UNIVERSIDADE FEDERAL DE LAVRAS

JORDANO SALAMANCA BASTIDAS

USE OF HERBIVORE-INDUCED PLANT VOLATILES FOR MANIPULATING NATURAL ENEMIES IN AGRO-ECOSYSTEMS

LAVRAS – MG
2016

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

Dra. Brígida Souza
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USE OF HERBIVORE-INDUCED PLANT VOLATILES FOR MANIPULATING NATURAL ENEMIES IN AGRO-ECOSYSTEMS

USO DE VOLÁTEIS DE PLANTAS INDUZIDOS PELA HERBIVORIA PARA A MANIPULAÇÃO DOS INIMIGOS NATURAIS EM AGROECOSSISTEMAS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

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A minha mãe Gloria Del Rosario Bastidas (*in memoriam*), por dar-me a vida, pelos momentos de alegria, por seu amor, apoio e confiança incondicional. Amo-te demais e você está sempre em meu coração.

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SINCERAMENTE AGRADEÇO

GENERAL ABSTRACT

Herbivore-induced plant volatiles (HIPVs) play an important role in the attraction of natural enemies of herbivores in agro-ecosystems. One such HIPV emitted by several plant species and often induced after herbivore attack is methyl salicylate (MeSA). This compound has been shown to attract biological control agents, including members of the families Coccinellidae, Chrysopidae, Syrphidae, Geocoridae, and Anthocoridae, in many agricultural crops. In addition, MeSA combined with companion plants in an attract-and-reward approach can enhance natural enemy ecosystem function. However, little is known whether or not manipulation of natural enemies by MeSA results in a reduction of pest populations, and ultimately results in higher crop yield. For this reason, in this work, I conducted studies to address the following two main hypotheses: (1) *Hippodamia convergens* Guérin-Méneville and *Chrysoperla rufilabris* Burmeister, two agriculturally-important predatory insects, respond physiologically and behaviorally to MeSA, which may lead to increased predation and oviposition, and (2) attraction of natural enemies to MeSA, alone or in combination with coriander (*Coriandrum sativum* L.) as a companion plant reduces pest populations and increases crop productivity in common bean (*Phaseolus vulgaris* L.). To test for my first hypothesis, I conducted laboratory studies using electro-antennography (EAG), behavioral studies in the greenhouse using cages and wind tunnels, and mark-release-recapture studies to investigate *H. convergens* and *C. rufilabris* attraction to MeSA over various distances in cranberry fields. I found that female *H. convergens* and *C. rufilabris* antennae can detect MeSA. Also, *H. convergens* females were attracted to, and arrested by, MeSA; this attraction increased predation of *Ostrinia nubilalis* Hübner eggs. Attraction of *C. rufilabris* females to MeSA also increased oviposition. In field studies, higher numbers of marked *H. convergens* and *C. rufilabris* were caught on yellow sticky cards placed close (0-30 m) to the predator release site than at further distances, regardless of whether the cards were baited with MeSA or not. For my second hypothesis, I used visual sampling to count the number of arthropods (natural enemies and herbivores) on (a) bean plants alone, (b) bean plants baited with MeSA, (c) bean plants intercropped with coriander, and (d) bean plants baited with MeSA and intercropped with coriander throughout two growing seasons (2015-2016). Sentinel aphids were also used as a measure of ecosystem function (i.e. predation). Plant damage and biomass as well as the number and weight of pods and seeds were measured as a proxy for ecosystem services. *At the natural enemy level*, I found that predators from six different insect families were attracted to MeSA and coriander, when alone or in combination. *At the herbivore level*, spider mite and thrips populations were reduced with MeSA with or without coriander; however, MeSA increased, while coriander reduced, leaf beetle (Chrysomelidae) abundance. Coriander increased aphid predation rate in both years 2015 and 2016. *At the plant level*, MeSA and coriander reduced damage by spider mites. However, the effects of MeSA and/or coriander on crop biomass and yield were mostly non-significant. Altogether, my results have important implications for conservation and augmentative biological control. They demonstrate that predator responses to HIPVs can be complex in real agricultural settings because laboratory/greenhouse results do not necessarily translate to the field, and that manipulation of natural enemies may not enhance their ecosystem services as increases in natural enemy abundance did not cascade down to increase crop productivity.

Keywords: Conservation biological control. *Chrysoperla rufilabris. Hippodamia convergens.* Methyl salicylate. Tritrophic interactions. Mark-release-recapture. Ecosystem services.

RESUMO GERAL

Voláteis de plantas induzidos pela herbivoria (HIPVs) tem um papel importante na atração de inimigos naturais dos herbívoros nos agroecossistemas. Salicilato de metila (MeSA) é um HIPV emitido por diversas espécies de plantas e induzido após o ataque dos herbívoros. Este composto tem mostrado atratividade de predadores das famílias Coccinellidae, Chrysopidae, Syrphidae, Geocoridae e Anthocoridae em diferentes culturas agrícolas. Além, o MeSA combinado com plantas companheiras enfocando-se na atração/recompensa pode aumentar as funções dos inimigos naturais nos agroecossistemas. No entanto, pouco é conhecido sobre a manipulação dos inimigos naturais pelo MeSA, visando reduzir populações de pragas, resultando assim na alta produtividade das culturas. Neste sentido, foram avaliadas as seguintes hipóteses: (1) *Hippodamia convergens* Guérin-Méneville e *Chrysoperla rufilabris* Burmeister, dois importantes insetos predadores tem uma resposta fisiológica e comportamental ao MeSA, levando ao incremento da predação e oviposição, e (2) a atração dos inimigos naturais ao MeSA sozinho ou combinado com coentro (*Coriandrum sativum* L.) como uma planta companheira, reduz populações de pragas e incrementa a produtividade do feijão (*Phaseolus vulgaris* L.). Para avaliar a primeira hipótese foram conduzidos estudos no laboratório usando o eletroantenograma (EAG), estudos de comportamento em casa de vegetação usando gaiolas e túnel de vento e estudos de marcação liberação e recaptura para avaliar a atração de *H. convergens* e *C. rufilabris* ao MeSA em diferentes distancias em cranberries. Foi encontrado que as antenas de fêmeas de *H. convergens* e *C. rufilabris* podem detectar o MeSA. Ainda fêmeas de *H. convergens* foram atraídas e detidas pelo MeSA e está atração incrementou a predação de ovos de *Ostrinia nubilalis* Hübner. O MeSA também atraiu as fêmeas de *C. rufilabris* incrementando sua oviposição. No campo, números maiores de *H. convergens* e *C. rufilabris* marcados foram capturados em armadilhas amarelas pegajosas colocadas perto (0-30 m) do lugar de liberação dos predadores que a longas distancias, independentemente se as armadilhas foram ou não iscadas com MeSA. Para a segunda hipótese, foram realizados monitoramentos visuais para contar o número de artrópodes (inimigos naturais e herbívoros) nas (a) plantas de feijão sozinhas, (b) plantas de feijão com MeSA, (c) plantas de feijão consorciadas com plantas de coentro e (d) plantas de feijão com MeSA e coentro durante toda a safra no 2015 e 2016. Afídeos sentinela foram usados para medir a função no ecossistema (predação). Dano e biomassa das plantas, como também o número e peso das vagens e sementes foram medidos como um representante dos serviços nos ecossistemas. *Ao nível dos inimigos naturais*, foram encontrados que predadores de seis família de insetos foram atraídos ao MeSA e coentro sozinhos ou combinados. *Ao nível dos herbívoros*, populações de ácaros e tripés foram reduzidas pelo MeSA com ou sem coentro, no entanto o MeSA incrementou a abundancia dos desfolhadores (Chrysomelidae) e o coentro influenciou sua redução. Coentro também incrementou a taxa de predação de afídeos no 2015 e 2016. *Ao nível da planta*, MeSA e coentro reduziram o dano pelos ácaros. No entanto, os efeitos do MeSA ou coentro na biomassa e a produção não foram significativas. De modo geral, estes resultados têm implicações no controle biológico conservativo e aumentativo. A resposta dos predadores aos HIPVs pode ser complexa em condições de campo. A manipulação dos inimigos naturais não pode aumentar os serviços dos ecossistemas no incremento da produtividade.

Palavras chave: Controle biológico conservativo. *Chrysoperla rufilabris. Hippodamia convergens.* Salicilato de metila. Interações tritróficas. Marcação-liberação-recaptura. Serviços ecossistêmicos.

SUMMARY

FIRST PART

GENERAL INTRODUCTION

Over time plants have evolved and improved different types of defenses against herbivore attack (PRICE et al., 2011; FÜRSTENBERG-HÄGG; ZAGROBELNY; BAK, 2013). Some of these defenses, including physical and chemical defenses, are constitutively present in plants. Physical defenses consist of morphological features such as spines, hairs, trichomes, thorns, and surface waxes (WAR et al., 2012). Chemical defenses include the production of secondary metabolites that are compounds that do not play a role in basic plant metabolism such growth, development and reproduction (e.g. alkaloids, glucosinolates, etc.) (HOWE; JANDER, 2008; HOPKINS; van DAM; van LOON, 2009; PRICE et al., 2011), and include protein defenses as protease inhibitors, α-amylase, lectins, and chitinasesnol oxidases (FALCO et al., 2001). All above-mentioned types of defenses can arrest, repel, or poison herbivores, or affect their growth rate and development (PRICE et al., 2011).

Other defenses are only induced after herbivore attack. These types of defenses include both physical (ALVES-SILVA; DEL-CLARO, 2016) and chemical defenses, and in this case, the plants increase the production of secondary metabolites, protease inhibitors, and toxins in response to herbivore feeding. Inducible indirect defenses comprise the emission of volatile organic compounds that may repel herbivores and attract carnivorous arthropods (DICKE; BALDWIN, 2010). These compounds are commonly called herbivore-induced plant volatiles (HIPVs), and involve mixtures of green leaf volatiles (C6 aldehydes, alcohols, and acetates) and terpenoids (monoterpenes and sesquiterpenes) (RODRIGUEZ-SAONA; BLAAUW; ISAACS, 2009; PICHERSKY; NOEL; DUDAREVA, 2006).

While physical (e.g. spines) and chemical defenses (e.g. protein defenses) are considered direct defenses, as they have a direct negative effect on the herbivore, HIPV production is considered both a direct and an indirect defense. Laboratory as well as field studies have shown that HIPVs can repel herbivores (DE MORAES; MESCHER; TUMLINSON, 2001), an example of a direct defense. Indirectly, HIPVs can protect plants from their enemies (herbivores) through the attraction of natural enemies such as predators and parasitoids (DICKE; SABELIS, 1988; PARÉ; TUMILSON, 1999; DICKE; BALDWIN, 2010). Thus, plants release HIPVs in relatively high quantities, offering detectability, reliability, and suitability to natural enemies to find their prey or hosts (DICKE, 1999; VET; DICKE, 1992).

An increasing number of studies have shown evidence of natural enemy attraction by HIPVs. For example, one of the first studies on this topic by Dicke and Sabelis (1988) showed under laboratory conditions that HIPVs released by spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae)-infested lima bean plants (*Phaseolus lunatus* L.), attracted the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). A second study showed that volatiles from corn seedlings (*Zea mays* L.) damaged by the armyworm larvae *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) emit volatile compounds that attract females of the parasitic wasp *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) (TURLINGS; TUMLINSON; LEWIS, 1990). Another early study, also performed under laboratory conditions, presented evidence that caterpillar-damaged cabbage plants (*Brassica oleracea* L.) are more attractive than undamaged plants to the larval parasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae), and concluded that volatile blends released by herbivore-damaged plants are a detectable and reliable source of information to this parasitoid to find its host (STEINBERG; DICKE; VET, 1993). Ninckovic, Abassi and Pettersson (2001) showed that the seven-spotted ladybeetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) responded to volatiles from aphids [*Rhopalosiphum padi* L. (Hemiptera: Aphididae)]-infested barley plants (*Hordeum vulgare* L.) in olfactometer bioassays. Other studies showed that HIPVs induced by *Tetranychus ludeni* Zacher (Acarina: Tetranychidae) in eggplant (*Solanum melongena* L.), okra (*Abelmoschus esculentus* L.), and peppers (*Capsicum annum* L.) attract the predatory lacewing *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) (REDDY, 2002). Clearly, various plant species emit HIPVs, and HIPVs are attractive to a wide range of predators and parasitoids in different families (KAPLAN, 2012; PINTO-ZEVALLOS et al., 2013).

Herbivore-damaged plants usually release multiple HIPVs, and in some occasions, a few compounds in these blends can be responsible for the attraction of natural enemies. For example, in the study described above by Dicke and Sabelis (1988), the authors identified four main volatile compounds from the headspace of *T. urticae*-infested plants: linalool, (*E*)-β-ocimene, 4,8-dimethyl-1,3(*E*),7-nonatriene, and methyl salicylate (MeSA). These four compounds were clearly associated with predator attraction in Y-tube tests. Hegde et al. (2011) found that methyl salicylate (MeSA), (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and 4,8,12-trimethyl-1,3,7,11-

tridecatetraene (TMTT) released by aphid [*Aphis gossypii* Glover (Hemiptera: Aphididae)]-infested cotton plants (*Gossypium hirsutum* L.) elicit the antennal responses of *Chrysoperla lucasina* Lacroix (Neuroptera: Chrysopidae), while (Z)-3-hexenyl acetate do not. Another study related methyl salicylate, a major component in the volatile blend of aphid [*Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae)]-infested rose plants (*Rosa* × *hybrida* L.), to oviposition of *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) females; the females showed a higher oviposition response to herbivore-infested plants than to uninfested plants (SALAMANCA et al., 2015).

There is an abundance of laboratory studies that use different techniques (e.g. electroantennogram, Y-tube olfactometer, 4-arm olfactometer, wind tunnel) to evaluate the effective responses of natural enemies to HIPVs (PRICE et al., 2011), and a wide variety of predators and parasitoids display attraction to HIPVs under these conditions. However, only a small number of studies show evidence of natural enemy attraction to HIPVs under realistic field conditions. Flint, Salter and Walters (1979) found that caryophyllene release in cotton crops increases *C. carnea* abundance. In tobacco (*Nicotiana attenuata* Torr), Kessler and Baldwin (2001) showed that three HIPVs (cis-3-hexen-1-ol, linalool, and cis-α-bergamotene) attract the predator *Geocoris pallens* Stäl (Hemiptera: Geocoridae), and that this attraction leads to an increase in *Manduca sexta* L. (Lepidoptera: Sphingidae) egg predation. Combining HIPVs, Braasch, Wimp and Kaplan (2012) found that methyl salicylate plus cis-3-hexen-1-ol, increased the attraction of the predator *Orius insidious* Say (Hemiptera: Anthocoridae) compared with either volatile alone in soybean (*Glycine max* L.) crops. Another study found evidence of significant attraction of *Orius tristicolor* White (Hemiptera: Anthocoridae) to (*Z*)-3-hexenyl acetate-baited hop crops (*Humulus lupulus* L.) (JAMES, 2003). Generally, most field studies have evaluated single HIPVs rather than volatile blends (KAPLAN, 2012), as it would be commercially interesting to find a single, easy-to-make, and cheap compound to manipulate natural enemy behavior. As mentioned above, it is known that not all volatile compounds in the plants' headspace are necessary to elicit a response from natural enemies. There is incredible potential for the use of HIPVs to manipulate the behavior of natural enemies in agro-ecosystems, as a method of sustainable pest control.

One of the most frequently used single HIPVs in agro-ecosystems is methyl salicylate (MeSA), a volatile emitted by many plant species. It is commonly induced by arthropod herbivores with different feeding habits, including sucking aphids and chewing beetles (NINKOVIC et al., 2003; SALAMANCA et al., 2015; BOLTER et al., 1997). MeSA is commercially available in different presentations and formulations, and serves a variety of functions. For example, it is an ingredient in a botanical insecticide spray (EcotrolTM, Ecosmart Technologies, Alpharetta, GA), it is available in slow-release dispensers to attract natural enemies of various agricultural pests (Predalure, AgBio, Westminster, CO), and is sold as an odor masking agent to mask undesirable pesticide odors (Odor-Mask, Monterey AgResources, Fresno, CA) (WOODS et al., 2011). Sigma-Aldrich also sells MeSA in bottles of different capacity. For agricultural or experimental use, this compound is usually placed in polyethylene vials to be released in the field.

Recent studies showed that MeSA is attractive to several families of natural enemies when released in different crops. For example, in strawberry (*Fragaria × ananassa* Duch), MeSA-release (PredaLure) increased the abundance of Chrysopidae and Anthocoridae species (LEE, 2010). James (2003) found that MeSA released in controlled-release dispensers in hop crops attract significant numbers of *G. pallens*, as well as Syrphidae species. In cranberry (*Vaccinium macrocarpon* Ait.), Rodriguez-Saona et al. (2011) showed that MeSA-baited cards (PredaLure) attracted numerous Coccinellidae and Chrysopidae species. Similarly, adults of *C. septempunctata* were significantly attracted to MeSA (controlled-release dispensers)-baited soybean crops (ZHU; PARK, 2005).

The above-mentioned studies show the importance of MeSA to increase the abundance of natural enemies in crop fields. However, only a few studies have so far provided evidence that this attraction increases predation or actual pest regulation. For example, Mallinger, Hogg and Gratton (2011) found that MeSA-release in soybean crops attract natural enemies, including Syrphidae and Chrysopidae species, and that this attraction leads to reduced soybean aphid [*Aphis glycines* Matsumura (Hemiptera: Aphididae)] populations. MeSA-release in hop crops attracted numerous *Stethorus* spp. (Coleoptera: Coccinellidae) individuals and this attraction led to reduced spider mite populations (JAMES; PRICE, 2004; WOODS et al., 2011). Importantly, other studies did not show an effect of MeSA on pest numbers, even though natural enemy numbers were increased (LEE, 2010; GADINO; WALTON; LEE, 2012).

Despite the promising results of MeSA as an attractant of natural enemies, the use of this HIPV under field conditions needs special attention, because it could potentially be disrupting biological control. Constitutively present MeSA could confuse natural enemies in the process of finding prey or host, and it could attract more herbivores into the crops (KAPLAN, 2012). Recent studies are combining strategies to counteract these negative effects. For example, Simpson et al. (2011) tested an “attract and reward” approach, and showed that MeSA (attractant) in combination with buckwheat, *Fagopyrum esculentum* Moench (food source as reward, and potential companion plant) in broccoli crops (*Brassica oleracea* L. cv. italica) increase the abundance of scelionid wasps, egg parasitoids of *Helicoverpa* spp. (Lepidoptera: Noctuidae). Wang et al. (2011) tested a similar approach in a system consisting of MeSA (attract) and the companion plant oilseed rape (*Brassica napus* L.) (as reward: alternative resources such as floral nectar and/or pollen) in a wheat crop (*Triticum aestivum* L.). The authors demonstrated an increased attraction of coccinellid species in the combination treatments, as well as a reduction of *Sitobion avenae* Fabricius (Hemiptera: Aphididae) aphids. Overall, this led to increased quality and weight of grains.

There is imminent potential for the use of HIPVs, especially MeSA, in agro-ecosystems to attract natural enemies, reduce pest populations, and increase crop yield. However, more research is needed to better understand the use of MeSA on manipulation of natural enemies to enhance their ecosystem function and services in agro-ecosystems. This thesis aims to evaluate the effectiveness of MeSA, alone or in combination with companion plants, to attract natural enemies. Also, this thesis aims to assess whether MeSA, besides attracting natural enemies, also enhances their ecosystem function and services in terms of reducing pest populations, and increase crop yield. Under laboratory, greenhouse, and field conditions, I studied natural enemy responses to MeSA in 2014, 2015, and 2016. In both 2014 and 2015, I evaluated whether the convergent ladybeetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), and the green lacewing, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), can detect and are attracted to, and arrested by, MeSA; and whether attraction to MeSA leads to increased predation and predator oviposition. These predators were used, because several studies (some of them described above) showed the powerful attraction of MeSA on Coccinellidae and Chrysopidae species. In addition, I assessed the range of attraction of *H. convergens* and *C. rufilabris* to MeSA (PredaLure)-baited sticky cards in cranberries. Cranberries have an economic importance in North America; they originate in New Jersey and are dominant in the Northeastern United States (AVERIL; SYLVIA, 1998). In 2015 and 2016, I evaluated the combination of

MeSA (controlled-release dispensers) with *Coriandrum sativum* L. in an attract-and-reward scenario to reduce pest populations and increase yield in bean plants (*Phaseolus vulgaris* L.). Bean is an important crop in Brazil, which is one of the largest producers in the world (GRAHAM; RANALLI, 1997; CABRAL et al., 2011). *C. sativum* is an important companion plant to attract natural enemies with attractive constitutive volatiles and providing food sources (pollen and/or nectar) (PATT; HAMILTON; LASHOMB, 1997; TOGNI et al., 2009, 2016; JANKOWSKA; WOJCIECHOWICZ-ŻYTKO, 2016). In the discussion, the data presented in this thesis are discussed in the context of conservation/augmentative biological control, and sustainable pest control in agro-ecosystems.

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SECOND PART – ARTICLES

ARTICLE 1 From laboratory to field: Electro-antennographic and behavioral responsiveness of two insect predators to methyl salicylate

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Abstract Methyl salicylate (MeSA) is a commonly-emitted herbivore-induced plant volatile (HIPV) known to attract insect predators in agricultural crops. However, thorough studies on whether MeSA can increase their ecological functioning or their attraction under field conditions are still lacking. Here, we conducted laboratory, greenhouse, and field studies to address the hypothesis that two agriculturally-important predatory insects (the ladybeetle *Hippodamia convergens* Guérin-Méneville and the lacewing *Chrysoperla rufilabris* Burmeister) respond physiologically and behaviorally to MeSA, which may lead to increased predation and oviposition. In laboratory studies using electro-antennography, we found that male and female *H. convergens* and *C. rufilabris* antennae can detect MeSA; however, for both species, female antennae responded more strongly. In greenhouse studies using cages and wind tunnels, *H. convergens* females were attracted to, and arrested by, MeSA; this attraction increased *Ostrinia nubilalis* sentinel egg predation. Attraction of *C. rufilabris* females to MeSA also increased oviposition. In field studies, mark-release-recapture experiments were performed to investigate *H. convergens* and *C. rufilabris* attraction to MeSA over various distances in cranberry fields. More *H. convergens* and *C. rufilabris* were caught on yellow sticky cards placed close (0-30 m) to the predator release site than at further distances, regardless of whether the cards were baited with MeSA or not. In conclusion, *H. convergens* and *C. rufilabris* adults detected, and were attracted to, MeSA under laboratory and greenhouse conditions, resulting in higher predation and oviposition, but this attraction was not observed under field conditions. These results, demonstrate that predator responses to HIPVs can be complex in real agricultural settings.

Keywords *Hippodamia convergens*, *Chrysoperla rufilabris*, Herbivore-induced plant volatiles, Electro-antennography (EAG), Mark-Release-Recapture, Cranberries.

Introduction

Plants increase emissions of volatiles after herbivore feeding damage, commonly known as herbivore-induced plant volatiles (HIPVs) (Dicke and van Loon 2000). These HIPVs are utilized by natural enemies, such as insect predators, to locate their prey (Vet and Dicke 1992). Methyl salicylate (MeSA) is an HIPV emitted from many plant species, particularly after attack by piercing-sucking herbivores (Dicke et al. 1990; Agrawal et al. 2002; Hegde et al. 2011; Melo Machado et al. 2014; Salamanca et al. 2015) that often serves as an important attractant to natural enemies of these herbivores in agro-ecosystems. For example, MeSA-baited sticky cards captured predatory insects of the families Anthocoridae, Chrysopidae, Coccinellidae, Syrphidae, Miridae, and Geocoridae in hops (James 2003), strawberries (Lee 2010), cranberries (Rodriguez-Saona et al. 2011a), soybeans (Mallinger et al. 2011), and vineyards (James and Price 2004, Gadino et al. 2012a). Despite the proven attraction of natural enemies to MeSA (e.g. De Boer and Dicke 2004; James and Price 2004; James and Grasswitz 2005; Zhu and Park 2005; Sznajder et al. 2010; Mallinger et al. 2011; Woods et al. 2011; Wang et al. 2011; Gadino et al. 2012b; Azandeme-Hounmalon et al. 2016; also see meta-analysis in Rodriguez-Saona et al. 2011a), this attraction has remained variable particularly in the field (Braasch et al. 2012); thus, there is the need for additional comprehensive studies that explore the response of natural enemies to MeSA, and that combine both laboratory and field studies under natural and agricultural conditions.

Ladybeetles (Coleoptera: Coccinellidae) are key predators regularly used in biological control of arthropod pests in many agricultural crops (Obrycki et al. 2009; Weber and Lundgren 2009), and some species are known to utilize MeSA as an olfactory cue for prey location. For example, *Coccinella septempunctata* L. showed electrophysiological (antennal) responses to MeSA emitted from aphid-infested soybean plants, and was highly attracted to MeSA-baited cards in the field (Zhu and Park 2005). Similarly, Gadino et al. (2012a) found that MeSA-baited cards placed in vineyards attracted *C. septempunctata*, *Stethorus* spp., and *Cycloneda polita* Casey. Rodriguez-Saona et al. (2011a) also showed that MeSA-baited cards attracted Coccinellidae species in cranberry fields. However, only few studies have shown whether this attraction leads to increased predation; an exception to this is a study by Woods et al. (2011) showing that *Stethorus* spp. are attracted to MeSA-baited cards in hop yards and that this attraction leads to reduced spider mite populations.

Similarly, lacewings (Neuroptera: Chrysopidae) are important predators of aphids, whiteflies, thrips, mites, and some caterpillars in agro-ecosystems (Principi and Canard, 1984; Carvalho and Souza, 2009), and several studies have shown attraction of lacewings adults to MeSA. For example, under field conditions, James (2003, 2006) found that MeSA-baited cards attracted *Chrysopa nigricornis* Burmeister adults in hop yards and *Chrysopa oculata* Say in vineyards, respectively. Lee (2010) and Rodriguez-Saona et al. (2011a) also found that Chrysopidae species were attracted to MeSA-baited cards in strawberry and cranberry fields, respectively. However, whether this attraction results in increased oviposition remains largely unknown. In a recent study, Salamanca et al. (2015) found that *Chrysoperla externa* Hagen adults are attracted to aphid-infested rose plants, and that this attraction increased the number of *C. externa* eggs on plants; the main compound identified as potentially mediating this tri-trophic level interaction was MeSA.

To further understand the responses of ladybeetles and lacewings to MeSA, in the present study, we tested the hypotheses that: (1) the convergent ladybeetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), and the green lacewing, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), can detect and are attracted to, and arrested by, MeSA; and (2) attraction to MeSA leads to increased predation and predator oviposition. Specifically, we conducted laboratory, greenhouse, and field studies to: (1) determine whether males and females of *H. convergens* and *C. rufilabris*, respond physiologically to different concentrations of MeSA using electro-antennography (EAG); (2) test whether different MeSA concentrations attract these predators, and if this attraction increases egg predation by *H. convergens* and increases oviposition by *C. rufilabris* in cage studies; (3) investigate whether MeSA arrests *H. convergens* and *C. rufilabris*, in more detailed behavioral (wind-tunnel) studies using video recordings; and, (4) measure the range of attraction of *H. convergens* and *C. rufilabris* to MeSA-baited sticky cards in a cranberry, *Vaccinium macrocarpon* Ait., field in a 2-year mark-release-recapture study using an immuno-marking technique.

Material and methods

Insects

Hippodamia convergens and *C. rufilabris* adults were purchased from ARBICO Organics (Oro Valley, AZ, USA) in 2014 and 2015. We used these predatory insect species because of their agricultural importance and because they are commercially available to farmers. Upon arrival, both insect predators were maintained in separate cages (30 × 30 × 30 cm) in the Entomology Laboratory at the P.E. Marucci Center, Rutgers University (Chatsworth, NJ, USA), under 24 ± 1°C, 65 ± 10% r.h., and 14:10 L:D photoperiod. *H. convergens* adults were fed with 1:4 yeast:honey, while *C. rufilabris* adults were fed with 1:1 yeast:honey. Eggs of the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) were purchased from Rincon-Vitova Insectaries (Ventura, CA, USA); these were used as sentinel eggs in predation experiments.

For electro-antennography (EAG) assays, we used *H. convergens* adults (males and females) starved for 24 h prior to the experiments; adult *C. rufilabris* males and gravid females were used. We considered a female to be gravid if its abdomen was swollen with eggs (see Fréchette et al. 2006). For greenhouse cage and wind tunnel assays, only *H. convergens* and *C. rufilabris* adult females were used because EAG responses to MeSA were similar (or even stronger) in females (see Results) for both species, and females need to make critical decisions during foraging for the survival of their offspring such as oviposition choice. For *H. convergens*, females were starved for 24 h before the experiments. For *C. rufilabris*, only gravid females were tested. In field experiments, adult males and females of *H. convergens* and *C. rufilabris* were used. All lab and greenhouse experiments were performed at the Entomology Laboratory at the P.E. Marucci Center, Rutgers University (Chatsworth, NJ, USA).

Electroantennogram (EAG) Assays

Antennal receptivity of *H. convergens* and *C. rufilabris* adults to five concentrations of MeSA (240826; Sigma-Aldrich, St. Louis, MO, USA): 0 (control; solvent alone), 1 µg/mL, 10 µg/mL, 0.1 mg/mL, and 1 mg/mL (neat) was determined by EAG (following methods similar to those described by Stelinski et al. 2003, Rodriguez-Saona et al. 2006). For *H. convergens* adults, dilutions were made with hexane (34859; Sigma-Aldrich) as solvent (Zhu and Park 2005); while for *C. rufilabris* adults, methylene chloride (650463; Sigma-Aldrich) was used as solvent (Zhu et al. 2005), because *C. rufilabris* antennae responded to hexane.

For *H. convergens*, the insect's head was carefully removed from the body. A capillary glass with reference electrode filled with physiological saline solution (7.5 g NaCl, 0.21 g $CaCl_2$, 0.35 g KCl, 0.2 g $NaHCO_3$ in 1 L H_2O) was inserted at the base of the head; whereas the tip of one, randomly chosen antenna, was inserted into the recording capillary glass electrode.

Humidified and charcoal-purified air was directed over the antenna through a 10-mm diameter glass tube. The odor source was released from Pasteur pipettes containing a piece of filter paper (Whatman No. 1; Whatman International Ltd, Maidstone, United Kingdom) prepared with 20 µL of each concentration or solvent (control) described above, which was exposed to air for 10 min prior to testing to allow solvent evaporation. The Pasteur pipette tip was placed into a holder located at the mid-point of the glass tube that provided the airflow to the antenna (0.5 L/min). The antennal response was amplified and recorded with a data acquisition controller and software (CS-05; Syntech, Hilversum, The Netherlands). Individual antennae received four stimulus pulses at 1-min intervals, and concentrations were tested in a random order. Fifteen male and fifteen female heads were tested for their response to each MeSA concentration.

For *C. rufilabris,* we used the same methodology as describe above except that we excised the antenna from the head and attached the electrodes to each end. Fifteen gravid female and fifteen male antennae were tested for their EAG response to each MeSA concentration. We considered a gravid female if its abdomen was swollen with eggs (see Fréchette et al. 2006).

Greenhouse Choice Tests

We conducted greenhouse choice experiments in cages to determine attraction of *H. convergens* and *C. rufilabris* females to MeSA and the effects of this attraction on egg predation and oviposition, respectively. Experiments were performed at 26 ± 1°C, 50 ± 10% r.h. and 14L:10D. We tested three MeSA concentrations that elicited strong EAG responses: 10 µg/mL, 0.1 mg/mL and 1 mg/mL (see Results).

Four artificial plants were placed inside 60 × 60 × 60 cm cages (Bugdorm-2120F; MegaView Science Co. Ltd, Taichung, Taiwan), diagonal from each other. Instead of natural plants, artificial plants were used to avoid any additional volatile cues. Each plant consisted of eight green-colored leaves, and was 30 cm tall. These plants were placed individually in plastic containers (250 mL) filled with sand. Two plants were baited with a septum (Precision Seal®, Sigma-Aldrich, St. Louis, USA) containing 20 µL of one of the MeSA concentrations, while the other two (controls) contained a septum with the solvent only (hexane for *H. convergens* or methyl chloride for *C. rufilabris*). Each septum was exposed to air for 10 min to allow for solvent evaporation before the tests.

For *H. convergens* choice assays, six adult females (that were starved 24 h before the assays) were released in the morning (10:00 h) in the middle of each cage, and their position on plants monitored every hour for 6 h/d for a total of 5 d. To measure egg predation all plants received one egg mass of *O. nubilalis*. The number of eggs per mass was counted before and after each experiment; eggs were examined under the microscope for signs of predation at the end of the experiment. *O. nubilalis* eggs were used as sentinel prey due to their commercial availability and because both ladybeetle adults and larvae and lacewing larvae can utilize these eggs for food (Richman et al. 1980; Musser and Shelton 2003). Each greenhouse choice assay was replicated 15 times for each MeSA concentration.

For *C. rufilabris* choice assays, eight gravid females were release in the morning (10:00 h) in the middle of each cage and their position on plants was monitored every hour for 6 h/d for a total of 5 d. To measure oviposition, the number of eggs on each plant was counted daily. Each greenhouse choice assay was replicated eight times for each MeSA concentration.

Wind Tunnel Assays

To further understand the behavioral response of *H. convergens* and *C. rufilabris* to MeSA, we conducted wind tunnel assays with video recordings. A concentration of 0.1 mg/mL MeSA was used in these studies because this concentration elicited physiological and behavioral responses in both predators in EAG and choice assays (see Results). Experiments were conducted in a greenhouse under the conditions described above. The experimental setup consisted of eight small wind tunnels made of 34 × 20 × 12 cm clear plastic containers (6.15 L ShoeBox; Iris USA Inc., Pleasant Prairie, WI, USA) filled with 1 cm of sand, with a 80 mm computer case fan (Antec Inc, Fremont, CA, USA) attached to one end and a 9 cm diam mesh at the other end to provide constant airflow; the top of the wind tunnels was covered with a transparent 35 × 25 cm Plexiglas sheet, so that the interior of the wind tunnels could be easily seen from above. Each wind tunnel was randomly assigned to one of two treatments; either MeSA or control. Above each wind tunnel, we placed a video camera (Night Owl, http://nightowlsp.com/), connected to an 8-channel DVR Surveillance System (Q-See, Anaheim, CA), and situated 10-15 cm above an odor station that was baited with either a septum (Precision Seal®, Sigma-Aldrich, St. Louis, MO, USA) containing 20 μL of 0.1 mg/mL MeSA or a septum containing 20 μL of the solvent alone (hexane or methylene chloride) (control). The odor station consisted of a single septum placed with a pin on a white 5 × 5 cm cardboard, positioned ~2 cm above the sand, and 5 cm from the fan.

Groups of eight (CO_2-anesthetized) *H. convergens* females or six gravid *C. rufilabris* females were released inside the wind tunnels, approx. 20 cm from the odor station. Upwind orientation of both predators to MeSA or control stations was video recorded for 6 h after their release (10:00-16:00 h). We recorded the total number of individual visits to the odor station (note: we could not differentiate between multiple versus single visits made by the same individual; thus, we report cumulative number of visits) and the time spent by each individual during a visit. The study was replicated nine times for each predator.

Release Rates. Release rates from each of the MeSA-treated septa used in the greenhouse choice assays described above (*n* = 3 per concentration) were measured by placing individual septa in 6 mL glass vials (Fisher Scientific Inc., Pittsburg, PA, USA). Headspace volatiles from vials were collected in 30 mg Super-Q adsorbent traps (Alltech, Deerfield, IL, USA) by pulling air at a rate of 600 mL/min with the aid of a 12 V vacuum pump (Sensidyne, Clearwater, FL, USA). Volatiles were collected for 3 h (11:00-14:00 h).

Volatiles were analyzed as described in Rodriguez-Saona et al. (2009) and Rodriguez-Saona et al. (2011b). The collected volatiles from Super-Q traps were eluted with methylene chloride (150 μL). A Hewlett Packard 6890 Series Gas Chromatograph (GC) equipped with a flame ionization detector and a HP-1 column (10 m × 0.53 mm × 2.65 μm; Agilent Technology, Santa Clara, CA, USA) under a helium flow of 5 mL/min (carrier gas, velocity = 39 cm/sec) was used for volatile analysis. The GC oven was programmed at an initial temperature of 40°C held for 1 min, and then raised at 14°C/min to 180°C where it was held for 2 min, and then increased at 40°C/min to a final temperature of 200°C, and held at this temperature for 2 min. The release rates were calculated based on a linear regression with known quantities of MeSA.

Field Experiments

Experiments were conducted in 2014 (no-choice) and 2015 (choice) to investigate the attraction of *H. convergens* and *C. rufilabris* to MeSA under field conditions and the range of this attraction. No pesticides were applied during the experiments.

In 2014, we conducted no-choice tests in four commercial cranberry *Vaccinium macrocarpon* Aiton (cvs. ‘Early black’ or ‘Stevens’) 1-2 ha beds (isolated, individuals fields) at two farms located in Chatsworth, New Jersey, USA (Farm A - Latitude: $39^o44'22.42''$N; Longitude: $74^o31'38.51''$W, and Farm B - Latitude: $39^o56'29.63''$N; Longitude: $74^o29'35.20''$W). In two beds, yellow sticky cards (23 × 28 cm; ISCA Technologies, Riverside, CA) were baited with MeSA lures (5 g load/lure; 90 d lure; average release rate: ~ 35 mg/d over a 4 week period at 30^oC; PredaLure; AgBio Inc., Westminster, Colorado; www.agbio-inc.com), following methods described in Rodriguez-Saona et al. (2011a). In the other two beds, cards were unbaited (without PredaLure). Both baited and unbaited cards were placed at five distances: 0, 5, 10, 15, and 20 m, extended in a linear transect to the south, north, east, and west from a release site. The release site was 2 × 2 m; 2,000 *H. convergens* adults (mixed sexes) were released at the release site in each of the beds. Cards were collected 24 h after release, and replaced with new ones, which were collected 24 h later; thus, the number of *H. convergens* adults captured on cards was counted 24 and 48 h after release. This experiment was repeated four times from June through mid-August, and a total of 32,000 *H. convergens* were released during this study. Before releasing, *H. convergens* adults were marked with a protein (10% chicken egg albumin) (Hagler et al., 2014), applied with a 500 mL hand sprayer (ULINE, Pleasant Prairie, WI, USA). The release site also was marked with this protein using a HDX 3.8 L backpack sprayer (The Home Depot; Atlanta, GA, USA), to ensure that the predators had contact with the protein before and at the moment of their release. Enzyme-Linked Immunosorbent Assay (ELISA) tests were performed to determine whether the captured *H. convergens* adults were marked with the protein (see Methods below).

In 2015, we conducted choice tests in four commercial cranberry beds of similar size as in 2014. Compared with 2014, we increased the distance between the cards and the release site such that, in each bed, unbaited yellow sticky cards and cards baited with MeSA (PredaLure) were placed at seven distances: 0, 5, 10, 15, 20, 40, and 60 m to the south, north, east, and west from the release site. Two transects, opposite of each other, contained the baited cards and the other two transects contained the unbaited cards; the position of the treatments in these transects was changed with each replication. Two thousand *H. convergens* and 800 *C. rufilabris* adults (mixed sexes) were released at the release site in each of the beds, and the number of individuals captured on cards was counted 24 and 48 h after release, as described above. The entire experiment was repeated three times from May through mid-June, for a total of 24,000 *H.*

convergens and 9,600 *C. rufilabris* adults released during this study. Before their release in the field, both predators, as well as the release site, were marked with chicken egg albumin protein as describe above, and ELISA tests were performed to determine whether the captured predators were marked with the protein (see below).

Enzyme-Linked Immunosorbent Assay (ELISA). ELISA tests were performed, following methods slightly modified from Hagler et al. (2014) and Blaauw et al. (2016), to determine whether *H. convergens* and *C. rufilabris* adults captured on cards were marked with the chicken egg albumin protein, and were thus the predators that were released. Captured insects were removed carefully from the sticky cards with a toothpick (a new toothpick was used for each insect), and both toothpick and insect were placed in individual 1.5 mL microcentrifuge tubes (Flex Tube; Eppendorf North America, Inc., Hauppauge, NY). One mL of an extraction buffer solution consisting of Tris Buffered Saline (TBS) (T6664; Sigma-Aldrich) and Sodium Ethylenediamine Tetra Acetate (EDTA) (S25311; Thermo Fisher Scientific, Waltham, MA, USA) was added to each tube for 3 min. After this time, insects and toothpicks were removed from the tube, and the solution was kept frozen until later analyses by ELISA. In addition, unmarked *H. convergens* and *C. rufilabris* obtained from the colony, as well as any wild (field) specimens of these species captured on cards, were processed as describe above; colony specimens were used as negative controls.

An 80 µL aliquot of each sample was placed with a pipette into 96-well ELISA microplates (M9410; Sigma-Aldrich; 8 × 12 wells). Samples in microplates were distributed as follows: (a) the extraction buffer solution (TBS+EDTA) was added to the first column (eight wells); (b) the solution that had the unmarked insects was added to the second column (negative control), (c) the solution that had the marked insects was added from the third to the tenth column; (d) deionized water was added to the eleventh column, and (e) a 10% chicken egg albumin solution was added to the twelfth column as the positive control. Microplates were incubated for 2 h at 37°C, emptied, and washed five times with 300 µL of Phosphate Buffered Saline (PBS; P4417; Sigma-Aldrich) and 0.09% Triton-X100 (X100; Sigma-Aldrich) (PBST), and then, the wells were blocked with 300 µL of PBS and 1,300 ppm Silwet L-77 (Momentive Performance Materials Inc., Columbus, OH) and 20% Bovine Serum (B9433; Sigma-Aldrich), and incubated for 1 h at 37°C. Microplates were then emptied and washed twice with 300 µl of

PBST solution. An 80 µL aliquot of Rabbit Anti-chicken Egg Albumin (primary antibody) (C6534; Sigma-Aldrich) diluted at 1:8,000 in a solution of PBS, 1,3000 ppm Silwet L-77, and 20% Bovine Serum was added to each well of the microplate, and incubated for 30 min at 37°C. Microplates were emptied and washed five times with 300 µl of PBST solution. An 80 µL aliquot of Donkey, anti-Rabbit IgG (H+L) conjugated to Horseradish Peroxidase (31458; Thermo Fisher Scientific) (secondary antibody) diluted at 1:32,000 in the same solution as describe above for the primary antibody was added to each well, and incubated for 2 h at 37°C. Microplates were emptied and washed three times with 300 µL of PBS and 2.3 g/L Sodium Dodecyl Sulfate (SDS; L4509; Sigma-Aldrich) (PBS-SDS), followed by three more washes with 300 µL of PBST. An 80 µL aliquot of Ultra TMB-ELISA Substrate Solution (34028; Thermo Fisher Scientific) was added to each well, and incubated for 5 min at 37°C. Afterwards, microplates were gently shaken for 5 min in a dark room. Following, an 80 µL aliquot of 2N H_2SO_4 (258105; Sigma-Aldrich) was added to each well to stop the reaction, and the optical density (OD) of each well (sample) was read with a BioTek Synergy^{TM} 4 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at 450 nm, using 490 nm as the reference standard.

Statistical Analyses

EAG data for both predators, *H. convergens* and *C. rufilabris,* were analyzed using two-way ANOVA, with sex, MeSA concentrations, and their interaction, as independent factors. A significant ANOVA was followed by Tukey's HSD test ($\alpha = 0.05$). Paired *t*-tests were used to analyze the data from greenhouse cage experiments to compare the number of visits and percent egg predation by *H. convergens* between plants baited with MeSA and unbaited plants. Percent of egg predation data were arcsine-square-root-transformed prior to analysis. The same statistical tests were used to compare the number of visits and number of eggs of *C. rufilabris* on baited and unbaited plants. Wind tunnel data were also analyzed with Paired *t*-test to compare the number of visits and time spent by *H. convergens* and *C. rufilabris* at each odor source (MeSA or solvent); data were ln(x + 0.5)-transformed prior to analysis.

Field data were analyzed using repeated measures ANOVA comparing the effects of treatment (MeSA-baited and unbaited cards), distance, and date (sampling time), and their interactions. A significant ANOVA was followed by Tukey's HSD test ($\alpha = 0.05$). Data on percent of insect captures were arcsine-square-root-transformed prior to analysis. To determine the range of predator attraction of MeSA-baited and unbaited cards, we fitted the percent capture data to a logarithmic equation, and then calculated the distance when captures equal zero, i.e., x values when $y = 0$. Data for marked insects were scored positive for the presence of the protein marker if the ELISA OD reading exceeded the mean negative control (unmarked insects) reading by three standard deviations (ELISA threshold value; based on Hagler et al. 2009; Hagler and Jones 2010; Blaauw et al. 2016). All analyses were conducted in R 3.1.3 (R Development Core Team, 2015).

Results

Electroantennogram (EAG) Assays

There were significant differences in EAG responses among MeSA concentrations for adult *H. convergens* ($F = 10.74$; df = 4, 140; $P < 0.001$) (Fig. 1A) and *C. rufilabris* ($F = 8.34$; df = 4, 140; $P < 0.001$) (Fig. 1B). We also detected significant differences between sexes of both predators (*H. convergens*: $F = 5.61$; df = 1, 140; $P = 0.01$; *C. rufilabris*: $F = 10.96$; df = 1, 140; $P = 0.001$). However, there was no interaction between sex and MeSA concentrations for *H. convergens* ($F = 0.83$; df = 4, 140; $P = 0.50$) or *C. rufilabris* ($F = 0.62$; df = 4, 140; $P = 0.64$). *H. convergens* males and females showed stronger EAG responses to MeSA concentrations of 10 µg/mL, 0.1 mg/mL, and 1 mg/mL than to the controls (Fig. 1A); however, female antennal responses were ~ 1.4 times greater than those of males (Fig. 1A). Females of *C. rufilabris* showed stronger EAG responses to MeSA concentrations at 1 µg/mL, 10 µg/mL, 0.1 mg/mL, and 1 mg/mL than to the controls, and their response was ~ 1.5 times greater than those of males (Fig. 1B); males of this predator only showed significantly stronger EAG responses to MeSA at 1 mg/mL than to the controls (Fig. 1B).

Greenhouse Choice Tests

Hippodamia convergens females showed more visits to plants baited with MeSA than to unbaited plants at all concentrations tested (10 µg/mL: $t = 2.75$, df = 14, $P = 0.01$; 0.1 mg/mL: $t = 2.19$, df = 14, $P = 0.04$; 1 mg/mL: $t = 2.17$, df = 14, $P = 0.04$) (Fig. 2A). Although percent egg predation was at least 1.9 times higher on the MeSA-baited plants compared with unbaited plants, this effect was significant only for the 0.1 mg/mL concentration ($t = 2.94$, df = 11, $P = 0.01$); egg predation on plants baited with the two other MeSA concentrations was not significantly different compared to unbaited controls (10 µg/mL, t = 1.45, df = 11, P = 0.17; 1 mg/mL, t = 1.11, df = 11, P = 0.28) (Fig. 2B).

The amount of visits by *C. rufilabris* gravid females to MeSA-baited plants was significantly higher at 10 µg/mL (t = 3.54, df = 7, P = 0.009) and 0.1 mg/mL (t = 2.60, df = 7, P = 0.03) compared with unbaited plants (Fig. 2C). Similarly, although not statistically significant (t = 0.69, df = 7, P = 0.50), MeSA-baited plants at 1 mg/mL showed ~ 1.5 times more visits by *C. rufilabris* than unbaited plants (Fig. 2C). Number of *C. rufilabris* eggs were also significantly higher on MeSA-baited plants at 1 mg/mL (t = 2.96, df = 7, P = 0.02) than unbaited plants (Fig. 2D), but only marginally higher at 10 µg/mL (t = 2.20, df = 7, P = 0.06) and 0.1 mg/mL (t = 2.0, df = 7, P = 0.08) (Fig. 2D).

Wind Tunnel Assays

Hippodamia convergens females showed higher numbers of visits to the MeSA than to the control treatment in (no-choice) wind tunnel experiments (t = 2.23, df = 8, P = 0.05) (Fig. 3A). *H. convergens* also spent significantly more time on the MeSA than on the control treatment (t = 2.62, df = 8, P = 0.03) (Fig. 3B), indicating that MeSA attracted and arrested *H. convergens* females.

The numbers of visits by *C. rufilabris* gravid females were not significantly different between the MeSA and control treatments (t = 0.89, df = 8, P = 0.39) (Fig. 3A). However, *C. rufilabris* spent ~2.5 more time on the MeSA treatment than on the control treatment, indicating possible effects of MeSA on *C. rufilabris* foraging behavior, although this effect was not significant (t = 1.17, df = 8, P = 0.27) (Fig. 3B).

Release Rates. For *H. convergens* assays, the releases rates from septa treated with 10 µg/mL, 0.1 mg/mL and 1 mg/mL MeSA were 44.7 ng/h, 418.31 ng/h, and 6.42 µg/h, respectively. For *C. rufilabris* assays, the releases rates for the 10 µg/mL, 0.1 mg/mL and 1 mg/mL MeSA concentrations were 27.35 ng/h, 469.79 ng/h, and 4.17 µg/h, respectively.

Field Experiments

In both study years (2014-2015), there were significant differences in *H. convergens* adult card captures in cranberry fields at different distances from the predator release site (Table 1). Cards near to the release site (0 m) captured ~70-80% of *H. convergens* adults; the number of captured *H. convergens* declined significantly at distances above 5 m, with very few individuals at distances greater than 20 m (Fig. 4A, B). However, captures of adult *H. convergens* did not differ between MeSA-baited and unbaited cards (no 'treatment' effect) or due to time of sampling (no 'date' effect) in cranberry fields. In 2015 (but not in 2014), there was a significant treatment-by-date and distance-by-date interaction on *H. convergens* adult captures (Table 1), indicating some variation on treatment and distance effects due to sampling time; however, there was no treatment × distance or a 3-way interaction (Table 1). Based on the logarithmic equations, in 2014, the range of attraction for MeSA-baited and unbaited cards was 28 and 22 m, respectively (Fig. 4A, B). Similarly, for 2015, the range of attraction was 27 m for both treatments (Fig. 4A, B).

Similarly, there were significant differences in card captures of *C. rufilabris* adults among different distances from the predator release site (Table 1); cards captured ~ 80% of *C. rufilabris* adults near to the release site (0 m), but these numbers declined to significantly at distances greater than 5 m (Fig. 4C). There was no effect of MeSA treatment or treatment × distance interaction on *C. rufilabris* adult captures on cards (Table 1; Fig. 4C), or other interactions except for a distance-by-date interaction (Table 1). Based on the logarithmic equations, the range of attraction for MeSA-baited and unbaited cards was 24 and 20 m, respectively (Fig. 4C).

In 2014, 83% of *H. convergens* (*n* = 256) scored positive for egg albumin protein by ELISA (Fig. 4D) [mean ± SD OD for the negative control (unmarked individuals) = 0.106 ± 0.041 (*n* = 32), with a critical positive threshold value of 0.230; mean ± SD OD for marked individuals = 0.693 ± 0.158, which was ~3 times higher than the negative control threshold value (Fig. 4D)]. In 2015, 83% of *H. convergens* (*n* = 384) scored positive for egg albumin protein by ELISA (Fig. 4E) [mean ± SD OD for the negative control (unmarked individuals) = 0.06 ± 0.01 (*n* = 48), with a critical positive threshold value of 0.09; mean ± SD OD for marked individuals = 0.628 ± 0.170, which was ~7 times higher than the negative control threshold value (Fig. 4E)].

In 2015, 89% of *C. rufilabris* (*n* = 256) scored positive for egg albumin protein by ELISA (Fig. 4F) [mean ± SD OD for the negative control (unmarked individuals) = 0.06 ± 0.01 (*n* = 16), with a critical positive threshold value of 0.122; mean ± SD OD for marked individuals = 1.062 ± 0.501, which was ~9 times higher than the negative control threshold value (Fig. 4F)].

Discussion

Based on laboratory, greenhouse, and field experiments, we demonstrated that: 1) *H. convergens* and *C. rufilabris* antennae were able to detect MeSA at various concentrations; 2) females of both predators were attracted to MeSA; 3) predator attraction to MeSA led to higher egg predation by *H. convergens* and oviposition by *C. rufilabris*; 4) MeSA also arrested *H. convergens*; and, 5) the effective range of attraction to MeSA for both insect predators was at short distances (<30 m).

Hippodamia convergens and *C. rufilabris* adults showed significant electro-antennographic responses to MeSA, confirming that the antennae of both predators can detect this compound. These results are consistent with previous studies showing the electrophysiological responses by many members of the families Coccinellidae and Chrysopidae to MeSA. For example, Zhu and Park (2005) found that female *C. septempunctata* antennae responds to MeSA levels found in volatile extracts from aphid-infested soybean plants. Female antennae of the lacewings *Chrysoperla lucasina* Lacroix (Hegde et al. 2011) and *Chrysopa phyllochroma* Wesmael (Xu et al. 2015) also responded positively to MeSA. In contrast to these previous studies that tested only females, here we tested the antennal response of both females and males, and found that female antennae responded more strongly to MeSA than male antennae. Females need to locate habitats of high prey quality and abundance for the optimal performance of their offspring (Kindlmann and Dixon 1993; Aldrich and Zhang 2016), which may explain their higher sensitivity to MeSA compared to males. For this reason, we focused our behavioral studies in the greenhouse on females.

Greenhouse choice experiments showed that *H. convergens* females were attracted to MeSA-baited plants when using the same MeSA concentrations as for the EAG studies, and this attraction led to an increase in egg predation. Field studies in soybean (Zhu and Park 2005), vineyards (Gadino et al. 2012a), and cranberries (Rodriguez-Saona et al. 2011a) have demonstrated attraction of various coccinellids to MeSA; however, few studies have shown whether this attraction increases a predator's ecological functioning. One example stems from James and Price (2004), who found that controlled-release plastic sachets of MeSA attached to yellow sticky cards in hop yards attracted different predators of spider mites, in particular *Stethorus punctum picipes* Casey; attraction of this predator to MeSA reduced spider mite populations. Another study showed that, the coccinellids *Stethorus* spp. were attracted to sticky cards baited with MeSA lures (PredaLure) in hop yards, and this attraction resulted in a 40-91%

reduction of spider mite populations (Woods et al. 2011). In cranberry fields, we also observed that attraction of predators to MeSA can increase *O. nubilalis* egg predation (J.S., unpublished data). The results presented here support these findings under controlled (greenhouse) conditions. In addition to attraction, our wind tunnel experiments showed that MeSA arrests *H. convergens* females; as far as we know, this is the first study to document an arrestment behavior in a predator in response to MeSA.

Similarly, in this study, we showed that *C. rufilabris* gravid females were attracted to MeSA-baited plants, and this attraction increased the number of eggs laid on these plants. Under field conditions, MeSA attracted several species of lacewings, such as *C. nigricornis* and, *C. oculata*, as well as other unidentified species (James 2003, 2006; James and Price 2004; Lee 2010; Mallinger et al. 2011; Rodriguez-Saona et al. 2011a); however, these studies did not show if attraction to MeSA affected their oviposition behavior. Previous studies have shown attraction and oviposition preference by Chrysopidae species to aphid-infested plants (Petersen and Hunter 2002; Kunkel and Cottrell 2007), and MeSA is often emitted by plants attacked by aphids (Pettersson et al. 1994; Zhu and Park 2005; Blande et al. 2010; Hegde et al. 2011). It is thus possible that MeSA serves as an important cue in guiding these predators to aphid-infested plants. For example, a recent study by Salamanca et al. (2015) showed, in a greenhouse experiment, that *C. externa* gravid females were attracted to aphid-infested rose plants and that this attraction led to an increase in oviposition; the most abundant and consistent HIPV emitted from infested plants was MeSA. Our data support the notion that MeSA attracts, and has a tendency to arrest, *C. rufilabris*, which in turn stimulates oviposition in this species.

Unlike our results from the greenhouse, we found no differences in *H. convergens* and *C. rufilabris* adult attraction to MeSA-baited cards as compared with unbaited cards in cranberry fields. These findings differ from those reported by Rodriguez-Saona et al. (2011a) who found that naturally present coccinellids and chrysopids were attracted to yellow sticky cards similarly baited with MeSA lures (PredaLure). Most (40%) coccinellids captured, in that study, belonged to the species *C. septempunctata* and only 11% belonged to the species *H. convergens*. Rodriguez-Saona et al. (2011a) did not identify the chrysopids to species. It is thus possible that attraction to MeSA depends on predator identity. For instance, some chrysopid species respond to MeSA in the field (James 2003; James and Price 2004; Lee 2010; Jones et al. 2011; Mallinger et al. 2011), while others, such as *Chrysoperla plorubunda* Fitch, do not (James 2003; James 2006;

Lee 2010). Another difference is that we used inexperience (naïve) adults in our study, whereas field individuals might be more responsive to MeSA due to learning. In fact, Drukker et al. (2000) showed that the attraction of the anthocorid predator *Anthocoris nemoralis* (Fabricius) to HIPVs is lost after being reared in the laboratory; however, this attraction was restored once the lab-reared individuals were exposed to MeSA in the presence of prey, indicating associative learning. This may explain the lack of response of *H. convergens* and *C. rufilabris* adults towards MeSA in the field, even though these predators did respond strongly to MeSA in the greenhouse. Although there were differences in the MeSA dose used in greenhouse and field experiments, we used a commercially-available lure (PredaLure) shown previously to attract natural enemies in cranberries (Rodriguez-Saona et al. 2011a) and other agro-ecosystems (Lee 2010; Mallinger et al. 2011; Gadino et al. 2012). The conditions in the field are more complex and unpredictable than in the greenhouse, with multiple cues simultaneously influencing predator foraging behavior. For instance, in the field, we combined both visual (i.e., yellow sticky cards) and chemical (i.e., MeSA) cues, while in the greenhouse we tested for attractiveness of MeSA alone; visual cues might be more critical than a single chemical cue like MeSA to attract *H. convergens* and *C. rufilabris* during foraging under field conditions. In addition, the physiological state of the predators might have also influenced their response to MeSA (e.g. Sznajder et al. 2010), i.e., in the field, they might have been more interested in migration than in host finding.

Despite of the lack of an attractive response to MeSA by *H. convergens* and *C. rufilabris* in the field, our mark-release-recapture data could be used to predict the maximum distance these predators travel to locate a visual/chemical cue, i.e., range of predator attraction. The maximum range of attraction for *H. convergens* and *C. rufilabris* to MeSA-baited and unbaited cards was 20-30 m, with that for *H. convergens* being a few meters longer than *C. rufilabris*. This is the first mark-release-recapture study to investigate the range of predator attraction to an HIPV, which will help determine the spacing needed for placing lures to attract predators to avoid interference among them. Previous field studies have shown that predators respond to MeSA at short distances. For example, Lee (2010), using withe sticky cards captured coccinellids at distances between 0 and 10 m from the MeSA point source, while chrysopids were attracted at 0 m from the MeSA source, in strawberry fields. In soybean, captures with yellow sticky cards showed that natural enemy abundance was high at a distance of 1.5 m from the MeSA volatile source (Mallinger et al. 2010). Similarly, MeSA attracted syrphids at very short distances from the emitting source in cranberries (Rodriguez-Saona et al. 2011a). Gadino et al. (2012) also using yellow sticky traps showed higher numbers of *Orius* spp. at distances of 0 and 5 m from the MeSA source than at 10 m. Altogether, these studies and ours suggest a short range of attraction of predators to MeSA. These findings are supported by Kelly et al. (2014), who reported that sentinel caterpillars were preyed upon by the predatory stink bug *Podisus maculiventris* Say at a higher rate on tomato plants located near (<3 m from) MeSA lures.

In summary, our results support the hypothesis that *H. convergens* and *C. rufilabris* can detect and are attracted to, and arrested by, MeSA under controlled laboratory and greenhouse conditions, and that these changes in their behavior may lead to increased predation and oviposition. To our knowledge, this is the first comprehensive study to show the role of MeSA on prey location and oviposition behavior in these predator species. Although our greenhouse results did not translate to the field, indicating that predator attraction to HIPVs can be complex in real agricultural settings, we showed that *H. convergens* and *C. rufilabris* travel short distances during foraging. These findings have implications for conservation/augmentation biological control of agricultural pests. For example, MeSA could be used to attract and retain predators in areas for longer durations, thus potentially increasing their ecosystem functioning (i.e., predation or oviposition) (Kelly et al. 2014). However, further studies are needed to better understand the inconsistencies in natural enemy responses to HIPVs under field conditions. Here we used a single chemical stimulus to attract natural enemies; however, use of multiple HIPVs might be more efficient in the field. For example, Jones et al. (2011) found greater attraction of chrysopids when MeSA was combined with a male aggregation pheromone (iridodial). Another strategy would be to combine HIPVs with companion plants as food sources for natural enemies in an attract-and-reward scenario. Orre Gordon et al. (2013), for example, showed that MeSA in combination with buckwheat, an important companion plant that provide food sources, increases the abundance of natural enemies in Brassica crops. We are currently conducting field studies to evaluate the combined effects of MeSA and companion plants (e.g. coriander) on the ecosystem services provided by natural enemies.

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Tables

Table 1 Results of repeated measures ANOVA comparing the effects of treatment (methyl salicylate (MeSA) baited and unbaited cards), distance, and date (sampling time), and their interactions on the number of *Hippodamia convergens* and *Chrysoperla rufilabris* adults captured in cranberry fields in 2014 and 2015.

Year	Predator Species	Variable	*df* [a]	*F*	*P* [b]
2014	*H. convergens*	Treatment	1, 120	0.04	0.83
		Distance	4, 120	17.51	<**0.001**
		Date	3, 120	0.30	0.82
		Treatment × Distance	4, 120	0.39	0.81
		Treatment × Date	3, 120	0.24	0.86
		Distance × Date	12, 120	0.31	0.98
		Treatment × Distance × Date	12, 120	0.27	0.99
2015	*H. convergens*	Treatment	1, 294	0.01	0.91
		Distance	6, 294	167.04	<**0.001**
		Date	2, 294	1.47	0.22
		Treatment × Distance	6, 294	0.57	0.74
		Treatment × Date	2, 294	3.94	**0.02**
		Distance × Date	12, 294	3.01	<**0.001**
		Treatment × Distance × Date	12, 294	0.34	0.97
	C. rufilabris	Treatment	1, 294	0.09	0.75
		Distance	6, 294	93.06	<**0.001**
		Date	2, 294	7.47	<**0.001**
		Treatment × Distance	6, 294	0.61	0.71
		Treatment × Date	2, 294	0.88	0.41
		Distance × Date	12, 294	7.74	<**0.001**
		Treatment × Distance × Date	12, 294	0.35	0.97

[a] Numerator, denominator (error).

[b] Numbers in bold are statistically significant.

Figures

Figure Captions

Fig. 1 Electroantennografic (EAG) response of adult *Hippodamia convergens* (A) and *Chrysoperla rufilabris* (B) males and females to different methyl salicylate (MeSA) concentrations. Different letters indicate significant differences at $\alpha = 0.05$ (Tukey's HSD test). Upper case letters indicate differences among females; lower case letters indicated differences among males.

Fig. 2 Effect of different methyl salicylate (MeSA) concentrations on number of visits (A) and percent egg predation (B) by *Hippodamia convergens* females, and number of visits (C) and number of eggs laid (D) by *Chrysoperla rufilabris* females. ***** = indicate significant differences between control and MeSA concentrations ($P \leq 0.05$; Paired *t*-tests); **+ =** indicate marginal differences between control and MeSA concentrations ($0.05 \leq P < 0.1$; Paired *t*-tests); n.s. = indicate no significant differences between control and MeSA concentrations ($P \geq 0.1$; Paired *t*-tests).

Fig. 3 Effect of methyl salicylate (MeSA) on the number of visits (A) and time spent (B) by *Hippodamia convergens* and *Chrysoperla rufilabris* females. n.s. = no significant difference between control and MeSA ($P > 0.05$; Paired *t*-tests); ***** = significant difference between control and MeSA ($P \leq 0.05$; Paired *t*-tests).

Fig. 4 Percentage of *Hippodamia convergens* (A and B) and *Chrysoperla rufilabris* (C) adults captured on methyl salicylate (MeSA) baited and unbaited cards at different distances in 2014 and 2015. Different letters indicate significant differences at $\alpha = 0.05$ (Tukey's HSD test). Upper case letters indicate differences in captures among unbaited cards; lower case letters indicate differences among MeSA-baited. Graphs show the fitted logarithmic equations and curves for unbaited (dotted lines) and MeSA-baited (solid lines) cards. ELISA optical density values of unmarked and marked *H. convergens* (D and E) and *C. rufilabris* (F) adults captured in cranberry fields. Dotted lines are the positive control ELISA threshold value (e.g., mean negative control

ELISA value plus three standard deviations). The percentage of predators that scored positive for egg albumin protein is given above each bar.

Fig. 1

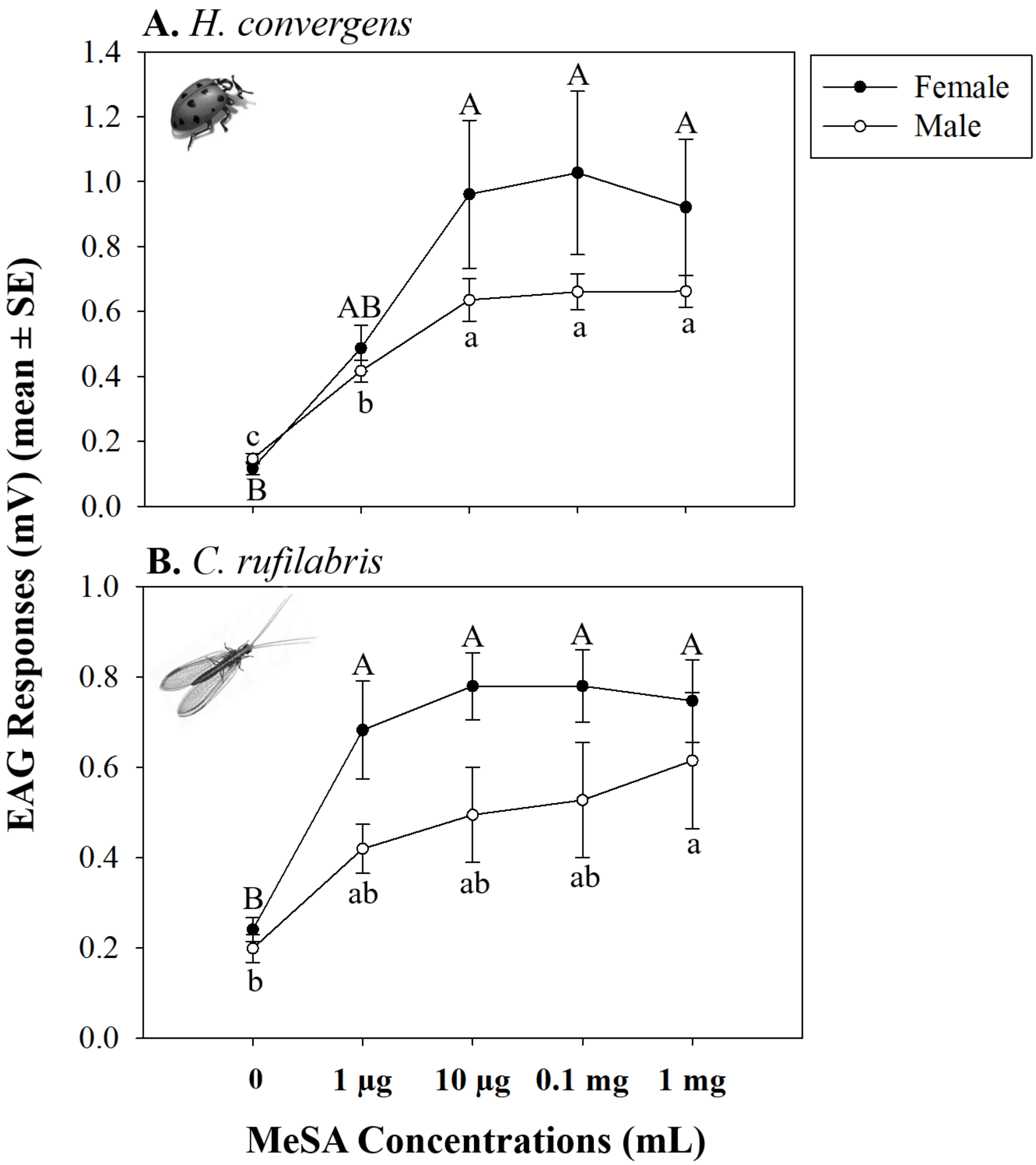


Fig. 2

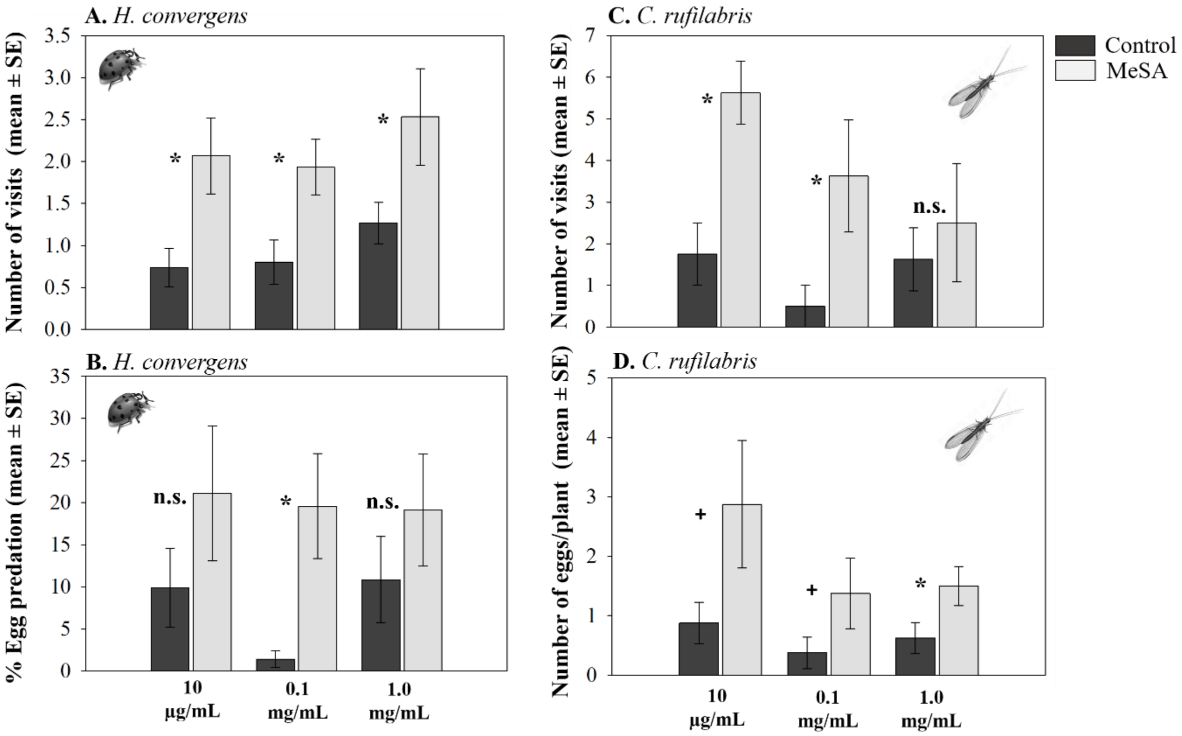

A. H. convergens
Number of visits (mean ± SE)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
C. C. rufilabris
Number of visits (mean ± SE)
7
6
5
4
3
2
1
0
*
*
n.s.
Control
MeSA
B. H. convergens
% Egg predation (mean ± SE)
35
30
25
20
15
10
5
0
n.s.
*
n.s.
D. C. rufilabris
Number of eggs/plant (mean ± SE)
5
4
3
2
1
0
+
+
*
10 µg/mL
0.1 mg/mL
1.0 mg/mL

Fig. 3

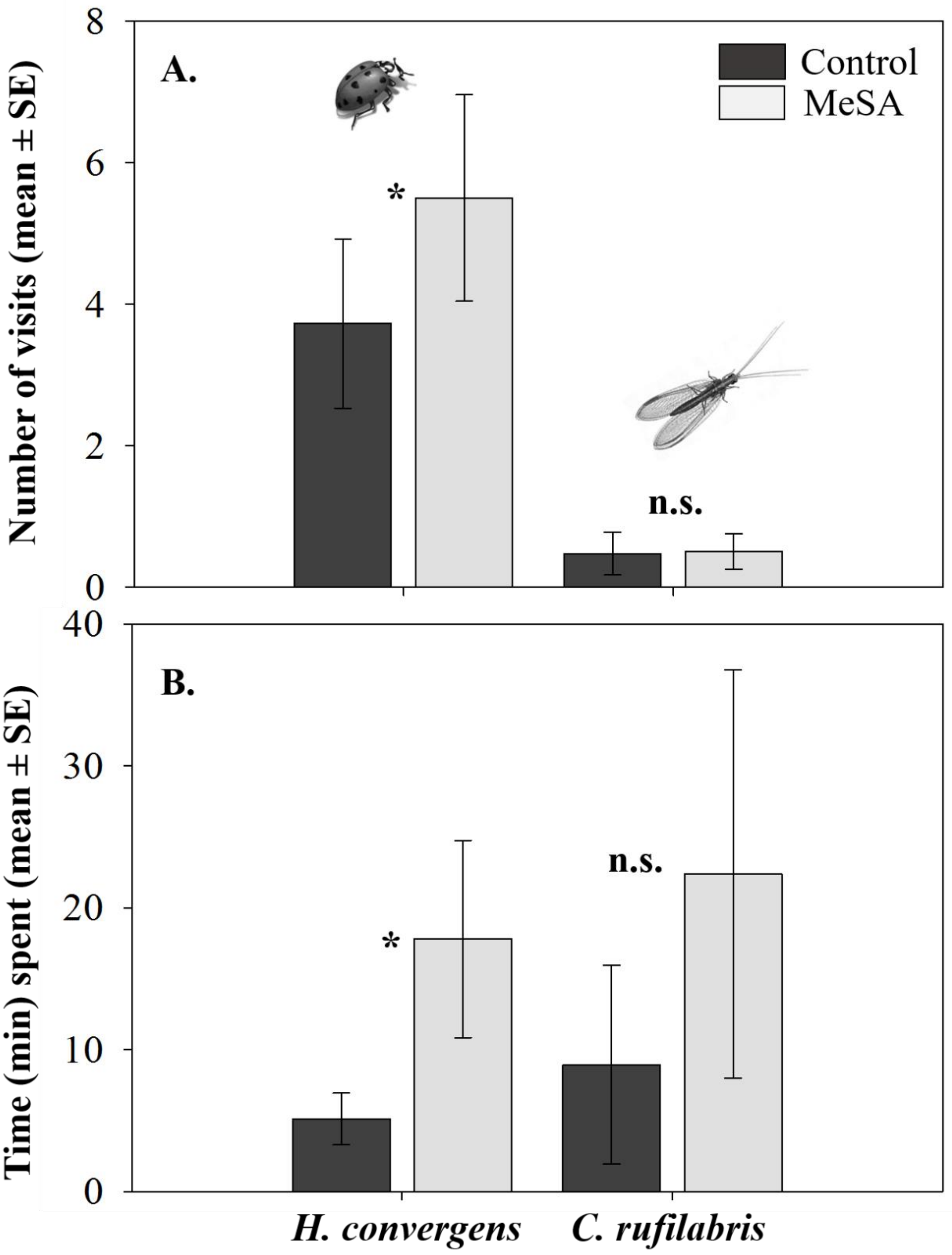
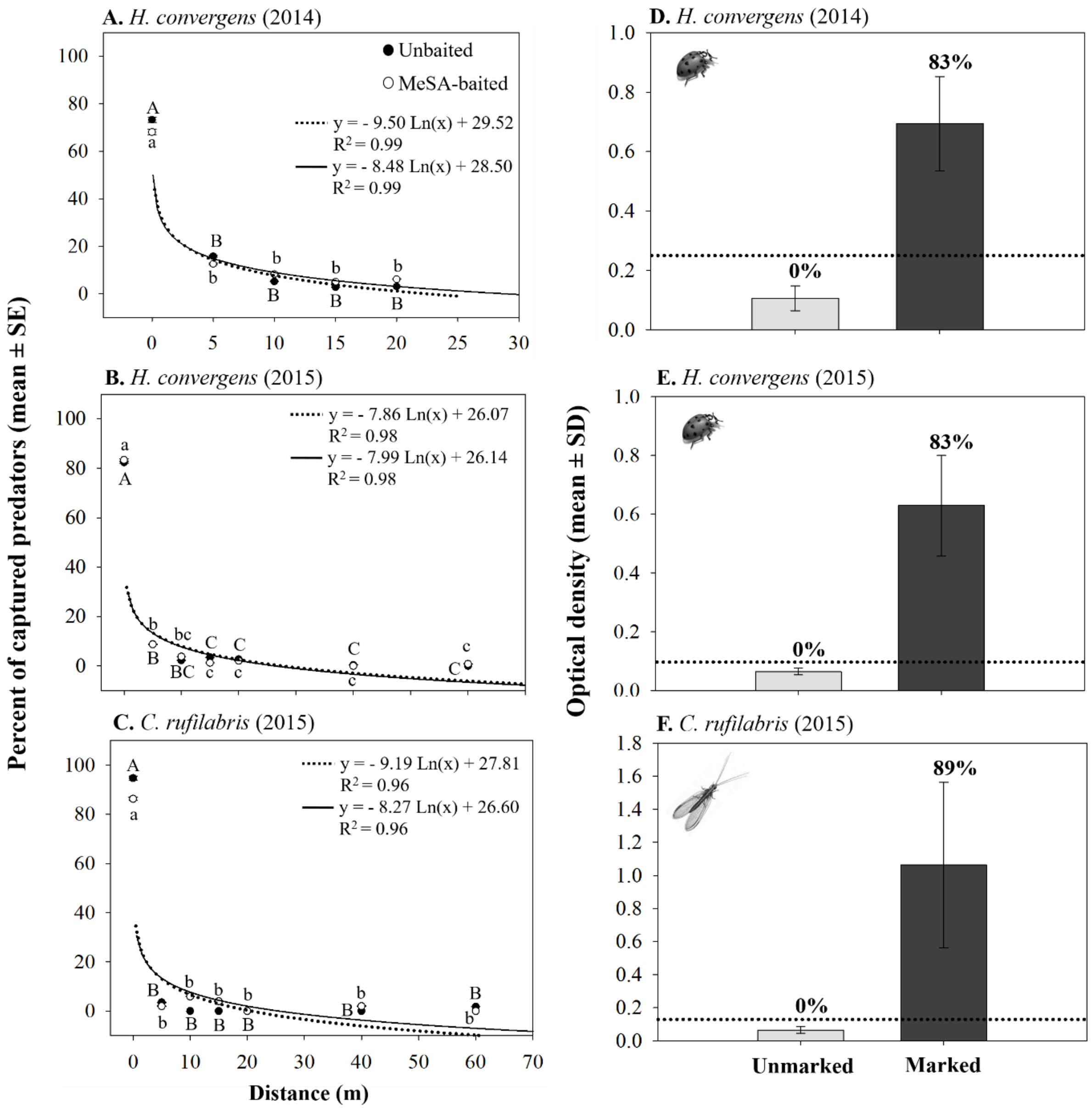

A.
B.
Control
MeSA
Number of visits (mean ± SE)
Time (min) spent (mean ± SE)
0
2
4
6
8
0
10
20
30
40
*
n.s.
*
n.s.
H. convergens
C. rufilabris

Fig. 4


A. H. convergens (2014)
● Unbaited
○ MeSA-baited
y = - 9.50 Ln(x) + 29.52
R² = 0.99
y = - 8.48 Ln(x) + 28.50
R² = 0.99
B. H. convergens (2015)
y = - 7.86 Ln(x) + 26.07
R² = 0.98
y = - 7.99 Ln(x) + 26.14
R² = 0.98
C. C. rufilabris (2015)
y = - 9.19 Ln(x) + 27.81
R² = 0.96
y = - 8.27 Ln(x) + 26.60
R² = 0.96
Percent of captured predators (mean ± SE)
Distance (m)
D. H. convergens (2014)
83%
0%
E. H. convergens (2015)
83%
0%
F. C. rufilabris (2015)
89%
0%
Optical density (mean ± SD)
Unmarked
Marked

ARTICLE 2 Cascading effects of combining herbivore-induced plant volatiles with companion plants to manipulate natural enemies in an agro-ecosystem

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Summary

1. Herbivore-induced plant volatiles (HIPVs) have been used to attract natural enemies of herbivores in agro-ecosystems. However, whether this attraction leads to enhanced ecosystem function and services provided by natural enemies remains debatable.

2. Field experiments were conducted to test the hypothesis that attraction of natural enemies to HIPVs, alone or in combination with companion plants, increases crop productivity. Our treatments consisted of common bean plants either alone; baited with methyl salicylate (MeSA; an HIPV known to attract natural enemies); with coriander (as a companion plant); or with both MeSA and coriander.

3. Number of arthropods (natural enemies and herbivores) were visually sampled throughout two growing seasons (2015-2016). 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.

4. *At the natural enemy level*, MeSA and coriander, when alone or in combination, increased abundance, diversity, and evenness of insect predators from six families. *At the herbivore level*, MeSA and coriander, alone or together, reduced spider mite and thrips populations and increased aphid predation; however, MeSA increased, while coriander reduced, leaf beetle abundance. *At the plant level*, MeSA and coriander reduced damage by spider mites. However, MeSA with or without coriander did not increase crop biomass or any yield parameters.

5. In conclusion, 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.

Key-words: methyl salicylate, HIPVs, attract-and-reward, conservation biological control, ecosystem function, ecosystem services.

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 (Dicke & Sabelis 1988; Turlings &Tumlinson 1992; Cortesero *et al*. 2000; Khan *et al*. 2008; Jonsson *et al*. 2008; Orre *et al*. 2010; Rodriguez-Saona *et al*. 2012; Heil 2014; Jones *et al*. 2015). As a result, various synthetic HIPVs have been tested to manipulate natural enemy behavior in agro-ecosystems (e.g., Dicke *et al*. 1990; Zhu & Park 2005; Kunkel & Cottrell 2007; Jones *et al*. 2011; Woods *et al*. 2011). However, the use of these 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, as well as the possibility of increasing ecological risks by unintentionally attracting the herbivores themselves (Kaplan, 2012).

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’. Under 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 (Simpson *et al*. 2011a; Simpson *et al*. 2011b; Orre Gordon *et al*. 2013). For example, Orre Gordon *et al*. (2013) found that intercropping buckwheat, *Fagopyrum esculentum* Moench, with brassica plants baited with the HIPV methyl salicylate (MeSA) increases 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*. (2011) 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.

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 criteria must be meet: (1) HIPVs need to attract natural enemies, and thus enhance their ecosystem structure (abundance and diversity); (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 (criteria #1) (e.g. James & Grasswitz 2005; Tóth *et al*. 2009; Lee 2010; Jones et al. 2011; Rodriguez-Saona *et al.* 2011; Woods *et al*. 2011; Maeda *et al.* 2015). For example, Flint *et al*. (1979), in an early study, showed that *Chrysoperla carnea* Stephens is attracted to the HIPV caryophyllene in cotton fields. More recently, James (2003a) 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 (Gadino *et al*. 2012). Less evidence currently exists in support of the 2nd criteria 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 parasitism rate of *Lygus lineolaris* Palisot de Beauvois eggs (Williams *et al*. 2008). In soybean, Mallinger *et al*. (2011) 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 3rd criteria). Wang *et al*. (2011) found that MeSA combined with oilseed rape (*Brassica napus* L.) 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*. (2011b) found no effects of an attract-and-reward approach on crop (wine grapes) yield. Therefore, studies on the impact of HIPVs alone, or in combination with companion plants (i.e., an ‘attract-and-reward’ scenario), on ecosystem structure, function, and services provided by natural enemies in agro-ecosystems, hence addressing all of the three criteria outlined above, are still lacking.

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 (criteria #1), reduce pest populations (criteria #2), and increase crop productivity (criteria #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. We predicted that MeSA and coriander will interact synergistically to alter the arthropod community, i.e., increase natural enemy abundance and reduce herbivore density, and that these effects will ultimately cascade down to increase crop productivity. Our specific objectives were to: (1) identify which beneficial arthropods 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.

Material and methods

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 (Graham & Ranalli 1997; Cabral *et al*. 2011). In Brazil, beans are grown throughout the year in three main seasons from November-February (‘summer’ crop, rainy season), March-June, and May-September (‘winter’ crop). Our studies were conducted in two of these growing seasons and in two consecutive years from 31 August through 11 December of 2015 (season 1) and from 7 April through 15 July of 2016 (season 2). Seeds were purchased from Mega grãos 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 (Schausberger *et al*. 2012). It is also induced after damage by herbivores from different feeding guilds including mites (Agrawal *et al*. 2002; van den Boom *et al*. 2004), aphids (Ninkovic *et al*. 2003; Salamanca *et al*. 2015), beetles (Bolter *et al*. 1997), and caterpillars (Geervliet *et al*. 1997; Kessler & Baldwin 2001). In addition, MeSA has been used in various agro-ecosystems such as soybeans (Mallinger *et al*. 2011), cranberries (Rodriguez-Saona *et al*. 2011), vineyards (Gadino *et al*. 2012), strawberries (Lee 2010), wheat (Wang *et al*. 2011), and hops (James 2003b) to attract beneficial insect predators such as members of the families Syrphidae, Chrysopidae, Anthocoridae, Coccinellidae, and Geocoridae. 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 (Resende *et al*. 2011), tomato (Togni *et al*. 2009), carrot (Jankowska & Wojciechowicz-Żytko 2016), eggplant (Patt *et al*. 1997), and rose plants (Salamanca *et al*. 2015) attracts natural enemies, for example lacewings (Chrysopidae), hoverflies (Syrphidae), and ladybeetles (Coccinellidae). Coriander emits volatiles throughout all vegetative stages known to be attractive to natural enemies (Potter & Fagerson 1990; Deng *et al*. 2003; Togni *et al*. 2016). In addition, coriander flowers provide a source of nutrient (nectar and pollen) to natural enemies (Patt *et al*. 1997; Resende *et al*. 2012). Coriander seeds were purchased from Horticeres Sementes Ltda. (Indaiatuba, SP, Brazil).

Study sites and experimental design

Field experiments were conducted at a research farm located in Ijaci, Minas Gerais, Brazil (Latitude: 21º09'54.7"S; Longitude: 44º55'04.3"W). We planted 28 (3 m × 1 m) plots of bean plants spaced 3 m apart (Fig. 1) on 31 August-1 September (2015, season 1) and on 7-11 April (2016, season 2). Each plot consisted of 20 plants planted in 5 rows (total of 100 plants/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.

Each plot received one of four treatments in a randomized complete block design, and each of the treatment plots was replicated in 7 blocks. 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 in the middle of the rows of bean plants (20 coriander plants/row for a total of 80 plants/plot), with a spacing of 5 cm between plants (Fig. 1). 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, in 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 entirety of the experiment, while MeSA-baited vials were placed in the field 30 days after planting, to allow time for plants to grow and plots to get 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).

Arthropod community sampling

To determine the effects of treatments on both beneficial (criteria #1) and detrimental (criteria #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 (season 1) and from 24 May until 1 July in 2016 (season 2), for a total of 5 sampling dates per season. 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/plot. All observations were done on sunny, calm days for approximately 10 min periods per plot on each of the sampling dates and carried out from 900 to 1400 h (season total = 50 min/plot or 280 min/sampling date or 1,400 min of total sampling time for the entire study). 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 the growing season following methods modified from Karlik *et al*. (1995) and Alston & Reding (2011). 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/plant/plot.

In 2016, thrips were the dominate herbivores in our plots. Thrips populations were sampled following methods modified from Santos-Amaya *et al*. (2012) and Gonzalez-Zamora & Garcia-Mari (2003). 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.

Aphid predation rate

To further test criteria #2, we used sentinel aphids to assess predation rates in each of the plots. In both years (2015 and 2016), four black square cardboards (6 x 6 cm) made of sandpaper (AC Parafusos Ltda.; Lavras, MG, Brazil) were placed in each plot (for a total of 112 cardboards in all plots). Just prior to deployment, five frozen aphids were glued onto each cardboard, which were then hanged with pins on wooden stakes at ~70 cm above ground; the stakes were placed near the center of each plot at least 20 cm apart from each other. 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 in each cardboard was counted after 24 h. These assays were repeated twice in both years on 9 October and 30 October in 2015 (season 1), and on 10 June and 1 July in 2016 (season 2).

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 (criteria #3). Spider mite damage was assessed visually by counting the number of leaves per plot expressing severe chlorosis or necrosis. Visual inspections were done 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 due to herbivory in 2016.

Crop yield and dry mass

At the end of the growing season, 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 (criteria #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 125ºC for 48 h to obtain total dry mass for each of the plants. Before cutting the plants into pieces, all pods were removed from plants, counted, and weighed. In addition, the number and weight of seeds in each pod were recorded. In 2015 (season 1), we harvested ten plants per plot on 7-11 December (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 = 1,120 plants for all plots). Plants in season 2 were harvested on 11-15 July in 2016.

Statistical analyses

All analyses were conducted in R 3.3.1 (R Development Core Team 2016). Prior to analyses, counts for each arthropod taxa (family/species) were averaged across all sampling dates to obtain the mean seasonal abundance per treatment/plot. These data were used to determine the effect of treatment on diversity (i.e., number of taxa; Shannon's H'), evenness (i.e., relative abundance of each taxa; J'), and abundance (i.e., number of individuals/taxa) 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. Diversity and evenness indices were calculated using the 'vegan' package in R (Oksanen *et al.* 2009), and analyzed using analysis of variance (ANOVA). A significant ANOVA was followed by Tukey's HSD test ($\alpha = 0.05$).

The effects of MeSA, coriander, and their interaction on the abundance of arthropods, natural enemies, and herbivores were analyzed using 2 × 2 factorial Multivariate Analysis of Variance (two-way MANOVA). 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 arthropod, natural enemy, and herbivore communities. MANOVA and PCA were also used to analyze the effects of treatment on crop yield parameters. Score and loading plots were drawn in R using the 'ggplot2' package (Wickham 2009). We followed these multivariate analyses with 2 × 2 factorial univariate analyses. 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.

Results

Arthropod community composition

A total of 9 arthropod taxa were recorded in 2015. These consisted of the herbivores: spider mites [*Tetranychus* sp. (Acarini: Tetranychidae)], leaf beetles [*Diabrotica* sp. (Coleoptera: Chrysomelidae)], and an unknown phytophagous stink bug sp. (Hemiptera: Pentatomidae); and the natural enemies (predators): hoverflies [*Toxomerus* sp. (Diptera: Syrphidae)], predatory stink bugs [*Podisus* sp. (Hemiptera: Pentatomidae)], convergent ladybeetles [*Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae)], multicolored Asian ladybeetles [*Harmonia axyridis* Pallas (Coccinellidae)], spotless ladybeetles [*Cyclonella sanguinea* L. (Coccinellidae)], and earwigs [*Doru* sp, (Dermaptera: Forficulidae)]. A total of 11 arthropod taxa was recorded in 2016, consisting of the herbivores: thrips [*Neohydatothrips* sp. (Thysanoptera: Thripidae)], as well as the *Diabrotica* sp. (Coleoptera: Chrysomelidae) and the phytophagous Pentatomidae observed in 2015; and the natural enemies (predators): *Toxomerus* sp., lacewings [*Chrysoperla* sp. (Neuroptera: Chrysopidae)], *H. convergens*, *H. axyridis*, predatory thrips [*Franklinotrips vespiformis* Crawford (Thysanoptera: Aeolothripidae)], minute pirate bugs [*Orius insidiosus* Say (Hemiptera: Anthocoridae)], spiders (Araneae), and ants (Hymenoptera: Formicidae). Both larvae and adults of ladybeetles, lacewings, thrips, and Chrysomelid beetles were recorded. Other functional groups such as parasitoids are not reported because they were rarely observed during visual inspections likely due to their small size and mostly cryptic behaviors.

In both years, arthropod diversity (2015: $F = 6.33$; df = 3, 18; $P = 0.004$; 2016: $F = 12.54$; df = 3, 18; $P < 0.001$) and evenness (2015: $F = 4.5$; df = 3, 18; $P = 0.01$; 2016: $F = 8.56$; df = 3, 18; $P < 0.001$) were affected by treatment. MeSA, coriander, and combining both MeSA and coriander increased arthropod diversity and evenness as compared with the control; however, this difference was not significant in 2015 for both parameters for the MeSA treatment and for evenness for the combined treatment (Table 1).

Arthropod abundance was also 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' $\lambda = 0.84$; $F = 2.56$; df = 1, 102; $P = 0.01$) 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; $P = 0.003$); 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 shows a differentiation in arthropod community composition according to the treatments, explaining ~53% of the variance (Fig. 2a). The first PC component 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 (Fig. 2a). In 2016, the PCA explained ~50% of the variation in arthropod community composition among the treatments (Fig. 2e). The first PC component explained 32% of the variation and separated the MeSA from the coriander treatments (Fig. 2e);

while the second PC explained 18% of the variation and separated the control from the other treatments (MeSA, coriander, and both) (Fig. 2e).

After finding that our treatments affected diversity, evenness, and 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.

Natural enemies. Natural enemy diversity (*F* = 3.80; df = 3, 18; *P* = 0.02) and evenness (*F* = 3.46; df = 3, 18; *P* = 0.03) were affected by treatment in 2015 but not in 2016 (diversity: *F* = 1.32; df = 3, 18; *P* = 0.29; evenness: *F* = 0.25; df = 3, 18; *P* = 0.85). In 2015, diversity and evenness of natural enemies were higher in coriander plots as compared with control plots; MeSA also increased evenness (Table 1).

In 2015, the abundance of natural enemies was affected by MeSA (Wilks' λ = 0.86; *F* = 3.69; df = 1, 102; *P* = 0.007) and coriander (Wilks' λ = 0.73; *F* = 8.92; df = 1, 102; *P* < 0.001), and there was a marginal interaction effect (MeSA × coriander: Wilks' λ = 0.91; *F* = 2.30; df = 1, 102; *P* = 0.06). The PCA for 2015 shows a distinct composition of the natural enemy communities according to treatments, explaining ~76% of the variance (Fig. 2b). The first PC component explained 51% of the variation and partially separated the coriander from the MeSA and control treatments; while the second PC explained only 25% of the variation (Fig. 2b). In 2016, the abundance of natural enemies was affected also by MeSA (Wilks' λ = 0.70; *F* = 7.26; df = 1, 126; *P* < 0.001), but not by coriander (Wilks' λ = 0.91; *F* = 1.54; df = 1, 126; *P* = 0.15); however, there was a marginal MeSA × coriander interaction (Wilks' λ = 0.89; *F* = 1.92; df = 1, 126; *P* = 0.07), indicating that the effect of MeSA was somewhat influenced by coriander. The PCA in 2016 explained ~54% of the variation of natural enemy communities among treatments (Fig. 2f). The first PC component explained 33% of the variation and separated the control from the other treatments; while the second PC component explained 21% of the variation and partially separated the control particularly from the MeSA and coriander treatments (Fig. 2f).

When each natural enemy was analyzed separately, the abundance of ladybeetles (Coccinellidae) was influenced by coriander, but not by MeSA, in both years (2015-2016) (Table 2; Fig. 3a, 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. 3a, e). MeSA and coriander increased independently, in an additive manner, the abundance of hoverflies, *Toxomerus* sp., in 2015 (Table 2; Fig. 3b). In 2016, MeSA also increased the abundance of hoverflies (Table 2), and interacted with coriander (i.e., non-additive effect; Table 2; Fig. 3f). Coriander, but not MeSA, increased the abundance of earwigs as compared with controls, and there was no MeSA × coriander interaction (Table 2; Fig. 3c). 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. 3d), 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. 3g). MeSA increased also the abundance of the minute pirate bug *O. insidiosus* (Table 2; Fig. 3h). There were no effects of treatment on the abundances of neither lacewings, spiders, nor ants (Table 2).

Herbivores. Similarly to natural enemies, herbivore diversity ($F = 5.06$; df = 3, 18; $P = 0.01$) and evenness ($F = 4.89$; df = 3, 18; $P = 0.01$) were affected by treatment in 2015 but not in 2016 (diversity: $F = 2.27$; df = 3, 18; $P = 0.11$; evenness: $F = 0.82$; df = 3, 18; $P = 0.49$). In 2015, diversity and evenness of herbivores were higher in coriander plots as compared with control plots (Table 1).

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; $P = 0.05$). The PCA for 2015 explained ~71% of the variance and shows differences in the composition of herbivore communities among treatments (Fig. 2c). The first PC component 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 (Fig. 2c). 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; $P = 0.36$). The PCA for 2016 explained ~83% of the variation in herbivore communities among treatments (Fig. 2g). The first PC component explained 48% of the variation and partially separated the coriander from the control treatments; while the second PC explained 35% of the variation and moderately separated the coriander and MeSA treatments from the control treatment (Fig. 2g).

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 (significantly MeSA-by-coriander interaction), as compared with the control treatment (Table 3; Fig. 4a). 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. 4b). 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. 4c). There was no effect of treatment on leaf beetle abundance in 2015, nor an effect of treatment on the abundance of phytophagous stink bugs in any of the sampled years (Table 3).

Aphid predation rate

In 2015, coriander increased significantly aphid predation rates (F = 4.75; df = 1, 47; P = 0.03) (Fig. 5a). 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. 5a).

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. 5b). There were no effects of MeSA alone (F = 0.03; df = 1, 48; P = 0.84) (Fig. 5b) or in combination with coriander (F = 0.36; df = 1, 46; P = 0.55) on aphid predation rates (Fig. 5b).

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. 6) 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. 6).

Crop yield and dry mass

There were no effects of MeSA (Wilks' λ = 0.96; *F* = 0.10; df = 1, 18; *P* = 0.98), coriander (Wilks' λ = 0.78; *F* = 0.77; df = 1, 18; *P* = 0.58), or their interaction (Wilks' λ = 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' λ = 0.55; *F* = 2.26; df = 1, 18; *P* = 0.10) nor coriander (Wilks' λ = 0.73; *F* = 1.01; df = 1, 18; *P* = 0.44) on yield parameters in 2016; however, there was a significant interaction effect (Wilks' λ = 0.47; *F* = 3.03; df = 1, 18; *P* = 0.04). The PCAs show large amount of overlap in yield parameters among treatments. The PCA for 2015 explained 90% of the variation, with the first PC explaining most of the variation (74%) and moderately separating the control from the MeSA/coriander combined treatment (Fig. 2d). Similarly, the PCA for 2016 explained 94% of the variation, with the first PC explaining again most of the variation (71%) and partially separating the coriander from the MeSA/coriander combined treatment (Fig. 2h).

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 (Fig. 7). No other yield parameters were influenced by treatment in 2016 (Table 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 (Rodriguez-Saona *et al*. 2012). 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 that: 1. The HIPV methyl salicylate (MeSA), alone or in combination with coriander as a companion plant, increases the abundance, diversity, and evenness of arthropods. 2. These manipulative approaches increased natural enemy abundance (in both years), as well as their diversity and evenness (in one of the two years), which supports criteria #1 outlined above that these strategies need to attract natural enemies and enhance their abundance and diversity. 3. 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 provide support for criteria #2 that these strategies need to enhance natural enemy ecosystem function. 3. Although in one of the field seasons 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 criteria #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. These 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 (Rodriguez-Saona *et al*. 2011), soybeans (Zhu & Park 2005; Mallinger *et al*. 2011), and hops (James 2003a). Similarly, MeSA-baited traps captured more *Orius similis* Zheng in cotton (Yu *et al*. 2008) and *Orius tristicolor* White in strawberries (Lee 2010) than unbaited traps. MeSA could have influenced recruitment of natural enemies in two ways: a) directly, by natural enemy attraction to the dispensers emitting MeSA, or 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 mites-infested bean plants (Ozawa *et al*. 2000). Rodriguez-Saona *et al*. (2011) also demonstrated that exposure to MeSA increases the emission of MeSA from cranberry vines.

Intercropping bean plants with coriander also increased the abundance of hoverflies, but this effect was inconsistent among seasons. Morris & Li (2000) 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 increases syrphid abundance compared with carrot alone (Jankowska & Wojciechowicz-Żytko 2016). Ladybeetle abundance was also higher in bean plots intercropped with coriander. Previous studies showed that coriander plants intercropped with carrot (Jankowska & Wojciechowicz-Żytko), tomato (Togni *et al*. 2009), cabbage (Resende *et al*. 2011), and eggplant (Patt *et al*. 1997) have higher abundance and diversity of coccinellids species than their respective monocrops, likely due to the attractiveness, and suitability as food, of coriander flowers to adult ladybeetles. Coccinellids also have an innate attraction to volatiles from coriander at the vegetative stage (Togni *et al*. 2016). Thus, in our studies constitutive volatiles and floral resources from coriander plants could have increased ladybeetle recruitment. Other natural enemies influenced by coriander include predatory pentatomids and earwigs. Patt *et al*. (1997) also found numerous predatory pentatomids in eggplant intercropped with coriander. Togni *et al*. (2010) showed a positive impact of coriander on earwigs, where Forficulidae populations were greater in tomato intercropped with coriander than in tomato monocultures.

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 analysis, 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 repel by coriander (e.g. Gomes *et al*. 2012). Orre Gordon *et al*. (2013) also reported that attraction of syrphids to MeSA was inhibited when brassica were combined with buckwheat as a companion plant. So far, our results and previous findings (Simpson *et al*. 2011a; Simpson *et al*. 2011b; Orre Gordon *et al*. 2013) 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, diversity, and evenness of herbivore communities were also affected by our manipulative treatments. MeSA and coriander reduced spider mite (*Tetranychus* sp.) and thrips (*Neohydatothrips* sp.) populations. On the other hand, 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 (Melo & Silveira 1998; Rodrigues *et al*. 2011; Romano 2013). Interestingly, low populations of spider mites in the MeSA and coriander treatments in 2015 correlated with high populations of ladybeetles in those treatments (see Fig. 4a versus Fig. 3a). In fact, spider mites are suitable prey for many coccinellid species (Lucas *et al*. 1997, 2002). James & Price (2004) also found high number of coccinellids and reduced numbers of spider mites in hops treated with MeSA. In 2016, low populations of thrips correlated with high abundance of predatory minute pirate bugs (*O. insidiosus*) in our manipulative treatments (see Fig. 4b versus Fig. 3h). Thrips are suitable prey for minute pirate bugs (Silveira *et al*. 2004; Xu *et al*. 2006). For instance, Resende *et al*. (2012) 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*. In addition, predatory thrips (*F. vespiformis*) attraction to MeSA could have contributed to the reduction of *Neohydatothrips* sp. populations. Previous studies have shown the importance of this predator in regulating thrips populations (Pizzol *et al*. 2008; Nammour *et al*. 2008). 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 is no clear link of this to predator abundance. Coriander attracted predatory earwigs and stink bugs; 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, most likely due to an increase in ladybeetle abundance; yet, the contribution of other predators on aphid predation cannot be discarded. 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 were 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 due to 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. A negative outcome of using strategies to manipulate natural enemy behavior in agro-ecosystems could be an unintended increase in herbivore populations due to 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*. (2011a, 2011b) also found attraction of thrips to MeSA in vineyards. Moreover, Hammack (2001) 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 lower number of leaf beetles as compared with the control plots and its presence ameliorated the potential ecological risk of attracting an herbivore such as leaf beetles to MeSA-treated plots. Gomes *et al*. (2012) also reported that coriander intercropped with tomato reduced the abundance of thrips, possibly due to 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 salicylic acid (SA), the non-volatile analog of MeSA, activates induced systemic resistance and reduces spider mite *Tetranychus urticae* Koch population in bean plants (*Ph. vulgaris*) (Farouk & Osman 2011). In choice assays, *Frankliniella occidentalis* Pergrande was repelled by bean and cucumber leaves treated with MeSA; MeSA also increased resistance against this thrips (Koschier *et al*. 2007). Further 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. Previous studies have shown that reduction of spider mite populations, via SA-mediated induction of plant defenses, increases yield in common bean plants (Farouk & Osman 2011). Bean plants can, however, tolerate high amount of damage (Graham & Ranalli 1997). For example, Blue *et al*. (2015) found that mechanical leaf damage reduces yield in lima bean plants; however, they report no differences in aboveground biomass, number of pods, and number of seeds between 33% and 66% defoliation. A possibility for a lack of an effect of our natural enemy manipulation treatments on crop yield is the 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 higher in the MeSA treatment. Leaf beetle damage could have caused a decrease in photosynthetic activity, lack of nutrients, and/or water intake, leading to lower mass of bean seeds (Blue *et al*. 2015). Overall, however, the 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 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 to the hypothesis that these effects cascade down to increase crop yield. Simpson *et al*. (2011b) also found no effects of combining HIPVs (MeSA and methyl anthranilate) and buckwheat in vineyards on grape yield. In contrast, Wang *et al*. (2011) found that an attract-and-reward that combines MeSA with oilseed rape (*B. napus*) increases natural enemy abundance, reduces aphid densities, and increases yield in wheat. In our studies we used relatively small size plots; future studies are needed to determine if plot size affects the outcome of using strategies to manipulate natural enemy behavior (Kaplan & Lewis 2015).

Our findings have important implications for conservation biological control of agricultural pest. 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 (Simpson *et al*. 2011a, 2011b; Orre Gordon *et al*. 2013) provide no evidence that combining two strategies in an attract-and-reward approach enhance natural enemy abundance, or their diversity, 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) enhance 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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Tables

Table 1. Effects of treatment (control, methyl salicylate (MeSA), coriander, and both) on the diversity (Shannon's H') and evenness (J') of total arthropod community and the communities of natural enemies and herbivores.

Year	Treatments	Arthropod communities[a]		Natural enemy communities		Herbivore communities	
		H'	**J'**	**H'**	**J'**	**H'**	**J'**
2015	Control	0.16 ± 0.06b	0.12 ± 0.04b	0.33 ± 0.12b	0.40 ± 0.15b	0.11 ± 0.04b	0.11 ± 0.04b
	MeSA	0.57 ± 0.13ab	0.45 ± 0.17ab	0.60 ± 0.22ab	0.91 ± 0.40a	0.29 ± 0.12ab	0.37 ± 0.16ab
	Coriander	1.01 ± 0.13a	0.66 ± 0.24a	0.90 ± 0.34a	0.88 ± 0.33a	0.37 ± 0.16a	0.44 ± 0.19a
	Both	0.67 ± 0.13a	0.44 ± 0.16ab	0.72 ± 0.27ab	0.82 ± 0.30ab	0.18 ± 0.06ab	0.19 ± 0.07ab
2016	Control	1.24 ± 0.46b	0.61 ± 0.23b	1.17 ± 0.44a	0.79 ± 0.29a	0.57 ± 0.21a	0.74 ± 0.27a
	MeSA	1.59 ± 0.60a	0.74 ± 0.27a	1.35 ± 0.51a	0.76 ± 0.28a	0.67 ± 0.25a	0.70 ± 0.26a
	Coriander	1.54 ± 0.58a	0.74 ± 0.27a	1.26 ± 0.47a	0.75 ± 0.28a	0.64 ± 0.24a	0.68 ± 0.25a
	Both	1.59 ± 0.60a	0.76 ± 0.28a	1.36 ± 0.51a	0.80 ± 0.30a	0.67 ± 0.25a	0.74 ± 0.27a

a Number are means ± SE; means with different letters within the same column are significantly different (Tukey's HSD test, α = 0.05).

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.

Year	**Taxa**[b]	**GLM results**											
		Block			**MeSA**			**Coriander**			**MeSA × Coriander**		
		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 taxa and developmental stages were pooled prior to analysis.

[c] Numerator, denominator (error).

[d] Numbers in bold indicate significant differences at α = 0.05.

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.

Year	**Taxa**[b]	**GLM results**											
		Block			**MeSA**			**Coriander**			**MeSA × Coriander**		
		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 taxa and developmental stages were pooled prior to analysis.

[c] Numerator, denominator (error).

[d] Numbers in bold indicate significant differences at α = 0.05.

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.

Year	**Parameters**	**GLM results**											
		Block			**MeSA**			**Coriander**			**MeSA × Coriander**		
		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
	Pods 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
	Seeds 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 α = 0.05.

Figures

Figure Captions

Fig. 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 7 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.

Fig. 2. Principal component analysis (PCA) on the effects of control (circles), methyl salicylate (MeSA) (squares), coriander (triangles), and both (cross) treatments on the abundance of arthropods (a,e), natural enemies (b,f), herbivores (c,g), and yield parameters (d,h) for years 2015 and 2016. In each PCA, lines going in the same direction indicate that those group of variables are associated with the same treatments.

Fig. 3. Abundance 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 in plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

Fig. 4. Abundance 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 in plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

Fig. 5. 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 in plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

Fig. 6. Amount 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 in plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

Fig. 7. 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 in plots with bean plants baited with MeSA and intercropped with coriander (+ MeSA, + Coriander).

Fig. 1

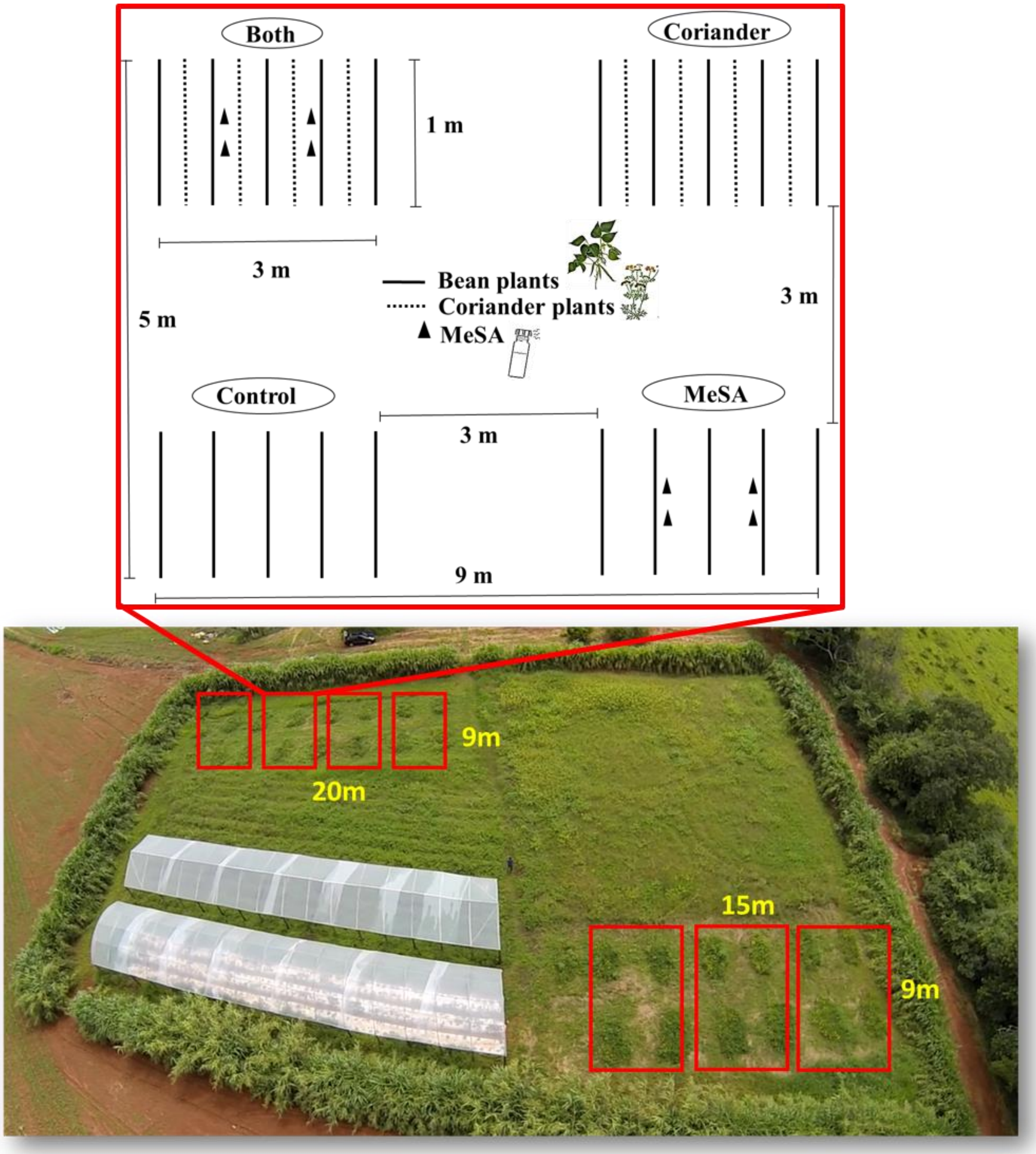

Both
Coriander
1 m
3 m
5 m
Bean plants
Coriander plants
MeSA
3 m
Control
MeSA
3 m
9 m
9m
20m
15m
9m

Fig. 2

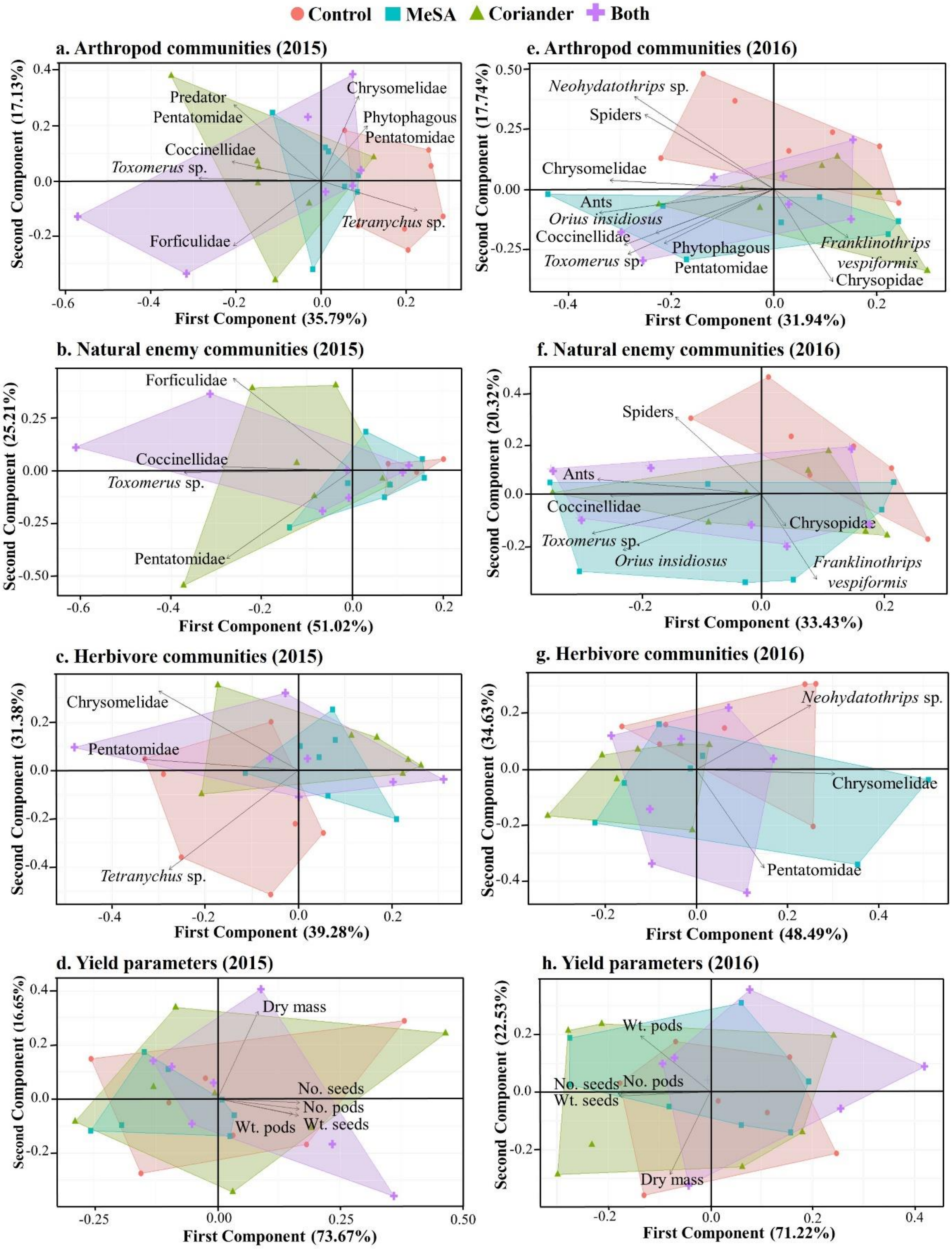
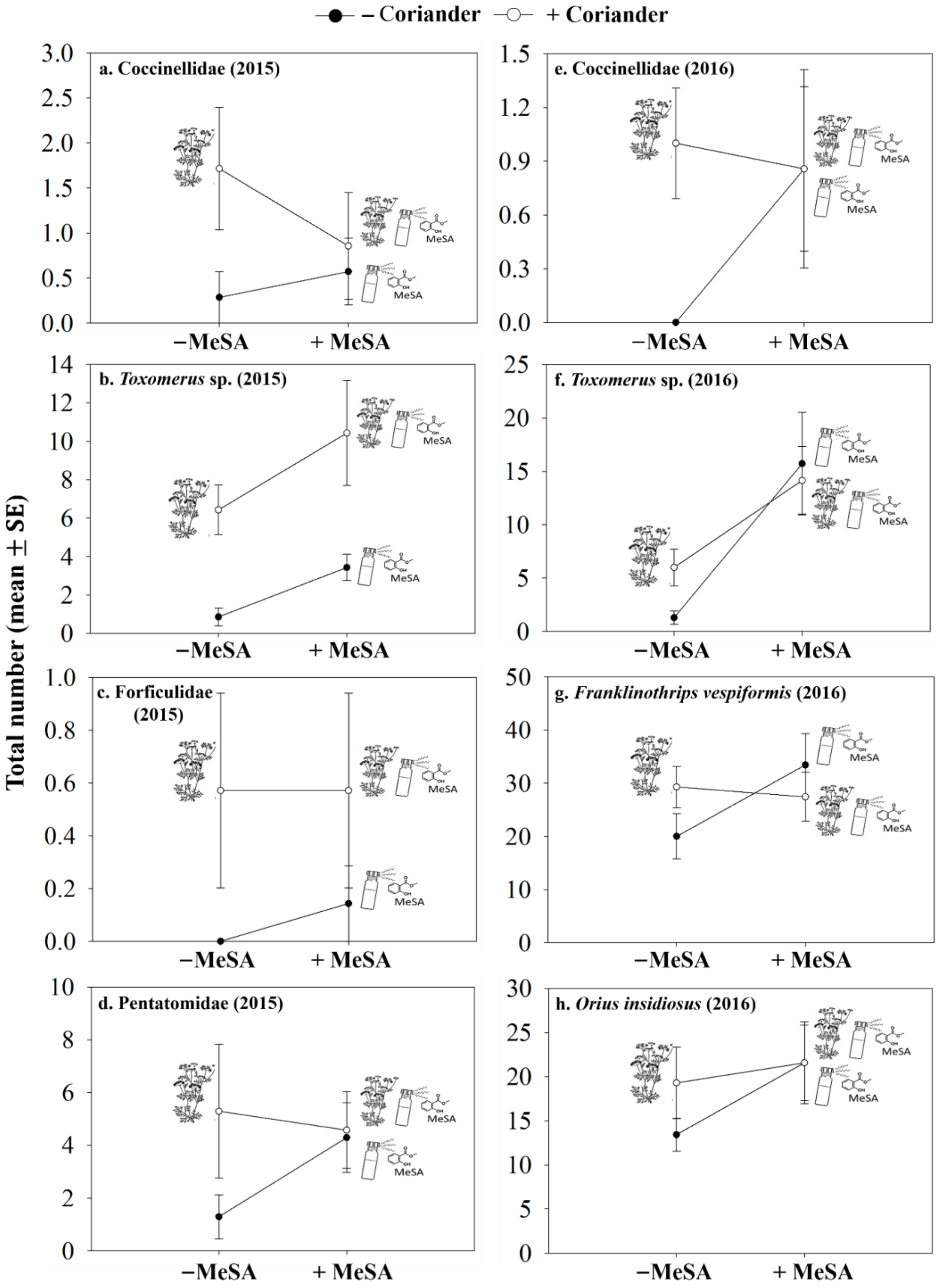

● Control ■ MeSA ▲ Coriander ✚ Both
a. Arthropod communities (2015)
Second Component (17.13%)
First Component (35.79%)
Predator Pentatomidae
Chrysomelidae
Phytophagous Pentatomidae
Coccinellidae
Toxomerus sp.
Tetranychus sp.
Forficulidae
b. Natural enemy communities (2015)
Second Component (25.21%)
First Component (51.02%)
Forficulidae
Coccinellidae
Toxomerus sp.
Pentatomidae
c. Herbivore communities (2015)
Second Component (31.38%)
First Component (39.28%)
Chrysomelidae
Pentatomidae
Tetranychus sp.
d. Yield parameters (2015)
Second Component (16.65%)
First Component (73.67%)
Dry mass
No. seeds
No. pods
Wt. pods
Wt. seeds
e. Arthropod communities (2016)
Second Component (17.74%)
First Component (31.94%)
Neohydatothrips sp.
Spiders
Chrysomelidae
Ants
Orius insidiosus
Coccinellidae
Toxomerus sp.
Phytophagous Pentatomidae
Franklinothrips vespiformis
Chrysopidae
f. Natural enemy communities (2016)
Second Component (20.32%)
First Component (33.43%)
Spiders
Ants
Coccinellidae
Chrysopidae
Toxomerus sp.
Orius insidiosus
Franklinothrips vespiformis
g. Herbivore communities (2016)
Second Component (34.63%)
First Component (48.49%)
Neohydatothrips sp.
Chrysomelidae
Pentatomidae
h. Yield parameters (2016)
Second Component (22.53%)
First Component (71.22%)
Wt. pods
No. seeds
No. pods
Wt. seeds
Dry mass

Fig. 3



– Coriander
+ Coriander
Total number (mean ± SE)
a. Coccinellidae (2015)
e. Coccinellidae (2016)
b. *Toxomerus* sp. (2015)
f. *Toxomerus* sp. (2016)
c. Forficulidae (2015)
g. *Franklinothrips vespiformis* (2016)
d. Pentatomidae (2015)
h. *Orius insidiosus* (2016)
−MeSA
+ MeSA
MeSA
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.2
0.9
0.6
0.3
14
12
10
8
6
4
2
0
25
20
15
5
0.8
0.4
0.2
50
40
30

Fig. 4

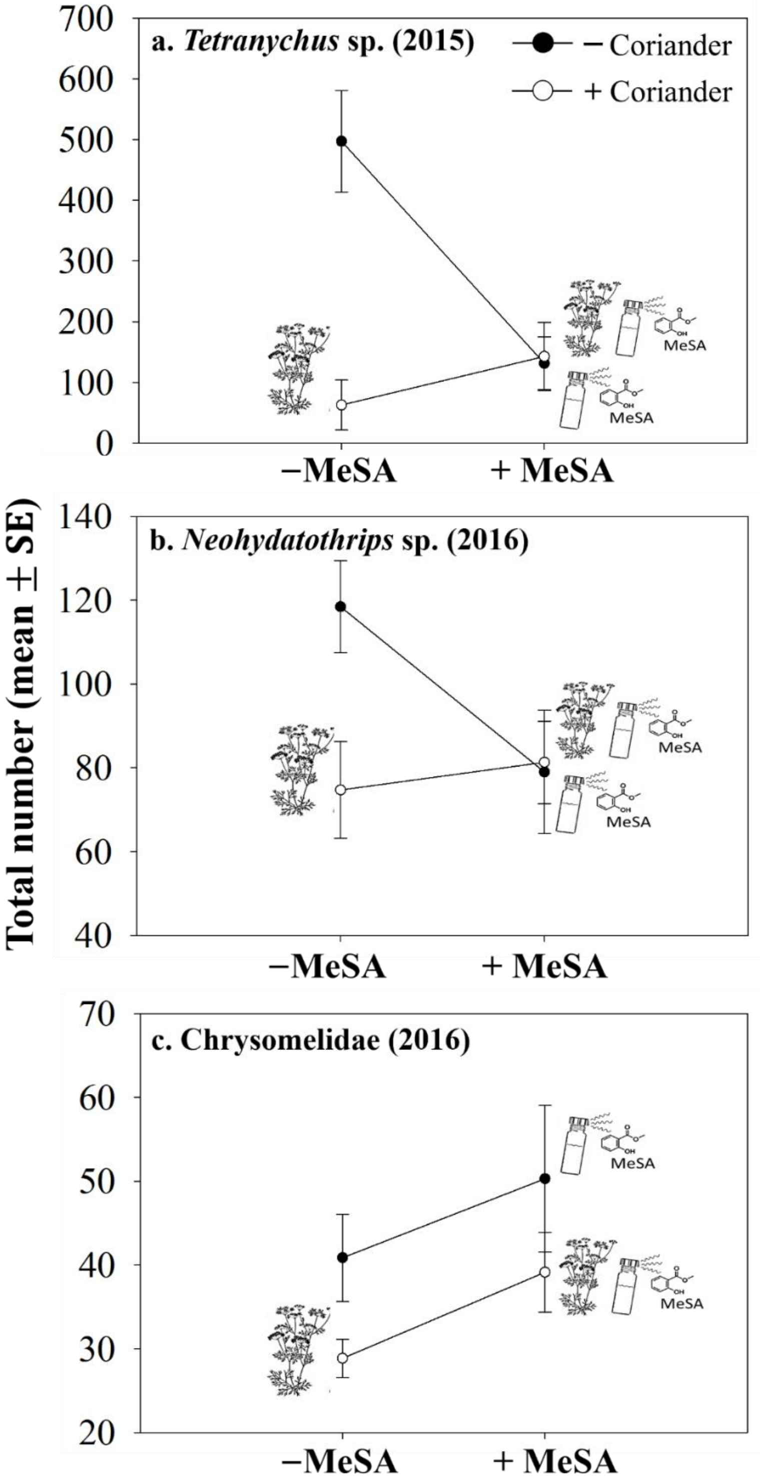

Total number (mean ± SE)
a. *Tetranychus* sp. (2015)
− Coriander
+ Coriander
700
600
500
400
300
200
100
0
−MeSA
+ MeSA
MeSA
MeSA
b. *Neohydatothrips* sp. (2016)
140
120
100
80
60
40
−MeSA
+ MeSA
MeSA
MeSA
c. Chrysomelidae (2016)
70
60
50
40
30
20
−MeSA
+ MeSA
MeSA
MeSA

Fig. 5

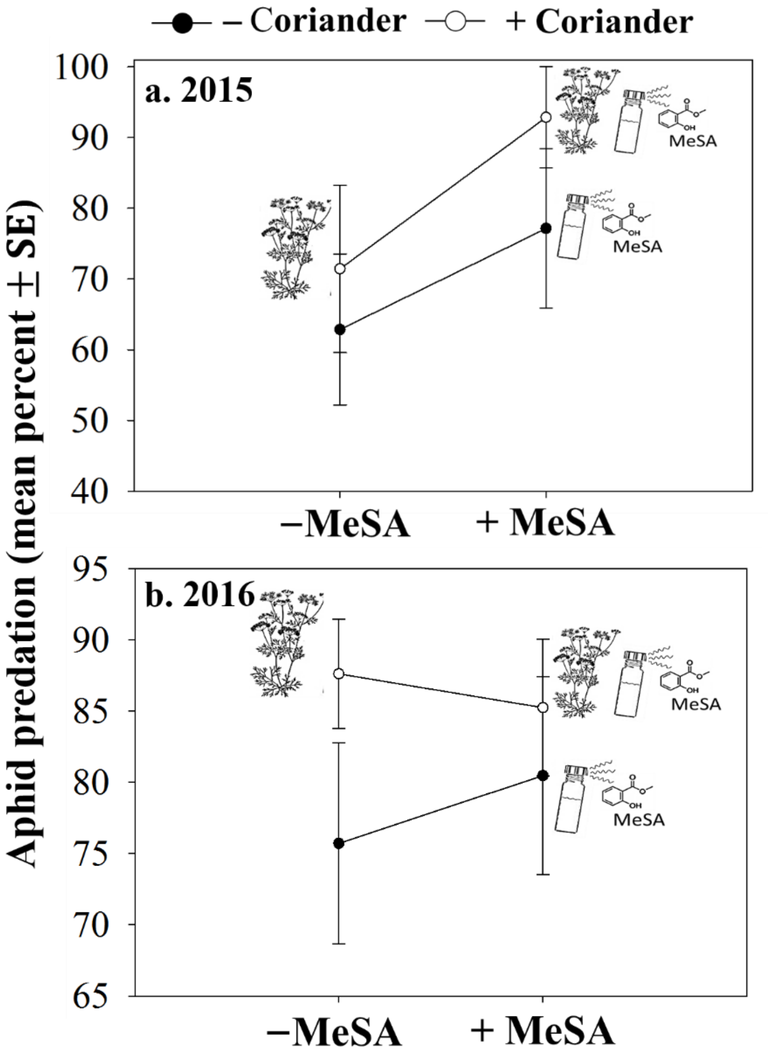

– Coriander
+ Coriander
a. 2015
b. 2016
Aphid predation (mean percent ± SE)
100
90
80
70
60
50
40
95
85
75
65
MeSA
−MeSA
+ MeSA

Fig. 6

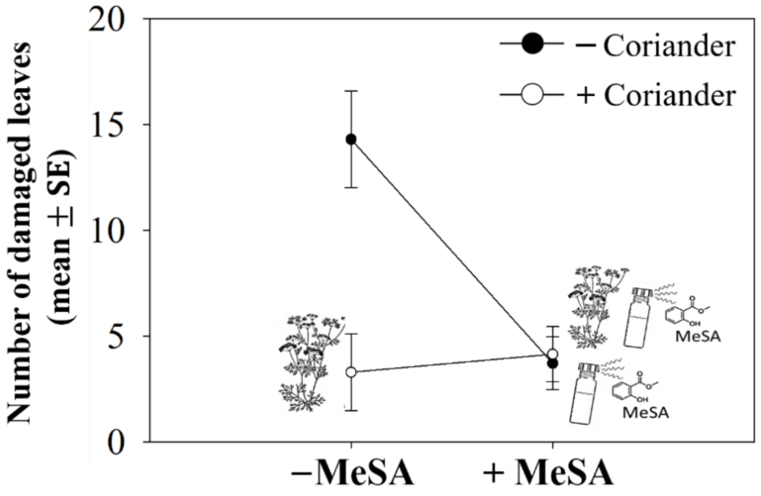
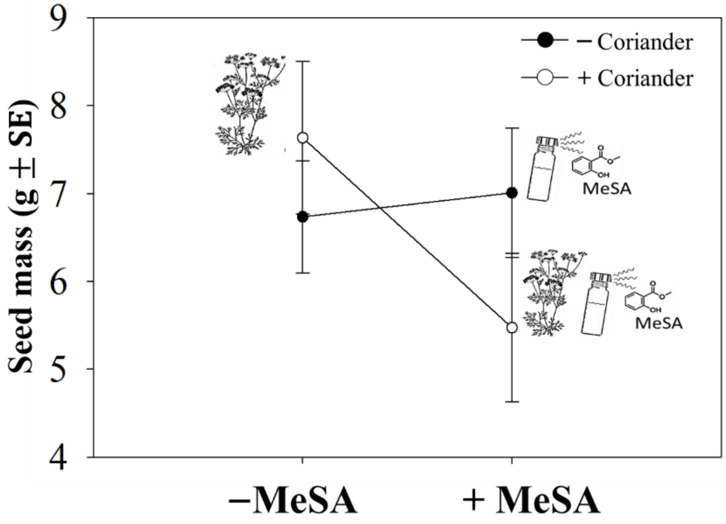

– Coriander
+ Coriander
Number of damaged leaves (mean ± SE)
20
15
10
5
0
MeSA
−MeSA
+ MeSA

Fig. 7


9
8
7
6
5
4
Seed mass (g ± SE)
● − Coriander
○ + Coriander
MeSA
MeSA
−MeSA
+ MeSA