Research Article

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Cascading effects of combining synthetic herbivore-induced plant volatiles with companion plants to manipulate natural enemies in an agro-ecosystem

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Abstract

BACKGROUND: Whether tactics to manipulate natural enemies in agro-ecosystems enhance their ecosystem function and services remains debatable. We conducted field experiments in 2015–2016 to test the hypothesis that attraction of natural enemies to herbivore-induced plant volatiles (HIPVs), alone or in combination with companion plants, increases crop productivity. Our treatments consisted of bean plants alone or baited with methyl salicylate (MeSA; an HIPV), or combined with coriander (a companion plant), or with both MeSA and coriander. Numbers of arthropods were visually sampled in each treatment. Sentinel aphids were used to measure ecosystem function (i.e. predation). Plant damage and biomass, and the number and weight of pods and seeds, were measured as a proxy for ecosystem services.

RESULTS: MeSA and coriander, when alone or combined, increased the abundance of insect predators from six families, reduced herbivore (e.g. spider mite and thrips) populations, and increased aphid predation. MeSA and coriander also reduced damage by spider mites. MeSA with or without coriander did not, however, increase crop biomass or any yield parameters.

CONCLUSIONS: MeSA alone or combined with coriander attracted different predator communities, altered pest communities, and reduced damage; however, these results did not cascade down to improve crop productivity.

Supporting information may be found in the online version of this article.

Keywords: methyl salicylate; herbivore-induced plant volatiles; attract-and-reward; conservation biological control

1 INTRODUCTION

In the past 30 years, research has shown that herbivore feeding induces the production of volatiles from plants, commonly referred to as herbivore-induced plant volatiles (HIPVs), and that, in turn, the natural enemies of herbivores can be attracted to these HIPVs during prey location.[1–9] As a result, various synthetic HIPVs have been tested to manipulate natural enemy behavior in agro-ecosystems.[10–15] However, the use of HIPVs for this purpose has remained controversial because there is the risk of disrupting biological control by confusing the natural enemies instead of helping them during prey or host location,[16] by possibly increasing ecological risks by unintentionally attracting the herbivores themselves,[16] or by attracting the enemies of the natural enemies, for example hyperparasitoids,[17] which in turn may reduce populations of the third trophic level.

A way to ameliorate the negative effects of HIPVs on biological control is by combining different tactics to conserve natural enemies, such as HIPVs and companion plants, in an approach known as 'attract-and-reward.' In this scenario, a synthetic HIPV is used to attract natural enemies while a floral resource is used to provide food and thus conserve their populations.[18–20] For example, Orre Gordon *et al.*[20] found that intercropping buckwheat, *Fagopyrum esculentum* Moench, with brassica plants baited with the HIPV methyl salicylate (MeSA) increased the recruitment of natural enemies from multiple (third and fourth) trophic levels; this natural enemy attraction to MeSA resulted in greater aphid parasitism rates. Wang *et al.*[21] also demonstrated that combining MeSA with oilseed rape, *Brassica napus* L., as a companion plant in wheat fields enhances the attraction of ladybeetles, and that this attraction leads to reduced aphid, *Sitobion avenae* F., populations. Similarly, in a recently study, Xu *et al.*[22] reported increased numbers of hoverflies (Syrphidae) and reduced aphid populations when wheat (*Triticum aestivum* L.) was intercropped with pea (*Pisum sativum* L.) and baited with either MeSA or *E*-β-farnesene.

If HIPVs are used to manipulate natural enemies of herbivores and enhance their ecosystem function and services for conservation biological control in agro-ecosystems, three cri-

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teria must be meet: (1) HIPVs need to attract natural enemies, and thus enhance their ecosystem structure (i.e. abundance); (2) this attraction should reduce pest populations, thus enhancing natural enemy ecosystem function; and (3) a reduction of pest populations should cascade down to reduce damage and increase crop productivity, thus enhancing ecosystem services provided by the natural enemies. Several studies have shown that natural enemies are attracted to HIPVs in agro-ecosystems (criterion 1).[14,15,23–27] For example, in an early study, Flint *et al.*[28] showed that *Chrysoperla carnea* Stephens is attracted to the HIPV caryophyllene in cotton fields. More recently, James[29] reported that beneficial insects from the families Syrphidae, Geocoridae, Anthocoridae, and Miridae are attracted to MeSA and (*Z*)-3-hexenyl acetate in hops. Similarly, coccinellids were attracted to MeSA-baited traps in vineyards.[30] Less evidence currently exists in support of the second criterion that natural enemy attraction to HIPVs will lead to reduced pest populations in agricultural systems. For example, in cotton, α-farnesene and (*Z*)-3-hexenyl acetate attracted the parasitoid *Anaphes iole* Girault, and this attraction increased the parasitism rate of *Lygus lineolaris* Palisot de Beauvois eggs.[31] In soybean, Mallinger *et al.*[32] reported that MeSA increases the attraction of predatory insects, such as members of the families Syrphidae and Chrysopidae, and reduces soybean aphid, *Aphis glycines* Matsumura, populations. As far as we know, only two studies have so far tested whether attraction of natural enemies to HIPVs can impact crop productivity (our third criterion). Wang *et al.*[21] found that MeSA combined with oilseed rape (*B. napus*) in wheat increased the attraction of Coccinellidae species, reduced populations of the aphid *S. avenae*, and improved yield parameters such as quality and weight of grains. In contrast, Simpson *et al.*[19] found no effects of an attract-and-reward approach on crop (wine grapes) yield. Therefore, it remains unclear whether manipulation of natural enemy behavior via HIPVs alone or in combination with companion plants (i.e. an 'attract-and-reward' scenario) increases their ecosystem structure, function, and services in agro-ecosystems – hence addressing all of our three criteria.

In the present study, we conducted a 2-year field study to test the hypothesis that HIPVs alone or in combination with companion plants attract natural enemies (criterion 1), reduce pest populations (criterion 2), and increase crop productivity (criterion 3). We tested this hypothesis in a system consisting of common bean (*Phaseolus vulgaris* L.) plants, the HIPV MeSA, and coriander (*Coriandrum sativum* L.) as a companion plant. Both MeSA[15,26] and coriander[33,34] have been shown to attract natural enemies of herbivores in agro-ecosystems (see below). We predicted that MeSA and coriander would interact synergistically to alter the arthropod community, i.e. increase natural enemy abundance and reduce herbivore density, and that these effects would ultimately cascade down to increase crop productivity. Our specific objectives were to: (1) identify which natural enemies are attracted to MeSA alone or in combination with coriander; (2) determine the effects of MeSA alone or in combination with coriander on herbivore abundance and predation rates; and (3) measure the cascading effects of MeSA alone or in combination with coriander on plant damage, biomass, and yield.

2 METHODS

2.1 Study system

Common bean, *Ph. vulgaris* cv. 'Carioquinha', plants were used in field experiments. *Phaseolus vulgaris* originated from the Americas, and Brazil (where this work was conducted) is one of the largest producers of dry beans in the world.[35,36] Seeds were purchased from Mega graõs Alimentos Ltda. (São Paulo, SP, Brazil).

Methyl salicylate (MeSA) was used because it is an HIPV commonly released by many plant species, including bean plants.[37–39] It is also induced after damage by herbivores from different feeding guilds including mites,[40,41] aphids,[42,43] beetles,[44] and caterpillars.[45,46] In addition, MeSA has been used in various agro-ecosystems such as soybeans,[32] cranberries,[26] vineyards,[30] strawberries,[25] wheat,[21] and hops[47] to attract beneficial insect predators such as members of the families Syrphidae, Chrysopidae, Anthocoridae, Coccinellidae, and Geocoridae, and a MeSA-based bait is commercially available to growers for this purpose (PredaLure; AgBio Inc., Westminster, CO, USA). MeSA was purchased from Sigma-Aldrich (>99% purity; São Paulo, SP, Brazil).

Coriander, *Coriandrum sativum* cv. 'Verdão', was used as a companion plant because previous studies have shown that coriander when intercropped with cabbage,[33] tomato,[34] carrot,[48] eggplant,[49] and rose plants[43] attracts natural enemies – for example, lacewings (Chrysopidae), hoverflies (Syrphidae), and ladybeetles (Coccinellidae). Coriander emits volatiles throughout all vegetative stages, which are known to be attractive to natural enemies.[50–52] In addition, coriander flowers provide a source of nutrient (nectar and pollen) to natural enemies.[49,53] Coriander seeds were purchased from Horticeres Sementes Ltda. (Indaiatuba, SP, Brazil).

2.2 Study sites and experimental design

Field experiments were conducted at a research farm located in Ijaci, Minas Gerais, Brazil (latitude 21°09′54.7′′ S, longitude 44°55′04.3′′ W) in 2015 and 2016. In each year, we planted 28 (3 m × 1 m) plots of bean plants spaced 3 m apart (Fig. 1). Each plot consisted of 20 plants planted in five rows (total of 100 plants per plot), with a spacing of 5 cm between plants and 70 cm between rows. Bean plant seeds were hand planted and a sprinkler system provided irrigation as needed. Plots were fertilized once, 30 days after planting, with cow manure, and no insecticides, fungicides, or herbicides were used during the study. Plots were weeded regularly by hand.

In each year, each plot received one of four treatments in a randomized complete block design, and each of the treatment plots was replicated in seven blocks ($N = 14$ replicates per treatment for the entire study). Treatments consisted of: (1) bean plants alone (referred to as 'control'); (2) bean plants baited with MeSA ('MeSA'); (3) bean plants intercropped with coriander ('coriander'); and (4) bean plants baited with MeSA and intercropped with coriander ('both') (Fig. 1). In treatments with coriander, coriander plants were planted between each of the rows of bean plants (20 coriander plants per row for a total of 80 plants per plot), with a spacing of 5 cm between plants (Fig. 1), which allowed us to test the effects of coriander as a 'companion' plant to the bean crop while maintaining the number of bean plants constant across all plots. In treatments with MeSA, four polyethylene vials (Comar LLC, Voorhees, NJ, USA) containing 3 mL of pure MeSA were placed in the center of each plot, between rows 2 and 3 and rows 3 and 4; two vials were placed per row, separated 20 cm from each other within rows (Fig. 1). Vials were tied to poles so that they were hanging ∼70 cm above ground. A small hole was poked through the lid of all vials using a needle to aid the release of MeSA from vials. Coriander seeds were planted by hand at the same time as the bean plants and remained in the field for the entire duration of the experiment. Coriander plants started to flower approx. 40 days after planting, which coincided with the beginning of our sampling period, and

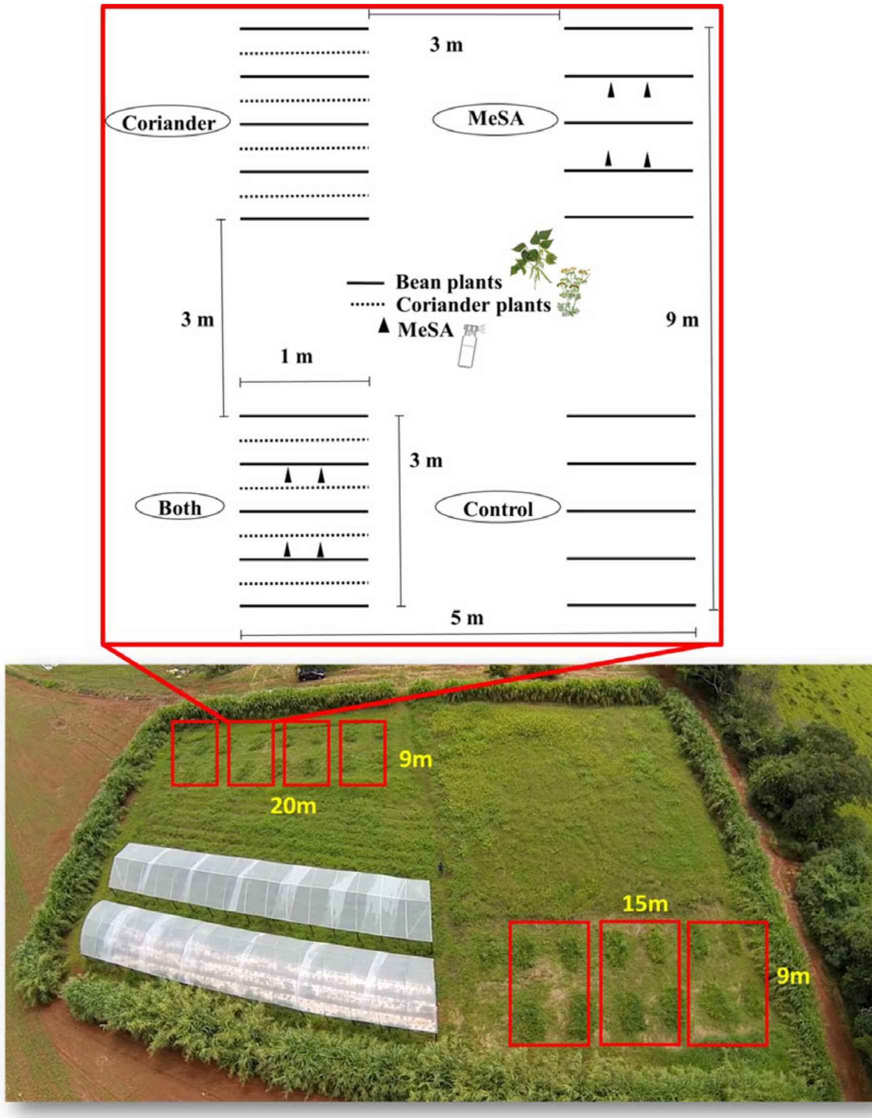

Figure 1. Aerial view of the research farm (Ijaci, MG, Brazil) and schematic representation of the field experiment. Plots were set up in a randomized complete block design with seven replicates (red rectangles) per treatment. There were four treatment plots in each block: (1) bean plants alone (referred to as 'control'); (2) bean plants baited with methyl salicylate ('MeSA'); (3) bean plants intercropped with coriander ('coriander'); and (4) bean plants baited with MeSA and intercropped with coriander ('both'). Treatments were randomly assigned to each plot within blocks.

flowers remained open during the whole sampling duration (see below). MeSA-baited vials were placed in the field 30 days after planting, to allow time for plants to grow and plots to be colonized by arthropods, and were replaced every 15 days until harvest. The release rate of MeSA was calculated by obtaining the mass of each vial before and after field deployment (each vial released ~21 mg of MeSA per day).

2.3 Arthropod community sampling

To determine the effects of treatments on both beneficial (criterion 1) and detrimental (criterion 2) arthropod communities, natural enemies and herbivores were sampled in each of the plots. Sampling was initiated a week after placing the MeSA vials in the field and continued every 15 days from 9 October until 30 November in 2015 and from 24 May until 1 July in 2016, for a total of five sampling dates per year. In each plot, 20 plants were randomly selected and visually inspected for the presence of arthropods (natural enemies and herbivores) to calculate the total number of natural enemies and herbivores per plant in each plot. Because plants within plots were sampled randomly, the same plant was not likely to be sampled multiple times throughout the growing season. All observations were made on sunny, calm days for approximately 10-min periods per plot on each of the sampling dates and carried out from 0900 to 1400 h (total per year = 50 min per plot or 280 min per sampling date or 1400 min of total sampling time for the entire study). This sampling period (i.e. 30 s per plant) allowed enough time to survey all arthropods on a single plant. Arthropods were identified to family or species. We did not use a trapping method of collection to avoid removal of arthropods in the field. However, a few specimens of the most representative arthropods were collected and taken to the laboratory for further identification, and are kept as vouchers at the Departamento de Entomologia, Universidade Federal de Lavras (Lavras, MG, Brazil).

In 2015, spider mites were the main herbivores of bean plants in our plots; for this reason, spider mite populations were monitored throughout each of the growing seasons following methods modified from Karlik *et al.*[54] and Alston and Reding.[55] Unlike sampling for other arthropods which was recorded on a per plant basis, spider mite sampling was performed on a per leaf basis. We used the same 20 plants described above and, for each plant, counted the number of mites on 10 leaves with at least one mite to estimate the number of mites per leaf, per plant and per plot.

In 2016, thrips were the dominant herbivores in our plots. Thrips populations were sampled following methods modified from Santos-Amaya *et al.*[56] and Gonzalez-Zamora and Garcia-Mari.[57] The same 20 plants described above were used to sample thrips per plot; each plant was tapped five times against a white tray and the number of thrips observed on the tray was counted. The numbers of all other arthropods (natural enemies and herbivores) on the tray were also recorded.

2.4 Aphid predation rate

To further test criterion 2, we used sentinel aphids to assess predation rates in each of the plots. In both years (2015 and 2016), four black squares (6 × 6 cm) made of sandpaper (AC Parafusos Ltda., Lavras, MG, Brazil) were placed in each plot (for a total of 112 squares in all plots). Just prior to deployment, five frozen aphids were glued onto each square, and the squares were then hung with pins on wooden stakes at ∼70 cm above the ground; the stakes were placed near the center of each plot at least 20 cm apart. The yellow rose aphid (*Rhodobium porosum* Sanderson), obtained from a colony maintained on rose plants at the Universidade Federal de Lavras (Lavras, Minas Gerais, Brazil), was used as sentinel prey. The number of aphids remaining on each square was counted after 24 h. These assays were repeated twice in each year.

2.5 Herbivore damage

In 2015, we saw severe damage to bean plants caused by spider mites. Thus, we investigated the effects of our treatments on crop damage (criterion 3). Spider mite damage was assessed visually by counting the number of leaves per plot expressing severe chlorosis or necrosis: we considered the damage as 'severe' if the entire leaf showed these symptoms. Visual inspections were performed on the same 10 leaves used for counting spider mite populations, and on the same dates as the arthropod counts (see above). This was done only in 2015 because we did not observe any obvious damage to bean plants caused by herbivory in 2016.

2.6 Crop yield and dry mass

At the end of each of the growing seasons, we harvested bean plants from each of the plots to assess the effects of our natural enemy manipulation treatments on crop yield and dry mass (criterion 3). To measure plant mass, the above-ground portion of plants was removed, cut into small pieces, and then placed in 4-L aluminum trays. These pieces were weighed and then dried in an oven at 125 °C for 48 h to obtain total dry mass for each of the plants. Before cutting the plants, all pods were removed from plants, counted, and weighed to obtain the number and mass of pods per plant. In addition, the number and mass of seeds in each pod were recorded for each plant. In 2015, we harvested ten plants per plot ($N = 280$ plants for all plots). Because we did not find any effects of treatment on crop yield or dry mass in the first year (see Results), we increased our sample size to 40 plants per plot in the second year ($N = 1120$ plants for all plots).

2.7 Statistical analyses

All analyses were conducted in R 3.3.1.[58] Prior to analyses, counts for each arthropod taxon (family/species) were averaged across all sampling dates to obtain the mean seasonal total abundance per leaf, per plant and per plot (spider mites) or per plant in each plot (all other arthropods) for each treatment. These data were first checked for normality using the Shapiro–Wilk test[59] and for homoscedasticity using Levene's test ('car' package in R), and then used to determine the effect of treatment (control, MeSA, coriander, and MeSA + coriander) on abundance (i.e. number of individuals per taxon) for the entire arthropod community (i.e. for both natural enemies and herbivores together), as well as for the natural enemy and herbivore communities separately.

We first conducted multivariate analyses to test the effects of treatments (i.e. presence or absence of MeSA and coriander) on arthropod, natural enemy, and herbivore abundances as separate groups; we then followed these analyses by using univariate statistics for each individual taxon within each of these groups. For the multivariate analyses, the effects of MeSA, coriander, and their interaction on the abundance of each group of arthropods, natural enemies, and herbivores were analyzed using 2 × 2 factorial multivariate analysis of variance (two-way MANOVA). MANOVA was also used to analyze the effects of treatment on crop yield parameters. In addition, principal component analysis (PCA) was used to visualize (score and loading plots) the effects of the different treatments (control, MeSA, coriander, and both) on the abundances of arthropod, natural enemy, and herbivore communities. Score and loading plots were drawn in R using the 'ggplot2' package.[60]

We followed our multivariate analyses with 2 × 2 factorial univariate analyses to investigate the effect of treatment (i.e. presence or absence of MeSA and coriander) on individual insect taxa and crop yield parameters. We used generalized linear models (GLMs) with a quasipoisson distribution and a logit-link function to test for the effects of MeSA, coriander, and their interaction on the abundances of each of the natural enemies and herbivores separately, as well as their effects on the number and mass of pods and seeds. GLMs were also used to analyze predation rate, crop damage, and crop dry mass. If needed, data were transformed prior to MANOVA and GLM analyses using $\ln(x + 0.5)$ to meet assumptions of normality. Untransformed data are presented in tables and figures.

In addition, we performed correlation analysis (Pearson correlation tests) to determine if the abundance of the most common natural enemies (e.g. ladybeetles, hoverflies, earwigs, minute pirate bugs, and predatory thrips and stink bugs; see Results) correlated with the abundance of the most common herbivores (e.g. spider mites, thrips, chrysomelids, and pentatomids; see Results) in each of the manipulative treatments (MeSA, coriander, and both) as compared with the abundances in the controls.

3 RESULTS

3.1 Arthropod community composition

A total of nine arthropod taxa were recorded in 2015 and 11 arthropods in 2016 (Table 1). Other functional groups such as parasitoids are not reported because they were rarely observed during visual inspections, probably as a consequence of their small size and mostly cryptic behaviors.

Arthropod abundance was affected significantly by MeSA, coriander, and their interaction in 2015 (MeSA: Wilks' $\lambda = 0.83$; $F = 2.68$; df = 1, 102; $P = 0.01$; coriander: Wilks' $\lambda = 0.71$; $F = 5.53$; df = 1, 102; $P < 0.001$; MeSA × coriander: Wilks' $\lambda = 0.84$; $F = 2.56$;

Table 1. Herbivore and natural enemy communities found in 2015 and 2016

Year	Common name	Genus/species	Order	Family
		Herbivores		
2015	Spider mites	*Tetranychus* sp.	Acarini	Tetranychidae
	Leaf beetles	*Diabrotica* sp.	Coleoptera	Chrysomelidae
	Stink bugs	Unknown	Hemiptera	Pentatomidae
		Natural enemies		
	Hoverflies	*Toxomerus* sp.	Diptera	Syrphidae
	Predatory stink bug	*Podisus* sp.	Hemiptera	Pentatomidae
	Convergent ladybeetles	*Hippodamia convergens* Guérin-Méneville	Coleoptera	Coccinellidae
	Multicolored Asian ladybeetles	*Harmonia axyridis* Pallas	Coleoptera	Coccinellidae
	Spotless ladybeetles	*Cyclonella sanguinea* L.	Coleoptera	Coccinellidae
	Earwigs	*Doru* sp.	Dermaptera	Forficulidae
		Herbivores		
2016	Thrips	*Neohydatothrips* sp.	Thysanoptera	Thripidae
	Leaf beetles	*Diabrotica* sp.	Coleoptera	Chrysomelidae
	Stink bugs	Unknown	Hemiptera	Pentatomidae
		Natural enemies		
	Hoverflies	*Toxomerus* sp.	Diptera	Syrphidae
	Lacewings	*Chrysoperla* sp.	Neuroptera	Chrysopidae
	Convergent ladybeetles	*Hippodamia convergens* Guérin-Méneville	Coleoptera	Coccinellidae
	Multicolored Asian ladybeetles	*Harmonia axyridis* Pallas	Coleoptera	Coccinellidae
	Predatory thrips	*Franklinotrips vespiformis* Crawford	Thysanoptera	Aeolothripidae
	Minute pirate bugs	*Orius insidiosus* Say	Hemiptera	Anthocoridae
	Spiders	Various spp.	Araneae	Unknown
	Ants	Various spp.	Hymenoptera	Formicidae

[a] Both immatures and adults of ladybeetles, lacewings, thrips, and Chrysomelid beetles were recorded.

Table 2. Results of general lineal model (GLM) analyses testing the effects of methyl salicylate (MeSA), coriander, and their interaction on different natural enemies sampled in common bean, *Phaseolus vulgaris*, field plots[a] in 2015 and 2016

		GLM results											
		Block			MeSA			Coriander			MeSA × coriander		
Year	Taxa[b]	*F*	df[c]	*P*[d]	*F*	df[c]	*P*[d]	*F*	df[c]	*P*[d]	*F*	df[c]	*P*[d]
2015	Coccinellidae	5.64	6, 105	**< 0.001**	1.10	1, 104	0.29	10.32	1, 103	**0.001**	3.37	1, 102	0.06
	Toxomerus sp.	2.50	6, 105	**0.02**	10.78	1, 104	**0.001**	41.50	1, 103	**< 0.001**	2.77	1, 102	0.09
	Forficulidae	3.81	6, 105	**0.001**	0.21	1, 104	0.64	11.70	1, 103	**< 0.001**	2.40	1, 102	0.12
	Pentatomidae	3.97	6, 105	**0.001**	1.52	1, 104	0.21	5.41	1, 103	**0.02**	6.36	1, 102	**0.01**
2016	Coccinellidae	3.87	6, 129	**0.001**	1.95	1, 128	0.16	3.88	1, 127	**0.05**	10.38	1, 126	**0.001**
	Toxomerus sp.	7.90	6, 129	**< 0.001**	56.93	1, 128	**< 0.001**	1.03	1, 127	0.31	12.09	1, 126	**< 0.001**
	Franklinotrips vespiformis	1.86	6, 129	0.09	2.16	1, 128	0.14	0.17	1, 127	0.67	4.00	1, 126	**0.04**
	Orius insidiosus	5.42	6, 129	**< 0.001**	4.23	1, 128	**0.04**	1.33	1, 127	0.25	1.77	1, 126	0.18
	Chrysopidae	3.42	6, 129	**0.003**	1.56	1, 128	0.21	0.17	1, 127	0.68	1.39	1, 126	0.24
	Spiders	2.75	6, 129	**0.01**	0.00	1, 128	0.92	0.00	1, 127	0.92	1.79	1, 126	0.18
	Ants	1.71	6, 129	0.12	0.01	1, 128	0.88	0.01	1, 127	0.88	0.05	1, 127	0.81

[a] See Figure 1 for details.
[b] All species within a taxon and developmental stages were pooled prior to analysis.
[c] Numerator, denominator (error).
[d] Numbers in bold indicate significant differences at $\alpha = 0.05$.

df = 1, 102; $P = 0.01$) (Supporting Information Fig. S1a) and in 2016 (MeSA: Wilks' $\lambda = 0.68$; $F = 5.48$; df = 1, 126; $P < 0.001$; coriander: Wilks' $\lambda = 0.80$; $F = 2.87$; df = 1, 126; $P = 0.003$) (Fig. S1d), although the interaction effect was only marginal in 2016 (Wilks' $\lambda = 0.86$; $F = 1.77$; df = 1, 126; $P = 0.07$). In 2015, the PCA showed a differentiation in arthropod community composition according to the treatments, explaining ~53% of the variance (Fig. S2a). The first principal component (PC) explained 36% of the variation and clearly separated the control treatment from the other treatments, while the second PC explained only 17% of the variation. In 2016, the PCA explained ~50% of the variation in arthropod community composition among the treatments (Fig. S2d). The first PC explained 32% of the variation and partially separated the MeSA from the coriander treatments, while the second PC explained

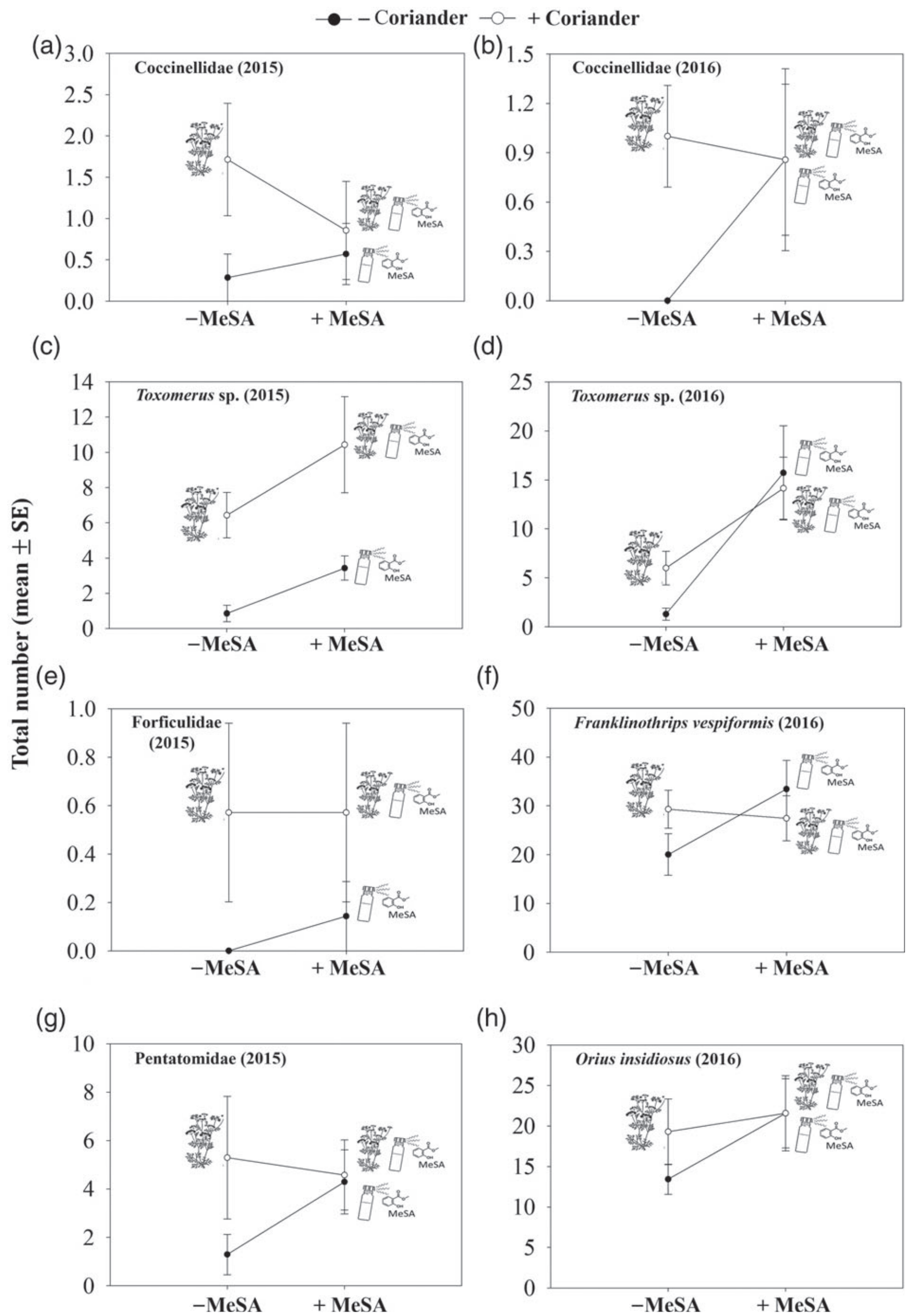

Figure 2. Abundance (total number per plant in each plot) of Coccinellidae spp. (a, e), *Toxomerus* sp. (b, f), Forficulidae spp. (c), Pentatomidae spp. (d), *Franklinothrips vespiformis* (g), and *Orius insidiosus* (h) in control plots [common bean, *Phaseolus vulgaris*, alone; − methyl salicylate (MeSA), − Coriander], plots with bean plants baited with MeSA (+ MeSA, − Coriander), plots with bean plants intercropped with coriander (− MeSA, + Coriander), and plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

18% of the variation and separated the control from the other treatments (MeSA, coriander, and both).

After finding that our treatments affected the abundance of arthropod communities in common bean, we conducted further analyses to investigate whether natural enemies and/or herbivores as a group, and which members within each of these trophic groups, were being affected by the treatments.

3.2 Natural enemies

In 2015, the abundance of natural enemies was affected by MeSA (Wilks' $\lambda = 0.86$; $F = 3.69$; df = 1, 102; $P = 0.007$) and coriander (Wilks' $\lambda = 0.73$; $F = 8.92$; df = 1, 102; $P < 0.001$), and there was a marginal interaction effect (MeSA × coriander: Wilks' $\lambda = 0.91$; $F = 2.30$; df = 1, 102; $P = 0.06$) (Fig. S1b). The PCA for 2015 showed a distinct composition of the natural enemy communities according to treatment, with the first two components explaining ~76% of the variance (Fig. S2b). The first PC explained 51% of the variation and partially separated the coriander from the MeSA and control treatments, while the second PC explained 25% of the variation. In 2016, the abundance of natural enemies was affected also by MeSA (Wilks' $\lambda = 0.70$; $F = 7.26$; df = 1, 126; $P < 0.001$), but not by coriander (Wilks' $\lambda = 0.91$; $F = 1.54$; df = 1, 126; $P = 0.15$);

Table 3. Results of general lineal model (GLM) analyses testing the effects of methyl salicylate (MeSA), coriander, and their interaction on different herbivores sampled in common bean, *Phaseolus vulgaris*, field plots[a] in 2015 and 2016

		GLM results											
		Block			MeSA			Coriander			MeSA × coriander		
Year	Taxa[b]	*F*	df[c]	*P*[d]	*F*	df[c]	*P*[d]	*F*	df[c]	*P*[d]	*F*	df[c]	*P*[d]
2015	*Tetranychus* sp.	0.71	6, 105	0.63	3.68	1, 104	**0.05**	8.29	1, 103	**0.004**	5.85	1, 102	**0.01**
	Chrysomelidae	1.90	6, 105	0.08	0.73	1, 104	0.39	0.35	1, 103	0.55	1.15	1, 102	0.28
	Pentatomidae	2.86	6, 105	**0.01**	1.16	1, 104	0.28	0.35	1, 103	0.55	0.24	1, 102	0.62
2016	*Neohydatothrips* sp.	1.51	6, 129	0.17	1.69	1, 128	0.19	2.70	1, 127	0.10	2.85	1, 126	0.09
	Chrysomelidae	4.29	6, 129	**< 0.001**	5.30	1, 128	**0.02**	7.31	1, 127	**0.007**	0.19	1, 126	0.65
	Pentatomidae	3.28	6, 129	**0.004**	2.07	1, 128	0.15	0.22	1, 127	0.63	0.09	1, 126	0.76

[a] See Figure 1 for details.
[b] All species within a taxon and developmental stages were pooled prior to analysis.
[c] Numerator, denominator (error).
[d] Numbers in bold indicate significant differences at $\alpha = 0.05$.

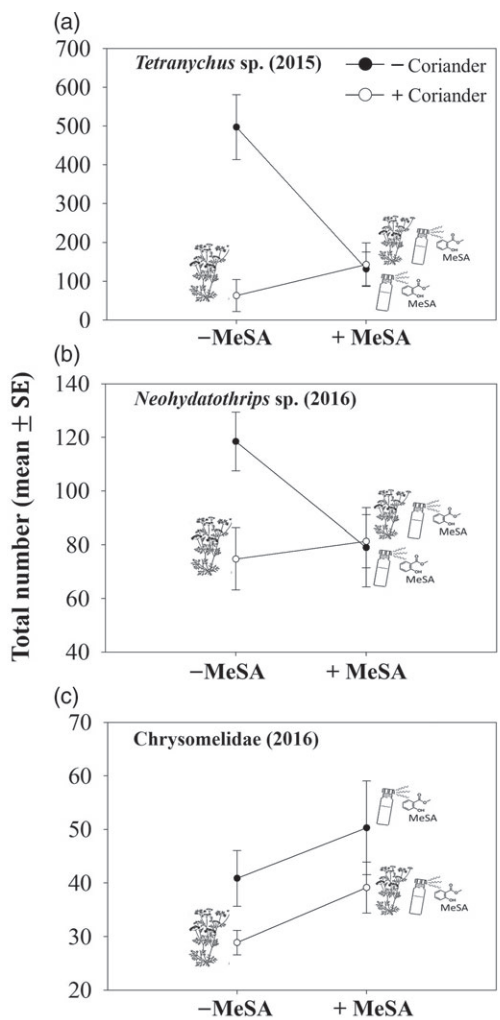

Figure 3. Abundance (total number per leaf, per plant and per plot or per plant in each plot) of *Tetranychus* sp. (a), *Neohydatothrips* sp. (b), and Chrysomelidae spp. (c) in control plots [common bean, *Phaseolus vulgaris*, alone; – methyl salicylate (MeSA), – Coriander], plots with bean plants baited with MeSA (+ MeSA, – Coriander), plots with bean plants intercropped with coriander (– MeSA, + Coriander), and plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

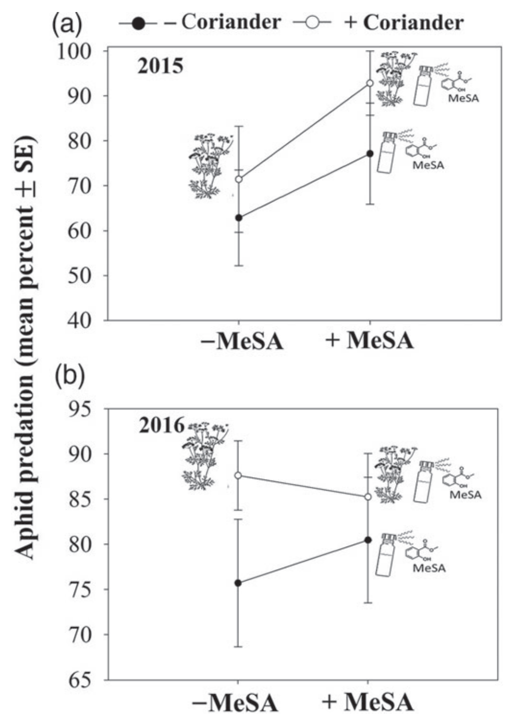

Figure 4. Percent aphid predation in control plots [common bean, *Phaseolus vulgaris*, alone; – methyl salicylate (MeSA), – Coriander], plots with bean plants baited with MeSA (+ MeSA, – Coriander), plots with bean plants intercropped with coriander (– MeSA, + Coriander), and plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

however, there was a marginal MeSA × coriander interaction (Wilks' $\lambda = 0.89$; $F = 1.92$; df = 1, 126; $P = 0.07$) (Fig. S1e), indicating that the effect of MeSA was somewhat influenced by coriander. In 2016, the first two PCs explained ∼54% of the variation of natural enemy communities among treatments (Fig. S2e). The first PC explained 33% of the variation and separated the control from the other treatments, while the second PC explained 21% of the variation and separated the control particularly from the MeSA and coriander treatments.

When each natural enemy was analyzed separately, the abundance of ladybeetles (Coccinellidae) was influenced by coriander,

Table 4. Results of general lineal model (GLM) analyses testing the effects of methyl salicylate (MeSA), coriander, and their interaction on various yield parameters for common bean, *Phaseolus vulgaris*, plants harvested from field plots[a] in 2015 and 2016

		GLM results											
		Block			MeSA			Coriander			MeSA × coriander		
Year	Parameters	*F*	df[b]	*P*[c]	*F*	df[b]	*P*[c]	*F*	df[b]	*P*[c]	*F*	df[b]	*P*[c]
2015	Dry mass	0.77	6, 21	0.60	0.07	1, 20	0.79	0.73	1, 19	0.40	0.24	1, 18	0.62
	Pod number	1.12	6, 21	0.38	0.10	1, 20	0.74	0.81	1, 19	0.37	0.92	1, 18	0.34
	Pod mass	1.28	6, 21	0.31	0.07	1, 20	0.78	2.15	1, 19	0.15	1.14	1, 18	0.29
	Seed number	0.91	6, 21	0.50	0.22	1, 20	0.64	1.38	1, 19	0.25	0.36	1, 18	0.55
	Seed mass	1.09	6, 21	0.40	0.43	1, 20	0.51	0.42	1, 19	0.52	1.00	1, 18	0.33
2016	Dry mass	3.19	6, 21	**0.02**	2.56	1, 20	0.12	0.08	1, 19	0.77	0.95	1, 18	0.34
	Number of pods	7.68	6, 21	**< 0.001**	0.17	1, 20	0.68	0.53	1, 19	0.47	1.81	1, 18	0.19
	Pod mass	2.03	6, 21	0.11	0.01	1, 20	0.90	0.00	1, 19	0.99	2.20	1, 18	0.15
	Number of seeds	8.58	6, 21	**< 0.001**	1.75	1, 20	0.20	0.00	1, 19	0.99	3.91	1, 18	0.06
	Seed mass	8.84	6, 21	**< 0.001**	4.07	1, 20	**0.05**	0.46	1, 19	0.50	7.09	1, 18	**0.01**

[a] See Figure 1 for details.
[b] Numerator, denominator (error).
[c] Numbers in bold indicate significant differences at $\alpha = 0.05$.

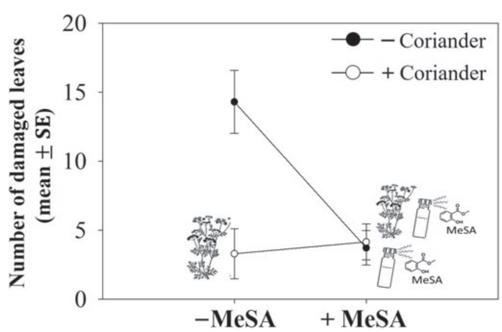

Figure 5. Amount (number of damaged leaves per plant in each plot) of herbivore damage in 2015 in control plots [common bean, *Phaseolus vulgaris*, alone; − methyl salicylate (MeSA), − Coriander], plots with bean plants baited with MeSA (+ MeSA, − Coriander), plots with bean plants intercropped with coriander (− MeSA, + Coriander), and plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

but not by MeSA, in both years (2015–2016) (Table 2; Fig. 2a and e). There was a marginal (in 2015) and significant (in 2016) MeSA-by-coriander interaction (Table 2), where the effect of coriander on ladybeetle abundance was similar when alone and when in combination with MeSA (Fig. 2a and e). MeSA and coriander increased independently, in an additive manner, the abundance of hoverflies, *Toxomerus* sp., in 2015 (Table 2; Fig. 2b). In 2016, MeSA also increased the abundance of hoverflies (Table 2), and interacted with coriander (i.e. non-additive effect; Table 2; Fig. 2f). Coriander, but not MeSA, increased the abundance of earwigs as compared with controls, and there was no MeSA × coriander interaction (Table 2; Fig. 2c). Coriander also increased the abundance of predatory stink bugs (*Podisus* sp.); however, there was a significant MeSA × coriander interaction effect on these predators (Table 2; Fig. 2d), indicating that MeSA influenced the coriander treatment in a non-additive manner. Although there were no effects of MeSA or coriander alone on the abundance of the predatory thrips *F. vespiformis*, they interacted to affect their abundance (significant MeSA × coriander interaction; Table 2; Fig. 2g). MeSA increased also the abundance of the minute pirate bug, *O. insidiosus* (Table 2; Fig. 2h). There were no effects of treatment on the abundances of lacewings, spiders, or ants (Table 2).

3.3 Herbivores

In 2015, the abundance of herbivores was not influenced by MeSA (Wilks' $\lambda = 0.98$; $F = 0.67$; df = 1, 102; $P = 0.56$) or coriander (Wilks' $\lambda = 0.96$; $F = 1.04$; df = 1, 102; $P = 0.37$), but it was influenced by their interaction (Wilks' $\lambda = 0.92$; $F = 2.65$; df = 1, 102; $P = 0.05$) (Fig. S1c). The first two PCs of the PCA for 2015 explained ~71% of the variance and showed differences in the composition of herbivore communities among treatments (Fig. S2c). The first PC explained 39% of the variation and separated the MeSA treatment from the control treatment, whereas the second PC explained 32% of the variation and partially separated the control treatment from the coriander and MeSA + coriander combined treatment. In 2016, coriander marginally (Wilks' $\lambda = 0.94$; $F = 2.41$; df = 1, 126; $P = 0.06$), but not MeSA (Wilks' $\lambda = 0.96$; $F = 1.39$; df = 1, 126; $P = 0.24$), influenced herbivore abundance; there was no MeSA × coriander interaction (Wilks' $\lambda = 0.97$; $F = 1.07$; df = 1, 126; $P = 0.36$) (Fig. S1f). In 2016, the first two components of the PCA explained ~83% of the variation in herbivore communities among treatments (Fig. S2f). The first PC explained 48% of the variation and partially separated the coriander from the control treatment, while the second PC explained 35% of the variation and moderately separated the coriander and MeSA treatments from the control treatment.

When we analyzed each of the herbivores separately, the abundance of spider mites (*Tetranychus* sp.) was significantly lower in the MeSA and coriander treatments, as well as in the combined MeSA + coriander treatment (significant MeSA-by-coriander interaction), as compared with the control treatment (Table 3; Fig. 3a). A similar pattern was observed for the abundance of the phytophagous thrips *Neohydatothrips* sp.; however, treatment effects were only marginal for the MeSA × coriander interaction (Table 3; Fig. 3b). In 2016, the abundance of leaf beetles (*Diabrotica* sp.) was higher in the MeSA plots but lower in the coriander plots as compared with the control plots; the effects of MeSA and coriander were additive in the combined treatment (no MeSA-by-coriander interaction; Table 3; Fig. 3c). There was no effect of treatment on

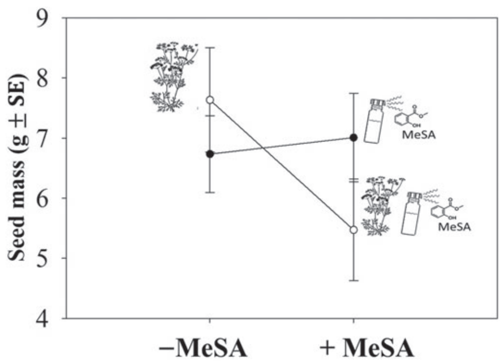

Figure 6. Mass of common bean, *Phaseolus vulgaris*, seeds harvested in 2016 from control plots [− methyl salicylate (MeSA), − Coriander], plots with bean plants baited with MeSA (+ MeSA, − Coriander), plots with bean plants intercropped with coriander (− MeSA, + Coriander), and plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

leaf beetle abundance in 2015, or an effect of treatment on the abundance of phytophagous stink bugs in any of the sampled years (Table 3).

In 2015, as compared with the control treatment, low populations of spider mites in the coriander treatment were correlated with high populations of ladybeetles, hoverflies, and predaceous pentatomids (Table S1). Low populations of chrysomelids (leaf beetles) in the MeSA treatment correlated with high numbers of earwigs. In the MeSA + coriander treatment, low populations of spider mites correlated with high numbers of hoverflies, while low populations of chrysomelids correlated with high populations of ladybeetles and earwigs. In 2016, the abundance of phytophagous thrips negatively correlated with the abundance of predatory thrips in both the coriander and MeSA + coriander treatments (Table S2). Interestingly, there was a positive correlation between the abundance of ladybeetles and two herbivores (chrysomelids and phytophagous pentatomids) in the MeSA treatment (Table S2), indicating that populations of both the natural enemy and these herbivores increased in this treatment as compared with the control. No other natural enemy/herbivore correlation was significant (Tables S1 and S2).

3.4 Aphid predation rate

In 2015, coriander increased significantly aphid predation rates ($F = 4.75$; df = 1, 47; $P = 0.03$) (Fig. 4a). There was no effect of MeSA alone ($F = 0.31$; df = 1, 48; $P = 0.57$) or in combination with coriander (MeSA× coriander interaction: $F = 2.99$; df = 1, 46; $P = 0.09$) on aphid predation rates (Fig. 4a).

In 2016, although coriander also increased aphid predation rates by ~1.2× as compared with the control, this effect was not significant ($F = 1.90$; df = 1, 47; $P = 0.17$) (Fig. 4b). There were no effects of MeSA alone ($F = 0.03$; df = 1, 48; $P = 0.84$) (Fig. 4b) or in combination with coriander ($F = 0.36$; df = 1, 46; $P = 0.55$) on aphid predation rates (Fig. 4b).

3.5 Herbivore damage

The damage to bean leaves caused by spider mites was significantly reduced by the MeSA ($F = 6.00$; df = 1, 104; $P = 0.01$) (Fig. 5) and coriander ($F = 7.14$; df = 1, 103; $P = 0.008$) treatments. There was also a significant MeSA × coriander interaction effect ($F = 4.60$; df = 1, 102; $P = 0.03$), such that MeSA affected spider mite damage similarly when alone as when in combination with coriander (Fig. 5).

3.6 Crop yield and dry mass

There were no effects of MeSA (Wilks' $\lambda = 0.96$; $F = 0.10$; df = 1, 18; $P = 0.98$), coriander (Wilks' $\lambda = 0.78$; $F = 0.77$; df = 1, 18; $P = 0.58$), or their interaction (Wilks' $\lambda = 0.85$; $F = 0.48$; df = 1, 18; $P = 0.78$) on any of the yield parameters in 2015. Similarly, there was no effect of MeSA (Wilks' $\lambda = 0.55$; $F = 2.26$; df = 1, 18; $P = 0.10$) or coriander (Wilks' $\lambda = 0.73$; $F = 1.01$; df = 1, 18; $P = 0.44$) on yield parameters in 2016; however, there was a significant interaction effect (Wilks' $\lambda = 0.47$; $F = 3.03$; df = 1, 18; $P = 0.04$).

To better explain these subtle effects of treatment on yield parameters, we conducted univariate statistical analysis for each of the parameters. There were no effects of treatment on any of the yield parameters in 2015 (Table 4). However, in 2016, there were significant MeSA and MeSA-by-coriander interaction effects on the mass of seeds (Table 4). Seed mass was lower in the MeSA + coriander combined treatment than in the coriander treatment alone, indicating that the mass of seeds was decreased by MeSA but only in the presence of coriander (Fig. 6). No other yield parameters were influenced by treatment in 2016 (Table 4).

4 DISCUSSION

Both herbivore-induced plant volatiles (HIPVs) and companion plantings, as a sole strategy or combined in an attract-and-reward approach, have been used to manipulate the behaviors of natural enemies in agro-ecosystems.[7,61] In the present study, we tested whether these behavioral manipulation tactics lead to changes in ecosystem structure and increases in ecosystem function and services provided by natural enemies. We demonstrated the following. (1) The HIPV MeSA, alone or in combination with coriander as a companion plant, increased the abundance of arthropods. These manipulative approaches increased natural enemy abundance (in both years), which supports criterion 1 outlined above that these strategies need to attract natural enemies and enhance their abundance. (2) MeSA and coriander, alone or combined, reduced populations of two important herbivores of common beans, spider mites and thrips, and increased aphid predation rates, which provides support for criterion 2 that these strategies need to enhance natural enemy ecosystem function. (3) Although in one of the years we found reduced herbivore damage to bean plants in the MeSA, coriander, and combined treatments, these effects did not cascade down to increase crop productivity (i.e. bean biomass and yield); thus, we found little support for criterion 3 that attraction of natural enemies to MeSA and/or coriander increases their ecosystem services.

The abundance of hoverflies (*Toxomerus* sp.) and minute pirate bugs (*O. insidiosus*) increased in MeSA-baited bean plots. The plant hormone salicylic acid (SA), the non-volatile analog of MeSA, is often induced in response to feeding by piercing-sucking herbivores like aphids.[62] Thus, it is not surprising that predators of aphids like hoverflies and minute pirate bugs responded positively to our MeSA treatment. While SA is induced by piercing-sucking herbivores, other herbivores (particularly those with different feeding habits like chewers) might not induce SA,[63] resulting in specificity in the HIPVs they induce.[64] The fact that not all predators in our study were affected in the same way by the addition of MeSA supports this specificity. Our results are consistent with previous studies using MeSA-baited sticky traps to assess attraction of natural enemies to MeSA. For example, MeSA-baited traps captured higher numbers of Syrphidae species than unbaited traps in cranberries,[26] soybeans,[11,32] and hops.[29] Similarly, MeSA-baited traps captured more *Orius similis* Zheng in cotton[65] and *Orius* *tristicolor* White in strawberries[25] than unbaited traps. MeSA is not ubiquitously produced by all plant species, or in the same amounts, but is emitted in significant amounts by bean (*Phaseolus* spp.) plants, including *Ph. vulgaris*.[37–39] Therefore, the applicability of MeSA may depend on the crop plant species.[66] In fact, volatiles emitted from these crops could influence the response of natural enemies to MeSA.[39] MeSA could have influenced recruitment of natural enemies in two ways: (a) directly, by natural enemy attraction to the dispensers emitting MeSA, or (b) indirectly, by MeSA inducing the release of volatiles from neighboring bean plants to increase natural enemy attraction. For example, lima bean plants exposed to MeSA emit volatile blends similar to the blend emitted by mite-infested bean plants.[67] Rodriguez-Saona *et al.*[26] also demonstrated that exposure to MeSA increases the emission of MeSA from cranberry vines. Future studies need to disentangle these two possibilities by using mutant plants that do not respond to HIPVs and herbivore feeding. Regardless of the mechanism, however, our study shows an attraction of natural enemies to plants baited with MeSA.

Intercropping bean plants with coriander also increased the abundance of hoverflies, but this effect was inconsistent among years. Morris and Li[68] also found that coriander used as a companion plant together with cabbage attracted large numbers of syrphid flies. Another study showed that intercropping carrot with coriander increased syrphid abundance compared with carrot alone.[48] Ladybeetle abundance was also higher in bean plots intercropped with coriander. Previous studies showed that coriander plants intercropped with carrot,[48] tomato,[34] cabbage,[33] and eggplant[49] had higher abundance and diversity of coccinellids species than their respective monocrops, likely as a result of the attractiveness, and suitability as food, of coriander flowers for adult ladybeetles. Coccinellids also have an innate attraction to volatiles from coriander at the vegetative stage.[52] Thus, although we used coriander as a 'reward' plant for natural enemies, this aromatic plant can attract natural enemies via emissions of constitutive leaf and floral volatiles. As a result, in our studies, constitutive volatiles and floral resources from coriander plants could have increased natural enemy recruitment. Other natural enemies influenced by coriander include predatory pentatomids and earwigs. Patt *et al.*[49] also found numerous predatory pentatomids in eggplant intercropped with coriander. Togni *et al.*[69] showed a positive impact of coriander on earwigs, where Forficulidae populations were greater in tomato intercropped with coriander than in tomato monocultures. In our study we added coriander while keeping the number of bean plants constant across all treatment plots. As a result, plots with coriander had more plants and higher diversity, which could explain the differences in natural enemy abundances. Future studies should test if our findings are the same under constant plant densities and variable degrees of plant diversity.

The combination of MeSA and coriander, in an attract-and-reward approach, affected the abundance of natural enemies in different, and often unexpected, ways. In general, as shown visually by the PCA, combining MeSA and coriander resulted in a natural enemy community that was a fusion of the communities found in the MeSA and coriander only treatments, resulting in natural enemy abundances that were similar to those found in the individual treatments (i.e. non-additive effects). An exception was observed in 2015 where hoverfly abundance increased in an additive manner in the combined MeSA + coriander treatment. In contrast, the abundance of predatory thrips, *F. vespiformis*, slightly decreased in the combined treatment as compared with the single treatments, suggesting that coriander could interfere with this predator's attraction to MeSA. Some evidence shows that thrips can be repelled by coriander (e.g. Gomes *et al.*[70]); thus, this interference could have been mediated by volatiles emitted from coriander. Orre Gordon *et al.*[20] also reported that attraction of syrphids to MeSA was inhibited when brassicas were combined with buckwheat as a companion plant. So far, our results and previous findings[18–20] suggest that HIPV and companion plants, as an attract-and-reward strategy, do not act synergistically to enhance the abundance of natural enemies in agricultural systems.

The abundance of herbivore communities was also affected by our manipulative treatments. MeSA and coriander reduced spider mite (*Tetranychus* sp.) and thrips (*Neohydatothrips* sp.) populations. In contrast, MeSA increased, and coriander reduced, populations of a leaf beetle (*Diabrotica* sp.). Spider mites, thrips, and leaf beetles are important pests of common bean, *Ph. vulgaris*, in Brazil.[71–73] Interestingly, we found that, in 2015, populations of spider mites were negatively correlated with populations of ladybeetles, hoverflies, and predaceous pentatomids. In fact, spider mites are suitable prey for many coccinellids[74,75] and syrphids,[76] but we found no reports in the literature that predatory stink bugs can utilize spider mites as a prey item. In 2016, populations of phytophagous thrips (*Neohydatothrips* sp.) were negatively correlated with the abundance of predatory thrips (*F. vespiformis*), and, although the effect was not significant, they also seemed to be associated with the abundance of predatory minute pirate bugs (*O. insidiosus*) (see Fig. 3b versus Fig. 2h). Previous studies have shown the importance of *F. vespiformis* in regulating thrips populations.[77,78] Thrips are also suitable prey for minute pirate bugs.[79,80] For instance, Resende *et al.*[53] associated low abundance of various thrips species, including *Frankliniella* sp., *Haplothrips gowdeyi* Franklin, *Thrips tabaci* Lindeman, and *Neohydatothrips* sp., in coriander to high abundance of *O. insidiosus*. The effects of our manipulative treatments on leaf beetles are less clear. Populations of leaf beetles were affected negatively by coriander but positively by MeSA, but there was no clear link of this to predator abundance. The attraction of ladybeetles and predatory earwigs to coriander in combination with MeSA was negatively correlated with the abundance of leaf beetles; however, whether these predators reduced leaf beetle populations remains unknown. Ladybeetles, hoverflies, and pirate bugs are predators of aphids. Although aphids were not abundant in our plots, we placed sentinel aphids to assess predation rates. Coriander increased aphid predation, probably as a consequence of an increase in ladybeetle abundance; yet, the contribution of other predators to aphid predation cannot be discounted. Similar to our findings with natural enemies, combining MeSA and coriander in an attract-and-reward approach had a non-additive, non-synergistic effect on ecosystem function, where the abundance of herbivores (e.g. spider mites and thrips), as well as aphid predation rates, was similar in the single treatments as compared with the combined treatment. Only the effect on leaf beetles was additive, where their abundance in MeSA-baited plots was reduced in the presence of coriander.

In addition to increased suppression of herbivore populations as a consequence of natural enemy recruitment, herbivore abundance in our manipulative plots could have been affected by changes in volatile emissions or by changes in the quality of the host plant. For instance, herbivores could have been repelled, or attracted, by MeSA itself and/or by volatiles emitted from coriander or from bean plants exposed to MeSA and/or coriander. Future studies need to be conducted where natural enemies are excluded to determine the relative contributions of natural enemy-mediated and plant-mediated effects of HIPVs on herbivore suppression. A negative outcome of using strategies to manipulate natural enemy behavior in agro-ecosystems could be an unintended increase in herbivore populations as a result of their attraction to HIPVs. In our case study, the abundance of chrysomelid leaf beetles in MeSA-treated plots was higher than in control plots in 2016. Simpson *et al.*[18,19] also found attraction of thrips to MeSA in vineyards. Moreover, Hammack[81] showed that the chrysomelid *Diabrotica virgifera virgifera* Leconte is more attracted to the HIPVs MeSA and linalool when combined than when alone, indicating that MeSA can interact with other HIPVs for increased herbivore attraction. Interestingly, coriander plots had a lower number of leaf beetles as compared with the control plots and the presence of coriander ameliorated the potential ecological risk of attracting a herbivore such as leaf beetles to MeSA-treated plots. Gomes *et al.*[70] also reported that coriander intercropped with tomato reduced the abundance of thrips, possibly as a consequence of a repellent effect. MeSA could have also activated defenses in bean plants against herbivores that resulted in their reduction in MeSA-baited plots. For example, application of SA activates induced systemic resistance and reduced spider mite *Tetranychus urticae* Koch populations in bean plants (*Ph. vulgaris*).[82] In choice assays, *Frankliniella occidentalis* Pergrande was repelled by bean and cucumber leaves treated with MeSA; MeSA also increased resistance against this thrips.[83] Therefore, additional studies are needed to elucidate the mechanisms underlying the observed impacts of MeSA on herbivore abundance in the field.

In 2015, reduction in the abundance of spider mites in the MeSA, coriander, and MeSA + coriander combined treatments resulted in less leaf damage to bean plants. This reduction in herbivore damage did not, however, translate to increases in crop yield, possibly because the difference in damage among treatments was not big enough to cause yield losses. Previous studies have shown that reduction of spider mite populations, via SA-mediated induction of plant defenses, increases yield in common bean plants.[82] Bean plants can, however, tolerate high amount of damage.[35] For example, Blue *et al.*[84] found that mechanical leaf damage reduced yield in lima bean plants; however, they reported no differences in above-ground biomass, number of pods, and number of seeds between 33% and 66% defoliation. Besides direct feeding damage by herbivores, factors such as competitive interactions with the companion plant or phytotoxicity effects of MeSA could have weakened the bean plants and, as a result, masked any positive effects of our natural enemy manipulation treatments on crop yield. Another possible explanation for this lack of effects on crop yield is sample size; thus, in 2016, we increased by 4-fold the number of harvested bean plants. In 2016, we only found an interactive effect of MeSA and coriander on seed mass. Although we did not observe any noticeable evidence of damage caused by leaf beetle (Chrysomelidae) feeding, a possible explanation for the effects of treatment on seed mass could be the lower abundance of leaf beetles (Chrysomelidae) in the coriander treatment, while abundance was higher in the MeSA treatment. Leaf beetle damage could have caused a decrease in photosynthetic activity, lack of nutrients, and/or a decrease in water intake, leading to lower mass of bean seeds.[84] Regardless of the mechanism and sample size, the overall effects of our treatments on the various yield parameters measured were largely minimal for both years.

In conclusion, in agreement with previous studies, we found strong support for the hypothesis that manipulation of HIPVs and companion plants, separately or together, increases natural enemy abundance. However, combining MeSA and coriander did not interact synergistically in an attract-and-reward approach. HIPVs and companion plants also caused an effect, mostly negative, on herbivores. We found lower herbivore abundance on bean plants baited with MeSA, coriander, or both – an indication of increased natural enemy function. However, we found no support for the hypothesis that these effects cascade down to increase crop yield. Simpson *et al.*[19] also found no effects of combining HIPVs (MeSA and methyl anthranilate) and buckwheat in vineyards on grape yield. In contrast, Wang *et al.*[21] found that an attract-and-reward that combines MeSA with oilseed rape (*B. napus*) increased natural enemy abundance, reduced aphid densities, and increased yield in wheat. In our studies, we used relatively small size plots; therefore, it is likely that insects were able to move among them and select those that attracted them the most, similar to a multiple choice set-up. At the moment it is difficult to assess the distance at which our plots would act independently because the range of attraction of coriander and MeSA is unknown; future studies are needed to determine if plot size affects the outcome of using strategies to manipulate natural enemy behavior.[85]

Our findings have important implications for conservation biological control of agricultural pests. We showed that the use of HIPVs alone or with a companion plant could increase natural enemy abundance and their function. However, our study together with others[18–20] provides no evidence that combining two strategies in an attract-and-reward approach enhances natural enemy abundance more than the use of a single strategy alone. Thus, the benefits of combining these two strategies to manipulate natural enemy behavior need further investigation. Furthermore, there is limited evidence so far that an increase in natural enemy abundance by HIPVs (alone and with companion plants) enhances ecosystem services. Future studies need to address if, and under what circumstances, integrating these two tactics to enhance biological control in agro-ecosystems may cascade down to improve crop productivity.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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