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Running title: Evaluation of pesticide mixtures for western corn rootworm

Evaluation of Pyrethroids and Organophosphates in Insecticide Mixtures for Management of Western Corn Rootworm Larvae

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Abstract

BACKGROUND: The western corn rootworm is an economically important pest of corn. Management tactics include pyrethroid and organophosphate insecticides, which may be applied as a mixture to protect corn roots. The goal of our study was to characterize the effects of pyrethroids and organophosphates alone and in combination on larval corn rootworm mortality and injury to corn roots. We evaluated two insecticide combinations: tebupirimphos with β-cyfluthrin, and chlorethoxyfos with bifenthrin. Using a soil-based, laboratory bioassay, we exposed larvae to five concentrations of the pyrethroid alone, the organophosphate alone, the combined formulation, and a water control. We calculated LC_{50} values and co-toxicity factors to determine synergism or antagonism between organophosphates and pyrethroids. We also measured adult emergence and root injury in a field experiment that tested tebupirimphos alone, β-cyfluthrin alone, the combined formulation, and an untreated control.

RESULTS: Bioassay results indicated antagonism between the pyrethroid and organophosphate at most concentrations for both insecticide combinations. In the field experiment, tebupirimphos alone or in combination with β-cyfluthrin significantly reduced adult emergence and root injury compared to the untreated controls, but β-cyfluthrin alone did not differ from the untreated control for either metric.

CONCLUSIONS: These results suggest that, at the concentrations tested, the pyrethroid component of pyrethroid-organophosphate mixtures may not contribute to a reduction of rootworm emergence or root injury. While these pyrethroids may confer a management benefit

for other pests, such as seedcorn maggot, the concentrations of pyrethroids present in current formulations of these mixtures are likely too low for effective rootworm management.

Keywords: antagonism, insecticide, organophosphate, pyrethroid, western corn rootworm

Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the most economically important pests of corn in the United States. This univoltine coleopteran primarily injures corn by feeding on root tissue in the larval stage, which can reduce the plant's ability to acquire water and nutrients.[1,2] Severe injury may result in loss of structural support and lodging, which can interfere with harvesting the corn.[3,4] In general, each node of root pruning results in approximately 15 to 17% loss in yield.[5,6]

Western corn rootworm populations are typically managed by a combination of rotation to a non-host crop (e.g., soybeans), soil insecticide application at planting, and transgenic corn that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt).[7–9] Beginning in 2003, the advent of Bt corn reduced the use of conventional insecticides in the midwestern United States.[10] However, recent cases of Bt resistance may cause farmers to increase their reliance on soil insecticide as a management tactic for this pest.[11] Thus, there is renewed interest in the agricultural community in understanding the efficacy and interactions of conventional insecticides used for rootworm management.

Multiple classes of insecticides have been used to manage rootworm. Early examples include chlorinated hydrocarbons such as aldrin and heptachlor in the 1940's and 1950's, with resistance to these insecticides reported by 1962.[12] Organophosphate and carbamate insecticides have been used to manage adults, but resistance had developed to both of these insecticide classes by the mid-1990's.[13] One carbamate, carbofuran, was shown to be susceptible to enhanced microbial degradation in certain field conditions, reducing its efficacy for rootworm management.[14] Pyrethroids and organophosphates are used currently, either alone or in combination, and a combination of these two insecticides applied in the furrow at planting has been widely shown to reduce adult emergence and root injury.[15] Combining multiple insecticides is a commonly used tactic in agriculture, and may increase pest mortality and delay the evolution of insecticide resistance.[16,17]

When multiple insecticides with differing modes of action are combined, redundant killing may be achieved where individuals not killed by one component are killed by the second component of the mixture.[18] This tactic can serve to delay resistance to both insecticides of the mixture.[19,20] However, the level of mortality achieved by soil-applied insecticides targeting larval rootworm is typically well below the level that would be needed to delay resistance beyond what would be achieved by using the insecticides sequentially rather than as a pyramid.[15,20–22]

Insecticides may sometimes act unpredictably when used in a mixture. The effect of the mixture may be stronger (synergism) or weaker (antagonism) than the sum of the individual component effects.[23–25] From a management perspective, synergism among pesticide mixture components is beneficial, as sufficient pest mortality can be maintained at lower doses. Conversely, antagonism between components would result in higher dose requirements to achieve the level of mortality imposed by a single component at a lower dose. The degree of interaction experienced by combined insecticides may be specific to the insecticides used and the target pest. For example, the organophosphate ethion was shown to be synergistic with the pyrethroid bifenthrin when used for management of cotton bollworm, *Helicoverpa armigera* Hübner, but antagonism was observed between another organophosphate, quinalphos, and bifenthrin for the same insect. [26] Thus, the exploration of potential synergistic and antagonistic effects of pesticide mixtures must be tailored to specific pest-insecticide combinations.

The goal of our research was to test whether synergistic or antagonistic activity exists in organophosphate and pyrethroid insecticide combinations used for management of western corn rootworm larvae in the soil. We used a soil-based bioassay to test the interaction between these insecticide classes, performing laboratory bioassays using larval western corn rootworm and two liquid pesticide mixtures: tebupirimphos with β-cyfluthrin, and chlorethoxyfos with bifenthrin. We examined rootworm mortality imposed by the combined formulation, as well as the two components individually. To more fully explore this interaction, we also performed a field experiment using granular formulations of tebupirimphos and β-cyfluthrin. The field experiment examined adult rootworm emergence and root injury to corn in plots with both insecticidal components and each component individually. We hypothesized that the effect of the organophosphate and pyrethroid components of these formulations would be additive, with no synergism or antagonism.

Methods

Laboratory bioassay

All insecticides used in the laboratory bioassay were non-commercial, technical-grade liquids, and were supplied by AMVAC Chemical Corporation (Newport Beach, CA). Liquid insecticides were used because they allowed for a homogeneous mixture in soil, thus ensuring a consistent exposure for all larvae. For organophosphate-alone formulations, the concentrations of active ingredients were 4.3% and 4.5% chlorethoxyfos and tebupirimphos, respectively. For pyrethroid-alone formulations, the concentrations of bifenthrin and β-cyfluthrin were 0.7% and 0.22%, respectively. For the formulation with both active ingredients, the formulations were 4.3% chlorethoxyfos with 0.7% bifenthrin, and 4.5% tebupirimphos with 0.22% β-cyfluthrin, which reflects the ratios in some commercial formulations. For bioassays of tebupirimphos and β-cyfluthrin, the concentrations used were a 0.042, 0.084, 0.21, and 0.42 ppm of formulated product and a deionized water control. For bioassays with chlorethoxyfos and bifenthrin, concentrations were the same with the exception that the 0.21 ppm concentration was replaced with a 0.168 ppm concentration. Final concentrations were based on concentrations found in commercially-available products (i.e., field-relevant application rates). In order to assess the contribution of each individual insecticide applied alone or in combination, the combined formulations of tebupirimphos and β-cyfluthrin or chlorethoxyfos and bifenthrin contained the same amount active ingredient as the individual formulations (e.g., the concentration of tebupirimphos in the tebupirimphos-only treatment was the same as the concentration of tebupirimphos in the tebupirimphos and β-cyfluthrin combination).

The strain of western corn rootworm used in laboratory bioassays was a non-diapausing strain which had been cultured in a laboratory setting since 1976.[27] This strain had been cultured at Iowa State University since October 2009, with insects originally received from the United States Department of Agriculture, Agricultural Research Service, North Central Agricultural Research Laboratory in Brookings, South Dakota, USA.

A soil-based bioassay was used to test effects of insecticides on mortality of larval western corn rootworm. Corn seeds were wrapped in moist paper towels for two days prior to the assay to induce germination. Soil was obtained from Agronomy Farm, an Iowa State University research and demonstration farm, in Boone County, Iowa. No insecticides had been used on this field for six years prior to soil collection. Soil from this field had previously been determined to be a Nicollet-Webster complex with 1.6% organic matter, 60% sand, 22% silt, 18% clay, with pH 7.0.[28] Cation exchange capacity of soil at this location, which was obtained from the United States Department of Agriculture Natural Resources Conservation Service (https://websoilsurvey.sc.egov.usda.gov /App/HomePage.htm), was 23.7 millequivalents (meq) / 100 g. Soil was dried in a greenhouse for more than 48h, crushed in a soil crusher, and sieved (<600 µm particle size).

Assay containers were prepared separately by combining 30g sieved soil (dry weight) with the appropriate liquid (i.e., insecticide treatment). All replicates received 4.5 mL of liquid (i.e., controls received 4.5 mL of de-ionized water, and insecticide treatments received de-ionized water plus the relevant amount of insecticide totaling 4.5 mL). The soil and liquid were mixed thoroughly using a steel scoopula to ensure that liquid was evenly distributed. Three germinated corn seedlings were then placed at the bottom of each 44-mL assay container (Dart Container Corporation, Mason, Michigan). Six neonate western corn rootworm larvae (<24h old) were added to each container by applying them directly to the radicle root of the newly sprouted corn seeds using a fine paint brush. Homogenized soil with insecticide treatment (i.e., 30g dry weight soil plus 4.5mL liquid) was then added on top of the seedlings. Lids were then placed on the containers. Small holes (approximately 3 mm diameter) were made in each lid to provide ventilation for the larvae and a layer of mesh poly chiffon fabric (Hobby Lobby Stores, Inc., Oklahoma City, Oklahoma, USA) was used to prevent larvae from escaping. Containers were placed in a biological incubator (Percival Scientific, Perry, Iowa, USA) at 25°C and 16:8 L:D cycle. Moist paper towels were placed beneath each container and on top of the lid to maintain humidity. One mL of de-ionized water was applied to each container on the seventh day to maintain moisture within each bioassay container. Containers were incubated for 10 days, at which time the contents were removed and placed onto Berlese funnels for 3 days, with surviving larvae of each bioassay container collected in 50mL vials of 85% ethanol and enumerated.

For tebupirimphos and β-cyfluthrin, assays were conducted in six blocks, with each block consisting of one replicate of each concentration of tebupirimphos, β-cyfluthrin, and the combination, and three replicates of a deionized water control. Blocks were started on 15 March, 22 March, 29 March, 31 March, 6 April, 8 April, with the final samples of block six removed from Berlese funnels on 21 April, 2016. There was a total of six replications for each insecticide concentration and 18 replications for the experimental control of water alone.

For chlorethoxyfos and bifenthrin, bioassays consisted of four blocks of two replicates per concentration and four replicates of controls. These were conducted starting 7 July, 14 July, 21 July, and 28 July, with the final samples removed from Berlese funnels on 10 August, 2017. The 0.84 ppm concentration was not initially included, as preliminary experiments indicated that 0.42 ppm would result in sufficient mortality for LC_{50} calculations. However, low mortality in the first two blocks prompted the inclusion of the 0.84 ppm concentration in the last two blocks to achieve a higher level of mortality on rootworm larvae. Thus, for each insecticide concentration there were eight replications, with the exception of 0.84 ppm, which had only four replications. There was a total of 16 replications for the experimental control of water alone.

Field experiment

A field experiment measuring adult rootworm emergence and rootworm feeding injury to corn was conducted at Iowa State University's Johnson Research and Demonstration Farm, in Story County, Iowa. Treatments were tebupirimphos and β-cyfluthrin in combination (4.67% a.i.), tebupirimphos alone (4.45% a.i.), β-cyfluthrin alone (0.22% a.i.), and an untreated control. The tebupirimphos and β-cyfluthrin combination treatment reflected the concentrations of active ingredient found in the commercially-available product. The tebupirimphos-alone and β-cyfluthrin-alone treatments had the same concentration of active ingredient as found in the combined formulation. Granular insecticide was used because the commercial product of combined tebupirimphos and β-cyfluthrin is used in this formulation. This differs from the laboratory experiment, which used liquid insecticides to ensure consistent exposure of larvae to insecticide, because the goal of the field experiment was to examine the effect of these insecticides under realistic field conditions. The study was a randomized complete block design with six blocks. Each treatment was randomly assigned to one of four plots within a block, for a total of 24 plots.

Plots were planted 14 May, 2018 using corn that lacked a rootworm active Bt trait (DKC 60-69 VT2P RIB, Monsanto Co. St. Louis, MO). The area of the field used had been planted to corn for at least 10 years, was not irrigated, had not had insecticides applied in the previous year, and contained an established corn rootworm population. Soil was analyzed in the year of planting (Agvise Laboratories, Northwood, ND). The soil at this location was primarily sandy clay loam, with 3.3% organic matter, 50% sand, 27% silt, and 23% clay, with pH 5.3. Cation exchange capacity was 11.4 meq/100 g.

All chemicals used in the experiment were granular formulations provided by AMVAC Chemical Corporation, and were applied at 85g/305 row m(3.0 oz/1000 row ft) in-furrow at time of planting. Plots consisted of four rows of corn that were 9.14 m long, with 0.76 m between rows. Seeds were planted with a spacing of 15.24 cm (six inches) between seeds, at a rate of 14, 600 seeds per hectare. Four plots were planted adjacent to one another (forming one block), with

1.5 m of fallow ground between blocks. The study was surrounded by 3 m of fallow ground, with additional corn planted on all sides of the study beyond the fallow ground.

Illinois-style emergence cages, constructed based on Fisher (1980),[29] were placed around individual corn plants on 21 and 22 June, 2018. Each plot received four cages placed on randomly chosen plants in the center two rows of corn, for a total of 96 cages. Cages were situated such that the corn plant could grow through the hole in the center of the cage, which was wrapped with mesh to prevent rootworm adults from escaping. The sides of the cage were buried in the soil. Rootworm emerging from the soil became trapped in a container with an inverted funnel that was located at the top of each cage. After collection from the field, containers were frozen for approximately 48 h to kill rootworm, after which time the number of rootworms in each container was recorded. These containers were changed weekly, with the first containers placed on 22 June, 2018 and the last containers collected on 7 September, 2018 (11 weeks). Only one rootworm adult was collected during the first week, and collections were stopped when no rootworm were found in the containers for three consecutive weeks. Thus, all, or nearly all, rootworm emergence in the field was captured in the timeframe of the experiment.

To assess root injury, five plants were randomly chosen from the interior two rows of each plot. These plants were removed from the soil using a spade and the top portion of the above-ground tissue was removed, leaving approximately 40 cm of stem above the roots. These roots were soaked in water for 24 h, then washed and rated on the 0-3 node injury scale following Oleson et al. (2005).[30] Roots were sampled on 9 August, 2018, and were rated on 10 August, 2018.

Data analysis

LC_{50} values for each formulation were calculated using a Probit model in SAS 9.4 (PROC PROBIT; SAS Institute, Cary, NC). A Pearson's chi-square test was conducted to assess goodness-of-fit in each model. If the Pearson's test indicated a lack of fit, the covariance matrix of the Probit model was multiplied by a heterogeneity factor, Pearson's chi-square ÷ degrees of freedom, and fiducial limits were calculated using a critical value from the *t* distribution.[31] For tebupirimphos alone, mortality in the 0.084 ppm concentration deviated from the Probit model and prevented the calculation of fiducial limits, and thus was removed from the LC_{50} analysis.

Co-toxicity factors between the organophosphate and pyrethroid components of each formulation were used to quantify synergy or antagonism between the insecticides.[32] Co-toxicity was calculated as:

$$\textit{Co-toxicity} = \frac{PM_C - (PM_O + PM_P)}{(PM_O + PM_P)} \times 100$$

Where: PM_C = Proportion mortality for the combined formulation

PM_O = Proportion mortality for organophosphate alone

PM_P = Proportion mortality for pyrethroid alone

Strongly negative co-toxicity values (< -20) indicate antagonism (i.e., the combined formulation imposes less mortality than would be expected from examining mortality imposed by the components individually). Strongly positive values (> 20) indicate synergism (i.e., the combined formulation imposes a greater degree of mortality than the two components individually). Values between -20 and 20 are indicative of additive effects (i.e., the mortality observed by the mixture is within the range expected from the components individually).[32]

To examine adult emergence in the emergence cage experiment, the total number of western corn rootworm that emerged from each cage throughout the 11 weeks of the experiment was calculated. A mixed-model analysis of variance (PROC GLIMMIX) was used to analyze adult emergence. The fixed factor in the model was treatment (untreated control, tebupirimphos-only, β-cyfluthrin-only, and tebupirimphos and β-cyfluthrin combination), and block and the block × treatment interaction were random effects. Due to overdispersion of the data (i.e., the variance of the data was larger than the mean), the model assumed a negative binomial response distribution, which is an appropriate method for analyzing overdispersed data.[33] The LSMEANS statement with a Bonferroni adjustment for multiple comparisons was used to test for differences among the treatments. Significance of random terms was tested by conducting a one-way probability test using the log-likelihood ratio statistic (-2 RES Log Likelihood), which follows a χ^2 distribution assuming 1 degree of freedom. Random terms were pooled if $P \geq 0.25$.[34]

Root injury was analyzed using a mixed-model analysis of variance (PROC MIXED) assuming a normal response distribution. The fixed factor was treatment, and the random effects were block and block × treatment. Comparisons among treatment means were made using the LSMEANS statement with a Bonferroni adjustment for multiple comparisons.

Results

Laboratory bioassay

The LC_{50} for tebupirimphos and β-cyfluthrin in combination was 0.41 ppm, and the value calculated for tebupirimphos alone was 0.08 ppm (Table 1). An LC_{50} value for β-cyfluthrin could not be calculated due to low mortality imposed by this insecticide at the concentrations tested. The LC_{50} for chlorethoxyfos and bifenthrin in combination was 0.43 ppm, and the value for chlorethoxyfos alone was 0.14 ppm (Table 1). Again, the LC_{50} value for the pyrethroid component, bifenthrin, could not be calculated due to low mortality of rootworm larvae at the concentrations tested. The 95% fiducial limits did not overlap for the tebupirimphos and β-cyfluthrin in combination compared to tebupirimphos alone, or for the chlorethoxyfos and bifenthrin in combination compared with chlorethoxyfos alone (Table 1). This result indicates a significantly higher LC_{50} for the combined formulation of an organophosphate with a pyrethroid versus an organophosphate alone.

In general, mortality imposed by pyrethroids was low compared to organophosphates alone or in the combined formulation at the rates and ratios tested (Fig. 1). Co-toxicity values between the pyrethroid and organophosphate components of both insecticide formulations were negative, with the exception of chlorethoxyfos and bifenthrin at 0.042 ppm, which was near zero (Table 2). Co-toxicity between tebupirimphos and β-cyfluthrin at 0.084 ppm was -10.00, and all other co-toxicity calculations exceeded -20.00, and mortality in the organophosphate-only treatments was higher than mortality in the combined formulations (Fig. 1). These results suggest the presence of antagonism between pyrethroid and organophosphate insecticides in the laboratory bioassays.

Field experiment

In total, 717 western corn rootworm adults and 20 northern corn rootworm adults were collected. Because northern corn rootworm accounted for < 3% of the rootworm captured in the field, they were not included in statistical analyses. There was a significant effect of treatment on adult western corn rootworm emergence ($P < 0.0001$; Table 3). A significantly greater number of rootworm emerged in the untreated control than the treatments of tebupirimphos alone ($P < 0.0001$) and the combination of tebupirimphos with β-cyfluthrin ($P = 0.001$). Emergence was significantly greater in the β-cyfluthrin-only treatment compared to the tebupirimphos-only treatment ($P = 0.0002$). Emergence was also higher in the β-cyfluthrin-only treatment than in the combination of tebupirimphos with β-cyfluthrin, and this difference was marginally significant ($P = 0.05$). Emergence in the β-cyfluthrin-only treatment did not differ from the untreated controls (Fig. 2A).

The results for root injury showed a similar trend to adult rootworm emergence. Root injury in the untreated control group was significantly higher than tebupirimphos alone (P = 0.01) or in combination with β-cyfluthrin (P = 0.02). Root injury in the β-cyfluthrin-only treatment was significantly higher than tebupirimphos alone (P = 0.007) or in combination with β-cyfluthrin (P = 0.01), and did not differ from untreated controls (Fig. 2B).

Discussion

The goal of this study was to characterize the insecticidal contributions of organophosphate and pyrethroid active ingredients to mortality in western corn rootworm larvae in laboratory bioassays and a field experiment. In the laboratory bioassays, we found evidence of antagonism between two pairs of organophosphate and pyrethroid insecticides at several tested concentrations (Table 2; Fig. 1), refuting our hypothesis that the effect of the mixture would be additive. However, antagonistic effects were not observed in a field setting using equivalent application rates to the laboratory experiment (Table 3; Fig. 2). Laboratory bioassays have previously shown antagonism between organophosphates and pyrethroids in other insect orders, including lepidopterans and hemipterans.[26,35] Here we have shown evidence that pyrethroids can antagonize organophosphates when used for management of a major coleopteran pest of corn, western corn rootworm, but such antagonism may not necessarily be present in field conditions.

In some cases, additive effects or synergism have been observed between organophosphates and pyrethroids.[36] However, antagonism between these two insecticide classes also has been experimentally demonstrated. Examples include quinalphos and bifenthrin with *H. armigera*,[26] chlorpyrifos and deltamethrin with *Spodoptera littoralis* Boisduval,[37] and profenofos and λ-cyhalothrin with *Bemisia tabaci* Gennadius.[35] In our bioassay experiments, the LC_{50} values for both organophosphate and pyrethroid combinations were significantly higher than their respective organophosphate alone (i.e., the 95% fiducial limits did not overlap) (Table 1). For both bioassay experiments, the co-toxicity factors between the pyrethroids and organophosphates tended to be strongly negative (Table 2). These results suggest antagonism between the pyrethroid and organophosphate under certain conditions.

Antagonism between organophosphates and pyrethroids may be caused by behavioral or biochemical mechanisms. Pyrethroids are known to have a repellent effect on a wide variety of insect orders, including Hymenoptera, Orthoptera, Diptera, and Coleoptera.[38–42] It is possible pyrethroid-mediated repellency caused reduced rootworm feeding or movement compared to non-treated larvae, such that exposure to the organophosphate component of the insecticide mixture was limited. This would have resulted in lower mortality imposed by the mixture compared to the organophosphate-only treatment, where a pyrethroid-mediated repellency effect was not present. Another possible mechanism is a change in detoxification of organophosphates by exposure to pyrethroids. Other studies have demonstrated that sub-lethal pesticide exposure can lead to an increase in detoxification enzyme levels in target organisms.[43,44] Such exposure can drastically affect how an insect responds to a subsequent insecticidal challenge. In this case it is possible that the low-level pyrethroid exposure lead to an increase in detoxification enzymes, which allowed for more effective degradation and excretion of organophosphate metabolites. The antagonism observed in our assays may have been caused by a combination of these two mechanisms, or by other unknown means. Future work is needed to identify the responsible mechanism.

In the field trial of tebupirimphos and β-cyfluthrin, root injury and adult emergence were lower in the tebupirimphos-alone treatment and the combined formulation compared to the untreated controls, while the β-cyfluthrin-alone treatment was similar to controls (Fig. 2). Because the tebupirimphos-alone and the combined formulation did not differ for either metric, these results do not directly imply antagonism. Instead, these results suggest that the dose of pyrethroid in this insecticide combination was too low to effectively reduce adult rootworm emergence or prevent injury to corn roots. In the laboratory assay, larvae were assured of a full dose of insecticide because their movement was limited to the assay arena, while larvae in the field could move away from the insecticide-treated area. In general, exposure in the field may be variable as larvae are able to move away from treated root tissue, and may complete development on more distal, untreated root tissue.[45] This difference may be why antagonism was observed in the laboratory but not in the field. This suggests that antagonism may exist between pyrethroids and organophosphates for western corn rootworm, but is only in areas where insecticide is present. An additional factor to consider is that the field experiment took place in more acidic soil (pH 5.3) compared to the laboratory bioassays (pH 7.0), and the soil in the field had a higher cation exchange capacity (11.4 meq/100 g) than soil used in bioassays (23.7

meq/100g). Soil conditions are known to influence the performance of insecticides,[46] and it is possible that differences in soil attributes may partially explain the difference in results between the laboratory and the field.

Root injury and emergence of western corn rootworm in the β-cyfluthrin-alone treatment was similar to untreated controls, and significantly higher than tebupirimphos-alone or the combination (Fig. 2). Pyrethroids used alone for management of western corn rootworm have been shown to reduce root injury.[47–49] However, the dose of β-cyfluthrin tested in this study does not appear to be high enough to protect roots from feeding (Fig. 2). These results suggest that the organophosphate component was the primary agent responsible for reducing corn root injury by western corn rootworm. It is worth noting that pyrethroids may be applied for management of multiple corn pests, including seedcorn maggot, wireworms, and cutworms.[50] Additionally, the effects observed here may vary as a function of rootworm density, soil properties and farming practices (e.g., irrigation) and additional field studies would be needed to more fully understand the potential interactions that could arise. Thus, the inclusion of the pyrethroid component in this insecticide mixture may still confer some management benefit, even if the effect on western corn rootworm larvae was negligible in this study.

The low dose of pyrethroid in this insecticide mixture may have consequences for western corn rootworm resistance management in the field. Such a low dose of one insecticide of a mixture undermines the basic principle of combining insecticides, which is that individuals not killed by one agent will be killed by the other.[18,19] In this case, individuals not killed by the organophosphate are unlikely to be killed by the pyrethroid, but instead will have received a sub-lethal dose. This provides selection pressure for resistance to the pyrethroid, and, a low dose of one insecticide of the mixture may speed adaptation to both insecticides.[16,18,51,52] However, this effect may be mitigated by any repellency effects of the pyrethroid. If larvae are repelled by the pyrethroid component of the mixture, this will have the effect of reducing exposure to the organophosphate, and consequently reduce selection for organophosphate resistance. In general, conditions that reduce pest mortality in this way are likely to delay resistance.[53,54] Thus, the effect of the pyrethroid and organophosphate mixture on resistance management is unclear, and should be explored in future research.

Our study found that two pyrethroids, β-cyfluthrin and bifenthrin, and two organophosphates, tebupirimphos and chlorethoxyfos, acted to antagonize each other in a laboratory bioassay with western corn rootworm larvae. A field trial with β-cyfluthrin and tebupirimphos showed that the organophosphate was the primary insecticide responsible for reducing adult emergence and root injury, while the pyrethroid alone had no effect compared to untreated controls. These findings underscore the importance of investigating potential interactions of insecticide mixtures. This is especially important in the case of western corn rootworm, as farmers may now be returning to conventional insecticides because of rootworm resistance to Bt corn.[11,55] A repellency effect by pyrethroids may act to reduce selection pressure for organophosphate resistance, but further research is necessary to confirm the presence and magnitude of this effect in the field. Ultimately, understanding the interactions of pyrethroids and organophosphates used for management of western corn rootworm will aid in preserving the efficacy of this important pest management tool for future use.

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Tables

Table 1. LC_{50} and Pearson χ^2 goodness-of-fit values for laboratory bioassays.

	N [a]	Slope ± SE	χ^2	DF	P [b]	LC50 (95% fiducial limits)
Tebupirimphos + β-cyfluthrin	216	2.04 ± 0.45	4.84	3	0.18	0.41 (0.26, 0.62)
Tebupirimphos [c]	180	1.72 ± 0.30	1.12	2	0.57	0.08 (0.04, 0.13)
β-cyfluthrin [d]	-	-	-	-	-	-
Chlorethoxyfos + bifenthrin	312	5.90 ± 1.50	1.85	3	0.60	0.43 (0.35, 0.51)
Chlorethoxyfos [e]	312	3.83 ± 0.97	8.8	3	0.03	0.14 (0.04, 0.29)
Bifenthrin [d]	-	-	-	-	-	-

[a] N = total number of larvae used in estimating LC_{50}
[b] P-value tests for goodness-of-fit of the Probit model, based on a Pearson's χ^2 statistic.
[c] The 0.084 ppm concentration did not fit the Probit model and was removed from analysis to ensure estimation of fiducial limits.
[d] LC_{50} values could not be calculated due to low larval mortality.
[e] To account for a significant lack of fit with the model, the covariance matrix was multiplied by a heterogeneity factor, Pearson $\chi^2 \div$ DF = 2.93, during the calculation of fiducial limits.

Table 2. Co-toxicity factors of tebupirimphos and β-cyfluthrin, and chlorethoxyfos and bifenthrin at five concentrations.

	Insecticide concentration (ppm)					
	0.042	0.084	0.168	0.21	0.42	0.84
Tebupirimphos / β-cyfluthrin [a]	-77.78	-10.00	-	-48.57	-43.24	-31.71
Chlorethoxyfos / bifenthrin [a]	0.00	-68.00	-87.23	-	-94.59	-95.74

[a] Negative values indicate antagonism between individual components

Table 3. Analysis of variance for rootworm emergence and root injury in the field experiment.

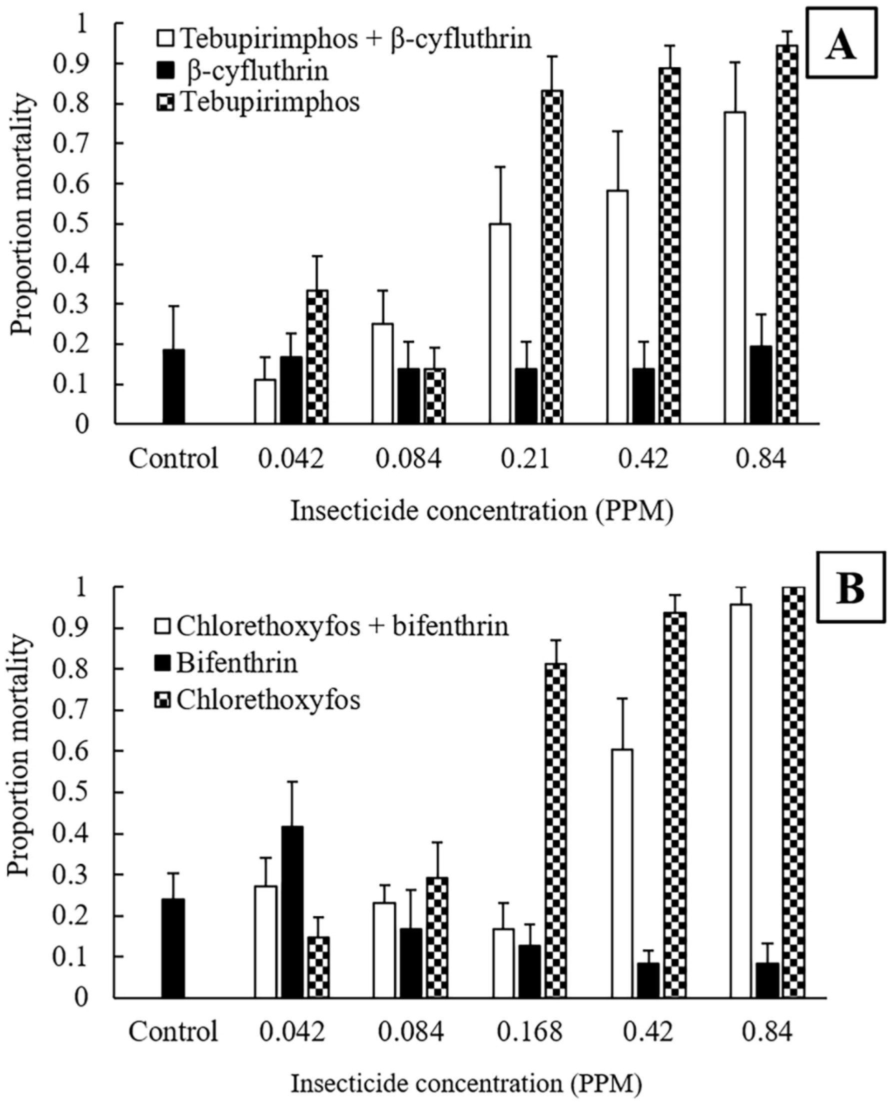
Effect	*DF*	*F*	*P*
Rootworm emergence [a]	3, 87	12.34	<0.0001
Root injury [b]	3, 15	9.27	0.001

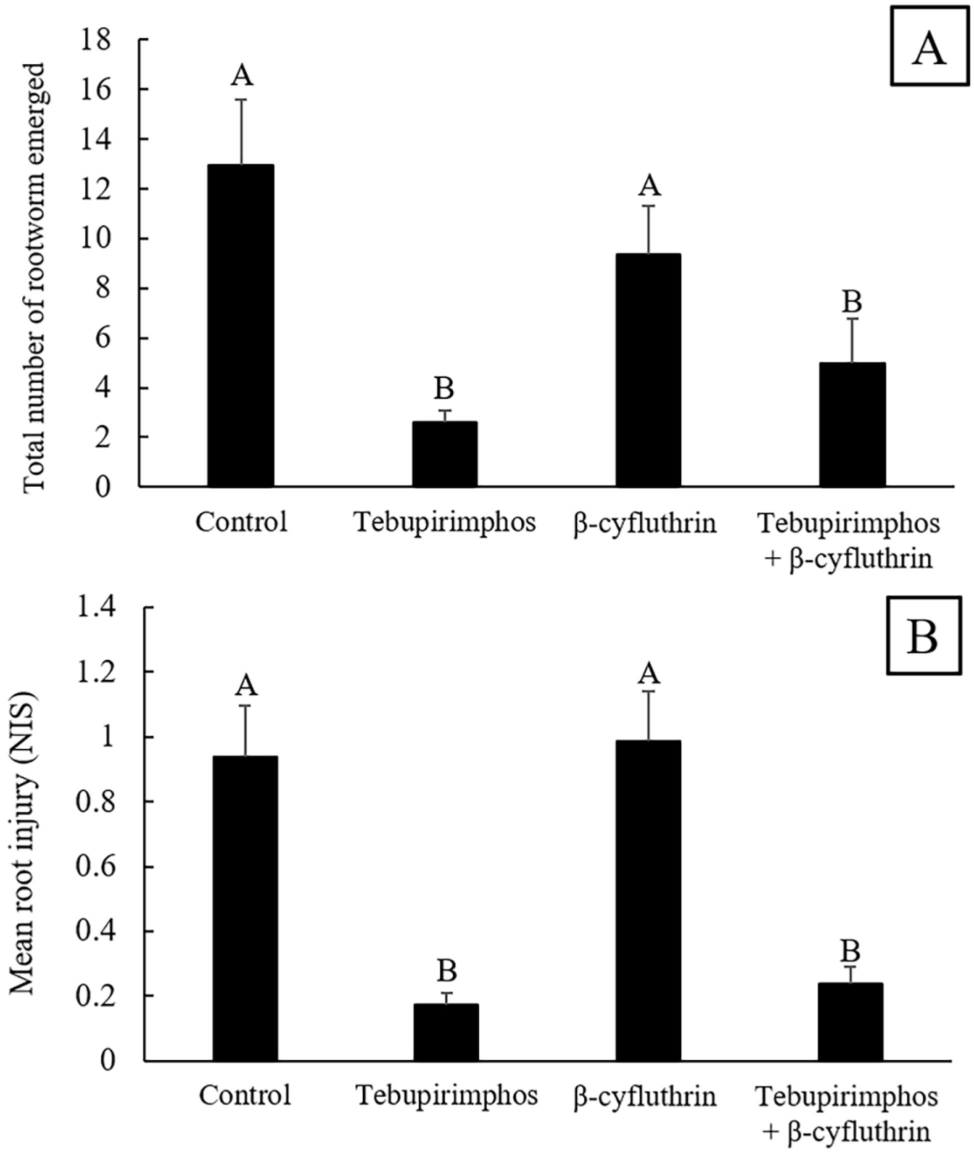
[a] Random factor: Block χ^2=6.93, DF=1, P=0.004
[b] Random factor: Block χ^2=0.1, DF=1, P=0.38; Block × Treatment χ^2=2.6, DF=1, P=0.05

Figure Legends

Figure 1. Mortality imposed on western corn rootworm larvae in soil-based laboratory bioassays by A) tebupirimphos and β-cyfluthrin in combination, β-cyfluthrin alone, and tebupirimphos alone, and B) chlorethoxyfos and bifenthrin in combination, bifenthrin alone, and chlorethoxyfos alone. Bar heights are sample means and error bars are the standard error of the means.

Figure 2. Adult emergence of western corn rootworm (A) and injury to corn roots (B) in the field experiment. Bar heights are sample means and error bars are standard error of the mean. Different letters indicate significant differences between means at the $P \leq 0.05$ level.


A
Tebupirimphos + β-cyfluthrin
β-cyfluthrin
Tebupirimphos
Proportion mortality
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
0.042
0.084
0.21
0.42
0.84
Insecticide concentration (PPM)
B
Chlorethoxyfos + bifenthrin
Bifenthrin
Chlorethoxyfos
Proportion mortality
Control
0.042
0.084
0.168
0.42
0.84
Insecticide concentration (PPM)


A
Total number of rootworm emerged
18
16
14
12
10
8
6
4
2
0
A
B
A
B
Control
Tebupirimphos
β-cyfluthrin
Tebupirimphos
+ β-cyfluthrin
B
Mean root injury (NIS)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A
B
A
B
Control
Tebupirimphos
β-cyfluthrin
Tebupirimphos
+ β-cyfluthrin