

Methyl Salicylate Fails to Enhance Arthropod Predator Abundance or Predator to Pest Ratios in Cotton

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Subject Editor: Brett Hurley

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Received 28 September 2020; Editorial decision 27 November 2020

Abstract

Conservation biological control is a fundamental tactic in integrated pest management (IPM). Greater biological control services can be achieved by enhancing agroecosystems to be more favorable to the presence, survival, and growth of natural enemy populations. One approach that has been tested in numerous agricultural systems is the deployment of synthetic chemicals that mimic those produced by the plant when under attack by pests. These signals may attract arthropod natural enemies to crop habitats and thus potentially improve biological control activity locally. A 2-yr field study was conducted in the cotton agroecosystem to evaluate the potential of synthetic methyl salicylate (MeSA) to attract native arthropod natural enemies and to enhance biological control services on two key pests. Slow-release packets of MeSA were deployed in replicated cotton plots season long. The abundance of multiple taxa of natural enemies and two major pests were monitored weekly by several sampling methods. The deployment of MeSA failed to increase natural enemy abundance and pest densities did not decline. Predator to prey ratios, used as a proxy to estimate biological control function, also largely failed to increase with MeSA deployment. One exception was a season-long increase in the ratio of *Orius tristicolor* (White) (Hemiptera: Anthocoridae) to *Bemisia argentifolii* Bellows and Perring (= *Bemisia tabaci* MEAM1) (Hemiptera: Aleyrodidae) adults within the context of biological control informed action thresholds. Overall results suggest that MeSA would not likely enhance conservation biological control by the natural enemy community typical of U.S. western cotton production systems.

Key words: conservation biological control, natural enemy, synthetic attractant, integrated pest management, *Bemisia argentifolii*, *Lygus hesperus*

Conservation biological control is a foundational element in integrated pest management (IPM) that strives to enhance agricultural ecosystems by making them more favorable to the abundance and activity of natural enemies. This goal of improved biological control services can be achieved either by improving the habitat through the addition of resources aiding attraction and retention of natural enemies and/or by mitigating adverse factors such as the use of broad-spectrum insecticides (Van den Bosch and Telford 1964, DeBach 1974, Rabb et al. 1976, Ehler 1998, Gurr and Wratten 1999, Landis et al. 2000, Zalucki et al. 2015, Shields et al. 2019). Conservation biological control can be an economically valuable pest management strategy that takes advantage of existing, naturally adapted species in the environment. Recent syntheses suggest that conservation biological control can be valued at \$36–470/ha depending on the general approach (use of selective insecticides, habitat engineering) and the underlying value of the crop (Naranjo et al. 2015, Naranjo et al. 2019).

Herbivore feeding can elicit the emission of an array of volatile compounds, some of which might attract natural enemies of the herbivore (Dicke et al. 1990, Turlings et al. 1990, Stowe et al. 1995). These so-called herbivore-induced plant volatiles (HIPVs) have been widely explored over the past three decades as an approach for attracting natural enemies to crop habitats to enhance biological control of arthropod pests (Khan et al. 2008, Rodriguez-Saona et al. 2011, Kaplan 2012). These HIPVs also may act as repellents or deterrents to pests (Ninkovic et al. 2003, Hegde et al. 2012, Allsopp et al. 2014, Pérez-Hedo et al. 2018). Prominent among these volatiles is methyl salicylate (MeSA), a phenolic compound produced in plants in response to herbivory (Dicke et al. 1990, Campbell et al. 1993, Geervliet et al. 1997, Kessler and Baldwin 2001, Agrawal et al. 2002). MeSA is commercially available in synthetic form for direct application, primarily in lures that can be deployed in the field (James 2003a,b; Lee 2010; Mallinger et al. 2011; Salamanca

et al. 2019). While meta-analyses suggest that many insect predators and parasitoids can be attracted with MeSA in various crop habitats (Rodríguez-Saona et al. 2011), results are highly variable, even for the same taxa. It remains unclear if the overall approach can be valuable for increasing mortality of target pests (James and Price 2004, Khan et al. 2008, Mallinger et al. 2011, Simpson et al. 2011a, Gadino et al. 2012, Kaplan 2012, Ingwell et al. 2018, Morrison et al. 2018).

IPM systems for the main cotton pests in the western U.S. (*Bemisia argentifolii* Bellows and Perring (= *Bemisia tabaci* MEAM1) [Hemiptera: Aleyrodidae]; *Lygus hesperus* Knight [Hemiptera: Miridae]) are well established and highly effective in providing growers with economical options for pest management (Naranjo and Ellsworth 2009b, Ellsworth et al. 2018, Romeis et al. 2019). Fundamental to the success of this program has been the conservation of the native arthropod predator community. This conservation is achieved through the use of selective insecticides based on a sound decision-making system (Naranjo and Ellsworth 2009b). Natural enemies and other natural forces inflict over 90% mortality in immature populations of *B. argentifolii* (Naranjo and Ellsworth 2005) and recent advances are attempting to further improve pest management decision-making and reduce grower risk by including arthropod predator abundance into threshold-based decisions (Vandervoet et al. 2018). Tools that could locally increase natural enemy populations by ‘herding’ them from source areas such as perennial alfalfa into cotton could provide additional flexibility and efficacy in managing pests biologically. It also could serve as a useful experimental tool for manipulating natural enemy populations to better understand pest control dynamics. To our knowledge, the use of MeSA in western cotton systems has not been examined.

The objectives of this research were to test the utility of deploying synthetic, slow-release lures of MeSA to enhance local populations of arthropod natural enemies and thereby increase the probability of biological pest control. Replicated large plot studies were conducted in Arizona cotton over a 2-yr period. Focus was placed on increasing abundance of a diverse natural enemy community and understanding if favorable predator to prey ratios could be realized to enable improved conservation biological control.

Materials and Methods

Study Site and Experimental Design

Studies were conducted over two cotton growing seasons at the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ. The cotton variety was Genuity Bollgard II with Roundup Ready Flex (now Bayer Crop Science, St. Louis, MO), conferring resistance to lepidopteran insects and tolerance to glyphosate herbicides. Fields were planted on 16 and 26 April in 2009 and 2010, respectively. The crop was grown following standard agronomic practices for the area except that no insecticides were applied nor were the seeds treated with insecticides.

In 2009, plots were established in the northern half of a 9.7-ha block of cotton (surrounded immediately by fallow land) planted in an 18 row (1.02 m row spacing), skip two row configuration. The experimental design was a randomized complete block replicated four times. The plots were 18 × 18 m with 2 m bare borders between plots east to west and 4 m bare borders north to south. Plots within a block were separated by 36 m and blocks were separated by 36–54 m. The treatments were MeSA lures (see below) and an untreated control (UTC). Populations of *B. argentifolii* were extremely low in prior years at the field site, so cantaloupes (Jumbo Hales Best,

Willhite Seed Inc., Poolville, TX) were planted along with the cotton to enhance populations. These cantaloupes were planted in four row strips on the east and west borders of the large cotton block and were irrigated along with the cotton. In mid-June irrigation of the cantaloupes was terminated, slowly forcing the whiteflies to move into the adjacent cotton field.

In 2010, 18 rows of cotton were planted on the east and west sides of a 252 × 183 m (4.6 ha) unsprayed alfalfa field. The experimental design was again a two treatment (MeSA lures and UTC) randomized complete block with two blocks within each of the 18 row strips of cotton (4 blocks total). Plots were 18 × 18 m and placed within the continuous strips of cotton, separated by 40 m north to south and 250 m east to west. Plots were 2 m from the alfalfa and the land immediately surrounding the cotton/alfalfa area was fallow.

Plots assigned to the MeSA treatment received 90 d slow-release packets (5 g each) of Predalure (AgBio, Westminster, CO). Nine packets (468/ha) were placed in each plot in an evenly spaced 3 × 3 grid in the central portion of the plot at least 3 m from any plot edge on 7 and 14 July in 2009 and 2010, respectively. Each packet was hung in the middle of the top third of individual cotton plants and was relocated over the course of the season so that their placement in the top third of the plant was maintained. The same packets were left in place for the duration of the study. The UTC plots received no packets.

Measurement of MeSA and Plant Volatiles

Measurements of MeSA and plant volatile emissions were made in situ. The top 30–40 cm of an individual cotton plant with or without a MeSA packet was enclosed with a clear-plastic Reynolds oven bag (482 × 596 mm) sealed tightly around the mainstem. A charcoal filter (6–14 mesh, 15 cm × 1 cm dia., purged with N₂ at 130°C for 24 h) was placed within this seal around the stem, half in and half out of the bag. Cotton volatiles were collected for 15 min by pulling outside air through the charcoal filter into the bag and through the cotton plant top into a 20 mm × 2.3 mm plug of Porapak Q (80/100 mesh, W.R. Grace & Co., Columbia, MD; hereafter Grace) inside of 0.32 cm Teflon tubing. No breakthrough of volatiles was observed with a second Porapak Q plug in preliminary work. The adsorbent plug was attached by Swagelok fittings to 1 m of tubing that exited the bag seal and connected to a flow meter (airflow 500 ml/min) and then to a 12 V vacuum pump powered by a lead-acid rechargeable 12V battery. After the assay, the Porapak plug with the ‘downstream’ Swagelok fittings was removed from the system and a glass tube (25 mm × 3.2 mm O.D.) was attached to the fitting to allow 200 µl of hexane with ethyl heptanoate and (+)-carvone internal standard (1 ng/µl; Sigma-Aldrich) to pass through the adsorbent and wash volatiles into a vial for subsequent analysis. Three plants were assayed in each of three replicate plots for each treatment on 8 dates between 8 July and 29 September 2009.

Volatile compounds were separated on a Varian 3800 gas-chromatograph (Varian, Inc., Palo Alto, CA) equipped with a 60 m × 0.25 mm Cyclodex-B fused-silica capillary column (J & W Scientific of Agilent Technologies, Santa Clara, CA) using a temperature program optimized for volatiles. The carrier gas was UHP helium set to a constant flow of 1.2 ml min⁻¹. Samples of 1 µl were injected into a port set to 250°C using a Varian CP-8400 auto-sampler. The injection mode was split-less for 0.75 min, thereafter split at 60:1 split for 5 min, then 20:1 for the remainder of the run. The oven/column temperature was initially held at 40°C for 2 min, then increased to 60°C at a rate of 10°C min⁻¹. After 10 min at 60°C, the oven temperature was increased by 3°C min⁻¹ to 150°C, then by 20°C min⁻¹

to 230°C where it was held 10 min. For chemical identification, the masses of the compounds separated by GC were determined with a Varian Saturn 2000 Mass Spectrometer (MS, ion trap, 70 eV EI) using the NIST08 (National Institute of Standards, USA) and Wiley7 (John Wiley & Sons, Inc., Hoboken, NJ) spectral databases and by comparison with commercial standards. Quantification was based on the MS response factors of the commercial standards (or similar compounds/isomers), the internal standard, and adjusted for collection time and the volume of solvent used for extraction.

In 2010, a MeSA lure was randomly taken from a cotton plot in the field and placed inside a 5 cm diameter × 11 cm high glass jar with a Teflon-lined lid for 2 min. prior to insertion of a solid phase microextraction (SPME) fiber (60 µm PDMS/DVB purple, Supelco, Bellefonte, PA) holder assembly (1 cm dia.). The MeSA volatiles were sampled for 1 min and the lure was returned to the plant. The process was repeated for a second lure from another plot. Samples were taken weekly from 23 July to 30 September.

Arthropod Sampling

In both years, *B. argentifolii* adults were counted on the underside of leaves from the fifth mainstem leaf node below the terminal (Naranjo and Flint 1995) and eggs and nymphs were counted on 3.88 cm² disks taken from this fifth node leaf in the laboratory under a dissecting microscope (Naranjo and Flint 1994). The sample size was 15 randomly selected leaves or disks per plot. In 2009, a standard 38 cm sweep net was used to sample the foliar dwelling arthropods, including *L. hesperus*. Two sets of 25 sweeps were taken along randomly selected rows from the center 14 rows of each plot. No samples were taken in the two outside rows, within 2 m of each edge perpendicular to the rows, or within rows where the MeSA packets were placed to avoid disturbance of the lures. Samples were returned to the laboratory, frozen and then later processed to identify approximately 20 arthropod taxa under a dissecting microscope. Adult and immature stages of arthropod taxa were combined for analyses.

In 2010, three methods were used to assess the density of foliar dwelling arthropods. For the first two methods, a beat bucket (Knutson et al. 2008) was used to sample arthropods within each plot generally and on plants directly surrounding the plant containing the MeSA packet. The beat bucket was constructed with an 18 liters white plastic painting pail (37 cm × 27 m dia.). The bucket bottom was cut out and a large plastic funnel (P- 06121-20, Cole Parmer Co., Vernon Hills, IL) was fastened over the bottom with metal brackets. A 120-ml plastic jar was attached to the base of the funnel for collecting the arthropods that could be detached and capped after each sample. A drawer handle was fastened to the side of the bucket to allow it to be tilted for sampling. The bucket was held at a 45° angle to the ground and an individual cotton plant was carefully grasped by the lower stem and quickly bent into the bucket where it was beaten against the sides a total of 10 times (ca. 3–4 s) to dislodge arthropods. After beating, the plant was then removed, the bucket was tilted upright and the sides were tapped sharply until all the arthropods fell into the collecting jar. For general plot sampling a total of 20 plants (sample unit) along a randomly selected row of the plot were individually beaten into the bucket. To minimize disturbance, individual plants were separated by two steps along the row. The second sample consisted of beating five individual plants immediately adjacent to each of two randomly selected MeSA lure stations within the plot (10 total plants per sample unit). Sampled plants were 0.5–1 m from the lure. In control plots, samples were collected around single flagged plants (two per plot) without a lure. Hereafter, these samples will be called lure samples. Samples were

returned to the laboratory for processing similar to sweep net samples described above. Finally, nonattractive sticky traps were placed in the center of each plot. The trap was a sheet of 0.64 cm hardware cloth (Keystone LG, Lowes, Chandler, AZ) formed into a cylinder 30.5 cm in height and 7.5 cm in diameter coated in sticky material (Pestick, Gempler's, Janesville, WI). Traps were attached to a wooden post at 1 m and 2 m above the ground with binder clips and exposed for 1 wk. Arthropods were extracted from the traps and identified as described above. Catches were combined for the two traps in each plot. In both years, arthropod samples were taken approximately weekly from mid-July to mid-September (2009: 9 dates, 2010: 6–9 dates). The first samples were taken the day after the MeSA packets were installed.

In 2010, samples for whitefly parasitoids (Aphelinidae: Hymenoptera) were collected on two dates (26 August and 9 September) using methods described by Naranjo and Li (2016). Briefly, 20 whole leaves from the seventh node below the mainstem terminal were randomly collected from each plot and returned to the laboratory where all larval and pupal parasitoids of *Eretmocerus* spp. and *Encarsia* spp. (Hymenoptera: Aphelinidae) and all unparasitized fourth instar *B. argentifolii* nymphs were counted under a dissecting microscope. Proportional parasitism was estimated as the quotient of all immature parasitoid stages and all unparasitized fourth-stage nymphs plus immature parasitoids.

Predator to Prey Ratios

Predator to prey ratios were estimated as a proxy for examining the biological control potential of the natural enemy community in response to the application of MeSA. Ratios were estimated for eggs/leaf disk, nymphs/leaf disk, and adults/leaf of *B. argentifolii* and adults plus nymphs of *L. hesperus* (per 50 sweeps in 2009, per beat bucket sample unit or per trap in 2010) using the total of all arthropod predator taxa per sample unit for each sample date.

Recent research indicates that six arthropod predator species can potentially reduce populations of *B. argentifolii* when present in sufficient abundance relative to prey density; *Misumenops celer* (Hentz) (Araneida: Thomisidae), *Collops* spp. (Coleoptera: Melyridae), *Geocoris* spp. (Hemiptera: Geocoridae), *Orius tristicolor* (White), (Hemiptera: Anthocoridae), *Drapetis* nr. *divergens* (Diptera: Empididae), and larvae of *Chrysoperla carnea* s.l. (Neuroptera: Chrysopidae) (Vandervoet et al. 2018). These authors developed action thresholds for each of these predator taxa per 100 sweeps for *B. argentifolii* nymphs/leaf disk and/or adults/leaf. In 2009, where sweep net samples were taken, the proportion of sample dates on which these threshold ratios were sufficient to provide whitefly control were estimated based on a total of 8 proposed ratios per experimental unit (Vandervoet et al. 2018).

Statistical Analyses

A mixed model within the generalized linear modeling platform of SAS V9.4 (GLIMMIX, SAS Institute, Cary, NC) was used to compare arthropod abundance between the MeSA treatment and the untreated control. Treatment was a fixed effect, date was a repeated measure (with a first-order autoregressive [AR1] covariance structure) and block and block by treatment interactions were random effects. Arthropod abundance was modeled with a negative binomial distribution, proportional parasitism and proportion of predator to prey ratios indicating control were modeled with a binomial distribution and total predator to prey ratios were modeled with a log-normal distribution. The Kenward–Roger formula was used to estimate corrected degrees of freedom. Due to different sampling

procedures, analyses were conducted separately for each year. The false discovery rate method (Benjamini and Hochberg 1995), with the false discovery rate set at 10%, was used to correct hypothesis testing for multiple tests of individual taxa within a year and sampling method. In 2009, the readings from the MeSA lures were compared with zero (UTC) using one-sided *t*-tests. Comparison of other volatile chemicals from MeSA and control plants were compared using a similar mixed-model repeated measure model as detailed above with a Poisson distribution. MeSA packet emission rates were measured in 2010, but with $n = 2$ and missing data on some dates, statistical analyses were not conducted.

Seasonal dynamics of the arthropod community were further examined with principal response curves (PRC), a time-dependent, multivariate analysis (Van den Brink and Ter Braak 1998, 1999). PRC is a partial redundancy analysis where information is extracted only from the variance explained by treatment effects. The method provides a simple and powerful means of visualizing and testing an overall response of the arthropod community to an environmental variable—in this case the addition of MeSA. The results are plotted to show changes in the response to MeSA relative to the untreated control, represented by the $y = 0$ line. The graphs are structured such

that a positive canonical coefficient (above the $y = 0$ line) would indicate a higher abundance in the MeSA treatment and a negative coefficient would denote higher abundance in the UTC. Analyses were performed with CANOCO 4.5 (Ter Braak and Smilauer 1998) for sweep net, beat bucket and sticky trap counts each year. *F*-type tests based on sample permutation (1,000 iterations) were used to compare seasonal treatment effects. Arthropod count data were transformed by $\ln(x + 1)$ before analysis.

Results

MeSA Lures and Plant Volatiles

The 90-d slow-release MeSA packets emitted an average of 240–450 $\mu\text{g/h}$ during the first 37 d after deployment in 2009 (Fig. 1). The absolute emission rate determined by air collection on Porapak was consistently above 200 $\mu\text{g/h}$ for 56 d and then dropped rapidly to below 40 $\mu\text{g/h}$ and then to below 10 $\mu\text{g/h}$ after 83 d. Emission rates were generally greater than zero for the first 56 d following deployment ($t > 2.12$, $df = 2$, $P < 0.08$). A different assay using SPME fibers was conducted in 2010, but the pattern was similar, with relative emission rates between 8 and 49 ng/fiber for the first 39 d after

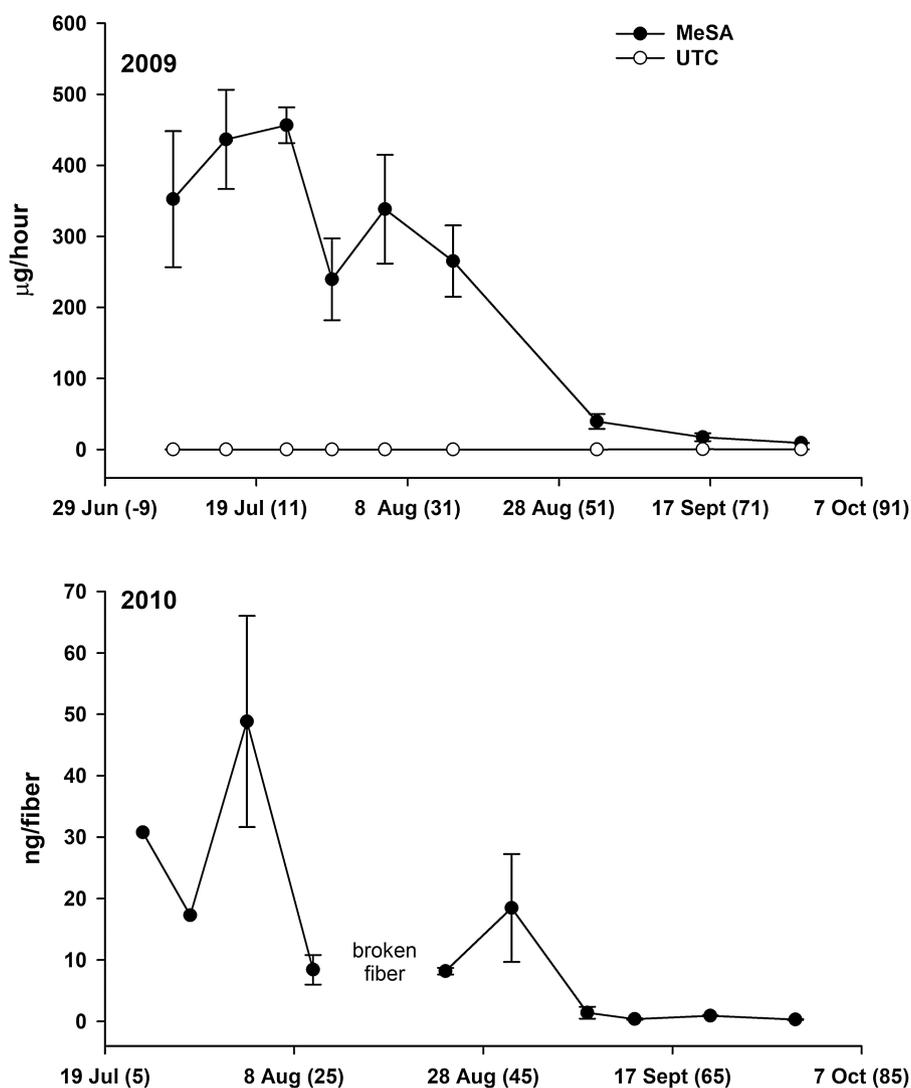


Fig. 1. Mean (\pm SE) emission rates of MeSA from 90-d slow-release lures attached to cotton plants in 2009 and 2010. The numbers in parentheses following the date denote the number of days after the initial deployment of MeSA packets, $n = 3$ and 2 in 2009 and 2010, respectively.

deployment and below 1.4 ng/fiber after 69 d (Fig. 1). Relatively high levels of several other plant volatiles were measured from the in-field assays in 2009, but there was no pattern of differential emission rates of these due to the deployment of MeSA ($F < 3.81$, $df = 1$, $7.1\text{--}12$, $P > 0.08$; Fig. 2). Low levels of other plant volatiles were measured but did not differ by treatment ($P > 0.05$, data not shown).

Arthropod Dynamics

Twenty taxa of arthropod natural enemies were cataloged using different sampling approaches over the 2-yr study period. Uniformly, there were no differences in arthropod density due to the addition of MeSA packets using sweep nets in 2009 (Table 1) or using beat buckets and sticky traps in 2010 (Table 2) after correcting for multiple hypothesis tests using a false discovery rate of 10%. This was despite a more targeted sampling approach centered around the packets in 2010 (lure sampling) and the presence of a large source reservoir of arthropods from an adjacent unsprayed alfalfa field in 2010. As expected, predator abundance varied over the season for most taxa (2009 sweeps, $F > 3.61$, $df = 8$, $19.2\text{--}54$, $P < 0.002$; 2010 beats, $F > 3.16$, $df = 8$, $18.2\text{--}54$, $P < 0.024$; 2010 lures, $F > 3.02$, $df = 8$, $33.4\text{--}54$, $P < 0.01$; 2010 traps $F > 3.38$, $df = 5$, $12.7\text{--}36$, $P < 0.014$), but no interactions between MeSA treatment and date were observed ($P > 0.09$) for any comparison indicating that differential abundance due to treatment did not change over time.

Likewise, the densities of the two key pests (*B. argentifolii* and *L. hesperus*) were not influenced by the addition of MeSA packets, nor was the level of parasitism on *B. argentifolii* by several species of native and exotic aphelinid parasitoids (Table 2). Again, results varied by date within the season ($F > 7.00$, $df = 8$, $24.5\text{--}39$, $P < 0.01$), but there were no interactions between MeSA treatment and date ($P > 0.20$). Parasitoid abundance and rates of parasitism did not vary over time ($P > 0.40$), and there was no interaction between treatment and date ($P > 0.55$). Given that MeSA emissions from the 90-d packets appeared to decline after about 50 d, all analyses above were re-ran after eliminating the final three dates in 2009 and two dates in 2010. Results did not change from those based on all sample dates.

These taxa-specific results were confirmed with more powerful multivariate analyses examining responses of the entire arthropod community. Temporal curves showed that community abundance in the MeSA treatments varied over time relative to the UTC ($y = 0$ line) but did not differ from the control in either year regardless of sampling approach (Fig. 3). The curves best represent taxa with high positive species weights while those with high negative weights represent an opposite pattern. Taxa with weights between -0.5 and 0.5 have relatively little influence on the shapes of the curves. Note that canonical coefficients above the $y = 0$ line indicate higher abundance in the MeSA treatment and vice versa. The arthropod groups best represented by the curves in each year and sampling method varied somewhat and included Anthicidae, *Spanogonicus albofasciatus* (Reuter) (Hemiptera: Miridae), *D. nr. divergens* and *G. punctipes* in 2009 and most of these taxa and *Collops* spp., *O. tristicolor* and Hymenoptera as a group in 2010. Those taxa associated with the curves, but showing an opposite pattern include *G. pallens* Stål, *C. carnea s.l.*, Anthicidae, *Dictyna reticulata* Gertsch & Mulaik (Araneida: Dictynidae) and *Zelus renardii* Kolenati (Hemiptera: Reduviidae). Consistent with univariate analyses for individual taxa, the redundancy analyses described 43.4–57.5% of the variation due to sampling date but only 4.5–7.9% of the variation due to the addition of MeSA. Again, elimination of the last three or two sampling dates in 2009 and 2010, respectively, did not alter the results of these multivariate analyses.

Predator to Prey Ratios as a Proxy for Potential Biological Control Function

Ratios of all arthropod predators to various life stages of *B. argentifolii* and *L. hesperus* were unaltered by the addition of MeSA packets based on multiple comparisons corrected for a false discovery rate of 10% (Fig. 4). This was true for sweep net samples in 2009, and general beat bucket samples, beat samples immediately surrounding the MeSA lures and sticky traps in 2010. Most ratios varied over the season each year (2009 sweeps, $F > 4.24$, $df = 8$, $29.5\text{--}35.4$, $P < 0.001$; 2010 beats, $F > 2.39$, $df = 8$, $26.6\text{--}35.3$, $P < 0.04$; 2010 lures, $F > 3.01$, $df = 8$, $16.8\text{--}35.9$, $P < 0.03$; 2010 traps $F > 4.09$,

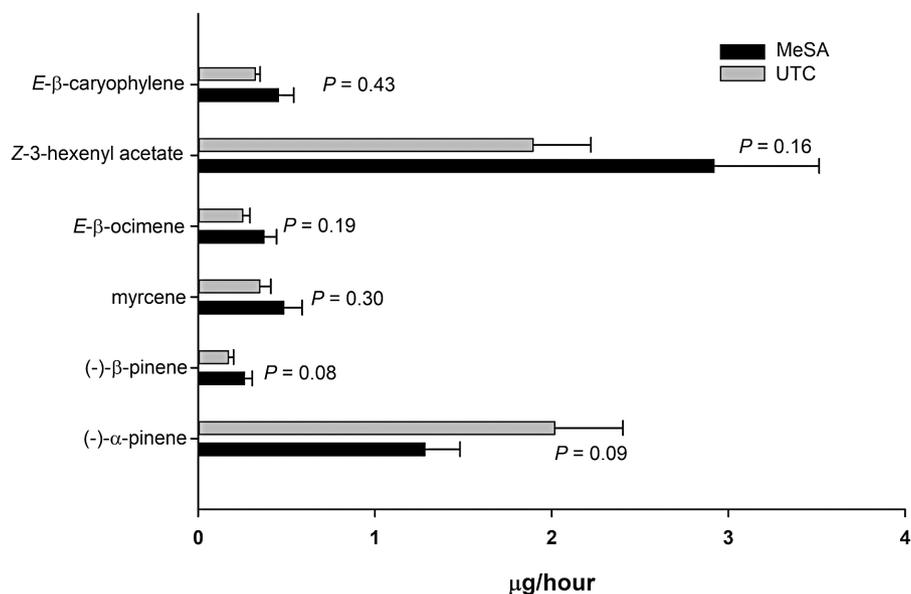


Fig. 2. Seasonal mean (\pm SE) emission rates of six of the most abundant volatiles emitted from cotton plants that contained MeSA lures or no lures in 2009. P-values denote the results of mixed-model, repeated-measures analysis of variance, $n = 3$.

Table 1. Seasonal mean densities (\pm SE) of arthropods in cotton with and without MeSA lures in 2009

Taxa	Number per 50 sweeps		
	MeSA	UTC	F-value (<i>P</i>) ^a
Natural enemies			
<i>Dictyna reticulata</i>	0.28 \pm 0.09	0.43 \pm 0.15	0.53 (0.56)
<i>Misumenops celer</i>	4.83 \pm 0.53	5.75 \pm 0.29	2.88 (0.27)
Salticidae	1.10 \pm 0.26	0.85 \pm 0.12	0.48 (0.51)
Other Araneida	0.40 \pm 0.11	0.40 \pm 0.13	0.59 (0.48)
<i>Collops</i> spp.	0.33 \pm 0.07	0.40 \pm 0.11	0.09 (0.81)
<i>Hippodamia convergens</i>	0.28 \pm 0.05	0.45 \pm 0.12	0.66 (0.42)
Anthicidae	0.95 \pm 0.33	0.63 \pm 0.43	0.50 (0.51)
<i>Geocoris punctipes</i>	2.08 \pm 0.30	2.03 \pm 0.29	1.26 (0.27)
<i>Geocoris pallens</i>	0.75 \pm 0.05	0.83 \pm 0.26	0.01 (0.90)
<i>Orius tristicolor</i>	3.75 \pm 0.30	3.43 \pm 0.22	0.13 (0.72)
<i>Nabis alternatus</i>	0.73 \pm 0.14	0.78 \pm 0.19	0.12 (0.73)
<i>Zelus renardii</i>	1.05 \pm 0.12	1.00 \pm 0.18	0.54 (0.46)
<i>Spanogonicus albofasciatus</i>	1.55 \pm 0.42	1.88 \pm 0.24	0.60 (0.53)
<i>Rhinacloa forticornis</i>	4.08 \pm 0.94	4.23 \pm 1.24	0.01 (0.92)
<i>Chrysoperla carnea</i> s.l. (larvae)	2.15 \pm 0.39	1.83 \pm 0.34	0.01 (0.95)
<i>Drapetis</i> nr. <i>divergens</i>	6.05 \pm 1.91	4.38 \pm 1.17	0.29 (0.60)
Other Hymenoptera	1.68 \pm 0.17	1.73 \pm 0.21	0.06 (0.81)
Pests			
<i>Lygus hesperus</i>	4.23 \pm 0.49	5.03 \pm 1.43	0.09 (0.81)
<i>Pseudatomoscelis seriatus</i>	5.40 \pm 0.94	5.63 \pm 0.21	0.05 (0.84)
<i>Bemisia argentifolii</i> eggs ^b	1.34 \pm 0.22	2.57 \pm 1.01	0.07 (0.79)
<i>Bemisia argentifolii</i> nymphs ^b	1.22 \pm 0.20	1.83 \pm 0.46	1.40 (0.37)
<i>Bemisia argentifolii</i> adults ^b	2.31 \pm 0.36	3.87 \pm 1.09	1.75 (0.33)

^adf = 1, 17.6–54.

^bNumbers per fifth mainstem node leaf disk (eggs, nymphs) or leaf (adults).

df = 5, 13.6–24.3, $P < 0.008$), but as with arthropod abundance, the relationship between treatments did not vary over time ($P > 0.29$).

Comparative predator to prey ratios were further examined through the perspective of pest management using biological control informed thresholds developed for *B. argentifolii*. The proportion of dates over the season in 2009, where predator densities would have been sufficient to provide biological control of whitefly, varied widely depending on the arthropod predator considered (Fig. 5). On over 80% of sample dates, the abundance of *M. celer* and *Geocoris* spp. would have been sufficient to negate the need for pest control. But only about 20 or 40% of the time would a decision to not control whitefly population have been made using the abundance of *Collops* spp. or *Drapetis* sp., respectively. Based on all predator taxa, 50–60% of the time, predator abundance would have been sufficient to provide biological control of *B. argentifolii*. For the most part, the deployment of MeSA packets did not change these patterns except for ratios based on *O. tristicolor*. For this predator species, the proportion of sample dates suggesting sufficient biological control doubled from around 30 to 60% with the addition of MeSA packets ($F = 11.7$, df = 1, 6, $P = 0.014$; Fig. 5). Predator:prey ratio results overall were unaltered by eliminating the last three and two sampling dates in 2009 and 2010, respectively.

Discussion

A diverse community of arthropod predators and parasitoids inhabit cotton in the western United States. (Van den Bosch and Hagen 1966) and they can provide effective biological control services if the crop habitat is managed to conserve important natural enemies (Naranjo and Ellsworth 2009a). Our goal was to determine which species of generalist predatory arthropods inhabiting

cotton in the western United States are attracted to synthetic MeSA, thereby potentially enhancing biological control services. We wanted to know if slow-release synthetic MeSA lures could be distributed in the field and provide general attraction to a local area. Using several common sampling methods over a 2-yr period, we failed to document an enhanced abundance of natural enemies via MeSA for any of 20 common taxa. In year 1, our plots were placed inside a large area of cotton surrounded mainly by fallow ground and other production crops that are generally managed with insecticides. Thus, there were no likely nearby sources of natural enemies other than the cotton in which our plots were embedded. In year 2, our plots bordered a five-hectare alfalfa field. Alfalfa in our region is mostly grown as forage crop and insecticide use is rare. Thus, alfalfa represents a large perennial reservoir for arthropods, many of them generalist natural enemies (personal observation). Even with this large source for natural enemies, MeSA failed to attract natural enemies as measured with beat bucket and nonattractive sticky trap sampling techniques. Additionally, in this second year, we sampled plants immediately surrounding (within 0.5–1 m) our deployed MeSA packets. Several studies have demonstrated that attraction to MeSA can be restricted to very short distances. For example, Lee (2010) showed that Chrysopidae were attracted to MeSA-baited traps in strawberry fields, but not on traps 5–10 m distant. Likewise, Mallinger et al. (2011) found attraction to MeSA on baited traps but not on traps only 1.5 m away. This short-range attraction appears to be a normal phenomenon for MeSA and many other attractive chemicals. Trap studies commonly use separation distances of 5–15 m and yet still demonstrate treatment effects (James 2003b, Zhu and Park 2005, Yu et al. 2008, Simpson et al. 2011b, Braasch et al. 2012, Dong and Hwang 2017). However, even with these samples adjacent to the lures we were unable to document enhanced natural

Table 2. Seasonal mean densities (\pm SE) of arthropods in cotton with and without MeSA lures, Maricopa, AZ, 2010

Taxa	Beat buckets – general			Beat buckets – lures			Sticky traps		
	MeSA	UTC	F-value (P) ^a	MeSA	UTC	F-value (P) ^a	MeSA	UTC	F-value (P) ^a
Natural enemies									
<i>Dictyna reticulata</i>	3.78 \pm 0.51	4.22 \pm 1.07	0.05 (0.82)	2.28 \pm 0.38	1.92 \pm 0.20	0.35 (0.56)	-	-	-
<i>Misumenops celer</i>	9.97 \pm 2.00	10.03 \pm 1.61	0.00 (0.99)	5.53 \pm 0.97	5.28 \pm 0.87	0.41 (0.52)	2.54 \pm 0.68	2.46 \pm 0.57	0.05 (0.86)
Salicidae	0.81 \pm 0.12	0.92 \pm 0.21	0.04 (0.85)	0.39 \pm 0.07	0.64 \pm 0.15	1.87 (0.23)	0.71 \pm 0.22	0.29 \pm 0.13	3.55 (0.07)
Other Araneida	-	-	-	-	-	-	0.54 \pm 0.54	0.25 \pm 0.25	0.28 (0.60)
<i>Collops</i> spp.	0.78 \pm 0.29	1.11 \pm 0.32	0.12 (0.76)	0.36 \pm 0.08	0.17 \pm 0.05	1.56 (0.22)	1.83 \pm 0.26	0.71 \pm 0.04	16.23(0.01) ^b
<i>Hippodamia convergens</i>	0.47 \pm 0.33	0.64 \pm 0.33	0.37 (0.55)	0.25 \pm 0.15	0.22 \pm 0.12	0.00 (0.96)	1.50 \pm 0.32	0.96 \pm 0.26	0.69 (0.41)
Anthicidae	3.50 \pm 1.11	3.03 \pm 1.11	0.42 (0.52)	1.56 \pm 0.20	1.19 \pm 0.46	0.13 (0.72)	51.46 \pm 13.5	45.95 \pm 15.14	1.52 (0.23)
Other Coccinellidae	2.17 \pm 0.49	2.52 \pm 0.45	0.12 (0.76)	0.92 \pm 0.15	0.53 \pm 0.17	1.88 (0.18)	3.83 \pm 0.59	3.17 \pm 0.95	0.02 (0.89)
<i>Geocoris punctipes</i>	5.19 \pm 0.98	6.47 \pm 0.78	4.08 (0.05)	3.58 \pm 0.44	2.86 \pm 0.35	1.35 (0.25)	0.46 \pm 0.14	0.46 \pm 0.04	0.00 (0.99)
<i>Geocoris pallens</i>	2.11 \pm 0.45	2.00 \pm 0.55	0.01 (0.95)	0.61 \pm 0.15	1.00 \pm 0.16	1.41 (0.24)	0.25 \pm 0.11	0.29 \pm 0.10	0.03 (0.89)
<i>Orius tristicolor</i>	7.31 \pm 0.94	7.92 \pm 0.45	0.00 (0.95)	3.94 \pm 0.66	3.44 \pm 0.27	0.02 (0.89)	3.50 \pm 0.92	2.13 \pm 0.82	1.16 (0.28)
<i>Nabis alternatus</i>	0.28 \pm 0.03	0.17 \pm 0.06	1.17 (0.28)	-	-	-	-	-	-
<i>Zelus renardii</i>	3.86 \pm 0.48	3.72 \pm 0.06	0.12 (0.73)	1.33 \pm 0.25	1.83 \pm 0.26	1.43 (0.24)	-	-	-
<i>Sinea</i> spp.	0.47 \pm 0.22	0.50 \pm 0.07	0.16 (0.69)	-	-	-	-	-	-
<i>Spanogonicus albofasciatus</i>	0.39 \pm 0.21	1.06 \pm 0.17	6.26 (0.11)	0.22 \pm 0.05	0.33 \pm 0.05	1.29 (0.26)	3.25 \pm 1.23	2.13 \pm 0.83	1.30 (0.26)
<i>Rhinacloa forticornis</i>	1.14 \pm 0.37	1.14 \pm 0.27	0.06 (0.85)	0.47 \pm 0.15	0.42 \pm 0.15	0.01 (0.93)	1.5 \pm 0.24	1.95 \pm 0.52	0.24 (0.63)
<i>Chrysoperla carnea</i> s.l. (larvae)	0.64 \pm 0.05	0.75 \pm 0.15	0.36 (0.55)	0.22 \pm 0.10	0.42 \pm 0.09	1.91 (0.22)	1.66 \pm 0.22 ^c	2.04 \pm 0.27 ^c	0.00 (0.98)
<i>Drapetis</i> nr. <i>divergens</i>	1.00 \pm 0.16	1.69 \pm 0.39	2.42 (0.13)	0.64 \pm 0.17	0.69 \pm 0.23	0.03 (0.86)	4.25 \pm 0.55	6.25 \pm 1.93	0.03 (0.89)
Aphelinid parasitoids ^c	0.35 \pm 0.05	0.28 \pm 0.03	3.78 (0.15)	-	-	-	-	-	-
Aphelinid parasitism ^d	0.39 \pm 0.07	0.33 \pm 0.08	0.35 (0.57)	-	-	-	-	-	-
Other Hymenoptera	1.94 \pm 0.42	1.97 \pm 0.21	0.10 (0.75)	0.72 \pm 0.23	0.78 \pm 0.19	0.02 (0.91)	45.58 \pm 7.78	48.95 \pm 5.01	0.00 (0.99)
Pests									
<i>Lygus hesperus</i>	2.72 \pm 0.67	2.92 \pm 0.55	0.04 (0.88)	1.47 \pm 0.18	1.61 \pm 0.375	0.01 (0.90)	6.95 \pm 1.65	5.25 \pm 0.39	0.46 (0.53)
<i>Pseudatoscelis seriatus</i>	15.64 \pm 1.11	14.92 \pm 1.61	0.02 (0.90)	6.67 \pm 1.24	8.92 \pm 1.82	0.06 (0.84)	4.00 \pm 0.66	3.83 \pm 0.98	1.05 (0.31)
<i>Bemisia argentifolii</i> eggs ^f	0.20 \pm 0.03	0.18 \pm 0.03	0.19 (0.66)	-	-	-	-	-	-
<i>Bemisia argentifolii</i> nymphs ^f	0.32 \pm 0.03	0.36 \pm 0.11	0.82 (0.37)	-	-	-	-	-	-
<i>Bemisia argentifolii</i> adults ^f	0.80 \pm 0.22	1.01 \pm 0.33	0.30 (0.59)	-	-	-	-	-	-

^adf = 1, 16.6–54 (beats); 1, 13.9–36 (traps); 1, 2.3–17.6 (parasitoids/parasitism).

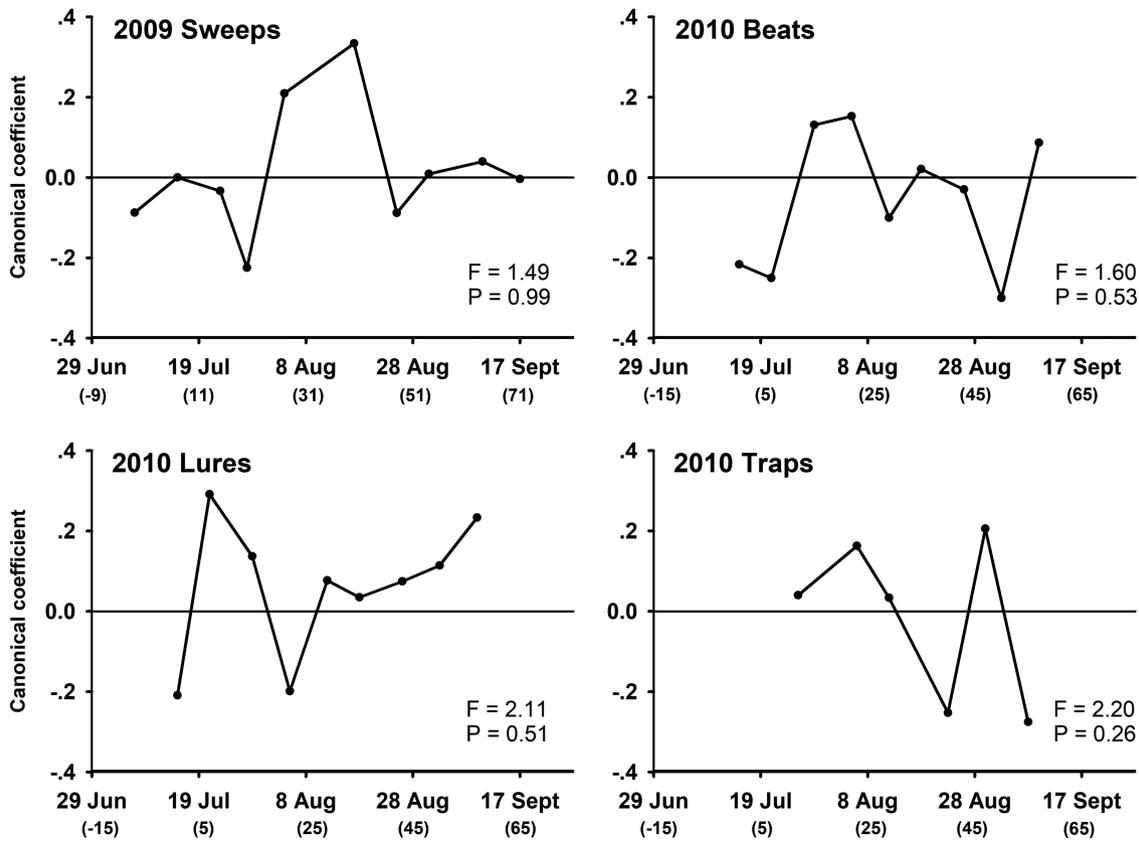
^bP-value above the threshold needed for significance as determined by the false discovery rate.

^cNumbers per seventh mainstem node leaf.

^dProportional parasitism (all immature parasitoid stages/all unparasitized fourth-stage nymphs plus all immature parasitoids).

^eAdult *Chrysoperla carnea* s.l.

^fNumbers per fifth mainstem node leaf disk (eggs, nymphs) or leaf (adults).



2009 Sweeps		2010 Beats		2010 Lures		2010 Traps	
Anthicidae	1.69	Collops spp.	1.97	G. punctipes	2.40	Hymenoptera	2.58
S. albofasc.	1.69	C. carnea	0.72	Other Cocc.	0.10	Drapetis sp.	2.44
Drapetis sp.	1.56	R. forticornis	0.71	M. celer	0.09	O. tricolor	1.28
G. punctipes	1.04	L. hesperus	0.66	D. reticulata	-0.10	S. albofasc.	1.14
Salticidae	0.99	O. tricolor	0.63	H. convergens	-0.17	C. carnea	0.59
O. tricolor	0.99	G. punctipes	0.57	L. hesperus	-0.24	Anthicidae	0.41
H. convergens	0.72	S. albofasc.	0.42	Collops spp.	-0.29	L. hesperus	0.41
Other Aran.	0.45	S. confusa	0.33	Drapetis sp.	-0.39	P. seriatus	0.12
R. forticornis	0.31	Drapetis sp.	0.08	S. albofasc.	-0.40	G. punctipes	0.11
Collops spp.	0.30	H. convergens	0.05	C. carnea	-0.60	M. celer	-0.01
N. alternatus	0.24	N. alternatus	-0.12	Salticidae	-0.62	Collops spp.	-0.30
M. celer	-0.11	Z. renardii	-0.22	R. forticornis	-0.68	Salticidae	-0.42
L. hesperus	-0.13	M. celer	-0.31	P. seriatus	-0.75	H. convergens	-0.44
Hymenoptera	-0.76	G. pallens	-0.42	O. tricolor	-1.10	G. pallens	-0.44
D. reticulata	-0.87	P. seriatus	-0.44	Hymenoptera	-1.16	Other Aran.	-0.51
Z. renardii	-0.96	Salticidae	-0.51	G. pallens	-1.48	Other Cocc.	-0.57
P. seriatus	-1.01	Other Cocc.	-0.67	Z. renardii	-1.83	R. forticornis	-0.86
G. pallens	-1.26	Hymenoptera	-0.73	Anthicidae	-1.89		
C. carnea	-1.40	Anthicidae	-1.99				
		D. reticulata	-2.99				

Fig. 3. PRC depicting patterns of natural enemy community abundance in cotton in MeSA-treated plots compared to the UTC standard ($y = 0$ line) for four sampling methods over 2 yr, Maricopa, AZ. The greater the species weight, the more the response for that species resembles the PRCs. Negative weights indicate an opposite pattern. The product of the species weight and the canonical coefficient for a given date estimates the natural log change in density of that species relative to the standard. The P -value denotes the significance of the PRC analysis over all dates based on an F -type permutation test; numbers in parentheses following the date denote the number of days after the initial deployment of MeSA packets.

enemy abundance. Natural enemy abundance changed over time, as expected, but there also was a consistent absence of treatment by time interaction indicating that the lack of differences between MeSA and control plots were stable over the entire season.

It has been suggested that attraction for a given species can be contextual - that is, a species might be attracted in one crop in a given year but not in a different crop and/or in a different year (Braasch et al. 2012). Kaplan (2012) noted that this could be due

to taxonomic (aggregation of taxa in some studies), exogenous (the plant matrix), and endogenous (biological) variables. Many of the species in our study have not been examined elsewhere and so direct comparisons with other studies are not possible. However, several of our species have shown to be attracted to MeSA in other systems. For example, *G. pallens*, *O. tricolor*, *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae), *Chrysoperla* spp., and Empididae, as a family, have shown attraction to MeSA in

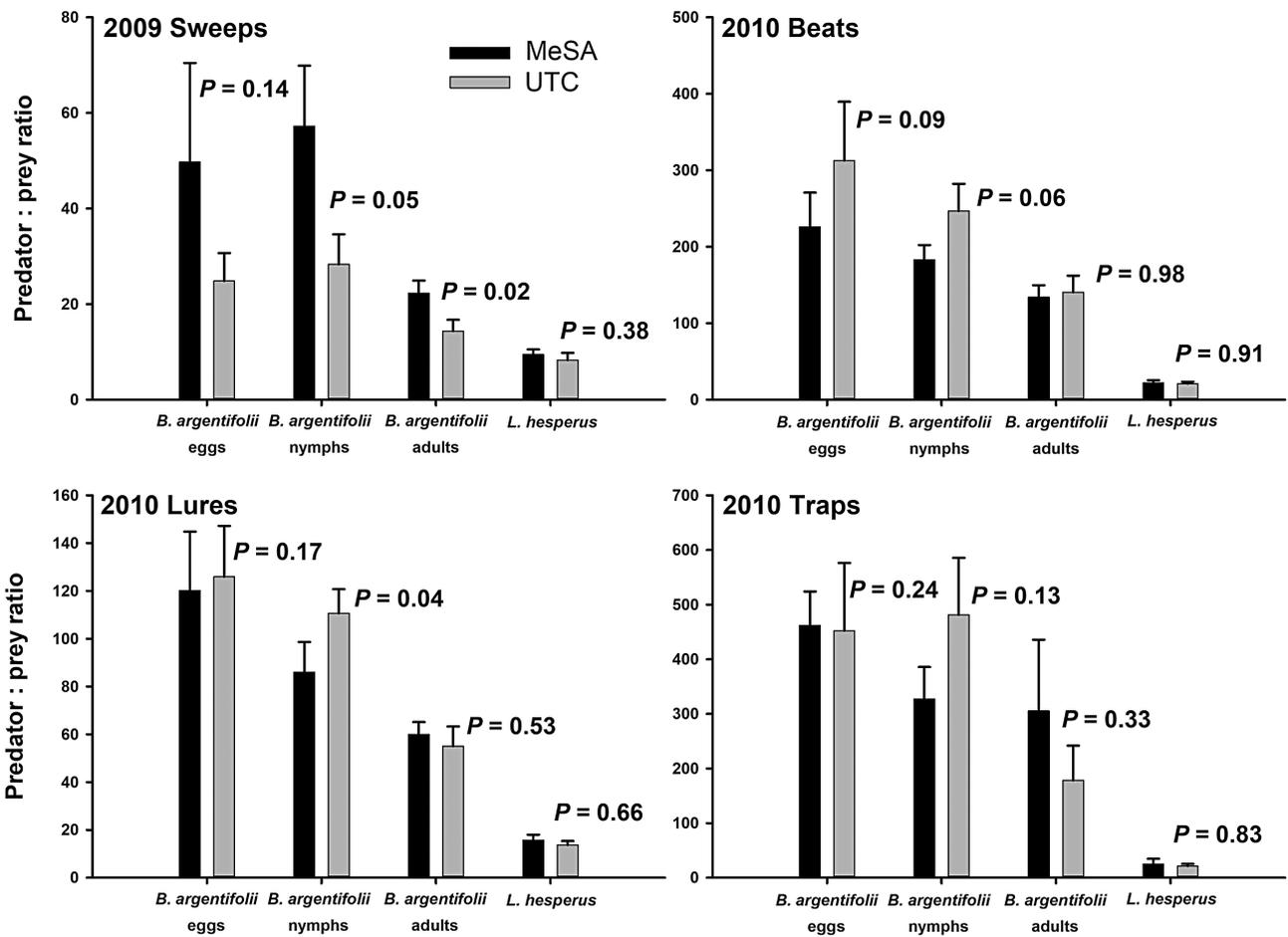


Fig. 4. Comparative seasonal mean (±SE) predator to prey ratios for various stages of *B. argentifolii* prey and for *L. hesperus* prey between MeSA-treated and UTC for four sampling methods over 2 yr. P-values denote the results of mixed-model repeated-measures analysis of variance. Results based on total arthropod predator abundance (n = 4).

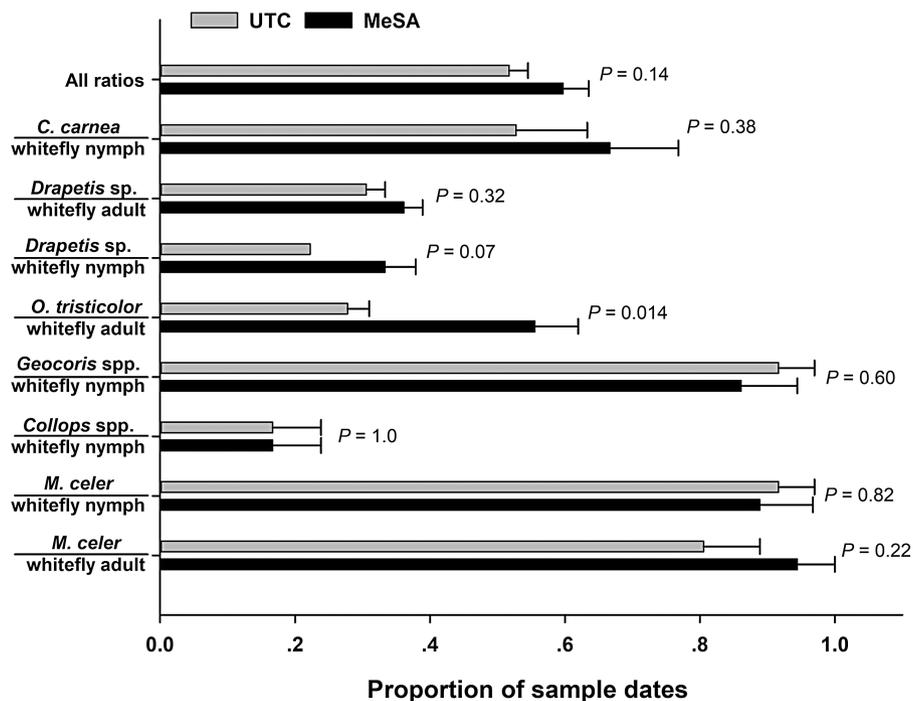


Fig. 5. Mean proportion of sampling dates (±SE) in which predator to prey ratios based on six key arthropod predator species were above levels that would indicate functioning biological control in Arizona cotton (Vandervoet et al. 2018), 2009. P-values denote the results of mixed-model analysis of variance, n = 4.

hops and grape (James 2003b, James and Price 2004, James 2005), strawberry (Lee 2010), soybean (Mallinger et al. 2011), cranberry (Salamanca et al. 2017), and potato (Wimer et al. 2014). Syrphidae are frequently attracted to MeSA (James 2003a, Jones et al. 2011, Mallinger et al. 2011, Orre Gordon et al. 2013, Xu et al. 2018, De Lange et al. 2019, Salamanca et al. 2019) and while syrphid species are found in our cotton system they occur at very low densities and were not included in our analyses. We also have several abundant spider taxa, but Arachnida in general (outside of spider mites) appear to have gotten little attention in studies of HIPVs. Our conflicting finding could have many causes including all those noted by Kaplan (2012) as well as differing experimental factors (discussed below). Further investigation would be needed to define and understand these discrepancies.

Although most studies have focused on attraction to MeSA, a well-known effect of the compound is repellency in some predatory and herbivorous species (Braasch et al. 2012, Lin et al. 2016, De Lange et al. 2019), including one of our primary pests, *B. argentifolii*. Shi et al. (2016) used salicylic acid to induce emission of MeSA in tomato plants and found repellency to non-viruliferous *B. tabaci*, especially with a low dose of salicylic acid. Pérez-Hedo et al. (2018) reported that *B. tabaci* were repelled by MeSA emissions from zoophytophagous mirid infested tomato. These repellent effects have not been demonstrated in the field, and we found no change in *B. argentifolii* abundance as a result of MeSA deployment in our studies. We also found no effects of MeSA on our other key pest, *L. hesperus*, and this is consistent with other reports (James 2003a, James and Grasswitz 2005). In the laboratory, both females and males of *L. hesperus* were repelled by MeSA in Y-tube olfactometer assays and electroantennography demonstrated they were moderately receptive to MeSA (Williams et al. 2010). However, there is no field evidence supporting this finding. Cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), appear to be repelled by HIPVs including MeSA (Hegde et al. 2011, Hegde et al. 2012), but this insect is typically only present when broad-spectrum insecticides disrupt its biological control in our system, and it was not present during the years of our study.

While many studies have shown that MeSA can attract various natural enemies, the ultimate goal of using HIPVs, in general, is to improve biological control effectiveness. Relatively few studies have directly examined biological control services, and even fewer still have demonstrated it can be enhanced with MeSA. Dong and Hwang (2017) found an association between attraction of coccinellid beetles and decreases in cotton aphid abundance up to 10 m from MeSA lures. Mallinger et al. (2011) demonstrated an association between natural enemy attraction to MeSA and declines in soybean aphid abundance, and exclusion cage studies supported the hypothesis that this decline in pest density was likely due to natural enemy activity. Salamanca et al. (2017) reported that predation on sentinel prey increased with attraction of coccinellids and chrysopids by MeSA in cranberry. One concern with using HIPVs to attract natural enemies is that they would not remain in the area of attraction if there was not a prey or other resource reward. Thus, several studies using an attract and reward approach (combination of attractants, including MeSA and flowering resources), have demonstrated moderate success in improving predation and parasitism (Simpson et al. 2011a, Orre Gordon et al. 2013, Jaworski et al. 2019). Other studies, however, have documented no impacts of MeSA on improved biological control activities (Gadino et al. 2012, Wimer et al. 2014, Ingwell et al. 2018, Morrison et al. 2018, Graham et al. 2020, Mercer et al. 2020). We did not directly measure biological control activity but instead used predator to prey ratios based on the two most important

and abundant pest species in our system as a proxy for biological control function. We found that ratios between all predator species and three stages of *B. argentifolii* prey and combined nymph and adult stages of *L. hesperus* were not altered by the addition of MeSA. This is consistent with the lack of change in either predator or pest abundance with the deployment of MeSA packets. Again, as with abundance, the lack of treatment by time interactions indicated a stable lack of difference in ratios over the season. However, when predator to prey ratios are viewed through the lens of IPM decision-making (Vandervoet et al. 2018), we found that biological control informed threshold for *O. tristicolor* more often resulted in a decision to delay or eliminate control actions with the deployment of MeSA. This suggests that focus should be placed on the comparative abundance of pest and predator species and not just solely on densities of either group in isolation, especially in the context of IPM decision-making.

Field studies introduce many more variables that cannot always be adequately controlled compared with laboratory bioassay approaches. There are several limitations worth noting here relative to our experimental approach when interpreting outcomes. First, the commercial packets we used were advertised as a 90-d slow-release formulation. Direct measurement of MeSA emissions demonstrated, however, that in our Arizona system the packets are good for about 50 d. This was unknown to us in real time and so the goal of a full season attraction study was not achieved. We did correct for this by re-analyzing the data after truncating sample dates beyond 50 d in both years. The results did not change, suggesting that the lack of treatment differences was not affected by the shorter life of our lures.

It is well known that synthetic MeSA can induce the emission of a number of other volatile compounds (including MeSA), that may have additional effects on the insect fauna (Kaplan 2012). We measured the emission of a number of additional volatile compounds from in situ headspace samples of cotton plants containing MeSA packets in 2009 and found no difference in emission profiles among the six most abundant chemicals or several other compounds emitted at lower concentrations. Although it has been suggested that even chemicals emitted at low concentrations and blends of volatiles can be important in affecting arthropod behavior (Clavijo McCormick et al. 2014), we found no differential patterns of attraction. Many of these chemicals are known to be released from uninfested cotton and at higher levels when plants were infested with *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) (Rodríguez-Saona et al. 2001). This suggests that MeSA may have failed to elicit emissions from our cotton in the field and that those chemicals emitted arose naturally or due to the feeding of herbivores in both treatment and control plants. It is unclear if plants adjacent to those with MeSA packets were affected, but in any case these additional volatiles did not appear to influence arthropod abundance at a plot scale.

Treatment plot separation is always a concern in-field studies given the dispersal capacity of many arthropods (Jepson and Thacker 1990, Duffield and Aebischer 1994, Prasifka et al. 2005, Macfadyen et al. 2014). As noted, many studies on HIPVs used baited traps with relatively small treatment separations (5–15 m) and were able to find treatment effects. This is consistent with what appears to be a relatively small range of attraction of HIPVs like MeSA as previously noted. An additional factor to consider is that many field studies used yellow sticky traps, which are well known to be attractive to many insects and the reason we chose to use a non-attractive trap (hardware cloth) as one of our sampling methods.

Field studies that distributed MeSA lures more broadly (beyond baited traps) tended to use larger inter-plot distances ranging from 10 to 100 m, with some demonstrating attraction to

MeSA and a few not, but without a clear relationship to distance in either case. For example, studies by Xu et al. (2018) had inter-plot distances of 14–16 m and showed attraction, while those by Mercer et al. (2020) and Wimer et al. (2014) had inter-plot distances of 1–30.5 m and showed no attraction. Those with inter-plot distances ranging from 15 to 100 m demonstrated attraction to at least a few of the natural enemies studied (James and Grasswitz 2005, Lee 2010, Orre et al. 2010, Simpson et al. 2011a, Gadino et al. 2012). And, of course, there are the large numbers of baited-trap studies showing attraction at ‘inter-plot’ distances as small as 5 m (Braasch et al. 2012). Our inter-plot distances ranged from 36 to 250 m and fell well within the range of past studies showing attraction. Because the use of HIPVs are more likely to result in a re-distribution of arthropods within the habitat (Kaplan 2012), one could assume that treatment differences should be more readily detected with smaller inter-plot differences as individuals move from plots without attractants to adjacent plots with attractants. A final factor to consider is lure density. For those studies reporting these data, values range from 260/ha (Gadino et al. 2012) to 2297/ha (James and Price 2004). Uncontrolled analyses suggest that lower densities may be more attractive than higher densities (Khan et al. 2008), but attraction has been demonstrated at all densities. Our densities were 468/ha, which fall somewhere in the middle range and certainly at a density where attraction has been demonstrated in other studies.

In conclusion, the deployment of HIPVs such as MeSA represent a potentially useful approach for improving biological control at the local scale. Attraction has been demonstrated in the field for a number of predator and parasitoid species and there is limited evidence that this increased density of natural enemies can lead to higher levels of predation and parasitism. Our 2-yr study failed to show differential attraction of 20 taxa of common natural enemies found in-field crops in the western United States. We also demonstrated that several important pest species were neither negatively nor positively affected by the deployment of MeSA. The one exception was an increased ratio of *O. tristicolor* to *B. argentifolii* adults in MeSA plots that would have delayed or possibly eliminated the need for insecticide intervention. Many of the challenges and gaps identified by Kaplan (2012) with the use of HIPVs such as MeSA remain unanswered and additional research is needed to make this approach useful for reliably improving biological control services in a wide range of crops.

Acknowledgments

We thank Melissa Stefanek, Scott Machtley, Gabriel Zilink, and LeAnne Elhoff for technical assistance, AgBio (Westminster, CO) for supplying the MeSA packets and Roberta Gibson for reviewing an earlier draft of this manuscript.

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