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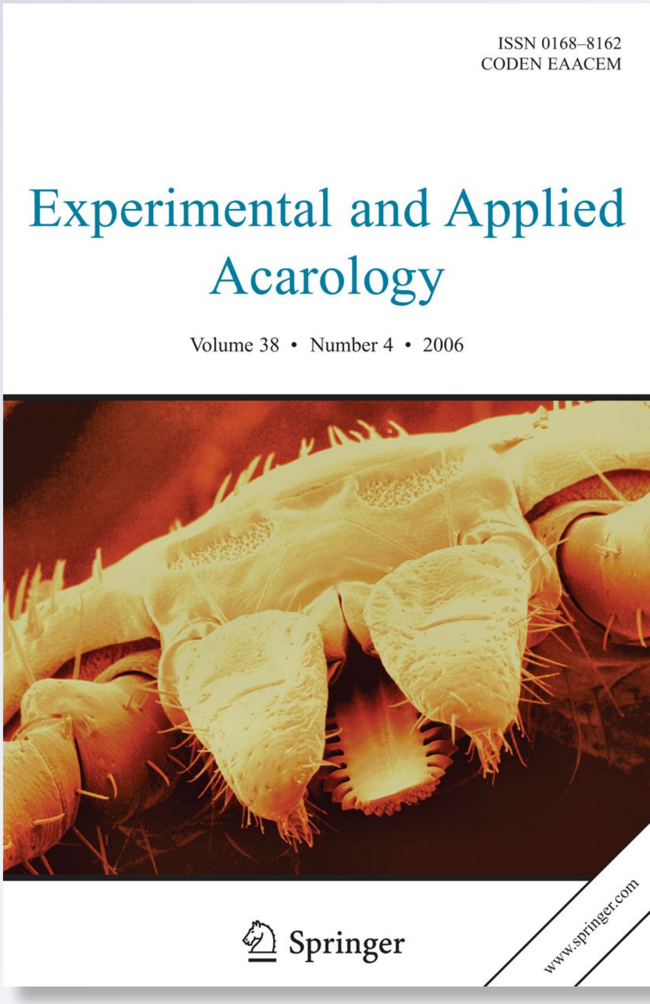

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Evaluation of airborne methyl salicylate for improved conservation biological control of two-spotted spider mite and hop aphid in Oregon hop yards

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Abstract The use of synthetic herbivore-induced plant volatiles (HIPV) to attract natural enemies has received interest as a tool to enhance conservation biological control (CBC). Methyl salicylate (MeSA) is a HIPV that is attractive to several key predators of two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and hop aphid, *Phorodon humuli* (Schrank) (Homoptera: Aphididae). A 2-year study was conducted to evaluate the recommended commercial use of MeSA in hop yards in Oregon. Slow-release MeSA dispensers were stapled to supporting poles in 0.5 ha plots and these plots were compared to a paired non-treated plot on each of three farms in 2008 and 2009. Across both years, there was a trend for reduced (range 40–91%) mean seasonal numbers of *T. urticae* in five of the six MeSA-baited plots. *Stethorus* spp., key spider mite predators, tended to be more numerous in MeSA-baited plots compared to control plots on a given farm. Mean seasonal densities of hop aphid and other natural enemies (e.g., *Orius* spp. and *Anystis* spp.) were similar between MeSA-treated and control plots. Variability among

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farms in suppression of two-spotted spider mites and attraction of *Stethorus* spp. suggests that the use of MeSA to enhance CBC of spider mites in commercial hop yards may be influenced by site-specific factors related to the agroecology of individual farms or seasonal effects that require further investigation. The current study also suggests that CBC of hop aphid with MeSA in this environment may be unsatisfactory.

Keywords Semiochemicals · *Humulus lupulus* · Integrated pest management

Introduction

Conservation biological control (CBC) was defined in the twentieth century as the "modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests" (Eilenberg et al. 2001). Within the hop production system, CBC recently has focused on greater reliance on attraction of natural enemies (James 2003b, c, 2005, 2006; James et al. 2003; James and Price 2004; Khan et al. 2008) to manage the two key arthropod pests of this crop in the Northern Hemisphere, two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and hop aphid, *Phorodon humuli* (Schrank) (Homoptera: Aphididae) (Cranham 1985; Neve 1991). Conventional management of these pests relies heavily on repeated applications of pesticides (James and Barbour 2009; Weihrauch 2009). Considerable efforts have been expended investigating augmentative biological control of spider mites, often focusing on phytoseiid mites (Pruszynski and Cone 1972; Strong and Croft 1995, 1996; Lilley and Campbell 1999). However, augmentative biological control of spider mites generally has not been successful in commercial production, likely due to the rapid growth habit of the hop plant and certain cultural practices that disturb refugia (Strong and Croft 1995, 1996). Judicious use of pesticides and conservation of crucial habitat for natural enemies can conserve endemic populations of natural enemies on hop, and provide suppression of spider mite and hop aphid populations (van de Vrie et al. 1972; Aveling 1981; Cranham 1985; Strong and Croft 1993, 1996; Campbell and Cone 1994; Gardiner et al. 2003; James et al. 2001, 2003; James and Price 2004).

Enhanced recruitment of natural enemies has been a prime motivation for research on the semiochemicals that plants emit in response to herbivore feeding, commonly termed herbivore-induced plant volatiles (HIPVs) (Dicke et al. 1998; Khan et al. 2008). Synthetic methyl salicylate (MeSA) has been identified as a HIPV that is attractive to certain natural enemies under laboratory (Dicke and Sabelis 1988; Drukker et al. 2000; Pickett et al. 2006; Shimoda 2009) and field settings (James 2003b, c, 2005; James and Price 2004; Khan et al. 2008; Yu et al. 2008). In a laboratory trial analyzing volatiles released from hop plants mechanically damaged or fed on by spider mites, MeSA was the primary novel volatile released in response to spider mite feeding (Van den Boom et al. 2004), indicating that the use of synthetic MeSA may be an appropriate candidate to enhance natural enemy recruitment in a field setting. Most field evaluations of synthetic MeSA generally have been limited to grape vineyards and hop yards in south-central Washington (James 2003b, c, 2005, 2006; James and Price 2004; Khan et al. 2008). Other studies have reported repellency of aphids due to MeSA (Lösel et al. 1996; Ninkovic et al. 2003), and attraction of *Orius similis* (Hemiptera: Anthorcoridae) and *Erigonidium graminicolum* (Araneidae: Micryphantidae) in cotton fields (Yu et al. 2008). These field evaluations of HIPVs provide critical data because controlled environments are unable to simulate environmental conditions and ecological differences among growing regions, differences which may greatly impact the efficacy of HIPVs (Dicke and Baldwin 2010; Hunter 2002; James 2003b).

Available formulations (or blends) of MeSA for field application include a sprayable botanical insecticide (Ecotrol™, Ecosmart Technologies, Alpharetta, GA), slow-release dispensers (Predalure®, AgBio, Westminster, CO) active for 30–90 days, and a liquid product used to mask pesticide odors (Odor-Mask®, Monterey AgResources, Fresno, CA). Preliminary work in Washington hop yards provided evidence for the attraction of *Stethorus punctum picipes* and *Orius tristicolor* to 5 g controlled-release plastic sachets of MeSA (98%) at a rate of 448/ha (James and Price 2004). Other studies (reported in Khan et al. 2008) suggested that lower deployment rates (180 sachets/ha) were more attractive to all predator groups sampled in Washington hop yards. A recent study in Oregon strawberry fields using 30-day 2 g Predalure® dispensers documented attraction of some natural enemies, particularly green lacewings (Chrysopidae) and *Orius tristicolor* at a range of approximately 10 m or less from the point source (Lee 2010).

The majority of commercial hop production in the United States occurs in the Pacific Northwest. Hop growing regions in western Oregon and northern Idaho generally have mild, wet maritime climates, while production regions in southern Idaho and south-central Washington are semi-arid (Barth et al. 1994). Spider mites reproduce rapidly in hot, dry, and dusty conditions (Cranham 1985; James and Barbour 2009), while hop aphid typically reproduce well in a cool, humid climate (Cranham 1985; Weihrauch 2009). Thus, the contrasting climates of south-central Washington and western Oregon result in differing severity of these pests. Since all previous field-based research for the use of MeSA in hop yards has been conducted in south-central Washington, this study sought to quantify the effects of MeSA deployment in commercial hop yards in Oregon on spider mites, hop aphids, and their natural enemies.

Materials and methods

Experimental design and treatment application

Experiments were conducted in 2008 and 2009 in commercial hop yards in Oregon to quantify pest and predator arthropod populations in response to deployment of MeSA in slow-release dispensers (Predalure®). Two paired hop yards of cultivar Willamette were selected on each of three farms (farms ‘A’, ‘B’, and ‘C’) in each year. Each farm represented an experimental replication, with blocking over farms, with MeSA deployed in one yard (termed “MeSA-baited plot”) and the other yard serving as a “control plot”. Blocking over farms was intended to minimize differences in farm-specific factors, such as cultural practices. Farms A, B, and C were located in Marion County, Oregon, approximately 1–2 km apart in 2008 and 2–10 km apart in 2009. Each treatment was not replicated within individual hop yards due to the potential for plot-to-plot interference from MeSA volatilization. Different hop yards were utilized in 2008 and 2009 studies to avoid potential carry-over effects from MeSA deployment the previous season.

Each plot within a hop yard was 0.5 ha and was established in yards with a “narrow” row spacing (plants arranged on a 2.1 m grid) or “wide” row spacing (plants arranged on a 2.1 × 4.2 m pattern). In 2008, both plots on farm B and C and the MeSA-baited plot on farm A were planted in a wide row configuration. The control plot on farm A was planted in a narrow-row configuration. Irrigation was supplied according to grower standard practices. Plots on farm A were irrigated by an overhead traveling sprinkler system, whereas plots on farms B and C were irrigated by a surface drip system. In 2009, plots on farm A were planted on a narrow-row configuration and plots on farm B and C were planted on wide-row spacing. Plots on farms A and C were irrigated with by an overhead traveling sprinkler, while plots on farm B were irrigated by surface drip. MeSA-baited and control plots on each farm were separated by 100–1,600 m, a distance adequate to minimize plot-to-plot interference from MeSA volatilization (Lee 2010). The yards utilized on a given farm were comparable in size and cultural practice so that potential treatment effects due to MeSA deployment could be evaluated. In 2008, Predalure dispensers (2 g, 30-day activity period) were deployed on 29 April at a rate of 185/ha by stapling the dispensers to poles at approximately 1.5 m from the ground in a regular pattern. This rate was determined based on previous research in Washington hop yards (Khan et al. 2008). In 2009, dispensers were deployed similarly on 29 April at a rate of 123 dispensers/ha. The deployment rate in 2009 was reduced compared to 2008 based on practical considerations of cost. Commercial deployment of MeSA dispensers could potentially replace one or more miticide applications, therefore we tested a lower rate (123/ha) that is near the typical cost of a single miticide application (approximately $120US/ha). Deployment in both years was timed to coincide with the natural occurrence of pest species, and corresponded with known activity of natural enemies in Oregon (Gent et al. 2009).

Cooperating growers were asked to avoid using broad-spectrum insecticides to conserve natural enemies, although given the practicalities of commercial crop production this request was not followed in all cases. Spider mites and aphids typically are managed with annual insecticide applications in commercial hop production (James and Barbour 2009), and most growers in the study applied an insecticide when pest levels exceeded their empirical economic thresholds or other criteria. Table 1 describes the insecticide and miticide applications made by the cooperating grower. To minimize confounding effects from these pesticide applications, data presented below (Tables 2, 3, 4) includes seasonal means up to the first application of a miticide or aphicide in an individual plot by the cooperating grower.

Arthropod sampling

Leaf samples were collected at biweekly intervals beginning with a pre-treatment assessment in late April and continuing until cone harvest during mid- to late August. On each sampling date, 30 leaves were collected arbitrarily at a height of approximately 1.5 m from the ground in each plot. Leaves were collected into paper bags, stored on ice in a cooler, and promptly transported to a laboratory. Motile spider mite stages, spider mite eggs, hop aphid nymphs and adults, predatory mites (Phytoseiidae), and mite-eating ladybeetles (*Stethorus* spp.) were identified and enumerated with the aid of a stereomicroscope. *Stethorus* spp. were not identified to the species level. Predatory mites were either not detected or were present at very low levels (total of 3 or less on all sampling dates) in all yards both years and were not identified to the species level.

To assess motile natural enemies, four yellow, unbaited sticky cards (23 × 18 cm, Trece Inc, Salinas, CA) were stapled to supporting poles at a height of approximately 1.5 m from the ground and approximately 3 m from the corners of each plot. Each sticky card faced a different compass direction, which was re-randomized on subsequent sampling dates. Predalure dispensers were not attached to poles where sticky cards were placed to avoid potential sampling bias. Sticky cards were collected and replaced at biweekly intervals in coordination with leaf samples. Beginning in mid-June, biweekly arthropod

Table 1 Miticide and aphicide active ingredient (AI), application date and rate/ha applied by growers to experimental plots in 2008 and 2009 in hop yards in Oregon

Grower	Treatment	Date	Miticide	Rate (kg AI/ha)	Date	Aphicide	Rate (kg AI/ha)
2008[a]							
A	MeSA	18 July	Spirodiclofen (Envidor® 2 SC, Bayer CropScience)	0.21	30 June	Imidacloprid (Pasada™ 1.6F, Makhteshim Agan of North America, Inc)	0.31
A	Control	25 July	Spirodiclofen (Envidor® 2 SC)	0.21	2 Aug	Bifenthrin (Fanfare® 2EC, Makhteshim Agan of North America, Inc)	0.11
B	MeSA	–	–	–	–	–	–
B	Control	–	–	–	–	–	–
C	MeSA	31 July	Bifenazate (Acramite® 50WS, Chemtura Corp)	0.71	25 June	Imidacloprid (Advise™ 2FL, Agriliance LLC)	0.31
C	Control	25 June	Abamectin (Epi-Mek® 0.15 EC, Syngenta Crop Protection)	0.02	25 June	Imidacloprid (Advise™ 2FL)	0.31
2009							
A	MeSA	6 July	Abamectin (Abba™ 0.15 EC, Makhteshim Agan of North America, Inc)	0.02	23 June	Imidacloprid (Pasada™ 1.6F)	0.31
A	Control	6 July	Abamectin (Abba™ 0.15 EC)	0.02	23 June	Imidacloprid (Pasada™ 1.6F)	0.31
B	MeSA	6 July	Hexythiazox (Savey® 50 DF, Gowan Co)	0.14	6 July	Spirotetramat (Ultor™, Bayer CropScience)	0.09
B	Control	–	–	–	30 June	Spirotetramat (Ultor™)	0.11
C	MeSA	30 June	Bifenazate (Acramite® 50WS)	0.22	8 June	Imidacloprid (Provado® 1.6 F, Bayer CropScience)	0.31
C	Control	30 June	Bifenazate (Acramite® 50WS)	0.22	8 June	Imidacloprid (Provado® 1.6 F)	0.31

[a] A dash indicates that no sprays were applied

Table 2 Mean seasonal density ± SEM (prior to first miticide application) of *Phorodon humuli*, Phytoseiidae, and *Tetranychus urticae* on hop leaves in relation to methyl salicylate (MeSA) deployment in hop yards in Oregon

Grower	Arthropod (mean ± SEM per leaf)[a,b]			
	Treatment	*Phorodon humuli*	Phytoseiidae	*Tetranychus urticae*
2008				
A	MeSA	0.15 ± 0.07	0	0.41 ± 0.19
A	Control	0.18 ± 0.08	0	4.13 ± 1.43
B	MeSA	0.08 ± 0.05	0	0.50 ± 0.16
B	Control	0.09 ± 0.05	0.004 ± 0.004	0.83 ± 0.26
C	MeSA	0.32 ± 0.24	0	0.21 ± 0.13
C	Control	0.06 ± 0.03	0	2.43 ± 1.17
2009				
A	MeSA	4.98 ± 0.82	0 ± 0	0.013 ± 0.013
A	Control	5.63 ± 1.39	0 ± 0	0.04 ± 0.03
B	MeSA	0.9 ± 0.26	0 ± 0	0.51 ± 0.19
B	Control	0.57 ± 0.2	0.004 ± 0.004	0.21 ± 0.07
C	MeSA	3.39 ± 0.55	0 ± 0	0.42 ± 0.42
C	Control	6.02 ± 0.63	0 ± 0	1.05 ± 0.49

[a] Data reported are from sampling dates after MeSA was deployed, but excludes data collected after the first miticide/insecticide application made by the cooperating grower

[b] Seasonal means on individual farms were not significantly different among treatments, as determined by the Mann–Whitney rank sum test ($\alpha = 0.10$)

samples were taken using a canopy 'shake' method as described in James and Price (2004). Hop bines were shaken for approximately 3 s over a 1-m^2 funnel, and natural enemies that fell into the funnel were brushed into a collection vial with 70% ethanol. Arthropods from shake samples from three bines were combined and considered a sub-sample. Three sub-samples were collected from each plot, thus a total of nine bines were sampled from each plot. Non-acarine, motile beneficial arthropods (macropredators) were identified and enumerated with the aid of a stereomicroscope.

Statistical analysis

Densities of spider mites, hop aphid, and *Stethorus* spp. in the control and MeSA-baited plots were each plotted over time to derive arthropod development curves. The area under the "population development curve" for each of these organisms was calculated using a macro available in SigmaPlot version 11.2.0 (Systat Software, 2009, San Jose, CA) to produce a variable integrating arthropod density over an entire season. The area under the population development curve was analyzed in SAS version 9.2 (SAS Institute 2002) using a linear mixed model (MIXED procedure) after either square-root or log transformation to normalize residuals. Blocks (farms) were considered a random effect in the analysis. In these analyses, the hypothesis tested was the MeSA-baited plots would have greater abundance of natural enemies and correspondingly a lower adundance of spider mites and hop aphid. Therefore, statistical tests were considered one-sided where H_0: $\mu_c = \mu_{MeSA}$ and $H_{A:}$ $\mu_c < \mu_{MeSA}$, where μ_c is the mean of area under the arthropod development curve for control plots and μ_{MeSA} is the mean for MeSA baited plots. The sign for the alternative

Table 3 Mean seasonal density ± SEM (prior to first miticide application) of arthropods collected in shake samples in relation to methyl salicylate (MeSA) deployment in hop yards in Oregon

Grower	Treatment	Arthropod (mean ± SEM per shake)[a,c]					
		Hypena spp.	*Anystis* spp.	Coccinellidae		Parasitic hymenoptera	Macropredators[b]
				Aphidophagous lady beetles	*Stethorus* spp.		
2008							
A	MeSA	1.17 ± 0.44	3.67 ± 0.83	0	0.33 ± 0.33	0.67 ± 0.33	4.83 ± 0.5
A	Control	0.33 ± 0.19	3 ± 1.21	0	0.11 ± 0.11	0.33 ± 0.19	4.22 ± 1.21
B	MeSA	28.27 ± 6.74	7.73 ± 2.17	0.02 ± 0.02	0.2 ± 0.13*	1.53 ± 0.40	10.73 ± 2.91
B	Control	31.67 ± 6.14	1.93 ± 0.60	0.02 ± 0.02	0.07 ± 0.07	2.4 ± 0.61	5.8 ± 1.31
C	MeSA	0.35 ± 1.14	2.78 ± 1.06	0	0.11 ± 0.11	2.22 ± 0.73	5.67 ± 1.201
C	Control[d]	–	–	–	–	–	–
2009							
A	MeSA	0 ± 0	0 ± 0	0.06 ± 0.06	0 ± 0	1.33 ± 0.88	1.5 ± 0.77
A	Control	0 ± 0	0.83 ± 0.44	0.06 ± 0.06	0.5 ± 0.5	0.17 ± 0.17	2.33 ± 1.18
B	MeSA	1.00 ± 0.33	7.67 ± 2.92	0 ± 0	0.50 ± 0.50	0.33 ± 0.33	9.00 ± 3.22
B	Control	0.17 ± 0.17	10.17 ± 0.83	0 ± 0	0.17 ± 0.17	0.17 ± 0.17	10.67 ± 0.78
C	MeSA	0 ± 0	0.17 ± 0.17	0 ± 0	0.17 ± 0.17	0.83 ± 0.33	1.337 ± 0.46
C	Control	0 ± 0	0 ± 0	0.06 ± 0.06	0 ± 0	1.83 ± 0.93	2.33 ± 1.20

[a] Data reported are from sampling dates after MeSA was deployed, but excludes data collected after the first miticide/insecticide application made by the cooperating grower. In 2008, Grower C applied a non-selective miticide to the control plot prior to the first shake sample of the season

[b] Macropredators encompass all winged or mobile natural enemies of spider mites and/or aphids observed in the samples

[c] Other seasonal means on individual farms were not significantly different among treatments, as determined by the Mann–Whitney rank sum test ($\alpha = 0.10$)

[d] A dash indicates the absence of data due to the application of a non-selective miticide prior to the first shake sample on 26 June

* Indicates a significant difference ($\alpha = 0.10$)

Table 4 Mean seasonal density (prior to first miticide application) ± SEM of arthropods on sticky traps in relation to methyl salicylate (MeSA) deployment in hop yards in Oregon

Grower	Treatment	Arthropod (mean ± SEM per sticky trap)[a,c]				
		Coccinellidae		Parasitic Hymenoptera	Cantharidae: soldier beetles	Macropredators[b]
		Aphidophagous lady beetles	*Stethorus* spp.			
2008						
A	MeSA	3.50 ± 1.05	4.00 ± 1.20	6.80 ± 1.49	1.45 ± 0.59	17.80 ± 2.63
A	Control	4.90 ± 1.17	2.88 ± 0.76	12.04 ± 2.90*	2.08 ± 0.38	27.58 ± 3.61
B	MeSA	3.25 ± 1.03	9.84 ± 1.42	19.63 ± 4.56	0.69 ± 0.32	38.06 ± 6.29
B	Control	3.19 ± 1.03	7.53 ± 1.68	18.56 ± 5.21	1.16 ± 0.39	39.31 ± 7.47
C	MeSA	2.29 ± 0.71*	1.92 ± 0.66	12.08 ± 2.31	0.79 ± 0.54	33.04 ± 8.04*
C	Control	1.81 ± 1.11	1.00 ± 0.65	5.25 ± 2.05	1.50 ± 0.56	10.88 ± 3.26
2009						
A	MeSA	0.90 ± 0.39	2.80 ± 1.09	8.25 ± 0.64	0.75 ± 0.23	16.40 ± 2.88
A	Control	1.56 ± 0.61	1.38 ± 0.89	8.00 ± 0.54	1.00 ± 0.24	13.15 ± 1.46
B	MeSA	1.96 ± 0.67	11.38 ± 3.24	6.52 ± 0.63	0.81 ± 0.46	21.19 ± 4.81
B	Control	1.55 ± 0.49	9.90 ± 2.68	7.15 ± 0.65	1.25 ± 0.59	19.63 ± 3.94
C	MeSA	2.31 ± 0.55	4.38 ± 1.61	5.44 ± 0.49	0.15 ± 0.15	12.44 ± 3.88
C	Control	2.19 ± 0.46	2.31 ± 1.03	7.44 ± 0.65	0 ± 0	12.50 ± 2.80

[a] Data reported are from sampling dates after MeSA was deployed, but excludes data collected after the first miticide/insecticide application made by the cooperating grower

[b] Macropredators encompass all winged or mobile natural enemies of spider mites and/or aphids observed in the samples

[c] Seasonal means on individual farms were not significantly different among treatments, as determined by the Mann–Whitney rank sum test ($\alpha = 0.10$)

* Indicates a significant difference ($\alpha = 0.10$)

hypothesis is reversed when considering pests not natural enemies. As described above, insecticide and miticide applications were in some instances applied to the sampled plots. Therefore, the analysis of the area under the arthropod abundance curve should be viewed as a measure of arthropod abundance when MeSA is or is not applied in addition to other routinely applied control measures.

Inspection of the data by farm indicated that suppression of two-spotted spider mite and attraction of *Stethorus* spp. varied among farms (detailed below), and therefore considering farms blocks in an analysis of variance potentially could obfuscate treatment differences. Thus, differences in the number of pest and beneficial arthropod species on leaves, sticky cards, and shake samples on individual dates, and also summed over the season, among paired plots on individual farms were determined using the Mann–Whitney rank sum test (SigmaPlot version 11.2.0). Counts were log-transformed before the analysis to stabilize variances. Differences were considered significant at $\alpha = 0.10$.

Results

A diversity of beneficial arthropods, identified on leaf samples, shake samples, and sticky cards were found in association with hop plants (Tables 2, 3, 4). The major beneficial arthropod families and species from these samples included: Coccinellidae (aphid-feeding ladybeetle species including *Harmonia axyridis* Pallas, *Coccinella septempunctata* L., *Coccinella transversoguttata* Falderman, and *Cycloneda polita* Casey) and the mite-eating ladybeetles, *Stethorus* spp., minute pirate bug, *Orius tristicolor*, predatory thrips, parasitic hymenoptera, predatory diptera (primarily flies from the families: Empididae, Dolichopodidae, Sarcophagidae, and Tachinidae), Cantharidae (generalist soldier-beetle species), brown and green lacewings (Chrysopidae and Hemerobiidae), and predatory mites (Phytoseiidae).

2008

There was a general trend for fewer spider mites in the MeSA-baited plot compared to the control plot on all three farms (Fig. 1). However, seasonal mean density of spider mites up to the time of the first miticide application was not significantly different between treatments across all three farms ($P \geq 0.171$) as determined by the Mann–Whitney rank sum test (Table 2). For spider mites, the area under the population development curve for the entire season was greater in the control plots than the MeSA-baited plots across farms ($F = 14.74$; $df = 1,2$; one-sided test $P = 0.031$) (Table 5). Additionally, the number of spider mites on individual sampling dates were significantly different ($P \leq 0.081$) on one to four individual sampling days, depending on the farm (Fig. 1). Spider mite populations were reduced on one to two of the eight total sampling dates in MeSA-baited plots compared to the control plots on a given farm. However, on two sampling dates, significantly ($P \leq 0.023$) more spider mites were observed in the MeSA-baited plot compared to the control plot on farm C (Fig. 1e). Seasonal mean density of hop aphid up to the time of first insecticide application was not significantly different ($P \geq 0.343$) on any of the farms (Table 2), nor were there consistent differences in populations over time between MeSA-baited and control plots. The area under the population development curve for the entire season for hop aphid was similar among MeSA-baited versus control plots across farms ($F = 2.34$; $df = 1,2$; one-sided test $P = 0.133$) (Table 5).

The area under the population development curve for *Stethorus* spp. was greater in the MeSA-baited plots than the control plots across farms ($F = 12.52$; $df = 1,2$; one-sided test $P = 0.035$) (Table 5). The number of *Stethorus* spp. on sticky card samples tended to be higher in MeSA-baited plots as compared to the control plots (Fig. 1), although seasonal mean densities of *Stethorus* spp. on sticky cards and in shake samples were not significantly different among treatments on all farms ($P \geq 0.181$) (Tables 3, 4). The number of *Stethorus* spp. trapped on sticky cards on individual sampling dates were significantly different ($P \leq 0.057$) on one to five sampling days, depending on the farm. On one sampling date, the number of *Stethorus* spp. in a control plot was significantly greater ($P = 0.029$) than the MeSA-baited plot on farm C (Fig. 1f). However, among other predators and predator groups collected from shake samples and sticky traps on all farms, seasonal mean densities were similar between MeSA-baited and control plots ($P \geq 0.181$) (Tables 3, 4).

2009

Over the season, there was a general trend for lower numbers of spider mites in the MeSA-baited plots on all three farms (Table 2). However, seasonal mean densities of spider mites up to the time of the first miticide application were similar between treatments on all farms ($P \geq 0.252$). Across farms, the area under the population development curve for spider

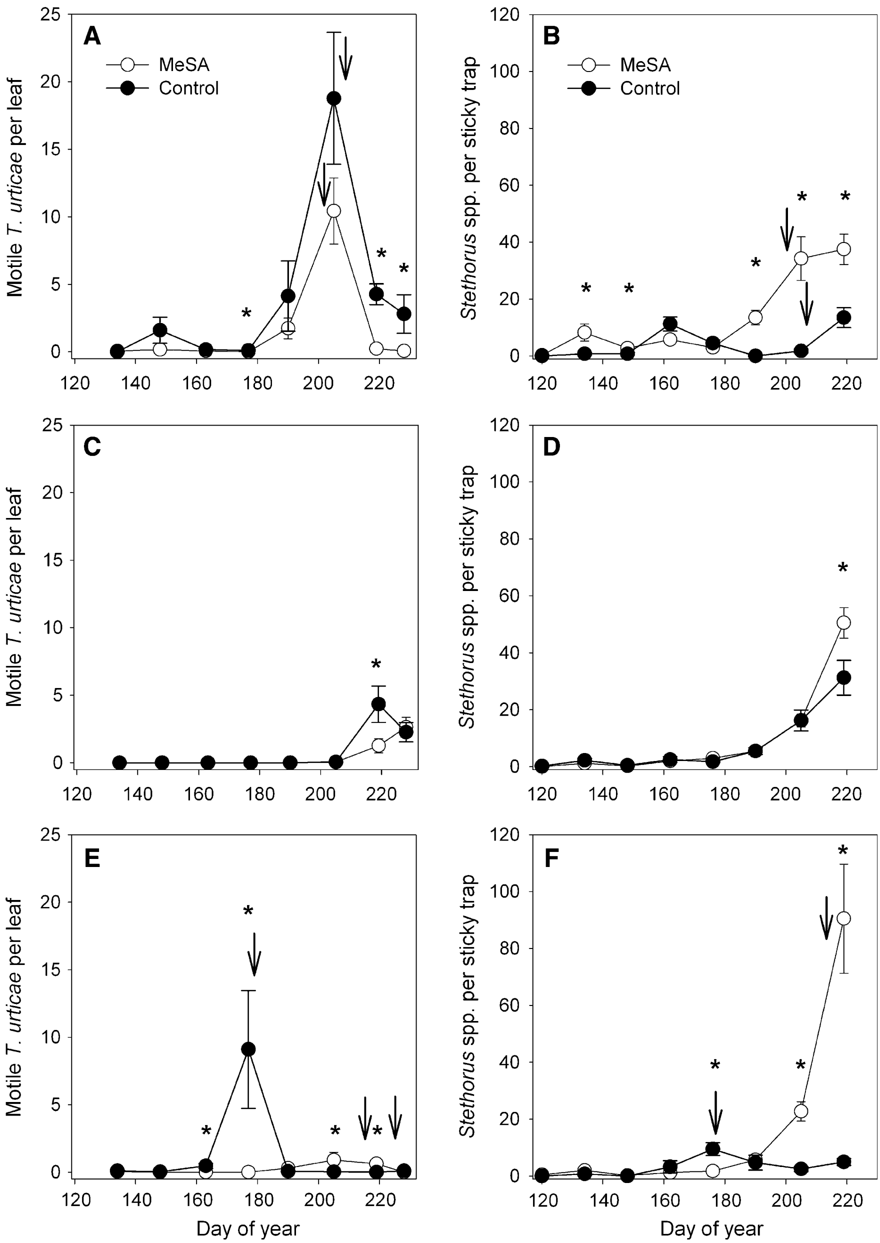

Fig. 1 Density of *Tetranychus urticae* (mean ± SEM) (**a**, **c**, **e**) per leaf and *Stethorus* spp. (mean ± SEM) per sticky trap (**b**, **d**, **f**) in MeSA-baited and control plots in 2008. Grower A, B, and C are represented in (**a**, **b**), (**c**, **d**) and (**e**, **f**), respectively. *Tetranychus urticae* data are means from 30 leaves per plot. *Stethorus* spp. data are means from four traps per plot. *Arrows* indicate the application of a miticide by the cooperating grower. Significantly different means on specific dates are based on a Mann–Whitney rank sum test ($\alpha = 0.10$) and are noted by an *asterisk*. Across farms, area under the population development curve for *T. urticae* and *Stethorus* spp. in the MeSA-baited plots was significantly different than the control plots (*T. urticae*: $F = 14.74$; $df = 1, 2$; one-sided test $P = 0.031$; *Stethorus* spp.: $F = 12.52$; $df = 1, 2$; one-sided test $P = 0.035$)

Table 5 Effect of synthetic methyl salicylate (MeSA) deployment on seasonal abundance of *Phorodon humuli, Stethorus* spp., and *Tetranychus urticae* on hop

Year	Treatment	Area under the population development curve[a,b]		
		Phorodon humuli	*Stethorus* spp.	*Tetranychus urticae*
2008	MeSA	13.8	1038.9[a]	78.2[a]
	Control	7.6	441.5[b]	204.1[b]
2009	MeSA	201.6	804.7	68.6
	Control	176.1	468.6	52.0

[a] The area under the population development curve for each arthropod was derived by plotting seasonal densities and calculating the area under the curve by integration. These data were analyzed using a linear mixed model with farms as blocks

[b] Data reported include all sampling dates. Means within a year and column followed by a different letter are significantly different (one-sided test $\alpha = 0.05$)

mites were similar for the MeSA-baited and control plots ($F = 0.18$; $df = 1, 2$; one-sided test $P = 0.358$) (Table 5). On individual sampling dates, spider mite populations varied and were reduced on up to two of the nine sampling days in MeSA-baited plots compared to the control plots among farms. On two sampling dates, on farms A and B, the number of spider mites per leaf was significantly greater ($P \leq 0.072$) in a MeSA-baited plot than the corresponding control plot (Fig. 2a, c). Seasonal mean densities of hop aphid were similar ($P \geq 0.548$) between treatments on all farms (Table 2), and there were no consistent differences in hop aphid populations over time as indicated by the area under the population development curve ($F = 0.07$; $df = 1, 2$; one-sided test $P = 0.410$) (Table 5).

There was again a trend for the seasonal means of *Stethorus* spp. trapped on sticky cards to be higher in MeSA-baited plots than control plots when averaged over farms (Table 4), although the area under the population development curve for *Stethorus* spp. was similar among treatments ($F = 3.63$; $df = 1,2$; one-sided test $P = 0.099$) (Table 5). Among individual farms, a significant difference was not detected among treatments for the seasonal means of *Stethorus* spp. captured on sticky cards or shake samples ($P \geq 0.171$) (Table 3). On individual sampling dates the number of *Stethorus* spp. on sticky cards was significantly different ($P \leq 0.057$) on up to two sampling days (Fig. 2b, d). Seasonal mean densities of other predators and predator groups were similar ($P \geq 0.284$) between the MeSA-baited and control plots (Tables 3, 4).

Discussion

In the 2 years of this study, mean seasonal densities of the key spider mite predator *Stethorus* spp. tended to be greater in all six MeSA-baited yards. When individual sample dates were compared, there were significantly greater numbers of *Stethorus* spp. on specific dates in four of the six MeSA-baited yards. Mean seasonal densities of spider mites were reduced 40–91% in five of the six yards, although these differences were statistically significant only in 2008. In turn, on specific individual dates there were significantly fewer spider mites in five of the six MeSA-baited yards. Previous work in Washington hop yards indicated significant attraction of *Stethorus* spp. to synthetic MeSA dispensers as well a corresponding reduction in the number of spider mites (James and Price 2004). Attraction of diverse predator groups (e.g., Chrysopidae, *Orius tristicolor*) to slow-release MeSA

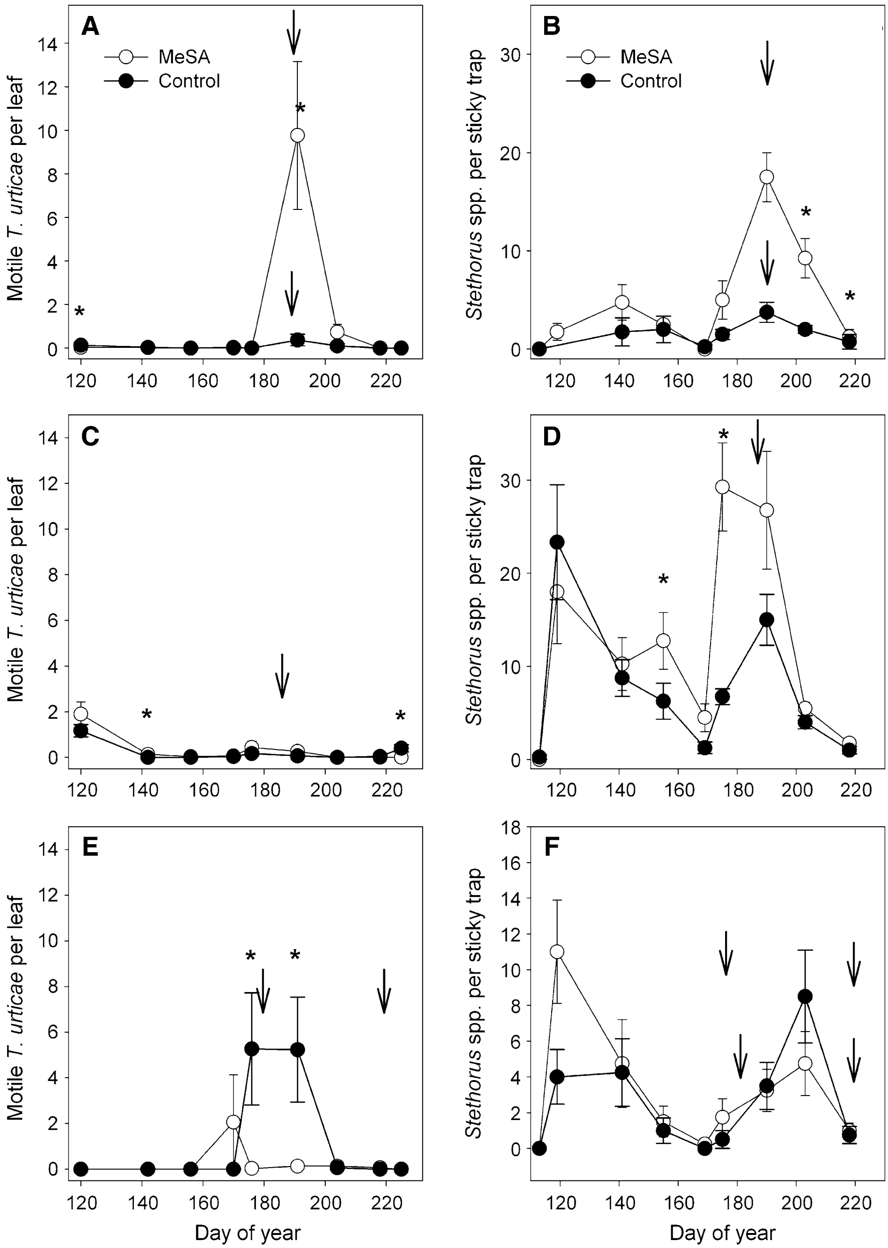

Fig. 2 Density of *Tetranychus urticae* (mean ± SEM) (**a, c, e**) per leaf and *Stethorus* spp. (mean ± SEM) per sticky trap (**b, d, f**) in MeSA-baited and control plots in 2009. Grower A, B, and C are represented in (**a, b**), (**c, d**) and (**e, f**), respectively. *Tetranychus urticae* data are means from 30 leaves per plot. *Stethorus* spp. data are means from four traps per plot. *Arrows* indicate the application of a miticide by the cooperating grower. Significantly different means on specific dates are based on a Mann–Whitney rank sum test ($\alpha = 0.10$) and are noted by an *asterisk*. Across farms, the area under the population development curve for *T. urticae* and *Stethorus* spp. were similar among MeSA-baited and control plots (*T. urticae*: $F = 0.18$; $df = 1, 2$; one-sided test $P = 0.358$; *Stethorus* spp.: $F = 3.63$; $df = 1, 2$; one-sided test $P = 0.099$)

dispensers as documented in Washington (James and Price 2004) and strawberry fields in Oregon (Lee 2010) was not observed in these studies. While the data from the current study are less robust in species abundance and cumulative treatment effect, there was a similar trend for attraction of *Stethorus* spp. and a coincident reduction in the number of spider mites.

The effect of synthetic MeSA deployment on natural enemy attraction and spider mite densities appeared to be site specific. Farm-to-farm variability in pest and predator densities observed in the current study could be attributed to environmental conditions, historical CBC practices, and other ecological and cultural factors that may vary between growers and sites. For instance, the practice of providing alternative food sources such as nectar has been suggested as a potential tool to retain natural enemy populations (Khan et al. 2008), and it is unlikely that nectar sources were the same among all the farms. An additional source of variability could result from local agro-ecology. Laboratory studies showed that HIPVs released from *Arabidopsis* and Brussels sprouts (*Brassica oleracea)* consumed by diamond-back moth larvae (*Plutella xylostella)* reduced attraction of a parasitoid, *Diadegma semiclausum* in the presence of isoprene, a volatile released by poplar *Populus* spp. (Loivamäki et al. 2008). The ecological complexity of HIPVs has been studied intensively, and it is likely that the performance of synthetic HIPVs could be affected by volatiles released from neighboring plants (Dicke and Baldwin 2010). The effect of other plant volatiles in the surrounding environment could potentially have affected the efficacy of MeSA at individual sites. Analyzing the effect of MeSA across all farms thus ignores the impact of ecological and volatile chemical diversity common across sites, potentially eliminating any treatment effect that could be seen by a farm-by-farm analysis. The farm-to-farm variability seen in this study suggests that site selection may be an important consideration in the use of synthetic MeSA.

The aim of this study was to evaluate the performance of slow-release MeSA dispensers in commercial hop yards in Oregon in conjunction with the current production practices used by growers. Commercial production practices in Oregon generally include the use of at least one annual miticide and aphicide application. Cooperating growers were asked to avoid broad-spectrum pesticides; however abamectin was used in the control plot on farm C in 2008 and both plots on farm A in 2009. Abamectin was toxic to *Stethorus punctum picipes* in laboratory bioassays (James 2003a). The use of imidacloprid, toxic to *S. punctum picipes*, *Harmonia axyridis,* and several species of predatory mites (James 2003a; James and Coyle 2001), occurred on all farms with the exception of farm B. The impact of the use of these compounds in this study is unknown, although their application likely interfered with MeSA treatment effects. Potentially, season-long data collected in the absence of broad-spectrum pesticides may have yielded larger differences between MeSA-baited and control plots, although data collected from farm B (unsprayed in 2008) did not indicate this occurred.

Several other factors may have influenced the relatively modest efficacy of MeSA in these studies, such as the timing and rate of MeSA deployment. The timing of MeSA deployment in this study was based principally on evidence that early season recruitment of natural enemies is key for successful biological control of spider mites in hop yards. Previous research in Washington indicated that spring colonization of hop yards by natural enemies is essential to contain localized areas of high spider mite abundance and thus mitigate outbreaks throughout a yard (James et al. 2003). However, conditioning predators to associate MeSA with a non-food source is potentially possible if MeSA packets are deployed too early and pest species have not yet colonized the crop. For example, research in The Netherlands indicated that *Anthocoris nemoralis*, a key predator of psyllids, could associate synthetic MeSA with either the presence or absence of food and respond accordingly (Drukker et al. 2000). The impact of deployment timing in this study are unknown, however the trend for greater numbers of *Stethorus* spp. in MeSA-baited yards does not suggest that this predator associated MeSA with a lack of food. Other predator species may respond differently to the presence of a HIPV in the absence of food. Thus the importance of deployment timing on other predator species is unclear, particularly given that other predators appeared to respond variably to MeSA among farms. The chemical ecology of semiochemicals is complex (De Boer and Dicke 2004; Dicke and Baldwin 2010; Halitschke et al. 2008), but direct measures of arthropod behavior were beyond the scope the current study, which sought simply to evaluate the efficacy of synthetic MeSA deployment in commercial hop yards.

Practicality of the cost of deploying synthetic MeSA as a replacement for a miticide application was considered in the experimental design and deployment rate. Laboratory studies using a Y-tube olfactometer showed that the predatory mite *Phytoseilus persimilis* response to MeSA was dose-dependent (De Boer and Dicke 2004), indicating that there may be an optimal concentration that is most attractive and higher concentrations may have a repellent effect on a predatory mite species, and possibly other predatory insects. In Washington hop yards, more predatory insects were attracted at lower deployment rates (Khan et al. 2008). Based on this evidence, the current study sought to evaluate the use of synthetic MeSA dispensers at similar rates confirmed as most attractive in trials in Washington and at rates competitive with common measures used to control spider mites in the Pacific Northwestern U.S. Clearly, more research is needed is define the optimum rate of synthetic MeSA required for field applications.

The impact of environmental and ecological conditions on the performance of synthetic MeSA remains largely unknown. Compared with trials conducted in Washington, the data collected from Oregon indicate that the efficacy of synthetic MeSA in hop yards was relatively modest, with growers' provisional spider mite action thresholds exceeded in four out of six MeSA-baited yards (Table 1). While trends for greater abundance of *Stethorus* spp. and suppression of spider mite populations were apparent, and significant in 2008, strong statistical support of differences between the treated and control plots were not observed in both years. Although the current study found that MeSA deployment did not substitute for a miticide and aphicide application in a commercial setting, there was evidence of attraction of one of the key spider mite predators in hop yards and a reduction in spider mite populations. Further research is needed to optimize timing, deployment rates, mode of action and to characterize the association of local farm agro-ecology on the performance of MeSA to improve its efficacy and reliability as an integrated pest management tool.

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