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Alfalfa Weevil (Coleoptera: Curculionidae) Resistance to Lambda-cyhalothrin in the Western United States

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Abstract

Forage alfalfa (*Medicago sativa* L. [Fabales: Fabaceae]) is a key agricultural commodity of the western region of the United States. The key insect pest of alfalfa, *Hypera postica* Gyllenhal (Coleoptera: Curculionidae), has developed resistance to the most common class of insecticide used to manage its damage. Alfalfa weevil samples from 71 commercial alfalfa fields located in Arizona, California, Montana, Oregon, Washington, and Wyoming were assayed for susceptibility to lambda-cyhalothrin during 2020–2022 using a laboratory concentration-response assay. Seventeen field sites representing all six states were highly resistant to lambda-cyhalothrin (resistance ratios >79.6) and bioassay mortality often did not exceed 50% even at the highest concentration tested (3.30 μg/cm^2 in 2020 and 10.00 μg/cm^2 in 2021–2022). Field sites assayed with more than one pyrethroid active ingredient indicated likely cross-resistance between lambda-cyhalothrin and zeta-cypermethrin (type II pyrethroids) and variable and/or limited potential cross-resistance to permethrin (type I pyrethroid). Thirty-two field sites representing five states were susceptible to lambda-cyhalothrin (resistance ratios ranging from 1 to 20). While resistance is widespread, integrated resistance management strategies including rotating mode of action groups, applying chemical control tactics only when economic thresholds have been met, and utilizing cultural control tactics can be employed to slow the further development of resistance.

Key words: insecticide resistance, forage crop, Curculionidae, integrated resistance management

Alfalfa, *Medicago sativa* L. (Fabales: Fabaceae), is the third most valuable row crop in the United States (US), and in 2019, the western region of the US produced 15.4 million tons (Nelson 2019, Rankin 2020). *Hypera postica* Gyllenhal (Coleoptera: Curculionidae), the alfalfa weevil, is the primary defoliating insect pest of alfalfa causing economic damage throughout the continental US (Peterson et al. 1992, Pellissier et al. 2017). The larval stage of all alfalfa weevil strains can cause yield and quality loss by feeding on alfalfa leaves (Onstad and Shoemaker 1984, Hutchins et al. 1990). Alfalfa weevil is an invasive species native to Eurasia and North Africa and was introduced to the US on three known occasions. Each introduction is recognized as a distinct strain: the western (Salt Lake City UT, 1904), Egyptian (Yuma AZ, 1939), and eastern (Maryland, 1952) strains (Titus 1910, Wehrle 1940, Poos and Bissell 1953, Wood et al. 1978, Hsiao 1993, Bundy et al. 2005).

Due to the economic injury caused by alfalfa weevils, the application of effective and inexpensive pyrethroid insecticides (mode of action [MoA] group 3A) has been the primary method of management (Wright et al. 2015, IRAC 2022). The use of some older organophosphate (MoA 1B) insecticides has been restricted, limiting producer access to active ingredients with alternative MoA groups (Wright et al. 2015, Pellissier et al. 2017, IRAC 2022). During the 1950s, organochlorine (MoA 2A) insecticides were used routinely for the control of alfalfa weevil, and by the early 1960s resistance to heptachlor had developed (Alder and Blickenstaff 1964, Bishop 1964). After several decades of pyrethroid use, alfalfa weevil resistance to lambda-cyhalothrin has been reported in Alberta Canada, California, and Montana (Glen 2015, Orloff et al. 2016, Rodbell and Wanner 2021, Wanner et al. 2022). These repeated reports of pyrethroid insecticide failure highlight a growing concern for alfalfa producers.

One major challenge facing alfalfa weevil management is the lack of alternative MoA insecticides. Six pyrethroid (MoA 3A) active ingredients (five type II pyrethroids and one type I pyrethroid) are registered for the control of alfalfa weevil in forage alfalfa. Other than indoxacarb (MoA 22A), few effective alternative MoA insecticides are available. Spinosad (MoA 5) is recommended only for suppression, while the use of chlorpyrifos (MoA 1B) has recently been restricted (UC-ANR 2020, EPA 2021). With the potential for cross-resistance within MoA group 3A, alfalfa producers could lose the ability to rely upon all currently available pyrethroid-based insecticides, significantly limiting their options.

Integrated resistance management (IRM) strategies are urgently needed to slow the development of alfalfa weevil resistance to pyrethroids. Integrated resistance management strategies would not only prolong the efficacy of pyrethroid active ingredients. They would also slow the development of resistance to indoxacarb because of repeated use of this MoA in areas where pyrethroids are no longer effective. The first step of IRM is the detection and monitoring of resistance, ideally early in resistance development (Croft 1990a). The goals of this research were to 1) determine the extent of lambda-cyhalothrin resistance in the western US and 2) assess the risk of cross-resistance between pyrethroid active ingredients. We used concentration-response bioassays to estimate lambda-cyhalothrin resistance ratios using larvae collected from Arizona, California, Montana, Oregon, Washington, and Wyoming. Commercial fields with highly resistant alfalfa weevils were identified from all six states surveyed in this study and preliminary evidence supports cross-resistance among type II pyrethroids.

Materials and Methods

Insect Collections

Field sampling in six western region states was partly targeted towards sites with suspected pyrethroid resistance, based on crop advisor, extension agent, and/or alfalfa producer reports, and included fields with no known history of insecticide resistance. Alfalfa weevil larvae from 71 commercial alfalfa fields were evaluated during a three-year period to assess the degree of lambda-cyhalothrin resistance in the western region.

Alfalfa weevil larvae were collected, and hand carried, or shipped overnight, to Montana State University or the University of California Davis where the bioassays were conducted. We made 34 weevil collections in 2020, including six counties in California, nine counties in Montana, one county in Washington, and one county in Wyoming (Table 2). Field collections extended from 21 February until 25 June 2020. In 2021, we made 32 collections from three counties in Arizona, five counties in California, three counties in Montana, one county in Oregon, one county in Washington, and two counties in Wyoming. Field collections extended from 9 February until 24 June 2021. In February 2022, alfalfa weevils were collected in two Arizona counties and Riverside County California.

Arizona field sites were generally located along the western border of the state with Pinal County the exception (9 sites total, an average of 4 per county). California field sites were generally located in the center of the state (24 sites total, an average of 3.7 per county). Distance between sites within the same county ranged from 0.40 to 88.5 kilometers (km). The commercial alfalfa fields were all irrigated and the majority conventionally managed.

Montana field sites were located across the southern region of the state with Richland County the exception (18 sites, an average of 2.3 per county). Wyoming field sites were generally located along the northern border with Montana and the south-eastern portion of the state (5 sites total, an average of 1.3 per county). Distance between sites within the same county ranged from 0.80 to 40 km. All field sites were conventionally managed, with a mixture of dryland and irrigated production systems.

Oregon and Washington sites were located along the border between the two states. Oregon field sites in 2021 were isolated to Umatilla County along the northern border (4 sites total). Washington field sites were located in two counties along the south-central portion of the state (3 sites total). Distance between sites within the same county ranged from 0.8 to 34 km. All field sites were conventionally managed and were irrigated.

Larvae were collected with a sweep net while walking in a zig-zag pattern through each field. Once collected, samples were placed in plastic buckets or paper bags filled with cut alfalfa from the sampled field and transported in coolers to the laboratory for bioassay. In the laboratory, 19-liter plastic buckets filled with untreated fresh alfalfa and covered with No-see-um number 20 mesh (Quest Outfitters, Sarasota, FL) were used to maintain larvae for 24–48 hours at room temperature (20–25°C). In 2020, visually estimated third to fourth instar larvae were sorted and used in the bioassay; 2021–2022 visually estimated second to third instar larvae were used.

Active Ingredients

Lambda-cyhalothrin, zeta-cypermethrin (type II pyrethroids), and permethrin (type I pyrethroid) were assayed using technical grade material. Lambda-cyhalothrin ([(R)-cyano-(3-phenoxyphenyl)methyl] (1S, 3S)-3-[(Z)-2-chloro-3,3,3,-trifluoroprop-1-enyl]-2,2-dimethylcyclo propane-1-carboxylate) was provided by Syngenta (Basel, Switzerland), 89% purity. Zeta-cypermethrin ([cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate) was provided by FMC Corporation (Newark, NJ), 92% purity. Permethrin ((3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2, 2-dimethylcyclopropane-1-carboxylate)) was purchased from Sigma-Aldrich (St. Louis, MO).

Concentration Response Bioassays

Detailed bioassay methods are reported in Rodbell and Wanner (2021). In brief, stock solutions of each active ingredient were prepared in 95% acetone. Using serial dilutions with 95% acetone, we generated solutions of seven concentrations. One milliliter (mL) of each concentration was added to the glass vials (Brindley 1975; Discount Vials, Madison, WI) and the acetone evaporated without heat on a commercial food roller at room temperature (21°C), yielding 0.0033–10.0 micrograms per centimeter squared (μg/cm^2) of vial surface area for lambda-cyhalothrin and zeta-cypermethrin, and 0.033–100.0 μg/cm^2 for permethrin. Treated vials were stored at room temperature in a dark cabinet and used during a two-week period for bioassay experiments. Each bioassay typically consisted of seven concentrations and a 95% acetone control ($n = 5$ replicated vials, 10 larvae per vial). After 24 hr in the dark at room temperature, the larvae were scored as dead or alive after exposure to a heated hot plate (43–50°C).

Probit analysis was used to quantify the lethal concentration of an active ingredient that generates 50% mortality (LC_{50}) using Probit Or LOgit analysis (POLO) software (LeOra Software, Parma, MO) (Robertson et al. 2017). Bioassays with control mortality >20% were omitted from analysis (Robertson et al. 2017). If mortality was observed in the control group, correction for natural response was estimated by the POLO Probit analysis (Robertson et al. 1980). Analyses with a t-ratio of the slope less than 1.96 were excluded from this study (Yu 2015, Robertson et al. 2017). Data sets that did not fit the probit model were omitted based on the chi-square (χ^2) goodness of fit test ($P < 0.05$) unless a single outlier was identified as the cause for the lack of fit. Outliers were identified through the graphical method of the residual values (Sarkar et al. 2011, Robertson et al. 2017). In this study, single outliers were removed from 22 lambda-cyhalothrin, 4 zeta-cypermethrin, and 7 permethrin bioassays (Supp Table 1 [online only]). Larval mortality from some highly resistant sites failed to reach 50% and did not fit the requirements of probit analysis. In these cases, the LC_{50} value was conservatively listed as greater than (>) the highest concentration tested (Brindley 1975, Haddi et al. 2018, Rodbell and Wanner 2021).

Assessing Cross-Resistance Between Active Ingredients

To assess cross-resistance between active ingredient types, alfalfa weevil larvae from individual fields in all sampled states were assayed with multiple active ingredients. We assayed weevils from 14 fields with lambda-cyhalothrin, zeta-cypermethrin, and permethrin and 11 additional fields with lambda-cyhalothrin and permethrin. The LC_{50} resistance ratios for lambda-cyhalothrin were calculated using the average of the nine most susceptible location samples as the common denominator (0.013 μg/cm^2) (Forrester and Cahill 1987, ffrench-Constant and Roush 1990, Burton Jr. et al. 1996). Due to limited bioassays, all resistance ratios for permethrin and zeta-cypermethrin were calculated using the most susceptible LC_{50} value for each active ingredient generated to descriptively illustrate patterns of cross-resistance (Tabashnik et al. 2011).

Evidence for cross-resistance was further tested by dividing the fields into two conservative categories based on their lambda-cyhalothrin resistance ratios (RR), fields with a RR $\leq$ 79.6 (considered susceptible or moderately resistant) and fields with a RR $\geq$ 79.6 (considered highly resistant, Table 1). If cross-resistance exists between lambda-cyhalothrin and zeta-cypermethrin and/or permethrin, responses to these active ingredients (mortality at a diagnostic-concentration) should correspond to the resistance category defined by the lambda-cyhalothrin RR (Menger et al. 2020). For example, high resistance to lambda-cyhalothrin predicts low mortality to zeta-cypermethrin if cross-resistance exists. A concentration of 1.0 μg/cm^2 was analyzed as a diagnostic-concentration for zeta-cypermethrin and 3.3 μg/cm^2 for permethrin that is generally less potent. Percent mortality was log-transformed to meet the normality assumption of the one-way analysis of variance (ANOVA) that was performed on RStudio statistical software version 1.3.1093 (RStudio Team, Boston MA). The Tukey HSD test (P = 0.05) was used to identify significant differences between treatments.

Results

Resistance Levels to Lambda-cyhalothrin in the Western United States

The 71 field sites were categorized into three general levels of resistance to lambda-cyhalothrin, to assess trends across the western US. Susceptible, moderately resistant, and highly resistant categories were defined relative to the higher label rate of lambda-cyhalothrin: <0.9x, 0.9–2.9x, and >2.9x, respectively (Kostromytska et al. 2017, Table 1). The slope of the probit relationship was approximately 1.0 for susceptible and moderately resistant field sites and approximately 0.6 for highly resistant fields (Table 2).

Of the 71 fields tested with lambda-cyhalothrin, 34 were categorized as susceptible, 18 as moderately resistant, and 17 as highly resistant, to lambda-cyhalothrin (Table 2; resistance levels as defined in Table 1; Fig. 1). With this sampling intensity alfalfa fields with highly resistant larvae as well as susceptible larvae were identified in every surveyed state but Wyoming (limited sites tested in WY yielded moderate and high resistance ratings, Fig. 1).

Concentration-dependent mortality caused by lambda-cyhalothrin varied greatly among the 71 fields tested, resulting in LC_{50} values ranging from 0.002 to 8.4 μg/cm^2 (Table 2). Eight field sites in five of the sampled states, did not exceed 8–50% mortality even at the highest concentration of lambda-cyhalothrin tested (3.3 or 10 μg/cm^2) (>3.3 μg/cm^2: Riverside County CA, field site 9; Big Horn County MT, field sites 1 & 2; Klickitat County WA, field site 1; and >10.0 μg/cm^2: La Paz County AZ, field site 7; Umatilla County OR, field site 4; Yakima County WA, field site 1; Table 2). For context, the label rate for lambda-cyhalothrin is 22.7–33.6 g of active ingredient per hectare (0.02–0.03 pounds of active ingredient per acre; Warrior II with Zeon Technology, Syngenta 2021), corresponding to 0.22–0.34 μg/cm^2 in the treated vials.

Seventeen of the 71 location samples tested were categorized as highly resistant and local agriculturalists suspected insecticide resistance based on control failures in the field. The 17 highly resistant fields represent two counties each in Arizona, Washington, and Wyoming, and one county each in California, Montana, and Oregon (Fig. 1). The majority of the remaining 48 field sites were selected arbitrarily, and not based on reports of suspected resistance. Thirty-four of these fields were categorized as susceptible (Tables 1 and 2), representing six counties in California, six counties in Montana, two counties in Arizona, and one county in Oregon and Washington. It is important to note that sampling was not intended to estimate the frequency of resistance in each state, but rather to initially characterize its extent in the western region of the US, and sampling was more extensive in California and Montana. Based on lambda-cyhalothrin LC_{50} values, 18 fields were categorized as moderately resistant, representing four counties in California and Montana, and one county in Arizona and Wyoming.

The LC_{50} estimates from field-sampled larvae were considered consistent based on regional patterns (as opposed to resampling the same field repeatedly). For example, there were four field sites (Riverside County CA, field site 1; Big Horn County MT, field site 1; Big Horn County, field site 2; and Madison County MT, field site 2) that were sampled in 2020 and 2021. All sampled sites but Riverside County, field site 1, were of the same resistance level across both years. The Riverside County CA, field site 1, LC_{50} value increased

Table 1. To inform integrated resistance management (IRM) recommendations three categories of lambda-cyhalothrin resistance were defined based on lethal concentrations causing 50% mortality (LC_{50}) and the equivalent pesticide label rate for commercial products registered for alfalfa weevil control: 1) Susceptible, 2) Moderate resistance, and 3) High resistance. For each category, the equivalent resistance ratio (RR) range is listed using the average LC_{50} value of the nine most susceptible location samples assayed in this study (Table 2)

Resistance Category	LC_{50} (μg/cm^2)	Resistance Ratio (LC_{50}/0.013)	Times (x) Higher Label Rate 0f 0.34 μg/cm^2
Susceptible	<0.30	<23.08	<0.9x
Moderate	0.30–1.0	23.08–76.9	0.9–2.9x
High	>1.0	>76.9	>2.9x

Table 2. Alfalfa weevil larval resistance (R) level to lambda-cyhalothrin in the western region of the United States based on assayed LC_{50} values from 71 location samples: S is susceptible; M is moderate resistance; and H is high resistance. Probit analysis statistics are listed for the *t*-ratio of the slope, the chi-square (χ^2) goodness of fit, and the degrees of freedom (df). Resistance ratios (RR) were calculated using the LC_{50} value of the average of the nine most susceptible location samples, 0.013 µg/cm².

State	County	Site $_{\text{year}}$	LC_{50} µg/cm²	*t*-ratio $_{\text{slope}}$	χ^2_{df}	*P*-value	RR	R Level
AZ	La Paz	1_{2021}	1.55	6.09	8.39_5	0.14	119	H
		2_{2021}	0.65	8.27	7.9_5	0.16	50	M
		3_{2021}	2.78	9.00	5.04_5	0.41	214	H
		4_{2021}	0.53	7.00	0.04_2	0.98	41	M
		6_{2022}	0.62	3.88	3.43_2	0.18	49	M
		7_{2022}	>10.0	3.25	2.62_2	0.27	>770	H
		8_{2022}	4.67	4.32	0.052_2	0.97	360	H
	Pinal	1_{2021}	0.15	8.52	6.08_4	0.19	12	S
	Yuma	1_{2020}	0.18	5.77	2.05_1	0.15	14	S
		2_{2021}	0.12	6.64	3.30_4	0.51	9	S
		3_{2021}	1.35	8.27	7.90_5	0.16	104	H
CA	Kern	1_{2020}	0.02	4.82	8.37_5	0.14	1	S
		2_{2021}	0.004	3.75	4.37_4	0.36	1	S
	Kings	1_{2020}	0.34	7.35	6.78_4	0.15	26	M
		2_{2020}	0.44	5.91	7.22_4	0.12	34	M
		3_{2020}	0.002	10.4	3.95_5	0.56	1	S
		4_{2020}	0.01	8.46	2.52_5	0.77	1	S
	Madera	1_{2020}	0.05	8.68	8.11_4	0.09	4	S
	Merced	1_{2021}	0.17	6.96	3.99_4	0.41	13	S
		2_{2020}	0.04	7.34	3.72_5	0.59	3	S
		3_{2021}	0.04	7.76	5.70_4	0.22	3	S
		4_{2021}	0.02	10.10	6.99_5	0.22	1	S
	Riverside	1_{2020}	0.26	7.95	6.58_5	0.25	20	S
		1_{2021}	0.92	6.54	6.07_5	0.30	71	M
		2_{2020}	0.53	5.59	0.12_1	0.73	41	M
		3_{2020}	0.51	4.51	0.38_1	0.53	40	M
		4_{2020}	0.12	4.64	1.19_3	0.76	9	S
		5_{2021}	0.01	8.63	3.09_4	0.54	1	S
		6_{2020}	0.63	4.35	0.25_1	0.62	48	M
		7_{2020}	0.22	4.51	0.39_1	0.53	17	S
		8_{2022}	1.82	3.87	3.69_1	0.055	140	H
		9_{2022}	>3.33	–	–	–	>256	H
	Yolo	1_{2021}	0.30	7.32	5.86_4	0.21	23	M
		2_{2021}	0.08	6.60	5.75_4	0.22	6	S
		3_{2020}	0.13	5.30	6.88_5	0.23	10	S
		4_{2020}	0.01	2.66	5.97_5	0.31	1	S
		5_{2021}	0.51	7.66	4.38_5	0.50	39	M

Table 2. Continued

State	County	Site $_{year}$	LC_{50} µg/cm^2	*t*-ratio $_{slope}$	χ^2_{df}	*P*-value	RR	R Level
MT	Beaverhead	1_{2020}	0.05	8.26	7.05_5	0.22	4	S
	Big Horn	1_{2020}^{a}	>3.33	4.12	9.78_5	0.08	>256	H
		1_{2021}	4.50	6.48	4.63_4	0.33	346	H
		2_{2020}^{a}	>3.33	4.08	7.62_4	0.11	>256	H
		2_{2020}^{a}	>1.00	2.51	3.09_2	0.21	>77	H
		2_{2021}	1.78	4.86	6.50_4	0.16	137	H
		3_{2021}	0.32	3.54	3.11_1	0.08	25	M
		3_{2021}	0.57	6.76	3.61_5	0.61	44	M
		4_{2021}	8.40	4.65	6.95_5	0.22	646	H
	Broadwater	1_{2020}^{a}	0.26	5.1	7.39_5	0.19	20	S
		2_{2020}^{a}	0.26	8.22	4.79_5	0.44	20	S
		3_{2020}^{a}	0.77	7.82	2.68_2	0.26	59	M
		4_{2021}	0.43	5.27	3.45_2	0.18	33	M
	Gallatin	1_{2020}	0.16	6.95	0.92_5	0.97	12	S
		2_{2020}	0.31	7.47	1.87_4	0.76	24	M
	Madison	1_{2020}^{a}	0.02	8.33	2.47_5	0.78	1	S
		2_{2020}^{a}	0.06	7.18	6.41_5	0.27	5	S
		2_{2021}	0.26	7.01	3.67_3	0.30	20	S
	Powder River	1_{2020}^{a}	0.03	4.58	2.73_2	0.26	2	S
		2_{2020}^{a}	0.1	7.14	5.51_4	0.24	8	S
		3_{2020}^{a}	0.09	7.83	6.58_4	0.16	7	S
	Ravalli	1_{2020}	0.42	3.92	4.65_5	0.46	32	M
	Richland	1_{2020}^{a}	0.09	6.58	5.13_5	0.40	7	S
OR	Umatilla	1_{2021}	0.06	5.48	5.89_4	0.21	5	S
		2_{2021}	0.17	5.88	7.9_4	0.10	13	S
		3_{2021}	0.01	3.38	3.45_5	0.63	1	S
		4_{2021}	>10.0	–	–	–	>770	H
WA	Klickitat	1_{2020}	>3.33	3.76	6.56_5	0.26	>256	H
	Yakima	1_{2021}	>10.0	3.76	6.56_5	0.65	>770	H
		2_{2021}	0.21	9.27	8.56_5	0.13	16	S
WY	Converse	1_{2021}	1.84	6.54	8.92_4	0.06	142	H
		1_{2021}	6.92	4.2	9.60_5	0.08	532	H
	Platte	1_{2021}	0.52	7.97	3.92_4	0.41	40	M
	Sheridan	1_{2020}	2.23	5.05	11.09_5	0.05	172	H

[a] Rodbell and Wanner 2021.

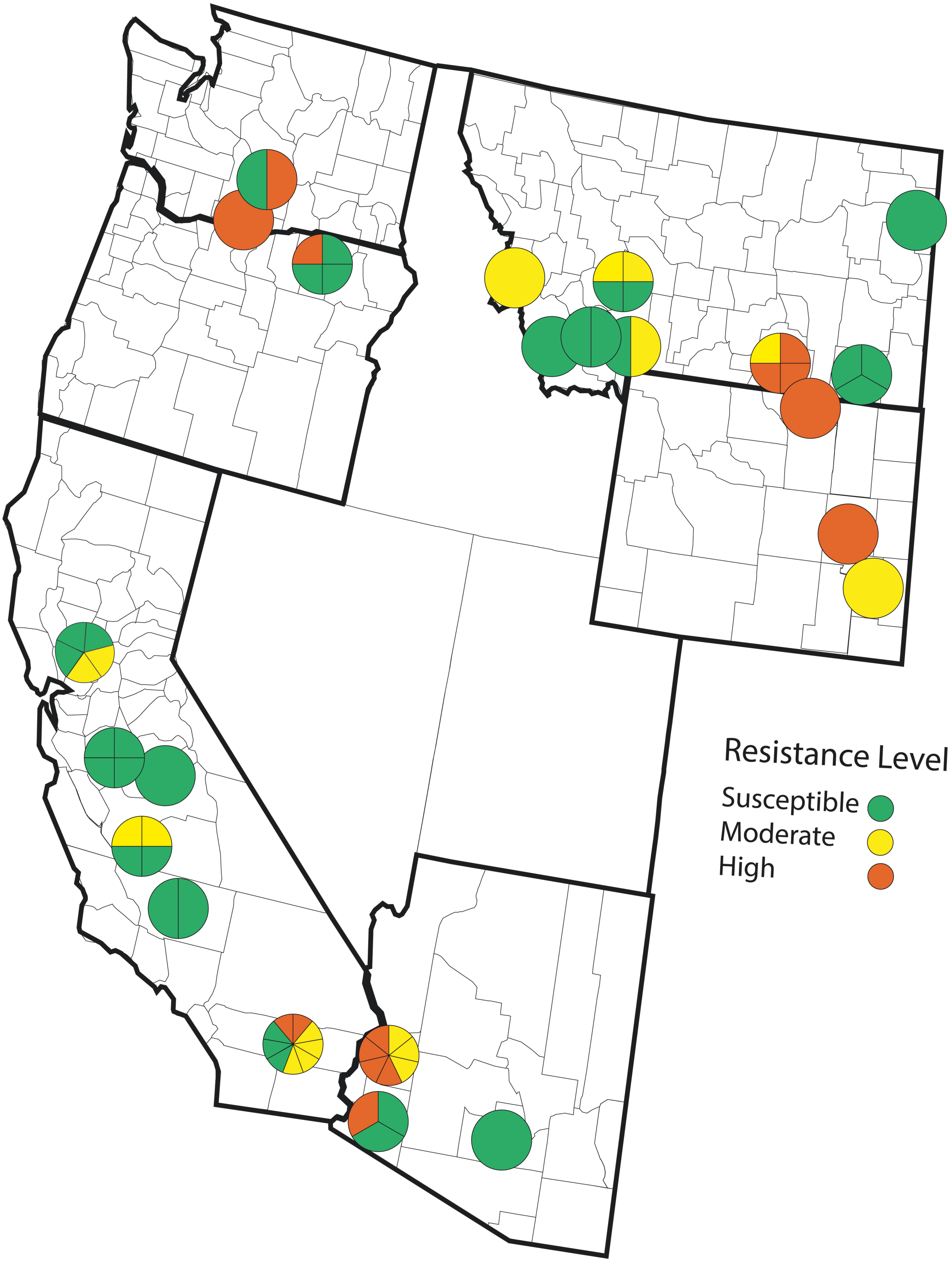

Fig. 1. Lambda-cyhalothrin resistance level categories for 71 commercial alfalfa field sites located in six western states: Arizona, California, Montana, Oregon, Washington and Wyoming. Pie charts represent location samples within a county (listed in Table 2). Each section of a pie chart represents a single alfalfa field site that has been categorized as highly resistant (LC50 >1.0 μg/cm²), moderately resistant (LC50 0.30–1.0 μg/cm²) or susceptible (LC50 value <0.30 μg/cm²). For context, the label rate for lambda-cyhalothrin is 0.22–0.34 μg/cm².

in 2021, indicating that the estimated resistance level changed from susceptible to moderately resistant (Table 2). This field site was managed organically in an area with alfalfa predominantly managed conventionally and may have been impacted by migrating resistant adults. Some field sites in Montana were evaluated multiple times within the same year, generating similar LC_{50} values between bioassays, including Big Horn County field sites 1_{2020}, 2_{2020}, and 3_{2021} (Table 2).

Cross-Resistance Between Type I and II Pyrethroid Active Ingredients

Our findings indicate cross-resistance between lambda-cyhalothrin and zeta-cypermethrin, however, there was limited cross-resistance between lambda-cyhalothrin and permethrin. Sixteen of the 72 field sites collected from 2020 to 2022 were assayed with zeta-cypermethrin (Table 3). LC_{50} values ranged from a low of 0.09 µg/cm² (Broadwater County MT, field site 4) to a high of 7.75 µg/cm² (Big Horn County Montana, field site 1). For context, the label rate for zeta-cypermethrin is 15.7–28.0 g of active ingredient per hectare (0.014–0.025 pounds of active ingredient per acre, Mustang Maxx Insecticide, FMC 2021), equivalent to about 0.16–0.28 µg/cm². LC_{50} values were not calculated for field sites where maximum larval morality failed to reach 50% and are conservatively reported as greater than the highest concentration tested (>3.3 µg/cm²: Big Horn County MT, field sites 1, & 2; and, >10 µg/cm² La Paz County AZ, field site 1, Umatilla County OR, field site 4, and Yakima County WA, field site 1). Four of five field sites that failed to reach 50% larval mortality after exposure to the highest concentration of zeta-cypermethrin also failed to reach 50% mortality after exposure to the highest concentration of lambda-cyhalothrin (Table 2). Mortality at Umatilla County OR field site 4 never exceeded 8% for any concentration tested. Larvae from alfalfa fields highly resistant to lambda-cyhalothrin also tended to be highly resistant to zeta-cypermethrin, supporting cross-resistance between these two type II active ingredients.

Lambda-cyhalothrin and zeta-cypermethrin cross-resistance is supported by diagnostic-concentration mortality between field sites that were grouped based on their lambda-cyhalothrin resistance ratio. Sixteen field sites were assayed with both lambda-cyhalothrin and zeta-cypermethrin; larvae from 6 fields had lambda-cyhalothrin $RR < 76.9$ (susceptible–moderate resistance) and 10 had $RR > 76.9$ (highly resistant to lambda-cyhalothrin). Generally, susceptibility to zeta-cypermethrin corresponded to susceptibility to lambda-cyhalothrin. Consistent with cross-resistance, diagnostic-concentration mortality resulting from zeta-cypermethrin was lower in the field sites highly resistant to lambda-cyhalothrin ($RR > 76.9$) and conversely, higher in the field sites susceptible to lambda-cyhalothrin (susceptible–moderate resistance, $RR < 76.9$) (97.9 vs. 48.2 %mortality, Fig. 2, $F = 49.67$; df = 5, 24; $P < 0.001$).

Evidence for cross-resistance between lambda-cyhalothrin and permethrin is variable. Thirty-one of the 72 field sites were also assayed with permethrin. The permethrin LC_{50} values ranged from a low of 0.1 µg/cm² (Powder River County MT, field site 2) to a high of 19.4 µg/cm² (Gallatin County MT, field site 2). The label rate for permethrin, 112.0–224.1 g per hectare (0.1–0.2 pounds of active ingredient per acre, Loveland Products Inc. 2013), is equivalent to 1.12–2.24 µg/cm² in the bioassay vials. Unlike zeta-cypermethrin, alfalfa fields highly resistant to lambda-cyhalothrin were not always associated with higher permethrin LC_{50} values (for example, Big Horn County MT). Additionally, some field sites susceptible to lambda-cyhalothrin appeared to be resistant to permethrin (for example, Umatilla OR, Yuma AZ, and Gallatin MT counties). These results suggest variable patterns of resistance to lambda-cyhalothrin and permethrin at these field sites, including partial (but not complete) cross-resistance (Supp Table 2 and Supp Fig. 1 [online only]).

Results from diagnostic-concentration assays with permethrin also support the lack of complete cross-resistance since it was high both in lambda-cyhalothrin susceptible and highly resistant field sites (Fig. 2; $F = 49.67$; df = 5, 24; $P < 0.001$). Unlike lambda-cyhalothrin and zeta-cypermethrin, permethrin maximum mortalities were always higher than 50%, enabling LC_{50} values to be calculated. Collectively, the evidence for cross-resistance between lambda-cyhalothrin and permethrin is mixed (Fig. 2, Supp Fig. 1 [online only]).

Discussion

Alfalfa weevil lambda-cyhalothrin resistance is established across the western United States and highly resistant locations were identified in all six surveyed states. Larval mortality from some surveyed alfalfa fields never reached 50% mortality at the highest concentration tested. Susceptible field sites were identified in every state surveyed, except Wyoming, where only four sites were sampled. Field sites that were tested with multiple pyrethroid active ingredients provided some evidence for cross-resistance between lambda-cyhalothrin and zeta-cypermethrin (type II pyrethroid), and variable and/or limited cross-resistance between lambda-cyhalothrin and permethrin (type I pyrethroid). Based on historical distributions, the field sites in southern Arizona and southern California correspond to the range occupied by Egyptian strain alfalfa weevils, suggesting resistance to lambda-cyhalothrin has developed in both the Egyptian and western strains (Titus 1910, Wehrle 1940, Clancy 1969).

Our findings of alfalfa weevil field sites highly resistant to lambda-cyhalothrin suggest that the mechanism(s) of resistance is widespread in the western region of the United States. However, further studies are required to identify the mechanism(s) of alfalfa weevil resistance to lambda-cyhalothrin and their geographic variation. In other insect species, mutations to the voltage gated sodium ion channel gene target site that reduce sensitivity to pyrethroid active ingredients (i.e., knockdown resistance (*kdr*) and *super-kdr*) as well as the utilization of detoxification enzymes and/or behavioral resistance have been identified (Feyereisen 1999, Soderlund 2005, Davies et al. 2007, Després et al. 2007, Scott et al. 2008, Rinkevich et al. 2013, Pereira et al. 2017, Haddi et al. 2018, Fujii et al. 2020, Keïta et al. 2021).

Susceptibility to an insecticide can depend on the size, life stage, and sex of an insect (e.g., instar stage). Thus, insecticide applications should coincide with the most susceptible life stage to maximize efficacy, one recommendation to slow the development of resistance (Yu 1983, Koehler et al. 1993, Glunt et al. 2011, Zhu et al. 2020). Future research is needed to characterize the susceptibility of adult alfalfa weevils and the four instar stages to determine optimal timing of pyrethroid and indoxacarb insecticide sprays in the western region of the United States.

Within insecticide naïve populations, susceptibility to an insecticide is variable, producing a range of LC_{50} values, prompting some studies to use an average LC_{50} value derived from several 'susceptible' location samples (Brown and Brogdon 1987, Forrester and Cahill 1987, Sawicki 1987, ffrench-Constant and Roush 1990, Burton Jr. et al. 1996). An average of the lowest lambda-cyhalothrin LC_{50} values (0.013 µg/cm², $n = 9$ independent field sites, three states) was used to represent susceptible alfalfa weevils in the western region (Forrester and Cahill 1987, ffrench-Constant and Roush 1990, Burton Jr. et al. 1996). Lambda-cyhalothrin LC_{50} resistance ratios in this study ranged from 1 to 770 (Table 3). When characterizing susceptibility in field populations, other studies have found similar broad ranges of RRs (Luttrell et al. 1999, Shelton et al. 2003, Snodgrass and Scott 2003, Wang et al. 2010, Kostromyska et al. 2017). Intense resistance, a natural range of toxicity and the method of calculating RRs can contribute to the observed broad range of values.

Interpreting the significance of RRs produced by laboratory bioassays to insecticide efficacy in the field can be challenging. Ideally, LC_{50} values from bioassays are correlated with insect mortality after field application, but this approach is laborious and not commonly employed (Ball 1981, Denholm et al. 1984, ffrench-Constant and Roush 1990). Rather, the RRs are commonly divided into discrete categories with inferred levels of field resistance, such as susceptible, moderate resistance, and high resistance (Table 1, Kostromytska et al. 2017). Such categories provide a framework for IRM recommendations with supporting field trials to verify bioassay-based categories. In this study, the moderate resistance category represents field sites transitioning from susceptible (effective larval control) to resistant (control failure) after field applications of lambda-cyhalothrin. Alfalfa weevil highly resistant to lambda-cyhalothrin, are associated with control failure in the alfalfa fields where these larvae were sampled (Table 2, Big Horn County MT, field site 1). Lambda-cyhalothrin applied at its highest label rate failed to reduce larval counts compared to untreated check plots in a field trial where bioassays recorded an $LC_{50} > 3.3$ μg/cm^2 (RR > 330; Rodbell and Wanner 2021, Wanner et al. 2022; Table 2, Big Horn County MT field site 1).

Cross-resistance between different active ingredients within the same MoA group is common among insect pests, and our preliminary data suggest alfalfa weevils are cross-resistant to lambda-cyhalothrin and zeta-cypermethrin, both type II pyrethroid active ingredients (Yu 2015; Fig. 2). A descriptive comparison of the RRs of several active ingredients that results in a parallel trend of increasing RRs suggests cross-resistance exists (DeVries and Georghiou 1981, Scott 1990, Kostromytska et al. 2017). To supplement this descriptive approach and gain preliminary insights into cross-resistance we simplified the analysis by dividing field sites into two categories based on their susceptibility to lambda-cyhalothrin (RR < 76.9 vs. RR >76.9). Alfalfa weevils resistant to lambda-cyhalothrin averaged less than 50% mortality in response to a discriminating dose of zeta-cypermethrin, supporting cross-resistance of these two active ingredients. Average mortality of resistant larvae after exposure to a discriminating dose of permethrin resulted in high mortality, supporting a lack of complete cross-resistance between lambda-cyhalothrin and permethrin (Fig. 2, Supp Fig. 1 [online only]).

In several cases, susceptible and highly resistant field sites were identified in the same county, suggesting lambda-cyhalothrin resistance may be localized. The relatively limited dispersal of adult weevils and the density and isolation of some alfalfa production areas, likely contribute to the size and severity of resistance pockets (Prokopy et al. 1967). For example, alfalfa is a primary economic crop grown in the Palo Verde Valley of southern California, receiving irrigation from the lower Colorado River, and surrounded by the Sonoran Desert. In this contiguous production area with intensively managed forage alfalfa, resistance to lambda-cyhalothrin is relatively uniform (Table 2). A pattern not seen in other counties included in this study (e.g., Yuma County, AZ; Umatilla County, OR; Yakima County, WA).

Soon after the advent of the 'insecticide era' in the 1950s, insecticide resistance became a serious and persistent problem associated with routine pesticide use (e.g., calendar spraying; Pedigo et al. 2021). In addition to the monetary impact of yield loss, economic impacts can include higher costs associated with alternative MoAs required for adequate pest control (Grafius 1997). In the case of alfalfa weevil, reports of cross-resistance to both heptachlor and dieldrin insecticides (MoA 2A) began to emerge in 1962 and were subsequently replaced by pyrethroids (MoA 3A) (Adler and Blickenstaff 1964). Alternative MoA groups currently available for the control of alfalfa weevil are limited, and several are considered ineffective and/or have increased restrictions. In some areas, indoxacarb (MoA 22A) appears to be the only effective active ingredient where alfalfa weevils are highly resistant to pyrethroids. Indoxacarb (i.e., Steward) provided 92–96% control of alfalfa weevil larvae in fields resistant to lambda-cyhalothrin (Rodbell and Wanner 2021, Wanner et al. 2022; Table 2, Big Horn County MT field site 1). Thus, the loss of efficacy of all MoA 3A pyrethroid active ingredients would significantly limit available insecticide options for alfalfa weevil control and impede IRM strategies based on rotating insecticide MoA groups.

Insecticide resistance management is an approach to proactively manage resistance. In the case of alfalfa weevil, insecticide resistance management aimed at pyrethroid resistance has variable potential for success and can achieve different goals. For example, alfalfa weevil populations highly resistant to lambda-cyhalothrin have been identified from every state included in this study. For these populations, the first two goals of IRM, 1) preventing resistance from developing and 2) slowing the rate of resistance development, have already been bypassed (Croft 1990a). IRM strategies in these locations should be designed to revert lambda-cyhalothrin resistance to more susceptible levels (Croft 1990a). However, once resistance genes have been established, their frequencies can increase quickly with the resumption of insecticide use (Yu 2015). After more than five decades of pyrethroid use in agriculture, pyrethroid-naïve alfalfa weevil populations are likely quite rare. However, the identification of numerous locations with lambda-cyhalothrin susceptible alfalfa weevils was a promising outcome of this study. In these areas, IRM strategies could prevent the development of pyrethroid resistance, at least locally, for some time.

Integrated pest management (IPM) strategies function as important components of IRM (Croft 1990b, Dara 2021, Gallagher 2021). Therefore, the first recommendation is to use IPM strategies that are currently available to reduce insecticide use. Monitoring alfalfa weevil populations and employing economic thresholds to guide insecticide applications is a founding principle of IPM (Peterson et al. 2018, Pedigo et al. 2021). When economic thresholds are exceeded, cultural control practices should be considered before opting for chemical control. Recommended cultural control tactics include maintaining healthy alfalfa stands and harvesting early (seven to ten days before normal harvest time) to salvage yield and increase larval mortality (Summers 1998, Pellissier et al. 2017, Pedigo et al. 2021).

Successful IRM strategies include reducing the constant pressure to select resistance to specific active ingredients by rotating to different MoA groups (Tabashnik 1990, IRAC 2022). Currently, only two MoA groups are considered effective for alfalfa weevils for seasonal rotation, MoA 3A (pyrethroids) and MoA 22A (indoxacarb). Until new MoA groups become available, a current recommendation is to apply MoA groups 3A and 22A no more than once every three years, as well as including cultural control tactics to limit insecticide use (IRAC 2022). If multiple insecticide applications are required during the same season or generation, the same MoA should be used within a year (Roush and Daly 1990). Other goals include preserving the efficacy of indoxacarb (MoA 22A) and maximizing the effectiveness of insecticide applications by optimizing operational variables (e.g., application timing, spray coverage, and equipment calibration) (Roush and Daly 1990). Furthermore, insects heterozygous for resistance can be relatively more susceptible compared to homozygous individuals (Rawlings et al. 1981, Roush and Daly 1990). Higher efficacy, including the use of the higher rates listed on the pesticide label, result in greater mortality of susceptible and heterozygous

Table 3. Lethal concentrations resulting in 50% mortality (LC_{50} values) from 30 location samples assayed with permethrin (type I pyrethroid) and 16 location samples assayed with zeta-cypermethrin (type II pyrethroid). Probit analysis statistics are listed for the *t*-ratio of the slope, the chi-square (χ^2) goodness of fit, and the degrees of freedom (df).

Active ingredient	State	County	Field site $_{year}$	LC_{50} µg/cm²	*t*-ratio $_{slope}$	χ^2_{df}	*P* value	No. Concn.
Zeta-cypermethrin	AZ	La Paz	3_{2021}	>10.00	3.45	1.03_4	0.90	6
			5_{2021}	4.12	4.67	1.63_4	0.80	6
		Pinal	1_{2021}	0.1	10.92	7.31_5	0.20	7
	MT	Big Horn	1_{2020}	>3.33	5.18	9.02_5	0.11	7
			1_{2021}	7.75	6.32	10.95_5	0.052	7
			2_{2020}	>3.33	5.57	3.12_5	0.68	7
			2_{2021}	5.82	5.2	10.62_5	0.06	7
			3_{2021}	0.53	5.13	0.0002_1	0.96	3
			4_{2021}	1.23	6.89	8.75_5	0.12	7
		Broadwater	4_{2021}	0.09	5.2	1.03_1	0.31	3
		Madison	1_{2021}	0.32	7.3	1.58_4	0.31	6
			2_{2021}	0.28	6.52	4.79_4	0.31	6
	OR	Umatilla	3_{2021}	0.32	7.35	3.80_5	0.58	7
			4_{2021}	>10.00	–	–	–	7
	WA	Yakima	1_{2021}	>10.00	4.3	6.23_5	0.27	7
	WY	Platte	1_{2021}	0.48	6.94	0.35_2	0.84	4
Permethrin	AZ	La Paz	3_{2021}	12.92	10.36	10.14_5	0.07	7
			5_{2021}	13.29	7.51	4.66_3	0.20	5
		Pinal	1_{2021}	0.74	6.81	3.29_4	0.51	6
		Yuma	2_{2021}	13.33	6.55	8.92_4	0.06	6
	CA	Riverside	1_{2021}	6.76	2.75	9.38_4	0.052	6
	MT	Big Horn	1_{2020}	1.18	4.96	4.96_2	0.08	4
			1_{2020}	4.74	2.82	0.004_1	0.95	3
			1_{2020}	1.54	7.9	3.22_5	0.67	7
			1_{2021}	6.44	4.49	5.10_3	0.16	5
			2_{2020}	7.05	5.56	3.90_4	0.42	6
			2_{2021}	3.55	8.55	5.61_4	0.23	6
			3_{2021}	2.81	5.59	0.19_1	0.67	3
			4_{2021}	3.83	5.11	0.62_2	0.73	4
		Broadwater	1_{2020}	1.28	6.12	5.19_3	0.16	5
			3_{2021}	2.67	6.63	8.90_4	0.06	6
			4_{2021}	0.47	5.80	0.26_1	0.61	3
		Gallatin	2_{2020}	19.36	5.10	8.64_4	0.07	6
		Madison	1_{2020}	0.17	7.5	5.45_3	0.14	5
			2_{2021}	2.08	7.23	3.12_4	0.54	6
		Powder River	1_{2020}	0.55	5.81	8.06_5	0.15	7
			2_{2020}	0.10	7.36	6.58_4	0.24	6
			3_{2020}	0.19	5.85	5.45_3	0.14	5
	OR	Umatilla	1_{2021}	1.73	6.24	8.15_4	0.09	6
			3_{2021}	1.98	6.52	5.55_3	0.14	5
	WA	Yakima	1_{2021}	7.18	5.27	7.55_3	0.06	5
			2_{2021}	2.11	8.42	8.83_4	0.07	6
	WY	Converse	1_{2021}	2.70	8.47	4.00_4	0.41	6
			2_{2021}	2.17	6.94	7.89_3	0.05	5
		Platte	1_{2021}	1.04	5.53	0.05_1	0.82	3
		Sheridan	1_{2020}	0.70	5.84	5.22_2	0.07	4
			2_{2020}	5.54	5.27	3.40_5	0.64	7

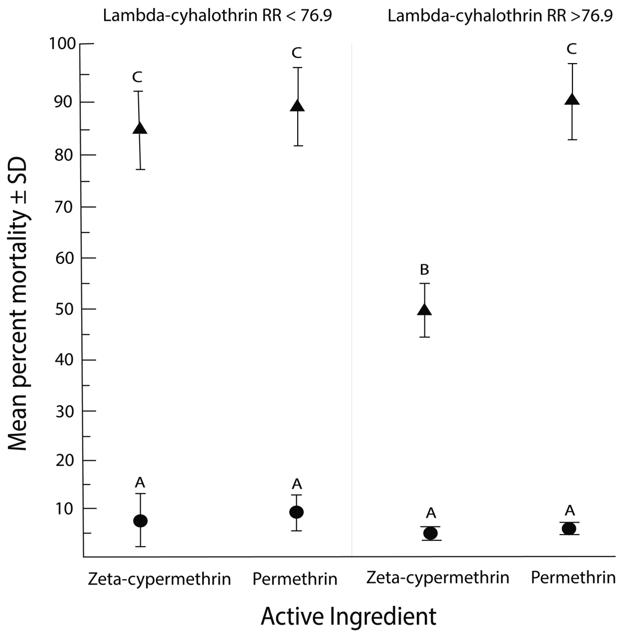

Fig. 2. Mean percent larval mortality exposed to diagnostic concentrations of zeta-cypermethrin (1.0 µg/cm^2) and permethrin (3.3 µg/cm^2), between location samples grouped based on lambda-cyhalothrin resistance ratios (RR) <76.9 and >76.9. Triangles represent the diagnostic concentration and circles represent the acetone control groups. Different letters signify significant differences ($P < 0.05$) between treatments ($F = 49.67$; df = 5, 24; $P < 0.001$).

individuals, thus delaying the gene(s) frequency from increasing (Rawlings et al. 1981, Roush and Daly 1990).

Further research efforts are needed to establish patterns of cross-resistance between the different pyrethroid active ingredients registered for alfalfa weevil management, including field efficacy, to provide IRM recommendations to alfalfa stakeholders. A better understanding of the physiological mechanisms of pyrethroid resistance may lead to the development of molecular markers to better monitor the spread of pyrethroid resistance and the effectiveness of IRM strategies. Emphasis should be placed on early efforts to monitor alfalfa weevil resistance to MoA 22A (i.e., indoxacarb), and to any new MoA group registered for the control of alfalfa weevil. Here, population genomics and next generation sequencing technology to detect early changes in genotype(s), coupled with laboratory bioassays that detect insecticidal activity patterns with greater sensitivity, may represent promising approaches.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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