Efficacy of Pesticide Mixtures Against the Western Flower Thrips (Thysanoptera: Thripidae) Under Laboratory and Greenhouse Conditions

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ABSTRACT Western flower thrips, *Frankliniella occidentalis* Pergande is a commonly encountered and economically important insect pest of greenhouses. Greenhouse producers typically apply pesticides as mixtures to mitigate western flower thrips populations; however, there is limited information available on the compatibility and efficacy of commonly used pesticide mixtures. This study assessed nine binary and three tertiary pesticide mixtures used in greenhouses which included pesticides containing abamectin, acephate, azadirachtin, bifenazate, bifenthrin, fenpropathrin, imidacloprid, novaluron, pymetrozine, and spinosad. Compatibility was determined for the binary pesticide mixtures using jar tests. In addition, the binary mixtures were applied to nine horticultural plant species to determine phytotoxicity based on visual appearance assessed 7 d after treatment. Bean-dip bioassays were performed in a laboratory using green bean (*Phaseolus vulgaris* L.) to determine LC_{50} values for each individual pesticide and the mixtures to establish whether the mixtures were synergistic, antagonistic, or there was no effect. The mortality of western flower thrips was assessed after 24 h, and LC_{50} values were calculated. Furthermore, semifield bioassays were performed in greenhouses for binary and tertiary mixtures to evaluate the efficacy (based on percent mortality) of the pesticide mixtures against western flower thrips. Results indicated that all binary mixtures were visibly compatible, and not phytotoxic to any of the plant species evaluated. Combination index calculations based on laboratory results indicated most of the binary mixtures were synergistic; however, the mixture containing spinosad + bifenazate appeared to be antagonistic against western flower thrips. The semifield bioassays demonstrated significantly reduced efficacy associated with mixtures containing azadirachtin, however, all binary mixtures provided ≈80% western flower thrips mortality.

KEY WORDS bioassays, synergism, antagonism, horticulture, pest management

Western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is an economically important insect pest of many horticultural crops grown in greenhouses worldwide (Robb and Parrella 1988, Robb et al. 1995, Tommasini and Maini 1995, Kirk 2002). They feed on all above ground plant parts causing both direct and indirect damage to plants (Ullman et al. 1993, Broadbent and Allen 1995, Mound 1996). Direct damage is caused when western flower thrips feed on plant cells, resulting in deformation of leaves and flowers, which makes plants unmarketable (Childers and Achor 1995, Cloyd and Lindquist 2001). Furthermore, indirect damage is caused when adults transmit the tospoviruses: impatiens necrotic spot virus (INSV) and/or tomato spotted wilt virus (TSWV) (Sether and DeAngelis 1992, Broadbent and Allen 1995, Daughtrey et al. 1997). Standards for greenhouse-grown crops are based primarily on aesthetics; thus, because of the potential of spreading viruses, the tolerance level for western flower thrips is near zero (Bethke and Cloyd 2009). Therefore, to mitigate populations of western flower thrips, greenhouse producers rely on insecticide applications (Parrella 1995, Brødsgaard and Albajes 1999). However, often times multiple arthropod (insect and/or mite) pest species including thrips, mealybugs, mites, aphids, whiteflies, and fungus gnats, are encountered simultaneously during a single cropping cycle (Bethke and Cloyd 2009). Therefore, using a variety of pesticides may be necessary to mitigate the multitude of arthropod pests that occur simultaneously in greenhouse environments because most newly registered pesticides have narrow-spectrum arthropod pest activity to comply with the Food Quality Protection Act (FQPA) standards (Sray 1997). Consequently, greenhouse producers apply pesticides as mixtures (Cloyd 2009).

A pesticide mixture is a combination of two or more pesticides into a single spray solution applied simultaneously (Brattsten et al. 1986, Roush 1993, O'Connor–Marer 2000, Cloyd 2011). Greenhouse producers apply pesticides as mixtures to reduce labor costs because fewer applications are required (Ca-

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bello and Canero 1994, O'Connor–Marer 2000, Cloyd 2009). In addition, pesticide mixtures may broaden the spectrum of pest activity; thus, mitigating populations of the multiple arthropod pests encountered in greenhouses simultaneously (Warnock and Cloyd 2005). Furthermore, there is a possibility of synergism occurring between the pesticides used in the mixture (Ware and Whitacre 2004, Warnock and Cloyd 2005, Cloyd et al. 2007). Synergism occurs when the toxicity of the pesticides used in the mixture is greater to the target pest when combined, compared with if the compounds were applied separately (Hewlett 1968, O'Connor–Marer 2000, Zhu 2004). In addition, pesticide mixtures have been recommended as a means of mitigating resistance as long as there is no cross-resistance (Brattsten et al. 1986; Roush 1989, 1993).

Despite the advantages, problems may occur when mixing pesticides including incompatibility, plant phytotoxicity, and antagonism (Cloyd 2001a). Pesticide incompatibility can cause problems for greenhouse producers by disrupting application equipment and inhibiting coverage. Incompatibility is evident when flakes, crystals, or clumps develop thus indicating the pesticides will not mix together uniformly (O'Connor–Marer 2000). Phytotoxicity or plant injury is another potential problem associated with pesticide mixtures, which may reduce crop marketability. Furthermore, antagonism occurs when the level of efficacy is reduced when pesticides are combined into a mixture (O'Connor–Marer 2000, Lindquist 2002). Despite these problems, greenhouse producers apply pesticide mixtures to manage arthropod pests although it is unclear if they provide any advantage compared with single components for western flower thrips control (Cloyd 2009). A study conducted by Warnock and Cloyd (2005) evaluated two-, three-, and four-way pesticide mixtures against western flower thrips under laboratory and greenhouse conditions. It was determined that mixtures containing spinosad, bifenazate, abamectin, imidacloprid, and azadirachtin had no antagonistic effects (based on percent mortality) when applied against adult western flower thrips. In addition, Cloyd et al. (2007) demonstrated that a number of pesticide mixtures provided ≥75% mortality of sweet potato whitefly B-biotype (*Bemisia tabaci* Gennadius [formally silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring]) nymphs 14 d after treatment, and ≥90% mortality of twospotted spider mite (*Tetranychus urticae* Koch) nymphs, 7 d after treatment under greenhouse conditions. These results indicate that the pesticide mixtures may not be antagonistic, based on percent mortality, against either arthropod pest. Furthermore, synergistic effects between carbamates and pyrethroids were observed in two field populations of western flower thrips with documented resistance to the pyrethroid, acrinathrin (Bielza et al. 2009). Based on these studies, pesticide mixtures applied under laboratory and greenhouse conditions may control western flower thrips populations without antagonism occurring. However, these studies were conducted without feedback from greenhouse producers on commonly used pesticide mixtures. Furthermore, no quantitative information associated with synergism and/or antagonism of widely used pesticide mixtures is available. Therefore, to obtain feedback from greenhouse producers on the most widely used pesticide mixtures, surveys were conducted twice in 2007 and once in 2008, requesting what pesticide mixtures are currently being applied in greenhouses (Cloyd 2009). Respondents indicated a wide-variety of pesticide mixtures including two-, three-, and four-way combinations (Cloyd 2009). Many of the binary mixtures reportedly used contained at least one pesticide either not registered or known to be not effective against adult western flower thrips.

Table 1. Commonly reported binary and tertiary pesticide mixtures including trade names and common names used in greenhouses (Cloyd 2009) that were evaluated in the pesticide compatibility, phytotoxicity, laboratory, and semi-field bioassays

Trade names[a]	Common names
Avid + Menace	Abamectin + bifenthrin
Avid + Conserve	Abamectin + spinosad
Avid + Azatin	Abamectin + azadirachtin
Avid + Ornazin	Abamectin + azadirachtin
Orthene + Tame	Acephate + fenpropathrin
Conserve + Endeavor	Spinosad + pymetrozine
Conserve + Pedestal	Spinosad + novaluron
Avid + Endeavor	Abamectin + pymetrozine
Conserve + Floramite	Spinosad + bifenazate
Abamectin + Orthene + Tame	Abamectin + acephate + fenpropathrin
Conserve + Floramite + Marathon II	Spinosad + bifenazate + imidacloprid
Orthene + Azatin + Tame	Acephate + azadirachtin + fenpropathrin

Tertiary mixtures were only evaluated in the semi-field bioassays.
[a] Company information: Avid (Syngenta Crop Protection Inc., Greensboro, NC); Azatin (OHP Inc., Mainland, PA); Conserve (Dow AgroSciences, LLC, Indianapolis, IN); Endeavor (Syngenta Crop Protection, Inc.); Floramite (OHP Inc.); Marathon II (OHP Inc.); Menace (Nufarm, Burr Ridge, IL); Ornazin (SePro Corp., Carmel, IN); Orthene (Valent U.S.A. Corporation, Walnut Creek, CA); Pedestal (OHP Inc.); and Tame (Valent U.S.A. Corporation).

The objectives of this study were to 1) examine compatibility and phytotoxicity of the most commonly used binary pesticide mixtures against western flower thrips based on survey results, 2) determine synergistic or antagonistic effects of the binary mixtures under laboratory conditions, and 3) evaluate the efficacy of currently used binary and tertiary pesticide mixtures against the western flower thrips under greenhouse conditions.

Materials and Methods

This study assessed efficacy of nine binary pesticide mixtures and three tertiary mixtures against western flower thrips in laboratory and greenhouse trials, respectively (Table 1). Voucher specimens of western flower thrips are deposited as accession number 223 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

Western Flower Thrips Colony. Laboratory colonies of western flower thrips were maintained on

Table 2. Common name, trade name, chemical class, labeled rates, rates used per 16 oz, percent active ingredient, and labeled rate (μg/ml) for each of the pesticides used in mixtures against the western flower thrips

Common name (abbreviation)	Trade name	Labeled rate per 100 gal	Rate per 16 oz[a]	Percent (%) active ingredient	Labeled rate (μg/ml)[b]
Abamectin (AB)	Avid	8.0 fl oz	0.30 ml	2.0	11.2
Acephate (A)	Orthene	10–2/3 oz	0.38 g	75.0	599.3
Azadirachtin [AZ(A)]	Azatin	16.0 fl oz	0.59 ml	3.0	39.7
Azadirachtin [AZ(O)]	Ornazin	8.0 fl oz	0.30 ml	3.0	20.2
Bifenazate (B)	Floramite	8.0 fl oz	0.30 ml	22.6	149.8
Bifenthrin (BI)	Menace	21.7 fl oz	0.80 ml	7.9	135.4
Fenpropathrin (F)	Tame	16.0 fl oz	0.59 ml	30.9	359.5
Imidacloprid (I)	Marathon II	1.7 fl oz	0.06 ml	21.4	31.8
Novaluron (N)	Pedestal	8.0 fl oz	0.30 ml	10.0	62.2
Pymetrozine (P)	Endeavor	5.0 oz	0.78 g	50.0	187.2
Spinosad (S)	Conserve	6.0 fl oz	0.22 ml	11.6	56.2
Tolfenpyrad (T)	Hachi–Hachi	22.0 fl oz	0.81 ml	15.0	257.4

[a] Rate used in pesticide incompatibility, phytotoxicity, and greenhouse experiments.
[b] Rate of each pesticide converted into μg/ml based off of the percent active ingredient in each formulation.

greenbean (*Phaseolus vulgaris* L.) under 24 ± 5°C, 50–60% relative humidity (RH), and a photoperiod of 14:10 (L:D) h in the Department of Entomology at Kansas State University (Manhattan, KS). These colonies had not been exposed to pesticides for at least 6 yr.

Pesticide Compatibility. Jar tests were performed to determine visual compatibility of the binary pesticide mixtures used in the laboratory and semifield bioassays. A procedure described by O'Connor–Marer (2000) was followed by mixing the pesticides in 500 ml Mason Ball (Broomfield, CO) jars. Each pesticide in the mixture was prepared using the highest labeled rate or the recommended rate for western flower thrips (Table 2). Each mixture remained in a controlled laboratory environment for ≈15 min. Compatibility was determined by visual observations.

Plant Phytotoxicity. Binary pesticide mixtures were prepared at the highest recommended labeled rates for western flower thrips, and for products not registered for western flower thrips, the highest recommended labeled rate was used (Table 2). Mixtures were applied to chrysanthemum (*Tanacetum grandiflorum* Thunberg), *Begonia* spp., *Petunia x hybrida* Hort. Wilm.-Andr., *Salvia* spp., *Tagetes* spp., *Impatiens* spp., *Vinca* spp., *Pansy* spp., and coleus (*Solenstemon scutellarioides* L. Codd.) plants to determine phytotoxicity of each binary mixture. All plants except chrysanthemum were planted into 10.2-cm containers using Fafard2 Mix growing medium (Agawam, MA) containing Canadian sphagnum peat moss (65%), perlite, vermiculite, starter nutrients, wetting agent, and dolomitic limestone. The leaves and flowers of each plant were sprayed using a 946 ml plastic spray bottle (The Home Depot, Manhattan, KS) with ≈15 ml of solution per plant. The chrysanthemum plants were potted into 15.2-cm containers using Fafard2 Mix growing medium and ≈63 ml of spray solution was applied per plant for each treatment. There were five replications per treatment and a water control was included. All plants were maintained in a greenhouse at Kansas State University (Manhattan, KS), and assessed visually for phytotoxicity 7 d after treatment.

Formulated Pesticide Bioassays. Bean-dip bioassays were conducted to determine LC_{50} values for the formulated pesticides containing abamectin (Avid: Syngenta Crop Protection Inc., Greensboro, NC); acephate (Orthene: Valent U.S.A. Corporation, Walnut Creek, CA); azadirachtin (Azatin: OHP Inc., Mainland, PA); azadirachtin (Ornazin: SePro Corp., Carmel, IN); bifenazate (Floramite: OHP Inc.); bifenthrin (Menace: Nufarm, Burr Ridge, IL); fenpropathrin (Tame: Valent U.S.A. Corporation); imidacloprid (Marathon II: OHP Inc.); novaluron (Pedestal: OHP Inc.); pymetrozine (Endeavor: Syngenta Crop Protection, Inc.); and spinosad (Conserve: Dow AgroSciences, LLC, Indianapolis, IN). Two azadirachtin formulations were evaluated because both products were reportedly used by greenhouse producers in pesticide mixtures according to the survey conducted by Cloyd (2009). Each pesticide was dissolved in deionized water and five serial dilutions were made using deionized water. Each experiment also included a control of deionized water. The control and each of five pesticide concentrations were repeated four times. Greenbeans were cut into 2-mm pieces, inserted into the designated solution for 10 s, and allowed to dry on FisherBrand (Pittsburgh, PA) qualitative-grade P8 9.0 cm circle filter paper. After drying, each greenbean slice was placed into a 7 ml glass vial. Then, ≈15, 7-d old adult female western flower thrips were counted and added to each vial. The vials were covered with Parafilm (Chicago, IL) with 50 holes in the top for ventilation. Vials were placed in an environmental growth chamber for 24 h at 25 ± 2°C and a photoperiod of 16:8 (L:D) h. Mortality was assessed by probing each individual with a needle and positioning the western flower thrips on the dorsal side. Those western flower thrips that did not move were considered dead.

Formulated Pesticide Mixture Bioassays. Bean-dip bioassays (as described previously) were used to determine LC_{50} values for each formulated binary pesticide mixture (Table 1). The procedure used was similar to the formulated pesticide experiment (described above); however, the pesticide mixtures were

prepared using a 1:1 volume ratio. The rate of each pesticide used in the mixture was based on the highest recommended labeled rates for western flower thrips, and for products not registered for western flower thrips, the highest recommended labeled rate was used. Concentrations of both pesticides were either increased or decreased proportionately to calculate an LC_{50} for each mixture. Each formulated pesticide was diluted with deionized water. Mortality was assessed similar to the previous laboratory experiment described above.

Semifield Bioassays. Semifield bioassays were conducted in greenhouses at Kansas State University to evaluate the efficacy of the formulated pesticides as well as the binary-and tertiary-pesticide mixtures (Table 1). There were three experiments with each consisting of individual pesticides and binary or tertiary mixtures. Experiment 1 consisted of individual pesticides and mixtures containing abamectin, azadirachtin, bifenthrin, pymetrozine, and spinosad. Experiment 2 consisted of individual pesticides and binary mixtures containing pymetrozine, bifenazate, spinosad, novaluron, acephate, and fenpropathrin. Experiment 3 consisted of individual pesticides and tertiary mixtures containing abamectin, azadirachtin, spinosad, bifenazate, imidacloprid, acephate, and fenpropathrin. In addition, a new pesticide, tolfenpyrad was evaluated in experiment 3. There were five replications per treatment for each experiment.

Yellow cut transvaal daisy (*Gerbera jamesonii* Bolus ex. Hook.f.) flowers were obtained from Koehler & Dramm of Missouri (Kansas City, MO). Each flower was cut ≈7.6 cm below the flower head and placed into a 22-mm glass vial containing tap water. Each vial was placed into a blue polypropylene container (250 ml) and surrounded with sand to ensure secure placement. Containers were placed on a wire-mesh greenhouse bench that had an open frame composed of polyvinyl chloride (PVC) pipe, which held a 50% black knit shade cloth (Hummert International, Earth City, MO) placed on top to protect the flowers from sunlight and preserve longevity.

After 2 d, ≈20 western flower thrips adults obtained from the laboratory-reared colonies were aspirated into vials, added to each flower, and allowed to establish for 2 d before pesticide applications. Pesticide treatments were mixed, with tap water, at the recommended labeled rates for western flower thrips, or for products not registered for western flower thrips, the highest labeled rate was used (Table 2). Applications were made using a 946 ml plastic spray bottle. Each flower received ≈15 ml of the designated spray solution, and after 5 d, western flower thrips mortality was assessed using destructive sampling. Efficacy of each pesticide and pesticide mixture was based on percent mortality of western flower thrips.

Evaluations of Pesticide Mixtures. Synergism or antagonism of the binary pesticide mixtures was evaluated based on combination index (CI) values as described by Chou and Talalay (1984). The following equation uses the LC_{50} values determined for the mixture and individual pesticide:

$$CI = \frac{LC_{50}^{1m}}{LC_{50}^{1}} + \frac{LC_{50}^{2m}}{LC_{50}^{2}} + \left(\frac{LC_{50}^{1m}}{LC_{50}^{1}} \times \frac{LC_{50}^{2m}}{LC_{50}^{2}}\right)$$

The numerator is the LC_{50} value for insecticides one and two used in the pesticide mixture. To obtain this value, a ratio was calculated by dividing LC_{50}^{1}, which is the LC_{50} value of the first pesticide in the mixture alone, by LC_{50}^{2}, which is the LC_{50} of the second pesticide in the mixture used alone. This ratio was factored into the LC_{50} value for the mixture to determine how much of the mixture was associated with each pesticide (Attique et al. 2006). The denominator is the LC_{50} value for each of the formulated pesticides when used individually. Based on the calculation, a CI value >1 indicates antagonism, < 1 synergism, and equal to one an additive effect.

Statistical Analysis. The LC_{50} values of individual formulations and mixtures of pesticides were calculated using a PROC PROBIT procedure (SAS Institute 2002). A Pearsons χ^2 value with $P > 0.05$ indicated no significant difference between the model and the observed regression lines. For the semifield bioassays, percent mortality was determined by dividing the number of dead western flower thrips per flower by the total number of western flower thrips recovered from each flower. For statistical purposes, percent mortality values among the treatments were transformed using an arcsine square-root transformation procedure and then analyzed using an analysis of variance (ANOVA) with treatment as the main effect. Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ was then used to identify significant differences among the treatments. In all cases, nontransformed data are presented.

Results

Pesticide Compatibility and Plant Phytotoxicity. Each of the nine binary pesticide mixtures displayed no visible signs of incompatibility. Furthermore, none of the nine binary pesticide mixtures were visibly phytotoxic to any of the horticultural plants tested.

Formulated Pesticide Bioassays. Results from the formulated pesticide bioassay using the bean-dip method are presented in Table 3. Ten individual pesticides were evaluated, with only four having a definitive LC_{50} value. Spinosad had the lowest LC_{50} value (0.44 μg/ml) indicating it was the most toxic to the baseline population of western flower thrips, followed by abamectin (148.8 μg/ml), acephate (720.9 μg/ml), and bifenthrin (1331.0 μg/ml). We were not able to obtain definitive LC_{50} values for the other pesticides because either there was no dose–response relationship or mortalities at the concentration of the maximum solubility was <50%. Therefore, the LC_{50} values for the pesticides were considered greater than the highest concentration tested. Low mortality (≤5%) was observed in the controls (vials treated with deionized water) indicating that the bean-dip method was appropriate for determining LC_{50} values.

Formulated Pesticide Mixture Bioassays. Results from the bean-dip pesticide mixture bioassays are pre-

Table 3. LC_{50} values, slope (± SEM), and P values for 10 formulated pesticides, and nine binary pesticide mixtures on western flower thrips in bean-dip laboratory bioassays; n = total no. of western flower thrips evaluated per treatment

Common name	n	Slope (± SEM)	$P > \chi^2$	LC_{50} μg/ml (95% CI)
Abamectin	300	0.72 (±0.19)	0.32[a]	148.8 (83.8, 609.2)
Acephate	299	4.02 (±0.54)	0.07	720.9 (603.8, 852.6)
Azadirachtin (Azatin)	303	0.13 (±0.42)	0.65	>634.0
Azadirachtin (Ornazin)	300	0.34 (±1.01)	0.83	>319.7
Bifenazate	303	0.08 (±0.44)	0.27	>2,396.5
Bifenthrin	312	2.14 (±0.26)	0.62	1,331.0 (1,100.0, 1639.0)
Fenpropathrin	296	0.74 (±0.41)	0.80	>5751.7
Novaluron	296	0.27 (±0.32)	0.84	>994.6
Pymetrozine	304	0.11 (±0.29)	0.36	>3,000.0
Spinosad	294	3.96 (±0.80)	0.06	0.44 (0.35, 0.54)
Abamectin + azadirachtin (Azatin)	297	0.57 (±0.18)	0.19	127.0 (69.2, 649.5)
Abamectin + azadirachtin (Ornazin)	305	0.83 (±0.18)	0.39	27.2 (12.7, 41.7)
Abamectin + bifenthrin	304	0.78 (±0.22)	0.09	157.6 (61.6, 282.4)
Abamectin + pymetrozine	298	0.28 (±0.19)	0.89	68.9 (53.4, 92.9)
Spinosad + abamectin	295	2.88 (±0.38)	0.07	0.37 (0.30, 0.46)
Spinosad + bifenazate	292	2.73 (±0.36)	0.20	1.79 (1.41, 2.14)
Spinosad + novaluron	301	3.24 (±0.39)	0.86	0.34 (0.30, 0.40)
Spinosad + pymetrozine	294	1.81 (±0.31)	0.35	0.38 (0.21, 0.53)
Acephate + fenpropathrin	295	2.71 (±0.42)	0.06	382.4 (301.3, 485.7)

There were four replications per treatment.
[a] $P > \chi^2 \geq 0.05$ indicates no significant difference between the observed regression line and the expected model.

sented in Table 3. Each mixture had a definitive LC_{50} value because they contained at least one pesticide that had an individual LC_{50} value. Those mixtures containing spinosad had the lowest LC_{50} values (≤1.79 μg/ml) indicating the highest toxicity to adult western flower thrips. Mixtures containing abamectin had a range of LC_{50} values from 27.2 μg/ml (abamectin + azadirachtin) to 157.6 μg/ml (abamectin + bifenthrin). The mixture containing acephate + fenpropathrin had the highest LC_{50} value (382.4 μg/ml) demonstrating it was the least toxic to adult western flower thrips. Calculations using the combination index equation (Chou and Talalay 1984) suggested that eight pesticide mixtures were synergistic and one (spinosad + bifenazate) was antagonistic (Table 4).

Semifield Bioassays. Results of the three semifield bioassays are presented in Figs. 1–3. For experiment 1, there were significant differences among the treatments ($F = 34.4$; df = 11, 48; $P \leq 0.0001$). Abamectin and spinosad when applied individually resulted in almost 100% mortality of western flower thrips. Although both azadirachtin (Azatin) and bifenthrin when applied individually had significantly higher western flower thrips mortality compared with the control, they were significantly less effective compared with abamectin and spinosad (Fig. 1). Mixtures of abamectin + azadirachtin (Azatin), abamectin + azadirachtin (Ornazin), and abamectin + bifenthrin had reduced efficacy compared with the other mixtures but still provided ≈80% western flower thrips mortality.

Results for experiment 2 are shown in Fig. 2. There was a significant difference among the treatments ($F = 78.74$; df = 10, 44; $P \leq 0.0001$). The individual pesticides spinosad and acephate, and the mixtures of spinosad + pymetrozine, spinosad + bifenazate, spi-

Table 4. Synergism and antagonism calculations for nine binary pesticide mixtures against laboratory-reared colonies of the western flower thrips based on a combination index (CI)

Mixture (common names)	$LC_{50}1$[a]	$LC_{50}2$[b]	Ratio[c]	$LC_{50}M$[d]	LC_{50} 1M[e]	LC_{50} 2M[f]	CI[g]
Abamectin + azadirachtin (Azatin)	148.8	634.0	0.23	127.0	102.9	24.1	0.76[h]
Abamectin + azadirachtin (Ornazin)	148.8	319.7	0.47	27.2	18.56	8.64	0.16[h]
Abamectin + bifenthrin	148.8	1331.0	0.11	157.6	141.75	15.85	0.98
Abamectin + pymetrozine	148.8	3000.0	0.05	68.9	65.64	3.26	0.44[h]
Spinosad + abamectin	0.44	148.8	0.003	0.37	0.37	0.001	0.84
Spinosad + bifenazate	0.44	2396.5	0.0002	1.79	1.79	0.0003	4.07[h]
Spinosad + novaluron	0.44	994.6	0.0004	0.34	0.347	0.0002	0.77[h]
Spinosad + pymetrozine	0.44	3000.0	0.0001	0.38	0.38	0.0001	0.86[h]
Acephate + fenpropathrin	720.9	5751.7	0.13	382.4	339.8	42.59	0.48[h]

Trade names of both azadirachtin products are in parentheses.
[a] $LC_{50}1$ = median lethal concn of the first pesticide alone in (μg/ml).
[b] $LC_{50}2$ = median lethal concn of the second pesticide alone (μg/ml).
[c] Ratio = $LC_{50}1/LC_{50}2$.
[d] $LC_{50}M$ = median lethal concn of the binary pesticide mixture (μg/ml).
[e] LC_{50} 1M = the part of the mixture attributed to $LC_{50}1$.
[f] LC_{50} 2M = the part of the mixture attributed to $LC_{50}2$.
[g] CI = combination index at LC_{50}.
[h] The combination indexes were estimated because one of the two pesticides in the mixtures did not provide a definitive LC_{50} value.

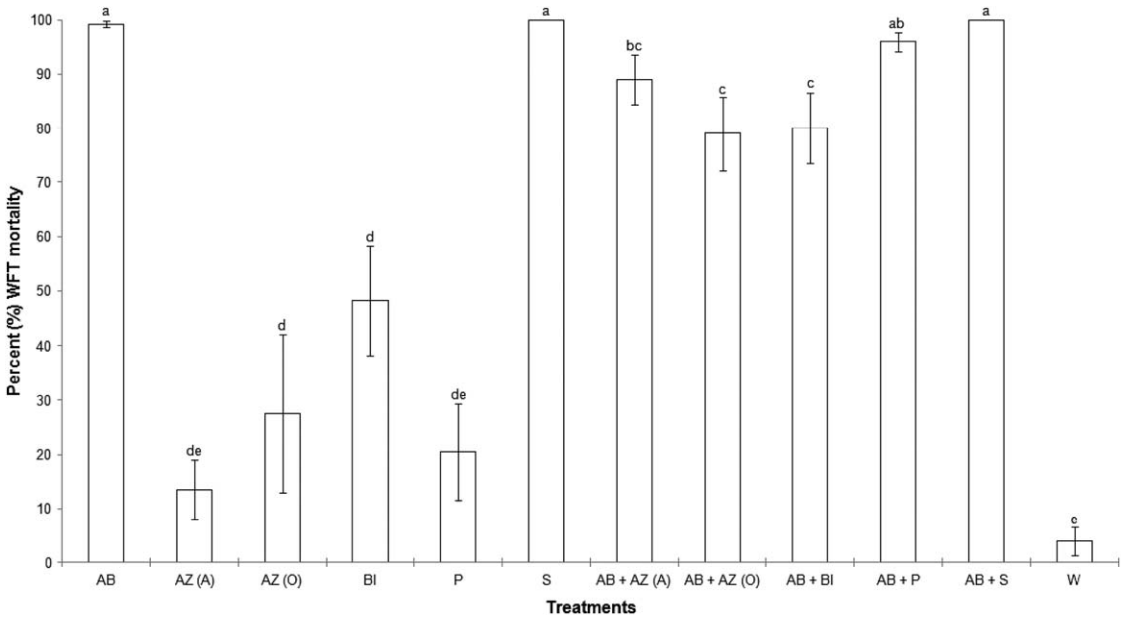

Fig. 1. Percent mortality of western flower thrips associated with six formulated pesticides and four pesticide mixtures applied as foliar sprays under greenhouse conditions. Bars with the same letter are not significantly different from each other ($P > 0.05$) based on Fisher's protected LSD mean separation test. Vertical lines indicate standard error of the mean (SEM). Treatment abbreviations are listed in Table 2.

nosad + novaluron, and acephate + fenpropathrin, all resulted in nearly 100% western flower thrips mortality. The remaining pesticides resulted in minimal western flower thrips mortality and were not significantly different from the water control (Fig. 2).

Results from experiment 3 are presented in Fig. 3. There was a significant difference among the treatments ($F = 56.45$; df = 11, 48; $P \leq 0.0001$). The individual pesticides abamectin, spinosad, acephate, and the three-way mixtures of spinosad + bife-

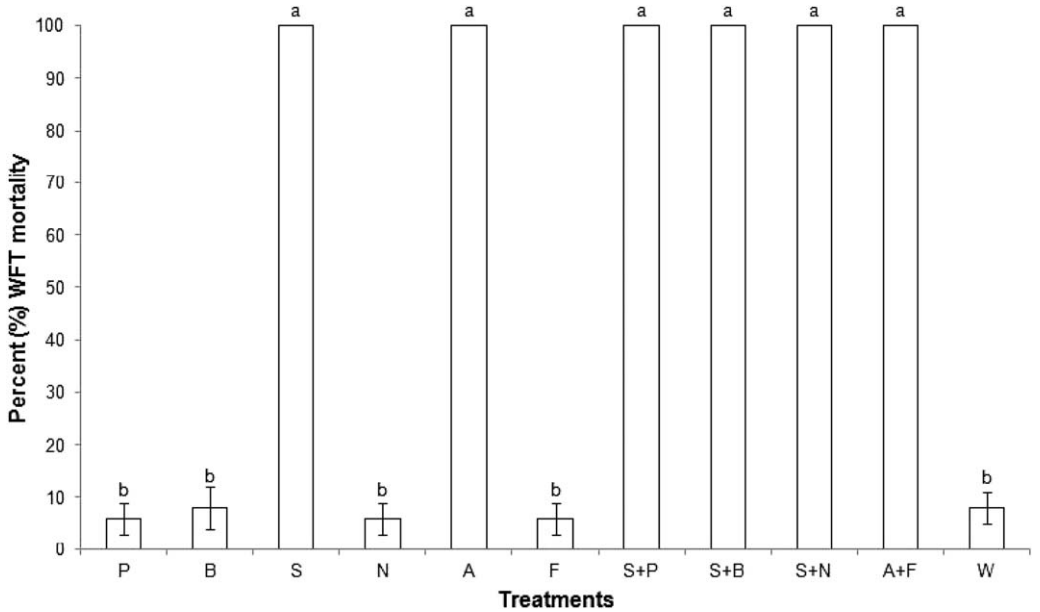

Fig. 2. Percent mortality of western flower thrips associated with six formulated pesticides and five pesticide mixtures applied as foliar sprays under greenhouse conditions. Bars with the same letter are not significantly different from each other ($P > 0.05$) based on Fisher's protected LSD mean separation test. Vertical lines indicate standard error of the mean (SEM). Treatment abbreviations are listed in Table 2.

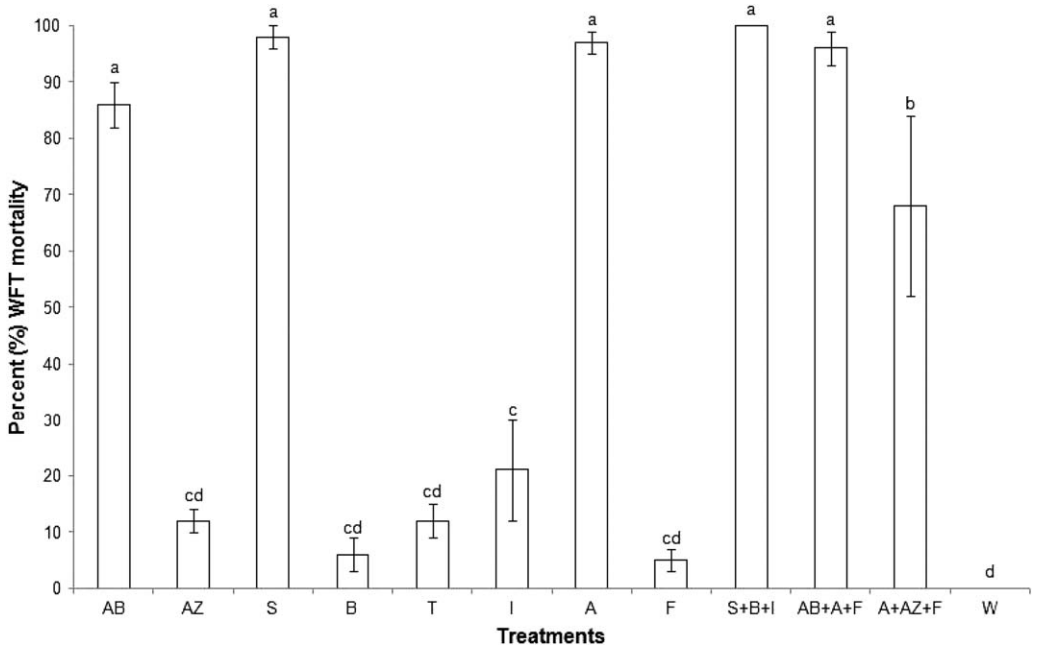

Fig. 3. Percent mortality of western flower thrips associated with eight formulated pesticides and three tertiary pesticide mixtures applied as foliar sprays under greenhouse conditions. Bars with the same letter are not significantly different from each other ($P > 0.05$) based on Fisher's protected LSD mean separation test. Vertical lines indicate standard error of the mean (SEM). Treatment abbreviations are listed in Table 2.

nazate + imidacloprid, and abamectin + acephate + fenpropathrin all provided ≥80% western flower thrips mortality. Azadirachtin (Azatin), bifenazate, tolfenpyrad, and fenpropathrin exhibited ≤15% western flower thrips mortality. Imidacloprid was significantly higher in western flower thrips mortality than the control although mortality was <25%.

Discussion

This study demonstrated that each of the binary mixtures were visibly compatible and showed no signs of phytotoxicity on the nine horticultural plant species evaluated. Information regarding pesticide mixtures that are safe to apply to plants is extremely valuable to greenhouse producers because if mixtures cause phytotoxicity then this negates their usefulness.

The CI has been widely used in pharmacology to determine toxicological interactions of drugs (Chou and Talalay 1984, Chou 2006). It has also been used to evaluate synergistic, additive, and antagonistic effects of pesticide mixtures in entomological research (Martin et al. 2003, Attique et al. 2006). In this study, we also used this technique to evaluate synergistic and/or antagonistic effects of pesticide mixtures used by greenhouse producers under laboratory conditions. Among the 10 pesticides evaluated using the bean-dip bioassay, only four (abamectin, acephate, bifenthrin, and spinosad) provided definitive LC_{50} values whereas the remaining six did not exhibit true LC_{50} values when bioassayed individually. Thus, the pesticide mixtures whose combination indexes can be reliably calculated were abamectin + bifenthrin and spinosad + abamectin. To provide information associated with synergism/antagonism trends of the pesticides that did not provide definitive LC_{50} values when they were evaluated individually, we included these mixtures in our calculations of the combination indexes as well. However, it should be noted that such data may indicate the synergism/antagonism trends when they are mixed, but the combination indexes calculated for these mixtures do not reflect their true values. In our study, except for the spinosad + bifenazate mixture, which showed an antagonistic effect, the remaining eight mixtures demonstrated synergistic effects (Table 4). Therefore, the trend associated with the synergistic effects of these pesticide mixtures will not change even for the mixtures whose individual pesticide bioassays did not provide definitive LC_{50} values simply because increasing the LC_{50} values of individual pesticides in the mixture will reduce the CI based on the equation provided in the Materials and Methods section.

Overall, our laboratory experiments using the individual formulated pesticide and formulated pesticide mixtures and our semifield bioassays demonstrated that spinosad, and those mixtures containing spinosad were the most toxic to susceptible populations of adult western flower thrips. These findings are similar to Warnock and Cloyd (2005) in which mixing spinosad with other pesticides did not affect the efficacy of spinosad against western flower thrips. However, the pesticide mixture of spinosad + bifenazate was considered antagonistic under laboratory conditions. The

reason there was no decrease in efficacy when using spinosad + bifenazate in the semifield bioassays, although antagonism was observed in the laboratory, was because the laboratory results evaluated LC_{50} values for western flower thrips whereas the semifield bioassays used the designated pesticide labeled rates, which were substantially higher than the LC_{50} value for spinosad (Table 2). Therefore, 100% mortality would be expected when susceptible populations of western flower thrips are exposed to mixtures containing spinosad. Spinosad is effective against susceptible populations of western flower thrips (Cloyd 2001b, Warnock and Cloyd 2005, Jones et al. 2005). However, spinosad resistance has been reported in field populations of western flower thrips (Loughner et al. 2005, Bielza et al. 2007). The western flower thrips population evaluated in this study was laboratory-reared; therefore, further research is necessary to determine the efficacy of pesticide mixtures against field populations of western flower thrips.

In both laboratory experiments, the mixtures of abamectin + azadirachtin (Azatin) and abamectin + azadirachtin (Ornazin) resulted in synergism, however, reduced efficacy was observed in the semifield bioassay when abamectin was combined with azadirachtin (Azatin or Ornazin) compared with abamectin alone. Reduced efficacy, when combining abamectin + azadirachtin has been observed against western flower thrips (Warnock and Cloyd 2005) and the beet armyworm (*Spodoptera exigua* Hübner) (Moar and Trumble 1987). The mechanism responsible for these antagonistic effects is currently unknown; however, it may be because of azadirachtin having repellent and antifeedant properties, which could reduce intake of the pesticides (Copping and Menn 2000).

The binary mixtures containing fenpropathrin + acephate were synergistic under laboratory conditions and there was no reduction in efficacy in the semifield bioassays. Synergism between pyrethroids and organophosphates has been observed when applied to a resistant strain of the cotton bollworm (*Helicoverpa armigera* Hübner) (Martin et al. 2003). The synergism between pyrethroids and organophosphates has been attributed to esterase inhibition, which prevents cleaving of the ester-linkage in pyrethroids; thus, allowing the pyrethroid to kill insect pests (Gaughan et al. 1980, Zhao et al. 1996, Martin et al. 2003).

Interestingly, when acephate + fenpropathrin were combined with azadirachtin (Azatin) in a tertiary mixture, there was significantly less western flower thrips mortality in the semifield bioassay. However, when acephate + fenpropathrin + abamectin were combined in a tertiary mixture, there was no reduced efficacy. As previously observed in the binary mixtures, pesticides combined with azadirachtin had reduced efficacy against western flower thrips. Further research is warranted to understand if the repellent and antifeedant properties of azadirachtin are responsible for reduced efficacy when azadirachtin is included in tertiary pyrethroid/organophosphate mixtures.

Active ingredients used in this study that were not effective against western flower thrips in the laboratory and semifield bioassays were azadirachtin, bifenazate, fenpropathrin, novaluron, and pymetrozine. This was expected as these pesticides are either insect growth regulators (IGRs) (novaluron and azadirachtin) (Yu 2008) or selective-feeding blockers (pymetrozine) (Harrewijn and Kayser 1997, Yu 2008), which would have minimal effect on western flower thrips adults. In addition, bifenazate and fenpropathrin are not registered for western flower thrips. However, when these pesticides were mixed with abamectin, spinosad, or acephate, which are registered for use against western flower thrips, the mixtures were effective. For instance, in all the semifield bioassays, >70% mortality was obtained.

In addition to the experiments presented above we also evaluated toxicity using technical grade material in glass residual bioassays (Willmott 2012). Although direct comparisons between glass residual bioassays and bean-dip bioassays may not be adequate it was interesting to note that there was no dose–response relationship observed using the technical grade abamectin; therefore, the LC_{50} value was considered >40,000 μg/ml. However, in the bean-dip bioassays we were able to calculate a LC_{50} value (148.8 μg/ml). This suggests that inert ingredients in the formulation such as butulated hydroxytoluene (BHT), n-methyl pyrrolidone, and mineral oil may be involved in enhancing mortality of western flower thrips. For instance, Stansly and Liu (1994) observed a toxic effect of mineral oil against the silverleaf whitefly, *B. argentifolii.*

In conclusion, this is the first study to demonstrate that the nine binary mixtures currently being used in greenhouses are visibly compatible and not phytotoxic when applied to a number of horticultural plants. Synergism and antagonism of binary pesticide mixtures were quantified using a CI associated with western flower thrips. Under laboratory conditions, eight of the nine most commonly used pesticide mixtures by greenhouse producers are synergistic. In addition, all nine of the evaluated binary pesticide mixtures provided >80% mortality of western flower thrips under greenhouse conditions. As such, eight of the binary pesticide mixtures may be used by greenhouse producers who are attempting to mitigate multiple arthropod pest populations simultaneously with no antagonistic effects against western flower thrips. The tertiary pesticide mixtures varied in regards to western flower thrips mortality under greenhouse conditions. Therefore, greenhouse producers should be cautious before applying tertiary mixtures of pesticides. In addition, future research is warranted to determine the efficacy of these mixtures against field populations of western flower thrips. Overall, this study will assist greenhouse producers interested in applying pesticide mixtures against western flower thrips populations.

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