LABORATORY BIOASSAYS FOR SCREENING BIFENTHRIN RESISTANCE IN WHITE CLOVER SEED WEEVIL (YEAR 2)

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Introduction

Clover seed weevil (CSW), *Tychius picirostris* Fabricius (Coleoptera: Curculionidae), is a key insect pest in white clover seed production systems, and it requires control in western Oregon. Besides white clover, CSW is also known to attack other clover species, including alsike, arrowleaf, and Ladino clovers (Anderson, 2020). CWS is a small, gray weevil about 0.1 inch in length. It has a characteristic long snout, and its body is covered with gray and white hair (Reeher et al., 1950). CSW has the potential to cause significant yield loss because larvae feed on developing clover seeds for a prolonged period during the growing season. In recent years, crop advisors in western Oregon have reported several cases of failed CSW control with bifenthrin (Brigade) insecticide. Testing for bifenthrin-resistance had not been investigated prior to a preliminary study conducted by Kaur et al. (2020). The objective of the present study was to further examine the extent to which bifenthrin insecticide resistance exists within CSW populations found in western Oregon white clover seed production systems.

Materials and Methods

In 2020, two laboratory studies were conducted to expose four field-collected adult CSW populations using two bioassays to test for the development of resistance against contact insecticides: (1) adult vial assay and (2) Potter spray tower assay.

<u>Adult vial assay</u>

In May 2020, four populations of CSW were collected from four different commercial white clover seed fields in Linn County, OR. Collected CSW adults were placed in separate, large, ventilated chambers for 24 hours prior to conducting bifenthrin dose-response assays according to methods described in Miller et al. (2010). Adult CSW were exposed to increasing bifenthrin rates (0, 17.5, 68.5, 112.5 g a.i./ha; 0, 1, 3.9, 6.4 fl oz a.i/acre) by treating the inside surface of 20-ml glass vials with 0.5 ml of commercially formulated Brigade in acetone. The treated vials were placed on a vial roller to dry and ensure uniform product distribution on each vial's interior surface. Ten field-collected CSW adults were then introduced to each treatment, with ten adults per vial. Vials were closed with a perforated stopper to allow air exchange. Each treatment was replicated five times (n = 50 adults per treatment).

Vials were then inspected for mortality (dead insects, often lying on their backs) after 12, 24, and 36 hours of bifenthrin exposure. Mortality data were subjected to log dose probit analysis using JMP Pro 15 (SAS Institute Inc., 2019) to generate estimates of a lethal dose that provides mortality to 50% (LD50) of the target populations. LD50 is the amount of test substance that is sufficient to kill 50% of a test population.

<u>Potter spray tower assay</u>

One field population of CSW adults was collected in late July. This method of screening simulated the exposure of the test population to bifenthrin by using a Potter spray tower (Burkard Manufacturing Co. Ltd., England). This method delivers the insecticide solution as a fine mist, which forms a uniform layer on the insects without creating any visible droplets. To expose the test population, adult insects (n = 10) were immobilized with a flash chilling treatment and placed in a 140-mm plastic petri dish. Each petri dish containing ten adult weevils was placed on the Potter spray tower stage and sprayed with 2 ml of the insecticide treatment (10 and 100 ppm concentration of bifenthrin (≤ 17.5 g a.i/ha; ≤ 1 fl oz a.i./acre). Water was used as untreated control or 0-ppm treatment. Treatments were applied using an air pressure of 47 kPa (6.8 psi) and a spray distance of 22 cm. Each spray treatment was replicated three times (n = 30 adults per treatment).

Insect mortality was recorded hourly for 8 hours. Total mortality was calculated at the end of the experiment for each replicate petri plate in all treatment and control groups. Kaplan-Meier log rank survival analysis was performed using GraphPad Prism Version 8.0.2 (GraphPad Software, San Diego, CA).

Results and Discussion

<u>Adult vial assay</u>

All four CSW populations exposed to bifenthrin in the adult vial assays resulted in 83.3 to 94.6% mortality after 36 hours of exposure (Figure 1). The dose probit analysis showed that a dose of 38.68–76.49 g a.i./ha caused 50% mortality (LD50) in 12 hours (Table 1). The LD50 value further decreased to 16.18–25.67 g bifenthrin/ha after 24 hours and to 8.79–16 g bifenthrin/ha after 36 hours. The subsequent decline of LD50 value as the exposure time progresses indicated a delayed mortality effect of bifenthrin on the adults.

The LD50 value can be used to establish baseline susceptibility of target CSW populations. In the future, these data can be used to determine whether the susceptibility of the target population has shifted. Actual LD50 values can be compared among populations by examining the 95% confidence intervals. If the upper and lower limits do not overlap, then it is likely that the population has experienced a significant change in susceptibility; in some situations this is an indication of developed resistance.

Potter tower assay

Control survival was ≥ 80% for the duration of the study (Figure 2). A log-rank test indicated significant differences in insect survival among experimental groups (Kaplan Meier, log rank test $\chi 2 = 239$, $df = 2$, and $P < 0.001$). The 10- and 100-ppm treatments did not significantly differ. After the 8-hour exposure period, 10% and 0% of adults survived the 10- and 100-ppm treatment, respectively.

In summary, these results from both the 2019 and 2020 studies did not indicate resistance development to bifenthrin. However, steps should be taken to avoid or delay the development of resistance in the future. We recommend managing crop canopy height and density to promote effective spray coverage and defoliating the crop to remove early-season inflorescences for better synchronization of bloom. Insecticide applications should be timed to coincide with economic thresholds for CSW, as listed in the *PNW Insect Management Handbook* (Anderson, 2020).

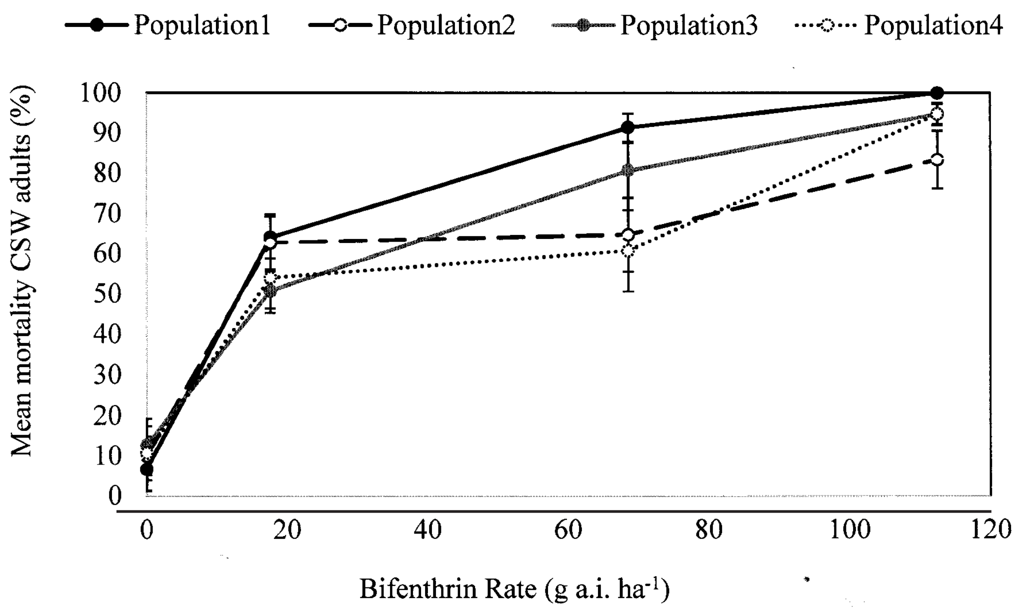

Figure 1. Mean mortality (%) of clover seed weevil adults exposed to different rates of bifenthrin in the adult vial test.

Table 1. The bifenthrin dose (g a.i./ha) that caused 50% mortality (LD50) of clover seed weevil adults within four field populations 12, 24, and 36 hours after exposure to treatment.

	12 hours			24 hours			36 hours		
		95% confidence interval			95% confidence interval			95% confidence interval	
Population	LD50	Lower CI	Upper CI	LD50	Lower CI	Upper CI	LD50	Lower CI	Upper CI
	(g a.i./ha)								
1	38.68	32.53	45.72	16.70	10.02	23.21	10.37	4.57	15.82
2	75.26	64.88	87.56	23.56	3.87	37.80	8.79	-13.71	23.56
3	70.33	62.24	78.95	16.18	6.51	24.44	11.60	3.69	18.46
4	76.49	67.34	87.21	25.67	13.01	36.22	16.00	6.15	24.26

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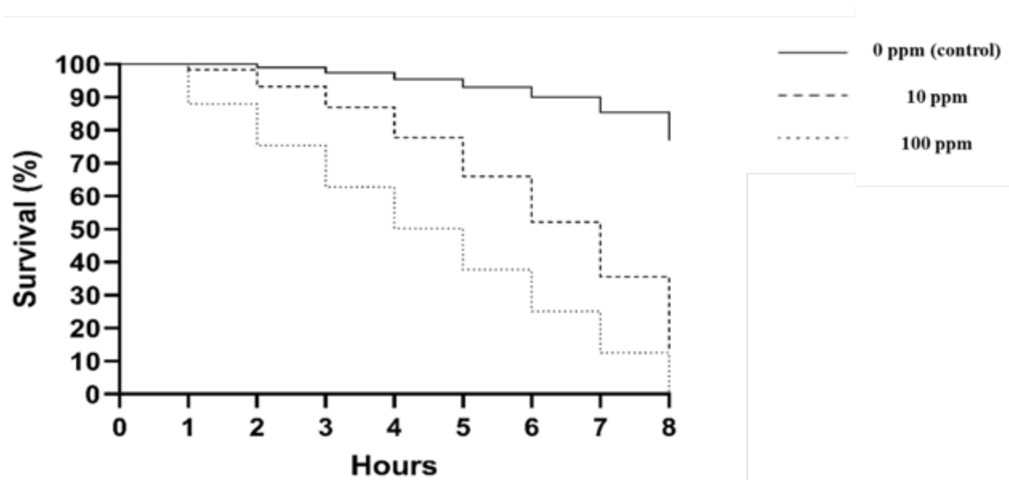

Figure 2. Clover seed weevil adult survival during the 8-hour observation period during the Potter spray tower experiment when exposed to different concentrations of bifenthrin.