Responses of *Liriomyza trifolii* (Diptera: Agromyzidae) to chemical and biorational insecticides

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Abstract

One of the most troublesome pests of snap beans is the American serpentine leafminer, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). Growers commonly use abamectin, spinosad, and cyromazine to manage *L. trifolii* populations; however, the biological insecticide azadirachtin and the fungus *Isaria fumosorosea* Wize offer promising alternatives. We tested the effectiveness of these five insecticides for controlling *L. trifolii* under field conditions in southern Florida. Abamectin and spinosad were generally the most effective for reducing *L. trifolii* mines, larvae, and/or pupae. Cyromazine and azadirachtin were less effective than abamectin or spinosad, although better than *I. fumosorosea* or the untreated control. *Isaria fumosorosea* was the least promising treatment; however, it still performed better than the control in reducing *L. trifolii* mines, larvae, and/or pupae. As pesticide effectiveness increased, differences in numbers of mines, larvae, and pupae appeared to be reduced among the 5 dates that pesticides were sprayed. Overall, mines appeared to be more effectively controlled 1 to 2 d after treatment than after 7 d, whereas larvae and pupae were controlled equally throughout the period. The pesticides employed can be classified into 3 general groups based on modes of action: abamectin and spinosad (disrupters of insect neural and muscular systems), cyromazine and azadirachtin (disrupters of molting), and *I. fumosorosea* (invades the insect, produces a toxin, and halts feeding). Pesticides with at least two modes of action were each able to provide effective control. Alternating pesticides may therefore control *L. trifolii* while limiting the development of resistance in *L. trifolii* populations.

Key Words: leafminer; mine; pesticide; spray date

Resumen

Un de los peores plagas de habichuelas es el minador serpentino Americano, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae). Frecuentemente, los productores utilizan abamectina, spinosad, y ciromazina para el control de *L. trifolii*. Sin embargo, azadiractina, una insecticida biológica, y el hongo, *Isaria fumosorosea* (Wize) AHS Br. Y G. Sm., pueden ofrecer alternativas eficaces. Pusimos a la prueba el rendimiento de todas estas insecticidas para el control de *L. trifolii* en el campo en el sur de Florida. Abamectina y spinosad fueron generalmente los más eficaces para la reducción de las minas, larvas, y/o pupas. Ciromazina y azadiractina aparecieron menos eficaz que abamectina o spinosad, pero más eficaz que *I. fumosorosea* o el control no tratado. *Isaria fumosorosea* fue el tratamiento menos eficaz, aunque más eficaz del control para la reducción de las minas, larvas, y/o pupas. Como la eficacia de pesticidas aumentó, las diferencias en los números de las minas, larvas, y pupas disminuyieron entre los 5 aerosoles. En general, las minas aparecieron controlado más eficaz a 1–2 d después de la rociada que después de 7 d, pero las larvas y pupas fueron controlados por igual en todo el período. Sobre la base de modos de acción, los plaguicidas se clasificaron en tres grupos generales: abamectina y spinosad (disruptores de sistemas neurales y musculares en insectos), ciromazina y azadiractina (interrumpen la muda), y *I. fumosorosea* (invade el insecto y detiene la alimentación). Cada de las pesticidas con modos de acción diferentes fué capaz de proporcionar un control eficaz. Alternando el uso de ellos puede controlar *L. trifolii* mientras illimitando el desarrollo de resistencia causada por el uso continuo de las pesticidas con un modo de acción único.

Palabras Clave: minador de la hoja; mina; plaguicida; fecha de rociar

Florida ranks first among states in the United States in total production of snap beans, *Phaseolus vulgaris* L. (Fabaceae), with 44% of the acreage, and Miami-Dade County ranks first among the 67 counties in Florida (Elwakil & Mossler 2012). The American serpentine leafminer, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), is one of the most problematic insect pest species attacking snap beans (Spencer 1965; Stegmaier 1966; Seal et al. 2002). It is a polyphagous fly that feeds on a large number of vegetable crops, and the adults and larvae cause considerable economic damage by making feeding punctures and leaf mines, respectively (Spencer 1981; Parrella et al. 1983; Seal et al. 2002). When adult females puncture the leaves to feed, they also oviposit in the punctures, whereas larvae damage the leaf mesophyll and reduce photosynthesis by mining the leaves (Schuster & Everett 1983; Parrella 1987). Although *L. trifolii* is considered a secondary pest of vegetable crops, it can become a primary pest in the absence of natural enemies.

To effectively manage *L. trifolii*, growers in southern Florida typically use abamectin and cyromazine, which are considered translaminar insecticides because they penetrate leaf surfaces (Weintraub 1999). Abamectin is a mixture of two avermectins (B1a and B1b), which are fermentation products of the soil bacterium *Streptomyces avermitilis* (ex Burg et al.) Kim and Goodfellow (Ananiev et al. 2002). Avermectins are classified in the IRAC group 6, or glutamate-gated chloride channel

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allosteric modulators (IRAC 2015), and they hinder neural and neuromuscular transmissions (Ananiev et al. 2002). Cyromazine, a cyclopropyl derivative of melamine, is in the IRAC group 17 (molting disruptors) and functions as a dipteran triazine insect growth regulator (El-Oshar et al. 1985; IRAC 2015).

Other reduced-risk insecticides that demonstrate potential for managing *L. trifolii* populations include azadirachtin, spinosad, and entomopathogenic fungi. Azadirachtin is a botanical insecticide from the neem tree, *Azadirachta indica* A. Juss. (Meliaceae); it is an insect growth regulator that prevents molting and hence is effective on immature insects (Koul 1999). Azadirachtin is in the IRAC group "UN," which are compounds of unknown or uncertain modes of action (IRAC 2015).

Alternatively, spinosad is a biological insecticide that comprises a mixture of spinosyn A and spinosyn D, which are fermented from the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Salgado 1998). Spinosad is in the IRAC group 5, or nicotinic acetylcholine receptor allosteric modulators (IRAC 2015). It enters the insect through contact or ingestion and affects the nervous system by acting on nicotinic acetylcholine receptors (Salgado 1998). The naturally occurring entomopathogenic fungus *Isaria fumosorosea* Wize is effective against all life stages of insects (Zimmermann 2008). Germinating spores produce hyphae that grow into the insect and release a toxin, which leads to reduced feeding and death (Zimmermann 2008).

Insecticide applications usually begin after mines are observed in the field, and they continue on a calendar basis every 10 to 14 d until the crops are harvested. However, continuous use of insecticides can lead to the development of resistance resulting in ineffective treatment against *L. trifolii* (Leibee 1981; Keil & Parrella 1983). Leibee & Capinera (1995) reported that some strains of *L. trifolii* are highly resistant to cyromazine, which Ferguson (2004) supported while noting that some strains also show resistance to abamectin and spinosad. These insecticides were among the most effective for managing *L. trifolii* populations. However, factors such as the short pest generation time and repeated application of pesticides with similar modes of action may have aided the selection of resistant genotypes.

Hence, the present study re-evaluated 3 industry standards (abamectin, spinosad, and cyromazine) and 2 potential alternatives, including a botanical insecticide (azadirachtin) and an entomopathogenic fungus (*I. fumosorosea*), each having a different mode of action. The objective was to determine the effectiveness of each insecticide in reducing the number of *L. trifolii* mines, larvae, and pupae and to determine the duration and extent of residual activity after each application. Pest control recommendations were made based on the findings.

Materials and Methods

The study was conducted at the Tropical Research and Education Center, Homestead, Florida, from 15 Oct to 1 Dec 2014 (48 d).

ENVIRONMENTAL CONDITIONS

At 60 cm above ground level, the Florida Automated Weather Network (FAWN) station at Homestead recorded mean monthly temperatures (minimum–maximum in parentheses) as 24.4 °C (14–34 °C), 20.6 °C (8–31 °C), and 19.4 °C (3–29 °C), for Oct, Nov, and Dec 2014, respectively. Relative humidity averaged 83, 82, and 84% for Oct, Nov, and Dec 2014, respectively (FAWN 2014).

FIELD PREPARATION AND EXPERIMENTAL DESIGN

The soil type was Krome gravelly loam (loamy-skeletal, carbonatic, hypothermic, lithic, udorthents), which had a pH of 7.4 to 8.4, was 34 to 76% limestone pebbles (>2 mm diameter), well drained, and had a low organic matter content (<2%) (Nobel et al. 1996; Li 2001). The field was 84 × 30 m and included 12 beds, each 84 × 0.9 m. Additional bare row spaces of 84 × 0.9 m separated beds within plots resulting in bed centers separated by 1.8 m and raised 15 cm. Each treatment plot included 3 parallel bed sections; thus, when including intervening row space, each plot was 8 m long, 4.5 m wide, and there was a buffer zone 1.5 m wide of non-planted bed space between plots. Treatment plots were arranged in a randomized complete block design with 4 replications.

Twenty-one days before planting, the herbicide halosulfuron methyl (Sandea®, Gowan Co., Yuma, Arizona) was applied at 51.9 g/ha to control the emergence of weeds. Granular fertilizer (N-P-K: 6-12-12) was applied at 1,345 kg/ha in a 10 cm wide band on each side of the bed centers and incorporated before placement of plastic mulch. Before planting seeds, the beds were covered with black-and-white polyethylene mulch (1.5 mil thick) with the white side facing upwards (Grower's Solution Co., Cookeville, Tennessee). Holes (13 cm diameter) were cut into the plastic and spaced 25 cm apart within the row. Then, on 15 Oct 2014, 2 to 3 seeds of snap beans (*P. vulgaris* 'Prevail') were directly planted 1.5 cm deep and later thinned to 2 plants per hole.

Plants were irrigated with the equivalent of 2.5 cm of precipitation delivered twice daily through 2 parallel drip-tube lines (T-systems, DripWorks, Inc., Willits, California). Liquid fertilizer (N-P-K: 4-0-8) was applied at 0.56 kg of N/ha/d through the drip-tube system at 3, 4, and 5 wk after planting. The fungicides chlorothalonil (Bravo®, Syngenta Inc., Greensboro, North Carolina) at 1.75 L/ha and copper hydroxide (Kocide® 3000, BASF Ag Products, Research Triangle Park, North Carolina) at 0.8 L/ha were sprayed every 2 wk to prevent fungal disease.

All treatment insecticides were sprayed weekly for 5 wk (12, 19, 26, 33, and 40 d after planting) using a CO_2 backpack sprayer with 2 nozzles delivering 234 L/ha at 172 kpa (kilopascals per acre). The 6 treatments included 3 industry standards 1) abamectin (Agri-Mek®, Syngenta Crop Protection LLC, Greensboro, North Carolina) at 9.7 g ai/ha; 2) spinosad (SpinTor®, Dow AgroSciences LLC, Indianapolis, Indiana) at 176 g ai/ha; and 3) cyromazine (Trigard®, Syngenta Crop Protection LLC, Greensboro, North Carolina) at 140 g ai/ha; and 2 potential alternatives: 4) azadirachtin (Neemix®, Certis USA LLC, Columbia, Maryland) at 20.8 g ai/ha; and 5) *I. fumosorosea* Apopka strain (PFR-97™, Certis USA LLC, Columbia, Maryland) at 448 g ai/ha. These treatments were compared with 6) an untreated control.

DATA COLLECTION

Leaflet samples were collected 1, 2, 5, and 7 d after each of the 5 pesticide applications, and the numbers of mines, larvae, and pupae were recorded. Sample collection involved randomly removing 5 leaflets (1 per plant) from the middle 6 m of the middle row of each treatment plot. Leaflets collected from each plot were placed into a 1L (10 × 15 cm), closed plastic bag, brought back to the laboratory, and kept at 24 ± 1.5 °C, 60 ± 10% RH, and a 14:10 h L:D photoperiod. Numbers of mines and larvae were recorded for each 5-leaflet sample, which was monitored daily until all the larvae molted into pupae; pupal numbers were also recorded. *Liriomyza trifolii* pupae were kept in a Petri dish (6 cm diameter) with moist filter paper on the bottom to avoid desiccation and observed daily to record numbers of emerging adults.

STATISTICAL ANALYSES

As described in the Discussion section, repeated measures ANOVAs were not appropriate because in addition to the pesticide treatments, there were 2 types of time intervals: individual sprays (time intervals after planting) and time lengths after each spray. Factorial analyses were initially performed and tested for interaction between 6 treatments, 5

spray dates, and 4 sample days following each spray date (SAS Institute 2014). Hence, there were three 2-way factorials: treatment × spray date (sample days pooled), treatment × sample day (spray dates pooled), and spray date × sample day (treatments pooled). Treatment × spray date analyses were followed by 1-way ANOVAs for interaction, whereas analyses of treatment × sample day and spray date × sample day were subjected to no-interaction 1-way ANOVAs. To determine the duration and extent of residual pesticide action on *L. trifolii* populations after each treatment, 1-way ANOVAs were used to compare means for sample days after each spray date. All 1-way ANOVAs were followed by mean separation using Waller–Duncan *K*-ratio *t*-tests ($\alpha < 0.05$; SAS Institute 2014).

Results

There were significant interactions ($P \leq 0.05$) between treatment and spray date for numbers of mines, larvae, and pupae per 5-leaflet sample ($F = 6.66$, df = 20, $P \leq 0.0001$; $F = 3.28$, df = 20, $P \leq 0.0001$; and $F = 7.76$, df = 20, $P \leq 0.0001$; respectively). However, there were no significant interactions for treatment × sample day or spray date × sample day when considering the data for mines, larvae, or pupae.

COMPARING SPRAY DATES WITHIN TREATMENTS AND TREATMENTS WITHIN SPRAY DATES (FROM A FACTORIAL ANALYSIS WITH INTERACTIONS)

Because there were significant interactions between spray date and treatment for numbers of mines, larvae, and pupae, two separate analyses were performed for these data sets: one for spray dates within treatments (Table 1) and another for treatments within spray dates (Table 2).

Spray Dates within Treatments

There were no significant differences between spray dates in the number of *L. trifolii* mines, larvae, or pupae for the abamectin treat-

Table 1. Mean numbers (± SD) of *Liriomyza trifolii* mines, larvae, and pupae per 5 bean leaflets. Data for individual spray dates were compared within treatments of chemical and biological insecticides and resulted from a factorial analysis with interaction.

Treatment	Spray date no.	Mines [a, b]	Larvae [a, b]	Pupae [a, b]
Abamectin	1	0.13 ± 0.34	0.00 ± 0.00	0.00 ± 0.00
	2	0.19 ± 0.54	0.00 ± 0.00	0.00 ± 0.00
	3	0.38 ± 0.89	0.19 ± 0.38	0.13 ± 0.50
	4	0.19 ± 0.54	0.00 ± 0.00	0.00 ± 0.00
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	P	NS	NS	NS
Spinosad	1	0.19 ± 0.54 b	0.06 ± 0.13	0.00 ± 0.00
	2	0.25 ± 0.77 b	0.00 ± 0.00	0.00 ± 0.00
	3	1.13 ± 1.82 a	0.13 ± 0.25	0.00 ± 0.00
	4	0.44 ± 0.81 ab	0.00 ± 0.00	0.00 ± 0.00
	5	0.19 ± 0.54 b	0.00 ± 0.00	0.00 ± 0.00
	P	0.0450	NS	NS
Cyromazine	1	3.44 ± 5.06 bc	3.06 ± 4.36	2.56 ± 4.03 a
	2	3.94 ± 2.54 b	1.31 ± 1.60	0.00 ± 0.00 b
	3	7.94 ± 4.22 a	1.31 ± 1.33	0.00 ± 0.00 b
	4	3.69 ± 2.91 b	0.63 ± 0.83	0.25 ± 1.00 b
	5	1.38 ± 0.81 c	0.00 ± 0.00	0.00 ± 0.00 b
	P	<0.0001	NS	<0.0001
Azadirachtin	1	0.44 ± 0.89 c	0.38 ± 0.48 b	0.19 ± 0.40 bc
	2	7.44 ± 8.41 b	3.13 ± 2.45 a	1.63 ± 4.80 abc
	3	9.69 ± 2.65 a	2.00 ± 1.10 a	0.19 ± 0.54 c
	4	5.63 ± 4.03 b	2.31 ± 0.85 a	1.75 ± 1.48 a
	5	4.44 ± 2.13 b	2.19 ± 0.97 a	1.19 ± 1.38 ab
	P	<0.0001	0.0208	0.0067
I. fumosorosea	1	3.13 ± 4.47 c	2.13 ± 3.07 b	1.44 ± 2.16 c
	2	11.56 ± 7.24 ab	4.94 ± 1.76 ab	2.13 ± 2.83 c
	3	13.50 ± 6.63 a	10.81 ± 5.21 a	7.69 ± 6.09 a
	4	10.75 ± 7.48 ab	6.50 ± 1.95 a	4.56 ± 4.32 b
	5	7.63 ± 3.30 b	4.81 ± 1.84 ab	2.44 ± 2.06 bc
	P	<0.0001	0.0169	<0.0001
Control	1	5.06 ± 7.22 d	4.44 ± 6.62 c	3.94 ± 5.25 b
	2	26.94 ± 20.72 a	20.01 ± 5.94 a	14.69 ± 16.05 a
	3	22.50 ± 9.39 a	13.06 ± 5.55 ab	11.00 ± 6.07 a
	4	13.94 ± 6.34 b	7.31 ± 3.40 bc	4.56 ± 2.78 b
	5	7.81 ± 4.00 c	5.25 ± 2.99 bc	2.94 ± 2.86 b
	P	<0.0001	0.0063	<0.0001

[a]Data were transformed by ($\sqrt{x} + 0.25$) before statistical analysis, but only non-transformed means and standard deviations (SDs) are shown.

[b]Means within a column followed by the same letter or no letter did not differ significantly based on analyses of variance followed by Waller–Duncan *K*-ratio *t*-tests ($P \leq 0.05$; NS = non-significant; SAS institute 2014).

Table 2. Mean numbers (± SD) of *Liriomyza trifolii* mines, larvae, and pupae per 5 bean leaflets treated with chemical and biological insecticides. Here, data for treatments were compared within spray dates based on results of a factorial with interaction.

Spray date no.	Treatment	Mines [a, b]	Larvae [a, b]	Pupae [a, b]
1	Abamectin	0.13 ± 0.34 b	0.00 ± 0.00	0.00 ± 0.00 d
	Spinosad	0.19 ± 0.54 b	0.06 ± 0.13	0.00 ± 0.00 d
	Cyromazine	3.44 ± 5.06 a	3.06 ± 4.36	2.56 ± 4.03 ab
	Azadirachtin	0.44 ± 0.89 b	0.38 ± 0.48	0.19 ± 0.40 cd
	I. fumosorosea	3.13 ± 4.47 a	2.13 ± 3.07	1.44 ± 2.16 bc
	Control	5.06 ± 7.22 a	4.44 ± 6.62	3.94 ± 5.25 a
	P	0.0005	NS	<0.0001
2	Abamectin	0.19 ± 0.54 d	0.00 ± 0.00 d	0.00 ± 0.00 c
	Spinosad	0.25 ± 0.77 d	0.00 ± 0.00 d	0.00 ± 0.00 c
	Cyromazine	3.94 ± 2.54 c	1.31 ± 1.60 cd	0.00 ± 0.00 c
	Azadirachtin	7.44 ± 8.41 c	3.13 ± 2.45 bc	1.63 ± 4.80 bc
	I. fumosorosea	11.56 ± 7.24 b	4.94 ± 1.76 b	2.13 ± 2.83 b
	Control	26.94 ± 20.72 a	20.01 ± 5.94 a	14.69 ± 16.05 a
	P	<0.0001	<0.0001	<0.0001
3	Abamectin	0.38 ± 0.89 d	0.19 ± 0.38 c	0.13 ± 0.50 c
	Spinosad	1.13 ± 1.82 d	0.13 ± 0.25 c	0.00 ± 0.00 c
	Cyromazine	7.94 ± 4.22 c	1.31 ± 1.33 bc	0.00 ± 0.00 c
	Azadirachtin	9.69 ± 2.65 c	2.00 ± 1.10 b	0.19 ± 0.54 c
	I. fumosorosea	13.50 ± 6.63 b	10.81 ± 5.21 a	7.69 ± 6.09 b
	Control	22.50 ± 9.39 a	13.06 ± 5.55 a	11.00 ± 6.07 a
	P	<0.0001	<0.0001	<0.0001
4	Abamectin	0.19 ± 0.54 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	Spinosad	0.44 ± 0.81 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	Cyromazine	3.69 ± 2.91 c	0.63 ± 0.83 c	0.25 ± 1.00 c
	Azadirachtin	5.63 ± 4.03 c	2.31 ± 0.85 b	1.75 ± 1.48 b
	I. fumosorosea	10.75 ± 7.48 b	6.50 ± 1.95 a	4.56 ± 4.32 a
	Control	13.94 ± 6.34 a	7.31 ± 3.40 a	4.56 ± 2.78 a
	P	<0.0001	<0.0001	<0.0001
5	Abamectin	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	Spinosad	0.19 ± 0.54 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	Cyromazine	1.38 ± 0.81 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	Azadirachtin	4.44 ± 2.13 b	2.19 ± 0.97 b	1.19 ± 1.38 b
	I. fumosorosea	7.63 ± 3.30 a	4.81 ± 1.84 a	2.44 ± 2.06 a
	Control	7.81 ± 4.00 a	5.25 ± 2.99 a	2.94 ± 2.86 a
	P	<0.0001	<0.0001	<0.0001

[a] Data were transformed by (v*x* + 0.25) before statistical analysis, but only non-transformed means and standard deviations (SDs) are shown.

[b] Means within a column followed by the same letter or no letter did not differ significantly based on analyses of variance followed by Waller–Duncan *K*-ratio *t*-tests ($P \geq 0.05$; NS = non-significant; SAS Institute 2014).

ment, larvae or pupae for spinosad, or larvae for cyromazine, but all the other treatments and data showed significant differences (Table 1). Spinosad resulted in significantly more *L. trifolii* mines after spray date 3 than after spray dates 1, 2, or 5, with spray date 4 statistically the same as the others. Treatment with cyromazine led to significantly more *L. trifolii* mines after spray date 3 than after all other spray dates. In turn, cyromazine spray date 5 had significantly fewer mines than spray dates 2, 3, and 4. However, cyromazine yielded significantly more *L. trifolii* pupae after spray date 1 than after the other spray dates.

Treatment with azadirachtin yielded significantly more *L. trifolii* mines following spray date 3 than after all the other spray dates, and spray dates 2, 4, and 5 each had significantly more mines than spray date 1. But spray date 1 for azadirachtin resulted in significantly fewer mines and larvae than all the other spray dates. Significantly fewer *L. trifolii* pupae were found on plants treated with azadirachtin following spray date 3 than spray dates 4 or 5, and spray date 4 yielded significantly more pupae than spray dates 1 or 3.

Treatment with *I. fumosorosea* led to significantly more *L. trifolii* mines after spray date 3 compared with spray dates 1 or 5. Spray date 5 yielded significantly more mines than spray date 1, which had significantly fewer mines than all the other spray dates. Spray date 1 with *I. fumosorosea* also resulted in the numerically lowest number of larvae with the numbers significantly lower than after spray dates 3 or 4. Similarly, treatment with *I. fumosorosea* led to significantly more *L. trifolii* pupae after spray date 3 than after all the other spray dates, whereas spray date 4 had significantly more than spray dates 1 or 2.

Considering the untreated control, the numerically highest numbers of *L. trifolii* mines, larvae, and pupae occurred after spray dates 2 and 3. Spray dates 2 and 3 each led to significantly more mines than all the other spray dates, and spray date 4 yielded significantly more mines than spray date 5, which had significantly more than spray date 1. The numerically lowest number of *L. trifolii* larvae for the control treatment occurred after spray date 1, with significantly fewer than after spray dates 2 or 3; and significantly fewer larvae were found following spray dates 1, 4, or 5 than after spray date 2. Numbers of *L. trifolii*

pupae for the untreated control followed a similar pattern with significantly more following spray dates 2 or 3 than spray dates 1, 4, or 5.

Treatments within Spray Dates

Spray date 1 yielded significant differences in numbers of mines and pupae between treatments, but non-significant differences for larvae (Table 2). All the other spray dates resulted in significant differences in numbers of mines, larvae, and pupae among treatments. Following spray date 1, significantly more mines were found in treatments of cyromazine, *I. fumosorosea,* and the control than azadirachtin, spinosad, or abamectin. Spray date 1 also yielded significantly more pupae from the control treatment than from *I. fumosorosea*, which had significantly more than from abamectin or spinosad. Abamectin, spinosad, and azadirachtin, in turn, yielded significantly fewer pupae than cyromazine or the control after spray date 1.

Numbers of mines following spray dates 2, 3, and 4 yielded the same statistical distribution among treatments: the untreated control led to significantly more mines than *I. fumosorosea*, which yielded significantly more mines than azadirachtin or cyromazine, which had significantly more than abamectin or spinosad.

After spray date 2, larvae and pupae each were found in significantly greater numbers in the control than in the *I. fumosorosea* treatment*,* which had significantly more than from treatments of cyromazine, abamectin, or spinosad. Also after spray date 2, the numbers of larvae and pupae in azadirachtin plots were significantly lower than in the control treatment but were statistically the same as from *I. fumosorosea* or cyromazine. Azadirachtin for spray date 2 also resulted in significantly more larvae than abamectin or spinosad, but statistically the same number of pupae as abamectin, spinosad, and cyromazine.

After spray date 3, numbers of larvae were significantly greater for *I. fumosorosea* and the control than for all the other treatments, and azadirachtin yielded significantly more larvae than abamectin or spinosad. Similarly, pupae after spray date 3 were found in significantly greater numbers in the control than in *I. fumosorosea* treatments*,* which had significantly more than the remaining 4 treatments.

Numbers of larvae and pupae following spray dates 4 and 5 all had the same distributions among treatments: the control and *I. fumosorosea* each yielded significantly more larvae or pupae than azadirachtin, which had significantly more than cyromazine, spinosad, or abamectin. Spray date 5 led to significantly more mines from *I. fumosorosea* or the control than from all the other treatments. In turn, azadirachtin yielded significantly more mines than cyromazine, which had significantly more than abamectin or spinosad.

COMPARING SPRAY DATES, SAMPLE DAYS, AND TREATMENTS BASED ON FACTORIAL ANALYSES WITH NO INTERACTION

A comparison of the 5 spray dates for numbers of mines, larvae, and pupae (sample days and treatments pooled) found significant differences between spray dates in numbers of mines and pupae, but not larvae (Table 3). Significantly more mines occurred following spray date 3 than spray date 4, which yielded significantly more than spray date 5, which in turn had significantly more than spray date 1. The number of mines following spray date 2 was statistically the same as those after spray dates 3 and 4, but was significantly greater than after spray dates 1 or 5. There were significantly more pupae following spray date 3 than spray dates 1 or 5, whereas spray dates 2 and 4 yielded statistically the same number of pupae as all the other spray dates.

Considering individual sample days after spraying (spray dates and treatments pooled), 2 identical data sets were produced by 2 factorials with no-interaction results: spray date × sample day and treatment × sample day. Therefore, results of these 2 factorials comparing sample days after spraying yielded the same numbers and statistical distributions of

Table 3. Mean numbers (± SD) of *Liriomyza trifolii* mines, larvae, and pupae per sample of 5 bean leaflets treated with chemical and biological insecticides. Data for spray date, sample day, and treatment were compared based on factorials yielding no interaction: spray date × sample day and treatment × sample day.

Factor	Level	Mines [a, b]	Larvae [a, b]	Pupae [a, b]
Spray date no. [c, d]	1	2.06 ± 4.40 d	1.68 ± 3.52	1.35 ± 3.15 b
	2	8.39 ± 13.19 ab	4.90 ± 7.55	3.07 ± 8.58 ab
	3	9.19 ± 9.10 a	4.58 ± 6.09	3.17 ± 5.65 a
	4	5.77 ± 6.74 b	2.79 ± 3.42	1.85 ± 2.96 ab
	5	3.57 ± 3.99 c	2.04 ± 2.65	1.09 ± 1.94 b
	P	<0.0001	NS	0.0438
Sample days after spraying [c, e, f]	1	5.17 ± 9.89 b	2.43 ± 5.02	1.83 ± 6.44
	2	4.90 ± 7.29 b	3.17 ± 4.90	1.70 ± 4.02
	5	6.20 ± 8.09 ab	3.18 ± 4.91	2.18 ± 4.36
	7	6.92 ± 8.96 a	4.02 ± 5.64	2.73 ± 5.27
	P	0.0283	NS	NS
Treatment [e, g]	Abamectin	0.18 ± 0.55 d	0.04 ± 0.17 d	0.03 ± 0.22 d
	Spinosad	0.44 ± 1.05 d	0.04 ± 0.12 d	0.00 ± 0.00 d
	Cyromazine	4.08 ± 3.98 c	1.26 ± 2.21 c	0.56 ± 2.07 cd
	Azadirachtin	5.53 ± 5.35 c	2.00 ± 1.51 c	0.99 ± 2.39 c
	I. fumosorosea	9.31 ± 6.94 b	5.84 ± 4.00 b	3.65 ± 4.36 b
	Control	15.25 ± 13.77 a	10.02 ± 7.51 a	7.43 ± 9.25 a
	P	<0.0001	<0.0001	<0.0001

[a]Data were transformed by (V*x* + 0.25) before statistical analyses, but only non-transformed means and standard deviations (SDs) are shown.

[b]Means within a column followed by the same letter or no letter did not differ significantly based on analyses of variance followed by Waller–Duncan *K*-ratio *t*-tests ($P \leq 0.05$; NS = non-significant; SAS Institute 2014).

[c]Based on spray date × sample day factorial results.

[d]Sample days and treatments were pooled.

[e]Based on treatment × sample day factorial results.

[f]Spray dates and treatments were pooled.

[g]Sample days and spray dates were pooled.

mines, larvae, and pupae. The factorials resulted in data for "sample days after spraying" (Table 3) showing that significant differences occurred for numbers of mines, but not larvae or pupae. Hence, there were significantly more mines 7 d after spraying than after 1 or 2 d with sample day 5 statistically the same as the other 3 sample days.

When comparing the 6 treatments with sample days and spray dates pooled, there were highly significant differences for numbers of mines, larvae, and pupae (Table 3). Distributions in numbers of mines and larvae among the 6 treatments (Table 3) were statistically the same as mine distributions among treatments following each of spray dates 2, 3, and 4 (Table 2), in which only sample days were pooled. For mines (Table 2, spray dates 2, 3, and 4) and for mines and larvae (Table 3, treatments), the control treatment led to significantly more mines and/or larvae than *I. fumosorosea*, which yielded significantly more than azadirachtin or cyromazine, which had significantly more than spinosad or abamectin. Pupal distributions were similar, with the control treatment having significantly more than *I. fumosorosea*, which yielded significantly more than azadirachtin, which had significantly more than abamectin or spinosad. In turn, the abamectin, spinosad, cyromazine, and azadirachtin treatments each led to significantly fewer pupae than *I. fumosorosea* or the control (Table 3).

RESIDUAL EFFECTS OF PESTICIDES

Among sample days within each treatment (pooled spray dates), significant differences occurred in numbers of mines for plants treated with abamectin and spinosad and numbers of pupae for treatments with cyromazine and *I. fumosorosea* (Table 4). However, there were no significant differences in numbers of larvae among sample days in any treatment, mines or pupae from azadirachtin or the control, mines from the cyromazine or *I. fumosorea* treatments, or pupae from abamectin or spinosad. Treating plants with abamectin significantly increased the number of mines on sample day 5 compared with sample days 1, 2, or 7. However, significantly more mines from spinosad and pupae from the cyromazine and *I. fumosorosea* treatments were found 7 d after spraying than after 1 or 2 d, with sample day 5 statistically the same as the other 3 sample days.

Discussion

To consider multiple applications of sprays often applied in the field and to test the objectives of this study, we compared not only treatments but also time lengths after each treatment and results for individual sprays. Hence, the study was more complex than a simple pesticide trial: it was a 3-way factorial testing 6 treatments × 5 sprays × 4 time lengths after each spray. Analyses by repeated measures ANOVAs were not appropriate because there were 2 types of time intervals: individual sprays (time intervals after planting) and time lengths after each spray. Also, each sample of 5 leaflets per plot typically was taken from different plants than those sampled during the other time intervals after each spray or sample date. Hence, our use of factorial analyses appeared well justified.

In the same field as the present study but earlier that year, Devkota (2015) found that *L. trifolii* activity started 12 d after snap bean planting and reached a maximum at 14 to 21 d. Hence, the first spray date began 12 d after planting, when bean plants had 2 fully unfolded primary leaves and pesticide-treated plots generally appeared to not be infested with *L. trifolii*. The presence of *L. trifolii* mines indicates feeding by larvae; therefore, similar patterns were found among the treatments in reducing numbers of mines and larvae after applying the pesticides. During statistical analyses, treatments were compared within spray dates (sample dates pooled) and when all the data were considered (sample and spray dates pooled). In either of these treatment comparisons, abamectin and spinosad generally resulted in the fewest mines per sample compared with the other 3 pesticides or the control treatment. Results for larvae and pupae followed a similar trend but tended to be less conclusive.

Studies by Hara (1986), Parrella et al. (1988), Cox et al. (1995), and Seal et al. (2002) similarly supported the effectiveness of abamectin and/or spinosad in controlling *L. trifolii*. In 1982, abamectin provided effective control for *L. trifolii* in vegetable crops such as celery (Trumble 1985; Leibee 1988; Parrella et al. 1988; Cox et al. 1995). Later, Seal et al. (2002) found that abamectin and spinosad provided better control of *L. trifolii* than the untreated control plants. Simultaneously, Webb (2002) reported that SpinTor® (spinosad) was effective in controlling *L. trifolii* populations and was relatively benign to natural enemies. Resistant strains of *L. trifolii* to abamectin, spinosad, and cyromazine have become susceptible in the absence of pesticide selection pressure, which was not the case for permethrin and chlorpyrifos (Parrella & Trumble 1989; Ferguson 2004).

Comparing treatments within spray dates suggested that cyromazine was less effective than spinosad in reducing numbers of mines, but results were less conclusive for larvae and pupae. Similar results were obtained when sample days and spray dates were pooled: cyromazine tended to allow more mines and larvae per sample than abamectin or

Table 4. Mean numbers (± SD) of *Liriomyza trifolii* mines and pupae per 5 bean leaflets treated with chemical and biological insecticides. The results compare individual sample days after spraying within treatments showing significant variation in numbers of mines or pupae over the post-application period.[a]

Treatment	Sample day	Mines[b,c]	Treatment	Sample day	Pupae[b,c]
Abamectin	1	0.00 ± 0.00 b	Cyromazine	1	0.00 ± 0.00 b
	2	0.00 ± 0.00 b		2	0.00 ± 0.00 b
	5	0.55 ± 0.76 a		5	0.50 ± 1.15 ab
	7	0.15 ± 0.67 b		7	1.75 ± 3.80 a
P		0.0004	*P*		0.0111
Spinosad	1	0.10 ± 0.31 b	*I. fumosorosea*	1	2.30 ± 2.64 b
	2	0.10 ± 0.31 b		2	2.90 ± 4.25 b
	5	0.60 ± 0.94 ab		5	4.15 ± 5.22 ab
	7	0.95 ± 1.73 a		7	5.25 ± 4.61 a
P		0.0196	*P*		0.0474

[a]Results were non-significant for all data with the control and azadirachtin treatments, all larvae for all treatments, pupae for abamectin and spinosad, and mines for cyromazine and *I. fumosorea*.

[b]Data were transformed for statistical analyses, but only non-transformed means and standard deviations (SDs) are shown.

[c]Means within a column followed by the same letter did not differ significantly based on 1-way analyses of variance followed by Waller–Duncan *K*-ratio *t*-tests ($P \geq 0.05$; SAS Institute 2014).

spinosad. However, the mines appeared smaller and the larvae usually aborted or died before pupation. Therefore, similarly few pupae were recovered from most plots treated with cyromazine, abamectin, or spinosad based on data collected following most spray dates (sample days pooled) or from all data (sample days and spray dates pooled). These results support the findings of Schuster & Everett (1983), Hara (1986), Saito et al. (1992), and Ferguson (2004). Ferguson (2004) found that cyromazine and abamectin resulted in relatively few cases of resistance and have been the most effective insecticides for *L. trifolii* control in vegetables and ornamentals.

Treatment by azadirachtin appears to have led to more intermediate effects. Comparison of azadirachtin with cyromazine treatments (sample days and spray dates pooled) yielded similar numbers of mines, larvae, and pupae per sample. Here, azadirachtin or cyromazine each tended to allow greater numbers of mines, larvae, and pupae per sample than abamectin or spinosad treatments, but less than from *I. fumosorosea* or the control. Similar results were obtained comparing treatments within spray dates (sample days pooled): most spray dates yielded this hierarchy for mines, but results were less conclusive for larvae or pupae. Overall, the number of pupae from azadirachtin plots was often similar to those from plots treated with abamectin or spinosad, but was always significantly lower than from the control. Azadirachtin does not work as an oviposition deterrent (Webb et al. 1983), yet it has strong larvicidal properties (Webb et al. 1983; Larew et al. 1985; Hossain & Poehling 2006). This may have led to the relatively large numbers of mines yet few pupae, which is suggested especially by comparing treatments within each spray date.

The fungus *I. fumosorosea* was less effective than the other insecticide treatments in reducing the numbers of mines, larvae, or pupae, although it was better than the control. There were more mines, larvae, and pupae found in plots treated with *I. fumosorosea* than in plots of the other 4 pesticides (spray dates and sample days pooled) or after most of the 5 spray dates (sample days pooled). Treatment with *I. fumosorosea* usually yielded fewer mines and pupae than the control, but similar numbers of larvae. Although the present field study using *I. fumosorosea* was not successful in reducing *L. trifolii* populations except when compared with the untreated control, several greenhouse or laboratory studies have demonstrated its potential (Vidal et al. 1998; Wraight et al. 2000; Ali et al. 2010; Wekesa et al. 2011). The endurance of quiescent conidia of *I. fumosorosea* is highly dependent on temperature and humidity conditions (Bouamama et al. 2010), and humidity fluctuations within 24 h of application of the fungus can lower its development rate (Landa et al. 1994). In the present study, the apparent lower ability of *I. fumosorosea* to control *L. trifolii* compared with the other 4 pesticides may have resulted from fluctuating temperatures and humidity levels in the field or possibly other unknown factors.

Sprays were applied at weekly intervals to reflect common field practices among growers in maintaining effective control of *L. trifolii*. To consider the effects of multiple spray applications, the 5 spray dates were compared in our analyses. Abamectin yielded no differences in numbers of mines, larvae, or pupae among the spray dates within each treatment, and spinosad had different numbers of mines, but not larvae or pupae. Different numbers of mines and pupae, though not larvae, were found when spray dates were compared for cyromazine, when treatments were compared within spray date 1 (each with pooled sample days), and when comparing spray dates generally (pooled treatments and sample days). The 3 treatments that appeared to be the most effective in reducing populations of *L. trifolii* were abamectin, spinosad, and cyromazine. They had non-significant differences between spray dates in one or more variables. Yet differences were recorded in numbers of mines, larvae, and pupae when comparing spray dates for the control treatment. Spinosad and abamectin seemed to be the most effective pesticides and resulted in the fewest differences between spray dates, whereas the control appeared to be the least effective yet most variable between spray dates. Hence, differences between spray dates appeared to be reduced as pesticide effectiveness increased. While the less effective pesticides allowed pest numbers to increase and more effective ones kept pest numbers down, the latter also seemed to reduce the variation in pest numbers between individual spray dates. The reasons why these differences were observed especially in the control treatment are unclear, but they may be related to a resident *L. trifolii* population in the field.

To determine the duration and extent of residual activity after each application, we tested a second, time-related factor by taking all samples at 1, 2, 5, or 7 d after each spray, hence at 4 time intervals after each spray date. The sample days were compared to assess the durability of each pesticide despite the no-interaction results from initial factorial analyses. With spray dates and treatments pooled, there were differences in numbers of mines, but not larvae or pupae, among the 4 sample dates. More mines were found at 7 d than 1 or 2 d after application. For most pesticide treatments, comparison of these sample days after pesticide application resulted in different numbers of mines or pupae, but not both, and not of larvae. Different numbers of mines were found for abamectin and spinosad and different numbers of pupae for cyromazine and *I. fumosorosea*. Treating plants with abamectin increased the number of mines on sample day 5 compared with the other sample days. However, more mines were found from treatments of spinosad and more pupae from cyromazine and *I. fumosorosea* 7 d after spraying than after 1 or 2 d. Abamectin, spinosad, cyromazine, and *I. fumosorosea* showed differences based on 1 kind of data (either mines or pupae), although 3 kinds of data were available. Some loss of effectiveness in most pesticides may have occurred, which is suggested by the occasionally greater numbers of mines or pupae found later (5–7 d) than at the beginning (1–2 d) during the first week after spraying. However, for azadirachtin and the control, differences did not occur in numbers of mines, larvae, or pupae. Hence, the pesticides appeared to have performed similarly to each other, though azadirachtin seems to have had longer residual activity with no differences between sample days. Similar to the control, azadirachtin may have appeared more "durable" because of its consistently poor performance, not its ability to last longer than the other products.

Similar to managing other pest species, alternating pesticides with different modes of action and limiting the duration for using a pesticide with a single mode of action can delay the onset of resistance in *L. trifolii*. Using abamectin to control *L. trifolii* in celery was permitted for only 2 consecutive applications to help limit the development of resistance during the growing season, which prompted the suggestion of a rotation program using abamectin and cyromazine (Leibee & Capinera 1995). Because we found that pesticides with different modes of action such as spinosad and azadirachtin can each be effective, they can be alternated to control *L. trifolii* while curbing the development of resistance caused by application of pesticides with a single mode of action.

In summary, the conventional industry standards, abamectin and spinosad, were the most effective products for managing *L. trifolii* populations by reducing mines, larvae, and/or pupae. Cyromazine and azadirachtin (a potential alternative) appeared more intermediate and were less effective or more variable than abamectin and spinosad. *Isaria fumosorosea* was the least effective treatment, although still usually more effective than the untreated control. It should not be considered for suppression of *L. trifolii* under field conditions unless an improved formulation is developed that is more resilient to environmental conditions. Among the 5 spray dates, the most effective treatments seemed to have reduced variation in addition to the expected benefit of keeping pest numbers lower compared with the least effective treatments.

These results may indicate increased variation in the field population of *L. trifolii* between spray dates when subjected to weak or no pesticides and left able to attack bean plants. Considering sample dates after pesticide application with spray dates and treatments pooled, *L. trifolii* mines appeared more effectively controlled 1 to 2 d following the spray dates than after 7 d, whereas larvae and pupae were controlled equally throughout the 7 d post-application period. However, examination of individual pesticides suggested that some degradation may have occurred by 5 to 7 d after application. Abamectin, spinosad, cyromazine, and *I. fumosorosea* appeared less durable over the 7 d period than azadirachtin, but this may have resulted from the consistently poorer performance of the latter, not its ability to last longer than the other pesticides.

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