Efficacy and Nontarget Effects of Reduced-Risk Insecticides on *Aphis glycines* (Hemiptera: Aphididae) and Its Biological Control Agent *Harmonia axyridis* (Coleoptera: Coccinellidae)

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ABSTRACT The efficacy of three reduced-risk insecticides (pyrethrins, insecticidal soap, and narrow-range mineral oil) was determined for nymphs and adults of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), an exotic pest of North American soybean, *Glycine max* (L.) Merr. These insecticides also were evaluated for nontarget effects on one of the aphid's key biological control agents, multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), including first and third instars, pupae, and adults. A Potter Spray Tower was used to conduct direct spray laboratory bioassays. Results indicated that although pyrethrins and narrow-range mineral oil caused 100% mortality to *A. glycines* nymphs and adults at 72 h posttreatment, insecticidal soap caused equivalent mortality to only the nymphs during the same time period. However, *A. glycines* adult mortality due to the insecticidal soap (83.3%) was significantly greater than the control. Pyrethrins were highly toxic to first instars of *H. axyridis* (98% mortality), but they had no effect on third instars, pupae, or adults. Mineral oil and insecticidal soap were moderately lethal to first (48.9 and 40% mortality, respectively) and third (31.9 and 38.8% mortality, respectively) instars of *H. axyridis*, but they had no effect on pupae and adults. Our results suggest that pyrethrins, insecticidal soap, and narrow-range mineral oil may prove useful for soybean aphid management in organic soybean due to efficacy against the aphid with differential nontarget effects on select stages of *H. axyridis*. Additional studies will be necessary to elucidate the efficacy of these insecticides under field conditions.

KEY WORDS *Aphis glycines*, *Harmonia axyridis*, organic, soybean, insecticides

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), first detected in Wisconsin in 2000, has rapidly spread to 21 states and three Canadian provinces, and it has become established as an economically important pest of soybean, *Glycine max* (L.) Merr., in the United States (Ragsdale et al. 2004). The aphid damages soybean plants directly by sucking phloem, resulting in plant stunting, reduced pod set, and smaller seed size (Wu et al. 2004); and indirectly through soybean virus transmission (Clark and Perry 2002). Soybean aphid is native to the temperate regions of Asia, where it is suppressed by numerous parasitoids and predators (Liu et al. 2004). In China, coccinellids are thought to play a key role in suppression of soybean aphid populations due to high predation rates and abundance in the field (Wang and Ba 1998). As an exotic pest in North America, the soybean aphid interacts with both native and naturalized predators, including several coccinellids, which were present in soybean agroecosystems before its arrival. One of the most abundant of these coccinellids, also from Asia, is the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Fox et al. 2004).

Although *H. axyridis* provides a suppressive effect on soybean aphid populations, aphid outbreaks may still cause economic damage, even in the aphid's native range (Sun et al. 2000). Periodic outbreaks require farmers to implement additional pest management tactics, such as insecticidal sprays, to prevent economic yield loss. However, many insecticides used in conventional soybean production systems, such as pyrethroids and organophosphates, have broad-spectrum activity. They are known to suppress or delay buildup of arthropod natural enemies in the field that prey on or parasitize the soybean aphid (van den Berg et al. 1997). In a study in China by Sun et al. (2000), suppression of the natural enemy population by broad-spectrum insecticides led to pest population resurgence and resulting high aphid density in soybean. After a broad-spectrum insecticide application, the pest-to-natural enemy ratio changes, and because of the aphid's rapid reproductive rate, population densities rebound quickly in a predator-free space (Myers et al. 2005). This demonstrates the importance of evaluating insecticidal inputs, which are effective in the management of soybean aphid, for nontarget effects

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and potential to conserve natural enemy populations and the suppressive effect that they provide.

Natural enemy conservation is especially important in organic soybean systems, where an emphasis is placed on multiple and/or varied tactics incorporated into the cropping system to prevent pests from reaching damaging levels (Zehnder et al. 2007). Organic farmers rely first on biological control, pest resistant or tolerant crop plant selection, and cultural practices such as crop rotation to suppress insect pests (McSorely 2002, USDA 2005). When a combination of these tactics have failed to adequately suppress pests below an economically damaging level, National Organic Program (NOP) standards state that organic farmers may apply an approved, reduced-risk insecticide (Delate et al. 2005, USDA 2005, OMRI 2007).

Examples of insecticides allowable under the NOP standards are pyrethrins, insecticidal soap, and narrow-range mineral oil. Pyrethrins are extracts of seed cases of chrysanthemum, *Chrysanthemum cinerariaefolium* Vis., which act by disrupting the sodium ion pump in insect nerve cells. Potassium salts of fatty acids, known as insecticidal soaps, act by penetrating the insect's cuticle and disrupting cell membranes, thereby causing the insect to desiccate. Narrow-range oils are mineral oils (also referred to as petroleum or horticultural oil), which act by smothering insects while not causing phytotoxicity.

Research has been conducted on control of soybean aphid by using synthetic insecticides (Ostlie 2001), and synthetic insecticides have been tested on *H. axyridis* for nontarget effects in other crops, such as hops (James 2003); corn, *Zea mays* L., (Galvan et al. 2005); and citrus (Michaud 2002). However, there are no studies that have considered NOP-compliant insecticides for soybean aphid control in organic production systems. Moreover, these insecticides have not been investigated for nontarget effects on *H. axyridis.* In this study, we determined the efficacy of pyrethrins, insecticidal soap, and narrow-range mineral oil against multiple life stages of the soybean aphid under laboratory conditions. We also establish possible nontarget effects of these insecticides against multiple life stages of *H. axyridis.*

Materials and Methods

***A. glycines* Colony and Bioassay Test Units.** An *A. glycines* colony was founded from individuals collected in summer 2005 from soybean at Arlington Agricultural Research Station, University of Wisconsin, Arlington, WI. Aphids were maintained on soybean plants ('Vinton 81'). Soybean plants were grown four plants per 15-cm pot in a 1:1 mix of 3M Metro Mix (Conrad Fafard, Inc., Agawam, MA) and sphagnum peat moss and seeded with *Bradyrhizobium japonicum* (EMD Crop Bioscience, Inc., Brookfield, WI) inoculum added per pot. Plants were grown in a greenhouse, supplemented with grow lights to attain a photoperiod of 16:8 (L:D) h. Plants were fertilized weekly using 20–9–20 water-soluble fertilizer (Technigro, Bellevue, WA) and irrigated as needed. As infested plants began to decline, leaves containing aphids were excised and placed on healthy plants. Once aphids had migrated to new plants, dead plant material was removed.

Adult aphids were randomly collected from the colony using a fine tip, no. 1 camel's-hair brush and placed on excised aphid-free soybean leaflets, with the underside of the leaf facing upward, in plastic petri dishes (100 by 15 mm). The leaflet petiole was wrapped in moistened cotton and kept moist until the leaflet was discarded. Petri dishes were kept in a temperature controlled chamber at 22 ± 1°C, with a photoperiod of 16:8 (L:D) h. Nymphs produced by these adults were removed daily, combined randomly into groups of 10 and placed on a soybean leaflet in a petri dish unit identical to the adults. If the desired stage for experimentation was nymphs, these groups were then used as the test unit for the bioassay. Thus, the bioassay was carried out on 10 first instars per replicate. If the desired stage was cohort adults, these groups were raised to adults, being provided fresh leaflets every 2 d. On the day of the adult aphid bioassay, the groups of 10 apterous F_1 adults per replicate were transferred to new petri dishes with fresh leaflets.

***H. axyridis* Colony and Bioassay Test Units.** The *H. axyridis* colony was founded from adults collected in autumn 2005 at Arlington Agricultural Research Station, University of Wisconsin, Arlington, WI. Beetles were held in 61- by 45- by 47-cm screened cages in a greenhouse supplemented with grow lights to attain a photoperiod of 16:8 (L:D) h. Cages also contained soybean plants infested with soybean aphids. Before experimentation, beetles were removed from cages and grouped into male/female pairs in petri dishes (100 by 15 mm). Sex was determined by examining the sexually dimorphic characteristics of the fifth abdominal segment (McCornack et al. 2007). Dishes were kept in growth chambers at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. These beetles were provided an ad libitum supply of soybean aphid-infested soybean leaflets and frozen *Ephestia kuehniella* Zeller eggs (Beneficial Insectary; Redding, CA).

Petri dishes containing the male and female pairs were checked daily for eggs, which were transferred to a new petri dish (100 by 15 mm) and kept at aforementioned conditions. After hatching, larvae were reared to the desired developmental stage on a diet of frozen *E. kuehniella* eggs.

Assays were conducted on eggs, first and third instars, pupae, and adults. However, due to experimental difficulties with the eggs, these data are not presented. Larvae and pupae were sprayed 24 ± 4 h after molting. Adults were sprayed 5–7 d after molting. Fifteen individuals of the larval stages or 10 individuals of pupal or adult stages (1:1 sex ratio) were considered a replication. Pupae were removed from their substrate with a razor blade. Replicate groups of larvae, pupae or adults were then placed into plastic petri dish bottoms (100 by 15 mm) for treatment application. Test units were kept in a temperature-controlled chamber at 25 ± 1°C and a photoperiod of 16:8 (L:D) h until time of treatment.

Insecticides. Treatments included pyrethrins (PyGanic EC 1.4 [3.51 liters/ha], McLaughlin Gormley King Co., Golden Valley, MN), potassium salts of fatty acids (insecticidal soap) (M-Pede [2% vol:vol], Dow Agrosciences, Indianapolis, IN), a narrow-range mineral oil (Omni Supreme [18.71 liters/ha], Helena Chemical Co., Collierville, TN), and a water (deionized) only control. All treatments, except pyrethrins, were administered at highest labeled field rate of each NOP-compliant insecticide for aphids on legume crops. Pyrethrins were administered at a medium rate within a wide range (1.4–5.61 liters/ha) given on the label. A carrier volume equivalent to 281 liters/ha (deionized water) was used to determine the ratio of insecticide to water needed to prepare treatments for laboratory bioassay. This application volume is similar to the range recommended to achieve optimal soybean canopy deposition with insecticides under commercial field conditions (Cullen 2007).

Bioassay. The experiment for each developmental stage of each insect consisted of the four treatments in a completely randomized block design with six replications through time. A Potter Precision Laboratory Spray Tower (Burkard Scientific Ltd., Uxbridge, United Kingdom) was used to deliver the insecticide treatment to each test unit. The total volume of carrier plus insecticide was converted from liters per hectare into grams per square centimeter to give a measurable unit for determining treatment rate delivered to the test unit. The spray tower was calibrated between each treatment, within each replicate, by manipulating spray pressure and weighing sprayed petri dishes until the target deposition was reached (Potter 1952). Five-ml aliquots were consistently used and spray pressure was adjusted between 54 and 80 kPa. Test units also were weighed after treatment to record precise deposition.

Treated *A. glycines* nymphs and adults were allowed to dry for 30 min uncovered, before being transferred using a fine, no. 1 camel's-hair brush to fresh soybean leaflets in fresh petri dishes. They were then covered and returned to the temperature-controlled chamber at 22 ± 1°C and a photoperiod of 16:8 (L:D) h.

Treated *H. axyridis* larvae and adults were transferred using a fine, no. 1 camel's-hair brush to individual covered petri dishes (60 by 15 mm) lined with filter paper and provided frozen *E. kuehniella* eggs ad libitum. Pupae were allowed to dry uncovered for 30 min before being transferred to fresh petri dishes (100 by 15 mm) and covered. All were returned to the controlled temperature chamber at 25 ± 1°C and a photoperiod of 16:8 (L:D) h.

Assessment. Mortality of the *A. glycines* adults and nymphs was recorded 24, 48, and 72 h posttreatment. Aphids were considered dead when they failed to respond to repeated gentle prodding with a no. 1 camel's-hair brush. Aphid nymphs produced by treated aphid adults were removed from the test unit.

H. axyridis larvae and pupae were checked every 24 h until they either molted to the next life stage or died. Adults were checked every 24 h for 7 d. Larval and adult mortality was determined if they failed to respond to repeated gentle prodding with a no. 1 camel's-hair brush. Pupae were assumed dead if they did not molt to adult stage in 10 d.

Analysis. Mean proportion mortality for each treatment replicate of aphid nymphs and adults at each time check were arcsine square-root transformed. Mean proportion mortality of all *H. axyridis* life stages also were arcsine square-root transformed. Transformed proportionate means were analyzed using analysis of variance (ANOVA), with replication and treatment as random and fixed factors, respectively (PROC Mixed, SAS Institute, 2006). Mean differences were compared using Tukey's test ($P < 0.05$) as appropriate, after a significant F-test.

Results

A. glycines. Pyrethrins and mineral oil caused significant nymphal mortality at the 24 h check compared with the control, whereas insecticidal soap did not cause significant mortality until the 48 h check (Fig. 1). By the 72-h check, insecticidal soap caused mortality that was statistically equivalent to that caused by pyrethrins and mineral oil (Fig. 1).

All three insecticidal treatments caused significant adult mortality compared with a water-only control by the 24-h posttreatment check (Fig. 2). At the 72-h check, pyrethrins and mineral oil caused the highest adult mortality, whereas mortality due to insecticidal soap was still statistically less (Fig. 2).

H. axyridis. Pyrethrins caused the greatest first instar mortality, at nearly 100% (Table 1). Mineral oil and insecticidal soap caused less first instar mortality than pyrethrins; yet, both were significantly higher than the control (Table 1). By contrast, third instar mortality due to pyrethrins did not differ statistically from the control. Third instar mortality due to the mineral oil and insecticidal soap were similar to mortality due to the same treatments on first instars. Pupal mortality ranged between 10.0 and 18.3%, with no significant difference ($P > 0.05$) between treatments (Table 1). We observed zero adult mortality in our study (Table 1).

Discussion

Due to farmer reliance on biological control for insect pest management in organic agriculture, it is essential to determine whether insecticides are compatible with suppressive effects of key biological control agents on the pest. One measure of testing for this compatibility is by conducting insecticide bioassays against not only the pest insect but also key biological control agents. Our study demonstrates that there are distinct effects of insecticides on different insects and even different life stages of the same insect.

Pyrethrins caused 100% mortality in both tested life stages of *A. glycines.* Similarly, a spray treatment of pyrethrins caused 100% mortality of a bean aphid, *Aphis fabae* Scopoli, colony 11 d after treatment (Foster and Kelly 1991). Pyrethrins also proved to reduce densities of another aphid colony, *Fimbriaphis fim-*

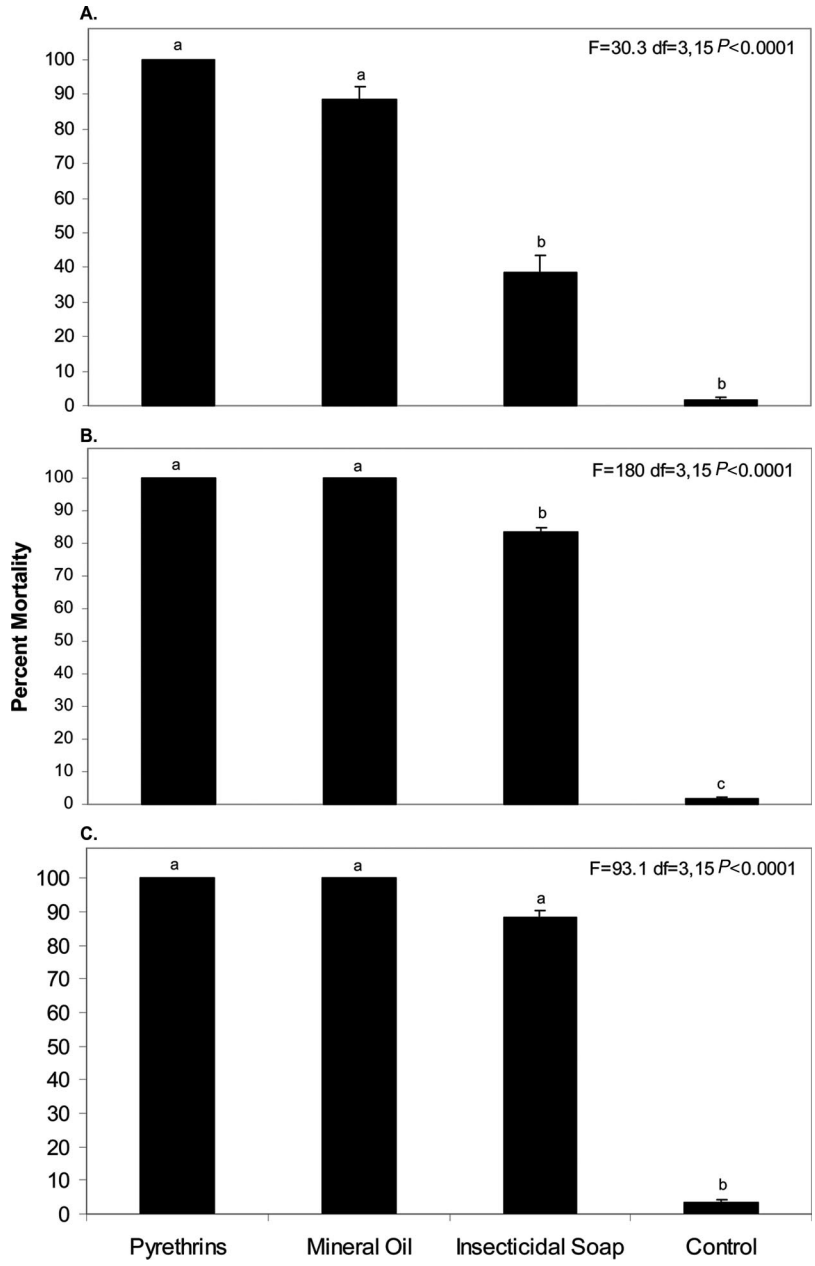

Fig. 1. Mean percentage of mortality of *A. glycines* first instars at 24 (A), 48 (B), and 72 (C) h after treatment with reduced-risk insecticides in Potter Spray Tower laboratory bioassay. Data are means ± SE of six replications. Within each time period, columns marked with the same letter do not differ significantly ($P < 0.05$; Tukey's test).

briata Richards, by >50% when foliar applied to strawberry plants, *Fragaria* × *ananassa* Duch., under field conditions (Lowery et al. 1993).

A. glycines and *H. axyridis* showed differential susceptibility to pyrethrins. This may be attributable to a difference in the rate with which the insecticide is able to penetrate each insect's cuticle. Zhuraravskaya et al. (1976) found that an organophosphate insecticide penetrated the cotton aphid, *Aphis gossypii* Glover, at a faster rate than either the adult or larval stage of one of its natural enemies, *Chrysoperla carnea* (Stephens). This difference in penetration rate further correlated with a differential insecticide toxicity between the insect species.

Another possible explanation for the differential susceptibility observed between insect species in our study may be differences in the ability of each insect to metabolize pyrethrins. Cho et al. (2002) reported significant differences between the amounts of a detoxifying enzyme, glutathione transferase, present in

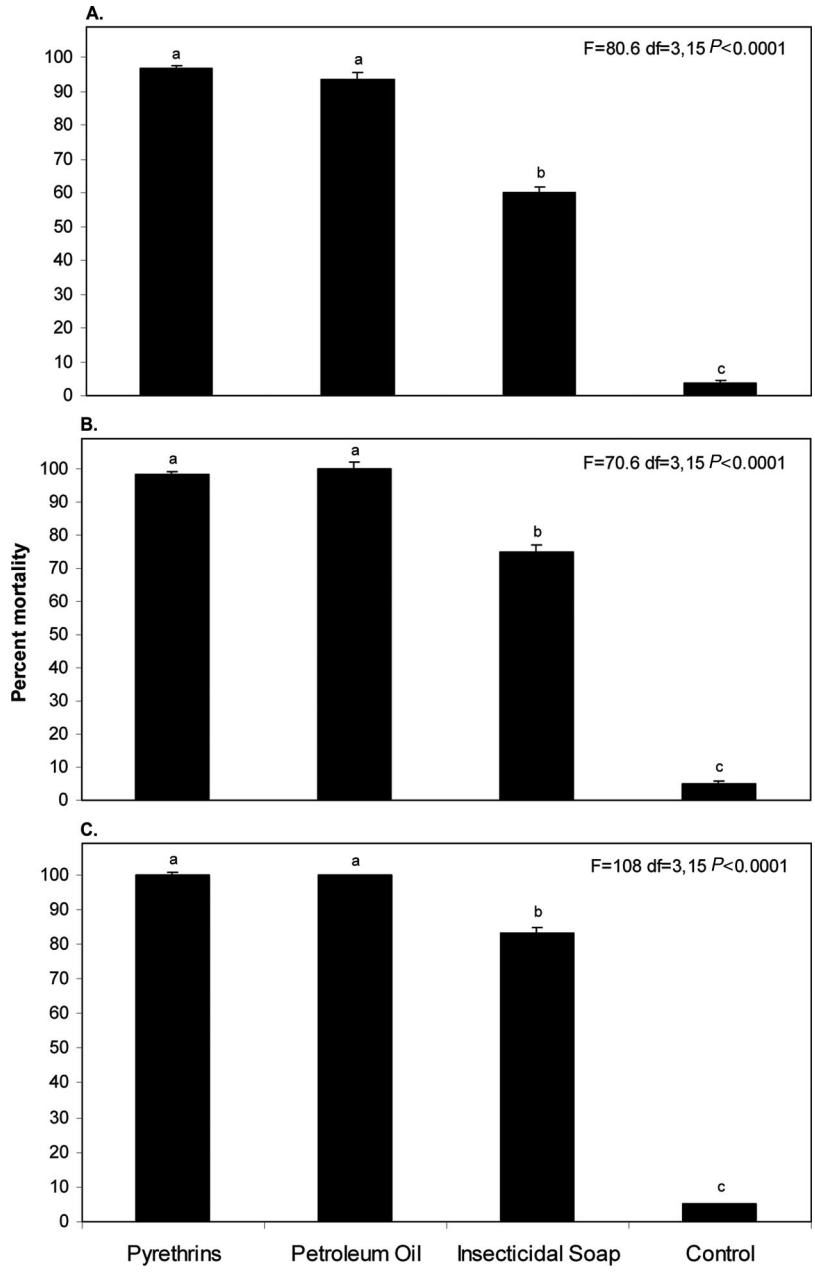

Fig. 2. Mean percentage of mortality of *A. glycines* adults at 24 (A), 48 (B), and 72 (C) h after treatment with reduced-risk insecticides in Potter Spray Tower laboratory bioassay. Data are means ± SE of six replications. Within each time period, columns marked with the same letter do not differ significantly ($P < 0.05$; Tukey's test).

the larval and adult stages of *H. axyridis* and two species of aphid prey, *Aphis citricola* van der Goot and *Myzus malisuctus* Matsumura. Specifically, *H. axyridis* adults produced >50 times the amount produced by each of the aphids and *H. axyridis* larvae produced over eight times more than the aphids. This difference in enzyme production correlates with relative susceptibility of each these insects to synthetic pyrethroids (Cho et al. 1997), suggesting glutathione transferase is capable of metabolizing this insecticide class.

Similarly, intraspecies differences in mortality caused by pyrethrins to the various life stages of *H. axyridis* in our study could also be explained by insecticide penetration rate of the insect's cuticle and/or glutathione transferase production. Christie and Wright (1990) attributed marked differences in relative toxicity of the insecticide abamectin between larval instars of *Spodoptera littoralis* (Boisduval) to differences in the insecticide's penetration rate. This suggests that between life stages of one species there

Table 1. Mean percentage of mortality ± SEM of *H. axyridis* life stages after treatment with reduced-risk insecticides in Potter Spray Tower-applied laboratory bioassay

Trade name	Active ingredient	*H. axyridis* developmental stages			
		First instar[a]	Third instar[b]	Pupae[c]	Adult
Control	Deionized water	3.34 ± 1.49a	0.0 ± 0.0a	18.33 ± 4.77a	0
Pyganic	Pyrethrins	98.89 ± 1.11c	9.57 ± 4.70a	10.0 ± 5.16a	0
Omni Supreme	Narrow-range mineral oil	48.89 ± 10.70b	31.90 ± 5.68b	16.67 ± 5.68a	0
M-Pede	Insecticidal soap	40.0 ± 10.03b	38.74 ± 7.30b	16.67 ± 3.33a	0

Means within a column followed by the same letter are not significantly different from each other ($P < 0.05$; Tukey's test).
[a] $F = 26.26$; df = 3, 15; $P < 0.0001$.
[b] $F = 21.86$; df = 3, 15; $P < 0.0001$.
[c] $F = 0.95$; df = 3, 15; $P = 0.44$.

may be enough cuticular differences to alter the insecticide's penetration rate and resulting toxicity. With regard to levels of glutathione transferase, Cho et al. (2002) found adult *H. axyridis* to produce six times more glutathione transferase than the larvae (instar not specified). Possibly, glutathione transferase production and accumulation increases as the insect ages. A similar pattern of differential life stage susceptibility in *H. axyridis* to several insecticide classes was observed by Galvan et al. (2005). In their study, first instar was most sensitive followed by third instar, adult, and finally pupae.

A third potential explanation for differential susceptibility of *H. axyridis* life stages in our study may be insect size. First instars are much smaller than later instars, pupae, and adults; yet, they all received the same insecticidal dose. Presumably, smaller insects with a lower body size to insecticide dosage ratio have increased susceptibility to insecticide toxicity.

Mineral oil also caused significant mortality to both tested life stages of *A. glycines.* Similarly, when bird-cherry oat aphids, *Rhopalosiphum padi* (L.), were sprayed on wheat (*Triticum* spp.) under field conditions with two different petroleum oil fractionations, CAPL1 and CAPL 2, there was a 75 and 92% reduction, respectively, in aphid densities 2 d after treatment (Hariry et al. 1997). Mineral oil also has proved capable of considerably reducing densities of wooly apple aphid, *Eriosoma lanigerum* (Hausmann), on apple, *Malus domestica* Borkhausen, seedlings under greenhouse conditions (Fernandez et al. 2005).

Mineral oil was moderately lethal to first and third instars of *H. axyridis.* Comparably lower larval mortality, 12.7% for small larvae and 26% for large larvae, of another coccinellid, *Nephaspis oculatus* (Blatchley), was found after treatment with a narrow-range mineral oil (Liu and Stansley 1996). However, these researchers used a concentration of 0.5% compared with 6.67% used in our study. This suggests that if a lower concentration of oil could be shown to cause sufficient mortality to soybean aphid, it may minimize impact on the larval stages of *H. axyridis.*

Insecticidal soap caused high rates of aphid mortality for both tested life stages. Due to insecticidal soap's mode of action, it is particularly effective against soft-bodied insects (Koehler et al. 1983). This suggests expected equivalent efficacy of insecticidal soap across various genera of aphids. A range of insecticidal soap concentrations were evaluated on three aphid species, *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer), and *Nasonovia ribisnigri* (Mosley), by using a hand-held spray bottle to treat 15–20 aphids placed on an excised lettuce leaf, *Lactuca sativa* L. (Fournier and Brodeur 2000). Results indicated that a 2% insecticidal soap solution caused ≈90 and 75% mortality at three days posttreatment for *M. ephorbiae* and *M. persicae,* respectively. However, *N. ribisnigri* seemed more tolerant of the same treatment with ≈20% mortality at the 3-d check. A 1% insecticidal soap solution was used with a hand held paint sprayer to treat 10 green peach aphids, *M. persicae,* placed on a shoot of *Verbena speciosa* Balazlav under greenhouse conditions and found to cause 55.2% mortality (Chiasson et al. 2004). Our results fit within a large range of mortality observed by other researchers across aphid genera. Such a wide range suggests that method of application and concentration, and species, may play a role in the efficacy of insecticidal soaps when treating aphids.

Aphid mortality due to the insecticidal soap treatment increased significantly during the time period of the study for both the first instar and adult stage. An observation made during the study, although not quantified in the results, was that due to this relatively slower lethal action, dying aphids continued to feed and adults continued to reproduce. This observation requires additional inquiry to establish effects delayed lethal action may have on aphid population growth rate and host plant damage.

Both instars of *H. axyridis* tested demonstrated similar rates of mortality when treated with the insecticidal soap. This level of mortality was analogous to that of early and late instars of the coccinellid *N. oculatus,* 24 h after treatment with insecticidal soap (Liu and Stansley 1996). Nonsignificant coccinellid pupal and adult mortality after treatment with soap was an additional similarity between our study and the work of Liu and Stansley (1996).

Our results indicate that pyrethrins, mineral oil, and insecticidal soap may prove useful in the management of soybean aphid in organic soybean while minimizing negative impacts on *H. axyridis.* Due to the slow lethal action of insecticidal soap and lower adult aphid mortality rate, this product may be less preferable in an organic management program where off-farm inputs must be kept to a minimum. On-farm trials are also

necessary to observe the effects of these insecticides on both *A. glycines* and *H. axyridis* under field conditions. Experimental variables should include application timing and frequency relative to soybean aphid density, carrier volume for optimal canopy deposition, residual activity of these materials in the field, and posttreatment predator-to-prey ratios. Other considerations of organic farmers before incorporation of insecticidal inputs into their organic system plan (USDA 2005) will include cost of treatment, value of the organic soybean crop, and soybean yield and/or quality response to treatment.

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