Research Article

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Susceptibility of *Alphitobius diaperinus* in Texas to permethrin- and β-cyfluthrin-treated surfaces

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Abstract

BACKGROUND: Effective control of the lesser mealworm beetle, *Alphitobius diaperinus*, relies heavily on insecticides. The susceptibility level of beetles to these insecticides can be dependent on active ingredient, population treated, formulation, surface treated and timing of observation. The susceptibility of adult beetles from six populations to β-cyfluthrin was determined up to 48 h after exposure. The susceptibility of adult beetles to the label rate of β-cyfluthrin and permethrin formulations on concrete, wood-chip-type particle board and pressure-treated wood was determined up to 48 h post-exposure.

RESULTS: Variation in LC_{50} values at 2 and 24 h was found within and between beetle populations from two regions of Texas. The permethrin formulation had lower mean mortality than the β-cyfluthrin formulation on all surfaces tested. The permethrin formulation had high levels of recovery on all surfaces tested after 2 h. Surface affected the efficacy of the insecticides tested on killing adult beetles.

CONCLUSION: Permethrin-based insecticide had lower knockdown and persistence on various surfaces over time than β-cyfluthrin-based insecticide. Beetle recovery in less susceptible populations may necessitate longer observation periods for efficacy evaluations. Our study also shows that surfaces chosen can affect the efficacy of the compound on killing adult beetles.

Keywords: darkling beetle; integrated pest management; poultry; surface treatments

1 INTRODUCTION

Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae), commonly known as the darkling beetle or lesser mealworm, is believed to originate from Sub-Saharan Africa.[1,2] The beetle is a cosmopolitan pest of poultry systems, particularly in broiler houses worldwide, including Brazil, Denmark, France, the United States and Australia.[3−9] It is also a common pest of grains and grain products, as well as oilseed products.[4]

The physical damage to poultry facilities by *A. diaperinus* is an important economic factor resulting in its classification as a poultry pest. Larvae of *A. diaperinus* damage poultry facility structures, as they burrow into walls, insulation and floors to pupate.[10] In addition, larvae and adults have been observed moving from the floor up unpainted wood posts of poultry houses.[6] The number of late-instar larvae that climb to pupate in the insulation is impacted primarily by the availability of soil as a pupation site and the density of beetles in the litter.[11] Damage to insulation from pupation of multiple generations can increase energy costs, particularly heating, by as much as 67%.[12]

Larvae and adults of *A. diaperinus* serve as a reservoir for several avian and food-borne disease agents.[10,13−15] Birds can become infected after consuming beetles containing pathogens such as *Salmonella enterica*, *Escherichia* sp., *Streptococcus* sp. and fungi.[10,14−16] Bates *et al.*[14] showed that *A. diaperinus* could be a reservoir for *Campylobacter jejuni*, one of the causal agents of human gastroenteritis.

Axtell[17] proposed that an integrated pest management (IPM) plan could be implemented for the management of *A. diaperinus* in broiler houses. Ideally, such an IPM program would have three main components with the goal of keeping the beetle population below the density where their damage incurs costs, while maximizing poultry production. These components are biological, chemical and cultural control methods. Monitoring of the pest populations in broiler houses is an important tool in the design of the IPM program, which includes damage assessments, knowledge of the areas of the house with highest pest numbers and knowledge of insecticide resistance levels of populations. Resistance level, or in the case of our study, susceptibility, monitoring is key to any

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chemical control of a pest population and can be achieved by conducting bioassays using either the active ingredient (AI) or commercial formulations.

To date, there have been a limited number of commercially available chemicals registered for use against *A. diaperinus*.[18] Several insecticides, such as permethrin, have been used for some time, possibly resulting in insecticide resistance and low knockdown rates. In addition, most of these compounds can be used only during cleanout when the birds are not present (every 6–8 weeks).

The objectives of the present study were to examine the possibility of population differences between regions and within a region, which may have implications in chemical management of *A. diaperinus* beetles in poultry houses. Additionally, this study compared the efficacy of β-cyfluthrin AI with a β-cyfluthrin commercial formulation and a permethrin formulation to determine whether differences between AI and formulations impact mortality. This study also compared the efficacy of formulations on varying surfaces by applying these two formulations to three surfaces commonly found inside a poultry house to see if surface type affects mortality.

2 MATERIALS AND METHODS

Adult lesser mealworms were collected from three farms in each of two regions, Mt Pleasant (farms A, B and C) and Franklin (farms D, E and F), in Texas. A distance of at least 0.31 km separated the Mt Pleasant farms, while the Franklin farms were approximately 25 km apart. Farms A to C instituted a chemical rotation involving a pyrethroid and a neonicotinoid insecticide. Farms D to F also instituted a rotation with a pyrethroid, neonicotinoid and an organophosphate insecticide. Three houses on each farm were randomly selected for collection of approximately 3000 adult *A. diaperinus* each. The beetles were collected from near feeder and water lines of the facility if litter was present. Beetles were also collected at random locations at cracks and crevices. For houses with the litter removed prior to harvesting of the beetles, the cracks and crevices were the sole area collected. Harvested beetles were then sifted out of litter with a kitchen colander. All individuals from multiple houses at one farm were combined into one container and represented the entire farm. The beetles were transported to the Forensic Laboratory for Investigative Entomological Sciences (FLIES) Facility at Texas A&M University and stored at 21.1 °C in tubs for at least 1 week before testing. The tubs contained litter from the whole-farm container as a substrate, and the beetles were provided sliced red apples (0.5 cm thick), fishmeal (Omega Protein, Inc., Hammond, LA) and sponges (approximately 6 cm^2) moistened with deionized water as needed.

2.1 β-Cyfluthrin AI filter paper bioassay

A preliminary assay was conducted using the equivalent dose of β-cyfluthrin (0.02 mg mL^{-1}) from a low-dose application of Tempo SC Ultra as the median dose of the assay to establish a dosage range of 18 concentrations for this experiment. Mortality was recorded at 2, 24 and 48 h post-exposure. Mortality was assessed as originally described by Lambkin[7] and then Lambkin and Rice[19] as beetles deemed alive could walk straight in a forward motion using all six legs with no jerky movements. Those individuals that did not meet these criteria were deemed dead as recorded at 2, 24 and 48 h. Therefore, mortality included individuals that were moribund, as well as those that were legitimately dead; at 24 and 48 h, individuals that no longer exhibited mortality (as defined by Lambkin[7]) were referred to as 'recovered'.

Methods for the assay were adapted from Tomberlin *et al.*[18] and Sheppard and Hinkle.[20] Eighteen concentrations ranging from 0.0 to 0.20 mg mL^{-1} of technical-grade β-cyfluthrin AI (Bayer Healthcare LLC, Shawnee Mission, KS) were tested. Each concentration was diluted in acetone. For each concentration, 1 mL was applied to a 9 cm diameter cellulose fiber filter paper (Fisherbrand Cat. No. 09-795C; Loughborough, UK). Acetone was used as the control. Under laboratory conditions (21.1 °C), the test populations consisting of 30 field-collected adult beetles per Petri dish were placed on the insecticide-treated or control filter paper for 2 h, then transferred to a clean 9 cm diameter Petri dish and mortality was recorded.

2.2 β-Cyfluthrin and permethrin formulation bioassay on varying surfaces

The β-cyfluthrin formulation, Tempo SC Ultra (Bayer Animal Science, Research Triangle Park, NC), and a permethrin formulation, Vector Ban Plus (Control Solutions Inc., Pasadena, TX), were selected on account of their commercial availability and current popularity for use by poultry producers in Texas. The test surfaces chosen were based on those surfaces commonly found inside poultry houses: concrete, pressure-treated wood and woodchip particle board.

The assay was performed inside an unused brooder barn at the Texas A&M University Poultry Center, College Station, Texas, to protect the surfaces from excessive dust and ultraviolet light degradation of the chemicals. The temperature was maintained at 21 °C, and lighting was set to 0 L:24D. Using protocols adapted from Kaufman *et al.*,[6] the surfaces were treated with tap water (control) or the low-dose insecticide label rate for *A. diaperinus*: 2.16 and 25.10 mL L^{-1} H_2O, respectively, for Tempo SC Ultra and Vector Ban Plus, with tap water used as the diluent. A chemical sprayer (B&G Equipment Company, Jackson, GA) was used with a fan spray pattern that distributes 100 mL of chemical in 10 s, and the surfaces were evenly sprayed for 1 s. After treatment, the surfaces were allowed to dry for 1 h at 21 °C.

Thirty field-collected adult beetles representing one farm were placed into a clean 9 cm diameter Petri dish, attached upside down against the test surface by a rubber band for the particle board and pressure-treated wood, and by packing tape for the concrete blocks. Three technical replicates for each surface and compound tested were conducted for each farm concurrently. After 2 h, the beetles were transferred to clean Petri dishes and mortality was recorded at 2, 24 and 48 h.

2.3 Statistical analysis

Statistical analysis was conducted using JMP Pro 11.0 software (SAS Institute Inc., Cary, NC). Probit analyses of LC_{50} were conducted using a generalized linear model with a binomial error structure and adjusted for overdispersion. The comparisons of LC_{50} between each farm population within each time point used the 95% Bonferroni simultaneous confidence interval.

In order to stabilize the variance of mean percentage mortality for ANOVA, arcsine square root transformation was applied. The transformed data were then analyzed by a generalized linear model with a normal error structure on a full factorial treatment design. The post hoc analyses for each treatment factor were performed using Tukey's HSD for controlling familywise type I error rate at $\alpha = 0.05$.

Table 1. The 2, 24 and 48 h LC_{50} values of adult *Alphitobius diaperinus* from three farms each in Mt Pleasant (A to C) and Franklin (D to F), Texas, with *N*, Slope ± SE and χ^2. Probit analysis conducted with data from filter paper bioassay treated with β-cyfluthrin AI at varying doses between 0 and 0.20 mg mL^{-1}

		2 h			24 h			48 h		
Farm	*N*	Slope ± SE	LC_{50}(95% CI)[a] (mg cm^{-2})	χ^2(df = 1)	Slope ± SE	LC_{50}(95% CI)[a] (mg cm^{-2})	χ^2(df = 1)	Slope ± SE	LC_{50}(95% CI)[a] (mg cm^{-2})	χ^2(df = 1)
A	1670	998.37 ± 67.48	0.0012 b (0.0011, 0.0014)	270.15	705.57 ± 54.19	0.0022 b (0.0020, 0.0026)	186.47	NA	NA	NA
B	1679	666.58 ± 58.13	0.0021 a (0.0019, 0.0024)	141.66	690.28 ± 60.06	0.0029 a (0.0027, 0.0033)	146.74	NA	NA	NA
C	1713	941.64 ± 65.14	0.0013 b (0.0012, 0.0014)	270.16	768.21 ± 68.14	0.0020 b (0.0018, 0.0023)	141.71	NA	NA	NA
D	1717	1998.61 ± 182.87	0.0006 c (0.0005, 0.0007)	281.09	1193.20 ± 114.70	0.0011 d (0.0009, 0.0012)	165.42	NA	NA	NA
E	1700	928.02 ± 69.84	0.0018 a (0.0016, 0.0020)	217.79	567.78 ± 65.27	0.0035 a (0.0031, 0.0042)	79.97	NA	NA	NA
F	1700	1749.44 ± 145.44	0.0009 b (0.0008, 0.0010)	312.21	1129.13 ± 77.07	0.0015 c (0.0013, 0.0016)	300.82	NA	NA	NA

[a] The 95% confidence intervals are Bonferroni simultaneous confidence intervals at a familywise alpha level of 0.05 and a pairwise alpha level of 0.05/6 = 0.0083. Values with different lower-case letters in each column indicate statistical significance (95% confidence intervals do not overlap, $P \leq 0.05$).

Table 2. Surface*Compound*Hour pooled mean percentage mortality ± SE for adult *Alphitobius diaperinus* beetles collected from six farms in Texas 2, 24 and 48 h after exposure to three surfaces treated with Tempo SC Ultra (β-cyfluthrin) or Vector Ban Plus (permethrin) for 2 h at their low-dose label rates (Tempo SC Ultra: 2.16 mL L^{-1} water; Vector Ban Plus: 25.10 mL L^{-1} water) under field conditions (21.11 °C)[a]

	Pressure-treated wood		Particle board		Concrete	
Exposure (h)	Tempo SC Ultra	Vector Ban Plus	Tempo SC Ultra	Vector Ban Plus	Tempo SC Ultra	Vector Ban Plus
2	98.9 ± 2.0 aA	66.1 ± 18.7 aA	88.1 ± 18.7 bB	70.9 ± 27.0 aA	95.2 ± 4.4 bAB	33.8 ± 2.4 aB
24	99.4 ± 1.3 aA	16.4 ± 18.1 bA	98.2 ± 4.2 aA	8.7 ± 8.6 bAB	98.7 ± 2.3 aA	3.3 ± 2.6 bB
48	99.4 ± 1.3 aA	12.1 ± 12.1 bA	94.0 ± 11.8 abA	11.8 ± 13.1 bA	98.9 ± 1.6 aA	2.1 ± 3.1 bB

[a] Treatments within a surface and time are always significantly different (HSD, $P \leq 0.05$). Values with different lower-case letters indicate statistical significance within a treatment and surface between hours (HSD, $P \leq 0.05$). Values with different upper-case letters indicate statistical significance between the same treatment and time compared across surfaces (HSD, $P \leq 0.05$).

3 RESULTS

3.1 β-Cyfluthrin AI filter paper bioassay

The 2, 24 and 48 h LC_{50} values for each farm are displayed in Table 1. The most susceptible population at 2 h was farm D (LC_{50} 0.0006 mg cm^{-2}) and the least susceptible population was farm B (LC_{50} 0.0021 mg cm^{-2}). At 24 h, farm D remained the most susceptible population, but farm E was the least susceptible (LC_{50} 0.0035 mg cm^{-2}). Significant differences in LC_{50} were seen between farms within the same region (farm B 0.0021 mg cm^{-2} and farm A 0.0012 mg cm^{-2}) at 2 h. The other region collected from in Texas also exhibited significant differences between all the farms tested at 2 and 24 h. Significant differences were also seen between the two regions (farm D 0.0006 mg cm^{-2} and farm B 0.0021 mg cm^{-2}) at 2 h.

3.2 β-Cyfluthrin and permethrin formulation bioassay on varying surfaces

A three-way Surface*Compound*Hour interaction ($F = 3.781$; df = 8, 414; $P = 0.0003$) was determined and pooled across farms (Table 2). The β-cyfluthrin-based insecticide always had significantly higher mortality than the permethrin formulation on each surface for 2, 24 and 48 h. The β-cyfluthrin formulation performed similarly between 2, 24 and 48 h on pressure-treated wood ($F = 0.579$; df = 2, 51; $P = 0.5643$), but significant differences were seen for particle board ($F = 3.638$; df = 2, 51; $P = 0.0334$) and concrete ($F = 6.598$; df = 2, 51; $P = 0.0028$). On particle board the highest mortality was at 24 h (98.2%) and the lowest at 2 h (88.1%), and on concrete the mortality was highest at 24 and 48 h.

When comparing the β-cyfluthrin formulation across surfaces, the lowest mortality at 2 h occurred on particle board (88.1%) and was significantly lower than on pressure-treated wood ($F = 5.276$; df = 2, 51; $P = 0.0083$). However, at 24 and 48 h all surfaces had similar mortality. With the permethrin formulation, lowest mortality was seen on concrete at 2 h (33.8%), which is 37.1% lower than particle board mortality. Concrete also had lowest mortality at 48 h, with the other two surfaces performing similarly to each other ($F = 7.841$; df = 2, 51; $P = 0.0011$). At 24 h the highest mortality (16.4%) was on pressure-treated wood, and concrete was significantly lower at 3.3% ($F = 7.149$; df = 2, 51; $P = 0.0018$).

The permethrin formulation had the highest mortality at 2 h (70.9%) for all surfaces. There was a marked reduction in mortality with this formulation that was not seen with the β-cyfluthrin formulation. For example, on particle board the 2 h mortality was 70.9%, whereas at 24 h mortality fell to 8.7%. The other two surfaces saw a 49.7% drop (pressure-treated wood) and a 30.5% drop (concrete).

Table 3. Surface*Compound*Farm pooled mean percentage mortality ± SE for adult *Alphitobius diaperinus* beetles collected from six farms in Texas (farms A to C represent Mt Pleasant, Texas, and farms D to F represent Franklin, Texas) 2, 24 and 48 h after exposure to three surfaces treated with Tempo SC Ultra (*β*-cyfluthrin) or Vector Ban Plus (permethrin) at their low-dose label rates (Tempo SC Ultra: 2.16 mL L^{-1} of water; Vector Ban Plus: 25.10 mL L^{-1} of water) under field conditions (21.11 °C)[a]

	Pressure-treated wood		Particle board		Concrete	
Farm	Tempo SC Ultra	Vector Ban Plus	Tempo SC Ultra	Vector Ban Plus	Tempo SC Ultra	Vector Ban Plus
A	99.7 ± 1.0 abA	30.4 ± 34.1 aA	93.0 ± 12.0 aA	21.7 ± 25.8 abcA	98.1 ± 2.4 aA	15.9 ± 24.9 aA
B	100.0 ± 0.0 aA	21.9 ± 28.9 aA	74.4 ± 21.4 bB	14.5 ± 21.1 cA	98.4 ± 3.9 aA	8.4 ± 12.6 aA
C	97.0 ± 2.1 cA	29.6 ± 28.6 aA	98.9 ± 1.7 aA	17.5 ± 23.1 bcA	97.1 ± 4.5 aA	9.6 ± 8.8 aA
D	100.0 ± 0.0 aA	50.6 ± 24.1 aA	99.2 ± 1.5 aA	46.4 ± 37.6 aA	96.0 ± 3.5 aB	24.3 ± 32.1 aA
E	98.9 ± 1.6 bA	17.3 ± 24.3 aA	95.6 ± 7.3 aA	31.9 ± 38.9 abcA	97.4 ± 3.6 aA	6.0 ± 12.0 aA
F	100.0 ± 0.0 aA	39.3 ± 31.7 aAB	99.6 ± 1.1 aA	50.9 ± 41.2 abA	98.5 ± 2.4 aA	14.3 ± 21.5 aB

[a] Treatments within a farm are always statistically significant within a surface (HSD, $P \leq 0.05$). Values with different lower-case letters indicate statistical significance between farms within a surface (HSD, $P \leq 0.05$). Values with different upper-case letters indicate statistical significance between the same treatment and farm across surfaces (HSD, $P \leq 0.05$).

A three-way Surface*Compound*Farm interaction ($F = 3.265$; df = 20, 414; $P < 0.0001$) was also determined and pooled across hours (Table 3). For all farms, mortality from the *β*-cyfluthrin formulation was always higher than mortality from the permethrin formulation for all surfaces.

On concrete, the permethrin formulation had similar mortality between all six farms ($F = 0.940$; df = 5, 48; $P = 0.4637$), and the same for the *β*-cyfluthrin formulation ($F = 0.769$; df = 5, 48; $P = 0.577$). The permethrin formulation had the same mortality on pressure-treated wood between the six farms ($F = 1.857$; df = 5, 48; $P = 0.1196$), but the *β*-cyfluthrin formulation had complete mortality on farms B, D and F and less mortality on the other farms. The lowest mortality seen was for farm C (97.0%). On particle board the *β*-cyfluthrin formulation had similarly high mortality rates across all farms except for farm B (74.4%) ($F = 9.167$; df = 5, 48; $P < 0.001$). The permethrin formulation had the highest mortality with farm D's population (46.4%) and the lowest on farm B (14.5%).

The *β*-cyfluthrin formulation had similar mortality across the three surfaces for farms A, C, E and F. Farm B had lower mortality on particle board ($F = 20.379$; df = 2, 24; $P < 0.001$), and farm D had lower mortality on concrete ($F = 8.235$; df = 2, 24; $P = 0.0019$). The permethrin formulation had similar mortality on all surfaces except for farm F, where the highest mortality was on particle board and the lowest on concrete ($F = 3.449$; df = 2, 23; $P = 0.0490$).

4 DISCUSSION

The selection of commercially available insecticides for suppressing *A. diaperinus* in poultry operations is limited, and control methods are further hindered by the fact that these compounds can be applied only during cleanout periods when birds are not present (about every 6–8 weeks).[18] Regular identification of insecticide susceptibility would allow producers to rotate chemicals based on the AI (mode of action) in order to reduce induction of resistance in a given population.[21,22] For example, *Bemisia argentifolii* (Homoptera: Aleyrodidae) treated with only bifenthrin developed a rapid increase in resistance by the 27th generation,[21] but when bifenthrin was used as part of a rotation with endosulfan and chlorpyrifos the resistance was 1–2% of that of the bifenthrin-only population at the 24th generation.

The estimated level of susceptibility of *A. diaperinus* adults to an insecticide, or an associated AI, is highly dependent on the amount of time allowed to pass after treatment. Previous studies assessed mortality of *A. diaperinus* 48 h after exposure to an AI or its formulated product.[6,23] Hamm *et al.*[23] determined that the LC_{50} for adult *A. diaperinus* exposed to cyfluthrin 48 h after treatment ranged from 0.04 to 0.16 μg cm^{-2}. Herein we observed mortality for *A. diaperinus* adults at 2, 24 and 48 h after exposure to filter papers treated with varying doses of *β*-cyfluthrin. Our 2 h mortality ranged from 0.6 to 2.1 μg cm^{-2} and increased to 1.5–2.9 μg cm^{-2}. The highest recovery by 48 h was 23.33%, thereby preventing an LC_{50} from being calculated.

Many studies have shown that application surface parameters matter with respect to insecticide efficacy.[24–27] In this study the *β*-cyfluthrin formulation saw significant increases in mortality after 2 h at 24 and 48 h on concrete, and on particle board from 2 to 24 h. The pressure-treated wood saw similar high mortality throughout the 48 h. As contact with a surface by the pest would be limited, having the highest mortality at 2 h and throughout the 48 h would be beneficial for controlling the pest. This study also found that at 2 h the pressure-treated wood treated with the *β*-cyfluthrin formulation had significantly higher mortality than particle board, but concrete had significantly lower mortality at 2 h compared with the other surfaces tested with the permethrin formulation. In our study, we conclude that spraying on pressure-treated wood would be beneficial to the grower with a *β*-cyfluthrin formulation, but spraying pressure-treated wood or particle board instead of concrete would benefit the grower when using a permethrin product.

While surface type may affect insecticide activity against lesser mealworm, so do the environmental conditions in which the surface is located.[27] High temperature and humidity can affect volatilization of the insecticide formulation applied. Gudrups *et al.*[27] also noted that higher respiration of insects in high temperatures could increase movement on surfaces and therefore increase contact with the compound. The conditions and surfaces described herein match those of the lesser mealworm inside poultry houses. Future research should focus on matching the environmental conditions with poultry house materials to improve the assessment of insecticide efficacy under real-world use conditions.

Variation in insecticide susceptibility was observed between adult beetles from the two regions assessed in this study. The 24 h

LC_{50} of farms in the Mt Pleasant area in Texas indicates that beetles from farm B had significantly lower susceptibility than beetles from farms A and C. The Franklin beetles from farm E had significantly lower susceptibility than farms D and F at 24 h. Overall, beetles from farm D (0.0011 mg cm^{-2}) had the highest susceptibility of the six farms, and those from farm E (0.0035 mg cm^{-2}) had the lowest susceptibility. Likewise, in Mt Pleasant, beetles from farm B (0.0029 mg cm^{-2}) had a much lower susceptibility than beetles from farm A (0.0022 mg cm^{-2}) and C (0.0020 mg cm^{-2}). This highlights the variation in susceptibility levels within a single region (Franklin) and the need to control the transfer of beetles between houses and farms and rotate the mode of action of insecticides. A study by Tomberlin *et al.*[18] found similar results using a bifenthrin AI insecticide, which demonstrated wide variation in knockdown and mortality rates in Titus County at 4 and 24 h post-exposure respectively. Similarly to the present study, Tomberlin *et al.*[18] remarked that mortality at 4 h was higher than that observed at 24 h. They therefore concluded that 4 h was more of a measure of knockdown. The same county used in the Tomberlin *et al.*[18] study contained the Mt Pleasant farms (A, B and C) used in the present study. Hamm *et al.*[23] observed wide variation in resistance ratios RR_{95} (1.7–9.5) for *A. diaperinus* beetles from across the East Coast of the United States.

Data collected from this study indicate that filter paper assays of AI are an important tool for toxicology studies, but the formulation has a notable impact on the efficacy of the product on surfaces, which requires assessment as well. The data presented here show a high recovery rate by 48 h when *A. diaperinus* beetles were subjected to β-cyfluthrin AI, but when a formulation was used containing β-cyfluthrin the mortality was 94.04% or higher at 48 h. This echoes Kaufman *et al.*,[6] who highlighted the importance of conducting both assay types. Caution is required to determine the appropriate observation times, as these are critical to the assessment. Recovery was seen in both assays conducted in this study. While moribund individuals were able to regain movement, this study did not take into account possible lasting physiological effects to beetles after they 'recovered'. These sublethal effects include behavior making them more prone to desiccation or predation,[28] and reproduction losses.[29,30] If the goal of IPM is to keep the mean population density below a threshold level,[17] then a bioassay study should factor in sublethal effects on individuals. In this study, a high level of recovery was measured with some compounds, and if these individuals have reduced reproductive capacity or will be unable to survive post-treatment for a prolonged period, then the treatment may still be judged a success when an IPM strategy is considered. Data from this study and others discussed above demonstrate that a failure to implement longitudinal observation (waiting 24–48 h post-treatment) could lead to false confidence in the ability of a compound to suppress an arthropod pest population while continuing added pressure for greater selection of resistance.

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