AN ABSTRACT OF THE DISSERTATION OF

Angela N. Gadino for the degree of Doctor of Philosophy in Entomology presented on December 8, 2010.
Title: Enhancing Pest Mite Biological Control by *Typhlodromus pyri* (Acari: Phytoseiidae) in Pacific Northwest Vineyards.

Abstract approved:

Vaughn M. Walton

The predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) is the predominant species in cool climate Pacific Northwest vineyards and a principal predator of pest mites including the grapevine rust mite *Calepitrimerus vitis* (Acari: Eriophyidae). In recent years vineyards have been experiencing *C. vitis* population outbreaks leading to increased economic damage from mite-related symptoms. It is believed that *T. pyri* is an effective biological control agent of *C. vitis*; however, information is limited regarding the factors that influence the success of *T. pyri* in Oregon vineyards. The goal of the current research is 1) examine the suitability of *T. pyri* to control *C. vitis* based on their respective temperature-related development and population parameters 2) determine the impact of commonly applied vineyard pesticides on *T. pyri* in laboratory bioassays and 3) evaluate the effect of synthetic methyl salicylate (MeSA) in laboratory and field experiments on the behavior and abundance of *T. pyri*.

The data presented from life table experiments conducted at seven constant temperatures displayed successful development from egg to adult at 15 to 30ºC. Upper, lower and optimal developmental temperatures were estimated in addition to determining

the intrinsic rate of population increase. Based on these biological parameters, *T. pyri* appears to be a suitable predator of *C. vitis* at temperatures below 25ºC.

Results from pesticide laboratory bioassays found lethal effects greater than 50% in *T. pyri* directly exposed to parafinnic oil. The five other compounds tested displayed predatory mite mortalities less than 50%, which were not significantly different from control treatments. Fecundity rates were reduced in adult female mites exposed to sulfur and mancozeb as developing juveniles. These results indicate that parafinnic oil and sulfur should be limited in integrated management programs to avoid negative effects on *T. pyri* field populations.

*T. pyri* adult females were significantly attracted to the herbivore induced volatile, MeSA at three doses in laboratory olfactometer bioassays. No repellency effect was observed, suggesting MeSA may be employed in vineyards to enhance the abundance of native *T. pyri* populations. Field experiments conducted using synthetic MeSA lures displayed variable responses of *T. pyri* to MeSA at the two experimental sites over two seasons. MeSA lures may be more effective in increasing predatory mite densities in vineyards with suitable prey resources.

Results from this research should assist the Pacific Northwest viticulture industry to develop integrated management programs geared toward conservation and enhancement of *T. pyri* and other beneficial arthropods.

# Enhancing Pest Mite Biological Control by *Typhlodromus pyri* (Acari: Phytoseiidae) in Pacific Northwest Vineyards

by
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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Angela N. Gadino, Author

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## CONTRIBUTION OF AUTHORS

Dr. Amy J. Dreves assisted with the design, implementation and writing of the laboratory bioassay research in chapter three.

Dr. Jana Lee provided the y-tube olfactometer and assisted in the design and implementation of the methyl salicylate bioassay experiments. She also provided statistical guidance and data analysis of in chapters four and five and was involved in the review and writing of chapter four.

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# Chapter I

# Literature Review

## Introduction

Phytoseiid mites play an important role in providing effective control of phytophagous mites in annual and perennial cropping systems (Helle and Sabelis 1985). Beneficial phytoseiids are economically important biological control agents in vineyards, apple orchards and hopyards in the Pacific Northwest (Croft and MacRae 1992, James et al. 2002, Prischmann et al. 2002).

In the last decade vineyards located in cool climate regions of Oregon and Washington have experienced increased symptoms of mite-related Short Shoot Syndrome (SSS) associated with the eriophyid grapevine rust mite, *Calepitrimerus vitis* Nalepa (Perez-Moreno and Moraza 1997, Bernard et al. 2005, Walton et al. 2007). The grapevine rust mite is a host-specific pest of *Vitis vinifera* and occurs in many grape-growing regions throughout the world (Duso and de Lillo 1996). Economic damage occurs from *C. vitis* feeding on susceptible young tissues and developing buds during the early part of the season, resulting in stunted shoots, shortened inter-nodal growth and yield loss due to cluster necrosis (Walton et al. 2010).

*Typhlodromus pyri* Scheuten has been documented as the predominant predatory mite species in western Oregon vineyards (Prischmann et al. 2002) and is a valuable predator in several agricultural systems due to its abundance, wide geographic distribution and polyphagous feeding habits (Helle and Sabelis 1985, Hadam et al. 1986, McMurtry and Croft 1997). Suitable prey for *T. pyri* includes *Tetranychus urticae*, *Panonychus ulmi*, *Aculus schlechtendali* and *C. vitis*. It is believed that *T. pyri* plays an integral role in managing *C. vitis* populations in western Oregon vineyards.

**Viticulture in Oregon**

The first vineyards in Oregon were established in the late 1960's in the northern Willamette and Umpqua Valleys (Hellman 2003). The current Oregon viticulture industry comprises approximately 850 vineyards totaling 19,300 acres planted and valued at $76 million (Mertz et al. 2008, ODA 2009). In 2008 there were 395 wineries operating in Oregon with case sales totaling $7.1 million domestically and $51,647 in export. There are six main American Viticultural Areas (AVAs) in Oregon with the Willamette Valley being the largest region in the state. Pinot Noir is the most popular wine grape varietal in the state totaling approximately 11,200 acres in 2008. The majority of Pinot Noir acres are planted in the Willamette Valley which contains about 8,500 acres. Pinot Noir production is valued at $46 million in Oregon and was sold for $2,600 per ton in 2008, making it the highest valued winegrape varietal grown in the state.

Basic vineyard management practices include vine pruning, canopy management, disease and pest control, and harvest. Cane-pruned vines on a vertically shoot positioned (VSP) trellis system is most commonly used in the cooler growing regions of Oregon (Hellman 2003). Grapevine fruiting canes are pruned to the trunk head every winter while leaving one or two strong renewal canes for the following year fruit production in cane-pruned vines. The damage from mite-related SSS is of particular concern in cane-pruned vines as grapevine rust mites tend to aggregate and feed on basal shoots near the vine trunk (Walton et al. 2007, Walton et al. 2010). This damage often leads to short, weak shoots at the vine head resulting in a lack of adequate renewal canes when pruning.

In all vineyards, active disease management programs are maintained with heavy reliance on sulfur sprays in order to control powdery mildew, *Erysiphe necator*, throughout the growing season. It is believed that these intense spray programs are a contributing factor to increased grapevine rust mite populations, causing secondary pest outbreaks, due to detrimental effects on predatory mite populations and other beneficial arthropods (Duso et al. 1992, Hanna et al. 1997, James and Price 2002, James et al. 2002).

Management of powdery mildew is the most intensive practice in vineyards. Additional control measures are however occasionally needed in order to manage weeds, insect pests and other diseases such as bunch rot. The viticulture industry in Oregon are strong supporters of sustainable agriculture and in 1999 a group of winegrowers established the Oregon Low Input Viticulture and Enology program (Hellman 2003). The program certifies sustainable viticulture production through a set of technical guidelines developed using current research and partnered with Salmon Safe, IOBC (International Organization for Biological and Integrated Control of Noxious Animals and Plants) and Oregon Certified Sustainable Wine (Oregon Live 2010). Currently Oregon LIVE has 216 vineyard members with 146 vineyards certified encompassing 4,427 acres (Oregon LIVE, 2010). LIVE certified and conventionally managed vineyards are in need of cost-efficient, effective and sustainable management options to control pest mite populations and reduce economic damage.

**Grapevine rust mite, *Calepitrimerus vitis***

In 2001, the first reports of short shoot damage in Oregon vineyards occurred, followed by the survey and detection of *C. vitis* populations in Willamette Valley, OR and Washington vineyards (Prischmann and James 2005, Walton et al. 2007). The association between SSS symptoms and *C. vitis* has been documented and described in several other grape growing regions in Europe, Australia, and California (Smith and Stafford 1948, Perez-Moreno and Moraza-Zorrilla 1998, de Lillo et al. 2004, Bernard et al. 2005). Common damage associated with mite-related SSS is stunted shoots, shortened inter-nodal growth, development of secondary buds and lateral shoots, cupped leaves, late season leaf bronzing, deformed or reduced fruit clusters and flower drop (Duso and de Lillo 1996, Duso et al. 2010). These symptoms may be misdiagnosed as damage related to other insect pests, viral infections, environmental factors or issues with vine physiology. In Australia SSS symptoms were initially thought to be the cause of environmental or physiological factors such as freezing injury or nutrient deficiencies but have now been correlated with eriophyid mite presence (Bernard et al. 2005). Appropriate diagnosis of mite-related SSS by monitoring vineyard populations is essential to implementing effective management tools.

Eriopyhid mite structure and identification

The grapevine rust mite adults are extremely small, ~ 0.2 mm in length, and contain four front legs with a worm-like body (Duso and de Lillo 1996). Other grapevine eriophyid pests include the bud mite, *Colomerus vitis* and the erineum blister mite which are morphologically similar but recently confirmed as two distinct species based on

genetic evidence (Carew et al. 2004). The leaf gall-forming erineum mite can be found in Oregon vineyards, but at present is not considered an economically important pest. The bud mite, *Col. vitis* although found in low numbers is not currently known at damaging levels in Oregon vineyards (Walton et al. 2007). It is possible to distinguish *C. vitis* adults from *Col. vitis* as they tend to be darker whitish-yellow shades with a broader frontal region, giving *C. vitis* a more kite-like appearance versus the cylindrical body shape of *Col. vitis* (Duso and de Lillo 1996, Bernard et al. 2000).

Seasonal phenology and development

The grapevine rust mite is classified as free-living, able to migrate over vine surfaces to feed, reproduce and move to over-wintering sites. Adult females have two distinct forms, the summer reproducing protogynes and the specialized over-wintering duetogynes. Female deutogynes overwinter in the outer bud scales and under bark crack and crevices of the vine (Duso and de Lillo 1996, Gabi and Meszaros 2001). In spring when buds begin to swell, populations of over-wintering females actively start to feed and lay eggs. Subsequent generations then feed on developing shoots and leaves throughout the remainder of the growing season. It has been reported that part of the mite population will move into new buds by early summer continuing to feed and increase in number (Duso and de Lillo 1996). The female deutogynes appear by late summer and start their migration toward over-wintering sites usually located in basal buds and canes near the vine head (Carmona 1973, Walton et al. 2007).

Developmental parameters for *C. vitis* in Oregon were recently determined in temperature controlled laboratory experiments. Development from egg to adult took

approximately 6 to 8 d at temperatures ranging from 25 to 28ºC. Rust mite longevity was approximately 25 d at 25ºC and estimates of 14 generations per season were reported (Walton et al. 2010). Damage from *C. vitis* infestations have been found to be more severe in varieties that require long periods of heat for rapid shoot growth and in climatic regions with long, cool spring seasons where susceptible young tissues are present for extended periods (Duso and de Lillo 1996, Bernard et al. 2005, Walton et al. 2010).

Mite damage and symptoms

A number of different factors may contribute to damaging outbreaks of *C. vitis* in vineyards. Environmental conditions with high temperatures (> 35ºC) and low humidity (~ 30%) reportedly increased *C. vitis* populations to the highest levels monitored over four consecutive growing seasons in Rioja, Spain (Perez-Moreno and Moraza-Zorrilla 1998). These findings are supported with developmental data reporting upper developmental thresholds of 39.2ºC for *C. vitis* collected in Oregon (Walton et al. 2010). Damaging outbreaks may also occur due to negative impacts from pesticide applications. Secondary outbreaks of mite pests can occur in crop systems where repeated or broad spectrum pesticide applications are common (Easterbrook 1996, James and Price 2002). The resurgence of *C. vitis* populations may potentially result from the lethal or sub-lethal effects of repeated fungicide applications on beneficial arthropods such as *T. pyri* (James and Price 2002, Prischmann and James 2003). The colonization and infestation of clean vines by mite dispersal may also lead to damaging pest outbreaks if comprehensive monitoring programs are not in place. Long-distance dispersal of *C. vitis* in vineyards

can occur through lifting into wind currents or by traveling on the cloths or hands of vineyard workers (Duffner et al. 2001).

Most economic damage occurs in the early part of the season from over-wintering deutogynes and their progeny. Severe vine damage has been correlated with 20-25 (Duso and de Lillo 1996) and 9 (Walton et al. 2007) mites per bud in cane pruned vines. Controlling *C. vitis* populations is critical during this period but may be difficult to achieve as mites are often hidden under bud-scales and not easily exposed to direct chemical treatments and predators. Previous research reported economic injury levels at 280 mites per leaf warranting treatment with acaracides in late summer monitoring (Hluchy and Pospisil 1992). Leaf-bronzing may occur in mid to late summer as mite populations' increase. This damage however is not directly linked to reduced fruit yields. When leaf bronzing becomes visible it is often too late for chemical controls as deutogyne females have already developed and migrated to protected areas on the vine (Walton et al. 2010). It has been suggested that monitoring *C. vitis* populations during this period may be essential in estimating infestation levels for the following year (Duffner et al. 2001).

Rust mite control

Early season applications of sulfur or acaricides are reportedly effective chemical controls of *C. vitis* in Europe and Australia (Bernard et al. 2005, Duso et al. 2010). Successful early season management of *C. vitis* in Pacific Northwest vineyards is still under investigation. Low temperatures and wet environmental conditions are believed to reduce the efficacy of wettable sulfur against *C. vitis* when applied at wooly-bud and

bud-break stages. The potential for chemical, cultural or biological control later in the season prior to deutogyne female migration is also under examination in Oregon vineyards. Mite outbreaks may be due to negative effects of pesticide treatments on beneficial arthropods, therefore increasing the potential for mite damage. An integrated approach that includes conserving and enhancing biological control agents in the vineyard should be considered.

***Typhlodromus pyri* (Acari: Phytoseiidae)**

The predatory mite *T. pyri* has been documented as a vital biological control agent of phytophagous mites in apple orchards and vineyards throughout the world. The control of key pest mites, *P. ulmi, T. urticae* and *A. schlechtendali*, by *T. pyri* has been documented in apple orchards from agricultural regions in Europe (Solomon and Fitzgerald 1984, Zacharda 1989, Hluchy and Pospisil 1991), New Zealand (Collyer 1980), Canada (Walde et al. 1992, Bostanian et al. 2006) and the United States (Watve and Lienk 1975, Croft et al. 1990, Croft and MacRae 1992). The importance of *T. pyri* in the control of pest mites *P. ulmi*, *T. urticae*, and *C. vitis* has been reported in vineyards from many grape growing regions throughout Europe (Boller et al. 1988, Duso et al. 1991, Hluchy and Pospisil 1991, Engel and Ohnesorge 1994a, Perez-Moreno and Moraza 1997). In Pacific Northwest vineyards *T. pyri* has been found as the predominant predatory mite species and associated as a control agent of the pest mite *T. urticae* (Prischmann et al. 2002).

Morphology and identification

The adult females of *T. pyri* are pear-shaped, translucent and creamy-white in color and approximately 0.4 mm in size (Collyer 1981). Adult males are smaller in size (< 0.3 mm), darker in color and narrower. Eggs are translucent white, barrel-shaped and about 0.2 mm in length. Small six-legged larvae emerge from hatched eggs and appear shiny white in color. Juvenile mites molt through two eight-legged stages, the protonymph and deutonymph, prior to becoming adult mites. Development rates in *T. pyri* are closely associated with temperature, prey type and resource availability similar to other phytoseiid species (Helle and Sabelis 1985).

Seasonal phenology, development and foraging behavior

Gravid adult females over-winter in cracks and crevices on host plants and emerge in spring when temperatures are sufficient for activity, feeding and egg laying. Several over-lapping generations can occur throughout the growing season, depending on factors such as geographic location, seasonal temperatures and food availability. Seasonal population dynamics of *T. pyri* from locations in New Zealand, Canada and Germany were estimated to have between 2 to 5 generations per year, with less generations occurring in cooler climatic regions (Chant 1959, Collyer 1976, Khan and Fent 2005).

Populations of *T. pyri* have been found on several different host plants, particularly deciduous trees and shrubs, in addition to apple and grape. Other common host plants include cherry, peach, pear, plum and hazelnut trees and blackberry, raspberry, strawberry, corn, and mint plants (Collyer 1981, Hadam et al. 1986). As

described previously *T. pyri* is a polyphagous predator that forages on a variety of phytophagous mites and is also able to survive on pollen, fungal tissues and sap-pearls when prey are scarce (Eichhorn and Hoos 1990, Engel and Ohnesorge 1994b, Zemek and Prenerova 1997). The reproductive success of *T. pyri* foraging on eriophyid mites has been established as greater than or equal to its success when foraging on tetranychid mites (Dicke et al. 1990a, Duso and Camporese 1991, Engel and Ohnesorge 1994b). Developmental rate from egg to adult was found to be longer in *T. pyri* when feeding exclusively on pollen, however oviposition rates of adult females were similar when fed a diet of either *P. ulmi* or pollen (Duso and Camporese 1991). Evaluation of *T. pyri* life history traits determined powdery mildew to be a nutritionally adequate food source allowing development, survival and reproduction to occur. Oviposition rates however were comparatively low on this diet alone (Zemek and Prenerova 1997, Pozzebon et al. 2009). These data suggest that *T. pyri* are likely to favor foraging on pest mites in order to optimize their reproductive potential but can utilize alternative food resources for population survival when phytophagous mites are not available. It has also been suggested that polyphagous predators do not exhibit the same functional response to pest numbers as specialist predators but are capable of regulating much lower pest densities (Croft et al. 1990). This trait is particularly advantageous in enabling *T. pyri* populations to survive long-term in specific areas as long as alternative food resources are available. Alternatively, a disadvantage may be a slower response time in population growth when pest mite outbreaks occur, thereby potentially decreasing the ability of *T. pyri* to control damaging populations.

**Biological control with *T. pyri*: Conservation and enhancement**

Previous research in European vineyards indicates *T. pyri* is a principle predator of *C. vitis* and able to keep pest populations below economic threshold levels when conditions are optimal (Duso and de Lillo 1996, Perez-Moreno and Moraza 1997). Conversely, it has also been noted that hot, dry seasonal conditions along with intensive pesticide applications negatively impacted *T. pyri* densities in Rioja vineyards where control of *C. vitis* was compromised (Perez-Moreno and Moraza 1997). Based on these findings it is supposed that *T. pyri* plays an important role in regulating *C. vitis* populations in Pacific Northwest vineyards under suitable conditions. Minimal research however has been conducted on the relationship between these two organisms in our vineyard systems and factors that may influence their population dynamics.

Influence of biological and population parameters

Knowledge of seasonal phenology and biological parameters of an organism are key components in the implementation and success of biological control programs. Detailed information on life history traits can assist in optimizing the effectiveness of a biological control agent and ultimately its ability to regulate pest populations. Biological parameters (developmental rates, intrinsic rate of population increase, and reproductive success) of *T. pyri* have been studied under laboratory conditions in relation to the pest mites' *P. ulmi*, *T. urticae,* and *A. schlechtendali* (Overmeer 1981, Hayes 1988, Dicke et al. 1990a, Genini et al. 1991). Research however is limited in detailing life history traits and population parameters of *T. pyri* in relation to *C. vitis* over a range of temperatures. Developmental times and reproductive rates have been assessed in two Italian strains of

*T. pyri* in relation to *P. ulmi, Eotetranychus carpini* (Tetranychidae), *Colomerus vitis* (Eriophyidae) and *Mesembryanthemum criniflorum* pollen. Experiments conducted at 26-27ºC reported shorter developmental periods (egg to adult female) when fed *E. carpini* and *Col. vitis* compared to pollen and no significant difference in oviposition rates (Duso and Camporese 1991). Intrinsic rate of population increase, oviposition rate and reproductive success have also been examined for a German strain of *T. pyri* fed on a diet of *C. vitis* at a constant temperature of 25ºC (Engel and Ohnesorge 1994b). Although developmental information was presented for two strains of *T. pyri* feeding on *C. vitis* and the related eriophyid *Cal. Vitis*, these studies did not provide data regarding the effect of temperature on these developmental parameters. Seasonal phenology and population parameters have recently been determined for *C. vitis* inhabiting Oregon vineyards (Walton et al. 2007, Walton et al. 2010). In order to optimize biological control in this system comprehensive research is needed to determine the developmental and population parameters for the *T. pyri* strain found in Oregon.

Non-target pesticide impacts

Another factor that may influence success of pest mite biological control is the impact of vineyard disease management programs on *T. pyri* and other natural enemies. The predatory mite *T. pyri* has been reported to be highly sensitive to several pesticide compounds (Candolfi et al. 1999). This susceptibility coupled with the intensive fungicide programs in Oregon vineyards have led to concerns regarding the side effects of these compounds, particularly sulfur which is commonly applied multiple times during the growing season. The lethal and sub-lethal effect of sulfur on these arthropods

remains controversial. Research has reported low toxicity values for sulfur (mortality <50%, 1.5% a.i.) in adult mite assays, whereas a similar study reported sulfur to be highly toxic (90% mortality, 0.25% a.i.) to this predator species (van de Vrie 1962, Zacharda and Hluchy 1991). Another study concluded that sulfur was also relatively harmless to adult *T. pyri* ($LC_{50}$ >4% a.i.) but showed greater toxicity ($LC_{50}$, 0.1% a.i.) to immature stages (Overmeer and van Zon 1981).

Testing standards exposing the test species to the dry residue of a pesticide have long been used to assess toxicity (Overmeer and van Zon 1982, Bluemel et al. 2000). This methodology however is argued to be an inaccurate predictor of pesticide safety which does not consider the proportion of the predator population or its food source which are directly exposed to pesticide sprays (Bernard et al. 2004a). Based on these findings it is critical to conduct direct exposure laboratory experiments to determine the impact of commonly applied fungicides on *T. pyri* found in Oregon vineyards.

Strategies to enhance predator populations

The conservation of biological control agents is a key component in developing effective management programs. Another aspect defined in conservation biological control (CBC) is the ability to enhance the activity and abundance of beneficial arthropod populations through cultural practices (Khan et al. 2008). These techniques include the employment of insectary plants to provide alternative food resources, push-pull strategies and most recently the modification of insect behavior through the exploitation of semio-chemicals. In nature, many tri-trophic interactions are regulated through chemical signals

that may benefit either the producer (allomone), the receiver (kairomone) or both producer and receiver (synomone) of the signal (Dicke and Sabelis 1988b, Price 1997).

It is well established that volatile kairomones play an integral role in the relationship between phytophagous and predatory mites (Sabelis and Baan 1983b, Dicke 1986, 1988, Takabayashi and Dicke 1992). These chemical signals influence predatory mite foraging behaviors such as dispersal, attraction, searching and prey location. It is now understood that the infested host plant plays a key role in the release and composition of volatile kairomones (Dicke et al. 1990c). Herbivore induced plant volatiles (HIPV) are believed to function as indirect plant defense mechanisms capable of attracting natural enemies and increasing biological control of pest populations. HIPV's usually contain several compounds in complex blends which vary in quality and composition based on a number of biotic and abiotic factors (Takabayashi et al. 1994). Methyl salicylate (MeSA), a phenolic compound, has been identified as one of the volatiles released from *T. urticae* infested lima beans (Dicke et al. 1990a, Ozawa et al. 2000a). MeSA has since been identified in the volatile blend for more than 13 different crop species, including grape and hops, when infested with *T. urticae* (James 2003a, van den Boom et al. 2004). It has also been detected in varying quantity and quality in other HIPV blends, such as cabbage fed on by caterpillars, *Pieris* spp (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).

Laboratory studies, conducted with an olfactometer, have reported significant attraction of the predatory mite *Phytoseiulus persimilis* and the generalist predator insect

*Anthocoris nemoralis* (Fabricius) toward isolated MeSA (Dicke et al. 1999b, Drukker et al. 2000b, De Boer and Dicke 2004b). Recently, research has begun to focus on the utilization of MeSA in field environments to attract and retain natural enemies to enhance CBC in different cropping systems. One vineyard experiment which used sachets releasing up to 60mg/day MeSA reported a significant increase in numbers of five beneficial species (*Stethorus punctum*, *Chrysopa nigricornis, Orius tristicolor, Hemerobius* spp and *Deraeocoris brevis*) along with an overall increase of natural enemy seasonal abundance (James and Price 2004). Increased abundance and significant attraction of beneficial arthropods were also found in strawberry plots using commercially available MeSA lures (Lee 2010). Although predatory mites were not among the arthropods sampled in these studies, it is believed that MeSA lures could enhance populations of *T. pyri* and other principle predators of pest mites in Oregon vineyards.

The main objectives of the current research is to 1) further the current knowledge of *T. pyri* life history traits and population parameters in relation to *C. vitis*, 2) investigate the lethal and sub-lethal impact of commonly applied vineyard pesticides on *T. pyri* and 3) evaluate the effect of synthetic MeSA on the *T. pyri* attraction, population density and activity in vineyard systems.

# Chapter II

## Temperature-related development and population parameters for *Typhlodromus pyri* (Acari: Phytoseiidae) found in Oregon vineyards.

**Abstract**

The beneficial mite *Typhlodromus pyri* is a key predator of grapevine rust mite *Calepitrimerus vitis* in Pacific coastal vineyards. Rust mite feeding has been associated with symptoms of Short Shoot Syndrome (SSS) causing stunted, deformed shoot growth and reductions in fruit yield. The life history traits of *T. pyri* were assessed at seven constant temperatures (12.5, 15, 17.5, 20, 25, 30 and 35ºC) to determine biological parameters and the suitability of this predatory mite in controlling *C. vitis* populations in vineyards.

Successful development from the egg to adult stage was observed at temperatures ranging from 15 to 30ºC. Constant exposure to minimum (12.5ºC) and maximum (35ºC) temperatures resulted in 100% mortality in immature *T. pyri*. Mite survival and fecundity rates were highest at 25ºC, and consistent with the estimated optimum developmental temperature of 26.1ºC. The minimum and maximum developmental thresholds were estimated at 8.74ºC and 35.2ºC, respectively. Intrinsic rate of increase ($r_m$) was positive from 15 to 30ºC indicating population growth within this range of temperatures. Net reproductive rate and intrinsic rate of increase were greatest at 25ºC suggesting optimal population growth for *T. pyri* at this temperature. Comparison of developmental parameters with published data suggests *T. pyri* is a more active and effective predator at temperatures below 20 ºC.

## Introduction

Phytoseiid mites play an important role in providing effective control of phytophagous mites in annual and perennial cropping systems (Helle and Sabelis 1985). Beneficial phytoseiids are considered economically important in vineyards, apple orchards and hopyards in the Pacific Northwest (Croft et al. 1992, James et al. 2002, Prischmann et al. 2002). *Typhlodromus pyri* Scheuten is documented as a predominant predatory mite in Oregon and Washington cropping systems (Hadam et al. 1986) and a valuable predator due to its abundance, wide distribution and capacity to feed on a variety of food resources (Helle and Sabelis 1985, McMurtry and Croft 1997).

In the last decade several vineyards in Pacific Northwest coastal regions have experienced increased symptoms of Short Shoot Syndrome (SSS) believed to be associated with feeding from the grapevine rust mite, *Calepitrimerus vitis* Nalepa (Perez-Moreno and Moraza-Zorrilla 1998, Bernard et al. 2005, Walton et al. 2007). This vineyard pest feeds on developing buds and young tissues during the early part of the season. Feeding damage results in stunted shoots, shortened inter-nodal growth and crop loss due to cluster necrosis. In vineyards throughout the Pacific Northwest and Europe *T. pyri* is considered an important predator of *C. vitis* and other Tetranychid pest mites (Hluchy and Pospisil 1991, Perez-Moreno and Moraza 1997, Prischmann et al. 2002).

Adult female *T. pyri* overwinter under bark and in cracks or crevices of the vine plant (Mathys 1958). Individuals emerge gradually in spring when temperatures are suitable and begin to actively feed on available prey and lay eggs on leaf undersides (Zacharda 1989, Khan and Fent 2005). The life cycle of *T. pyri* includes four immature

stages (egg, six-legged larvae, and eight-legged protonymph and deutonymph) prior to reaching adulthood (Helle and Sabelis 1985). The progression to each developmental stage is obtained through molting whereby a visible exuvia is present after each molt. Larvae of *T. pyri* almost never feed and remain inactive until development to the protonymph stage where they voraciously feed on mites, pollen, fungal spores and plant fluids (Croft and Croft 1993, McMurtry and Croft 1997). It has been suggested that at approximately 10º C, *T. pyri* begins actively searching for available prey or other food resource (Mathys 1958). Another study conducted by MacRae and Croft (1993) also suggests that *T. pyri* is more active compared to the phytoseiid *Metaseiulus occidentalis* at low temperatures (~ 15º C) representative of average spring temperatures in the Pacific Northwest.

The grapevine rust mite *C. vitis* has a free-living lifestyle and overwinters as adult female deutogynes under bud-scales or in bark crevices (Duso and de Lillo 1996). Over-wintering mites become active in early spring and begin to feed on swollen vine buds. Substantial economic damage can result from mite feeding in the early growth stages of grapevines (Duso and de Lillo 1996). Research in Oregon has demonstrated an increase in degree-days and development of *C. vitis* during early season vine growth indicating an increase in mite feeding on young, susceptible vine tissues (Walton et al. 2010). Research in Europe has described the ability of *T. pyri* to provide effective control of moderate to high population densities of *C. vitis* in vineyards (Hluchy and Pospisil 1991, Perez-Moreno and Moraza 1997). Moreover, data tracking seasonal population dynamics have displayed a functional response of *T. pyri* populations to *C. vitis* abundance in

European vineyards (Duso and de Lillo 1996). Although there is strong evidence supporting the ability of *T. pyri* to regulate *C. vitis*, there is a lack of published data on this association in Oregon vineyards. Trends observed in vineyard field data collected from the northern Willamette Valley, Oregon suggest that *T. pyri* population levels moderately track population densities of *C. vitis*. These trends are however variable between years and vineyard location (unpublished data, Walton). Due to the increased incidence of economic damage related to rust mite associated SSS in coastal Pacific Northwest vineyards it is necessary to further investigate the relationship of these two species in their current environment.

It is particularly important to determine and compare biological parameters such as lower and upper developmental temperature thresholds, intrinsic rate of population increase and net reproductive rate. Life history parameters were recently determined for the *C. vitis* strain present in Oregon vineyards enhancing our basic biological knowledge of this pest (Walton et al. 2010). Researchers in New Zealand, Europe and Canada have published developmental and reproductive data for *T. pyri* in relation to pest mites *P. ulmi*, *Tetranychus urticae*, *Eotetranychus carpini*, *Colomerus vitis* and *Aculus schlechtendali*. In these studies developmental or population parameters were however either calculated for a single temperature only (Herbert 1961, Overmeer 1981, Duso and Camporese 1991, Genini et al. 1991) or not estimated from developmental and reproductive data (Hayes and McArdle 1987, Hayes 1988, Hardman and Rogers 1991). Intrinsic rate of population increase, oviposition rate and reproductive success have been examined for a German strain of *T. pyri* feeding on *C. vitis* at a constant temperature of

25ºC (Engel and Ohnesorge 1994b). No data has been reported regarding the effect of variable temperatures on these developmental parameters under similar conditions. For these reasons it is important to determine comprehensive biological parameters for the *T. pyri* strain found in Oregon vineyards in relation to the pest mite *C. vitis* in order to investigate the suitability of *T. pyri* as an effective biological control agent.

**Materials and Methods**

Rearing and collection

Predatory mites were collected from northern Willamette Valley vineyards (Yamhill Co., OR) and a stock colony was maintained in the laboratory at 22 ± 2º C, 65% RH and a 16:8 (L:D) photoperiod using methods described by McMurtry and Scriven (1965). Each plastic tray contained a 12 ×12 cm black plastic sheet over sponges placed in a water moat. The perimeter of the plastic tile was lined with damp paper towel and a sticky barrier (Tanglefoot, USA) along each edge to prevent mite escape from the rearing unit. Each unit contained a cotton string (~10 cm) threaded up through a hole in the plastic substrate supplying water to predatory mites. A folded plastic tent (~2 $cm^2$) was placed on each unit to provide a site for oviposition. Mites were reared on a mixed diet associated with Type III (McMurtry and Croft 1997) generalist predatory mites consisting of pollen (*Tilia* spp. and *Typha* spp.) and spider mites (*T. urticae*).

Spider mite colonies were reared on live bean plants (*Phaseolus vulgaris* cv. Roma) under controlled conditions at 25 ± 1º C, 75% RH and a 16:8 (L:D) photoperiod. Clean bean plants were introduced every 5 to 7 days and infested with *T. urticae* by placing 4 to 5 mite colonized leaves onto newly introduced plants. Infested leaves were

collected daily, brushed onto a clean glass plate and offered as prey *ad libitum* in both stock colonies and temperature experiments.

Pinot Noir grapevine leaves (100 +) containing known infestations of *C. vitis* were collected from two north Willamette Valley vineyards (Yamhill Co., Oregon) from July 15 to August 15 in 2009. Leaves were stored in cold rooms at 4 ± 1º C until offered to *T. pyri* during temperature experiments. To collect *C. vitis*, leaves were brushed at room temperature onto a clean glass plate and transferred singly using a fine camel hair brush onto experimental units containing developing *T. pyri* individuals.

Influence of temperature on development, fecundity and survival

Plastic rearing trays as described previously were utilized as experimental units to assess the effect of temperature on *T. pyri* development and reproduction. Each rearing tray was divided into 16 cells (~ 8 $cm^2$) with a sticky barrier (Tanglefoot, USA). Every cell contained a single cotton thread to provide water and an oviposition tent (~ 2 $cm^2$) as described previously. One gravid adult female mite was placed in each of 16 cells on a rearing tray with a fine-haired brush, provided *T. urticae* prey *ad libitum* and checked every 12 h until an egg was oviposited. Rearing trays were placed in controlled growth chambers at seven different temperatures (12.5, 15, 17.5, 20, 25, 30 and 35º C) and 60-70% RH under a 16:8 (L:D) photoperiod. Experimental units were replicated three times at each temperature.

Assessments were performed every 12 h to monitor mite development. A molt to the next developmental stage was determined by the presence of exuvia and removed at each observation. The sex of each predatory mite was determined at the deutonymph

(fourth) developmental stage. Male mites were no longer observed after reaching adulthood. A single male mite was added to cells with adult female mites to allow mating. Immature mites were offered a mix of food types consisting of an estimated 50% *T. urticae* (all stages), 30% *C. vitis* (adult stage) and 20% pollen. Since juvenile *T. pyri* do not feed, mites in the protonymph and deutonymph stages were fed *ad libitum* once daily.

Adult female mites were observed every 24 h to determine pre-oviposition, oviposition and post-oviposition periods. Eggs oviposited per surviving female were recorded daily and removed at each observation. Missing or dead male mites were replaced throughout the oviposition period as subsequent mating is necessary for optimal reproduction in *T. pyri* females (Overmeer et al. 1982). Adult female mites were fed once daily with a mix consisting of 70% *T. urticae* and 30% pollen. The prey *C. vitis* was not offered to adult females due to limited access to leaves with high population numbers and difficulties in rearing this pest mite.

Life table parameters and threshold determination

Survival and fecundity data were used to estimate the intrinsic rate of population increase, $r_m$, described as the capacity of increase in a population under optimal conditions. This value was determined by using the equation $r = \log_e R_o / T$ (Price (1997), where $R_o$ is the net reproductive rate and $T$ is the mean generation time. Net reproductive rate was obtained using, $R_o = \Sigma\ l_x m_x$ and mean generation time estimated by $T = \Sigma\ l_x m_x x / R_o$, where $x$ = age in days, $l_x$ = the proportion of females surviving at day $x$, and $m_x$ = the mean number of eggs produced on day $x$.

Development rate was modeled as a function of temperature using nonlinear and linear models to calculate developmental parameters. Linear regression was used to estimate lower developmental threshold by regressing the reciprocal of development time in days ($t$) against temperature and then solving the regression equation for $1/t = 0$. The nonlinear Briere (1999) rate model for temperature dependant development was used to estimate upper and optimal thresholds. The expression of the user-specified regression model is: $r(T) = nT(T - T_b)(T_L - T)^{1/m}$ , where $r(T)$ is the rate of development at temperature T; $T_L$ is the upper temperature developmental threshold; $T_b$ is the lower temperature developmental threshold and $n$ and $m$ are empirical constants. In order to estimate the influence of temperature on the seasonal occurrence and development rate of *T. pyri* field populations, the thermal constant ($k$) in degree days from birth to adult was calculated as $k = 1/b$ (Liu and Meng 1999). The thermal constant ($k$) was then applied to estimate the number of potential generations per season in Oregon based on the daily 20-year average temperatures.

**Results**

Adult females transferred to experimental units for initial oviposition survived and oviposited at all temperatures. Survival from egg to adults occurred at all temperatures except at the extreme low (12.5ºC) and high (35ºC) temperatures tested (Figure 2.1). Egg hatch was approximately 45% at 12.5ºC. Subsequent exposure to this temperature resulted in 100% mite mortality during the protonymph stage. Eggs subjected to 35ºC displayed 100% egg mortality in all experimental units. Survival at the

remaining five temperatures was greater than 70%, with the lowest percent mortality occurring at 25 ºC.

The longest development period from egg to adult occurred at 17.5ºC (17 d) and the shortest (6.5 d) at 30ºC (Table 2.1). Immature mites spent less time in the non-feeding (juvenile) stage compared to all other developmental stages. A higher mean number of female mites survived to adulthood compared to male mites with a ratio of 1.3:1 at all temperatures except 30ºC where twice as many male mites developed to the adult stage compared to female mites.

Mean lifespan for adult females ranged from 33 to 63 d. An increase in temperature resulted in a decrease in longevity (Table 2.2). The minimum longevity (60 d) for mites developing to adulthood was recorded at 30ºC (Figure 2.2). One individual lived to 120 d at 15ºC as an adult female. Maximum longevities observed at the remaining temperatures were 80, 100 and 90 d at 17.5, 20 and 25ºC respectively. Pre-ovipostition period was shortest at 25ºC (~2 d) and the oviposition period was longest (~31 d) at this temperature. The maximum fecundity rate of 1.4 eggs/day was recorded at 25ºC.

The function obtained by multiple linear regression using lower temperatures (15, 17.5, 20, 25) was y = 0.0089x – 0.07737 ($R^2$ = 0.89, $F$ = 15.55, $P$ = 0.058, $df$ = 1, 2) with an estimated lower developmental threshold of 8.7ºC (Figure 2.3). Upper (35.2 ºC) and optimal (26.1ºC) developmental thresholds were obtained using non-linear estimation (Briere et al. 1999) resulting in the equation y = (0.00000490) * (x-12.079) * (35.22-x) $^{(1/0.44)}$ ($R^2$ = 0.95, $F$ = 19.47, $P$ = 0.049, $df$ = 2, 4). The thermal threshold, k = 112.4 dd,

was obtained using the minimum temperature threshold from the linear regression. We estimate that there will be 13.6 generations per season using 20 year daily means.

Generation time ($T$) for *T. pyri* ranged from 23 to 37 d and decreased with an increase in temperature (Table 2.3). The net reproductive rate ($R_o$) and intrinsic rate of population increase ($r_m$) both increased as temperature increased to 25ºC, after which it decreased at 30ºC. The rate of increase was above zero in all cases indicating positive population growth over this range of temperatures. The highest developmental parameters, $R_o$ = 48.3 and $r_m$ = 0.127, were displayed at 25ºC with a generation time of approximately 30 d. The lowest $R_o$ and $r_m$ occurred at 15ºC with values estimated at 11.2 and 0.072 respectively.

**Discussion**

The data presented in this study provides a comprehensive set of developmental parameters for the Oregon strain of *T. pyri* over a range of temperatures. The life history traits displayed in this study are comparable to findings from previous research conducted at temperatures ranging from 18 to 30ºC. Duration of development from egg to adult took place in 6.6 d and 6.4 d (26 -27ºC; diet of *P. ulmi* and *Colomerus vitis*) (Duso and Camporese 1991) and in 13.5 to 17 d (18ºC) fed a diet of P. *ulmi* (Overmeer 1981). A Swiss strain of *T. pyri* fed on a diet of *T. urticae* developed from egg to adult mites in 13.1, 8.3, and 6.6 d at constant temperatures of 20, 25 and 30ºC respectively (Genini et al. 1991) and were similar to the rates of development observed in this study at the same constant temperatures. Females took longer to develop compared to males at all temperatures. These findings are similar to those reported by Duso and Camporese

(1991) and Genini et al. (1991). In general, studies that tested the effect of temperature on *T. pyri* development demonstrated an increase in the rate of development with increasing temperature regardless of prey type and density (Overmeer 1981, Hayes and McArdle 1987, Genini et al. 1991). These results are consistent with results reported for several other phytoseiid mite species (Gotoh et al. 2004).

The development of male and female mites from egg to adult at 15ºC however occurred in approximately half the number of days (~15 d) in our study compared to the 32 d reported by Genini et al. (1991) suggesting that the Oregon *T. pyri* strain is well adapted to cool climate regions. The minimum development threshold for *T. pyri* was estimated to be 8.7ºC for the Oregon strain and 9.8ºC for the Swiss strain (Genini et al. 1991) indicating increased adaptation of Oregon *T. pyri* to colder temperatures.

Development of *T. pyri* to reproductive maturity occurred at all constant temperatures ranging from 15 to 30ºC. The duration of oviposition and the mean number of eggs oviposited per female each day increased with increasing temperatures up to 25ºC, indicating this as an optimal temperature for *T. pyri* fecundity. Oviposition rates of 0.72 (18ºC), 0.90 (20ºC) and 1.02 eggs per day (26-27ºC) have been documented in three separate studies and support the reproductive rates displayed in our experiments (Duso and Camporese 1991, Genini et al. 1991, Zemek 1993). Engel and Ohnesorge (1994b) however reported mean oviposition rates of 0.90 (*T. urticae* prey), 0.95 (*C. vitis* prey) and 0.56 (*P. ulmi* prey) at a constant 25ºC temperature and were lower than the 1.4 mean eggs/day displayed in our life history parameters at this temperature. One factor that may have influenced the increased egg production of *T. pyri* females in our study was the

availability of three prey resource types during development and reproduction that consisted of *T. urticae*, *C. vitis* and pollen compared to the single prey species offered in the experiments of Engle and Ohnesorge (1994).

Net reproductive rates and intrinsic rate of population increase were greater than zero at temperatures from 15 to 30ºC indicating positive population growth of *T. pyri* over this temperature range. The rate of population increase and net reproductive rate calculated at 20ºC for Oregon *T. pyri* was comparable to the 0.103 rate of increase and 21.8 net reproductive rate observed in the Swiss strain at this temperature (Genini et al. 1991). The highest rate of population increase ($r_m$ = 0.127) occurred at 25ºC and is consistent with the optimal developmental temperature of 26.1ºC found in our study. This value falls between the $r_m$ estimates of 0.100 and 0.159 reported for *T. pyri* reared on *T. urticae* and *C. vitis* respectively at 25ºC (Engel and Ohnesorge 1994b). Similar population parameters between the present study and previous research suggests that positive population growth will occur with the availability of several types of food resources including *C. vitis* and pollen.

It is also important to ascertain the suitability of *T. pyri* as an effective biological control agent for *C. vitis* in Oregon vineyards by comparing biological parameters. Successful development of *C. vitis* from egg to adult stages occurred at similar temperatures to those of *T. pyri* and the rate of development also increased with increasing temperature (Walton et al. 2010). Maximum net reproduction, intrinsic rate of increase and fecundity rates occurred at 25ºC for both *T. pyri* and *C. vitis*. Optimal developmental temperatures were estimated at 26ºC for both mite species pointing toward

similar temperature requirements. Population increase at 25ºC were $r_m$ = 0.141 for *C. vitis* and $r_m$ = 0.127 for *T. pyri*. These results signify a potential advantage in rust mite population growth at these higher temperatures, especially in agricultural systems applying pesticides that may disrupt the ecological balance between insect populations (James et al. 2002). Comparison of the intrinsic rate of increase at approximately 17ºC displayed the opposite trend where *T. pyri* populations have a greater capacity to increase to levels above those of *C. vitis* (Walton et al. 2010) supporting previous evidence that *T. pyri* is highly active and potentially more effective in controlling *C. vitis* populations at lower temperatures.

The minimum developmental threshold (10.51ºC) of *C. vitis* is above that of *T. pyri* indicating this predatory mite is likely active and foraging for prey prior to the activation of over-wintering *C. vitis* development in the early part of the growing season. The maximum developmental threshold (39.19ºC) of *C. vitis* however, appears to exceed the 35.2ºC upper threshold for *T. pyri* by four degrees. These results suggests that *T. pyri* may not have the capacity to increase population levels enough to control *C. vitis* populations when high temperatures occur in the latter part of the growing season. Additionally, Sengonca et al.(2003) reported a significant decrease in *P. ulmi* prey consumption by *T. pyri* when the temperature increased from 25 to 30ºC. Seasonal population dynamics in Oregon vineyards show an increase in *C. vitis* populations from late July to August prior to adult female movement to over-wintering sites (Walton et al 2010). Controlling pest mite numbers during this period can potentially reduce damaging populations the following year. The current data indicates that native *T. pyri* populations

alone may not be able to control increasing *C. vitis* populations if temperatures exceed 25ºC. This phenomenon was observed in the seasonal population dynamics occurring in Rioja vineyards where high temperatures and low humidity favored *C. vitis* populations but were detrimental to *T. pyri* populations (Perez-Moreno and Moraza 1997).

Results from these studies support our assertion that *T. pyri* is a highly active predator at lower temperatures and is likely to provide more effective control during the cooler spring season. The potential for native populations of *T. pyri* to control grapevine rust mite in Oregon vineyards may however be compromised due to several factors. These include unfavorable temperatures that may lead to late season *C. vitis* resurgence and negative impacts of disease management programs. Future research should be conducted to determine if early season augmentation of *T. pyri* populations through mass-release would aid in controlling *C. vitis* populations. The optimal release rates and timing should also be investigated. The establishment of an alternative native predatory mite species such as *Amblyseius andersoni* should be explored as prospective late season biological control agents.

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Table 2.1. Duration of *Typhlodromus pyri* developmental stages (days) at six constant temperatures.

| Life Stage | Temperature ºC | | | | | |
|---|---|---|---|---|---|---|
| | 12.5 | 15 | 17.5 | 20 | 25 | 30 |
| *Females* | n = 15[a] | n = 19[b] | n = 20[b] | n = 22[b] | n = 24[b] | n = 11[b] |
| Egg | 8.7 ± 0.3 | 6.0 ± 0.2 | 5.7 ± 0.1 | 3.3 ± 0.1 | 2.4 ± 0.1 | 2.0 ± 0.1 |
| Larvae | 3.1 ± 0.3 | 1.4 ± 0.1 | 1.8 ± 0.1 | 1.0 ± 0.1 | 0.6 ± 0 | 0.8 ± 0.2 |
| Protonymph | - | 3.9 ± 0.1 | 5.1 ± 0.2 | 2.4 ± 0.1 | 2.0 ± 0 | 1.8 ± 0.1 |
| Deutonymph | - | 4.3 ± 0.2 | 4.4 ± 0.2 | 2.2 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.2 |
| Egg-Adult | - | 15.7 | 17.0 | 9.0 | 7.0 | 6.5 |
| *Males* | | n = 14 | n = 17 | n = 18 | n = 19 | n = 23 |
| Egg | - | 6.5 ± 0.2 | 6.4 ± 0.2 | 3.6 ± 0.1 | 2.4 ± 0.1 | 1.9 ± 0.1 |
| Larvae | - | 1.5 ± 0 | 1.8 ± 0.1 | 0.9 ± 0 | 0.8 ± 0.1 | 0.6 ± 0.1 |
| Protonymph | - | 3.9 ± 0.2 | 4.8 ± 0.3 | 2.3 ± 0.1 | 1.8 ± 0.1 | 1.9 ± 0.1 |
| Deutonymph | - | 4.0 ± 0.2 | 4.0 ± 0.2 | 2.0 ± 0.1 | 1.8 ± 0.1 | 1.7 ± 0.1 |
| Egg-Adult | - | 15.9 | 17.0 | 8.9 | 6.8 | 6.2 |

[a]Total individuals molted to juvenile stage prior to 100% mortality at this temperature. Sex of each mite was not determined due to mortality during the larval stage.
[b]Total number of individuals completing development to adult stage.

**Table 2.2.** Adult *Typhlodromus pyri* average fecundity and survival parameters (days) at five constant temperatures.

| Parameter (Mean ± SE) | Temperature ºC | | | | |
|---|---|---|---|---|---|
| | 15 | 17.5 | 20 | 25 | 30 |
| | n = 15 | n = 18 | n = 21 | n = 23 | n = 11 |
| Pre-oviposition | 6.7 ± 1.2 | 6.0 ± 0.3 | 3.2 ± 0.3 | 2.2 ± 0.3 | 3.5 ± 0.9 |
| Oviposition | 25.7 ± 3.7 | 27.1 ± 2.1 | 31.1 ± 3.4 | 31.3 ± 2.8 | 20.1 ± 4.6 |
| Post-oviposition | 19.5 ± 5.8 | 5.4 ± 1.4 | 15.7 ± 4.1 | 6.6 ± 1.5 | 5.4 ± 2.4 |
| Eggs/day | 0.7 ± 0.1 | 0.7 ± 0 | 1.2 ± 0.1 | 1.4 ± 0.1 | 1.1 ± 0.1 |
| Adult longevity | 51.9 ± 6.3 | 38.4 ± 3.0 | 50.0 ± 6.0 | 40.0 ± 3.3 | 29.1 ± 6.0 |
| Total life span | 63.4 ± 7.0 | 50.2 ± 4.0 | 56.4 ± 6.2 | 45.0 ± 3.3 | 33.2 ± 5.0 |

**Table 2.3.** Parameters of population increase for *Typhlodromus pyri* at five constant temperatures ($R_o$ = the net reproductive rate; $T$ = mean generation time (days); and $r_m$ = intrinsic rate of population increase)

| Temperature ºC | Developmental and Reproductive Parameters | | |
|---|---|---|---|
| | $R_o$ | $T$ | $r_m$ |
| 15 | 11.2 | 33.5 | 0.072 |
| 17.5 | 15.5 | 37.0 | 0.074 |
| 20 | 22.2 | 33.2 | 0.093 |
| 25 | 48.3 | 30.6 | 0.127 |
| 30 | 17.7 | 23.8 | 0.121 |

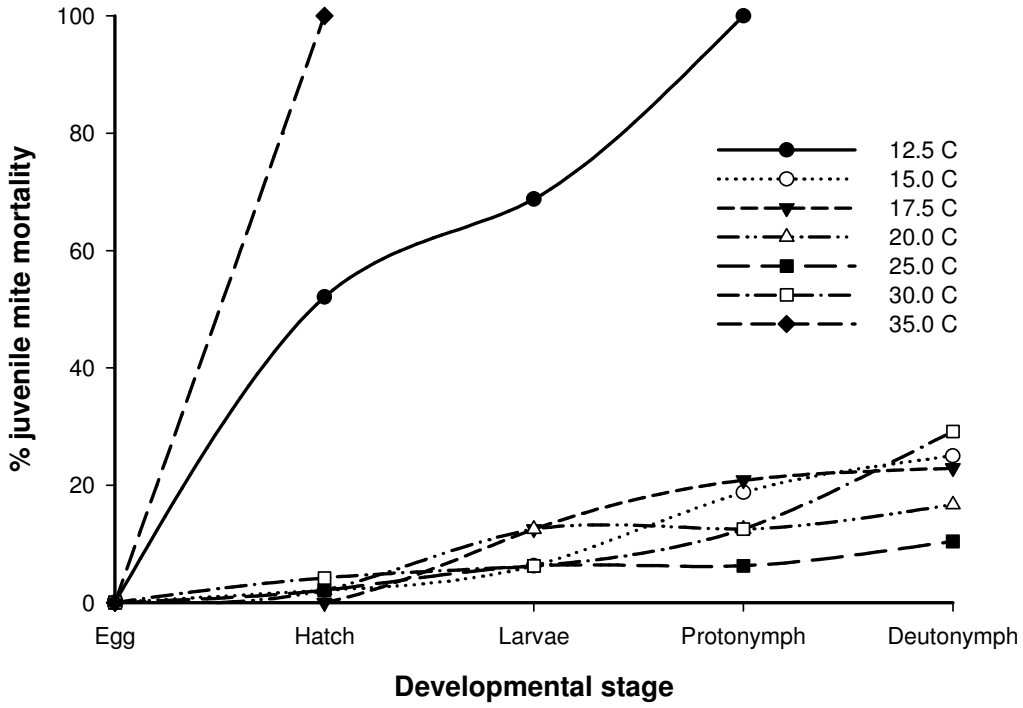


**Figure 2.1**. Percent mortality of immature *Typhlodromus pyri* during four developmental stages at seven constant temperatures.

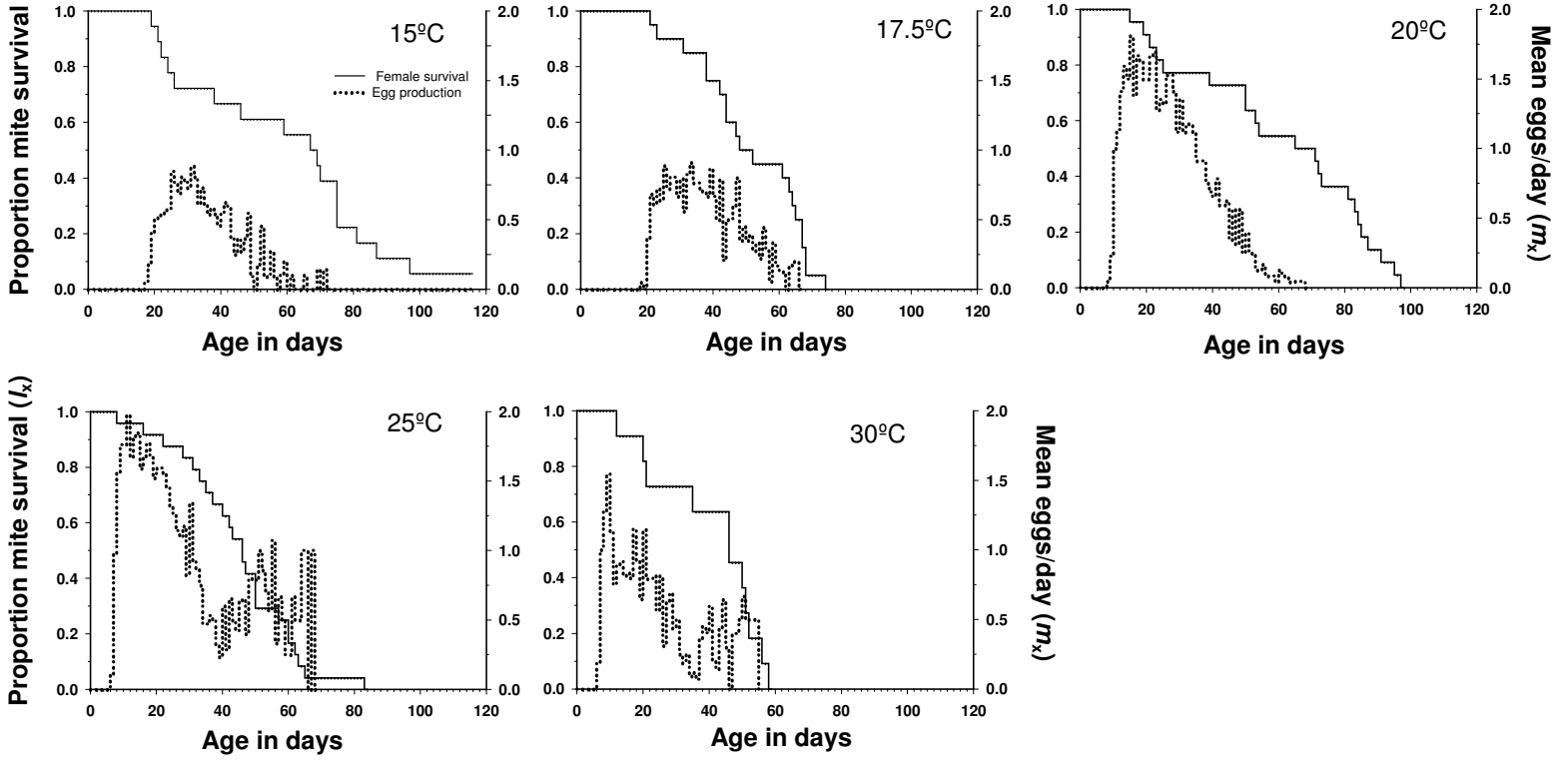


**Figure 2.2**. Mean proportion of *Typhlodromus pyri* female survival ($l_x$) (solid line) and mean egg production ($m_x$) (dotted line) at five different temperatures.

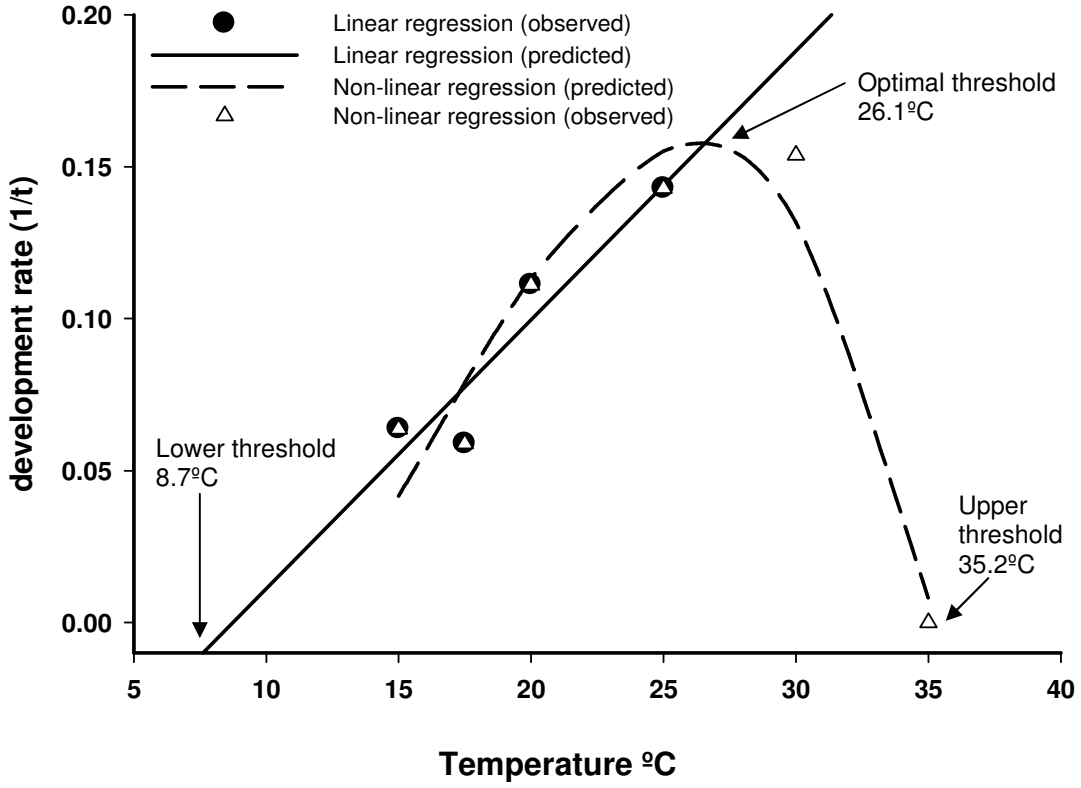


**Figure 2.3**. *Typhlodromus pyri* development thresholds and developmental rates as a function of temperature.

# Chapter III

## Impact of vineyard pesticides on a beneficial arthropod *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) in laboratory bioassays

**Abstract**

Laboratory bioassays were conducted to assess the effects of six vineyard pesticides on *Typhlodromus pyri* Scheuten, a key predator of grapevine rust mite *Calepitrimerus vitis* in Pacific coastal vineyards. Materials tested were 25% boscalid + 13% pyraclostrobin (Pristine), 40% myclobutanil (Rally), micronized sulfur (92% WP), 75% ethylene bisdithiocarbamate (Manzate), 91.2% paraffinic oil (JMS Stylet) and whey powder were applied at three different concentrations. Pesticide dilutions were directly sprayed onto *T. pyri* adult females and juveniles to determine effects on direct mortality and fecundity.

Five of the six pesticides tested resulted in less than 50% mortality to both life stages at all concentrations 7-d after treatment. Paraffinic oil treatments resulted in mortality rates greater than 50% in both adult and juvenile assays. Sub-lethal effects were more pronounced than acute pesticide toxicity, particularly in juvenile mite bioassays. Fecundity rates were lowered by exposure to sulfur and mancozeb treatments compared to the control in juvenile tests. The relative percent fecundity reduction for juvenile mites was highest when applying mancozeb (> 70%), sulfur (> 25%) or myclobutanil (> 20%). Adult mites displayed the greatest reductions in fecundity when treated with paraffinic oil (> 20%) or mancozeb (> 15%). Boscalid (+ pyraclostrobin) and whey displayed the least effect on fecundity across all bioassays. These results can be used to develop management guidelines in vineyard IPM practices to help protect and enhance predatory mite populations as biological control agents.

## Introduction

Predatory mites (Acari: Phytoseiidae) play an integral role in providing effective control of phytophagous mites in multiple annual and perennial cropping systems (Helle and Sabelis 1985, Croft et al. 2004). Beneficial phytoseiids are economically important in Pacific Northwest vineyards, fruit orchards and hop yards with regards to pest mite biological control (Croft and MacRae 1992, James et al. 2002, Prischmann et al. 2002).

Recently, many vineyards throughout the state of Oregon, parts of Western Washington and coastal California grape growing regions, such as Napa, have reported mite-related Short Shoot Syndrome (SSS) (Walton et al. 2007). This damage is associated with the presence of the grapevine rust mite, *Calepitrimerus vitis* Nalepa (Acari: Eriophyoidea), and susceptible overwintering bud tissues (Perez-Moreno and Moraza 1997, Bernard et al. 2005). This vineyard-specific pest feeds on developing buds during the early part of the season, resulting in stunted shoots, cupped leaves, shortened internodal growth and crop loss due to cluster necrosis. Pacific Northwest vineyards fungicide programs are implemented to control powdery mildew, *Erysiphe necator* Schw., throughout the growing season. It is hypothesized that these fungicide programs, especially programs which employ repeated sulfur applications, may contribute to increased pest mite populations due to detrimental effects on predatory mite populations and other beneficial arthropods (Hanna et al. 1997, James and Price 2002, James et al. 2002).

The predatory mite *Typhlodromus pyri* Scheuten has been documented as a predominant species in Oregon and Washington vineyards (Hadam et al. 1986,

Prischmann et al. 2002). Due to its abundance, wide distribution and ability to feed on a variety of food resources, *T. pyri* is a valuable and important predator in numerous agricultural systems (Dicke and deJong 1988, Camporese and Duso 1996, Zemek and Prenerova 1997). *T. pyri* feeds primarily on pest mites including *Tetranychus urticae*, *Panonychus ulmi* and *C. vitis* in orchards and vineyards worldwide (Duso et al. 1991, Hluchy and Pospisil 1991, Croft and MacRae 1992). A previous study found the population dynamics between *T. pyri* and *C. vitis* to be similar in vineyards indicating this predatory mite is integral in regulating grapevine rust mite densities (Perez-Moreno and Moraza 1997). Additionally, Hluchy and Pospisil (1991) reported that a inundative release of 10,000 *T. pyri* into a vineyard with extremely high *C. vitis* populations was able to stabilize and control pest mite populations by the following year without the application of miticides.

The predatory mite *T. pyri* is reported to be highly sensitive to many pesticides, including sulfur or mancozeb (Candolfi et al. 1999). This sensitivity to individual pesticides coupled with the rigorous fungicide programs for powdery mildew have led to concerns regarding side effects to non-target organisms from compounds often employed in Pacific Northwest vineyards. Many grape growers in these regions are heavily reliant on sulfur, synthetics and horticultural oils for control of powdery mildew during the growing season. Several field studies conducted in western U.S. vineyards have reported decreases in *T. pyri* and other predatory mite densities due to repeated pesticide applications, especially repeated exposure to sulfur (Calvert and Huffaker 1974, Hanna et al. 1997, James et al. 2002, Prischmann et al. 2005). However, it has also been reported

in laboratory studies that certain pesticides, sulfur included, are not directly toxic to *T. pyri* (Easterbrook 1984, Hassan et al. 1987). Based on these inconsistent results it was important to conduct laboratory bioassays as a first step to assess the impacts of commonly employed pesticides on *T. pyri* found in Oregon. The objective of our research was to test the lethal and sub-lethal effects of six synthetic and organic pesticides in controlled laboratory bioassays.

**Materials and Methods**

Rearing

Predatory mites were collected from a single vineyard in the Willamette Valley (Yamhill Co., Oregon) and maintained in a stock culture in the laboratory using the methods described by McMurtry and Scriven (1965) one year prior to initiating bioassay experiments. Plastic trays contained a 12 x 12 cm black plastic sheet over sponges placed in a water moat. The perimeter of the plastic square was lined with a dampened paper towel and a sticky barrier (The Tanglefoot Company, Grand Rapids, MI) applied along each edge to prevent mite escape. A cotton string (~10 cm) threaded through a hole in the plastic substrate provided moisture to the predatory mites. Clear plastic tents approximately 1-$cm^2$ were folded and placed onto the rearing arenas providing shelter and a place for oviposition.

Adult female and juvenile test units

The assay experimental unit consisted of a single bean (*Phaseolus vulgaris* cv. Roma) leaf placed adaxial side down on damp cotton in a sterilized glass Petri dish. A circular sticky barrier approximately 3- cm in diameter, was applied to each test leaf to

prevent predator mites from escaping. Both juvenile and adult female mites were directly exposed to a test pesticide and assessed over time to determine effects on mortality and fecundity.

Each test unit contained 15 juvenile mites (1-3 d old) or 15 mated adult female mites, transferred from the rearing trays. Experimental units were sprayed with test compounds and immediately placed under a fume hood for approximately 15 minutes to ventilate until the spray fully dried. Test units were held at 22 ± 2ºC, 55-65% RH and 16:8 (L:D) photoperiod. Predatory mites were fed daily with 20 - 40 spider mites, *T. urticae,* reared on bean plants in the laboratory.

Pesticides and spray application

Six compounds were tested at three concentrations: recommended field concentration (1×), 1.5×, and a 2× increase from recommended field concentration (Table 1). Each material spray was replicated five times per concentration on a test unit. The pesticides tested were whey powder (Tillamook Dairy, Tillamook, OR), 25% boscalid + 13% pyraclostrobin (Pristine, BASF, Research Triangle Park, NC), 40% myclobutanil (Rally 40W, Dow AgroSciences, Indianapolis, IN), micronized sulfur 92% WP (ProNatural, Wilbur-Ellis Co., Fresno, CA), 75% ethylene bisdithiocarbamate (mancozeb; Manzate, du Pont, Wilmington, DE) and 91.2% paraffinic oil (JMS Stylet-oil, JMS Flower Farms Inc., Vero Beach, FL). Water and unsprayed treatments were also included for each tested pesticide concentration. Fractions of water volume used in pesticide dilutions were based on 153 liter/ha application field concentrations. A Precision Potter Spray Tower (Burkard Mfg. Co Ltd, Ricksmansworth, UK) was used for

all laboratory spray applications and calibrated to deposit spray quantities of 2.0 ± 0.2 mg/cm$^2$ per 1-ml water at 6.8 psi with a 0.275 atomizer.

Assessment of lethal effects

*Adult females.* Mated adult females were assessed at 1, 3, 5, and 7 d after treatment to determine direct mortality. Visibly dead mites were counted and removed at each observation time. Adult predatory mites that perished in the sticky barrier were counted and removed at each observation date. Numbers of dead mites and mites perished in the sticky barrier were combined and included in the data analysis.

*Juveniles.* Mortality assessments for juvenile mites were conducted as recommended by IOBC working group guidelines (Blumel et al. 2000b). In order to collect juvenile nymph and protonymphs of similar age, eggs were transferred to a separate rearing tray four days prior to the start of each bioassay. Cohorts of mites were then collected on the day of the bioassay ensuring 1-3 d old individuals at the start of each experiment. Mortality of developing juveniles was assessed at 1, 3 and 7 d after treatment. Dead mites were counted and removed at each observation. Mites that perished in the sticky barrier were also counted, removed at each examination date and included in the final analysis. Mites surviving on day 7 were counted and male to female sex ratios were determined.

Assessment of sub-lethal effects

A*dult females*. Fecundity of gravid adult females was measured in order to assess sub-lethal effects for each treatment. Observations were conducted on days 1, 3, 5 and 7 after treatment. The number of eggs and adult females present on each leaf disc were

recorded on each observation day. Hatched larvae also were counted and recorded on each observation date and included in calculations. Eggs and larvae were removed after each examination date to ensure accurate counts on subsequent assessment days. Data was used to determine the number of eggs laid per day per female and to calculate the cumulative mean reproduction (CMR) per treatment.

*Juveniles*. Fecundity assessments of adult females from juvenile bioassays were performed as described above. However, assessments occurred on days 10, 12 and 14 after the final molt of juveniles to adult females. Data were analyzed to obtain the same parameters described in the previous section.

Data analysis

Cumulative lethal effects on juvenile and adult mites were analyzed as percent mortality. These values were calculated using the formula [(100-(number alive/15)*100)] (Beers et al. 2009). The unsprayed and water treatments were analyzed using ANOVA to compare mortality means and to determine if either treatment exceeded the accepted 20% mortality threshold (Blumel et al. 2000a). The analysis displayed no significant differences between the unsprayed control and water treatments in all bioassay tests, suggesting there was no water effect on mortality. As a result, the unsprayed treatment was chosen to represent the untreated control for adult and juvenile bioassays as mortality levels exceeded the 20% thresholds in certain water treatments.

Sub-lethal effects were measured as the impact on fecundity from the pesticide test compounds. The CMR per adult female was calculated over a 7-d period to quantify fecundity in both juvenile and adult sprayed bioassays. Juvenile bioassay CMR were

calculated using the IOBC formula guidelines based on eggs oviposited per day, whereas adult female bioassay fecundity was calculated using a modified version of the formula that adjusted for the different assessment days (Blumel et al. 2000b). The percent reduction in fecundity from the test compounds (*Rt*) relative to the untreated controls (*Rc*) were calculated as [1 - (*Rc*/*Rt* ) * 100] for adult and juvenile bioassays (Blumel et al. 2000b).

Mortality data were analyzed within each pesticide group for each individual day after treatment using analysis of variance (ANOVA) and employing Fisher's LSD procedure to compare treatment means (SAS Institute 2006). Data were transformed for statistical analysis using arcsine (sqrt ($x$ + 0.375) / 100) when ANOVA assumptions (normality and/or homogeneity of variance) were not met. Mean percent mite mortality presented in tables 1 and 2 is based on untransformed data. Fecundity data (CMR) were analyzed within and between each pesticide group using ANOVA and treatment means compared using Fisher's LSD procedure (SAS Institute 2006).

**Results**

Lethal effects

*Adult females*. Five of the six pesticides tested in adult female assays resulted in less than 50% direct mean mortality, at the three concentrations (Table 3.1). Only paraffinic oil treatments resulted in mortality levels greater than 50%. Overall, direct mortality ranged from 1.3 to 86.6% for all tested concentrations. Percent mortality displayed in paraffinic oil (1×; 15.0 g/liter, 2×; 30.0 g/liter) treatments were significantly greater then the untreated control at 1-d after treatment ($P$ = 0.002). Myclobutanil

treatments (1×; 0.30g/liter, 1.5×; 0.45g/liter), resulted in significantly greater toxicity compared to the untreated control ($P$ = 0.002), and percent mortalities, 28% and 19% respectively (7-d after treatment) were approximately 20% lower compared to the paraffinic oil treatments. Percent mite mortality at 7-d after treatment ranged from 20% (mancozeb) to 37% (whey powder) among the remaining five pesticides displaying less than 50% mortality at the recommended field concentration (1×). Direct mortality levels in adult females treated with sulfur were not significantly different from the untreated control ($P > 0.05$).

*Juveniles*. Juvenile mortality trends were similar to those observed in the adult assays. Percent mortality was less than 50% in the sulfur, whey powder, boscalid + pyraclostrobin, myclobutanil and mancozeb treatments at all tested concentrations (Table 3.2). Paraffinic oil-treated mites displayed significantly higher mortality at all concentrations compared to the untreated control ($P$ = 0.0001). At 3-d after treatment boscalid + pyraclostrobin resulted in significant differences ($P$ = 0.009) in juvenile mortality (1×; 0.94 g/liter, 2×; 1.89 g/liter) compared to the untreated control. Similar to the adult assays, cumulative mortality increased over time in all treatments. The paraffinic oil treatment caused the most mortality within the first 24-h after treatment. In contrast to the adult assays, juvenile untreated controls exceeded 20% mortality in the sulfur (22%) and myclobutanil (33%) tests by 7-d after treatment and therefore statistical comparisons were only acceptable up to 3-d after treatment due to established thresholds mentioned previously (Blumel et al. 2000a). Percent mortality, not including paraffinic oil, at 7-d after treatment ranged from 21 (myclobutanil) to 39% (mancozeb) at the 1×

concentration; from 21 (boscalid) to 49% (mancozeb) at the 1.5× concentrations; and from 23 (whey) to 37% (sulfur and mancozeb) at the 2× concentrations.

Sub-lethal effects

*Adult females.* No differences in female fecundity were detected within treatments at the three concentrations compared to their respective untreated controls (Table 3.3). In addition there were no significant differences detected in CMR between comparable concentrations of the six pesticide compounds tested ($P > 0.05$). Mancozeb and paraffinic oil treatments however displayed greater than 20% reduction in predatory mite fecundity relative to the untreated control. Paraffinic oil (1× and 2× concentration) treated mites displayed the lowest CMR and highest percent fecundity reductions compared to all other treatments. The remaining pesticide compounds tested did not exceed greater than 18% fecundity reductions relative to the untreated control at the three concentrations. Myclobutanil treatments resulted in the least effect on predatory mite CMR potential with a zero percent reduction in fecundity across all tested concentrations. Whey powder and sulfur-treated mites exhibited < 20% percent fecundity reductions.

*Juveniles* (young adults at day 7-14). Differences in fecundity were found between pesticide concentrations within each treatment compared to the untreated control (Table 3.4). The CMR ranged on average from 0.7 to 5.8 eggs per female across the pesticide concentrations tested. Overall percent fecundity reduction relative to the untreated controls ranged from 0 to 83%. Mancozeb and sulfur treatments both showed significantly less CMR potential compared to their respective untreated controls ($P < 0.05$). Sulfur treated mites exhibited a significantly lower CMR and > 50% reduction in

fecundity at the 1.5× concentration ($P$ = 0.031). Mancozeb and whey treatments displayed the highest and lowest percent fecundity reductions at > 70% and < 15%, respectively. Whey powder, boscalid + pyraclostrobin, and myclobutanil spray applications did not result in significantly lower CMR compared to their respective untreated control. Percent fecundity reductions less than 15% were seen in mites treated with whey and boscalid. Mancozeb, sulfur and myclobutanil applications resulted in a wide range of fecundity reductions of > 15% and ranging up to 80%. We were unable to assess sub-lethal effects for paraffinic oil treatments (all concentrations) in juvenile bioassays at day 7-14 due to high mortality during the initial seven days after treatment (Table 3.2).

No significant differences were detected between pesticide treatments delivered in comparable 1× concentrations ($P$ = 0.911), however there were significant differences between treatments at the 1.5× and 2× concentrations (Table 3.4). Whey powder had significantly greater CMR per adult female ($F$ = 7.09, df = 16, $P$ < 0.001) compared to all other pesticides at the 1.5× concentrations. At the 2× concentration, whey powder showed significantly greater CMR compared to boscalid + pyraclostrobin or mancozeb, but not different from sulfur or myclobutanil ($F$ = 6.00, df = 16, $P$ = 0.003).

**Discussion**

Overall five of the six pesticides resulted in less than 50% direct contact mortality to both adult and juvenile *T. pyri*. Paraffinic oil treated mites displayed the most consistent significant differences from the untreated control and resulted in mortality greater than 50% for both adult and juvenile predatory mites at the three concentrations

tested. Our statistical analysis revealed smaller differences in percent mortality between concentrations and were difficult to detect due to unexplained variability caused by underlying sources of error such as natural variation in insect populations and variability in human operators. The low mortality of mites exposed to the five other pesticides, especially at recommended field concentrations, suggest that other factors may be involved with decreasing populations of *T. pyri* observed in agricultural production. Although acute pesticide toxicity is a critical issue, additional factors such as sub-lethal effects, pesticide repellency, dispersal, repeated pesticide exposure, application timing and food availability are potential contributors to reduced field populations.

In this study, sub-lethal effects measured as fecundity were more pronounced in juvenile bioassays with significant decreases in CMR evident when applying sulfur and mancozeb. Adult female assays did not result in significant reductions in fecundity for the tested compounds, however, paraffinic oil and mancozeb applications reduced fecundity by more than 20% compared to the untreated control. Our bioassays indicate that sulfur, mancozeb, and paraffinic oil have the ability to reduce the average number of eggs oviposited by *T. pyri* and potentially can affect population growth dynamics in the vineyard. As an illustration, one study reported a decrease in population growth rate of a predatory mite, *Iphiseiodes zuluagai*, with increasing pesticide concentration (Teodoro et al. 2005). Population extinction was reached in 7 days when exposed to the $LC_{25}$ concentration of wettable sulfur (estimated 0.64 g a.i./ liter). In addition, other studies have documented similar sub-lethal effects on predatory mites from pesticides (Alston and Thomson 2004, Auger et al. 2004).

Pesticide repellency is another factor that can potentially alter the emigration behavior of predatory mites. A pesticide may act as a repellent causing movement off of a leaf surface or from the plant canopy. Repellency effects were reported for mancozeb and low dose pyrethroid applications on *T. pyri* where an increased run-off rate from a leaf surface illustrated a change in behavior away from the pesticide (Bowie et al. 1999, Blumel et al. 2000a, Bowie et al. 2001). The phytoseiid *Galendromus occidentalis* exhibited repellent behavior from dry flowable sulfur where one half of the leaf disc was treated and the other half left untreated (Beers et al. 2009). Results reported that adult females were on the untreated leaf disc section at 4.3× higher levels compared to the treated half. Observations in our bioassays show a slight, although insignificant, increase in the number of *T. pyri* that perished in the sticky barrier in sulfur treatments compared to the untreated control, suggesting attempted movement away from sulfur residues. The repellency effect of specific pesticides may result in decreased predatory mite densities due to behavioral changes as opposed to direct or indirect mortality factors.

Whey powder has recently gained attention for its potential use as an effective powdery mildew fungicide in grapes (Crisp et al. 2006, Gadino 2007). Whey powder is a food grade by-product and can be applied in organic agricultural systems with minimal environmental and health risks. Nominal research and literature exist for this novel pesticide, particularly with regards to impacts on beneficial arthropods. The bioassays conducted in this study resulted in low mortality rates for adults and juvenile predatory mites. Whey powder treatments had no negative effect on predatory mite fecundity and

CMR. These results suggest that whey powder applications will pose no significant impacts on direct mortality and fecundity for predatory mite populations in the field.

Boscalid + pyraclostrobin and myclobutanil, resulted in low mortality levels when tested on *G. occidentalis* in two separate research studies (Alston and Thomson 2004, Bernard et al. 2004b). Direct sprays of boscalid + pyraclostrobin applied to juvenile stage *G. occidentalis* and *Euseius victoriensis* displayed no significant mortality (< 22%) or sub-lethal effects (< 18% reduction in fecundity) (Bernard et al. 2004b). The sub-lethal effects on juvenile mites treated with boscalid in our bioassays displayed no significant effects on fecundity with results comparable to Bernard et al. (2004). The direct effects displayed in our bioassays however resulted in higher mortality rate than those found in the previous study. Although this pesticide showed greater mortality on *T. pyri*, it is important to note that levels remained below 50%.

Our adult female bioassays on *T. pyri* treated with myclobutanil found similar results to those reported on *G. occidentalis* by Alston and Thomson (2004) showing relatively low mortality rates and non-significant impacts on fecundity. Overall, these results suggest that both synthetic compounds are likely non-toxic to *T. pyri* with minor sub-lethal effects.

Sulfur is often implicated as a major culprit in the reduction of predatory mite populations in vineyards, hop yards and fruit orchards (Sanford 1967, Calvert and Huffaker 1974, Hanna et al. 1997, James et al. 2002). Inconsistent results however have been reported regarding sulfur toxicity to *T. pyri* in laboratory assays. Micronized sulfur (1.5% A.I.) was reported as harmless (10-40% mortality) to adult females exposed to

residue and assessed over a seven day trial (Zacharda and Hluchý 1991) and were comparable to results displayed in our adult mite bioassays. In addition, micronized sulfur has been classified as non-toxic in several previous studies assessing mortality of both adult and juvenile *T. pyri* (Mathys 1958, Watve and Lienk 1975, Overmeer and van Zon 1981, Hassan et al. 1987). In contrast to these findings, results from a different laboratory study reported sulfur (0.25% A.I.) as toxic to adult female *T. pyri* reporting 90% mortality compared to 6% in the control at seven days after treatment (van de Vrie 1962).

Additional research has reported immature stages of *T. pyri* and other phytoseiids as highly sensitive to sulfur exposure (50-81% mortality) and have concluded that sulfur is harmful to juvenile mites (Overmeer and van Zon 1981, 1982, Beers et al. 2009). Juvenile mites in our bioassays did show levels of sensitivity to sulfur with mortality ranging from 32 to 42% however these values are lower than those reported in other studies that conducted similar bioassays. The method of application (i.e. residue versus direct spray), concentration and susceptible life stage may explain some of the variability in predatory mite response to sulfur.

Another explanation for the variability found in our results may be related to the volatilization of sulfur at higher temperatures. Bioassays testing the effects of temperature and humidity on sulfur sprayed spider mites reported increased egg and adult female mortality with increasing temperatures (> 27.5ºC) and relative humidity above 75% (Auger et al. 2003). Our bioassays were conducted at lower temperatures (22 ± 2ºC)

which may partially explain the variable mortality levels detected in both juvenile and adult female assays.

Results from our bioassays suggest that sulfur is relatively non-toxic toward both stages of *T. pyri* over a 7 to 14 day period. Predatory mites exposed to repeated applications of micronized sulfur could potentially result in greater mortality levels, but this remains to be investigated. Negative sub-lethal effects found in juvenile bioassays and the potential for pesticide repellency previously discussed may contribute to the suppression of predatory mite field populations. Additional applications of sulfur to control pest mite populations in Oregon vineyards, thereby decreasing the available prey resource, also may play a role in reducing predatory mite populations.

Mancozeb, a broad-spectrum pesticide, is primarily applied to control bunch rot but not commonly used in Pacific Northwest vineyards. It is however important to test the effects of mancozeb on the strain of *T. pyri* found in Oregon vineyards as this compound has been reported as moderately to highly toxic to this species in multiple lab and field experiments (Hassan et al. 1987, Blumel et al. 2000a, Auger et al. 2004, Hardman et al. 2006). Results from our bioassays indicate potential negative impacts from mancozeb applications, especially toward juvenile mites. Fecundity levels in the immature bioassays were significantly reduced in mancozeb treatments compared to the untreated control at the two higher concentrations and are consistent with previous research that reported acute effects on predatory mite reproductive potential (Angeli and Ioriatti 1994, Blumel et al. 2000a, Auger et al. 2004).

Paraffinic oil, a petroleum based product, is often used in vineyards for early season powdery mildew control and in certain cases for pest mite control. Horticultural oils have also been utilized for mealybug and leafhopper control displaying its toxic effects on various arthropod species. Petroleum oils were reported in other studies as low to moderately toxic to adult female *T. pyri* in slide-dip residue tests; field evidence has indicated a range of toxicity to predatory mites depending on seasonal spray timing (Bartlett 1964, Watve and Lienk 1975). Our bioassays displayed acute toxicity when mites were directly exposed to paraffinic oil, indicating an increase in mortality when direct contact occurs versus residue contact. These findings suggest that when predatory mites are able to find refuge (i.e. foliage, cracks in vine cane) and avoid direct contact with oil applications the negative effects can be mitigated. This could assist in defining optimal application timing, such as avoiding early season sprays when the canopy is open and predatory mites are more vulnerable, helping to enhance integrated pest management strategies.

Fully understanding the impact of pesticides on *T. pyri* and other beneficial organisms in the field is difficult to extrapolate from laboratory bioassays alone. Accurately estimating the lethal and sub-lethal effects of a pesticide from lab results to field applications continues to be a subject for debate. Studies that have documented high levels of pesticide toxicity in laboratory bioassays but found lower toxicity in the field, contend that lab tests designed to approximate the worst case scenario will likely over-estimate the negative effects of a pesticide (Blumel et al. 2000a). Three recommendations employed in our methodology to handle over-estimation include using

1) a natural substrate to provide refuge, 2) testing the least susceptible stage, and 3) testing a range of concentrations.

Knowledge regarding the lethal and sub-lethal impacts of commonly applied pesticides is integral in developing sustainable vineyard pest management programs. Results from these bioassays will assist Pacific vineyard producers in forming management plans that utilize lower risk pesticides in carefully designed rotation schedules. Key findings from our bioassays indicate that applications of horticultural oils and sulfur should be limited in the rotation plan for seasonal powdery mildew control. For example, oil applications should be avoided when vines are in early season growth stages and when the canopies are too open to provide adequate refuge for active predatory mites. These two materials however will remain important management tools, as they are effective and inexpensive. The negligible impact of whey powder on *T. pyri* is another significant discovery and highlights the value of whey powder as an alternative material for powdery mildew control, especially in organic systems. Continued research is needed to test the additive effects of these compounds on predatory mite and beneficial insect populations in large-scale vineyard field experiments. Future research should include studying behavioral effects such as dispersal and repellency caused by specific pesticides, particularly sulfur, to beneficial arthropods and to further understand the impacts on vineyard field populations.

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**Table 3.1** Direct mortality effects of six pesticides on the adult predator mite, *Typhlodromus pyri*.

| Day after treatment | Treatment[b] Concn (g/liter) | Mean % mortality (± SEM)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Whey | | | Water | Control | *F, P* |
| | | 12.0 (1×) | 18.0 (1.5×) | 24.0 (2×) | | | |
| 1 d | | 14.7 ± 8.0a | 9.3 ± 4.9a | 4.0 ± 1.6a | 3.6 ± 1.5a | 0.8 ± 0.5a | 1.13, 0.376 |
| 3 d | | 24.0 ± 9.1a | 14.7 ± 5.3a | 9.3 ± 5.0a | 11.1 ± 2.5a | 5.8 ± 2.4a | 1.23, 0.334 |
| 5 d | | 33.3 ± 7.9a | 20.0 ± 6.7a | 17.3 ± 6.9a | 14.2 ± 3.5a | 11.1 ± 4.2a | 1.98, 0.145 |
| 7 d | | 37.3 ± 9.3a | 24.0 ± 7.5a | 26.7 ± 8.7a | 20.0 ± 4.0a | 15.6 ± 3.9a | 0.95, 0.456 |
| | | Boscalid + pyraclostrobin | | | Water | Control | *F, P* |
| | | 0.94 (1×) | 1.42 (1.5×) | 1.89 (2×) | | | |
| 1 d | | 2.7 ± 3.7a | 9.3 ± 3.4a | 10.7 ± 6.9a | 2.0 ± 1.3a | 2.0 ± 1.3a | 1.19, 0.352 |
| 3 d | | 10.7 ± 3.4a | 20.0 ± 6.3a | 14.7 ± 8.0a | 7.3 ± 2.7a | 10.0 ± 3.5a | 0.93, 0.467 |
| 5 d | | 14.7 ± 3.9a | 25.3 ± 4.9a | 21.3 ± 9.7a | 11.3 ± 2.5a | 13.3 ± 2 .8a | 0.99, 0.441 |
| 7 d | | 24.0 ± 6.5a | 33.3 ± 3.7a | 25.3 ± 9.3a | 16.7 ± 3.0a | 17.3 ± 2.7a | 1.14, 0.370 |
| | | Myclobutanil | | | Water | Control | *F, P* |
| | | 0.30 (1×) | 0.45 (1.5×) | 0.60 (2×) | | | |
| 1 d | | 6.7 ± 3.0a | 12.0 ± 4.9a | 2.7 ± 1.6b | 0b | 0b | 5.18, 0.007 |
| 3 d | | 17.3 ± 4.5a | 16.0± 3.4a | 5.3 ± 2.5b | 2.7 ± 1.6b | 4.0 ± 2.7b | 4.11, 0.017 |
| 5 d | | 20.0 ± 4.2a | 17.3 ± 4.5a | 9.3 ± 2.7a | 5.3 ± 2.5a | 8.0 ± 3.9a | 2.82, 0.059 |
| 7 d | | 28.0 ± 2.5a | 18.7 ± 5.0a | 12.0 ± 1.3b | 6.7 ± 2.1b | 13.3 ± 2.1b | 6.42, 0.002 |
| | | Sulfur | | | Water | Control | *F, P* |
| | | 5.40 (1×) | 7.20 (1.5×) | 9.50 (2×) | | | |
| 1 d | | 8.0 ± 3.9a | 14.7 ± 7.1a | 12.0 ± 6.1a | 4.0 ± 1.8a | 2.2 ± 1.0a | 1.05, 0.411 |
| 3 d | | 18.7 ± 7.4a | 20.0 ± 7.6a | 13.3 ± 5.6a | 13.3 ± 2.5a | 8.0 ± 1.5a | 0.68, 0.615 |
| 5 d | | 26.7 ± 7.3a | 22.7 ± 7.5a | 14.7 ± 5.3a | 16.4 ± 2.9a | 12.9 ± 3.3a | 0.85, 0.511 |
| 7 d | | 33.3 ± 9.7a | 25.3 ± 6.5a | 25.3 ± 3.9a | 22.2 ± 2.9a | 17.3 ± 2.2a | 0.90, 0.483 |

**Table 3.1** Direct mortality effects of six pesticides on the adult predator mite, *Typhlodromus pyri*.

| | Treatment[b] | Mean % mortality (± SEM)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mancozeb | | | Water | Control | *F, P* |
| | Concn | 2.40 (1×) | 3.60 (1.5×) | 4.80 (2×) | | | |
| 1 d | (g/liter) | 1.3 ± 1.3a | 2.7 ± 2.7a | 1.3 ± 1.3a | 2.7 ± 1.2a | 1.3 ± 1.3a | 0.28, 0.884 |
| 3 d | | 8.0 ± 3.3a | 8.0 ± 3.9a | 5.3 ± 2.4a | 9.3 ± 2.4a | 2.0 ± 1.3a | 0.94, 0.465 |
| 5 d | | 14.7 ± 3.9a | 16.0 ± 5.4a | 13.3 ± 2.1a | 12.7 ± 2.4a | 7.3 ± 4.3a | 0.87, 0.500 |
| 7 d | | 20.0 ± 7.6a | 18.7 ± 5.3a | 22.7 ± 4.5a | 16.7 ± 2.8a | 12.0 ± 4.3a | 0.76, 0.564 |
| | | Paraffinic oil[c] | | | Water | Control | *F, P* |
| | | 15.0 (1×) | 22.5 (1.5×) | 30.0 (2×) | | | |
| 1 d | | 52.0 ± 17.5ab | 34.7 ± 19.0ac | 80.0 ± 7.3b | 8.0 ± 2.5c | 1.3 ± 1.3c | 6.69, 0.002 |
| 3 d | | 53.3 ± 16.7ab | 41.3 ± 16.7ac | 82.6 ± 7.2b | 10.7 ± 3.4c | 10.7 ± 3.4c | 6.38, 0.002 |
| 5 d | | 54.6 ± 16.1ab | 46.7 ± 14.4ac | 85.3 ± 5.7b | 14.7 ± 4.4c | 13.3 ± 2.1c | 6.92, 0.001 |
| 7 d | | 56.0 ± 15.3ab | 47.7 ± 14.4ad | 86.6 ± 5.9b | 14.7 ± 4.4c | 20.0 ± 4.2cd | 7.27, 0.001 |

Treatment means across columns (within treatment by each day) that are not followed by the same letter are significantly different ($P \leq 0.05$, Fishers LSD pairwise comparison). For all analyses, df = 4,4,16.

[a]Data transformed [arcsin (sqrt ($x$ + 0.375)/100)] for statistical analysis due to unequal variances and non-normality.

[b]Pesticides tested were formulated commercial products.

[c]Concentration is vol:vol (ml/liter).

**Table 3.2.** Direct mortality effects of six pesticides on the juvenile (0-3 d old) predatory mite, *Typhlodromus pyri*.

| Day after treatment | Treatment[b] | Mean % mortality (± SEM)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Whey | | | Water | Control | *F, P* |
| | Concn | 12.0 (1×) | 18.0 (1.5×) | 24.0 (2×) | | | |
| 1 d | (g/liter) | 17.3 ± 6.2a | 20.0 ± 2.1a | 12.0 ± 6.1a | 14.2 ± 3.0a | 6.2 ± 3.3a | 1.49, 0.251 |
| 3 d | | 21.3 ± 7.7a | 28.0 ± 4.9a | 16.0 ± 5.4a | 17.3 ± 2.9a | 15.1 ± 4.5a | 0.74, 0.575 |
| 7 d | | 29.3 ± 7.8a | 36.0 ± 5.4a | 22.7 ± 4.5a | 24.4 ± 3.7a | 20.0 ± 4.2a | 1.49, 0.249 |
| | | Boscalid + pyraclostrobin | | | Water | Control | *F, P* |
| | | 0.94 (1×) | 1.42 (1.5×) | 1.89 (2×) | | | |
| 1 d | | 12.0 ± 3.9a | 5.3 ± 2.5a | 16.0 ± 5.0a | 10.0 ± 3.2a | 2.7 ± 1.2a | 2.05, 0.134 |
| 3 d | | 26.7 ± 6.9a | 9.3 ± 3.4b | 22.7 ± 4.9a | 16.7 ± 3.5ab | 8.0 ± 3.3b | 4.80, 0.009 |
| 7 d | | 37.3 ± 8.8a | 21.3 ± 3.9a | 29.3 ± 5.0a | 26.0 ± 3.7a | 19.3 ± 4.3a | 2.93, 0.053 |
| | | Myclobutanil | | | Water | Control | *F, P* |
| | | 0.30 (1×) | 0.45 (1.5×) | 0.60 (2×) | | | |
| 1 d | | 4.0 ± 1.6a | 16.0 ± 5.0a | 17.3 ± 3.4a | 18.7 ± 4.9a | 10.7 ± 4.0a | 2.09. 0.129 |
| 3 d | | 12.0 ± 4.4a | 18.6 ± 3.9a | 20.0 ± 2.1a | 22.7 ± 6.2a | 20.0 ± 4.7a | 0.68, 0.613 |
| 7 d | | 21.3 ± 3.9a | 30.7 ± 6.9a | 36.0 ± 3.4a | 29.3 ± 5.4a | 33.3 ± 6.3a | 1.08, 0.398 |
| | | Sulfur | | | Water | Control | *F, P* |
| | | 5.40 (1×) | 7.20 (1.5×) | 9.50 (2×) | | | |
| 1 d | | 16.0 ± 5.0a | 21.3 ± 6.8a | 25.3 ± 6.8a | 12.0 ± 3.8a | 5.3 ± 2.3a | 1.84, 0.169 |
| 3 d | | 24.0 ± 5.0a | 26.7 ± 5.6a | 30.7 ± 6.7a | 17.8 ± 3.4a | 13.3 ± 3.1a | 2.02, 0.138 |
| 7 d | | 32.0 ± 6.5a | 41.3 ± 3.3a | 37.3 ± 8.6a | 24.0 ± 2.7a | 22.2 ± 2.7a | 2.01, 0.141 |
| | | Mancozeb | | | Water | Control | *F, P* |
| | | 2.40 (1×) | 3.60 (1.5×) | 4.80 (2×) | | | |
| 1 d | | 25.3 ± 11.3a | 13.3 ± 3.7a | 22.6 ± 3.7a | 16.0 ± 2.4a | 6.0 ± 2.4a | 1.56, 0.231 |
| 3 d | | 29.3 ± 11.6a | 37.3 ± 10.4a | 32.0 ± 16.5a | 18.0 ± 2.5a | 12.0 ± 3.1a | 1.47, 0.255 |
| 7 d | | 38.7 ± 11.4a | 49.3 ± 14.0a | 37.3 ± 16.1a | 24.7 ± 5.0a | 19.3 ± 2.9a | 1.74, 0.189 |

**Table 3.2.** Direct mortality effects of six pesticides on the juvenile (0-3 d old) predatory mite, *Typhlodromus pyri* (Continued).

| | | Mean % mortality (± SEM)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | Treatment[b] | Paraffinic oil[c] | | | Water | Control | *F, P* |
| | Concn | 15.0 (1×) | 22.5 (1.5×) | 30.0 (2×) | | | |
| 1 d | (g/liter) | 82.7 ± 7.8a | 93.3 ± 6.7ab | 100 ± 0b | 12.0 ± 3.3c | 8.0 ± 3.3c | 36.2, 0.000 |
| 3 d | | 89.3 ± 5.4a | 94.7 ± 5.3a | 100 ± 0a | 9.3 ± 3.9b | 9.3 ± 4.9b | 40.8, 0.000 |
| 7 d | | 93.3 ± 3.7a | 96.0 ± 4.0a | 100 ± 0a | 14.6 ± 6.7b | 13.3 ± 5.9b | 38.0, 0.000 |

Treatment means across columns (within treatment by each day) that are not followed by the same letter are significantly different ($P \leq 0.05$, Fishers LSD pairwise comparison). For all analyses, df = 4, 4, 16.

[a]Data transformed [arcsin (sqrt ($x$ + 0.375)/100)] for statistical analysis due to unequal variances and non-normality.

[b]Pesticides tested were formulated commercial products.

[c]Concentration is vol:vol (ml/liter).

**Table 3.3.** Sub-lethal (fecundity) effects of six pesticides on the adult female predatory mite, *Typhlodromus pyri*.

| Treatment [a] | Concn (g/liter) | Cumulative mean reproduction (± SEM)[b] | % fecundity reduction[c] | *F, P* |
|---|---|---|---|---|
| Whey | 12.0 (1×) | 4.3 ± 0.6a | 17.9 | |
| | 18.0 (1.5×) | 5.5 ± 0.3a | 0.0 | |
| | 24.0 (2×) | 5.0 ± 0.8a | 3.7 | |
| Water | - | 5.3 ± 0.4a | 0.0 | |
| Untreated Control | - | 5.2 ± 0.5a | - | 1.12, 0.378 |
| Boscalid + | 0.94 (1×) | 4.3 ± 0.4a | 16.0 | |
| pyraclostrobin | 1.42 (1.5×) | 4.7 ± 0.4a | 8.4 | |
| | 1.89 (2×) | 5.4 ± 0.4a | 0.0 | |
| Water | - | 5.1 ± 0.2a | 1.9 | |
| Untreated Control | - | 5.2 ± 0.2a | - | 2.58, 0.077 |
| Myclobutanil | 0.30 (1×) | 6.1 ± 0.5a | 0.0 | |
| | 0.45 (1.5×) | 6.0 ± 0.5a | 0.0 | |
| | 0.60 (2×) | 5.5 ± 0.6a | 0.0 | |
| Water | - | 6.0 ± 0.4a | 0.0 | |
| Untreated Control | - | 5.2 ± 0.5a | - | 1.11, 0.381 |
| Sulfur | 5.40 (1×) | 4.5 ± 0.6a | 3.8 | |
| | 7.20 (1.5×) | 5.1 ± 0.4a | 0.0 | |
| | 9.50 (2×) | 5.5 ± 0.4a | 0.0 | |
| Water | - | 5.3 ± 0.3a | 0.0 | |
| Untreated Control | - | 4.7 ± 0.6a | - | 0.96, 0.455 |
| Mancozeb | 2.40 (1×) | 6.0 ± 0.7a | 0.0 | |
| | 3.60 (1.5×) | 4.6 ± 0.3a | 16.8 | |
| | 4.80 (2×) | 4.4 ± 0.4a | 20.5 | |
| Water | - | 5.0 ± 0.2a | 10.1 | |
| Untreated Control | - | 5.6 ± 0.2a | - | 2.64, 0.071 |
| Paraffinic oil[d] | 15.0 (1×) | 3.8 ± 1.1a | 27.3 | |
| | 22.5 (1.5×) | 4.8 ± 0.5a | 7.6 | |
| | 30.0 (2×) | 4.0 ± 1.2a | 23.3 | |
| Water | - | 5.5 ± 0.2a | 0.0 | |
| Untreated Control | - | 5.2 ± 0.2a | - | 1.08, 0.398 |

Treatment means within columns not followed by the same letter are significantly different ($P \leq 0.05$, Fishers LSD pairwise comparison). For all analyses, df = 4, 4, 16.

[a]Pesticides tested were formulated commercial products.

[b]Cumulative mean reproduction is calculated to determine the average number of eggs per female over the seven day trial period.

[c]Percent fecundity reduction of treatment relative to the untreated control is calculated as (1- *Rt*/*Rc*) * 100, where R represents the absolute values of the treatments and control.

[d]Concentration is vol:vol (ml/liter).

**Table 3.4.** Sub-lethal (fecundity) effects of five pesticides on the juvenile predatory mite, *Typhlodromus pyri*.

| Treatment[a,b] | Concn (g/liter) | Cumulative mean reproduction (± SEM)[c] | % fecundity reduction[d] | *F, P* |
|---|---|---|---|---|
| Whey | 12.0 (1×) | 3.4 ± 0.4a | 15.2 | |
| | 18.0 (1.5×) | 5.8 ± 0.6b | 0.0 | |
| | 24.0 (2×) | 4.8 ± 0.7a | 0.0 | |
| Water | - | 3.7 ± 0.2a | 6.5 | |
| Untreated Control | - | 4.0 ± 0.1a | - | 4.55, 0.011 |
| Boscalid + | 0.94 (1×) | 3.6 ± 1.0a | 2.1 | |
| pyraclostrobin | 1.42 (1.5×) | 3.4 ± 0.5a | 8.7 | |
| | 1.89 (2×) | 2.8 ± 0.6a | 23.3 | |
| Water | - | 4.2 ± 0.5a | 0.0 | |
| Untreated Control | - | 3.7 ± 0.5a | - | 0.84, 0.515 |
| Myclobutanil | 0.30 (1×) | 3.7 ± 0.6a | 24.7 | |
| | 0.45 (1.5×) | 2.7 ± 0.9a | 45.7 | |
| | 0.60 (2×) | 4.1 ± 0.7a | 18.2 | |
| Water | - | 4.6 ± 1.0a | 8.6 | |
| Untreated Control | - | 5.0 ± 0.9a | - | 1.24, 0.331 |
| Sulfur | 5.40 (1×) | 3.0 ± 0.6ab | 28.4 | |
| | 7.20 (1.5×) | 2.0 ± 0.5b | 51.2 | |
| | 9.50 (2×) | 3.4 ± 0.2a | 17.8 | |
| Water | - | 3.5 ± 0.4a | 14.9 | |
| Untreated Control | - | 4.1 ± 0.3a | - | 3.47, 0.031 |
| Mancozeb | 2.40 (1×) | 3.4 ± 0.3a | 21.8 | |
| | 3.60 (1.5×) | 0.7 ± 0.6b | 83.2 | |
| | 4.80 (2×) | 1.3 ± 0.9b | 70.0 | |
| Water | - | 3.9 ± 0.5a | 9.7 | |
| Untreated Control | - | 4.3 ± 0.2a | - | 8.17, 0.000 |
| Paraffinic oil[e] | 15.0 (1×) | - | - | |
| | 22.5 (1.5×) | - | - | |
| | 30.0 (2×) | - | - | |

Treatment means within columns not followed by the same letter are significantly different ($P \leq 0.05$, Fishers LSD pairwise comparison). For all analyses, df = 4, 4, 16.

[a]Assessments were carried out from day 8 to day 14 once treated juveniles molted to adult stage.

[b]Pesticides tested were formulated commercial products.

[c]Cumulative mean reproduction is calculated to determine the average number of eggs per female over the seven day trial period.

[d]Percent fecundity reduction of treatment relative to the untreated control is calculated as $(1- Rt/Rc) * 100$, where R represents the absolute values of the treatments and control.

[e]Concentration is vol:vol (ml/liter). Fecundity values were not available for this treatment due to 100% mortality of female mites during initial 7 days of trial.

# Chapter IV

## Olfactory response of a predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae) to methyl salicylate in laboratory bioassays

**Abstract**

The response of *Typhlodromus pyri*, a key predator of grapevine rust mite, *Calepitrimerus vitis*, to MeSA was tested using a Y-tube olfactometer in laboratory bioassays. Six doses ranging from 0.002 to 200 µg of diluted MeSA were tested. Significantly higher proportions of *T. pyri* preferred MeSA at doses 0.02, 0.2, and 20 µg. No differences were detected at the highest (200 µg) and lowest (0.002 µg) doses. The response to dose was not significant, suggesting there is no relationship between dose quantity and *T. pyri* response. Subsequent analysis confirmed no correlation between dose level and mite response, however, an overall positive attraction of *T. pyri* to MeSA was found. Results indicate that MeSA may be applied to attract and retain predatory arthropod populations in vineyards to enhance biological control of pest mites.

## Introduction

Plants defend themselves from damaging herbivore attack through direct and indirect defense strategies (Dicke and Sabelis 1988a, Vet and Dicke 1992). One indirect defense mechanism involves the release of herbivore induced plant volatiles (HIPV's), believed to elicit top-down control by signaling to natural enemies (Price 1981, Dicke et al. 1990b).

The phenolic compound methyl salicylate (MeSA) has been identified as an important HIPV and is present in more than 13 different crop plants, including grapevines when infested with the spider mite, *Tetranychus urticae* (van den Boom et al. 2004). MeSA has also been detected in other HIPV blends, such as cabbage fed on by caterpillars, *Pieris* spp (Geervliet et al. 1997), and hops fed on by hop aphid, *Phorodon humuli* Schrank (Campbell et al. 1993).

The attraction of MeSA to various phytoseiid mites and natural enemies has also been documented (Dicke et al. 1990c, Drukker et al. 2000a, Shimoda et al. 2002, De Boer and Dicke 2004b, Zhu and Park 2005). *Typhlodromus pyri* is the predominant predatory mite in Pacific Northwest vineyards and considered an important natural enemy of phytophagous mites, including the grapevine rust mite *Calepitrimerus vitis* (Hadam et al. 1986, Hluchy and Pospisil 1991). It is believed that *T. pyri* utilizes HIPV's to aid in prey detection and foraging decisions (Dicke 1988). The attraction of *T. pyri* however to the widely present volatile MeSA, has not yet been determined.

In the present study we investigated the olfactory response of *T. pyri* to different quantities of synthetic MeSA in laboratory bioassays to determine the potential for attraction to synthetic lures employed in the field.

**Materials and Methods**

Predatory mites were collected from northern Willamette Valley vineyards (Yamhill Co., OR) and a stock colony maintained on plastic rearing units at 22 ± 2ºC using methods described by McMurtry and Scriven (1965). All mites used in the experiment were fed a mixed diet consisting of pollen (*Tilia* spp. and *Typha* spp.) and spider mites (*T. urticae*), reared on bean plants (*Phaseolus vulgaris* cv. Roma). Cohorts of approximately 100 deutonymphs were collected one week prior to the start of each olfactometer bioassay. Satiated, gravid adult female mites (2-5 d after final molt) were individually collected and placed into Eppendorf vials approximately 2 hours prior to olfactometer bioassays.

A y-tube olfactometer was employed to test the response of *T. pyri* in a two-way bioassay (Sabelis and Baan 1983a). Compressed air was blown at 1.3 liters/minute through a glass y-tube approximately 3 cm in diameter and an inert copper wire was present as a walking platform for the mites. The predatory mite response to methyl salicylate was tested at six doses (0.002µg, 0.02µg, 0.2µg, 2.0µg, 20µg, 200µg) at a volume of 0.1 ml of diluted MeSA (99% pure, diluted in hexane) placed on filter paper. The adjacent arm of the tube held filter paper with 0.1 ml hexane representing the control. All tests were conducted at 23 ± 2ºC and under similar light conditions. The doses were each replicated 5 times on different days, where eighteen individual adult

females were observed for response to one dose for each replicate-day. Mites were allowed five minutes to make a decision (MeSA diluted in hexane or hexane only) and once the time limit passed a 'no decision' was recorded for that individual. The arms and odor sources were interchanged after a progression of five mites had been observed to correct for any unexpected asymmetry in the y-tube design.

Contingency tables (2 × N, where N = replicates) were analyzed prior to pooling the data for further analysis to establish that no significant differences occurred between tests run on different days with different cohorts of predatory mites ($P > 0.05$). A binomial analysis tested for within-dose differences using a 50:50 distribution. The relationship between dose and response was first analyzed using logistic regression with log-dose and square log-dose as factors.

Subsequent analyses were conducted using linear regression to further evaluate mite response to dose. Binomial analysis was then performed combining data from all dose levels to determine the overall response to MeSA (SAS Institute 2007). Predatory mites that made no decision (7% in total) were excluded from all statistical analyses.

**Results**

A significant preference for MeSA was displayed within three (0.02, 0.2 and 20 µg) of the six concentrations ($P < 0.05$) (Figure 4.1). In all six doses tested, no significant preference was displayed by *T. pyri* toward the control source. Both the lowest (0.002 µg) and highest (200 µg) MeSA doses resulted in no significant difference in mite response. Approximately 70% of predatory mites responded positively to the 0.2 µg and 20 µg dose of MeSA.

The percent response of predatory mites to different quantities of MeSA was not significant ($P = 0.057$; $P = 0.739$) using the square of log-dose and log-dose prediction factors. This relationship is represented as a bi-modal curve with peak responses to MeSA occurring at 0.02 µg (63%), 0.2 µg (68%) and 20 µg (74%) (Figure 4.2). Percent mite response was highest at the 20 µg dose when excluding mites that made no decision.

Additional analysis of mite response to dose displayed no significant linear correlation ($P = 0.217$, $R^2 = 0.05$, $df = 1, 29$) between dose level and mite response to MeSA. When combining data from all dose levels, *T. pyri* showed significant preference (62%, $P < 0.0001$) to MeSA.

**Discussion**

*Typhlodromus pyri* displayed a significant response toward the volatile MeSA. This response supports the hypothesis that this predatory mite utilizes HIPV's as chemical cues to search for and locate prey. Significant attractive doses for *T. pyri* were 0.02, 0.2 and 20 µg. These results are comparable to those of De Boer and Dicke (2004b) in MeSA olfactometer experiments with the predatory mite *Phytoseiulus persimilis*. The attractive dose of 0.2 µg found for both predatory mite species is known to be similar to the estimated MeSA release rate of 0.2-0.4 µg emitted from *T. urticae* infested leaves over a 30 minute period (Dicke et al. 1999a, De Boer and Dicke 2004b). The 20 µg dose attracted the highest percentage of mites from those that made a decision, and the 0.2 µg dose elicited the highest percent response from all mites tested including those that made no choice. When accounting for individuals that made no decision, the percent response

was 67% (60 out of 90) at the 0.2 µg dose, and 60% at the 20 µg dose (60%, 54 out of 90).

*Typhlodromus pyri* did not respond significantly to 2 µg doses, which was unexpected with no clear explanation. Each cohort tested at this dose was mostly consistent over the five replicate-days with 0-2 mites not making a choice. This result differs from *P. persimilis* response reported by De Boer and Dicke (2004b) where significant attraction to the 2 µg dose of MeSA was documented. In our bioassays *T. pyri* was not attracted to nor repelled by the lowest (0.002 µg) and highest (200 µg) dose whereas *P. persimilis* was reported to display a significant repellent response to the highest MeSA dose (200 µg) (De Boer and Dicke 2004b).

The response to dose displayed in this study was not significant, indicating that there is no relationship between dose level and mite response. The response of *P. persimilis* to the same range of doses displayed a significant relationship between MeSA dose and response indicating that dose level is critical in affecting the behavior of this predatory mite (De Boer and Dicke 2004b). The variability observed between the two predatory mite species' response to different levels of MeSA can be attributed to factors such as 1) qualitative or quantitative differences in volatile blends, 2) learning to associate the volatile with the presence or absence of prey, 3) previous foraging experiences, 4) starvation level and prey preferences and 5) generalist or specialist classification (Dicke 1999, De Boer and Dicke 2004a).

MeSA is an important volatile component in the foraging behavior and prey discrimination in a number of plant-herbivore-natural enemy systems. Although several

researchers have provided evidence on the significance of MeSA, others argue that the HIPV blend as a whole is more important (Ishiwari et al. 2007, Van Wijk et al. 2008). Results from the current study indicate that MeSA is attractive to *T. pyri,* however further investigations are needed to determine additional volatile components that may contribute to the response of this predatory mite.

In summary, we found that *T. pyri* is attracted to synthetic MeSA in olfactometer bioassays. The use of synthetic MeSA lures in vineyards may have the potential to attract and increase populations of *T. pyri* thereby enhancing pest mite biological control. Synthetic MeSA dispensers were recently reported to increase the mean seasonal abundance of various natural enemies for enhanced biological control in commercial cropping systems such as hops and strawberry (James 2003a, James and Price 2004, Lee 2010).

Field experiments are currently in progress to evaluate the role of MeSA in Oregon vineyards. Additional research should include investigation of the attraction of *T. pyri* to additional synthetic HIPV's or combinations of volatiles.

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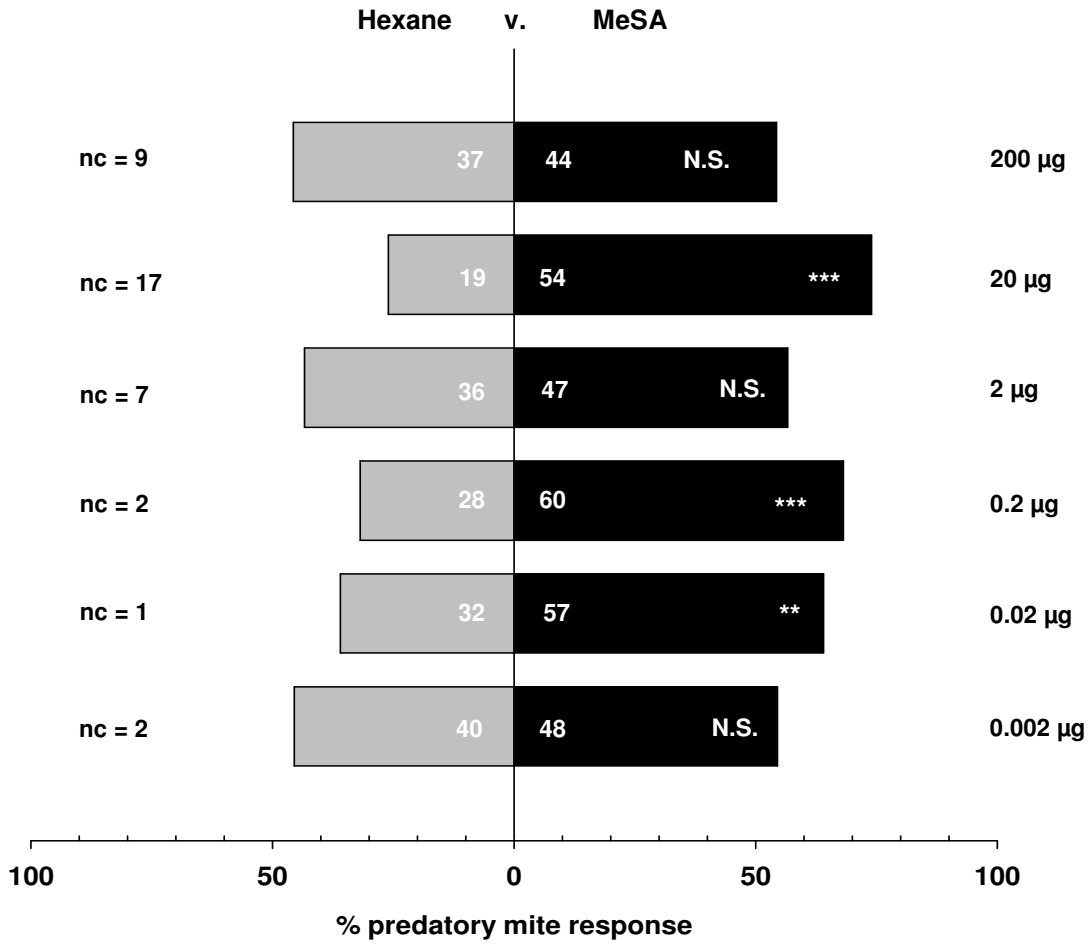


**Figure 4.1**. Percent response of adult female predatory mites, *Typhlodromus pyri*, in a two-choice bioassay to MeSA (black bar). Values within each bar represent the total number of mites responding to the treatment. MeSA doses are adjacent to the corresponding MeSA bars. Total number of mites per treatment that made no choice are represented to the left of each control bar (nc values) and were excluded from statistical analysis. A binomial analysis was used to analyze significant differences in choice between odor sources (N.S. $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$).

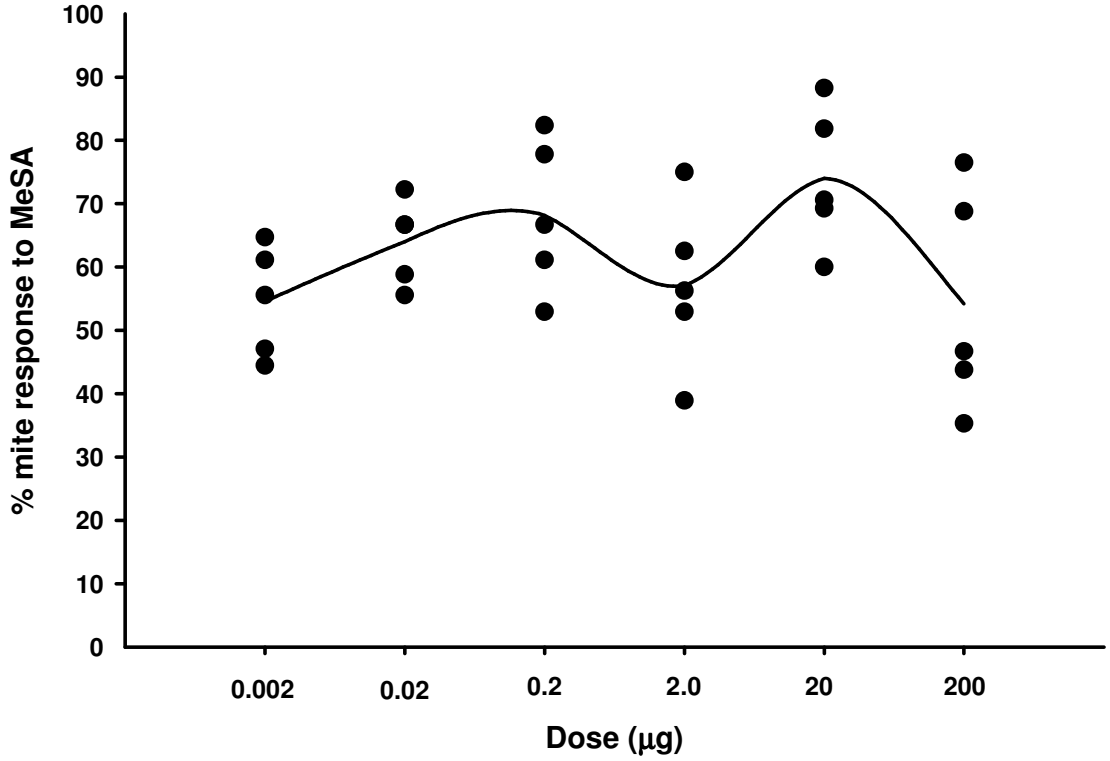


**Figure 4.2**. Dose-response of adult female *Typhlodromus pyri* to MeSA. The percent mites choosing MeSA per replicate-day is represented by vertical black dots within each dose. The black line represents the overall treatment (dose) average.

# Chapter V

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

**Abstract**

The predatory mite *Typhlodromus pyri* is considered a key biological control agent of the grapevine rust mite, *Calepitrimerus vitis*. Methyl salicylate (MeSA), an herbivore induced plant volatile, can potentially elicit top-down control of pests through attraction of beneficial arthropods. This study evaluates the effect of synthetic MeSA lures (PredaLure) in two vineyards on arthropod populations during 2009-2010 seasons. MeSA lures were deployed at a low (4/plot) and high (8/plot) rate in ~ 152 $m^2$ plots while control plots contained no lure. Each treatment contained three replicates per site. Leaf samples were collected to assess *T. pyri*, Eriophyid, Tetranychid and Thripidae densities. Yellow sticky traps were used to monitor other key arthropods. Overall *T. pyri* population densities at Salem were higher in control plots compared to MeSA plots. Comparisons of treatment by date revealed significantly lower numbers of *T. pyri* in treated plots in the middle (2009) and early (2010) growing season. In contrast, mean seasonal abundance of *T. pyri* was higher in MeSA plots at Dayton during both seasons. Significantly higher counts of *T. pyri* were shown in high rate MeSA plots in 2010 early in the season. Coccinellidae mean seasonal trap counts were significantly higher in MeSA plots in both years at the Dayton vineyard. MeSA plots did not show significantly higher or lower *C. vitis* populations. In 2009 at Salem, pest thrips densities were significantly lower in low rate MeSA plots late in the season although no trend of decreased seasonal abundance was evident.

## Introduction

Predatory mites (Acari: Phytoseiidae) and other beneficial arthropods play an integral role in regulating phytophagous mite populations in Pacific Northwest vineyards and hop yards (James et al. 2002, Prischmann et al. 2002). In the past decade, vineyards in cooler coastal regions of the Pacific Northwest and California have experienced increased economic damage from symptoms associated with grapevine rust mite, *Calepitrimerus vitis* Nalepa (Acari: Eriophyidae) (Walton et al. 2007). This host specific pest feeds on susceptible bud and shoot tissues in the early part of the season resulting in stunted shoots, shortened inter-nodal growth and crop loss due to cluster necrosis (Bernard et al. 2005, Duso et al. 2010). *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) is the predominant predatory mite found in these vineyard systems (Hadam et al. 1986) and is considered an important biological control agent of Eriophyid and Tetranychid pest mites (Duso and de Lillo 1996, Prischmann et al. 2002). Conservation and enhancement of beneficial arthropod populations is an essential component in achieving successful biological control in pest management.

The release of herbivore induced plant volatiles (HIPV's) as a indirect defense mechanism in plants, elicits top-down control of damaging pests through attraction and recruitment of natural enemies (Dicke et al. 1990b, Dicke 1999). The phenolic compound methyl salicylate (MeSA) has been identified as a valuable HIPV released by more than 13 different crop plants, including grape and hops, when infested with *Tetranychus urticae* (Dicke et al. 1990c, van den Boom et al. 2004). It is also a compound in HIPV blends released in cabbage infested by caterpillars, *Pieris* spp

(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).

Several studies have provided evidence demonstrating the positive response of beneficial arthropods toward MeSA and other herbivore induced volatiles in laboratory experiments (Ozawa et al. 2000b, Shimoda et al. 2002, De Boer and Dicke 2004b, Ishiwari et al. 2007, Shimoda 2010). Recent experiments found attraction of *T. pyri* adult females to MeSA (99% diluted in hexane) in y-tube olfactometer bioassays (Gadino 2010, unpublished data). Current research is focused on the deployment of synthetic HIPV's in agricultural crops as a strategy to augment natural enemies with the aim of increasing biological control in these systems. Sticky traps baited with MeSA dispensers (98-99% in solution) attracted an array of beneficial insects in hop yards (James 2003b, a) and resulted in attraction of *Coccinella septempunctata* in soybean (Zhu and Park 2005). Vineyard field evaluations demonstrated an increase in abundance of several natural enemy species in plots using controlled release MeSA sachets (James and Price 2004, James and Grasswitz 2005). These data suggest that MeSA may attract and retain beneficial insects within close vicinity of the lures and not solely to individual dispensers

Field and laboratory studies have also documented repellent effects of MeSA on pest insects, highlighting the potential for infochemical-based pest management. For example, the bird cherry oat aphid, *Rhopalosiphum padi* responded negatively to MeSA treated oats in laboratory experiments (Glinwood and Pettersson 2000) while field research demonstrated delays in establishment and decreased maximum abundance of the bird cherry oat aphid in barley (Ninkovic et al. 2003). In hopyards, Losel et al. (1996)

reported a decrease in aphid (*Phorodon humuli*) densities captured in water traps baited with MeSA.

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. In one field study, jasmonic acid (JA)-induced tomato plants increased parasitism rates of the lepidopteran pest *Spodoptera exigua* by the parasitoid *Hyposoter exiguae* two-fold compared to non-JA treated plants (Thaler 1999). Another experiment demonstrated an increase in parasitism rates of the rice brown planthopper, *Nilaparvata lugens* by the egg parasitoid *Anagrus nilaparvatae* in JA-induced rice plants which emitted elevated levels of MeSA along with other volatiles (Lou et al. 2005). Conversely, strawberry fields baited with synthetic MeSA (2 g lures) found no impact on Tetranychid, Aphididae or Thripidae populations regardless of the elevated abundance reported in six natural enemy groups (Lee 2010).

In the present study, we employed commercially available MeSA-based lures (PredaLure, AgBio Inc., Westminster, CO, USA) in Oregon vineyards and examined 1) the density of *T. pyri* and *C. vitis* populations; 2) the density of additional key natural enemy species and other pests present and; 3) the spatial and temporal effects of MeSA lures over the growing season in two consecutive years. The key beneficial arthropods evaluated in this experiment included *T. pyri* as well as Coccinellidae, Staphylinidae, Syrphidae, Anthocoridae, Geocoridae, Rhaphidiidae, Chrysopidae, Anystidae, Thripidae, Aeolothripidae and Araneae. These arthropods are considered important predators of Eriophyid, Tetranychid and Thripidae pests occurring in Oregon vineyards.

## Materials and Methods

Site location and experimental design

Field experiments were conducted during 2009 and 2010. The goal of the study was to assess the effect of MeSA lures on the seasonal population dynamics of key natural enemies and pests within two commercial vineyards in Oregon. Vineyards were located in Salem, OR (Marion Co., 45º01'46N; 123º08'17W, alt. 73 m) and Dayton, OR (Yamhill Co., 45º14'23N; 123º04'28W, alt. 66 m). Vineyards were planted with *Vitis vinifera* cv. Pinot Noir between 2003 and 2005, with 3 × 1 m row spacing and cane-pruned. Management programs were similar in the two sites with both vineyards following recommendations from Oregon Low Input Viticulture and Enology guidelines (Oregon LIVE; liveinc.org).

A randomized complete block design was used to establish experimental plots. Each plot covered an area approximately 152 $m^2$ and were four vine rows wide and 25-30 vines long. Treatments included MeSA lures (5 g lure) at a low rate (4 lures/plot), a high rate (8 lures/plot) and an untreated control. All treatments were replicated three times totaling 9 plots per location. The number of lures employed in the low density treatment was based on 260 lures/hectare and the high density treatment a 2× increase from this amount. 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). Samples were collected from plot center (0 m) and at 5 and 10 m laterally down the vine row to assess potential spatial trends in insect response.

Treatment plots were spaced approximately 60-100 m apart with lure plots located downwind of the prevailing wind direction to avoid volatile drift between treatments.

Arthropod sampling

In 2009-2010 MeSA lures were first deployed in both vineyards on May 6 and April 19 and then replaced on August 3 and July 19. Sampling was conducted every two weeks from April to October in each vineyard. Secondary shoot samples were collected at the start of the growing season and analyzed under a dissecting microscope in the laboratory to establish presence of *T. pyri* and *C. vitis* in experimental sites.

*Leaf samples*. Samples of 10 leaves were collected at each location, 0, 5 and 10 m, in each plot, and transported back to the laboratory in an insulated cooler. Arthropods were brushed onto a glass plate containing a thin film of detergent using a leaf brushing machine (Leedam Engineering, Twin Carte, CA, USA). The glass plate was placed on a black and white grid to assist with counting specimens using a dissecting microscope. Numbers from leaf assessments were used to determine *T. pyri* life stages (eggs, mobiles), pest mites (Eriophyid, Tetranychid) and thrips (Thripidae) density per leaf for each treatment and distance.

*Sticky trap captures*. Yellow sticky traps (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 key predatory arthropods for each plot and distance. Cicadellidae were counted during 2010 to determine the potential impact of MeSA on these pest populations. Counts from

sticky traps were divided by the number of days elapsed from trap placement to sample collection and presented as 14 d counts.

Statistical analysis

Individual analyses were conducted using per leaf density of *T. pyri* (mobile and egg stages), *C. vitis*, Thripidae and Tetranychid when arthropods were present. Specimens counted from sticky traps were grouped and analyzed by taxonomic family when insect abundance was sufficient for individual analysis. Taxa with insect abundance too low for separate comparisons were included into the macro-predator and micro-predator groups for analysis. Coccinellidae, *Stethorus* spp., *Coccinella* spp., and *Cycloneda* spp. were most often present on sticky traps. *Orius* spp. was the most abundant taxa in Anthocoridae (voucher specimens stored in Cordley Hall, Oregon State University, Corvallis, OR 97330).

The treatment effect of MeSA lures deployed at two rates on all major arthropod groups was determined using a split-plot repeated measure analysis which 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. Significant main factors and interactions ($P < 0.1$) were analyzed in a forward stepwise approach to further determine treatment differences. When treatment or treatment × date terms were significant, an analysis of variance (PROC GLM, SAS 2006) 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 (PROC GLM, SAS 2006) 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.

**Results**

Salem vineyard location

*Leaf samples*. During 2009 the mean density of *T. pyri* (mobiles and total) was higher in control plots compared to plots baited with MeSA lures (Table 5.1). Treatment by date analysis found significantly higher *T. pyri* density in control plots (Figure 5.1a) during fruit development (July 17, $F = 15.17$, $P < 0.001$, $df = 2, 26$) and berry ripening periods (September 9, $F = 7.64$, $P = 0.007$, $df = 2, 26$). A significant decrease of pest thrips (Thripidae) occurred later in the season during berry ripening in control plots compared to low rate MeSA plots (September 9, $F = 10.68$, $P = 0.002$, $df = 2, 26$). Additionally, mean seasonal abundance of pest thrips was higher in control versus MeSA baited plots during 2009.

During 2010, seasonal abundance of predatory mites was approximately 4× smaller compared to 2009 with lower densities of both *T. pyri* mobiles and eggs in all plots (Table 5.1). Higher *T. pyri* densities (May 11, $F = 4.83$, $P = 0.029$; June 21, $F = 5.35$, $P = 0.022$; $df = 2, 26$) were found on leaves sampled in control plots during early season bloom time when predatory mite abundance was highest (Figure 5.1b). During 2010 mean densities of pest thrips (Thripidae) was lower compared to 2009 in all plots

but did not decrease significantly in MeSA plots. Rust mite pest populations were similar both seasons with no observation of Eriophyid mites at this location.

*Trap captures*. Natural enemies appeared frequently on sticky trap captures in all treatments throughout the 2009-2010 sampling season. In 2009, Coccinellidae trap capture seasonal means were higher in both MeSA treatments but were not significantly higher than in the control plots (Table 5.1). Temporal analysis of macro predator abundance displayed greater natural enemy populations in high rate MeSA plots late in the growing season during berry ripening (September 22, $F = 6.32$, $P = 0.013$; October 9, $F = 8.71$, $P = 0.005$; $df = 2, 26$) which was the reverse trend of significantly lower densities of macro predators captured in MeSA plots earlier that same season during bloom (June 29, $F = 9.88$, $P = 0.003$, $df = 2, 26$).

Mean seasonal abundance of macro and micro predators were lower in 2010 compared to 2009 across all treatments. Spider counts were significantly higher in low rate MeSA plots during mid-season fruit development stages (July 6, $F = 6.62$, $P = 0.012$, $df = 2, 26$). Coccinellidae and total macro predators displayed a pattern of increased mean seasonal abundance in MeSA baited plots compared to control plots (Table 5.1). No significant differences in Cicadellidae numbers were detected between treatments. A slightly higher mean density was however evident in low rate MeSA plots.

Dayton vineyard location

*Leaf samples*. In 2009, mean arthropod density of beneficials and pests did not differ between treatments. Mean density of *T. pyri* mobile stages appeared higher, but not significantly, in MeSA baited plots compared to control plots (Table 5.2). A greater

mean number of *T. pyri* eggs were however evident in high rate MeSA plots. Pest mites, *C. vitis* and Tetranychids, and pest thrips (Thripidae) were present in all treatment plots.

During 2010, higher seasonal mean *T. pyri* densities were found in high rate MeSA plots compared to other treatments (Table 5.2). *T. pyri* densities were higher in MeSA (high rate) plots in early season bloom time with an average 0.98 mites per leaf (May 24, $F = 26.2$, $P = <.0001$, $df = 2, 26$) (Figure 5.1c). In 2010, lower mean abundance of *C. vitis* and Thripidae were found compared to 2009. No significant differences in pest density however occurred between baited and control treatments. Tetranychid pest mites were present in all treatment plots during 2010 with densities too low to allow statistical analysis.

*Trap captures*. Higher seasonal mean numbers of Coccinellidae were recorded during 2009 ($P = 0.029$) and 2010 ($P = 0.040$) with the highest counts recorded in high rate MeSA plots (Table 5.2). Temporal trends showed higher numbers of coccinellids in the high rate MeSA plots during bloom and fruit development months. Peak mean captures of coccinellids per trap per 14 d occurred in early June (> 4.5 ) and late July(> 2.0 ) in 2009 (Figure 5.2a). Anthocoridae and mean seasonal abundance of macro predators from sticky traps were higher in MeSA baited plots than control plots but these differences were not significant. Higher numbers of Anthocoridae were found at 5 m ($P < 0.05$) from lures, with lower numbers recorded at 0 m and 10 m from MeSA dispensers (Figure 5.3).

In 2010, seasonal mean abundance of Coccinellidae were more than 2× higher in MeSA treatments and displayed significant attraction of coccinellids to MeSA (Table

5.2). Peak captures of coccinellids occurred during bloom (> 2.0 per trap per 14 d) and again during fruit development (> 1.5 per trap per 14 d) in low and high rate MeSA plots (Figure 5.2b). A significant date by treatment response of hover flies (Syrphidae) to MeSA (low rate) during early season bloom was found (June 7, $F = 7.42$, $P = 0.008$, $df = 2, 26$) and mean seasonal captures ranged from 0.36 – 0.62 syrphids per trap per 14 d in baited plots. Total macro predator counts were significantly greater ($P = 0.029$) in baited plots (Table 5.2). Cicadellidae mean abundance ranged from 2.14 to 5.69 during 14 d intervals in all treatments. Significantly higher numbers of leafhoppers were trapped in high rate MeSA treatments earlier in the season during bloom time (May 24, $F = 15.32$, $P = 0.001$, $df = 2, 26$) but low rate MeSA and control plots were not significantly different from one another during this period.

**Discussion**

A variable response of *T. pyri* to synthetic MeSA was evident between the two experimental locations. At Salem a trend of decreased seasonal abundance of *T. pyri* in MeSA treated plots were shown during both seasons. Conversely, *T. pyri* populations at the Dayton vineyard displayed a pattern of higher seasonal abundance in MeSA plots during both 2009 and 2010. These inconclusive results were unexpected as *T. pyri* displayed significant attraction to MeSA in laboratory bioassays (Gadino 2010, unpublished data).

One possible explanation for the variable response pattern of *T. pyri* observed in our study may be due to the relative presence or absence of the prey resource *C. vitis* at the two vineyard locations. Research has documented that predatory arthropods are

capable of learning to associate and discriminate between prey by using chemical cues (Drukker et al. 2000b, De Boer et al. 2005, De Boer and Dicke 2006). Evidence for learning to associate chemical information with prey availability was documented in Anthocorid predators where individuals exposed to MeSA in the absence of prey resulted in avoidance of MeSA in subsequent tests (Drukker et al. 2000a). Based on these findings, it is plausible that *T. pyri* learned to associate MeSA with the availability of *C. vitis* prey at the Dayton vineyard where a positive response to MeSA occurred whereas the reverse trend took place at the Salem vineyard where *C. vitis* was virtually nonexistent.

The conflicting responses of *T. pyri* to MeSA in this study may also have been the result of genetic differences in sensitivity to semiochemicals between the two local, but separate populations. Maeda and Liu (2006) noted differences in the olfactory response to spider mite-infested kidney bean volatiles of both distinct and inbred strains of *Neoseiulus womersleyi* These results suggest that chemical cues may be perceived and utilized differently by predatory mites even among similar strains in local populations.

The lack of a strong positive response of *T. pyri* to MeSA is also potentially due to the dispersal, colonization and foraging habits of this predatory mite. Previous research documented low dispersal rates in *T. pyri* and although they are able to immigrate and colonize new agricultural areas, their movement appears to be limited compared to other predatory mite species (Boller et al. 1988, Dunley and Croft 1990 1994). Additionally, *T. pyri* is classified as a Type III generalist predator (McMurtry and Croft 1997) whose spatial distribution does not appear dependant on a single prey

resource (Nyrop 1988). These behavioral and feeding traits in *T. pyri* are likely to impact the level of immigration to an area emitting synthetic MeSA, particularly if food resources are readily available in their current location. For example, lures tested in preliminary field trails were unable to encourage emigration of *T. pyri* from surrounding blackberry hedgerows into vineyards plots (~ 25 m distance) where no predatory or pest mites were present (Gadino, personal observation).

Finally, the response of *T. pyri* to MeSA may increase in the presence of additional volatile compounds also produced in the HIPV blend of mite-infested grape leaves. It was documented that four HIPV's from mite-infested kidney bean leaves, including MeSA, were necessary to attract *N. womersleyi* in olfactometer bioassays and that the absence of a single compound resulted in no mite response (Ishiwari et al. 2007). It has also been suggested that synthetic HIPV's may prime neighboring plants or induce emission of their own volatile blends (James and Grasswitz 2005, Turlings and Ton 2006). Identifying the volatile or volatile blends that may trigger this effect is an important factor in utilizing these compounds effectively.

Higher mean seasonal abundance of Coccinellidae were found in the MeSA baited plots in both locations during both years. These findings are comparable with other field experiments where coccinellids were shown to be significantly attracted to synthetic MeSA. James and Price (2004) reported significantly greater numbers of *Stethorus punctum picipes* in MeSA baited vineyard plots and hop yards compared to untreated areas. Additionally, higher numbers of coccinellids were reported in MeSA treated strawberry plots from 3 - 7 d after lure placement (Lee 2010). Significant

coccinellid attraction to MeSA was shown at Dayton where pest species were more diverse and abundant. These results suggest that an adequate prey resource base is again an essential component in the system if using synthetic HIPV's as a strategy to enhance beneficial arthropod populations.

The responses of all other natural enemy groups to MeSA showed no clear patterns in our study. Mean seasonal abundance of *Orius* spp. was greater in MeSA plots at the Dayton site, 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 overall positive response of *Orius tristicolor* to MeSA lures was found in grape (cv. Concord), hops and strawberry. James (2003b) however reported greater attraction of *Orius* spp. to individual traps baited with (*Z*)-3-hexenyl acetate compared to MeSA, indicating this predator may show an increased response when other HIPV's are present. In our study, Anthocorid trap captures were higher at 0 and 5 m compared to 10 m and differences between treatments at 0 m were also found in 2009 suggesting the higher trap captures at Dayton may have been affected by the distance of sticky traps from the lures.

No significant effect of MeSA on *C. vitis* populations were displayed in our study which differed from the large reduction of spider mite numbers in hops observed by James and Price (2004). We did however find significantly lower thrips densities per leaf in MeSA plots late season in 2009 at Salem, this trend however was not evident at Dayton where no effect of MeSA on thrips was apparent. Results from Dayton are comparable with those found by Lee (2010) and James and Price (2004) where no

response in pest thrips to MeSA was demonstrated. One potential explanation for the inconsistency in results may be due to sampling techniques. In our study, pest thrips density was counted from leaf samples whereas previous field experiments sampled thrips primarily using sticky traps. Trap location and color are reported to be important factors in accurately sampling thrips populations (Hoback et al. 1999, Chu et al. 2000). It has also been found that concurrent sampling of leaf and sticky traps is most effective in estimating population density, as larval density is higher on leaf samples and sticky traps capture a greater number of flying adults (Higgins 1992).

Our studies indicate that MeSA may result in increased abundance and activity of certain beneficial arthropods when adequate prey resources are present. These findings will likely influence the practical application of commercially available MeSA lures in agricultural systems. The decision to deploy synthetic HIPV's should be based on careful monitoring of pest populations which will aid in appropriate lure placement and timing of application. This is reasonable as commercial growers often base pest management decisions on economic thresholds and control costs. Thereby employing MeSA lures would not make economic sense unless detectable pest levels were present.

Manipulating the release of HIPV's in agricultural and natural systems has proven beneficial in several cases, (Kessler and Baldwin 2001, Khan et al. 2008), however it is important to consider the ecological and evolutionary consequences of employing synthetic volatiles without sufficient application knowledge. The potential for predators and parasitoids in learning to associate volatiles with the absence of prey may have profound effects on their behavior in systems experiencing constant release of HIPV's.

The impact of synthetic volatiles on plant priming and fitness also needs further research before wide-spread application of these materials is adopted (Dicke and Baldwin 2009). Other possible uses for synthetic HIPV's in agricultural systems should also be explored. For example, MeSA lures may assist in the retention of mass-released *T. pyri* into a plot with damaging *C. vitis* populations in an effort to augment the existing natural population of predatory mites. Attract and reward strategies to mitigate the potential negative impact of prey absence should also be investigated.

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**Table 5.1**. Repeated measure analysis of natural enemy and pest populations sampled from vineyard site at Salem location over two seasons (2009-2010).

| Species | Mean ± SE | | | Treatment df = 2, 4 | Distance 2, 12 | Date 10, 180 | Block 2, 4 | Interaction terms if analyzed |
|---|---|---|---|---|---|---|---|---|
| | Control | MeSA LR | MeSA HR | | | | | |
| | ***Leaf samples*** | | | | | | | |
| ***2009*** | | | | | | | | |
| *Typhlodromus pyri* | | | | | | | | |
| Mobile stages | 2.10 ± 0.14 | 1.91 ± 0.13 | 1.43 ± 0.11 | 0.298 | 0.664 | <.0001 | 0.481 | **0.055** (tx*date) |
| Eggs | 0.89 ± 0.15 | 0.99 ± 0.24 | 0.56 ± 0.13 | 0.099 | 0.263 | <.0001 | 0.307 | |
| Total | 2.99 ± 0.23 | 2.91 ± 0.33 | 1.99 ± 0.21 | 0.178 | 0.527 | <.0001 | 0.361 | **0.007** (tx*date) |
| Thripidae | 0.44 ± 0.09 | 0.32 ± 0.07 | 0.30 ± 0.06 | 0.231 | 0.301 | <.0001 | 0.554 | **0.042** (tx*date) |
| ***2010*** | | | | | | | | |
| *Typhlodromus pyri* | | | | | | | | |
| Mobile stages | 0.50 ± 0.07 | 0.38 ± 0.06 | 0.31 ± 0.05 | 0.438 | 0.551 | <.0001 | 0.972 | **0.066** (tx*date) |
| Eggs | 0.19 ± 0.04 | 0.15 ± 0.04 | 0.15 ± 0.04 | 0.634 | 0.738 | <.0001 | 0.371 | |
| Total[1] | 0.69 ± 0.10 | 0.53 ± 0.09 | 0.47 ± 0.07 | 0.400 | 0.585 | <.0001 | 0.921 | |
| Thripidae | 0.21± 0.04 | 0.20 ± 0.04 | 0.15 ± 0.05 | 0.292 | 0.255 | <.0001 | 0.923 | |
| | ***Sticky trap captures*** | | | | | | | |
| ***2009*** | | | | | | | | |
| Coccinellidae | 0.37 ± 0.08 | 0.42 ± 0.07 | 0.56 ± 0.11 | 0.667 | **0.052** | <.0001 | 0.404 | |
| Anthocoridae | 0.42 ± 0.11 | 0.58 ± 0.20 | 0.28 ± 0.07 | 0.721 | 0.278 | <.0001 | 0.370 | |
| Araneae (spiders) | 2.14 ± 0.19 | 2.03 ± 0.25 | 2.31± 0.22 | 0.739 | 0.999 | <.0001 | 0.925 | |
| Macro predators[2] | 3.04 ± 0.26 | 3.19 ± 0.34 | 3.23 ± 0.25 | 0.746 | 0.091 | <.0001 | 0.907 | **0.001** (tx*date) |
| Micro predators[3] | 1.68 ± 0.37 | 1.29 ± 0.28 | 1.27 ± 0.31 | 0.531 | 0.183 | <.0001 | 0.989 | |
| ***2010*** | | | | | | | | |
| Coccinellidae | 0.30 ± 0.05 | 0.41 ± 0.09 | 0.49 ± 0.08 | 0.417 | 0.151 | <.0001 | 0.927 | |
| Syrphidae | 0.10 ± 0.03 | 0.06 ± 0.02 | 0.14 ± 0.04 | 0.320 | 0.146 | 0.0002 | 0.977 | |
| Araneae (spiders) | 0.99 ± 0.15 | 1.09 ± 0.14 | 0.99 ± 0.13 | 0.768 | 0.826 | <.0001 | 0.455 | **0.094** (tx*date) |
| Macro predators | 1.98 ± 0.21 | 2.30 ± 0.23 | 2.27 ± 0.20 | 0.783 | 0.072 | <.0001 | 0.588 | |
| Micro predators | 1.15 ± 0.21 | 1.04 ± 0.17 | 0.82 ± 0.18 | 0.485 | 0.269 | <.0001 | 0.222 | |
| Cicadellidae | 1.00 ± 0.13 | 1.24 ± 0.13 | 0.84 ± 0.12 | 0.172 | 0.971 | 0.001 | 0.462 | |

**Table 5.1**. Repeated measure analysis of natural enemy and pest populations sampled from vineyard site at Salem location over two seasons (Continued).

Significant p-values in bold.
[1]Total *T. pyri* calculated (mobile stages + eggs) per sample.
[2]Total counts of easily visible key natural enemies in Oregon vineyards (Coccinellidae, Staphylinidae, Syrphidae, Anthocoridae, Geocoridae, Rhaphidiidae, Chrysopidae and Araneae).
[3]Total counts of microscopic key natural enemies in Oregon vineyards (Anystidae, Thripidae and Aeolothripidae).

**Table 5.2**. Repeated measure analysis of natural enemy and pest populations sampled from vineyard site at Dayton location over two seasons (2009-2010).

| Species | Mean ± SE | | | Treatment df = 2, 4 | Distance 2, 12 | Date 10, 180 | Block 2, 4 | Interaction terms if analyzed |
|---|---|---|---|---|---|---|---|---|
| | Control | MeSA LR | MeSA HR | | | | | |
| | ***Leaf samples*** | | | | | | | |
| ***2009*** | | | | | | | | |
| *Typhlodromus pyri* | | | | | | | | |
| Mobiles | 0.75 ± 0.08 | 0.81 ± 0.11 | 1.17 ± 0.11 | 0.506 | 0.258 | <.0001 | 0.203 | |
| Eggs | 0.37 ± 0.07 | 0.37 ± 0.08 | 0.53 ± 0.10 | 0.734 | 0.143 | <.0001 | 0.241 | |
| Total | 1.12 ± 0.13 | 1.18 ± 0.17 | 1.7 ± 0.19 | 0.555 | 0.442 | <.0001 | 0.192 | |
| *Calepitrimerus vitis* | 4.33 ± 1.21 | 4.05 ± 1.14 | 4.44 ± 1.20 | 0.471 | 0.593 | <.0001 | **0.004** | |
| Thripidae | 0.47 ± 0.10 | 0.45 ± 0.08 | 0.53 ± 0.09 | 0.864 | 0.216 | <.0001 | 0.258 | |
| Tetranychid | 0.04 ± 0.02 | 0.05 ± 0.01 | 0.08 ± 0.02 | 0.345 | 0.718 | 0.038 | 0.152 | |
| ***2010*** | | | | | | | | |
| *Typhlodromus pyri* | | | | | | | | |
| Mobiles | 0.34 ± 0.06 | 0.28 ± 0.04 | 0.45 ± 0.07 | 0.208 | 0.658 | <.0001 | **0.007** | **0.072** (tx*time) |
| Eggs | 0.34 ± 0.06 | 0.25 ± 0.05 | 0.42 ± 0.08 | 0.257 | 0.696 | <.0001 | **0.003** | |
| Total[1] | 0.68 ± 0.10 | 0.53 ± 0.07 | 0.87 ± 0.14 | 0.304 | 0.666 | <.0001 | **0.002** | |
| *Calepitrimerus vitis* | 0.04 ± 0.01 | 0.08 ± 0.03 | 0.05 ± 0.02 | 0.604 | 0.912 | <.0001 | **0.020** | |
| Thripidae | 0.12 ± 0.03 | 0.10 ± 0.02 | 0.13 ± 0.03 | 0.644 | 0.207 | <.0001 | 0.156 | |
| | ***Sticky trap captures*** | | | | | | | |
| ***2009*** | | | | | | | | |
| Coccinelidae | 0.72 ± 0.10 | 0.94 ± 0.11 | 1.46 ± 0.18 | **0.029** | 0.909 | <.0001 | 0.952 | |
| Anthocoridae | 0.24 ± 0.07 | 0.47 ± 0.11 | 0.57 ± 0.17 | 0.401 | **0.021** | <.0001 | 0.782 | |
| Araneae (spiders) | 1.52 ± 0.16 | 1.20 ± 0.13 | 1.21± 0.13 | 0.313 | 0.759 | <.0001 | 0.182 | |
| Macro predators[2] | 2.50 ± 0.21 | 2.63 ± 0.22 | 3.22 ± 0.29 | 0.226 | 0.270 | <.0001 | 0.352 | |
| Micro predators[3] | 2.45 ± 0.58 | 1.16 ± 0.21 | 2.62 ± 0.70 | 0.374 | 0.430 | <.0001 | 0.039 | |
| ***2010*** | | | | | | | | |
| Coccinelidae | 0.41± 0.07 | 0.97 ± 0.13 | 0.91± 0.15 | **0.040** | 0.933 | <.0001 | 0.068 | |

**Table 5.2**. Repeated measure analysis of natural enemy and pest populations sampled from vineyard site at Dayton location over two seasons (Continued).

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Syrphidae | 0.18 ± 0.04 | 0.62 ± 0.17 | 0.36 ± 0.11 | 0.131 | 0.133 | <.0001 | 0.107 | **0.012** (tx*date) |
| Araneae (spiders) | 0.60 ± 0.10 | 0.77 ± 0.11 | 0.97 ± 0.12 | 0.093 | 0.913 | <.0001 | 0.037 | |
| Macro predators | 1.35 ± 0.15 | 2.51 ± 0.23 | 2.41± 0.24 | **0.029** | 0.255 | <.0001 | 0.071 | |
| Micro predators | 0.43 ± 0.10 | 0.41± 0.10 | 0.46 ± 0.11 | 0.965 | 0.098 | <.0001 | 0.840 | |
| Cicadellidae | 2.76 ± 0.37 | 2.14 ± 0.31 | 5.69 ± 1.30 | 0.395 | 0.862 | <.0001 | 0.638 | **<.0001**(tx*date) |

Significant p-values in bold.

[1]Total *T. pyri* calculated (mobile stages + eggs) per sample.

[2]Total counts of easily visible key natural enemies in Oregon vineyards (Coccinelidae, Staphylinidae, Syrphidae,Anthocoridae, Geocoridae, Rhaphidiidae, Chrysopidae and Araneae).

[3]Total counts of microscopic key natural enemies in Oregon vineyards (Anystidae, Thripidae and Aeolothripidae

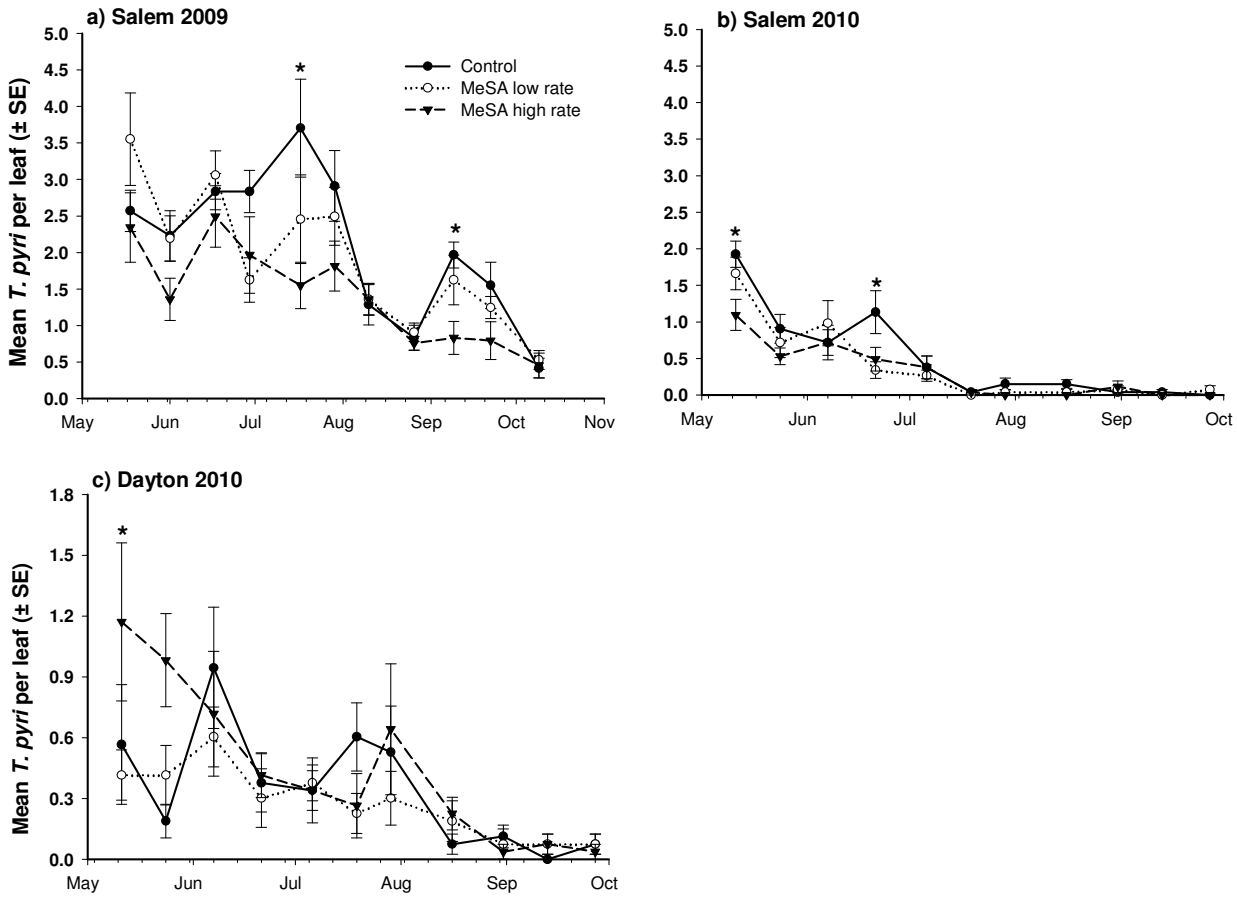


**Figure 5.1**. Mean *Typhlodromus pyri* per leaf at Salem during 2009 (a), 2010 (b) and Dayton during 2010 (c). Sampling dates marked with an asterisk indicate significant ($P < 0.05$) mean differences (Tukey's HSD) between control and MeSA plots for that given date.

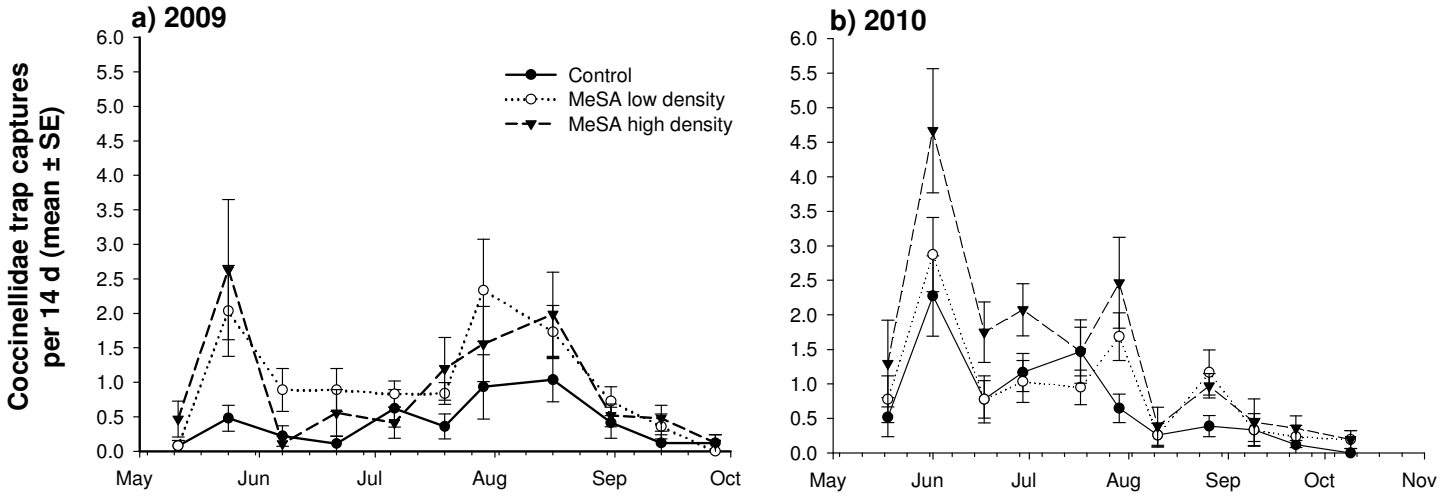


**Figure 5.2**. Mean seasonal counts of Coccinellidae trap captures based on 14 d intervals at Dayton location in 2009 (a) and 2010 (b).

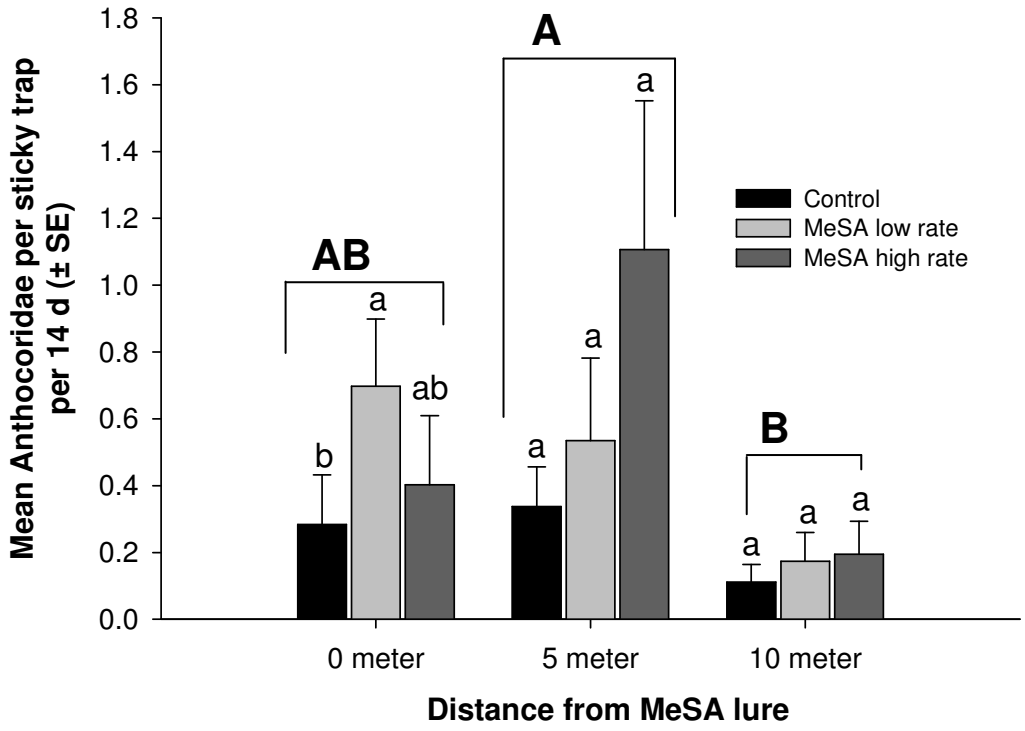


**Figure 5.3**. Mean seasonal sticky trap captures of Anthocoridae (*Orius* spp.) at three distances from MeSA lure (plot center) in all treatments at Dayton in 2009. Bars with different lower case letters show significant mean comparisons between treatments within each distance and groups with different upper case letters indicate significant differences between distances using Tukey's HSD ($P < 0.05$).

# Chapter VI

## Summary and Conclusions

**Temperature-related developmental parameters**

The biological parameters in this study indicate that *T. pyri* is an adequate biological control agent of the grapevine rust mite, *C. vitis* at cooler temperatures (< 25ºC) typically found during spring and early summer. The intrinsic rate of population increase was greater for *T. pyri* compared to *C. vitis* at temperatures below 20ºC indicating that predatory mite populations are able to increase to levels needed for *C. vitis* control at these and lower temperatures under optimal conditions. The upper developmental threshold and the intrinsic rate of increase is however lower at temperatures ≥ 25ºC suggesting biological control will be compromised when high temperatures occur later in the summer season. It is possible that supporting *T. pyri* populations through inundative releases may aid in controlling mid to late season *C. vitis* resurgences and reduce overwintering females. Releasing a compatible predatory mite that demonstrates a higher intrinsic rate of increase during warmer temperatures should be investigated. Further research is needed to determine the efficacy of mass-releases, the optimal number to release and the best timing for effective control.

**Pesticide impacts**

In determining the lethal and sub-lethal effects of commonly applied vineyard pesticides in labSoratory bioassays we found that five of the six compounds tested were not directly toxic to *T. pyri* adult female or juvenile mites. Paraffinic oil treatments however resulted in consistent mite mortalities greater than 50%. Sulfur and mancozeb treatments significantly reduced the reproductive potential and fecundity of female mites exposed as juveniles. From these results it can be concluded that paraffinic oil and sulfur

should be limited in the rotation plan for seasonal powdery mildew control. Direct contact with oils are most detrimental to *T. pyri* and applications should be avoided when seasonal vine or canopy growth does not provide adequate refuge for active predatory mites (i.e. minimal leaf cover at bud-break).

Decreases in predatory mite populations have been linked to repeated sulfur applications in vineyards to control powdery mildew. Results from this research suggest that predatory mite suppression is potentially due to sub-lethal impacts affecting the fecundity of adult female mites exposed as juvenile mites. It is likely however that other factors, such as pesticide repellency and *T. pyri* dispersal due to food scarcity, may also result in reductions of predatory mites.

Continued research is needed to determine the impact of pesticide sprays on *T. pyri* behavior and to test the additive effects from repeated exposure to these compounds on beneficial arthropod populations in large-scale vineyard field experiments. Finally, whey powder and the two synthetic fungicides bioassays resulted in negligible impacts on *T. pyri* and should be considered when developing integrated control programs for powdery mildew management in vineyards.

**Enhancing beneficials with MeSA**

Laboratory and field experiments determined the potential of employing MeSA lures to attract and retain *T. pyri* to enhance biological control in vineyards. Laboratory research indicated a significant attraction of *T. pyri* to synthetic MeSA at three dose levels in a two-choice test. There was no indication of predatory mite repellency to any of the six doses of MeSA tested in the olfactometer bioassays. Results from the

laboratory bioassays indicated that *T. pyri* responds positively to MeSA and will potentially increase predatory mite activity and abundance when employed in vineyards.

Field experiments using synthetic MeSA lures at two rates (low or high lures per plot) resulted in variable results in *T. pyri* population density between the two vineyard sites. One site displayed increased seasonal abundance of predatory mites in MeSA plots while the other site contained higher numbers of predatory mites in control plots. A potential explanation for these unexpected results may be the differences in pest species and abundance between the two sites. In particular, the availability of *C. vitis* prey may have influenced *T. pyri* behavior as rust mites were virtually absent in the site where predatory mites showed no response to MeSA. Predatory mites are known to learn to associate chemical cues with pest presence and host suitability in laboratory studies and this may explain the inconsistent results between the two vineyards. Other potential explanations include genetic variability within local populations, limited dispersal distance in *T. pyri* and the identification of additional HIPV's present in mite infested grape that may be necessary to elicit a positive response. Additional research is necessary to identify important volatiles released from *C. vitis* infested grape leaves and the impact these compounds may have on *T. pyri* behavior.

The effect of MeSA lures on other beneficial arthropods and pests were also evaluated during these experiments. An overall positive response of Coccinellidae was found with higher mean seasonal abundance at both sites in both years. MeSA lures did not appear to increase or decrease populations of *C. vitis*, Tetranychid mites or pest thrips. In conclusion the decision to deploy synthetic HIPV's should be based on the

careful monitoring of pest population abundance as the presence or absence of particular pest species is likely to influence the response of target beneficial arthropods. Further research is needed to determine the ecological impacts of releasing synthetic MeSA on plant fitness and associative learning in predators. The application of MeSA during inundative releases of *T. pyri* in vineyards in order to retain predator populations in infested areas should also be explored.

In summary, *T. pyri* is an important predator in Pacific Northwest vineyards during the early part of the growing season. Predatory mite populations appear to be negatively impacted by high temperatures and commonly used horticultural oils and sulfur. MeSA was attractive to *T. pyri* in laboratory olfactometer experiments but its impact on predatory mite populations in field experiments was variable. It is believed that conservation and enhancement of *T. pyri* populations is achievable through appropriate pesticide choices, well-timed spray rotations and the employment of MeSA lures when adequate food resources are available. Future work should include, researching the impact of mass released *T. pyri* during pest mite outbreaks, as well as the release of other compatible predatory mites such as *Amblyseius andersoni*.

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# Appendices

## Appendix A

### Chapter III – Determining mortality thresholds for control group

Pesticide laboratory bioassays: thresholds used to determine which group, no spray or water treated would represent the control group. Accepted mortality threshold of 20% was used to determine cut-off point.

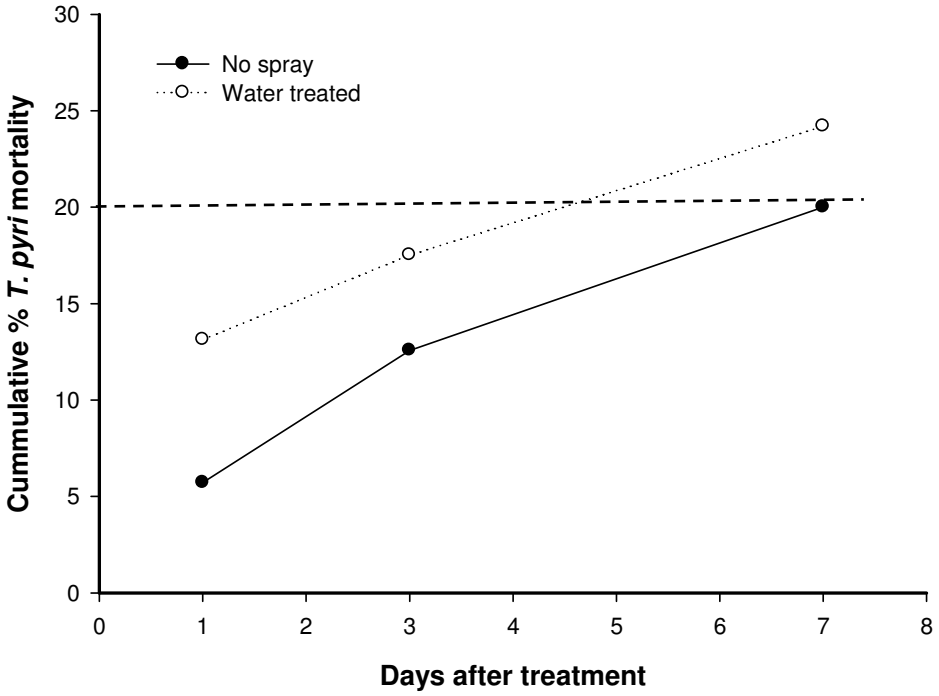


**Figure A.1**. Juvenile *Typhlodromus pyri* cumulative mortality in untreated and water treated bioassays. Mortality greater than 20% (dashed line) is above the accepted threshold to represent the control group.

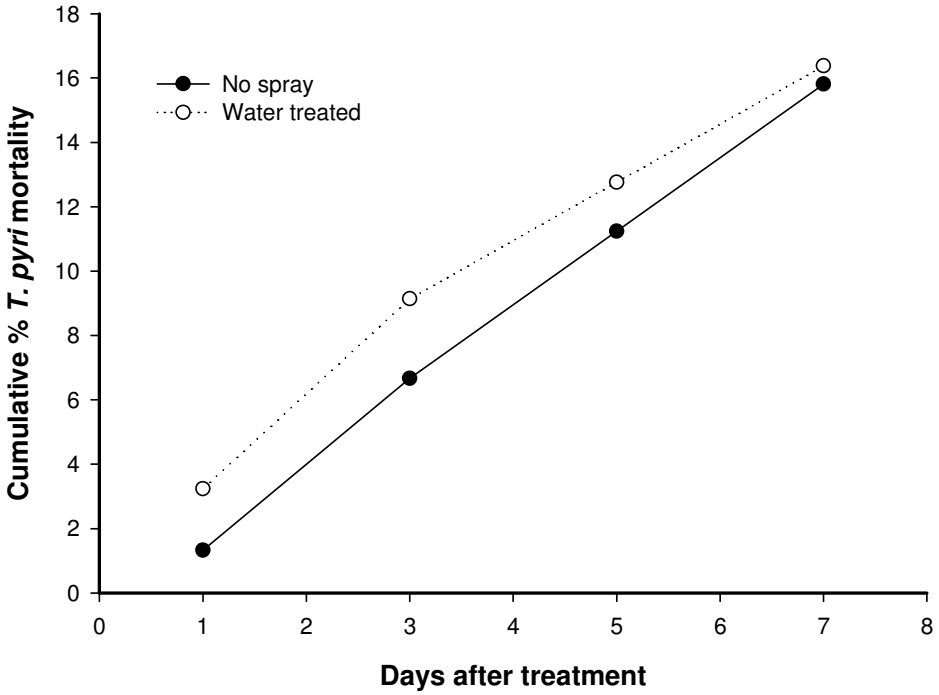


**Figure A.2**. Adult female *Typhlodromus pyri* cumulative mortality in untreated and water treated bioassays. Mortalities did not exceed accepted 20% threshold in adult bioassays.

## Appendix B

### Chapter IV – Preliminary tests for y-tube symmetry

Preliminary experiments conducted to test for bias in y-tube olfactometer setup

Methods: The glass olfactometer (Figure B-1, 2) was fitted with a copper wire walking platform and set to blow compressed air through both arms of the y-tube at 1.3 liters/minute. Clean air with no odor source was blown through each arm of the y-tube. Four replications of 18 adult female mites were introduced into the set-up and arm choice recorded for each individual mite. Data analysis was conducted to determine the proportion of mites choosing between the two arms.

Results: No predatory mite bias to either the right or left arm was detected (P = 0.453) when no odor source was present. These results suggested symmetry in the y-tube setup that would not bias mite response during MeSA experiments.

**Table B.1**. Contingency table analysis to determine differences between test replicates.

| Statistic | DF | Value | Probability |
|---|---|---|---|
| Chi-Square | 3 | 2.39 | 0.495 |
| Likelihood Ratio Chi-Square | 3 | 2.43 | 0.488 |

**Table B.2**. Binomial test using 50:50 distribution to test for differences in arm choice.

| Arm choice | Percent | Frequency |
|---|---|---|
| Left | 51.4 | 37 |
| Right | 48.6 | 35 |
| | | |
| P-value | 0.453 | |
| Sample size | 72 | |

## Appendix C

## Chapter V – Preliminary results from 2008 MeSA field trials

Methods: Experiments were conducted at a single vineyard site located in Yamhill Co., Oregon. Vineyard plots approximately 150 $m^2$ were replicated three times. Treatments included synthetic PredaLure MeSA lures (4/plot) or no lure control plots. Predatory mite densities were determined by leaf counts (n = 30/plot). Yellow sticky traps (6/plot) were used to count population abundance of other beneficial arthropod taxa including Hymenoptera (parasitic wasps), Coccinellidae, Staphylinidae, Syrphidae, Anthocoridae, Geocoridae, Rhaphidiidae, Chrysopidae, Anystidae, Thripidae, Aeolothripidae and Araneae.

Results: Leaf counts showed no presence of *T. pyri* predator mites or pest mites (Tetranychid or Eriophyid) throughout the sampling season in both control and MeSA baited plots. On October 22, 2008 a brief assessment of blackberry leaves from hedgerows bordering MeSA plots (~ 25 m distance) found *T. pyri* mites (approximately 3 per15 leaves) in addition to Tetranychid pest mites. Based on these results experiments were conducted in two new vineyards with known *T. pyri* populations in 2009-2010. Total counts of other beneficial arthropods captured on sticky cards (Figures C-1, 2, 3) display higher counts in MeSA plots on approximately seven given sample dates out of thirteen.

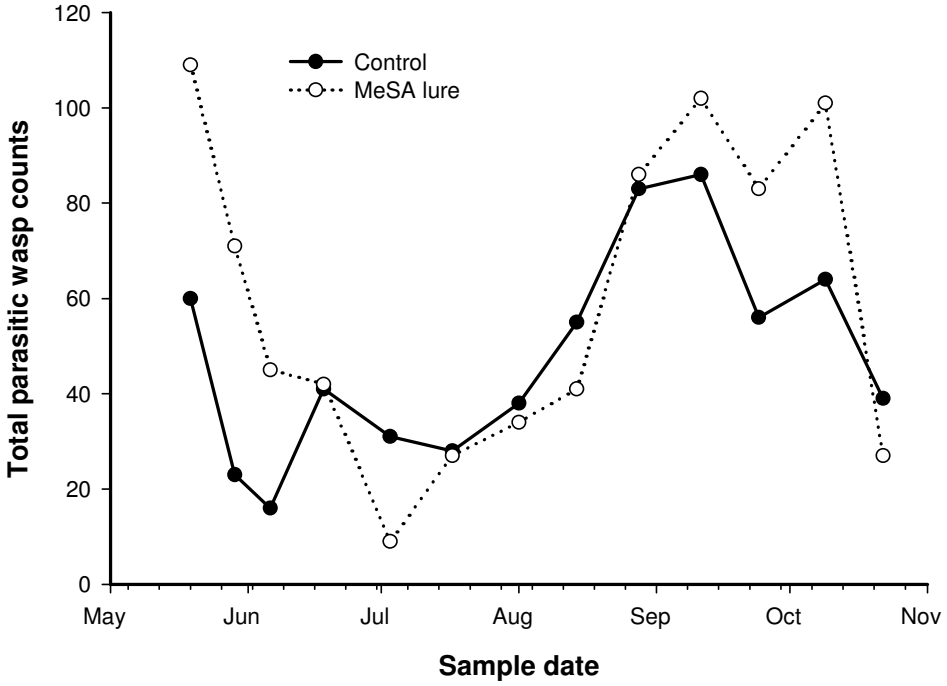


**Figure C.1**. Total parasitic wasp (Hymenoptera) counts on sticky traps in MeSA baited and control blocks in 2008.

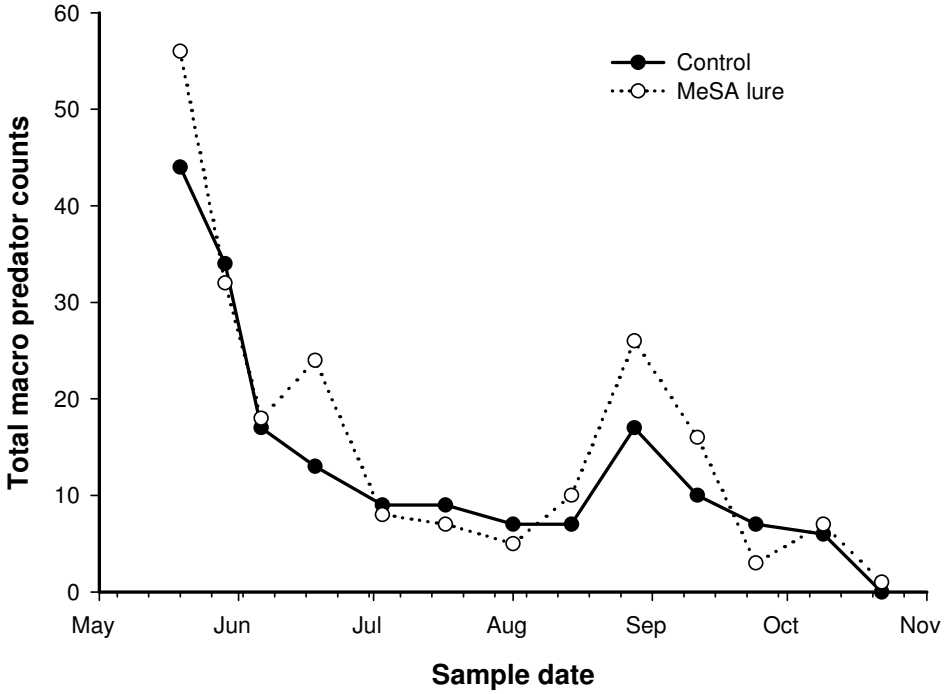


**Figure C.2**. Total macro predator counts on sticky traps in MeSA baited and control blocks in 2008.

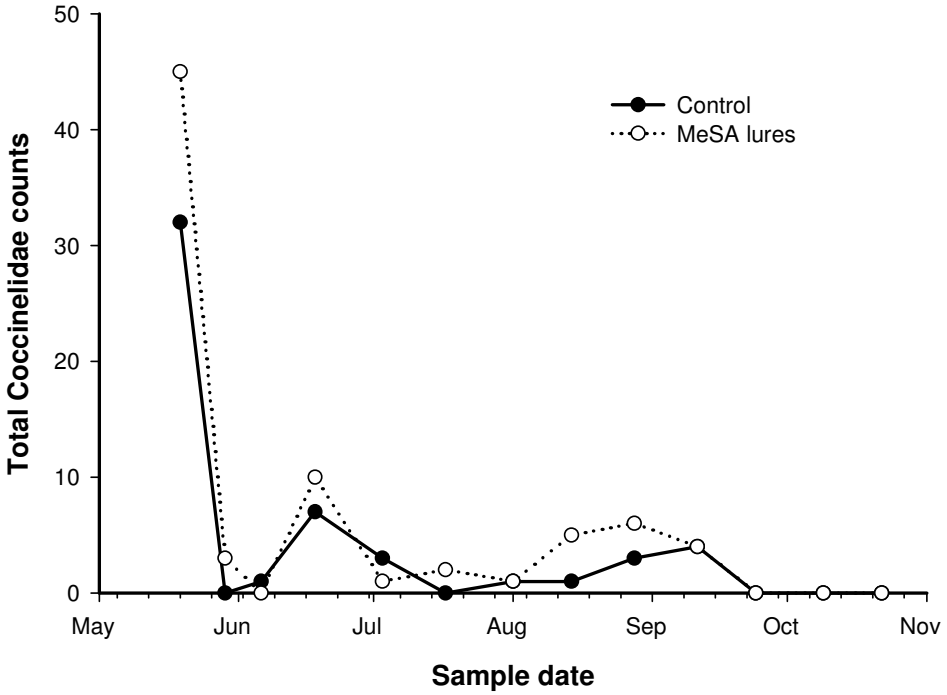


**Figure C.3**. Total Coccinellidae counts on sticky traps in MeSA baited and control blocks in 2008.