



## Evaluation of methyl salicylate lures on populations of *Typhlodromus pyri* (Acari: Phytoseiidae) and other natural enemies in western Oregon vineyards

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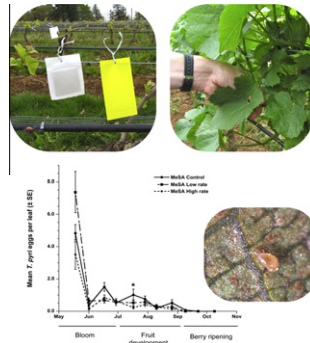
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### HIGHLIGHTS

- ▶ The effect of methyl salicylate (MeSA) was evaluated on natural enemies and pests.
- ▶ Attraction to MeSA was not consistent for *Typhlodromus pyri* between vineyards.
- ▶ Coccinellids were attracted to MeSA treatments showing higher seasonal abundance.
- ▶ MeSA lures did not impact pest populations in the investigated vineyards.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Methyl salicylate (MeSA), a herbivore-induced plant volatile, can elicit control of pests through attraction of beneficial arthropods. This study evaluates the effect of synthetic MeSA lures (PredaLure) on arthropod populations during the 2009 and 2010 seasons in two Oregon vineyards (Dayton and Salem). MeSA lures were deployed at a low (4/plot or 260 lures/ha) and high (8/plot or 520 lures/ha) rate in ~152 m<sup>2</sup> plots while control plots contained no lure. The predatory mite *Typhlodromus pyri* Scheuten is considered to be a key biological control agent of the grapevine rust mite, *Calepitrimerus vitis* Nalepa in Oregon vineyards. Leaf samples were collected to assess *T. pyri*, *C. vitis*, spider mite (Tetranychidae) and thrips (Thripidae) population densities in MeSA treated plots compared to control plots. Yellow sticky traps were used to monitor other key predator groups including Anthocoridae, Araneae, Coccinellidae and Syrphidae. Our data did not display consistent trends in *T. pyri* response to MeSA between treatments at the two field sites over two seasons. Mean seasonal coccinellid counts were significantly higher in MeSA treatments in both years at Dayton. No differences in *C. vitis* population densities were found between treatments in both years. In 2009 at Salem, significantly lower pest thrips densities occurred in low rate MeSA treatments in the latter part of the season although no trend of decreased seasonal abundance was evident.

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### 1. Introduction

The release of herbivore-induced plant volatiles (HIPV's) as a indirect defense mechanism in plants, elicits top-down control of

damaging pests through recruitment of natural enemies (Dicke, 1999; Dicke et al., 1990a). The phenolic compound methyl salicylate (MeSA) has been identified as a important HIPV released by more than 13 different crop plants, including grapes when fed on

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by *Tetranychus urticae* Koch (Dicke et al., 1990b; Van Den Boom et al., 2004). MeSA is also found in the HIPV blends released in cabbage infested by *Pieris* spp. caterpillars (Geervliet et al., 1997), pear infested with Psyllidae (Scutareanu et al., 1997) and hops fed on by hop aphid, *Phorodon humuli* (Schrank) (Campbell et al., 1993).

The positive response of beneficial arthropods toward MeSA and other herbivore-induced volatiles has been demonstrated in a number of laboratory assays (De Boer and Dicke, 2004; Ishiwari et al., 2007; Ozawa et al., 2000; Shimoda, 2010; Shimoda et al., 2002). Recent experiments have also described significant attraction of the female predatory mite, *Typhlodromus pyri* Scheuten, to MeSA (99% diluted in hexane) in  $\gamma$ -tube olfactometer bioassays (Gadino et al., 2012).

Predatory mites and other beneficial arthropods play an integral role in regulating phytophagous mite populations in Pacific Northwest (PNW) vineyards and hop yards (James et al., 2002; Prischmann et al., 2002). Vineyards in the PNW and California have been experiencing increased economic damage from symptoms associated with grapevine rust mite, *Calepitrimerus vitis* Nalepa (Walton et al., 2007; Prischmann and James, 2005). *T. pyri* is the predominant predatory mite found in vineyard systems of the Willamette Valley in Oregon (Hadam et al., 1986) and is considered to be an important biological control agent of eriophyid and tetranychid pest mites (Duso and de Lillo, 1996; Prischmann et al., 2002). Large populations of thrips can cause damage to developing shoots, leaves and fruit in vineyards during spring (De Villiers and Pringle, 2007). There are a variety of other beneficial predators found in western Oregon vineyards that feed on pest mites and thrips, including coccinellids, particularly *Stethorus* spp., anthocorids, spiders, and other predatory mites (Biddinger et al., 2009; Hagen et al., 1999; Prischmann et al., 2002). The conservation and enhancement of beneficial arthropod populations is essential to implementing successful biological control programs in PNW vineyards.

Recent research has focused on the potential to increase natural enemies in crop systems through the application of MeSA. Sticky traps baited with synthetic MeSA dispensers (98–99% in solution) attracted an array of beneficial insects in hop yards (James, 2003a,b) and resulted in attraction of *Coccinella septempunctata* L., in soybean (Zhu and Park, 2005). Vineyard evaluations also demonstrated an increase in natural enemy abundance in plots using controlled release MeSA lures (James and Grasswitz, 2005; James and Price, 2004).

An important consideration when employing synthetic HIPV's is evaluation of the impact on damaging pest populations in the presence of increased natural enemy abundance and activity. Thaler (1999) and Lou et al. (2005) found increased parasitism rates of herbivorous pests in plants treated with synthetic jasmonic acid (JA) that emitted elevated levels of MeSA along with other HIPV's. Soybean aphid abundance was also shown to be significantly lower in MeSA treated plots and in exclusion cage field studies (Mallinger et al., 2011). In PNW hop yards, a trend of increased *Stethorus* spp. attraction in MeSA baited plots coincided with reduced spider mite abundance although results varied by experimental site, year and time of season (James and Price, 2004; Woods et al., 2011). Conversely, strawberry fields in the PNW baited with synthetic MeSA (2 g lures) found no impact on spider mite, aphid or thrips populations regardless of the elevated abundance reported in six natural enemy groups (Lee, 2010).

Repellent effects of MeSA on pest insects have also been found, highlighting the potential for infochemical-based pest management. The bird cherry oat aphid, *Rhopalosiphum padi* L., responded negatively to MeSA treated oats in laboratory experiments (Glinwood and Pettersson, 2000) while field research demonstrated delays in establishment and decreased abundance of bird cherry oat

aphid in barley (Ninkovic et al., 2003). In hop yards, Losel et al. (1996) reported a decrease in aphid (*Phorodon humuli*) densities in water traps baited with MeSA.

In the present study, we employed commercially available MeSA-based lures (PredaLure, AgBio Inc., Westminster, CO, USA) in Oregon vineyards in order to evaluate, (1) the response of *T. pyri* and other valuable predators to treated MeSA plots; (2) the potential effect of MeSA on enhanced conservation biological control of *C. vitis*, spider mite and pest thrips populations; and (3) the spatial and temporal effects of MeSA lures over the growing season in two consecutive years.

## 2. Materials and methods

### 2.1. Site location and experimental design

Field experiments were conducted during 2009 and 2010. Vineyards were located in Salem, OR (Marion Co., 45°01'46N; 123°08'17W, alt. 73 m) and Dayton, OR (Yamhill Co., 45°14'23N; 123°04'28W, alt. 66 m). Vineyards were planted with *Vitis vinifera* cv. Pinot Noir between 2003 and 2005, with 3 × 1 m row spacing and were cane-pruned. Management programs were similar at both vineyards and used a mixture of synthetic fungicides and sulfur in rotation for powdery mildew control, starting at the wooly bud stage (April) until veraison (August). No insecticides were applied at either vineyard during the two seasons.

A randomized complete block design was used and each treatment plot was approximately 152 m<sup>2</sup>, encompassing four vine rows in width and 25–30 vines long. Treatments included MeSA lures (5 g each, 90 d activity; 35 mg/d release rate at constant 30 °C; Rodriguez-Saona et al., 2011) at a low rate (4 lures/plot; 260 lures/ha), a high rate (8 lures/plot; 520 lures/ha) and an untreated control. All treatments were replicated three times totaling 9 plots per location. MeSA lures were tied to the fruiting wire of the trellis system (~1 m above ground) at the center of each plot (approximately 12.5 m from plot edge and across all four vine rows). Lures were replaced one time mid-season in both years. Treatment plots were spaced approximately 60–100 m apart with the treated plots located downwind of the prevailing wind direction to minimize volatile interference between treatments.

### 2.2. Arthropod sampling

Sampling was conducted every 14 days from April to October in each vineyard. Samples were collected from the plot center (0 m) and at 5 and 10 m laterally down the vine row to assess potential spatial trends on arthropod attraction. Secondary shoot samples were collected in April (2009 and 2010) and analyzed under a dissecting microscope in the laboratory to establish presence of *T. pyri* and *C. vitis* in experimental sites.

#### 2.2.1. Leaf samples

Ten leaves at each distance (0, 5 and 10 m) were collected for each location in all plots and transported back to the laboratory in an insulated cooler. Arthropods were brushed onto a glass plate containing a thin film of clear detergent using a leaf-brushing machine (Leedom Enterprises, Mi Wuk Village, CA, USA). The glass plate was placed on a black and white grid to assist in counting using a dissecting microscope. Counts from leaf samples were used to determine arthropod density for *T. pyri* egg and mobile stages, *C. vitis*, tetranychid spp. and thrips (Thripidae) for each treatment and distance.

### 2.2.2. Yellow sticky trap captures

Sticky cards (7.5 × 12.5 cm) were placed at 0, 5 and 10 m (1 trap/distance) in the vine canopy approximately 1 m from the ground. The trap at 0 m was located within 30 cm of the MeSA lure in the baited plots. The entire surface area of each sticky trap was searched using a dissecting microscope to obtain counts of Aeolothripidae, Anthocoridae, Anystidae, Araneae, Chrysopidae, Coccinellidae, Geocoridae, Raphidiidae, Staphylinidae, and Syrphidae. Sticky trap counts were divided by the number of days elapsed from trap placement to sample collection and presented as 14 d counts.

### 2.3. Statistical analysis

Individual analyses of *T. pyri*, *C. vitis*, thrips and tetranychid mites were conducted using leaf density counts. Specimens counted from sticky traps were grouped and analyzed by taxonomic family. Taxa with abundance too low for comparisons were included into the macro-predator (easily visible, mobile arthropods) and micro-predator (mobile arthropods viewed most easily with magnification) groups for analysis (see Tables 1 and 2 for taxonomic list).

MeSA treatment effects on individual arthropod species or taxa were determined using a split-plot repeated measure analysis and included treatment, block, date and distance as factors (PROC MIXED, SAS, 2006). Analyses were separated by location and year. Treatment was the whole factor, with distance within a plot as a split factor, and date as the repeated measure. All main factors and marginal or significant interactions ( $P < 0.10$ ) were analyzed in a backward stepwise approach. When treatment or treatment × date terms were significant ( $P < 0.05$ ), an analysis of variance was conducted for individual species on that given date and means separated with Tukey's HSD procedure ( $P < 0.05$ ). Temporal trends observed over the growing season are defined by vine

phenology and significant results referred to in the bloom (May 1–June 30), fruit development (July 1–August 31) or berry ripening (September 1–October 31) periods. Significant distance and treatment × distance factors were further analyzed and spatial differences separated by Tukey's HSD ( $P < 0.05$ ). Data were transformed using natural log ( $x + 1.0$ ) to normalize distribution when necessary.

## 3. Results

### 3.1. Effects of MeSA on natural enemies

#### 3.1.1. Leaf samples

The number of *T. pyri* egg and mobile stages are presented for all analyses. Population age structure was generally consistent in all experimental treatments, years and vineyards with approximately 70% in the mobile stage and 30% in the egg stage.

During 2009, the mean seasonal density of *T. pyri* eggs and mobiles in Salem was not significantly different in MeSA treated plots compared to control plots (Table 1). Treatment by date interaction for *T. pyri* eggs was significant with greater densities displayed in control plots during the fruit development period (Fig. 1). In Dayton, *T. pyri* densities for eggs and mobiles were not significantly different between treatments, distances or dates (Table 2).

During 2010, no significant differences in *T. pyri* seasonal egg or mobile densities were found between treatments in Salem or Dayton (Tables 1 and 2). Mean seasonal abundance of *T. pyri* mobiles in Dayton was numerically, but not significantly, higher in the high rate MeSA treatments.

#### 3.1.2. Yellow sticky trap captures

Natural enemies were frequently found on sticky traps in all treatments and vineyards during both sampling seasons. In the taxa Coccinellidae, *Stethorus* spp. and *Cycloneda polita* Casey were

**Table 1**  
Mean seasonal abundance and repeated measure analysis (PROC MIXED, SAS) of natural enemy and pest populations sampled at Salem, OR during 2009 and 2010.

Species	Mean ± SE			Treatment (tx) <i>df</i> = 2, 4	Distance <i>df</i> = 2, 12	Date <i>df</i> = 10, 180	Interaction terms if analyzed
	Control	MeSA-low rate <sup>a</sup>	MeSA-high rate				
<i>Leaf samples</i>							
<i>2009</i>				<i>P-value</i>			
<i>Typhlodromus pyri</i>							
Mobiles	2.10 ± 0.14	1.91 ± 0.13	1.43 ± 0.11	0.298	0.664	<.0001	
Eggs	0.89 ± 0.15	0.99 ± 0.24	0.56 ± 0.13	0.099	0.263	<.0001	<b>0.002</b> (tx*date)
Thripidae	0.44 ± 0.09	0.32 ± 0.07	0.30 ± 0.06	0.231	0.301	<.0001	<b>0.042</b> (tx*date)
<i>2010</i>							
<i>Typhlodromus pyri</i>							
Mobiles	0.50 ± 0.07	0.38 ± 0.06	0.31 ± 0.05	0.438	0.551	<.0001	
Eggs	0.19 ± 0.04	0.15 ± 0.04	0.16 ± 0.04	0.634	0.738	<.0001	
Thripidae	0.21 ± 0.04	0.20 ± 0.04	0.15 ± 0.05	0.292	0.255	<.0001	
<i>Yellow sticky trap captures</i>							
<i>2009</i>							
Coccinellidae	0.37 ± 0.08	0.42 ± 0.07	0.56 ± 0.11	0.667	0.052	<.0001	
Anthocoridae	0.42 ± 0.11	0.58 ± 0.20	0.28 ± 0.07	0.721	0.278	<.0001	
Araneae (spiders)	2.14 ± 0.19	2.03 ± 0.25	2.31 ± 0.22	0.739	0.999	<.0001	
Macro predators <sup>b</sup>	3.04 ± 0.26	3.19 ± 0.34	3.23 ± 0.25	0.746	0.091	<.0001	<b>0.001</b> (tx*date)
Micro predators <sup>c</sup>	1.68 ± 0.37	1.29 ± 0.28	1.27 ± 0.31	0.531	0.183	<.0001	
<i>2010</i>							
Coccinellidae	0.30 ± 0.05	0.41 ± 0.09	0.49 ± 0.08	0.417	0.151	<.0001	
Syrphidae	0.10 ± 0.03	0.06 ± 0.02	0.14 ± 0.04	0.320	0.146	0.0002	
Araneae (spiders)	0.99 ± 0.15	1.09 ± 0.14	0.99 ± 0.13	0.768	0.826	<.0001	
Macro predators	1.98 ± 0.21	2.30 ± 0.23	2.27 ± 0.20	0.783	0.072	<.0001	
Micro predators	1.15 ± 0.21	1.04 ± 0.17	0.82 ± 0.18	0.485	0.269	<.0001	

Significant *p*-values in bold ( $P < 0.05$ ).

<sup>a</sup> MeSA = methyl salicylate.

<sup>b</sup> Total counts of easily visible key natural enemies in Oregon vineyards (Coccinellidae, Staphylinidae, Syrphidae, Anthocoridae, Geocoridae, Raphidiidae, Chrysopidae and Araneae).

<sup>c</sup> Total counts of microscopic key natural enemies in Oregon vineyards (Anystidae, Thripidae and Aeolothripidae).

**Table 2**

Mean seasonal abundance and repeated measure analysis (PROC MIXED, SAS) of natural enemy and pest populations sampled at Dayton, OR during 2009 and 2010.

Species	Mean $\pm$ SE			Treatment (tx) <i>df</i> = 2, 4	Distance <i>df</i> = 2, 12	Date <i>df</i> = 10, 180	Interaction terms if analyzed
	Control	MeSA-low rate <sup>a</sup>	MeSA-high rate				
<i>Leaf samples</i>							
<i>2009</i>				<i>P-value</i>			
<i>Typhlodromus pyri</i>							
Mobiles	0.75 $\pm$ 0.08	0.81 $\pm$ 0.11	1.17 $\pm$ 0.11	0.506	0.258	<.0001	
Eggs	0.37 $\pm$ 0.07	0.37 $\pm$ 0.08	0.53 $\pm$ 0.10	0.734	0.143	<.0001	
<i>Calepitrimerus vitis</i>	4.33 $\pm$ 1.21	4.05 $\pm$ 1.14	4.44 $\pm$ 1.20	0.471	0.593	<.0001	
Thripidae	0.47 $\pm$ 0.10	0.45 $\pm$ 0.08	0.53 $\pm$ 0.09	0.864	0.216	<.0001	
Tetranychid	0.04 $\pm$ 0.02	0.05 $\pm$ 0.01	0.08 $\pm$ 0.02	0.345	0.718	0.038	
<i>2010</i>							
<i>Typhlodromus pyri</i>							
Mobiles	0.35 $\pm$ 0.06	0.28 $\pm$ 0.04	0.45 $\pm$ 0.07	0.208	0.658	<.0001	
Eggs	0.34 $\pm$ 0.06	0.25 $\pm$ 0.05	0.42 $\pm$ 0.08	0.257	0.696	<.0001	
<i>Calepitrimerus vitis</i>	0.04 $\pm$ 0.01	0.08 $\pm$ 0.03	0.05 $\pm$ 0.02	0.604	0.912	<.0001	
Thripidae	0.12 $\pm$ 0.03	0.10 $\pm$ 0.02	0.13 $\pm$ 0.03	0.644	0.207	<.0001	
<i>Yellow sticky trap captures</i>							
<i>2009</i>							
Coccinellidae	0.72 $\pm$ 0.10	0.94 $\pm$ 0.11	1.46 $\pm$ 0.18	<b>0.029</b>	0.909	<.0001	
Anthocoridae	0.24 $\pm$ 0.07	0.47 $\pm$ 0.11	0.57 $\pm$ 0.17	0.401	<b>0.021</b>	<.0001	
Araneae (spiders)	1.52 $\pm$ 0.16	1.20 $\pm$ 0.13	1.21 $\pm$ 0.13	0.313	0.759	<.0001	
Macro predators <sup>b</sup>	2.50 $\pm$ 0.21	2.63 $\pm$ 0.22	3.22 $\pm$ 0.29	0.226	0.270	<.0001	
Micro predators <sup>c</sup>	2.45 $\pm$ 0.58	1.16 $\pm$ 0.21	2.62 $\pm$ 0.70	0.374	0.430	<.0001	
<i>2010</i>							
Coccinellidae	0.41 $\pm$ 0.07	0.97 $\pm$ 0.13	0.91 $\pm$ 0.15	<b>0.040</b>	0.933	<.0001	
Syrphidae	0.18 $\pm$ 0.04	0.62 $\pm$ 0.17	0.36 $\pm$ 0.11	0.131	0.133	<.0001	<b>0.012</b> (tx*date)
Araneae (spiders)	0.60 $\pm$ 0.10	0.77 $\pm$ 0.11	0.97 $\pm$ 0.12	0.093	0.913	<.0001	
Macro predators	1.35 $\pm$ 0.15	2.51 $\pm$ 0.23	2.41 $\pm$ 0.24	<b>0.029</b>	0.255	<.0001	
Micro predators	0.43 $\pm$ 0.10	0.41 $\pm$ 0.10	0.46 $\pm$ 0.11	0.965	0.098	<.0001	

Significant *p*-values in bold ( $P < 0.05$ ).<sup>a</sup> MeSA = methyl salicylate.<sup>b</sup> Total counts of easily visible key natural enemies in Oregon vineyards (Coccinellidae, Staphylinidae, Syrphidae, Anthocoridae, Geocoridae, Rhabdiniidae, Chrysopidae and Araneae).<sup>c</sup> Total counts of microscopic key natural enemies in Oregon vineyards (Anystidae, Thripidae and Aeolothripidae).

the dominant species on sticky traps followed by *C. septempunctata*. *Orius* spp. was the most abundant found on traps in the family Anthocoridae.

In Salem, mean seasonal coccinellid trap counts during 2009 were numerically higher in both MeSA treatments but were not significant (Table 1). Here, temporal analysis of macro predators displayed greater natural enemy populations in high rate MeSA plots during the berry ripening period in 2009 (Table 3). Significantly lower numbers of macro predators however were found in MeSA treated plots earlier in the season during bloom.

Mean seasonal abundance of Coccinellidae in Dayton was significantly greater in high rate MeSA treatments in 2009 (Table 2). Temporal trends showed greater numbers of coccinellids in the high rate MeSA treatments (Fig. 2a), with peak captures occurring in early June (>4.5 per trap) during bloom and late July (>2.0 per trap) during fruit development. Mean seasonal abundance of Anthocoridae and macro predators from sticky traps was numerically higher in MeSA baited plots but treatment differences were not significant (Table 2). Distance was a significant factor for Anthocoridae however treatment by distance analysis did not show significant differences in trap captures. Significantly greater numbers of anthocorids (*Orius* spp., Fig. 3) were found for all treatments at 0 and 5 m compared to lower numbers at 10 m.

During 2010, Coccinellidae and total macro predators displayed a pattern of increased mean seasonal abundance in MeSA-baited compared to control treatments in Salem but were not significant (Table 1). Mean seasonal abundance of Coccinellidae in Dayton during 2010 was more than 2  $\times$  higher in treated plots and displayed significant attraction of coccinellids to MeSA (Table 2). Peak captures of coccinellids occurred in late May (>2.0 per trap) during

bloom and again in August (>1.5 per trap) during fruit development in low and high rate MeSA treatments (Fig. 2b). Treatment by date analysis also displayed significantly higher Syrphidae abundance in low rate MeSA plots during bloom period (Table 3). Here, mean seasonal abundance of total macro-predators was significantly greater ( $P = 0.029$ ) in MeSA-baited plots in this year (Table 2).

### 3.2. Effects of MeSA on vineyard pests

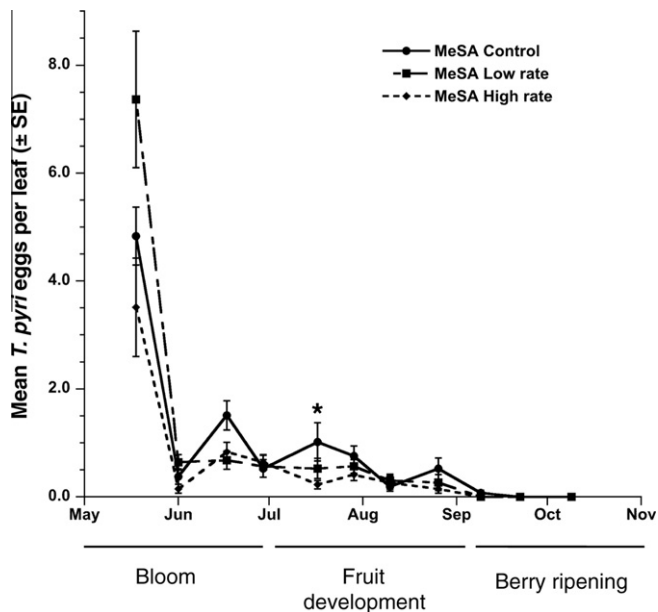
#### 3.2.1. Leaf samples

No *C. vitis* or tetranychid mite populations were recorded in Salem during 2009 or 2010. Mean seasonal counts of pest thrips were higher, but not significantly, in control compared to MeSA baited plots during 2009 (Table 1). However, a significant treatment by date interaction displayed lower pest thrips density occurring later in the season in low rate MeSA treatments during berry ripening (Table 3). During 2010 no significant differences in pest thrips densities were found between treatments, distances or dates (Table 1).

Pest mites (*C. vitis* and tetranychids) and pest thrips were present in all treatment plots in Dayton but no significant differences were found between treatments during both seasons (Table 2). Tetranychid pest mites were present in all treatment plots during 2010 but densities were too low for statistical analysis.

## 4. Discussion

Our data did not show consistent trends in *T. pyri* population response to synthetic MeSA in two field sites and seasons. Attraction



**Fig. 1.** Mean *Typhlodromus pyri* eggs per leaf at Salem during 2009. Mean comparisons (Tukey's HSD) displayed significant differences between control and methyl salicylate (MeSA) plots on July 17 ( $F = 5.39, P < 0.021, df = 2, 26$ ). Significant date denoted with (\*).

to MeSA lures was expected as *T. pyri* adult females displayed a significant attraction to MeSA in laboratory bioassays (Gadino et al., 2012). Data from the present study however, suggests that these results do not translate to a similar response under field conditions. Generally, seasonal mean abundance of *T. pyri* mobile and egg stages in Salem tended to be greater in control plots compared to treated plots, however the opposite trend was observed in Dayton.

The low levels or lack of key prey resources such as, *C. vitis* and other spider mites, in the studied vineyards may have prevented a clear response of *T. pyri* to synthetic MeSA lures. Predatory arthropods have displayed behaviors suggesting they learn to discriminate between prey types or presence and absence of prey through chemical cues (De Boer and Dicke, 2006; De Boer et al., 2005; Drukker et al., 2000a). For example, anthocorid individuals exposed to MeSA in the absence of prey resulted in the avoidance of MeSA in subsequent tests (Drukker et al., 2000b). The lack of significant *T. pyri* attraction to MeSA could, in part, be due to a learned association of volatile presence with limited prey availability.

The dispersal, colonization and foraging habits of *T. pyri* may also account for the lack of a strong positive response to MeSA in

the field. Compared to other predatory mite species, *T. pyri* has low dispersal rates and are less likely to emigrate when their current habitat is suitable (Boller et al., 1988; Dunley and Croft, 1990, 1994). *T. pyri* is also classified as a Type III generalist predator (McMurtry and Croft, 1997) whose spatial distribution does not appear dependent on a single prey resource (Nyrop, 1988). Croft and Jung (2001) suggest generalists with a broad diet are less likely to leave, or will delay leaving a foraging area, when prey numbers are low because they are able to survive on alternate food resources such as pollen unlike specialist predators that rely on a single food type. These behavioral and feeding traits could be inhibiting the response and recruitment of *T. pyri* to areas of elevated MeSA levels.

The response of predatory arthropods to synthetic MeSA may also be influenced by the presence of additional volatile compounds. In laboratory bioassays, attraction of two predatory mites, *Neoseiulus womersleyi* Schicha and *Phytoseiulus persimilis* Athias-Henriot, to HIPV blends as a whole was greater than their response to individual compounds (Ishiwari et al., 2007; van Wijk et al., 2008). Also, Jones et al. (2011) found that the addition of iridodial (a male-produced aggregation pheromone) to MeSA increased the attraction of chrysopids twofold compared to either compound tested individually in Washington apple orchards. It is possible that the magnitude of *T. pyri* attraction to MeSA could be amplified in the presence of additional volatiles and should be further investigated.

Higher mean seasonal abundance of Coccinellidae was found in the MeSA baited treatments in both locations during both years. These findings support other field experiments where coccinellids, particularly *Stethorus* spp. and *C. septempunctata* L, were shown to be significantly attracted to MeSA in various crop plants (James and Price, 2004; James, 2005; Zhu and Park, 2005; Lee, 2010; Rodriguez-Saona et al., 2011; Woods et al., 2011). Synthetic MeSA lures likely function as direct attractants for coccinellids, however, it is possible that exposure to HIPV lures induces a volatile response from the plants themselves that also attracts beneficial insects (Khan et al., 2008; James and Grasswitz, 2005). A recent study found an increase in MeSA volatile emissions from vegetative cranberry vines when exposed to MeSA lures in a greenhouse environment (Rodriguez-Saona et al., 2011). Although volatile responses from plants exposed to synthetic HIPV's has been observed, the association needs further investigation under field conditions and among different crop types.

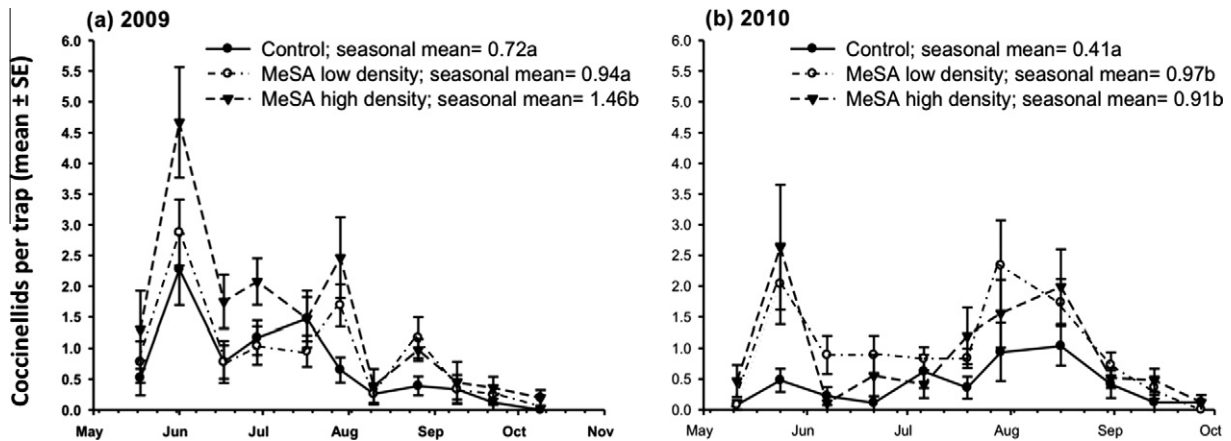
The responses of all other natural enemy groups to MeSA showed no clear temporal patterns in our study. Mean seasonal abundance of *Orius* spp. was numerically greater in MeSA plots at Dayton, but not Salem in 2009 and populations were too low to compare individually during 2010. These results differed from those found by James and Price (2004) and Lee (2010) where an

**Table 3**  
ANOVA of interactions (treatment by date) for natural enemy or pest densities in response to synthetic methyl salicylate (MeSA) at both vineyards during 2009 and 2010.

Species	Mean (±SE)			Date	Vine phenology	F, P df = 2, 26
	Control	MeSA- low rate	MeSA- high rate			
Salem 2009 Thripidae	Leaf samples 0.41 ± 0.14b	0.04 ± 0.03a	0.22 ± 0.15ab	September 9	Berry ripening	10.68, 0.002
Salem 2009 Macro predators <sup>a</sup>	Yellow sticky traps 5.64 ± 0.70b	2.98 ± 0.91a	2.72 ± 1.10a	June 29	Bloom	9.88, 0.003
	1.44 ± 0.80a	1.33 ± 0.44a	2.78 ± 0.22b	September 22	Berry ripening	6.32, 0.013
	1.31 ± 0.61a	0.35 ± 0.36a	2.03 ± 0.36b	October 9	Berry ripening	8.71, 0.005
Dayton 2010 Syrphidae	Yellow sticky traps 0.33 ± 0.17a	3.11 ± 1.21b	1.22 ± 0.86a	June 7	Bloom	7.42, 0.008

Values within each row followed by different letters are significantly different ( $P < 0.05$ ).

<sup>a</sup> Total counts of easily visible key natural enemies in Oregon vineyards (Coccinellidae, Staphylinidae, Syrphidae, Anthocoridae, Geocoridae, Raphidiidae, Chrysopidae and Araneae).



**Fig. 2.** Mean yellow sticky trap counts (14-day intervals) in methyl salicylate (MeSA) and control plots for Coccinellidae at Dayton during 2009 (a) and 2010 (b). Seasonal mean abundance presented in legend. Means not followed by the same letter are significantly different ( $P < 0.05$ ).

overall positive response of *Orius tristicolor* White to MeSA lures was found in grape (cv. Concord), hops and strawberry. James (2003b), however, reported greater attraction of *O. tristicolor* to individual traps baited with (Z)-3-hexenyl acetate compared to MeSA, indicating this predator may show an increased response to other HIPV's.

Spatial orientation of MeSA lures did not appear to influence the response of the majority of natural enemies or pests in our study. Anthocorid predators in Dayton during the first season were more abundant at distances closer to plot center regardless of treatment. Higher anthocorid captures were found in low rate MeSA plots at point center compared to control plots but this pattern was not confirmed due to insignificant treatment by distance effects. Similar spatial trends were found in MeSA-baited strawberry fields for *O. tristicolor* although sticky trap spatial arrangement and density differed from our study (Lee, 2010). In other field studies, a clear attraction to MeSA point sources was displayed by chrysopids in strawberry (Lee, 2010) and soybean (Mallinger et al., 2011); and by syrphids in cranberry (Rodriguez-Saona et al., 2011) and soybean (Mallinger et al., 2011). MeSA lures did not result in similar attraction from these two taxa in our vineyards. One potential explanation could be that these predators are strongly associated with aphids, which do not occur in large numbers on wine grapes in the PNW and may have influenced predator abundance due to prey preferences.

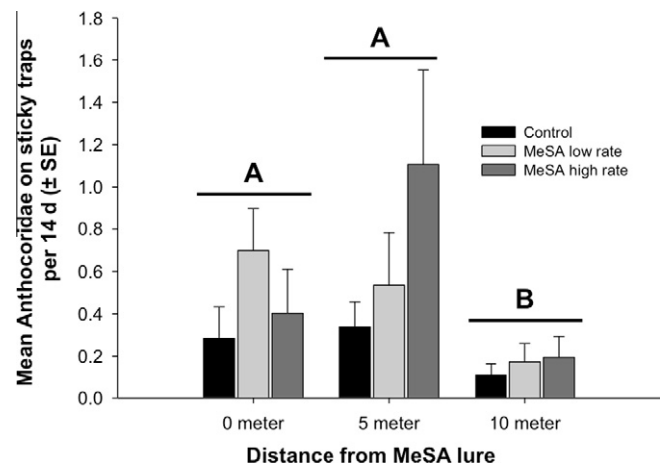
Overall, the attraction of natural enemies to MeSA in our sampled vineyards was variable between sites, sample date and years and is similar to findings by Woods et al., (2008) in Oregon hop yards. Several observations displayed a trend of greater predator abundance in MeSA baited plots but results were not always significant. These results differ from the wide range of natural enemies attracted to MeSA lures in Washington grape juice vineyards where treatments were separated by a minimum of 100 m (James and Price, 2004; James and Grasswitz, 2005). In our study, it is possible that a more considerable degree of predator attraction may have occurred with increased distance between plots (>60–100 m). There is, however, little evidence to suggest that increased plot distances aid in reducing chemical interference, as various field studies have employed distances from 15 to 150 m with minimal knowledge regarding the spatial biological activity of these volatiles (Kaplan, 2012).

Another difference between the Washington and Oregon vineyard field studies was the release rate, or density, of MeSA lures employed. In Washington, 2297 lures/ha and 586 lures/ha were used in the 2004 and 2005 studies, respectively, both of which were higher than the two densities used in the current study.

James and Grasswitz (2005) suggest the higher density used in the 2004 vineyard experiment may have inhibited or reduced the attraction of two parasitic wasp species to MeSA. Indeed, similar observations have been noted in other field experiments in hop yards with lower lure densities (~180/ha) attracting a greater number of natural enemies (Khan et al., 2008; Kaplan, 2012). Based on these findings, we could expect that the densities used in our field experiments would have resulted in significant predator attraction, and that the lower lure density would have attracted a greater level of natural enemies. However, these results were not displayed, suggesting that further research is needed to elucidate optimal release rates for field use.

The addition of MeSA to vineyard plots did not appear to have an impact on target pest populations. No direct (i.e. increased *T. pyri* predation) or repellent effects on *C. vitis* populations were evident in our study. Our results differed from James and Price (2004) and Woods et al. (2011) where a trend of reduced spider mite numbers was observed in hop yards treated with MeSA. We did find significantly lower thrips densities in MeSA plots in 2009 at Salem, but the results were not consistent. Use of MeSA lures also did not have any significant effect on pest thrips populations in studies by Lee (2010), James and Price (2004).

Manipulating the release of HIPV's in agricultural and natural systems has proven beneficial in certain cases (Kessler and Bald-



**Fig. 3.** Mean seasonal yellow sticky trap captures of Anthocorids (*Orius* spp.) at three distances from methyl salicylate (MeSA) lure (plot center) in all treatments at Dayton in 2009. Groups with different upper case letters indicate significant differences between distances using Tukey's HSD ( $P < 0.05$ ).

win, 2001; Khan et al., 2008), however it is important to consider the ecological and evolutionary consequences of employing synthetic volatiles without sufficient understanding of the effect on natural enemies, pests and their host plants (Dicke and Baldwin, 2009). As reviewed by Kaplan (2012) the use of HIPV's to enhance biological control in agricultural systems is a very complex process with many of the underlying mechanisms not yet fully understood, providing a wide range of areas for future research. Learning to associate volatiles with the absence of prey may have profound effects on the behavior of natural enemies in systems experiencing constant release of HIPV's. Attract and reward strategies, where supplemental resources such as nectar are provided (Khan et al., 2008), to mitigate the potential negative impact of prey absence should be further investigated. This strategy was evaluated in vineyards but results found no major impacts on natural enemy activity and the authors also suggest the need for further studies in this area (Simpson et al., 2011).

In summary, we found no clear pattern in the response of *T. pyri* to MeSA in the studied vineyards but did see a greater mean seasonal abundance of coccinellids in the MeSA treatments. The decision to deploy synthetic HIPV's should be based on careful pest monitoring to aid in appropriate lure placement and timing of application. The use of synthetic HIPV's to assist in the retention of mass-released natural enemies is also an important area further exploration. Finally, it is essential to continue to examine and quantify the direct benefits of attracting natural enemies as a pest management strategy with the intent of enhancing biological control.

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