

# USING SYNTHETIC HERBIVOR-INDUCED PLANT VOLATILES TO ENHANCE CONSERVATION BIOLOGICAL CONTROL: FIELD EXPERIMENTS IN HOPS AND GRAPES

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## ABSTRACT

The potential of using synthetic herbivore-induced plant volatiles (HIPV) as a cultural tool to enhance conservation biological control of insects and mites is being researched in hops and grapes in Washington State. Results to date indicate that a number of natural enemy species in the families, Chrysopidae, Hemerobiidae, Anthocoridae, Geocoridae, Miridae, Coccinellidae, Syrphidae, Braconidae, Empididae and Mymaridae, are attracted to sticky traps baited with aqueous methyl salicylate (MeSA), hexenyl acetate, farnesene or octyl aldehyde. Hop yards and grape blocks baited with controlled release sachet (CRS) dispensers of MeSA recruit larger populations of some insect predators (e.g., *Stethorus punctum picipes* Casey (Coleoptera: Coccinellidae), *Orius tristicolor* White (Hemiptera: Anthocoridae), *Chrysopa nigricornis* Burmeister (Neuroptera: Chrysopidae) than unbaited blocks, resulting in some instances, in improved biological control of spider mites and aphids. CRS dispensers of methyl salicylate, methyl jasmonate and hexenyl acetate increased populations of two parasitic wasp genera (*Anagrus*, *Metaphycus*) in grapes, possibly by 'signalling' to the plants to produce HIPVs. The use of synthetic HIPVs/plant-signalling compounds as 'Herbivore-Induced Plant Protection Odors' (HIPPOs) has the potential to provide a novel yet practical strategy for improving the efficacy and reliability of conservation biological control in a variety of agricultural ecosystems.

## INTRODUCTION

The use of conservation biological control (CBC) as a component of integrated pest management in agriculture is a strategy that is increasing in importance and popularity (Barbosa 1998). Concurrent with the increasing use of CBC in agriculture has been a realization that 'generalist' natural enemies (i.e., those that have a broad prey preference) can often play a major role in pest suppression (Symondson *et al.* 2002). Thus CBC as a strategy that enhances guilds or communities of both specialist and generalist natural enemies is now viewed as a pest management strategy, very likely to improve crop protection. Another factor that has encouraged and enhanced the use of CBC in many crop systems is the availability and use of

pesticides that are narrow-spectrum and safe to many beneficial insects and mites (James 2002; 2003d; 2004).

CBC research in many crop systems is focused on improving reliability by strengthening the natural enemy community both in terms of population density and species diversity (Cardinale *et al.* 2003). Inevitably there are two aspects of this problem that need to be addressed; 1) attraction of beneficial arthropods to the crop during early cropping phases and 2) maintenance of these populations throughout the life of the crop. Kean *et al.* (2003) identified 'spatial attraction' of natural enemies as the best way of enhancing CBC. Their results suggested an almost linear relationship between natural enemy attraction and prey equilibrium. The use of semiochemical attractants (e.g., host/prey-derived chemicals) to increase recruitment and retention of beneficial arthropods in crop ecosystems, is an area of opportunity for enhancement of CBC.

Herbivore-Induced Plant Volatiles (HIPV) offer the best potential for developing effective and practical semiochemical-based strategies for manipulating natural enemy populations. Plants attacked by herbivores emit specific chemical signals. These are the 'words' of a complex language used to 'warn' other plants of impending attack and to recruit predatory/parasitic arthropods for 'bodyguard' services. Such plant 'bodyguards' respond to plants in distress, and benefit from the food/host resources available (Sabelis *et al.* 1999). A voluminous body of literature now exists on this phenomenon, first demonstrated in a series of elegant laboratory investigations based on a bean plant-spider mite-predatory mite system (Sabelis and Dicke 1985; Sabelis and Van de Baan 1983; Sabelis *et al.* 1984). The qualitative and quantitative characteristics of HIPV can vary according to the herbivore involved, the plant species and even genotype (Takabayashi *et al.* 1994; Turlings *et al.* 1993). HIPV may function as direct attractants and/or as plant signals. Airborne or topically applied methyl jasmonate (MeJA) can act as a plant signal by causing the emission of volatiles in some plants mimicking those produced in response to herbivore damage (Hunter 2002). There is some evidence that methyl salicylate and hexenyl acetate may also function as plant signals (Engelberth *et al.* 2004; Ozawa *et al.* 2000; Shulaev *et al.* 1997). The use of HIPV as signalers or elicitors of 'correct' and complete blends of natural enemy attracting emissions, is an attractive possibility for manipulating predator and parasitoid populations in pest management.

Compared with the abundance of laboratory studies on HIPV, there is a dearth of field-based studies (Hunter 2002). The first demonstration of the impact of HIPV in the field came from research on biocontrol of psyllids in pear orchards in the Netherlands (Drukker *et al.* 1995), which showed that densities of predatory bugs (Anthocoridae) increased with the density of caged psyllids. Shimoda *et al.* (1997) recorded more predatory thrips on sticky cards near spider mite-infested bean plants than on traps near uninfested plants. Bernasconi *et al.* (2001) trapped more natural enemies near plants damaged and treated with caterpillar regurgitant, than near undamaged, untreated plants.

The first direct evidence for the potential of synthetic HIPV as field attractants for beneficial insects came from this research group (James 2003a,b,c) which demonstrated attraction of a number of insect species and families to methyl salicylate (MeSA) and (Z)-3-hexenyl acetate (HA) in Washington hop yards. Insects attracted to MeSA included *Chrysopa nigricornis* Burmeister (Chrysopidae), *Geocoris pallens* Stal. (Geocoridae), *Stethorus punctum picipes*

(Casey) (Coccinellidae) and species of Syrphidae. Three species were attracted to HA, a predatory mirid, *Deraeocoris brevis* (Uhler), an anthocorid, *Orius tristicolor* (White) and *S. punctum picipes*. Subsequent synthetic HIPV/trapping studies revealed at least 13 species or families of beneficial insects responded to one or more synthetic HIPV (James 2005). Thirteen HIPV attracted one or more species/family of beneficial insect.

Evidence for recruitment and retention of beneficial insects in grapes and hops using controlled-release (CR) dispensers of MeSA, was presented by James and Price (2004). In a replicated experiment conducted in a juice grape vineyard, sticky cards in blocks baited with MeSA captured significantly greater numbers of five species of predatory insects (*C. nigricornis*, *Hemerobius* sp., *D. brevis*, *S. punctum picipes*, *O. tristicolor*) than unbaited blocks. Four insect families (Syrphidae, Braconidae, Empididae, Sarcophagidae) were also significantly more abundant in the MeSA-baited blocks, as indicated by sticky card captures. Monitoring conducted in a MeSA-baited hop yard indicated development and maintenance of a beneficial arthropod population that was nearly four times greater than that in an unbaited reference yard. The large population of predatory insects in the MeSA-baited hop yard was associated with a dramatic reduction in spider mite and aphid numbers, and sub-economic populations were maintained for the rest of the season. The evidence presented in James and Price (2004) is highly suggestive that the use of controlled-release MeSA in a crop could increase recruitment and residency of populations of beneficial insects. Here, we report additional data from field experiments in 2004 on the use of synthetic HIPVs to enhance CBC.

## MATERIALS AND METHODS

### RECRUITMENT OF BENEFICIAL INSECTS TO HOP YARDS AND VINEYARDS

CR dispensers containing MeSA (5 g, 98%, Chem-Tica International, Costa Rica) were deployed in four hop yards and three vineyards (one juice grape, two wine grape) in south-central Washington State during May-September 2004. A control yard with similar characteristics (size, variety etc) was also established, 1-2 km from each MeSA site. Dispensers were stapled to supporting posts (~ 0.5 m above ground) in the yards. Dispenser deployment density for the vineyards was A – 336, B – 432 and C – 642, and for the hop yards, A – 180, B – 447, C – 516 and D – 556 per hectare. Beneficial and pest arthropod populations were monitored weekly at each site (including controls) by examining leaf samples, conducting canopy shake sampling and by retrieving/replacing four yellow sticky cards stapled to poles (see James and Price 2004) for full sampling methodology). Insecticide and miticide applications were kept to a minimum at all sites and where possible, chemicals known to have minimal effect on beneficial arthropods were used. Sticky cards were positioned randomly within each hop yard or vineyard and separated by at least 10 m. After collection, they were examined in the laboratory and all beneficial insects identified and counted. Trap data were analyzed using either the Mann-Whitney Rank –Sum Test, or the Kruskal-Wallis ANOVA on ranks.

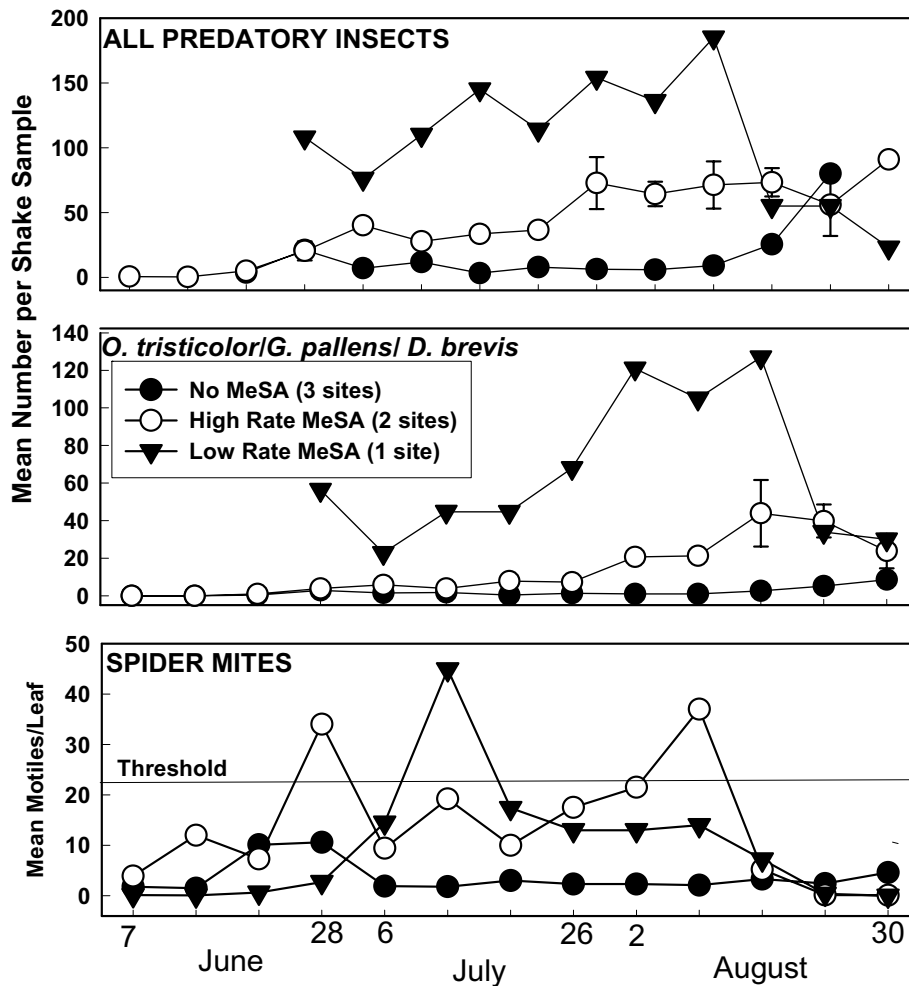
## ATTRACTION OF PARASITIC WASPS TO HIPV-BAITED GRAPES

A field experiment was conducted in an unsprayed 10 ha juice grape (cv. Concord) vineyard in south central Washington State during May-September 2004, to determine the attraction of synthetic formulations of three HIPV, MeSA, methyl jasmonate (MeJA) and (Z)-3-hexenyl acetate (HA) to parasitic wasps. CR dispensers containing 5 g (MeSA), or 1 g (HA, MeJA) were prepared and supplied by Chem-Tica Internacional (Costa Rica). The dispensers for each HIPV were deployed in three 8 X 30 m blocks distributed in a completely randomized design with three unbaited blocks in the vineyard. Each block contained 65 grapevines (5 rows of 13 vines) and blocks were separated by a minimum of 100-m. In the HIPV blocks, 14 dispensers were deployed on April 29 with half stapled at the base of supporting posts and the remainder attached to trellis wires at approximately 1.5 m above the ground. Both deployment types were evenly and alternately distributed in each block giving an approximate density equivalent to 586/ha. Dispensers were replaced on July 15. Populations of parasitic wasp species were monitored using three yellow sticky cards per block, equally spaced (8 m apart) along the center row. Cards were collected and replaced weekly. Parasitic wasps (Mymaridae, Encyrtidae) were identified to genus (*Anagrus* and *Metaphycus*) and counted in the laboratory under a stereomicroscope. At two weekly intervals small samples (~25 individuals) of trapped *Anagrus* spp. were identified to species using a compound microscope and the key of Triapitsyn (1998). Trapping data were analyzed using either the Kruskal-Wallis ANOVA on ranks (KW), with means separated by Dunn's method, or the Mann-Whitney Rank-Sum Test (MW).

## RESULTS

### RECRUITMENT OF BENEFICIAL INSECTS TO HOP YARDS AND VINEYARDS

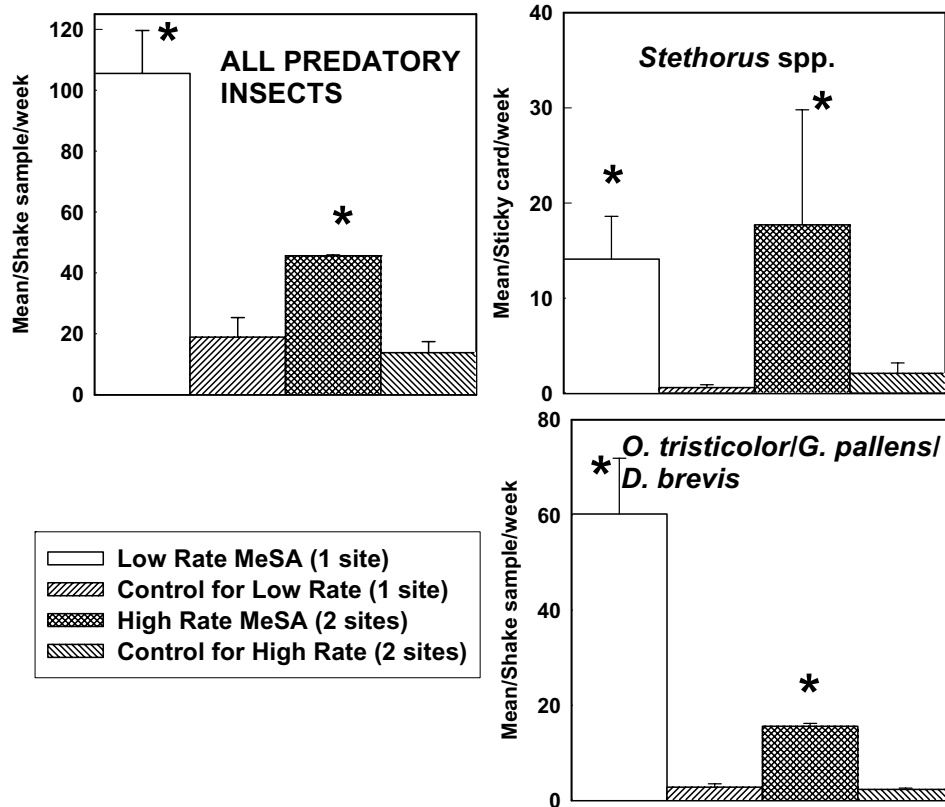
Hop yards. All of the unbaited control yards were treated with at least one miticide (for twospotted spider mite, *Tetranychus urticae* Koch) and/or insecticide (for hop aphid, *Phorodon humuli* Schrank). The control yard for site B was treated with abamectin and imidacloprid, pesticides known to be harmful to some beneficial insects (James 2001; James and Voge 2001), thus this pair was not used in this analysis. The remaining control yards were treated with bifenthrin and/or pymetrozine, both of which are considered safe to most beneficial arthropods in hops (James 2002). MeSA sites A and D were not treated with any insecticide/miticide, while site C received one application of the aphicide, pymetrozine and another of *Bacillus thuringiensis*. The MeSA-baited hop yards developed larger populations (3-5 X) of predatory insects than corresponding unbaited yards (Figs. 1-2). Predatory insect species that were significantly more abundant in MeSA-baited yards than unbaited yards included *O. tristicolor*, *G. pallens*, *D. brevis* and *Stethorus* spp (Fig. 2). Other species and families recorded and quantified but pooled here as 'predatory insects' included lacewings (Chrysopidae, Hemerobiidae), hover flies (Syrphidae), aphidophagous lady beetles (Coccinellidae), predatory thrips (Thripidae) and damsel bugs (Nabidae). Numbers of the predatory hemipterans, *O. tristicolor*, *G. pallens* and *D. brevis* were combined and were six times more abundant in the high deployment rate MeSA yards than in the corresponding control yards (Fig. 2). The



**Figure 1.** Mean ( $\pm$  SE) abundance and phenology of predatory insects and spider mites in hop yards baited with low (180 dispensers/ha) and high (516/556 dispensers/ha) deployment rates of MeSA or left unbaited.

difference was even greater between the low deployment rate yard and corresponding control yard (21.5 X). Similarly, mite-eating lady beetles (*Stethorus* spp.) were 23.5 X more abundant in the low rate MeSA yard than in the control yard (sticky card data) (Fig. 2). Predatory insect abundance was greater and earlier in establishment in the low rate yard than in the high rate yards (Fig. 1). Spider mite populations in the MeSA-baited yards, briefly exceeded the recommended miticide spray threshold (Fig. 1).

In the low rate MeSA yard spider mites exceeded 40 motiles/leaf for a week in early July but stayed below the threshold for the rest of the season. Similarly, populations in the high rate yards briefly climbed above 30 motiles/leaf in late June and again in mid-August. However, hop cone yield and quality were not affected in the MeSA yard and were comparable to those obtained in the non-MeSA yards. Large populations of predatory insects remained in the MeSA-baited yards during August despite the relatively low numbers of spider mites and aphids (e.g., in the low rate MeSA yard there was a mean of  $4.9 \pm 2.1$  mites/aphids per leaf/week in August). Other arthropod prey (e.g., thrips) were also present and may have helped sustain the generalist predator community.



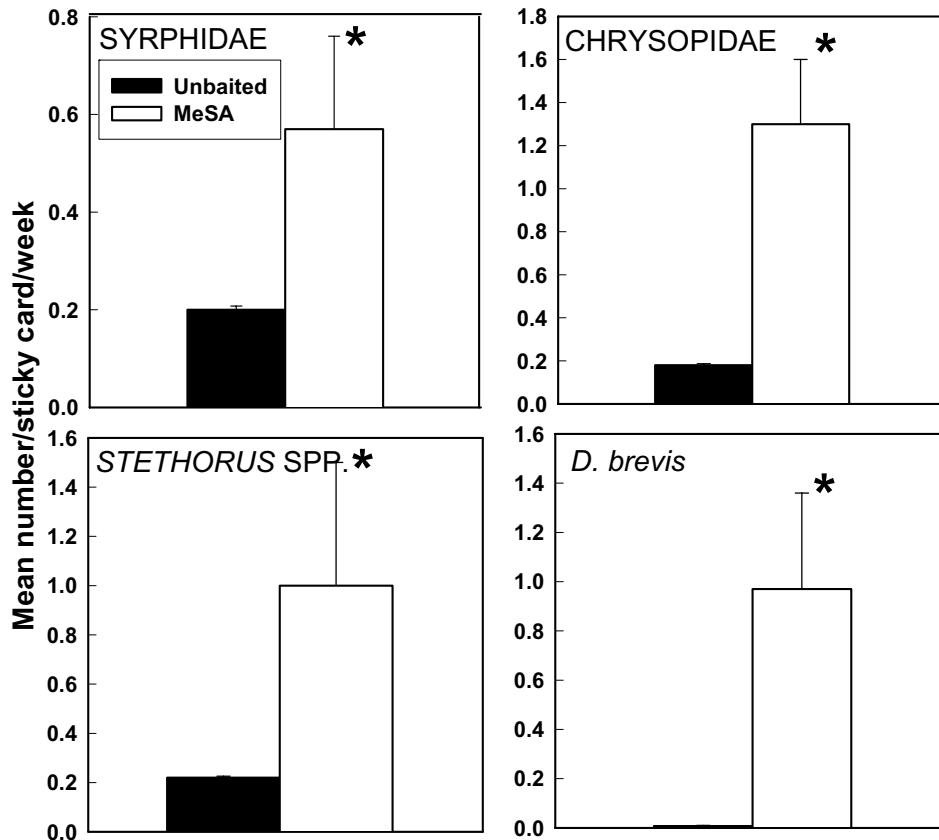
**Figure 2.** Mean ( $\pm$  SE) abundance of predatory insects (all taxa combined), *Stethorus* spp., *O. tristicolor*, *G. pallens* and *D. brevis* in MeSA-baited and unbaited hop yards during May-September 2004. Columns marked with asterisks are significantly greater than the corresponding control column ( $P < 0.05$ ).

197

Vineyards. Predatory insect populations in vineyards were much smaller than in hop yards. However, significant differences in the abundance of some species or families (e.g., Chrysopidae, *D. brevis*, *Stethorus* spp.) between MeSA-baited and unbaited sites were indicated by sticky card captures (Fig. 3).

#### ATTRACTION OF PARASITIC WASPS TO HIPV-BAITED GRAPES

Only two genera of Hymenoptera occurred commonly; *Anagrus* spp. (Hymenoptera: Mymaridae) and *Metaphycus* sp. (Hymenoptera: Encyrtidae). *Metaphycus* sp. appeared to be a parasitoid of scale insects, primarily *Parthenolecanium corni* (Bouche), present on vines in the vineyard. *Anagrus* spp. trapped in this study represented at least three species (*A. erythroneuræ* Triapitsyn and Chiappini, *A. daanei* Triapitsyn, *A. tretiakovæ* Triapitsyn), all important in biological control of grape leafhoppers in Washington State (Storm 2002). Leafhopper and scale insect populations appeared to be evenly distributed throughout the vineyard. Analyses conducted for the entire sampling period showed that significantly greater numbers of *Metaphycus* sp. were trapped in MeSA, MeJA and HA-baited blocks than in unbaited blocks (Fig. 4) ( $P < 0.001$ ,  $H = 63.68$ ,  $df = 3$ , KW). Greater numbers were trapped in MeJA and HA-baited blocks than in MeSA-baited blocks ( $P < 0.001$ ,  $T = 3531$  (MeJA),  $T =$



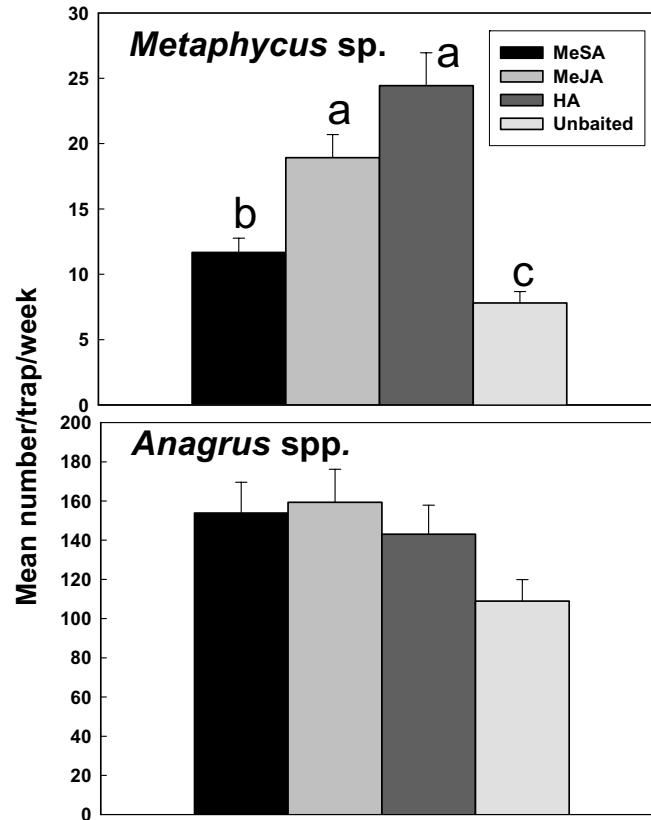
**Figure 3.** Mean ( $\pm$  SE) abundance of Syrphidae, Chrysopidae, *Stethorus* spp. and *D. brevis* in MeSA-baited and unbaited vineyards during May-September 2004 as indicated by sticky card captures. Columns marked by an asterisk significantly different from corresponding unmarked column ( $P < 0.05$ ).

198

3449 (HA), MW), with no difference between MeJA and HA-baited blocks ( $P = 0.33$ ,  $T = 3819$ , MW).

Greater numbers of *Metaphycus* sp. were trapped in MeJA and HA than in unbaited blocks in all months (May  $P < 0.001$ ,  $H = 21.30$ ,  $df = 3$ ; June  $P < 0.005$ ,  $H = 12.99$ ,  $df = 3$ ; August  $P = 0.001$ ,  $H = 53.82$ ,  $df = 3$ ; September  $P = 0.001$ ,  $H = 32.41$ ,  $df = 3$ , KW) except July when wasp abundance was low ( $P = 0.47$ ,  $H = 2.55$ ,  $df = 3$ , KW) (Fig. 5). Numbers of *Metaphycus* sp. trapped in MeSA blocks were significantly greater than in unbaited blocks during August and September only (Fig. 5).

Numbers of *Anagrus* spp. trapped were not significantly different between baited and unbaited blocks when analyzed over the whole trapping period (Fig. 4) ( $P = 0.22$ ,  $H = 4.39$ ,  $df = 3$ , KW). However, when analyzed on a monthly basis, significantly greater numbers of *Anagrus* spp. were trapped in MeSA-baited and MeJA-baited blocks than in unbaited blocks during August-September (August  $P = 0.05$ ,  $H = 10.68$  September  $P = 0.001$ ,  $H = 35.38$   $df = 3$ , KW) (Fig. 6). Traps in HA-baited blocks caught more *Anagrus* spp. than unbaited blocks during September only ( $P < 0.001$ ,  $T = 176$ , MW) (Fig. 6).

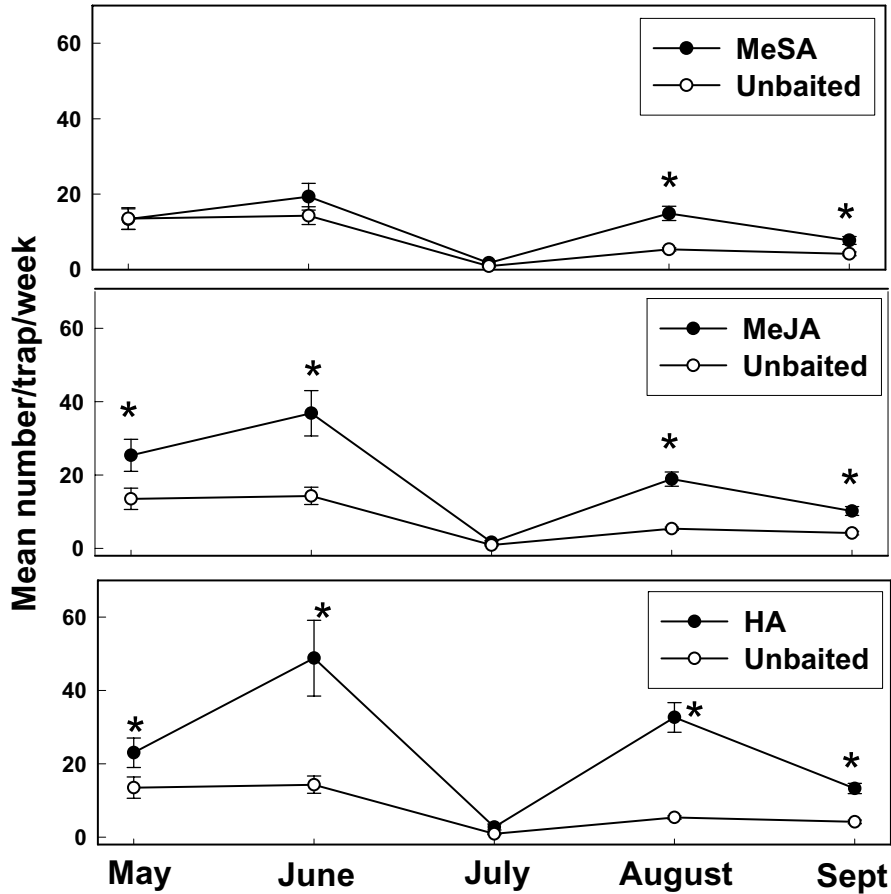


**Figure 4.** Mean ( $\pm$  SE) abundance of *Metaphycus sp.* and *Anagrus spp.* on sticky cards in methyl salicylate (MeSA), methyl jasmonate (MeJA), (Z) – 3 – hexenyl acetate (HA)-baited or unbaited grape blocks during May-September 2004. Different letters on columns denote significant differences for *Metaphycus sp.* ( $P < 0.001$ ). No significant differences for *Anagrus spp.* ( $P = 0.22$ ).

## DISCUSSION

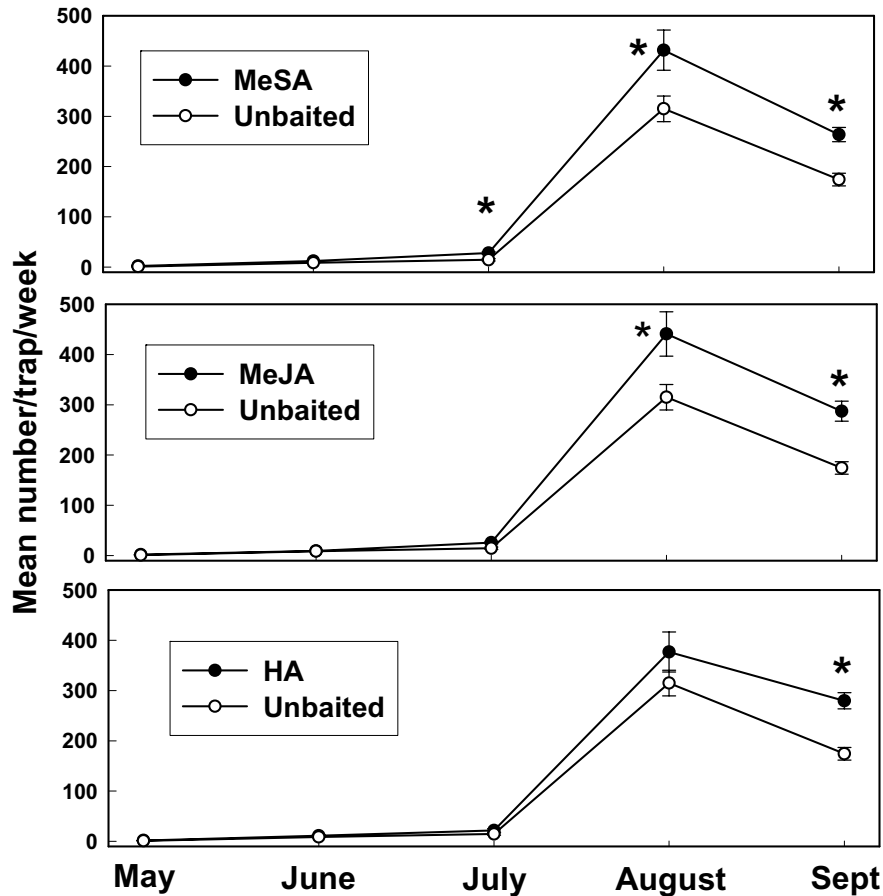
The potential of CR dispensers of MeSA for enhancing the recruitment and retention of predatory insects in crops (James and Price 2004), is further supported by the field data presented here. In addition, the attraction of parasitic wasps (not attracted to MeSA, MeJA or HA-baited sticky cards) to blocks of grapevines containing CR dispensers of MeSA, MeJA and HA, raises the possibility that these synthetic HIPV may also have practical application for signaling plants to produce their own HIPV.

Hop yards and vineyards baited with CR dispensers of MeSA at deployment rates of 180-642/hectare harbored larger populations of predatory insects than nearby and comparable yards without dispensers, as indicated by canopy shake sample, sticky card and leaf sample data. The data presented here are only a portion of the total data sets collected for each yard, but are typical of the results obtained. Species that appeared to respond most strongly to MeSA included *Stethorus spp.*, *O. tristicolor*, *G. pallens*, and *D. brevis*. During August up to 200 *Stethorus spp.* per sticky card/week were recorded from MeSA-baited hop yards, and 100-150 predatory bugs (*O. tristicolor*, *D. brevis*, *G. pallens*, Nabidae) could be shaken from each hop plant sampled. The largest populations of predators occurred in the hop yard baited with the lowest number of MeSA dispensers (180/ha), suggesting that the higher deployment rates used (447-642) may have been sub-optimal. It is possible that the atmospheric concen-



**Figure 5.** Monthly mean ( $\pm$  SE) abundance of *Metaphycus* sp. on sticky cards in methyl salicylate (MeSA), methyl jasmonate (MeJA), (Z) – 3 – hexenyl acetate-baited or unbaited grape blocks during May-September 2004. Asterisks denote significant differences from unbaited blocks ( $P < 0.05$ ).

tration of MeSA in these yards, particularly during spring and early summer, was too high for optimal predator attraction. Predator populations in these yards during July, although higher than in non-MeSA yards, were not as high as in the low deployment rate yard. None of the MeSA-baited hop yards were treated with a miticide and despite short-lived increases in mite populations above the spray threshold, acceptable commercial outcomes in terms of hop cone yield and quality were achieved. The best result was achieved in the low deployment rate yard where mite numbers remained below the spray threshold for all of the cone maturation period (late July-September). The slightly larger populations of spider mites permitted to develop in the MeSA-baited yards (compared to the miticide-treated unbaited yards) may have aided predator recruitment during the summer by direct (numerical aggregation) or indirect means (natural production of HIPV). However, early season spider mite populations in baited and unbaited yards were similar, but the size of predator populations was already differing by late June. The majority of predatory insect species/families attracted to MeSA-baited hops and grapes were generalist feeders. This community of predators will develop and maintain populations even if target prey (e.g., mites), are not abundant as long as alternative prey are available. Another possible advantage of not attracting specialist predators is avoiding selection against responding to synthetic MeSA if nutritional rewards are inadequate.



**Figure 6.** Monthly mean ( $\pm$  SE) abundance of *Anagrus* spp. on sticky cards in methyl salicylate (MeSA), methyl jasmonate (MeJA), (Z) – 3 – hexenyl acetate-baited or unbaited grape blocks during May-September 2004. Asterisks denote significant differences from unbaited blocks ( $P < 0.05$ ).

This study also provided evidence for attraction of two genera of specialist parasitic wasps to grapevines baited with MeSA, MeJA or HA. James (2005) reported micro-Hymenoptera (primarily the families Scelionidae, Encyrtidae, Mymaridae (excluding *Anagrus* spp.)) were attracted to MeSA, indole and *cis*- 3- hexen – 1 – ol –baited traps. However, *Anagrus* spp. and *Metaphycus* sp. were not attracted to MeSA, MeJA or HA-baited traps in James (2005) or James (2003 b). In the current study, blocks of grapevines baited with controlled-release dispensers of MeSA, MeJA or HA, recruited significantly larger populations of *Metaphycus* sp. and *Anagrus* spp. than unbaited blocks, as judged by their incidence on sticky yellow cards. The effect was strongest for *Metaphycus* sp. with significant responses to the three compounds in all months that these wasps were common. MeJA and HA were more attractive than MeSA. The response by *Anagrus* spp. to baited blocks was confined to late summer and early autumn and greatest responses were seen in the MeSA and MeJA-baited blocks. Numbers of trapped *Anagrus* spp. were small during May-July (means of 1-20 individuals/trap/week) and may have obscured any differences during this period. There was also a shift in species abundance during the season with *A. erythroneuræ* and *A. tretiakovæ* dominating during May-July (65%) and *A. daanei* dominating during August-September (70%).

*Anagrus* spp. and *Metaphycus* sp. do not respond to sticky traps baited with MeSA, MeJA or HA (James 2003; 2005), although *A. daanei* was attracted to traps baited with *cis*-3-hexen-1-ol, farnesene or octyl aldehyde (James, 2005). James and Price (2004) using controlled-release dispensers of MeSA in the same vineyard used here, noted no difference in *Anagrus* spp. abundance between baited and unbaited blocks. However, almost twice as many MeSA dispensers were used in each block. There is laboratory evidence for a repellent effect of high rates of MeSA on predatory mites (De Boer and Dicke 2004) and it is possible that *Anagrus* spp. were repelled or at least inhibited in the James and Price (2004) study as well as in the field screening work reported in James (2003 b) and James (2005) when undiluted MeSA was used to bait sticky traps.

Given the earlier non-responsiveness of *Anagrus* spp. and *Metaphycus* sp. to MeSA, MeJA and HA-baited sticky cards, it is possible that the positive responses to grapevine blocks baited with these HIPV reported here, may have been mediated by plant-signaling. Dispersion of MeSA, MeJA or HA volatiles in the vineyard may have signaled the plants to produce their own HIPV blend(s) which attracted *Anagrus* spp. and *Metaphycus* sp. James and Price (2004) suggested that the attraction of a wide variety of insect families to synthetic MeSA deployed in controlled-release dispensers in a vineyard experiment (Chrysopidae, Hemerobiidae, Coccinellidae, Geocoridae, Anthocoridae, Miridae, Syrphidae, Braconidae, Empididae, Sarcophagidae), was also perhaps a consequence of gaseous MeSA acting as a plant signal. Complete blends of natural HIPV from plants would be expected to attract a wider spectrum of pest natural enemies than synthetic MeSA alone.

202

Evidence of a signaling function for MeSA, MeJA and HA in helping plants to recruit 'bodyguards' does exist. Rodriguez-Saona *et al.* (2001) in laboratory studies showed that cotton plants exposed to gaseous MeJA emitted elevated levels of volatiles similar but not identical, to those produced by plants exposed to herbivore attack. Shimoda *et al.* (2002) provided evidence that gaseous MeSA elicits the production of volatiles from bean leaves that are attractive to a predatory thrips species. Arimura *et al.* (2001) demonstrated that gaseous HA induced the expression of defense genes in bean leaves and Engelberth *et al.* (2004) presented evidence of a 'priming' role of green leaf volatiles (GLV) including HA, which enabled corn seedlings to produce greater amounts of HIPV after herbivore attack, than seedlings not previously exposed to GLV. No published studies have shown signaling effects of gaseous MeSA, MeJA or HA in the field, but this is clearly a promising area of research. The current study while raising the possibility of plants stimulated to produce HIPV in the presence of synthetic MeSA, MeJA or HA, does not provide the evidence needed to confirm this. Measuring and analyzing the volatiles produced by grapevines and hops exposed or not exposed to synthetic HIPV should provide definitive evidence for the existence or not of this mechanism and such studies are planned.

The possible use of synthetic HIPV either as direct or indirect enhancers of natural enemy population levels in crops (Herbivore-Induced Plant Protection Odors: HIPPO), is an exciting prospect. Recent studies (James, 2003 a,b; 2005; James and Price 2004) as well as the present work have provided evidence for the potential use of synthetic HIPV as aids to enhancing conservation biological control in crop ecosystems. However, many questions surrounding the use of these materials in integrated pest management remain to be answered.

For example, what are the ecological consequences of providing synthetic HIPV to predators and parasitoids in the absence (or relative absence) of their prey? Will this ‘misinformation’ result ultimately in non-response by natural enemies to HIPV? As noted above, most if not all of the predatory insects attracted to synthetic MeSA are generalist-feeding species (James 2003 a,b; James 2005; James and Price 2004) and the misinformation issue may not be as important with these species as it might be to specialist parasitoid species like *Anagrus* spp. which only parasitize leafhoppers. Defining and understanding the mechanism(s) of attraction and recruitment of predatory and parasitic insects by synthetic HIPV, will be of paramount importance in the effective use of these materials in crop pest management. The data in this study suggest that using synthetic HIPV to signal plants to produce their own HIPV blends is a possibility, but more extensive laboratory and field experimentation is required before this can be confirmed. Optimal deployment (release rates, dispenser density) of synthetic HIPV for natural enemy recruitment and retention, will require a good understanding of the precise mechanisms mediating attraction of predators and parasitoids. Comprehensive studies are planned and will be reported in due course.

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### REFERENCES

- Arimura, G., Ozawa, R., Horiuchi, J., Nishioka, T. and Takabayashi, J. 2001. Plant-plant interactions mediated by volatiles emitted from plants infested by spider mites. *Biochem. Syst. Ecol.* **29**, 1049-1061.
- Barbosa, P. 1998. “Conservation Biological Control,” Academic Press, San Diego, U.S.A.
- Bernasconi Ockroy, M. L., Turlings, T. C. J., Edwards, P. J., Fritzsche-Hoballah, M. E., Ambrosetti, L., Basetti, P., and Dorn, S. 2001. Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.). *Agricultural and Forest Entomology* **3**, 201-209.
- Cardinale, B. J., Harvey, C. T., Gross, K., and Ives, A. R. 2003. Biodiversity and biocontrol: Emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters* **6**, 857-865.
- De Boer, J. G., and M. Dicke, 2004. The role of methyl salicylate in prey searching behavior of the predatory mite, *Phytoseiulus persimilis*. *Journal of Chemical Ecology* **30**, 255-271.
- Drukker, B., Scutareanu, P., and Sabelis, M. W. 1995. Do anthocorid predators respond to synomones from *Psylla*-infested pear trees under field conditions? *Entomologia Experimentalis et Applicata* **77**, 193-203.

- Engleberth, J., Alborn, H. T., Schmelz, E. A., and Tumlinson, J. H. 2004. Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences USA* **101**, 1781-1785.
- Hunter, M. D. 2002. A breath of fresh air: beyond laboratory studies of plant volatile-natural enemy interactions. *Agricultural and Forest Entomology* **4**, 81-86.
- James, D. G. 2002. Selectivity of the miticide, bifentazate and aphicide, pymetrozine, to spider mite predators in Washington hops. *International Journal of Acarology* **28**, 175-179.
- James, D. G. 2003a. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: Methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *Journal of Chemical Ecology* **29**, 1601-1609.
- James, D. G. 2003b. Synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Environmental Entomology* **32**, 977-982.
- James, D. G. 2003c. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. Proceedings of the British Crop Protection Council International Congress-Crop Science and Technology 2003, Glasgow, Scotland pp. 1217-1222
- James, D. G. 2003d. Pesticide susceptibility of two coccinellids (*Stethorus punctum picipes* (Casey) and *Harmonia axyridis* Pallas) important in biological control of mites and aphids in Washington hops. *Biocontrol Science and Technology* **13**, 253-259.
- 204 James, D. G. 2004. Effect of buprofezin on survival of immature stages of *Harmonia axyridis*, *Stethorus punctum picipes* (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Anthocoridae) and *Geocoris* spp. (Hemiptera: Geocoridae). *Journal of Economic Entomology* **97**, 900-904.
- James, D. G. 2005. Further evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Journal of Chemical Ecology* **31**, 481-495.
- James, D. G., and Price, T. S. 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology* **30**, 1613-1628.
- James, D. G., and Vogele, B. 2001. The effect of imidacloprid on survival of some beneficial arthropods. *Plant Protection Quarterly* **16**, 58-62.
- Kean, J., Wratten, S., Tylianakis, J., and Barlow, J. 2003. The population consequences of natural enemy enhancement and implications for conservation biological control. *Ecology Letters* **6**, 604-612.
- Ozawa, R., Arimura, G., Takabayashi, J., Shimoda, T., and Nishioka, T. 2000. Involvement of jasmonate and salicylate-related signaling pathways for the production of specific herbivore-induced volatiles in plants. *Plant Cell Physiology* **41**, 391-398.
- Rodriguez-Saona, C., Crafts-Brandner, S. J., Pare P. W., and Henneberry, T. J. 2001. Exogenous methyl jasmonate induces volatile emissions in cotton plants. *Journal of Chemical Ecology* **27**, 679-695.

- Sabelis, M. W. and Dicke, M. 1985. Long-Range Dispersal and Searching Behavior. *In* "Spider Mites: Their Biology, Natural Enemies and Control" (W. Helle, and M. W. Sabelis, Eds.) pp141-160. Elsevier, Amsterdam. Vol 1B.
- Sabelis, M. W., and Van de Baan, H. E. 1983. Location of distant spider mite colonies by phytoseiid predators: Demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi* (Acari: Phytoseiidae, Tetranychidae). *Entomologia Experimentalis et Applicata* **33**, 303-314.
- Sabelis, M. W., Afman, B. P., and Slim, P. J. 1984. Location of Distant spider Mite Colonies by *Phytoseiulus persimilis*: Localization and Extraction of a Kairomone. *In* "Acarology VI" (D. A. Griffiths. and C. E. Bowman Eds.) pp. 431-440. Halsted Press, New York.
- Sabelis, M. W., Janssen, A., Pallini, A., Venzon, M., Bruin, J., Drukker, B., and Scutareanu, P. 1999. Behavioral Responses of Predatory and Herbivorous Arthropods to Induced Plant Volatiles: From Evolutionary Ecology to Agricultural Applications. *In* "Induced Plant Defenses Against Pathogens and Herbivores" (A. A. Agrawal, S. Tuzun, and E. Bent Eds.) pp. 269-296. APS Press, St Paul, Minnesota.
- Shimoda, T., Takabayashi, J., Ashira, W., and Takafuji, A. 1997. Response of a predatory insect, *Scolothrips takahashi* towards herbivore induced plant volatiles under laboratory and field conditions. *Journal of Chemical Ecology* **23**, 2033-2048.
- Shimoda, T., Ozawa, R., Arimura, G., Takabayashi, J., and Nishioka, T. 2002. Olfactory responses of two specialist insect predators of spider mites toward plant volatiles from lima bean leaves induced by jasmonic acid and/or methyl salicylate. *Applied Entomology and Zoology* **37**, 535-541.
- Shulaev, V., Silverman, P., and Raskin, I (1997). Airborne signaling by methyl salicylate in plant pathogen resistance. *Nature* **385**, 718-721.
- Storm, C. P. 2002. Identity, abundance and phenology of *Anagrus* spp. (Hymenoptera: Mymaridae) and their role in biological control of grape leafhoppers in Washington State. Masters Thesis, Washington State University, Pullman, Washington, U.S.A.
- Symondson, W. O. C., Sunderland, K. D., and Greenstone, M. H. 2002. Can generalist predators be effective biocontrol agents? *Annual Review of Entomology* **47**, 561-594.
- Takabayashi, J., Dicke, M., and Posthumus, M. A. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: Variation caused by biotic and abiotic factors. *Journal of Chemical Ecology* **20**, 1329-1354.
- Turlings, T. C. J., Wackers, F. I., Vet, L. E. M., Lewis, W. J., and and Tumlinson, J. H. 1993. Learning of Host-Finding Cues by Hymenopterous Parasitoids. *In* "Insect Learning" (D. R. Papaj, and W. J. Lewis Eds.) pp. 51-78. Chapman and Hall, New York.
- Triapitsyn, S. V. 1998. *Anagrus* (Hymenoptera: Mymaridae) egg parasitoids of *Erythroneura* spp. and other leafhoppers (Homoptera: Cicadeliidae) in North American vineyards and orchards: a taxonomic review. *Transactions American Entomological Society* **124**, 77-112.