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Efficacy of Several Pesticide Products on Brown Widow Spider (Araneae: Theridiidae) Egg Sacs and Their Penetration Through the Egg Sac Silk

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Abstract

Information on pesticide effects on spiders is less common than for insects; similar information for spider egg sacs is scarcer in the open literature. Spider egg sacs are typically covered with a protective silk layer. When pesticides are directly applied to egg sacs, the silk might prevent active ingredients from reaching the eggs, blocking their insecticidal effect. We investigated the impact of six water-based pesticide sprays and four oil-based aerosol products against egg sacs of brown widow spiders, *Latrodectus geometricus* C. L. Koch. All water-based spray products except one failed to provide significant mortality to egg sacs, resulting in successful spiderling emergence from treated egg sacs at a similar rate to untreated egg sacs. In contrast to water-based sprays, oil-based aerosols provided almost complete control, with 94–100% prevention of spiderling emergence. Penetration studies using colored pesticide products indicated that oil-based aerosols were significantly more effective in penetrating egg sac silk than were the water-based sprays, delivering the active ingredients on most (>99%) of the eggs inside the sac. The ability of pesticides to penetrate spider egg sac silk and deliver lethal doses of active ingredients to the eggs is discussed in relation to the chemical nature of egg sac silk proteins. Our study suggests that pest management procedures primarily relying on perimeter application of water-based sprays might not provide satisfactory control of brown widow spider eggs. Determination of the most effective active ingredients and carrier characteristics warrant further research to provide more effective control options for spider egg sacs.

Key words: brown widow spider, *Latrodectus*, Arachnida, pesticide barrier, pesticide efficacy

The body of literature regarding pesticide research on spiders pales in comparison with that known for insects [although spider pesticide research is less common, it is still not trivial; see Pekár (2012) for a review]. However, even scarcer and possibly nonexistent in the open scientific literature is information regarding the effects of pesticides on spider eggs. Two significant obstacles contribute to this paucity of research on spider eggs: 1) the difficulty in obtaining sufficient numbers of impregnated female spiders of the same species to produce a sample size that is meaningful, and 2) the difficulty in procuring sufficient numbers of eggs from these females in a short period such that experiments can be efficiently conducted using the groups of eggs with similar age composition. Eggs are likely to be one of the most important life stages that are responsible for spider infestation in urban environments, especially when aided by human-mediated dispersal and reintroduction. Moreover, considering the large number of spiderlings produced by a single egg sac, the management of egg sacs is critical in the reduction or elimination of pest spiders around structures. Thus, a toxicological study involving egg sacs is an important addition to provide insights for practical pest management of spiders in urban settings.

The brown widow spider, *Latrodectus geometricus* C. L. Koch, is a pantropical invasive species (Garb et al. 2004) inhabiting many Caribbean and Pacific Ocean islands and all continents except the obviously nontropic Antarctic. The female brown widow spider has prolific fecundity; she can produce 22 egg sacs on average throughout her lifetime and can produce an egg sac every 4 d during the early portion of her reproductive life (Bouillon and Lekie 1961). Field-collected brown widow spider egg sacs in southern California average around 135 eggs per sac, with a range of 23–282 (Vetter et al. 2012, Danielsen et al. 2014). Upon discovery of brown widow spider egg sacs, members of the general public often express a strong desire for effective control methods. Even though most pest management professionals typically utilize water-based pesticide sprays for spiders and spider webbing around structures (D.-H. Choe, unpublished data), the efficacies of various pesticide products for brown widow spider egg sacs remain to be determined.

Flores et al. (2007) recently examined the lethal effect of two commercial pesticide products (deltamethrin and cypermethrin) on the brown widow spider eggs that were dissected from egg sacs. However, the eggs of brown widow spider are always found within a protective egg sac. It has been suggested that the egg sac silk protects the eggs from various biotic and abiotic challenges such as predators, parasites, temperature, and humidity (Austin 1984, 1985). The unique physical and chemical properties of the egg sac silk might present a layer of complexity when chemical control of spider egg sacs is desired. For example, the egg sac silk layer could provide protection against pesticides by rebuffing water-based treatments owing to the hydrophobic nature of silk (Zhao et al. 2006). Alternatively, the active ingredients of pesticide could strongly bind to the egg sac silk so that the active ingredients are unable to penetrate the silk cover, being unavailable to negatively affect development of the eggs and spiderlings inside. Because of these reasons, chemical characteristics of the pesticide carriers might be also important to determine the efficacy of the pesticide products in addition to the inherent toxicity of their active ingredients.

In the current study, we examined the efficacy of several pesticide products with different carriers (e.g., water-based sprays and oil-based aerosols) targeting the egg sacs of brown widow spiders. In the first study (product efficacy study), a group of egg sacs was treated using the method by which the products would be used during practical pest management procedures. Using a group of egg sacs containing equal proportions of three different developmental stages, we investigated which products would provide complete prevention of spiderling emergence from the treated egg sacs regardless of the developmental stages of egg sacs. In the second study (penetration study), we investigated the penetration efficacies of selected pesticide products through the egg sac silk, and their relationship to the resulting mortality of the eggs inside of the treated egg sacs. The hydrophobic property of *Latrodectus* spider egg sac silk and its implications for practical pest management of the spider egg sac are discussed.

Materials and Methods

Spiders

Mature female brown widow spiders were collected throughout southern California and maintained in 163-ml plastic cup containers (550PC, Dart Container Corporation, Mason, MI) with lids (PL4, Dart Container Corporation). A Y-shaped piece of cardboard (2 mm wide with two arms of 35 mm length and the third arm of 52 mm length) was placed inside each container to provide a substrate from which the spider built a web. The containers with spiders were maintained on a laboratory bench top at 25–26°C. A nearby window provided a natural photoperiod [changed from 14:10 to 11:13 (L:D) h over the experimental period]. Each spider was provided with a beetle larva (*Tenebrio molitor* L.) as prey every 7 to 10 d. All containers were checked daily for newly produced egg sacs. Once producing an egg sac, the female spider was moved to a new container. The egg sacs were typically attached to the web or the Y-shaped cardboard substrate. Egg sacs with abnormal shape or unusual construction (i.e., exceptionally thin silk covering) were determined by visual inspection and not used for the study. Each egg sac was marked with the date of discovery to track its age. All egg sacs were maintained in the original plastic containers placed in an incubator at 29–31°C without a photoperiod until being used for the experiments. Under these conditions, the eggs typically hatch into the first instar around day 9 post-oviposition. The first-instar spiderlings have little pigmentation, being barely mobile with their short legs. The first-instar spiderlings undergo one molt within the egg sac by day 17 post-oviposition, whereupon the second-instar spiderlings develop longer legs, more pigmentation, and better mobility to prepare for emergence from the egg sac (R. S. V. et al., unpublished data). Most (98%) of the egg sacs used in the experiment were obtained from the females between June and October, a period during which field populations of brown widow spiders in southern California actively produce viable egg sacs (Danielsen et al. 2014). A few egg sacs were collected in November and December as replacements for egg sacs used early in the study whose contents were either unknowingly absent (i.e., no eggs inside the egg sac) or dead from causes other than the pesticide treatment (i.e., defective eggs forming a tight “egg ball”).

Product Efficacy Study

Ten pesticide products were selected for the product efficacy study. Six of them had water as a major carrier (hereafter referred to as water-based sprays): CyKick CS (BASF corporation, St. Louis, MO), Suspend Polyzone (Bayer Environmental Science, Research Triangle Park, NC), Temprid Ready Spray (Bayer Environmental Science), Harmonix (Bayer Environmental Science), Eco PCO WPX (Prentiss Inc., Alpharetta, GA), and Essentria IC3 (Envincio LLC, Cary, NC). Temprid Ready Spray was a water-based ready-to-use product available in a pressurized container, unlike the other spray products where the final sprays were prepared in the laboratory by mixing the concentrated materials with water. The other four were oil-based pressurized aerosol products (hereafter referred to as oil-based aerosols): CyKick aerosol (BASF corporation), D-Force HPX aerosol (Waterbury Companies, Inc., Waterbury, CT), Phantom aerosol (Phantom pressurized insecticide, BASF corporation), and Gentrol aerosol (Wellmark International, Schaumburg, IL). The aerosol products used in the current study contained combustible, oil-based carriers (e.g., petroleum distillates, dimethyl ether, or acetone) based on their material safety data sheets. The active ingredients, test rates, and major carrier types (i.e., water-based vs oil-based) for the pesticide products are listed in Table 1.

In total, 265 egg sacs obtained from 169 female spiders were used for the product efficacy study. Because the spider egg sacs can be at different developmental stages when treated with a pesticide product, we used a group of egg sacs ($n = 18$) representing identical proportions of three different developmental stages [i.e., egg (1 d post-oviposition), early stage spiderling (12 d post-oviposition), and pre-emergent spiderling (17 d post-oviposition)] to test each product. The egg sacs were treated with the pesticides while they were still attached to the Y-shaped cardboard inside the plastic container. For the water-based pesticide sprays, one or two applications with a plastic spray bottle (Greenbrier International Inc., Chesapeake, VA) held 20 cm from the plastic cup container (top surface area 44.15 cm^2) provided the label application rates of the pesticide products (i.e., 1 gal/1,000 ft^2 or 2 gal/1,000 ft^2). Temprid Ready Spray was also used in a similar fashion, but with the original spray container. The oil-based aerosols were briefly (1 s) dispensed on the egg sacs through the spray nozzles attached to the original containers. For all treatments, the pesticide products were topically applied to one side of the egg sacs. Because egg sacs are typically placed close to a substrate in the field, this one-sided treatment was considered to simulate the realistic amount and pattern of the pesticide residues that would be delivered to the egg sac surface during the pesticide application. The treated egg sacs were left in a fume hood for 1 h until the

Table 1. Pesticide products used for the efficacy studies

Pesticide product	Active ingredient	Label rate (wt/wt)	Rates tested (wt/wt)	Carrier type
CyKick CS	Cyfluthrin	0.05–0.1%	0.05% (product efficacy) 0.1% (penetration)	Water
Suspend Polyzone	Deltamethrin	0.01–0.06%	0.03% (product efficacy) 0.06% (penetration)	
Temprid Ready Spray	Imidacloprid β-Cyfluthrin	N/A	Imidacloprid 0.05% β-Cyfluthrin 0.024%	
Harmonix	Pyrethrins	0.03–0.24 %	0.06%	
Eco PCO WPX	2-Phenethyl propionate, thyme oil, pyrethrins	0.5–2 oz in 1 gal of water (0.0125–0.05%)	0.05%	
Essentria IC3	Rosemary oil, geraniol, peppermint oil	1–8 oz in 1 gal of water (0.13–1.06%)	1.06%	
CyKick aerosol	Cyfluthrin	N/A	0.1%	Oil
D-Force HPX aerosol	Deltamethrin	N/A	0.06%	
Phantom aerosol	Chlorfenapyr	N/A	0.5%	
Gentrol aerosol	(S)-Hydroprene	N/A	0.36%	

List of the active ingredients, label rates, test rates, and carrier types are provided.

pesticide residues dried. The egg sac was then carefully removed from the Y-shaped piece of cardboard (with forceps and scissors) and placed inside a new 163-ml container with a new Y-shaped cardboard. The egg sacs were attached to the Y-shaped cardboard using a few silk strings from the egg sacs. New lids were affixed and the containers were placed in the incubator described above. Of 265 egg sacs, 180 were used for the pesticide treatments. The untreated control egg sacs ($n = 85$) were obtained during the same period and prepared in an identical method without pesticide treatments. The egg sacs were checked daily for emergence of spiderlings.

If emergence of spiderlings occurred, the container was removed from the incubator and allowed to sit for 2 d to ensure that any stragglers were given a chance to emerge from the egg sac. The egg sacs and spiderlings in the container were frozen in a −20°C freezer for 12–24 h, and the emerged spiderlings were subsequently counted. The egg sacs and silk were first wet with ethyl alcohol, and a few drops of commercial bleach (6% sodium hypochlorite) were applied on them. This process dissolved the spider silk (Vetter et al. 1996) to facilitate counting of the clutch inside the egg sac or dead spiderlings attached to silk in the container. After dissolution, which takes about 5 min, ethyl alcohol was squirted onto the remains to eliminate the bubbles caused by bleach action, and the contents of the egg sacs (i.e., unhatched eggs and spiderlings) were counted under a microscope. In containers where no spiderlings emerged by day 30, the egg sacs were removed and the contents were examined but not counted (i.e., unhatched eggs, dead spiderlings, etc.).

Penetration Study

A subset of the pesticide products used in the product efficacy study was subsequently tested to determine their effectiveness in penetrating the egg sac silk. In particular, water-based sprays and oil-based aerosols containing pyrethroids [e.g., water-based CyKick CS vs oil-based CyKick aerosol (active ingredient: cyfluthrin) and water-based Suspend Polyzone vs oil-based D-Force HPX aerosol (active ingredient: deltamethrin)] were compared. The product efficacy study indicated that there was a significant difference between water- or oil-based products even though they contained similar active ingredients. CyKick CS and Suspend Polyzone sprays were tested at 0.1 and 0.06% (wt/wt) rates, respectively, to allow the comparison with the corresponding aerosol products containing the same active ingredients at those rates (i.e., CyKick aerosol and D-Force HPX aerosol).

To determine if different pesticide products have different levels of effectiveness in penetrating the silk layer of the egg sac, the egg sacs were treated with the pesticide products and the percentages of eggs receiving the pesticide inside the egg sacs were compared. Forty egg sacs in their early stage of development (i.e., <8-d-old, containing eggs only) were obtained from 40 female brown widow spiders, and a group of 10 egg sacs was used to test each product. To visualize the pesticide residue on the egg surface, we mixed pesticide preparations with red dyes. Neutral Red (Sigma Aldrich, St. Louis, MO) (4 mg/ml) was used to visualize the water-based sprays (e.g., CyKick CS and Suspend Polyzone). For the aerosol formulations (e.g., CyKick aerosol and D-Force HPX aerosol), Oil Red O (J. T. Baker Chemical Co., Phillipsburg, NJ) was used as a dye (4 mg/ml). Preliminary tests indicated that all of the pesticide products mixed with dye were effective in staining the surface of the eggs when they were directly applied to the eggs.

First, the egg sacs were carefully removed from the web or cardboard substrate with forceps and scissors. For the treatment with water-based sprays, the egg sacs were briefly (for 1 s) submerged in the pesticide preparation mixed with dye (18 ml contained in a glass vial) while gently holding them with forceps. For the aerosol formulations, an aliquot of pesticide mixed with dye was first prepared in a glass vial, and 10 µl of the mixture was topically applied to one side of an egg sac with a micropipette. The egg sacs were individually weighed with an analytical scale (Mettler AE 204, Mettler-Toledo, LLC., Columbus, OH) before and after the treatment to determine the weight gain by the pesticide application. The increase of weight was considered to be a result of the adherence (on the surface of the egg sac) or absorbance (penetration into the egg sac) of the pesticide product. The treated egg sacs were placed in the cells (16 mm diameter by 19 mm depth) of 24-well plastic culture plates (Corning Inc., Corning, NY), and left inside a fume hood without the lids for ventilation. After 24 h, the treated egg sacs were visually inspected to determine if the excess water or solvent had disappeared from the surface. Then, the egg sacs were gently opened with forceps and scissors, and the total numbers of stained and non-stained eggs were recorded under a microscope. Eggs stained partially with red dyes were considered stained.

After counting, the eggs were placed in a plastic petri dish (45 mm diameter by 8 mm depth) with a tight lid and maintained in an incubator described above for 14 d to determine mortality. For the untreated controls, 10 egg sacs were obtained during the same period and prepared in an identical method without pesticide treatments.

Statistics

For the product efficacy study, a pesticide treatment was considered to be successful only if it completely prevented the emergence of spiderlings in the treated egg sacs regardless of their developmental stages. With this dichotomous data set (e.g., emergence vs no emergence) obtained from randomly selected 265 egg sacs, a contingency table analysis was used to determine if the spiderling emergence rate was dependent on the kind of treatment received (Analytical Software 2008). If the null hypothesis was rejected, the comparison of a control to each other treatment was conducted with one-sided Dunnett-type test (Zar 1999).

For the penetration study, Wilcoxon rank sum tests were used to compare the weight increase after the pesticide treatment and the level of penetration (i.e., number of eggs dyed/total number of eggs × 100) between the treatments with same active ingredients (Analytical Software 2008). The mortality levels at day 14 [i.e., (total number of eggs − number of spiderlings at day 14)/total number of eggs × 100] were also compared among the treatments with same active ingredients and control using Kruskal–Wallis one-way nonparametric ANOVA. All pairwise comparison tests were conducted at $\alpha = 0.05$ (Analytical Software 2008).

Results

Product Efficacy Study

Untreated egg sacs had an emergence failure rate of 27% (23 of 85). For 62 egg sacs with spiderling emergence, 91.8 ± 2.2% (mean ± SEM) of eggs in the egg sacs successfully emerged as spiderlings by day 30. The incubation period between oviposition and spiderling emergence was 20.5 ± 0.3 d.

The contingency table analysis on the emergence data across three different developmental stages of egg sac indicated that the distributions of successful emergence and emergence failure were significantly different among 10 pesticide treatments and untreated egg sacs ($\chi^2 = 100.38$, df = 10, $P < 0.0001$) (Table 2). Based on the multiple comparisons for proportions, the levels of emergence failure were similar among untreated control group and all pesticide treatments except for four aerosol treatments (CyKick, D-Force HPX, Phantom, and Gentrol aerosols) and Temprid Ready Spray treatment, which significantly prevented spiderling emergence (Table 2). For example, 94–100% of egg sacs were prevented from having successful spiderling emergence when they were treated with the four aerosol products. The egg sacs treated with Temprid Ready Spray showed 78% emergence failure. In contrast, the egg sacs treated with water-based pesticide sprays (CyKick CS, Suspend Polyzone, Harmonix, Eco PCO WPX, and Essentria IC3) had only 28–56% emergence failure, not being significantly different from the untreated control group that had 27% natural emergence failure (Table 2). The egg sacs without spiderling emergence contained various compositions of dead eggs, dead spiderlings, or live spiderlings when opened for examination at day 30 (Table 2).

Penetration Study

Egg sacs averaged 111.8 ± 5.3 eggs (mean ± SEM, $n = 50$). The eggs dissected from the untreated egg sacs had an overall 19.7 ± 3.5% ($n = 10$) mortality at the end of the 14-d incubation period.

Two pyrethroid aerosol products penetrated the egg sac silk more effectively compared with the other pyrethroid products with aqueous carriers. For water-based CyKick CS and oil-based CyKick aerosol (AI = cyfluthrin), the amount of weight increase after the pesticide treatments was not significantly different between them [9.3 ± 0.5 and 8.7 ± 0.1 mg (mean ± SEM) for CyKick CS and CyKick aerosol, respectively] (Wilcoxon rank sum test: $z = 0.57$, $P = 0.57$). However, the percentage of eggs dyed was significantly higher in the CyKick aerosol treatment compared with the CyKick CS treatment [99.1 ± 0.6 and 0.0 ± 0.0 %, respectively] (Wilcoxon rank sum test: $z = 4.15$, $P < 0.0001$). The final mortality levels of CyKick CS and CyKick aerosol treatments, and untreated control were significantly different (Kruskal–Wallis ANOVA: $H = 20.24$; df = 2, 27; $P < 0.0001$). A multiple comparison analysis indicated that mortality level achieved by CyKick aerosol treatment (93.3 ± 4.9%) was significantly higher compared with those found in CyKick CS treatment (25.3 ± 3.7%) or untreated control (19.7 ± 3.5%) (Kruskal–Wallis all pairwise comparison test, $\alpha = 0.05$).

Similarly, for water-based Suspend Polyzone and oil-based D-Force HPX aerosol, the amount of weight increase after the pesticide treatments was not significantly different between the treatments [9.1 ± 0.9 and 7.8 ± 0.1 mg (mean ± SEM) for Suspend Polyzone and D-Force HPX aerosol, respectively] (Wilcoxon rank sum test: $z = 0.80$, $P = 0.43$). The percentage of eggs dyed was significantly higher for the D-Force HPX aerosol treatment compared with the Suspend Polyzone treatment [99.9 ± 0.1 and 0.1 ± 0.1% respectively] (Wilcoxon rank sum test: $z = 4.13$, $P < 0.0001$). The final mortality levels for Suspend Polyzone and D-Force HPX aerosol treatments, and untreated controls were significantly different (Kruskal–Wallis ANOVA: $H = 19.64$; df = 2, 27; $P = 0.0001$). A multiple comparison analysis indicated that mortality level achieved by D-Force HPX aerosol treatment (97.8 ± 2.2%) was significantly higher compared with those found in Suspend Polyzone treatment (26.1 ± 8.0%) or untreated control (19.7 ± 3.5%) (Kruskal–Wallis all pairwise comparison test, $\alpha = 0.05$).

Discussion

In nature, spiders use their silk for several purposes such as building webs, wrapping of prey, protection of their offspring, dispersal, and escaping from predators (Lewis 2006, Römer and Scheibel 2008). Depending on their uses, a single species of spider is able to produce several different types of silks from different glands, and the different silks might be distinctive in their mechanical properties, such as tensile strengths, extensibilities, and toughness (Lewis 2006, Garb et al. 2010). This mechanical diversity associated with the different silk types is believed to largely stem from variation in the molecular composition of the silk proteins, called spidroins (Gosline et al. 1999, Garb et al. 2010).

Hydrophobicity of egg sac silk was studied for tubuliform spidroins (TuSp1) of two *Latrodectus* spiders. TuSp1 is the dominant protein in the large diameter silks of *Latrodectus* egg sac (Garb and Hayashi 2005, Hu et al. 2005, Casem et al. 2010). Hydrophobicity indices of brown widow spider TuSp1 (GenBank AAY28940, AAY28950) and western black widow spider, *L. hesperus* Chamberlin & Ivie, TuSP1 (AAY28931) were calculated with General Protein Mass Analysis for Windows program (GPMAW lite; http://www.alphalyse.com/gpmaw_lite.html, last accessed 12 October 2015). For comparison with another silk type, hydrophobicity values were also calculated for the dominant proteins of dragline silk (i.e., the silk for making safety lines and frame lines of the webs), major ampullate spidroin 1 (MaSp1; *L. geometricus*

Table 2. Number of egg sacs without spiderling emergence after pesticide treatment and their contents upon dissection at day 30

Pesticide product	Number of egg sacs without spiderling emergence and their contents			Total (%)
	1-d-old egg sac	12-d-old egg sac	17-d-old egg sac	
CyKick CS	6 DE, DS	1 DS	1 DE, DS	8 (44)
Suspend Polyzone	3 DE, DS, LS	2 DS	1 DS	6 (33)
Temprid Ready Spray	6 DE, DS	4 DS, LS	4 DS	14 (78)*
Harmonix	4 DE, DS	3 DS	3 DS, LS	10 (56)
Eco PCO WPX	3 DE, DS	2 DS, DE	0	5 (28)
Essentria IC3	1 DE	0	4 DE, DS	5 (28)
CyKick aerosol	6 DE	6 DE, DS	6 DS	18 (100)*
D-Force HPX aerosol	6 DE	6 DE, DS	6 DE, DS	18 (100)*
Phantom aerosol	6 DE, DS	6 DS	6 DS	18 (100)*
Gentrol aerosol	6 DE, LS	6 DS, DE	5 DE, DS, LS	17 (94)*
Untreated control	N/A	N/A	N/A	23 (27)

DE, dead eggs; DS, dead spiderlings; LS, a few live spiderlings. A group of 18 egg sacs (6 egg sacs per age) was used for each pesticide treatment. Age information for the untreated control ($n = 85$) is not applicable because of the absence of the pesticide treatment for the group. For Total column, the numbers with asterisks indicate significant difference from the untreated control group. See text for the statistical analyses.

GenBank AAK30602; *L. hesperus* ABR68856) and major ampullate spidroin 2 (MaSp2; *L. geometricus* AAK30604; *L. hesperus* ABR68855). Hydrophobicity index values above zero indicate hydrophobicity and values below zero indicate hydrophilicity (Kyte and Doolittle 1982). The egg sac silk protein of brown widow spider (TuSp1) was hydrophobic, with scores of 0.19 for the repetitive region (GenBank AAY28950), which makes up the majority of the full-length protein, and 0.52 for the carboxy-terminal region (AAY28940). By contrast, brown widow spider dragline silk proteins were hydrophilic, with scores of −0.02 and −0.07 for MaSp1 and MaSp2, respectively. The same pattern was found for western black widow spider silk proteins. The egg sac silk protein of the western black widow spider (TuSp1) was hydrophobic with a 0.23 hydrophobicity index, while the dragline silk proteins were hydrophilic with −0.09 for MaSp1 and −0.11 for MaSp2 (C. Y. H., unpublished data).

While assuming the hydrophobic nature of the widow spider egg sac silk in general, the current study clearly showed that a range of pesticide products have differential efficacy when tested on the egg sacs of brown widow spiders. Overall, the pesticide products that are formulated or prepared in aqueous carriers were less effective compared with the pesticide products containing oil-based carriers. For five of six water-based pesticide sprays, the mortality rates of the treated egg sacs were low and similar to that of the untreated egg sacs. In contrast, the egg sacs treated with oil-based aerosols consistently showed high mortality (94–100%). The water-based Temprid Ready Spray was an exception from this pattern, which achieved overall 78% mortality of the treated egg sacs. The current study did not determine why this neonicotinoid/pyrethroid mixture product performed slightly better than did the other water-based pesticide sprays. However, the higher concentration of the active ingredients in the Temprid Ready Spray [i.e., 0.074% (imidacloprid 0.05%, β-cyfluthrin 0.024%)] compared with the other water-based products containing pyrethroids or pyrethrins (0.03–0.06%) could be one of the possible reasons for the efficacy difference observed between Temprid Ready Spray and the other water-based pesticide sprays. In any case, careful determination of inherent toxicity of neonicotinoid/pyrethroid mixture against the spider eggs in comparison with other pesticide active ingredients warrants further research.

Although not explicitly tested in the current study, the data separately collected for the different developmental stages of egg sacs (i.e., 1, 12, and 17 d post-oviposition) might provide additional insights for the product efficacy study (Table 2). For example, CyKick CS was found to be effective for all 1-d-old sacs but less effective in preventing the spiderling emergence when used for the egg sacs at their later developmental stages (i.e., killed only one each of the later age cohorts). Similarly, Temprid Ready Spray provided complete inhibition of spiderling emergence when they were used for the 1-d-old egg sacs, but failed to provide complete control when used for the egg sacs at their later developmental stages. Relatively higher efficacies of CyKick CS and Temprid Ready Spray on 1-d-old egg sacs could be explained by the longer exposure time of the egg sac contents to the active ingredients compared with the treatment of 17-d-old egg sacs, in which exposure to the active ingredients may only have lasted about 3 d on average prior to emergence of spiderlings. Also, even though CyKick CS and Temprid Ready Spray effectively prevented spiderling emergence when it was used for 1-d-old egg sacs, 83% (CyKick CS) and 100% (Temprid Ready Spray) of the treated 1-d-old egg sacs had dead spiderlings when opened after 30 d, indicating that the treatments could not prevent the eggs from developing into the spiderlings inside the egg sac. By contrast, all of the 1-d-old egg sacs treated with CyKick and D-Force HPX aerosols were found to have dead eggs as sole contents when opened after 30 d, indicating the aerosols effectively stopped the further development of the eggs inside the treated egg sacs (Table 2). Phantom aerosol treatment was an exception for this pattern, in which only dead spiderlings were found in 83% of 1-d-old egg sacs. Chlorfenapyr, the active ingredient of Phantom aerosol, is a pro-pesticide that needs to be converted to a toxic form by a target organism via an oxidative process probably catalyzed by P450s (Black et al. 1994). This additional step necessary for toxicity of chlorfenapyr might be responsible for the lack of immediate mortality of the chlorfenapyr-treated egg sacs.

The penetration study with the pyrethroid products indicated that the ability of a pesticide to control the egg sacs is positively correlated with the ability of the pesticide to penetrate the hydrophobic silk layer of the egg sac. Two different pesticide products containing same active ingredients at a same rate performed differently depending on the type of carrier (i.e., water-based vs oil-based). Thus, it would appear that the relatively low efficacy observed for the water-based pesticide sprays is not due to the ineffectiveness of the active ingredients but is related to the inability of the carrier to penetrate through the hydrophobic silk layer and bring the active ingredient in contact with the eggs. Penetration of the egg sac silk by the active ingredients might still occur for some of the water-based pesticide sprays, but perhaps at much slower rates compared with the oil-based aerosols. As previously discussed, CyKick CS and Temprid Ready Spray both resulted in 100% mortality for 1-d-old egg sacs, suggesting that their active ingredients might have taken a longer time to penetrate through the egg sac and reach the egg sac contents.

Spider egg sacs exhibit great variation in structure and silk coverage [e.g., see Figs. 1 –19 in Vetter and Carroll (2013)]. For example, some spider species cover their egg sacs with just a few strands of silk (e.g., *Oecobius navus* Blackwall), while others like the widow spiders often produce egg sacs with a tough, fabric-like surface. The current study result can be viewed as a general model among several *Latrodectus* widow spider species because the properties of their protective egg sac silk may be highly conserved due to the similar pressures of protecting the eggs from abiotic and biotic factors (Garb and Hayashi 2005). The water-based pesticide sprays are among the favored options by the pest control industry for treating pest insects and spiders around the structure partly because of their lack of oily residue and obnoxious odor (D.-H. Choe, unpublished data). Although these water-based spray products might be effective in controlling other household pests around the structures, these products may be less than optimal for providing the complete control for the brown widow spider eggs when briefly applied on the egg sacs, following the current label rates. The information generated by this study provides benefit for the pest control industry in addressing an issue regarding control of spider egg sac contents that has not received much previous attention whereby incorporating oil-based pesticides for spider control might improve control efficacy of widow spiders in the urban environment. Determination of the most effective active ingredients and carrier characteristics warrants further research to provide more effective control options for the spider egg sacs.

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