalupe LD, Luna-rudloff M, and Figueroa CC, Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae) II: Costs and benefits *PLoS ONE* **7**:e36366 (2012).

5 Alyokhin A and Chen YH, Adaptation to toxic hosts as a factor in the evolution of insecticide resistance. *Curr Opin Insect Sci* **21**:33-38 (2017).

6 Chen C, Liu Y, Shi X, Desneux N, Han P and Gao X, Elevated carboxylesterase activity contributes to the lambda-cyhalothrin insensitivity in quercetin fed *Helicoverpa armigera* (Hübner). *PLoS ONE* **12**:e0183111 (2017).

7 Tao XY, Xue XY, Huang YP, Chen XY and MAO YB, Gossypol-enhanced P450 gene pool contributes to cotton bollworm tolerance to a pyrethroid insecticide. *Mol Ecol* **21**:4371-4385 (2012).

8 Hafeez M, Liu S, Jan S, Ali B, Shahid M, Fernández-Grandon GM, Nawaz M, Ahmad A and Wang M, Gossypol-induced fitness gain and increased resistance to deltamethrin in beet armyworm, *Spodoptera exigua* (Hübner). *Pest Manag Sci* **75**:683-693 (2019).

9 Liu D and Trumble JT, Interactions of plant resistance and insecticides on the development and survival of *Bactericerca cockerelli* [Sulc] (Homoptera: Psyllidae). *Crop Prot* **24**:111-117 (2005).

10 Xie W, Wang S, Wu Q, Feng Y, Pan H, Jiao X, Zhou L, Yang X, Fu W, Teng H and Xu B, Induction effects of host plants on insecticide susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag Sci* **67**:87-93 (2011).

11 Zhu G, Luo Y, Xue M, Zhao H, Sun X and Wang X, Effects of feeding on different host plants and diets on *bradysia odoriphaga* population parameters and tolerance to heat and insecticides. *J Econ Entomol* **110**:2371-2380 (2017).

12 Duke SO, Hormesis with pesticides. *Pest Manag Sci* **70**:10-1002 (2014).

13 Kendig EL, Le HH and Belcher SM, Defining hormesis: evaluation of a complex concentration response phenomenon. *Int J Toxicol* **29**:235-246 (2010).

14 Jager T, Barsi A and Ducrot V, Hormesis on life-history traits: is there such thing as a free lunch? *Ecotoxicology* **22**:263-270 (2013).

15 Guedes RNC and Cutler GC, Insecticide-induced hormesis and arthropod pest management. *Pest Manag Sci* **70**:690-697 (2014).

16 Guedes RNC, Walse SS and Throne JE, Sublethal exposure, insecticide resistance, and community stress. *Curr Opin Insect Sci* **21**:47-53 (2017).

17 Alleman R, Grau C and Hogg D, Soybean aphid host range and virus transmission efficiency. *Proceedings: Wisconsin Fertilizer Agline Pest Management Conference* (2002). Available: https://soilsextension.triforce.cals.wisc.edu/wp-content/uploads/sites/68/2016/07/Alleman-Conf-2002.pdf [11 March 2019].

18 Ragsdale DW, Landis DA, Brodeur J, Heimpel GE and Desneux N, Ecology and management of the soybean aphid in North America. *Annu Rev Entomol* **56**:375-399 (2011).

19 Tilmon KJ, Hodgson EW, O'Neal ME and Ragsdale DW, Biology of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. *Integr Pest Manag* **2**:1-7 (2011).

20 Beckendorf EA, Catangui MA and Riedell WE, Soybean aphid feeding injury and soybean yield, yield components, and seed composition. *Agron J* **100**:237-246 (2008).

21 Pierson LM, Heng-Moss TM, Hunt TE and Reese JC, Categorizing the resistance of soybean genotypes to the soybean aphid (Hemiptera: Aphididae). *J Econ Entomol* **103**:1405-1411. (2010).

22 Ragsdale DW, McCornack BP, Venette RC, Potter BD, MacRae IV, Hodgson EW, O'Neal ME, Johnson KD, O'Neil RJ, DiFonzo CD and Hunt TE, Economic threshold for soybean aphid (Hemiptera: Aphididae). *J Econ Entomol* **100**:1258-1267 (2007).

23 Qu Y, Xiao D, Li J, Chen Z, Biondi A, Desneux N, Gao X, and Song D, Sublethal and hormesis effects of imidacloprid on the soybean aphid *Aphis glycines*. *Ecotoxicology*, **24**:479-487 (2015).

24 Qu Y, Xiao D, Liu J, Chen Z, Song L, Desneux N, Benelli G, Gao X and Song D. Sublethal and hormesis effects of beta-cypermethrin on the biology, life table parameters and reproductive

potential of soybean aphid *Aphis glycines*. *Ecotoxicology*, **26**:1002-1009 (2017).

25 Michel AP, Zhang W, Kyo Jung J, Kang ST and Mian MR, Population genetic structure of *Aphis glycines*. *Environ Entomol* **38**:1301-1311 (2009).

26 Michel AP, Mittapalli O, Mian MR and Sudaric A, Evolution of soybean aphid biotypes: understanding and managing virulence to host-plant resistance, in Soybean – Molecular Aspects of Breeding, ed. by Sudaric A. In Tech, Rijeka, Croatia, pp. 355–372 (2011).

27 Orantes LC, Zhang W, Mian MR and Michel AP, Maintaining genetic diversity and population panmixia through dispersal and not gene flow in a holocyclic heteroecious aphid species. *Heredity* **109**:127 (2012).

28 Cooper SG, Concibido V, Estes R, Hunt D, Jiang GL, Krupke C, McCornack B, Mian R, O'Neal M, Poysa V and Prischmann-Voldseth D, Geographic distribution of soybean aphid biotypes in the United States and Canada during 2008–2010. *Crop Sci* **55**:2598-2608 (2015).

29 Alt J, Ryan M, Onstad DW. Geographic distribution and intrabiotypic variability of four soybean aphid biotypes. *Crop Sci* **59**:84-91 (2019).

30 Alt J and Ryan-Mahmutagic M, Soybean aphid biotype 4 identified. *Crop Sci* **53**:1491-1495 (2013).

31 Hesler LS, Chiozza MV, O'Neal ME, MacIntosh GC, Tilmon KJ, Chandrasena DI, Tinsley NA, Cianzio SR, Costamagna AC, Cullen EM and DiFonzo CD, Performance and prospects of *Rag* genes for management of soybean aphid. *Entomol Exp Appl* **147**:201-216 (2013).

32 Li Y, Zou J, Li M, Bilgin DD, Vodkin LO, Hartman GL, Clough SJ, Soybean defense responses to the soybean aphid. *New Phytol* **179**:185-95 (2008).

33 Bansal R, Mian MAR, Mittapalli O and Michel AP, RNA-Seq reveals a xenobiotic stress response in the soybean aphid, *Aphis glycines*, when fed aphid-resistant soybean. *BMC genomics* **15**:972 (2014).

34 Dermauw W, Pym A, Bass C, Van Leeuwen T and Feyereisen R, Does host plant adaptation lead to pesticide resistance in generalist herbivores? *Curr Opin Insect Sci* **26**:25-33 (2018).

35 IRAC - Insecticide Resistance Action Committee, IRAC test methods (2018). Available: https://www.irac-online.org/methods/aphids-adultnymphs/ [19 June 2018].

36 Fehr WR, Caviness CE, Burmood DT and Pennington JS, Stage of development descriptions for soybeans, *Glycine Max* (L.) Merrill. *Crop Sci* **11**:929-931 (1971).

37 Hanson AA, Menger-Anderson J, Silverstein C, Potter BD, MacRae IV, Hodgson EW and Koch RL, Evidence for soybean aphid (Hemiptera: Aphididae) resistance to pyrethroid insecticides in the upper midwestern United States. *J Econ Entomol* **110**:2235-2246 (2017).39

38 Zhang X, Study on the instars of soyabean aphid (*Aphis glycines* Matsumura) in Jilin Province. *Acta Agric Univer Jilinensis* **10**:15-17 (1988).

39 Voegtlin DJ, Halbert SE and Qiao G, A guide to separating *Aphis glycines* Matsumura and morphologically similar species that share its hosts. *Ann Entomol Soc Am* **97**:227-232 (2004).

40 Ritz C, Baty F, Streibig JC and Gerhard D, Dose-response analysis using R. *PLoS ONE* **12**:e0146021 (2015).

41 Chi H and Liu H, Two new methods for the study of insect population ecology. *Acad Sin Bull Inst Zool* **24**:225–240 (1985).

42 Chi H, Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol* **17**:26–34 (1988).

43 Chi, H. TWOSEX-MSChart: A computer program for the age-stage, twosex life table analysis. [Online]. National Chung Hsing University, Taichung, Taiwan: http://140.120.197.173/ecology/products.htm. [18 December 2018].

44 Huang YB and Chi H. Life tables of *Bactrocera cucurbitae* (Diptera: Tephritidae): with an invalidation of the jackknife technique. *Appl Entomol* **137**:327-39 (2013).

45 McKenzie CL and Cartwright B, Susceptibility of *Aphis gossypii* (Glover) to insecticides as affected by host plant using a rapid bioassay. *J Entomol Sci* **3**:289-301 (1994).

46 O'Neal ME, Varenhorst AJ and Kaiser MC, Rapid evolution to host plant resistance by an invasive herbivore: soybean aphid (*Aphis glycines*) virulence in North America to aphid resistant cultivars. *Curr Opin Insect Sci* **26**:1-7 (2018)

47 Yates AD and Michel A, Mechanisms of aphid adaptation to host plant resistance. *Curr Opin Insect Sci* **26**:41-49 (2018).

48 Heidel-Fischer HM and Vogel H, Molecular mechanisms of insect adaptation to plant secondary compounds. *Curr Opin Insect Sci* **8**:8-14 (2015).

49 Tang Q, Ma K, Chi H, Hou Y and Gao X, Transgenerational hormetic effects of sublethal dose of flupyradifurone on the green peach aphid, *Myzus persicae* (Sulzer)(Hemiptera: Aphididae). *PLoS ONE* **14**:e0208058 (2019).

50 Liang PZ, Ma KS, Chen XW, Tang CY, Xia J, Chi H and Gao XW, Toxicity and sublethal effects of flupyradifurone, a novel butenolide insecticide, on the development and fecundity of *Aphis gossypii* (Hemiptera: Aphididae). *J Econ Entomol*. **112**:852-8 (2018).

51 Chen X, Ma K, Li F, Liang P, Liu Y, Guo T, Song D, Desneux N and Gao X, Sublethal and transgenerational effects of sulfoxaflor on the biological traits of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). *Ecotoxicology* **10**:1841-8 (2016).

52 Wang S, Qi Y, Desneux N, Shi X, Biondi A and Gao X, Sublethal and transgenerational effects of short-term and chronic exposures to the neonicotinoid nitenpyram on the cotton aphid *Aphis gossypii*. *J Pest Sci* **90**:389-96 (2017).

53 Gressel J, Low pesticide rates may hasten the evolution of resistance by increasing mutation frequencies. *Pest Manag Sci* **67:**253-257 (2011).

54 Brevik K, Lindström L, McKay SD and Chen YH, Transgenerational effects of insecticides—implications for rapid pest evolution in agroecosystems. *Curr Opin Insect Sci* **26**:34-40 (2018).

55 Olson KD, Badibanga T and DiFonzo CD, 2008. Farmers' awareness and use of IPM for soybean aphid control: report of survey results for the 2004, 2005, 2006, and 2007 crop years. *Staff Pap. P08-12. Dep. Appl. Econ., Univ. Minn., St. Paul.*

56 McCarville MT, O'Neal ME, Potter BD, Tilmon KJ, Cullen EM, McCornack BP, Tooker JF and Prischmann-Voldseth DA, One gene versus two: a regional study on the efficacy of single gene versus pyramided resistance for soybean aphid management. *J Econ Entomol* **107**:1680-1687 (2014).

Table 1. Concentration-mortality response and corrected mortality of both virulent and avirulent adult *Aphis glycines* to lambda-cyhalothrin.

Population	Slope±SE[a]	LC_{50} (95% FL)[b]	LC_{25} (95% FL)	$\chi 2$ (d.f.)[c]	Mortality (%)[d]
Avirulent	2.20±0.654	0.40 (0.23 - 0.57) b	0.25 (0.07 - 0.41)	9.30 (6)	23.70
Virulent	2.77±0.480	0.78 (0.67 - 0.88) a	0.53 (0.40 - 0.64)	10.35 (5)	25.05

[a] SE = standard error.

[b] LC_{50} values designated by different letters within a column are significantly different from each other through non-overlap of 95% fiducial limits.

[c] Chi-square testing linearity of concentration-mortality responses.

[d] LC_{25} induced mortality; Henderson-Tilton correction.

Table 2. Longevity and fecundity of adult *Aphis glycines* treated with the LC_{25} of lambda-cyhalothrin and untreated control treatments for 48 h post exposure.

Population	Longevity (days) of soybean aphid adults ± SE					Fecundity (number of nymphs per aphid) ± SE				
	Control	LC_{25}	df	t[a]	*P*	Control	LC_{25}	df	t[a]	*P*
Avirulent	10.06±0.49	8.82±0.41	198	1.913	0.057	20.49±1.23	15.43±1.10	198	3.045	0.002
Virulent	7.31±0.42	7.71±0.43	198	-0.651	0.515	18.06±1.23	17.8±1.20	198	0.150	0.880

[a]Student's *t* test for differences between LC_{25} and control treatments for each biotype.

Table 3. Biological and demographic parameters of avirulent and virulent soybean aphid exposed to the LC_{25} of lambda-cyhalothrin.

Biological parameter	Avirulent		Virulent	
	Control	LC_{25}	Control	LC_{25}
N1 (days)	2.34±0.06a	2.67±0.07a	1.55±0.00b	2.08±0.00a
N2 (days)	1.53±0.06a	1.54±0.07a	1.38±0.04a	1.26±0.04a
N3 (days)	1.36±0.06a	1.50±0.06a	1.07±0.02b	1.21±0.04a
N4 (days)	1.20±0.04a	1.24±0.06a	1.21±0.04a	1.19±0.04a
APOP	1.12±0.06a	0.98±0.03b	0.52±0.00a	0.45±0.00a
TPOP	7.55±0.11b	7.94±0.13a	5.75±0.07b	6.20±0.05a
Oviposition period (days)	10.73±0.37a	11.82±0.42a	11.28±0.30b	12.76±0.25a
Adult longevity (days)	18.13±0.78a	19.62±0.85a	14.66±0.53b	16.19±0.37a
Fecundity (no. nymphs per female)	34.42±1.31a	38.42±1.56a	45.43±1.45b	51.51±1.00a
Demographic parameter				
Net reproductive rate (Ro)	29.84±1.64a	31.49±2.27a	42.25±1.78b	47.91±1.61a
Finite rate of increase (λ, d^{-1})	1.32±0.00a	1.30±0.00a	1.44±0.00a	1.41±0.00b
Intrinsic rate of increase (r, d^{-1})	0.27±0.00a	0.26±0.00a	0.36±0.00a	0.34±0.00b
Mean generation time (T, days)	12.16±0.16b	12.94±0.16a	10.16±0.06b	11.07±0.07a
GRR	39.79±1.12a	41.26±0.99a	51.03±0.95b	54.46±0.87a

Mean ± standard error (SE) were estimated using 100,000 bootstrap replications. Different letters within the same row for avirulent and virulent soybean aphid, indicates significant differences between the control and LC_{25} group at $P < 0.05$ level, with a paired bootstrap test. APOP: Adult pre-oviposition period; TPOP: Total pre-oviposition period; GRR: Gross reproductive rate.

Figure 1. Age-stage specific survival rate (S_{xj}) of *Aphis glycines* exposed to control and LC_{25} of lambda-cyhalothrin. (A) avirulent control, (B) avirulent LC_{25}, (C) virulent control, (D) virulent LC_{25}.

Figure 2. Age-specific survival rates (l_x), age-specific fecundity (m_x) and net maternity ($l_x m_x$) of *Aphis glycines* exposed to control and LC_{25} of lambda-cyhalothrin. (A) avirulent control, (B) avirulent LC_{25}, (C) virulent Control, (D) virulent LC_{25}.