

AN ABSTRACT OF THE THESIS OF

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Title: Tactics for Enhancing Conservation Biological Control of Twospotted Spider Mites, *Tetranychus urticae* Koch, in Pacific Northwest Hop Yards.

Abstract approved:

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The twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a worldwide pest of numerous agronomic and horticultural plants. Conservation biological control of this pest can be unreliable due to asynchrony of natural enemies and certain horticultural practices that have a direct or indirect affect on natural enemies and the pest, such as sulfur fungicides applied for control of powdery mildew diseases. In this research, means to enhance the reliability of conservation biological control in hop through deployment of synthetic herbivore-induced plant volatiles and altered sulfur fungicide timing were investigated. Methyl salicylate (MeSA) is an herbivore-induced plant volatile (HIPV) that is attractive to several key predators of twospotted spider mite and hop aphid, *Phorodon humuli* (Schrank) (Homoptera: Aphididae). A two-year study was conducted to evaluate the recommended commercial use of synthetic MeSA in hop yards in Oregon. Slow-release MeSA dispensers were stapled to supporting poles in 0.5 ha plots and these plots were compared to a paired non-treated plot on each of three farms in 2008 and 2009. Across

both years, there was a trend for reduced (range 40 to 91%) mean seasonal abundance of *T. urticae* in five of the six MeSA-baited plots. *Stethorus* spp., key spider mite predators, tended to be more numerous (range 13 to 51%) in MeSA-baited plots compared to control plots on a given farm. Mean seasonal densities of hop aphid and other natural enemies (e.g., *Orius* spp. and *Anystis* spp.) were similar between MeSA-treated and control plots. Variability among farms in suppression of twospotted spider mites and attraction of *Stethorus* spp. suggests that the use of MeSA to enhance CBC of spider mites in commercial hop yards may be influenced by site-specific factors related to the agroecology of individual farms or seasonal effects that require further investigation. The current study also suggests that CBC of hop aphid with MeSA in this environment may be unsatisfactory.

Other studies were conducted during 2007 to 2009 in Oregon and Washington hop yards to evaluate the effect of timing of sulfur applications on twospotted spider mites and their predators. In both regions, applications of sulfur made relatively late in the growing season (mid-June to mid-July) were associated with the greatest exacerbation of twospotted spider mite outbreaks, particularly in the upper canopy of the crop. The severity of outbreaks was closely associated with sulfur applications made during a relatively narrow time period coincident with the early exponential phase of twospotted spider mite increase and rapid host growth. A nonlinear model relating mean cumulative mite days during the time of sulfur sprays to the percent increase in cumulative mite days (standardized to a non-treated plot) explained 58% of the variability observed in increased mite severity related to sulfur spray timing. Spatial

analysis of twospotted spider mites counts and their eggs in the Oregon plots indicated patterns of motile stages of twospotted spider mites were similar among leaves treated with sulfur versus nontreated leaves; however, in two of three years eggs were less aggregated on leaves of sulfur-treated plants, pointing to enhanced dispersal. Apart from one experiment in Washington, relatively few predatory mites (Phytoseiidae) were observed during the course of these studies and sulfur-induced mite outbreaks generally occurred irrespective of predatory mite abundance. These studies indicate that sulfur applications induce outbreaks in hop through direct or indirect effects on twospotted spider mites, mostly independent of predatory mite abundance or toxicity to these predators. Avoidance of negative side effects of sulfur sprays was achieved by carefully timing applications to periods of low spider mite abundance and slower host development, which is generally early to mid-spring for hop. Together, these findings point to strategies to enhance the reliability and efficacy of conservation biological control of twospotted spider mites in hop and perhaps other systems.

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October 6, 2011

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Tactics for Enhancing Conservation Biological Control of Twospotted Spider Mites,
Tetranychus urticae Koch, in Pacific Northwest Hop Yards

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Agriculture

Presented October 6, 2011

Commencement June 2012

Master of Agriculture thesis of Joanna L. Woods presented on October 6, 2011.

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Joanna L. Woods, Author

ACKNOWLEDGEMENTS

The creation of this thesis involved many people, and I hope I can adequately extend my appreciation. Firstly, I would like to offer my heartfelt appreciation to Dr. David Gent for his continuous belief in my capabilities and his never-ending drive to challenge and motivate me to remain unsatisfied with 'okay.' Thank you also to Dr. Glenn Fisher for being a source of encouragement and providing the kudos a graduate student often needs. I would also like to thank Dr. Greg Thompson for always knowing I was going to get my masters, even though it took longer and looks different than the one originally planned in his office. Thank you also to Dr. Mike Borman for his willingness to serve as my graduate student representative. A special thanks to Dr. Amy Dreves for the quality time spent in the hop yard - you were a shining example of an enthusiastic entomologist! Thank you also to Dr. Jerry Krantz for identifying phytoseiid species. Many people were involved in data collection and I would like to thank Chase Annen, Suzy Kropf, Leah Mancino, Katie Roseboro, the Jana Lee lab, the David James lab, and especially Kazandra Lewis and Tora Brooks for their countless hours cheerfully spent scanning sticky cards. The funding for this thesis research was provided by the Oregon Hop Commission, Oregon State University, Washington State University, USDA-ARS CRIS Project 5358-21000-035-00, USDA-ARS CRIS Project 5358-22000-032-00D, and USDA-CSREES Western IPM Center Award No. 2007-34103-18579. Thank you to the Department of Crop and Soil Science, Botany and Plant Pathology, and Agricultural Education for providing an avenue for me to pursue all of my interests and educational goals. Also, many thanks go to everyone in the

Gent lab for their support and making so many of my days a true joy. I am so thankful for my family, for their love and enthusiasm for all I was doing. And to my husband, Shawn: I can't even begin to describe the level of support, sacrifice, and love you have provided – I am so blessed to have you in my life, and this masters is as much yours as it is mine. And most importantly, may all the glory go to my Lord and Savior Jesus Christ, who is able to do exceedingly abundantly more than we can ask or imagine.

CONTRIBUTION OF AUTHORS

Dr. David Gent was involved with all aspects of these studies. Chapter 2 is a result of a collaborative study with Drs. David Gent, Jana Lee and David James. Chapter 3 is a result of collaborative study with Drs. David Gent, Amy Dreves, Glenn Fisher, David James and Larry Wright. All authors were involved in the data collection, experimental design of the studies, and interpretation of the results. Dr. Glenn Fisher and Larry Wright were involved with the treatment administration for the study described in Chapter 3.

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GENERAL INTRODUCTION

Arthropod pests cause substantial damage to plants in managed and natural ecosystems throughout the world (Oerke et al. 1994, Ehler 1998). Crop losses from arthropod pests in the U.S. were estimated at 15.6% due to reductions in both the quantity and quality of yield (Oerke et al. 1994). In the past 40 years global food production has doubled, and use of pesticides have played a significant role in this increase as crop loss has been abated through their application (Oerke 2006). Elevated pesticide usage to meet the demands of an increasing world population can disrupt the natural pest and predator equilibrium (Wratten et al. 2007). While pest management has been necessary for crop production for thousands of years, the advent and use of synthetic pesticides have been indicted in the generation of new pest problems, including pesticide resistance, pest resurgence, and environmental impacts (Huffaker et al. 1969, Hardin et al. 1995, Lewis et al. 1997, Ehler 1998).

Integrated pest management (IPM) describes a decision-making process that considers multiple tactics for the management of all pests in a manner that reduces environmental and ecological impact (Ehler 2006). IPM systems, when designed and deployed appropriately, are predicted to minimize issues related to pesticide resistance, pest resurgence, and negative environmental impacts (Ehler, 2006). A potential component of IPM is the use of conservation biological control (CBC), which seeks to enhance the resident environment for natural enemies to improve biological control of pest species (Barbosa et al. 1998). Numerous CBC tactics have

been developed to provide farmers with tools to reduce pesticide use with little impact on crop quality or yield (Barbosa et al. 1998, Jonsson et al. 2008). Successful utilization of CBC requires a thorough and intelligent investigation of crop production practices that may favor or suppress natural enemies and pest arthropods to best mitigate yield and quality losses while reducing pesticide use.

The twospotted spider mite, *Tetranychus urticae* Koch, is a cosmopolitan pest that plagues numerous cropping systems worldwide (van de Vrie et al. 1972). In natural conditions, twospotted spider mites generally are effectively regulated by predatory arthropods (Huffaker et al. 1970, James et al. 2001), suggesting that this pest can be effectively suppressed by CBC in managed ecosystems. In many cropping systems, the twospotted spider mite only reached pest status consistently with the advent of synthetic pesticides post World War II (Boudreaux 1963, Huffaker et al. 1969). The sometimes indiscriminate use of pesticides has been implicated in the destruction of twospotted spider mite natural enemies and the subsequent release of twospotted spider mite populations from predator regulation (McMurtry et al. 1970). Routine use of chemical intervention for pest management also has led to problems such as human and environmental health concerns, pesticide resistance, outbreaks of secondary pests, and pest resurgence (Lewis et al. 1997). Therefore, development of stable, reliable, and practical biologically-based approaches are needed to enable rational and durable management approaches for this pest.

Pest Resurgence

Pest resurgence, as defined by Hardin et al. (1995) is an increase in the ‘target’ arthropod pest species. Pesticide use has been indicted as a major cause of pest resurgence and secondary pest outbreaks in agriculture (Boudreaux 1963, Hardin et al. 1995). A consequence associated with pesticide use includes the disturbance of natural enemy populations. Certain broad-spectrum synthetic pesticides, used widely after World War II have been shown to be extremely toxic to natural enemies (Boudreaux 1963, Huffaker et al. 1969). DeBach (1964) cites the use of more selective pesticides as essential in the conservation of natural enemies and the avoidance of ‘biological explosions,’ e.g., outbreaks of secondary pests and/or pest resurgence. Arthropod surveys of wild and feral plant populations have indicated that in many undisturbed natural systems, common pest arthropods (e.g., spider mites and aphids) occur at non-damaging levels (Huffaker et al. 1970), and this was observed on feral and wild hop plants (*Humulus lupulus* L.) in both Washington and Idaho (James et al. 2001, Gardiner et al. 2003). These surveys provide circumstantial evidence to suggest that certain practices or conditions in managed agroecosystems are favorable to arthropod pests and/or limiting to beneficial arthropods.

In addition to the potential for the disturbance of natural enemies from pesticide use, pest resurgence could be attributed to other factors including enhanced fecundity, reduced herbivore-herbivore competition, sublethal behavioral effects, and alteration of host plant quality, among other potential mechanisms (Bartlett 1968, van de Vrie et al. 1972, Hardin et al. 1995). While pesticide use is often correlated with

increased pest problems, an explanation of natural enemy disturbance often may be too simplistic since many interrelated biological events also could be associated with natural enemy abundance (Hardin et al. 1995). Numerous factors likely are involved in pest resurgence and research to identify these factors could aid in the development of reliable CBC tactics and increase the efficiency of biologically-based pest management (Jonsson et al. 2008).

In a review of arthropod pest resurgence, Hardin et al. (1995) provided several mechanisms associated with arthropod pest resurgence and pesticide use: a direct effect of the pesticide(s) on the pest and predatory arthropods; an effect on the plant; and/or a combination of these factors. A review of some of these mechanisms is detailed below. Conclusively determining the cause of pest resurgence is problematic and many field studies may offer correlative evidence but have not establish causality (Hardin et al. 1995), and an incorrect explanation for pest resurgence may lead to an incorrect response to a pest outbreak.

Hormoligosis

Stimulation of fecundity due to a sublethal dose of a pesticide is frequently termed hormoligosis or hormesis (Luckey 1968, Cohen 2006). Hormoligosis describes an increase in fecundity that could be due to a shortened life cycle of immature stages, an increased adult life span, and/or a female-biased sex ratio (Cohen 2006). The basis of hormoligosis is a stimulation induced by a mild stressor that in turn induces enhanced growth and/or reproduction. For example, Luckey (1968) postulated that

hormoligosis was responsible for the elevated reproduction in locusts and could be a contributing factor to swarming behavior (Cohen 2006).

It is well known that hormoligosis in spider mites can be induced by several pesticides (Dittrich et al. 1974). James and Price (2002) observed increased fecundity (up to 26%) when twospotted spider mites were sprayed with field rates of imidacloprid, a systemic neonicotinoid pesticide. Similar results occurred in mites that received imidacloprid through ingestion, however there was a lag time of 6 days when the compound was ingested compared to direct exposure. In a similar study by Ako et al. (2004), imidacloprid did not increase fecundity in twospotted spider mites, although differences in experimental design may have contributed to these differences. On elm trees, twospotted spider mite fecundity also was enhanced when treated with imidacloprid. When two primary predators (*Stethorus punctillum* Weise and *Chrysopa rufilabris* Burmeister) were fed prey exposed to imidacloprid, there was evidence of reduced mobility in the predators (Szczepaniec et al. 2011). Establishing hormoligosis as the primary cause of pest resurgence can be strongly influenced by experimental methods, and this is well highlighted by the range of results in the literature.

Sublethal Behavioral Affects

An indirect effect from pesticide use may be increased plant-to-plant dispersal because a pesticide acts as an irritant with repellent and/or repulsive effects on an arthropod (Rodriguez and Rodriguez 1987, Walsh and Grove 2005). In spider mites, dispersion among or within plants occurs in response to plant age as spider mites

move to more nutritionally suitable leaves or other plant tissues (Rodriguez and Rodriguez 1987). Intra-plant dispersal in spider mites is often in response to a stressor, such as plant desiccation and/or food shortage due to large numbers of mites feeding on a plant (Kennedy and Smitley 1985, Li and Margolies 1993). Pre-reproductive females are the most likely colony members to disperse, and females may delay approximately half of their accumulation of biomass until they reach new food resources (Mitchell 1973, Kennedy and Smitley 1985). Intra-plant dispersion of spider mites is commonly through aerial transport, and twospotted spider mites are known to exhibit a dispersal posture in which they raise their forelegs and catch wind currents (Kennedy and Smitley 1985, Rodriguez and Rodriguez 1987). Studies conducted in peanut indicated that twospotted spider mites could be dispersed on winds of only 8 km/h (Boykin and Campbell 1984).

Once spider mites disperse to a suitable host plant or tissue, females display a pseudo-colonial behavior since their reproductive potential is no longer inhibited and the food source is rapidly exploited (Boudreaux 1963, Kennedy and Smitley 1985). The cycle of host exploitation and subsequent dispersal or migration characterizes spider mites; however this cycle can be amplified through the influence of an irritant, such as a pesticide residue (Kennedy and Smitley 1985). This may result in more rapid dispersal and in turn uninhibited reproduction, as more spider mites disperse onto new host tissue and exploit the food resource (Walsh and Grove 2005). In an agricultural setting, the implications for pest management can be substantial when spider mite

dispersal is enhanced and mite reproductive potential is released (Kennedy and Smitley 1985).

The dispersal patterns of spider mites within a specific host can also influence the predator-prey dynamics and influence the efficacy of biological control, as well as inform sampling strategies (Wright and Cone 1999). In hop, a plant that has a bine-like growth habit and is commercially produced on a 5 m trellis system, twospotted spider mites disperse vertically in the hop canopy (Sites and Cone 1985). Studies characterizing the inter- and intra-plant dispersal of twospotted spider mites in hop yards suggest that the rapid growth habit of the hop plant may lead to population instability in predatory mites because of empty prey patches (a site where the spider mites have taken up residence and no predators are present) due to superior dispersal of twospotted spider mite compared to predatory mites (Strong et al. 1997, Strong et al. 1999). For example, the cultural practice of removing basal foliage in hop for control of foliar diseases reduced the efficiency of predator mite dispersal, but did not influence spider mite resource assimilation and dispersion (Strong et al. 1999). The authors suggest that through careful management, the entire hop yard could become a metapopulation landscape, thus enhancing the stability of biological control.

Nutritional Quality of Host Plant

The application of certain pesticides can alter the nutritive quality of the host plant as documented in compounds such as DDT and carbofuran (Boudreaux 1963, Hardin et al. 1995). However, alteration of plant quality in conjunction with a change

in herbivory behavior or fecundity has not been well documented (Hardin et al. 1995). Other stressors, such as drought, incite a change in plant quality that is known to induce arthropod outbreaks, a phenomenon that has been documented in spider mite outbreaks on both drought-stressed bean and grape (English-Loeb 1990, Stavrinides et al. 2010).

Plant size and age can influence host plant nutrition and arthropod population dynamics. A study conducted on motherwort, *Leonurus cardiaca*, indicated that there was a density-dependent relationship between twospotted spider mite population growth and plant size. On smaller plants there was a negative relationship between population growth rate and pest density, but on larger plants population growth rate was unaffected by density dependence (Rotem and Agrawal 2003). Plant age also can have an effect on arthropod population dynamics. In chrysanthemum, younger leaves had lower densities of twospotted spider mites than older leaves, and this was attributed to higher levels of phenolic compounds in younger leaves, even though these leaves had superior nutritive value than older leaves (Kielkiewicz and van de Vrie 1990).

Host plant quality also can influence arthropod behavior. Certain insect species may develop a predilection for a certain degree of host plant quality. This was demonstrated when cerambycid beetles showed a preference for varying degrees of host plant quality through preferential selection of ovipositioning sites (Hanks 1999). In spider mites, large numbers of actively feeding spider mites are known to reduce

host plant quality and exponentially increase intra-plant dispersion behavior (Kennedy and Smitley 1985, Li and Margolies 1993, Nachman and Zemek 2002).

In cotton, photosynthetic rates were positively correlated with spider mite population growth (Karban and Thaler 1999). The authors suggested that levels of chlorophyll are potentially involved in spider mite growth rates and that plant developmental changes (e.g., reproductive to vegetative development) could be directly related to population growth and resistance to herbivory. In strawberry, spider mite densities were higher on fruit-bearing plants than on vegetatively-growing plants, and the onset of vegetative growth was postulated as a determinate of the seasonal decline of twospotted spider mites (Poe 1971, Shanks and Doss 1989). Other research in strawberry indicated that the onset of plant sexuality (flowering) enhanced plant nutritional quality and was linked to seasonal twospotted spider mite outbreaks on this crop (Walsh et al. 1998).

Destruction of Natural Enemies

The destruction of natural enemies from the use of pesticides has been inferred as the cause of pest outbreaks because pest populations are released from regulation (reviewed in Hardin et al. 1995). While harmful effects on natural enemies from the use of pesticides have been well documented in both field and laboratory tests (Croft 1990), many of the published studies may simply be correlative and may not establish a direct causal link to a pest outbreak (Hardin et al. 1995). Quantifying the impact of pesticides on natural enemies is complex as there are both direct and indirect effects

that need to be evaluated over time to determine their short and long-term effects (Johnson and Tabashnik 1999). Laboratory bioassays commonly are used to isolate the effect (direct or indirect) of a chemical on various life stages of an arthropod of interest (Croft 1990). A field setting is limited by the experimental controls that can be exerted, and typically laboratory experiments are undertaken as a first step to determine pesticide toxicity (Croft 1990).

Conservation Biological Control

Enhancing the resident environment through practices that conserve natural enemy abundance and diversity has been termed conservation biological control (Stern et al. 1959, DeBach 1964, Ehler 1998). In the broadest sense, CBC seeks to attract natural enemies and enhance the longevity and efficacy of resident natural enemy populations to suppress a pest or pests (Barbosa 1998, Kean et al. 2003). CBC differs from classical biological control in that arthropods are not released into the environment; rather the primary effort is directed towards encouraging the resident natural enemy population to thrive through selective pesticide applications, provision of refugia, and selection of cultural practices that have reduced impact on these arthropods (DeBach 1964, Şengonca 1998, Eilenberg et al. 2001). As such, successful CBC requires an in-depth understanding of the ecological system and determinates of pest resurgence to select a combination of appropriate tactics to provide stable pest suppression (Jonsson et al. 2008). The correct identification and study of the biological and ecological requirements of potentially important natural enemies is key

prior to successful implementation of CBC practices. This information allows for the selection of agronomic practices that promote natural enemy populations and the elimination or modification of practices that limit successful biological control (Naranjo 2001).

Some practitioners have argued that CBC is most successful when there is a paradigm shift toward habitat management, rather than placing the primary emphasis on one or a few pests (Lewis et al. 1997, Tscharntke et al. 2007). Given the spatial scale where processes may affect pests and natural enemies, as well as the various factors that are considered in CBC (e.g., predator-prey ratios, refugia, management practices, surrounding habitat, etc.), management of an agroecosystem at a spatial scale larger than an agricultural plot is often considered requisite. Management generally needs to occur at the landscape or whole-system scale (Lewis et al. 1997, Landis et al. 2000, Tscharntke et al. 2007). Habitat management as a component of CBC and the concepts of managing at the whole-system and landscape level are integrated into CBC implementation strategies (Landis et al. 2000, Tscharntke et al. 2007, Jonsson et al. 2010). When the whole system is considered, the complexity of trophic interactions, resource provisioning, and the functional traits of natural enemies are taken into account in identifying appropriate CBC strategies and tactics for a given agroecosystem (Straub et al. 2008). Considering these interactions enables a practitioner to focus efforts on enhancing components of CBC that foster natural enemies. Having a conceptual landscape perspective increases the likelihood for long-term success (Jonsson et al. 2008).

The management of a whole-system also requires consideration of all management decisions made to manage arthropods, pathogens, weeds, plant nutrition, as well as cultural practices and irrigation management (Lewis et al. 1997). Lewis et al. (1997) suggests that the long-term solution to the problem of the ‘pesticide treadmill’ is to economize on ‘built-in prevention,’ not chemical approaches to management. However, the management of both arthropods and plant diseases is frequently incompatible as applications of pesticides made for one pest can have non-target effects on another pest or its natural enemies. The principles of IPM and CBC seek to address this incongruence (Stern et al. 1959, Barbosa 1998, Lewis et al. 1997).

Resource Provisioning

The lack of a stable and consistent food source for predators can limit the effectiveness of CBC particularly when host plant growth and food resource availabilities are asynchronous, as often occurs within agricultural cropping systems (Barbosa 1998, Landis et al. 2000, Tschardtke et al. 2007, Grasswitz et al. 2009, Jonsson et al. 2010). The lack of plant diversity within agricultural monocultures may lead to reduced natural enemy diversity due to local extinction related to resource availability (Jonsson et al. 2010). Additionally, typical agroecosystems are fragmented mosaics of habitats that may not enable predators to find adequate food sources throughout the growing season (Tschardtke et al. 2007). Consequently, lack of sufficient refugia in these systems results in diminished predator populations that can lead to local seasonal extinction (Tschardtke et al. 2007). Increasing plant biodiversity

in cropping systems with non-crop plants and border plantings, as well as providing food sprays (e.g., sugar) for predators, can partially alleviate habitat fragmentation (Landis et al. 2000).

The provision of floral nectar and pollen resources as a means of habitat manipulation has been attempted in numerous systems, including vineyards, orchards, numerous row crops, turf grass, ornamental plants, and hop (Landis et al. 2000, Grasswitz et al. 2009, Jonsson et al. 2010). The degree of predation or parasitism reported is variable among studies, and each specific system may require a particular type of floral nectar resource to ensure positive effects on CBC. In a study conducted by Grasswitz et al. (2009), a blend of flowering ‘insectary’ plants was planted between hop rows and pest and predator levels were assessed on the hop plants over three growing seasons. In two of the three years of the study, twospotted spider mite abundance was reduced in the cover-cropped plots, however hop looper (*Hypena humuli* Harris) abundance was greater; hop aphid (*Phorodon humuli* Schrank) abundance was highly variable. The abundance of natural enemies was variable among plots; however there were greater numbers of spiders and *Nabidae* spp. in all three years of the study. For the hop system, it is likely that more study is needed to determine the correct blend and type of cover crop that best enhances CBC since a ‘multi-trophic approach’ is important to avoid negative impacts from the provisioning of floral resources to pest species (Winkler et al. 2010).

Early Season Recruitment and Herbivore-Induced Plant Volatiles

Early season recruitment of beneficial arthropods is thought to be an integral aspect of successful CBC (Barbosa 1998, Kahn et al. 2008, Simpson et al. 2011a). Because of the ephemeral nature of crops in most agricultural cropping systems, the establishment of predators early in the season prior to the development of high numbers of pest arthropods can be difficult to achieve.

Deployment of synthetic semiochemicals to enhance early season recruitment of natural enemies has been investigated recently as a means to improve the reliability of CBC. In response to plant injury or herbivory, most plants release specific semiochemicals, termed herbivore-induced plant volatiles (HIPVs), which can be repellent to herbivores or attractive to predators and/or parasitoids (Barbosa and Wratten 1998, James 2003b, Kahn et al. 2008, Orre et al. 2010). The use of semiochemicals as an attractant to predatory arthropods was first studied for recruitment of natural enemies in a laboratory setting, where predatory mites (Phytoseiidae) were attracted to plants fed on by twospotted spider mites (Dicke and Sabelis 1988, Dicke et al. 1998, De Boer and Dicke 2004). Since these seminal studies, CBC practitioners have attempted to isolate and utilize various chemical compounds and different experimental approaches to use HIPVs as a component of CBC (van den Boom et al. 2004, Pickett et al. 2006, Kahn et al. 2008). In a field setting, HIPVs such as methyl salicylate (MeSA), methyl jasmonate (MeJA), and others have been deployed on lures, controlled-release dispensers, and as liquid formulations sprayed directly onto the crop (James 2003a, James 2003b, James and

Price 2004, James 2005, Jones et al. 2011, Simpson et al. 2011a, Woods et al. 2011). Based on an 'attract and reward' approach, Simpson et al. (2011b) reported a potential synergistic response between the use of HIPVs and floral provisioning. HIPVs were deployed in sweet corn, broccoli, and grapevine in conjunction with buckwheat plantings and an increase in natural enemies as well as reduced pest damage was observed in some treatments. The results of some of these studies have been positive, while others have yielded mixed data (Woods et al. 2011, Orre et al. 2010), perhaps pointing to site-specific ecological factors and/or trophic interference that can influence the success of HIPVs as a CBC tactic.

Field testing of HIPVs has yielded variable results, and differences in experimental approaches (e.g., availability and consistency of lures, sachets, and sprayable formulations) may confound interpretation of results (Jones et al. 2011). It is also possible that certain crops or crop stages may be better suited to the use of HIPVs, as it is known that plant species, plant condition, and growth stage, among other factors, can interact with HIPVs, impacting efficacy (Khan et al. 2008, Dicke and Baldwin 2010, Simpson et al. 2011a). Another complicating factor is that trophic competition may occur due to non-target attraction of both pest and predatory arthropods that can negate the effects of HIPV deployment (Dicke and Baldwin 2010, Orre et al. 2010). Finally, attraction of enemies of natural enemies as well as hyperparasitoids by HIPVs could lessen the pest control benefits of these products in field studies (Prasad and Snyder 2006, Dicke and Baldwin 2010).

Limitations of Conservation Biological Control

A primary goal of CBC is to encourage an abundant and diverse natural enemy population, although the success and benefits can be difficult to quantify in practice (Wratten et al. 2007). In some circumstances, arthropod diversity can inhibit CBC due to intraguild predation among predators and reduce efficiency of pest suppression (Straub et al. 2008, Coll 2009). The complexity of the trophic interactions and the knowledge required to target the appropriate type of arthropod diversity may not be available, leading to inconsistent pest suppression by season or crop (Wratten et al. 2007). A study involving predatory beetles reported that intraguild predation and consumption of non-target prey reduced the efficiency of CBC, indicating that targeting generalist predators may reduce the success of CBC in some systems (Prasad and Snyder 2006). A diverse arthropod population can be ineffective due to intraguild predation, niche complementarities, and functional redundancy, and Straub et al. (2008) suggests that CBC should focus on the functional traits of natural enemies to better determine the 'right' type of diversity for a given system. While enhancing arthropod diversity and abundance is fundamental to CBC, the entomological and ecological knowledge needed to be successful may increase, potentially posing a barrier to CBC implementation (Jonsson et al. 2008).

Another practical limitation to implementing CBC is the perception by growers and pest managers (correct or incorrect) that CBC is unreliable and too complex, and hence the economic risk associated with CBC and IPM can be perceived to outweigh the benefits (Wearing 1988, Ehler 1998). The efficiency and economics of production

agriculture in developed countries is a potential barrier to CBC implementation due to the risk (either real and perceived) of financial loss (Ehler 1998). After conducting an extensive survey of research and extension specialists, Wearing (1988) concluded that the implementation of CBC and IPM should provide simple techniques that provide short-term financial advantages that fit the local needs of growers. Cullen et al. (2008) further suggested that farmers are more likely to implement and invest in a CBC practice if they are involved in the development. The authors proposed that farmer involvement enhances the perceived 'relative advantage' (Rodgers 2003) of the CBC practice in comparison to conventional practices, thereby increasing farmer adoption (Cullen et al. 2008).

The use of pesticides as a therapeutic treatment of pest problems provides a tangible sense of assurance that the pest will be brought under control and the perceived risk of not applying pesticides can be one of the largest barriers to farmer adoption of CBC practices (Wearing 1988). Uncertainty, low trialability, unacceptable risk, and long delay before payoff are key barriers to adoption and implementation of CBC (Cullen et al. 2008). While there are inherent risks associated with pesticide use such as pesticide resistance, environmental toxicity, and grower hazard due to exposure, the perceived and real costs of CBC may outweigh the benefits to an individual, since the externalities associated with pesticide use are subsidized in part by society (Wearing 1988, Cullen 2008).

Conservation Biological Control of *Tetranychus urticae*

Predators of twospotted spider mite consist of many generalist arthropods from the following taxa: Acarina (e.g., Anystidae) and the insect orders Coleoptera, Neuroptera, Hemiptera, Thysanoptera, and Diptera (McMurtry et al. 1970). Specialist predators include species of Acarina (Phytoseiidae), as well as mite-feeding lady beetles (Coccinellidae), *Stethorus* spp. (Huffaker et al. 1969, McMurtry et al. 1970, Biddinger et al. 2009). CBC and classical biological control of twospotted spider mite has placed a primary emphasis on the use of phytoseiids (Croft 1990, Strong and Croft 1995, Strong and Croft 1996, McMurtry and Croft 1997), and voluminous literature is devoted to biological control of twospotted spider mites by phytoseiids (Chant 1985, McMurtry and Croft 1997, Nyrop et al. 1998).

Phytoseiidae possess four developmental life stages: egg, larvae, protonymph, and deutonymph. The minimum developmental time for many phytoseiid species is approximately six days, however this is greatly influenced by temperature and species (McMurtry et al. 1970, Sabelis 1985). Members of the Phytoseiidae are considered effective predators of twospotted spider mites, with the number of prey consumed during development being, on average, less than 20 (Chant 1985, McMurtry et al. 1970). The density and distribution of prey influences the efficiency of phytoseiid feeding and when prey are abundant, less searching time is needed to locate suitable prey items (Sabelis 1985). Experimental evaluations of prey consumption varies greatly among phytoseiid species, however complete development can occur on as little as one spider mite larvae per day (McMurtry et al. 1970). Prey type and quality

influences ovipositioning rates and some species require a specific food source in sufficient quantity to reproduce (McMurtry et al. 1970). Among species, mean total eggs produced per female varies from 30 to 50 eggs (McMurtry et al. 1970).

Phytoseiid species that are capable of feeding on alternative food sources such as pollen, plant sap, and/or alternative prey have the potential to survive more readily in environments where tetranychid mites are limiting and these phytoseiid species may play a considerable role in effective biological control in agroecosystems (McMurtry et al. 1970, Sabelis 1985, McMurtry and Croft 1997, Nyrop et al. 1998).

Conservation of phytoseiids within a cropping system generally involves the careful selection of chemicals and cultivation practices that provide refugia during major disturbances such as pesticide applications and crop harvest (Croft 1990). Nyrop et al. (1998) concluded that the phytoseiids themselves must possess certain characteristics to become persistent and efficient biological control agents in the system, including the tolerance of some level of pesticides, utilization of alternative food sources for survival, and a high tolerance to adverse abiotic conditions. Predators are only effective if they are able to survive the ephemeral nature of a cropping system and natural enemies that are unable to use alternative food sources may lead to predator starvation and subsequent pest outbreaks (Croft 1990, McMurtry and Croft 1997). While specificity is advantageous, natural enemies that face extinction due to decline in a primary food source may not provide consistent control in a dynamic pest-predator system (McMurtry and Croft 1997, Nyrop et al. 1998).

Based on these factors, perennial cropping systems have been suggested as a more suitable environment for phytoseiids compared to annual cropping systems due to less habitat disturbance and provision of refugia (Nyrop et al. 1998). However, phytoseiids are known to be sensitive to broad-spectrum pesticides (Croft 1990, Nyrop et al. 1998), which still presents challenges in perennial cropping systems in modern agriculture. Some strains of phytoseiids have shown resistance to certain insecticides and have shown promise as biological control agents in IPM systems (Hoy 1985, Croft 1990, Nyrop et al. 1998). While selection for pesticide-resistant strains is feasible in a controlled setting, the effectiveness of releasing and establishing pesticide-resistant strains in the field has been mixed due to potential interbreeding, application of other deleterious pesticides, and/or inadequate food sources (Hoy 1985, Nyrop et al. 1998).

In addition to Phytoseiidae, many investigations of CBC of spider mites have placed emphasis on members of the family Coccinellidae and tribe Stethorini (e.g., *Stethorus* and *Parastethorus* spp.) (McMurtry et al. 1970, Chazeau 1985, Biddinger et al. 2009). As a group, Stethorini possess characteristics that make them successful biological control agents: long-lived adults, rapid migratory potential of adults, ability to search out small prey patches, and ability to survive on alternative food sources (Biddinger et al. 2009).

Stethorini possess six immature life stages including the egg, four larval stages and the pupa (Chazeau 1985). The four larval stages are considered voracious feeders and total prey consumption during larval development can be in excess of 200 mites (McMurtry et al. 1970, Chazeau 1985). Development time from egg to adult can occur

in as little as two weeks in high temperatures (28-35 °C), with an average development time of approximately three weeks at moderate temperatures (McMurtry et al. 1970). In certain climates, Stethorini can produce as many as three generations per year and may survive longer than a year (McMurtry et al. 1970, Chazeau 1985). When environmental conditions are favorable, hibernation can be avoided or shortened (Chazeau 1985). Adult members of Stethorini have a high prey consumption capacity and can consume as many as 30-60 spider mites per day (McMurtry et al. 1970, Biddinger et al. 2009). Reproduction varies among species and lifetime egg-laying are documented to range from 123 to 501, however the common species in the Pacific Northwest, *S. punctum* (LeConte) and *S. punctillum* Weise, have a reproductive potential of approximately 221 and 279 eggs, respectively (Biddinger et al. 2009). In a review of Stethorini, Biddinger et al. (2009) stated that while Stethorini have long been considered good biological control agents when spider mite densities are high, Stethorini actually have the ability to search out and eliminate smaller patches of prey, an observation not noted in previous reviews (Chazeau 1985).

Enhancing Conservation Biological Control in Hop Production Systems

The hop plant, *Humulus lupulus* L., is a dioecious perennial plant with annual shoots that can climb to heights greater than 4 to 5m in a single growing season (Neve 1991). Female strobiles, termed cones, are produced on lateral branches and contain the economically valuable bittering acids that act as a preservative and flavoring of beer (Neve 1991, Barth et al. 1994). In this crop, the rapid growth habit of the plant

and production of copious amounts of succulent leaf tissue make it favorable to several arthropod pests and foliar diseases (Mahaffee et al. 2009). The two primary arthropod pests are the hop aphid and the twospotted spider mite, and the primary foliar diseases are powdery mildew, caused by *Podosphaera macularis* (Wallr.:Fr.) U. Braun & S. Takamatsu, and downy mildew, caused by *Pseudoperonospora humuli* (Miyabe and Takah.) (Mahaffee et al. 2009). Management of the arthropod pests typically requires the annual use miticide and aphicides, and numerous applications of foliar fungicides are made to manage diseases (Turechek et al. 2001, Mahaffee et al. 2003, Mahaffee et al. 2009, Gent et al. 2008).

Hop is a highly acceptable and preferred host of twospotted spider mites (van den Boom et al. 2003). Twospotted spider mites feed by piercing the host plant and removing plant sap through stylet-like mouthparts, which can result in a speckled or 'burned' appearance of the plant tissue (van de Vrie et al. 1969). On hop, severe infestations of twospotted spider mites can cause bronzing and an overall necrosis of the leaves and feeding on the cones can result in red, dry, and brittle cones that are unmarketable (Cranham 1985, James and Barbour 2009). Left uncontrolled in a favorable environment, feeding damage by twospotted spider mites can result in complete crop rejection (Cranham 1985, Neve 1991). Twospotted spider mite infestations can reduce α -acid content in the cones, as well as overall cone yield, although an economic threshold for twospotted spider mites has not been definitively determined on hop (Weihrauch 2005). However, in a study conducted on three hop cultivars in Germany, Weihrauch (2005) observed no economic damage (e.g., cone

yield, α -acid content, or cone color defects) from as many as 90 twospotted spider mites per leaf at harvest. Environmental conditions in Germany are similar to those of western Oregon, but the findings of this study may be specific to this region (Neve 1991). Hops grown in dry and arid climates (e.g., south-central Washington) frequently suffer from severe spider mite outbreaks, and it is unknown if comparable levels of spider mites as reported by Weihrauch (2005) would impact crop quality and yield in other environments (Cranham 1985, Neve 1991).

Photosynthetic rates and plant phase (i.e., vegetative or reproductive development) are indicators of high levels of plant quality and in other crops, such as strawberry, have been correlated with the onset of twospotted spider mite population increases (Poe 1971, Shanks and Doss 1989). When hop leaves are at 75% leaf expansion, photosynthetic rates have peaked (Neve 1991, Kenny 2005). Transpiration rates, or the loss of water vapor, can be an indirect measurement of photosynthesis (Donahue et al. 1997). The highest transpiration rates in hop occur at bloom and the lowest have been measured to occur at harvest (Pokorný et al. 2011), indicating that plants may be most nutritionally suitable for spider mite development near bloom. In hop, there are additional factors such as leaf pubescence that are involved with spider mite host acceptance, and certain cultivars of hop with reduced leaf pubescence have higher levels of resistance to spider mite infestation (Peters and Berry 1980a, Peters and Berry 1980b).

The whole-system approach to pest management within the hop system involves some obstacles, as tactics used to suppress powdery mildew and downy

mildew can be disruptive to CBC of hop aphid and in particular, spider mites (James and Coyle 2001, James et al. 2003, Walsh and Grove 2005, Gent et al. 2009, James and Prischmann 2010). Predatory mites (Phytoseiidae) are known to be active predators of twospotted spider mites on hop (Pruszyński and Cone 1972, Strong and Croft 1993), however the annual growth habit of the crop may limit stable biological control via augmentative release of predatory mites, which were largely unsuccessful in controlling twospotted spider mites (Cranham 1985, Strong and Croft 1995, Strong and Croft 1996). Additionally, cultural practices employed for disease management also may disrupt biological control of twospotted spider mites. As mentioned above, basal foliage is removed from hop plants to reduce pathogen inoculum for disease management, but this practice may interfere with twospotted spider mite biological control because of the destruction of these refugia for predatory mites (Strong et al. 1997, Strong et al. 1999).

Additionally, fungicides utilized for disease management may disrupt biological control. In a study by Walsh and Grove (2005), fungicides commonly used on hop and grape were tested in laboratory bioassays to measure both the repellent and repulsive nature of each product to spider mites. In these studies, repellency was defined as movement from a pesticide treated surface to a non-pesticide treated surface, and repulsion was avoidance (non-movement) from a non-treated surface to a pesticide treated surface. While the results varied with each fungicide tested, the data indicate that certain fungicides can irritate and potentially enhance twospotted spider mite dispersion. The authors postulated that the enhanced dispersion observed in the

bioassays could be an indicator of the potential for dispersal of twospotted spider mites within a crop canopy. Walsh and Grove (2005) also included a sulfur fungicide in their studies, as sulfur is a common fungicide used extensively in both grapes and hop for the management of powdery mildew diseases (Mahaffee et al. 2009, Gent et al. 2009). Sulfur was among the most repulsive and repellent to spider mites in the bioassays (Walsh and Grove 2005). Sulfur fungicides are essential management tools for powdery mildew diseases on several host crops due to their excellent efficacy, low risk of pathogen resistance, and low cost. Integrating powdery mildew management with sulfur fungicides into a stable CBC system for twospotted spider mites presents a unique challenge because there are limited alternatives to sulfur fungicides.

Sulfur is the oldest known fungicide and in Homeric historical records sulfur was reportedly burned as a fumigant as early as 1000 B.C. (Holton 1926, Ware 2001, Williams and Cooper 2004). The use of sulfur continued throughout history as both a fungicide and an insecticide (Ware 2001). The mode of action of sulfur against fungi is not completely known, however it is speculated that sulfur is taken into the cytoplasm of the fungal cell and interferes with the mitochondrial respiratory chain (Ware 2001). Elemental sulfur is not overly effective as an insecticide; however sulfur formulations of lime-sulfur, dry lime sulfur, and 'soluble' sulfur have improved insecticidal properties compared to elemental sulfur (Holton 1926, Williams and Cooper 2004).

Deleterious effects from the use of sulfur and sulfur formulations were first documented in the early 1900s when lime-sulfur, used for the control of scale insects,

was the first documented case of pesticide resistance in an insect species (Melander 1914). Other deleterious effects from the use of sulfur include predatory mite toxicity, as documented in apple (Collyer and Kirby 1955, Childers and Enns 1975) and pecan (Ball 1981), and reduced predatory insect abundance in vineyards (English-Loeb et al. 1986, Hanna et al. 1997). Outbreaks of the European red mite, *Panonychus ulmi*, (Koch), a tetranychid mite, were exacerbated from the use of sulfur and sulfur dust in apple and peach orchards (Cutright 1944, Putman and Herne 1959, Lathrop and Hilborn 1967). In cotton, the control of the strawberry spider mite (*T. atlanticus* McGregor) was achieved from the use of sulfur dust, however, the twospotted spider mite and the Pacific spider mite (*T. pacificus* McGregor) were not completely controlled, and repeated applications of sulfur were necessary to obtain adequate control (Jackson and Leigh 1967).

The frequent use of sulfur in multiple crops has stimulated research on the effects of different sulfur formulations on pest and predatory mites. Bioassays conducted on a common predatory mite, *Galendromus occidentalis* (Nesbitt), have indicated that dry flowable formulations of sulfur are somewhat innocuous to adults, but toxic to larvae (Beers et al. 2009, Bostanian et al. 2009). Additionally, a dry flowable sulfur formulation was essentially innocuous to adult spider mites (Beers et al. 2009). Bioassays conducted by Auger et al. (2003) considered the effect of temperature and humidity on the acaricidal nature of a wettable sulfur, and the authors noted that mortality increased with temperature and humidity. However, in bioassays conducted by Beers et al. (2009), higher temperatures did not significantly impact the

mortality of pest or predatory mites exposed to a dry flowable sulfur. When a wettable sulfur formulation was applied to spider mites their population growth was suppressed by reducing mite longevity, however this formulation did not have an effect on any life stage of *G. occidentalis* (Price and James 2006, Stavrinides and Mills 2009). Alternatively, the application of lime-sulfur was toxic to both spider mites and *G. occidentalis* (Beers et al. 2009).

More recent field research on the negative impacts from the use of sulfur in grape vineyards indicated that season-long applications suppressed both pest and predatory mites (Prischmann et al. 2005, James and Prischmann 2010). In another vineyard study, the number of spider mites increased after sulfur applications were ceased early during the growing season (before bloom) and this increase seemed to occur irrespective of predatory mite abundance (Costello 2007). However, sulfur had little effect on spider mite abundance when applications were made only post-bloom. Sulfur is used extensively on hop for the management of powdery mildew, and repeated use of the sulfur up to bloom is deleterious to predatory mites and may exacerbate twospotted spider mite outbreaks (Gent et al. 2009). Gent et al. (2009) found that weekly sprays of a micronized wettable sulfur formulation on hop in both Oregon and Washington resulted in greater twospotted spider mite abundance compared to sprays of only synthetic fungicides, a horticultural oil, or water. Interestingly, similar levels of exacerbation of the spider mite outbreaks occurred in the experiments independent of the abundance of predatory mites. In both years of the study in Oregon, sulfur-induced spider mite outbreaks occurred in the near absence of

predatory mites in the plots (Gent et al. 2009). While it is quite clear that several species of predatory mites can be negatively impacted by sulfur, this finding and that of other recent studies indicate that a mechanism other than or in conjunction with natural enemy destruction may be involved in sulfur-induced spider mite outbreaks.

Gaps in Knowledge

The reliability of CBC for control of spider mites in hop is limited by a lack of understanding on how production system components influence the favorability (positively or negatively) of a hop yard to pest outbreaks. While spider mite outbreaks commonly occur in hop, it is unclear which mechanisms of pest resurgence are involved, their relative contribution to spider mite resurgence, and how these mechanisms interact. It is these factors that have resulted in a system where CBC of spider mites has been unreliable in commercial hop production, necessitating the annual application of miticides.

The successful commercial production of hop typically utilizes a large volume of inputs including fertilizer, water, and pesticides (Neve 1991, Gingrich et al. 2000, Mahaffee et al. 2009). The management of these inputs has a direct impact on arthropods and diseases. The interaction of application timing, plant health and growth stage, and pest and predator density requires a disentangling of these practices to identify mechanisms associated with pest outbreaks and resurgence. It is mostly unknown how these factors impact both pests and predators. Questions still remain regarding how to best enhance CBC through careful selection, application, and timing

of crop production inputs. Additionally, utilizing CBC tactics such as HIPV deployment and floral resource provisioning within the hop production system has yielded variable results, and further research is needed to validate and optimize these CBC tactics for optimum utility (Grasswitz et al. 2009, Woods et al. 2011).

The use of sulfur in numerous cropping systems is known to exacerbate spider mite outbreaks (Costello 2007, Beers et al. 2009, Gent et al. 2009), however the mechanisms underlying sulfur-induced mite outbreaks are unclear. Direct and indirect effects on natural enemies, alteration of plant quality, and/or a direct or indirect effect on the pest behavior may be involved with sulfur-induced spider mite outbreaks.

Natural enemy destruction from the use of sulfur has been observed in both field and bioassay settings (Prischmann et al. 2005, Beers et al. 2009), however these effects may only be correlative. Field and greenhouse studies in grape and hop have documented spider mite outbreaks in the absence or near-absence of predatory mites; little effect or no effect on other predators have been reported (Costello 2007, Gent et al. 2009). Sub-lethal behavioral effect of sulfur on spider mites are known to include repellency and repulsion, both potentially enhancing dispersion of spider mites within or among leaves and thereby indirectly increasing fecundity (Walsh and Grove 2005). How and to what degree these potential mechanisms interact remains unknown in hop and in other plant systems.

Research is needed to clarify the potential mechanisms behind sulfur-induced spider mite outbreaks, as well as develop and validate practical CBC tactics for the control of twospotted spider mites within a systems context. To this end, the purpose

of this thesis was to 1) validate the efficacy of methyl salicylate deployment as a means to enhance early season recruitment of natural enemies to hop yards and improve CBC in hop yards; and 2) characterize the impact of sulfur fungicide application timing on pest and predatory arthropods in hop.

CHAPTER 2:
EVALUATION OF AIRBORNE METHYL SALICYLATE FOR IMPROVED
BIOLOGICAL CONTROL OF TWOSPOTTED SPIDER MITE AND HOP
APHID IN OREGON HOP YARDS

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Experimental and Applied Acarology

Springer

223 Spring St.

New York, New York 10013

In Press

Abstract

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

Introduction

Conservation biological control (CBC) was defined in the 20th century as the ‘modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests’ (Eilenberg et al. 2001). Within the hop production system, CBC recently has focused on greater reliance on attraction of natural enemies (James 2003b, 2003c, 2005, 2006; James et al. 2003, James and Price 2004, Khan et al. 2008) to manage the two key arthropod pests of this crop in the Northern Hemisphere, twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and hop aphid, *Phorodon humuli* (Schrank) (Homoptera: Aphididae) (Cranham 1985, Neve 1991). Conventional management of these pests relies heavily on repeated applications of pesticides (James and Barbour 2009, Weihrauch 2009). Considerable efforts have been expended investigating augmentative biological control of spider mites, often focusing on phytoseiid mites (Pruszyński and Cone 1972, Strong and Croft 1995, Strong and Croft 1996, Lilley and Campbell 1999). However, augmentative biological control of spider mites generally has not been successful in commercial production, likely due to the rapid growth habit of the hop plant and certain cultural practices that disturb refugia (Strong and Croft 1995, 1996). Judicious use of pesticides and conservation of crucial habitat for natural enemies can conserve endemic populations of natural enemies on hop, and provide suppression of spider mite and hop aphid populations (van de Vrie et al. 1972, Aveling 1981, Cranham 1985, Strong and Croft 1993, 1996; Campbell and Cone 1994, Gardiner et al. 2003, James et al. 2001, 2003; James and Price 2004).

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

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

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

south-central Washington, this study sought to quantify the effects of MeSA deployment in commercial hop yards in Oregon on spider mites, hop aphids, and their natural enemies.

Materials and Methods

Experimental Design and Treatment Application

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

Each plot within a hop yard was 0.5 ha and was established in yards with a 'narrow' row spacing (plants arranged on a 2.1 m grid) or 'wide' row spacing (plants

arranged on a 2.1 x 4.2 m pattern). In 2008, both plots on farm B and C and the MeSA-baited plot on farm A were planted in a wide row configuration. The control plot on farm A was planted in a narrow-row configuration. Irrigation was supplied according to grower standard practices. Plots on farm A were irrigated by an overhead traveling sprinkler system, whereas plots on farms B and C were irrigated by a surface drip system. In 2009, plots on farm A were planted on a narrow-row configuration and plots on farm B and C were planted on wide-row spacing. Plots on farms A and C were irrigated with by an overhead traveling sprinkler, while plots on farm B were irrigated by surface drip. MeSA-baited and control plots on each farm were separated by 100 to 1600 m, a distance adequate to minimize plot-to-plot interference from MeSA volatilization (Lee 2010). The yards utilized on a given farm were comparable in size and cultural practice so that potential treatment effects due to MeSA deployment could be evaluated. In 2008, Predalure dispensers (2g, 30-day activity period) were deployed on 29 April at a rate of 185/ha by stapling the dispensers to poles at approximately 1.5 m from the ground in a regular pattern. This rate was determined based on previous research in Washington hop yards (Khan et al. 2008). In 2009, dispensers were deployed similarly on 29 April at a rate of 123 dispensers/ha. The deployment rate in 2009 was reduced compared to 2008 based on practical considerations of cost. Commercial deployment of MeSA dispensers could potentially replace one or more miticide applications, therefore we tested a lower rate (123/ha) that is near the typical cost of a single miticide application (approximately \$120US/ha). Deployment in both years was timed to coincide with the natural

occurrence of pest species, and corresponded with known activity of natural enemies in Oregon (Gent et al. 2009).

Cooperating growers were asked to avoid using broad-spectrum insecticides to conserve natural enemies, although given the practicalities of commercial crop production this request was not followed in all cases. Spider mites and aphids typically are managed with annual insecticide applications in commercial hop production (Barbour and James 2009), and most growers in the study applied an insecticide when pest levels exceeded their empirical economic thresholds or other criteria. Table 1.1 describes the insecticide and miticide applications made by the cooperating growers. To minimize confounding effects from these pesticide applications, data presented below (Tables 1.2 to 1.4) includes seasonal means up to the first application of a miticide or aphicide in an individual plot by the cooperating grower.

Arthropod Sampling

Leaf samples were collected at biweekly intervals beginning with a pre-treatment assessment in late April and continuing until cone harvest during mid- to late August. On each sampling date, 30 leaves were collected arbitrarily at a height of approximately 1.5 meters from the ground in each plot. Leaves were collected into paper bags, stored on ice in a cooler, and promptly transported to a laboratory. Motile spider mite stages, spider mite eggs, hop aphid nymphs and adults, predatory mites (Phytoseiidae), and mite-eating ladybeetles (*Stethorus* spp.) were identified and enumerated with the aid of a stereomicroscope. *Stethorus* spp. were not identified to

the species level. Predatory mites were either not detected or were present at very low levels (total of 3 or less on all sampling dates) in all yards both years and were not identified to the species level.

To assess motile natural enemies, four yellow, unbaited sticky cards (23 x 18 cm, Trece Inc, Salinas, CA) were stapled to supporting poles at a height of approximately 1.5 meters from the ground and approximately 3 meters from the corners of each plot. Each sticky card faced a different compass direction, which was re-randomized on subsequent sampling dates. Predalure dispensers were not attached to poles where sticky cards were placed to avoid potential sampling bias. Sticky cards were collected and replaced at biweekly intervals in coordination with leaf samples. Beginning in mid-June, biweekly arthropod samples were taken using a canopy ‘shake’ method as described in James and Price (2004). Hop bines were shaken for approximately 3 seconds over a 1-m² funnel, and natural enemies that fell into the funnel were brushed into a collection vial with 70% ethanol. Arthropods from shake samples from three bines were combined and considered a sub-sample. Three sub-samples were collected from each plot, thus a total of nine bines were sampled from each plot. Non-acarine, motile beneficial arthropods (macropredators) were identified and enumerated with the aid of a stereomicroscope.

Statistical Analysis

Densities of spider mites, hop aphid, and *Stethorus* spp. in the control and MeSA-baited plots were each plotted over time to derive arthropod development

curves. The area under the “population development curve” for each of these organisms was calculated using a macro available in SigmaPlot version 11.2.0 (Systat Software Inc, 2009, San Jose, CA) to produce a variable integrating arthropod density over an entire season. The area under the population development curve was analyzed in SAS version 9.2 (SAS Institute 2002) using a linear mixed model (MIXED procedure) after either square-root or log transformation to normalize residuals. Blocks (farms) were considered a random effect in the analysis. In these analyses, the hypothesis tested was the MeSA-baited plots would have greater abundance of natural enemies and correspondingly a lower abundance of spider mites and hop aphid. Therefore, statistical tests were considered one-sided where $H_0: \mu_c = \mu_{\text{MeSA}}$ and $H_A: \mu_c < \mu_{\text{MeSA}}$, where μ_c is the mean of area under the arthropod development curve for control plots and μ_{MeSA} is the mean for MeSA-baited plots. The sign for the alternative hypothesis is reversed when considering pests not natural enemies. As described above, insecticide and miticide applications were in some instances applied to the sampled plots. Therefore, the analysis of the area under the arthropod abundance curve should be viewed as a measure of arthropod abundance when MeSA is or is not applied in addition to other routinely applied control measures.

Inspection of the data by farm indicated that suppression of twospotted spider mite and attraction of *Stethorus* spp. varied among farms (detailed below), and therefore considering farms blocks in an analysis of variance potentially could obfuscate treatment differences. Thus, differences in the number of pest and beneficial arthropod species on leaves, sticky cards, and shake samples on individual

dates, and also summed over the season, among paired plots on individual farms were determined using the Mann-Whitney rank sum test (SigmaPlot version 11.2.0). Counts were log-transformed before the analysis to stabilize variances. Differences were considered significant at $\alpha = 0.10$.

Results

A diversity of beneficial arthropods, identified on leaf samples, shake samples, and sticky cards were found in association with hop plants (Table 1.2, 1.3 & 1.4). The major beneficial arthropod families and species from these samples included: Coccinellidae (aphid-feeding ladybeetle species including *Harmonia axyridis* Pallas, *Coccinella septempunctata* L., *Coccinella transversoguttata* Falderman, and *Cycloneda polita* Casey) and the mite-eating ladybeetles, *Stethorus* spp., minute pirate bug, *Orius tristicolor*, predatory thrips, parasitic hymenoptera, predatory Diptera (primarily flies from the families: Empididae, Dolichopodidae, Sarcophagidae, and Tachinidae), Cantharidae (generalist soldier-beetle species), brown and green lacewings (Chrysopidae and Hemerobiidae), and predatory mites (Phytoseiidae).

2008

There was a general trend for fewer spider mites in the MeSA-baited plot compared to the control plot on all three farms (Fig. 1.1). However, seasonal mean density of spider mites up to the time of the first miticide application was not significantly different between treatments across all three farms ($P \geq 0.171$) as

determined by the Mann-Whitney rank sum test (Table 1.2). For spider mites, the area under the population development curve for the entire season was greater in the control plots than the MeSA-baited plots across farms ($F = 14.74$; $df = 1,2$; one-sided test $P = 0.031$) (Table 1.5). Additionally, the number of spider mites on individual sampling dates were significantly different ($P \leq 0.081$) on one to four individual sampling days, depending on the farm (Fig. 1.1). Spider mite populations were reduced on one to two of the eight total sampling dates in MeSA-baited plots compared to the control plots on a given farm. However, on two sampling dates, significantly ($P \leq 0.023$) more spider mites were observed in the MeSA-baited plot compared to the control plot on farm C (Fig. 1.1E). Seasonal mean density of hop aphid up to the time of first insecticide application was not significantly different ($P \geq 0.343$) on any of the farms (Table 1.2), nor were there consistent differences in populations over time between MeSA-baited and control plots. The area under the population development curve for the entire season for hop aphid was similar among MeSA-baited versus control plots across farms ($F = 2.34$; $df = 1,2$; one-sided test $P = 0.133$) (Table 1.5).

The area under the population development curve for *Stethorus* spp. was greater in the MeSA-baited plots than the control plots across farms ($F = 12.52$; $df = 1,2$; one-sided test $P = 0.035$) (Table 1.5). The number of *Stethorus* spp. on sticky card samples tended to be higher in MeSA-baited plots as compared to the control plots (Fig. 1.1), although seasonal mean densities of *Stethorus* spp. on sticky cards and in shake samples were not significantly different among treatments on all farms ($P \geq$

0.181) (Table 1.3 & 1.4). The number of *Stethorus* spp. trapped on sticky cards on individual sampling dates were significantly different ($P \leq 0.057$) on one to five sampling days, depending on the farm. On one sampling date, the number of *Stethorus* spp. in a control plot was significantly greater ($P = 0.029$) than the MeSA-baited plot on farm C (Fig. 1.1F). However, among other predators and predator groups collected from shake samples and sticky traps on all farms, seasonal mean densities were similar between MeSA-baited and control plots ($P \geq 0.181$) (Table 1.3 & 1.4).

2009

Over the season, there was a general trend for lower numbers of spider mites in the MeSA-baited plots on all three farms (Table 1.2). However, seasonal mean densities of spider mites up to the time of the first miticide application were similar between treatments on all farms ($P \geq 0.252$). Across farms, the area under the population development curve for spider mites also were similar for the MeSA-baited and control plots ($F = 0.18$; $df = 1,2$; one-sided test $P = 0.358$) (Table 1.5). On individual sampling dates, spider mite populations varied and were reduced on up to two of the nine sampling days in MeSA-baited plots compared to the control plots among farms. On two sampling dates, on farms A and B, the number of spider mites per leaf was significantly greater ($P \leq 0.072$) in a MeSA-baited plot than the corresponding control plot (Fig. 1.2A & 1.2C). Seasonal mean densities of hop aphid were similar ($P \geq 0.548$) between treatments on all farms (Table 1.2), and there were no consistent differences in hop aphid populations over time as indicated by the area

under the population development curve ($F = 0.07$; $df = 1,2$; one-sided test $P = 0.410$) (Table 1.5).

There was again a trend for the seasonal means of *Stethorus* spp. trapped on sticky cards to be higher in MeSA-baited plots than control plots when averaged over farms (Table 1.4), although the area under the population development curve for *Stethorus* spp. was similar among treatments ($F = 3.63$; $df = 1,2$; one-sided test $P = 0.099$) (Table 1.5). Among individual farms, a significant difference was not detected among treatments for the seasonal means of *Stethorus* spp. captured on sticky cards or shake samples ($P \geq 0.171$) (Table 1.3). On individual sampling dates the number of *Stethorus* spp. on sticky cards was significantly different ($P \leq 0.057$) on up to two sampling days (Fig. 1.2B and 1.2D). Seasonal mean densities of other predators and predator groups were similar ($P \geq 0.284$) between the MeSA-baited and control plots (Table 1.3 & 1.4).

Discussion

In the two years of this study, mean seasonal densities of the key spider mite predator *Stethorus* spp. tended to be greater in all six MeSA-baited yards. When individual sample dates were compared, there were significantly greater numbers of *Stethorus* spp. on specific dates in four of the six MeSA-baited yards. Mean seasonal densities of spider mites were reduced 40 to 91% in five of the six yards, although these differences were statistically significant only in 2008. In turn, on specific individual dates there were significantly fewer spider mites in five of the six MeSA-

baited yards. Previous work in Washington hop yards indicated significant attraction of *Stethorus* spp. to synthetic MeSA dispensers as well a corresponding reduction in the number of spider mites (James and Price 2004). Attraction of diverse predator groups (e.g., Chrysopidae, *Orius tristicolor*) to slow-release MeSA dispensers as documented in Washington (James and Price 2004) and strawberry fields in Oregon (Lee 2010) was not observed in these studies. While the data from the current study are less robust in species abundance and cumulative treatment effect, there was a similar trend for attraction of *Stethorus* spp. and a coincident reduction in the number of spider mites.

The effect of synthetic MeSA deployment on natural enemy attraction and spider mite densities appeared to be site specific. Farm-to-farm variability in pest and predator densities observed in the current study could be attributed to environmental conditions, historical CBC practices, and other ecological and cultural factors that may vary between growers and sites. For instance, the practice of providing alternative food sources such as nectar has been suggested as a potential tool to retain natural enemy populations (Khan et al. 2008), and it is unlikely that nectar sources were the same among all the farms. An additional source of variability could result from local agro-ecology. Laboratory studies showed that HIPVs released from *Arabidopsis* and Brussels sprouts (*Brassica oleracea*) consumed by diamond-back moth larvae (*Plutella xylostella*) reduced attraction of a parasitoid, *Diadegma semiclausum* in the presence of isoprene, a volatile released by poplar *Populus* spp. (Loivamäki et al. 2008). The ecological complexity of HIPVs has not been studied intensively, and it is

likely that the performance of synthetic HIPVs could be affected by volatiles released from neighboring plants (Dicke and Baldwin 2010). The effect of other plant volatiles in the surrounding environment could potentially have affected the efficacy of MeSA at individual sites. Analyzing the effect of MeSA across all farms thus ignores the impact of ecological and volatile chemical diversity common across sites, potentially eliminating any treatment effect that could be seen by a farm-by-farm analysis. The farm-to-farm variability seen in this study suggests that site selection may be an important consideration in the use of synthetic MeSA.

The aim of this study was to evaluate the performance of slow-release MeSA dispensers in commercial hop yards in Oregon in conjunction with the current production practices used by growers. Commercial production practices in Oregon generally include the use of at least one annual miticide and aphicide application. Cooperating growers were asked to avoid broad-spectrum pesticides; however abamectin was used in the control plot on farm C in 2008 and both plots on farm A in 2009. Abamectin was toxic to *Stethorus punctum picipes* in laboratory bioassays (James 2003c). The use of imidacloprid, toxic to *S. punctum picipes*, *Harmonia axyridis*, and several species of predatory mites (James 2003c, James and Coyle 2001), occurred on all farms with the exception of farm B. The impact of the use of these compounds in this study is unknown, although their application likely interfered with MeSA treatment effects. Potentially, season-long data collected in the absence of broad-spectrum pesticides may have yielded larger differences between MeSA-baited

and control plots, although data collected from farm B (unsprayed in 2008) did not indicate this occurred.

Several other factors may have influenced the relatively modest efficacy of MeSA in these studies, such as the timing and rate of MeSA deployment. The timing of MeSA deployment in this study was based principally on evidence that early season recruitment of natural enemies is key for successful biological control of spider mites in hop yards. Previous research in Washington indicated that spring colonization of hop yards by natural enemies is essential to contain localized areas of high spider mite abundance and thus mitigate outbreaks throughout a yard (James et al. 2003).

However, conditioning predators to associate MeSA with a non-food source is potentially possible if MeSA packets are deployed too early and pest species have not yet colonized the crop. For example, research in The Netherlands indicated that *Anthocoris nemoralis*, a key predator of psyllids, could associate synthetic MeSA with either the presence or absence of food and respond accordingly (Drukker et al. 2000). The impact of deployment timing in this study are unknown, however the trend for greater numbers of *Stethorus* spp. in MeSA-baited yards does not suggest that this predator associated MeSA with a lack of food. Other predator species may respond differently to the presence of a HIPV in the absence of food. Thus the importance of deployment timing on other predator species is unclear, particularly given that other predators appeared to respond variably or not at all to MeSA among farms. The chemical ecology of semiochemicals is complex (De Boer and Dicke 2004, Dicke and Baldwin 2010, Halitschke et al. 2008), but direct measures of arthropod behavior were

beyond the scope the current study, which sought simply to evaluate the efficacy of synthetic MeSA deployment in commercial hop yards.

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

The impact of environmental and ecological conditions on the performance of synthetic MeSA remains largely unknown. Compared with trials conducted in Washington, the data collected from Oregon indicate that the efficacy of synthetic MeSA in hop yards was relatively modest, with growers' provisional spider mite action thresholds exceeded in four out of six MeSA-baited yards (Table 1.1). While trends for greater abundance of *Stethorus* spp. and suppression of spider mite populations were apparent, and significant in 2008, strong statistical support of

differences between the treated and control plots were not observed in both years. Although the current study found that MeSA deployment did not substitute for a miticide and aphicide application in a commercial setting, there was evidence of attraction of one of the key spider mite predators in hop yards and a reduction in spider mite populations. Further research is needed to optimize timing, deployment rates, mode of action and to characterize the association of local farm agro-ecology on the performance of MeSA to improve its efficacy and reliability as an integrated pest management tool.

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Table 1.1. Miticide and aphicide active ingredient (AI), application date and rate/ha applied by growers to experimental plots in 2008 and 2009 in hop yards in Oregon

Grower	Treat- ment	Date	Miticide	Rate (kg AI/ha)	Date	Aphicide	Rate (kg AI/ha)
2008*							
A	MeSA	18 July	spirodiclofen (Envidor® 2 SC, Bayer CropScience)	0.21	30 June	Imidacloprid (Pasada™ 1.6F, Makhteshim Agan of North America, Inc)	0.31
A	Control	25 July	spirodiclofen (Envidor® 2 SC)	0.21	2 Aug	bifenthrin (Fanfare® 2EC, Makhteshim Agan of North America, Inc.	0.11
B	MeSA	-	-	-	-	-	-
B	Control	-	-	-	-	-	-
C	MeSA	31 July	bifenazate (Acramite® 50WS, Chemtura Corp)	0.71	25 June	imidacloprid (Advise™ 2FL, Agrilience LLC)	0.31
C	Control	25 June	abamectin (Epi-Mek® 0.15 EC, Syngenta Crop Protection)	0.02	25 June	imidacloprid (Advise™ 2FL)	0.31

2009

A	MeSA	6 July	abamectin (Abba™ 0.15 EC, Makhteshim Agan of North America, Inc)	0.02	23 June	imidacloprid (Pasada™ 1.6F)	0.31
A	Control	6 July	abamectin (Abba™ 0.15 EC)	0.02	23 June	imidacloprid (Pasada™ 1.6F)	0.31
B	MeSA	6 July	hexythiazox (Savey® 50 DF, Gowan Co)	0.14	6 July	spirotetramat (Ultor™, Bayer CropScience)	0.09
B	Control	-	-	-	30 June	spirotetramat (Ultor™)	0.11
C	MeSA	30 June	bifenazate (Acramite® 50WS)	0.22	8 June	imidacloprid (Provado® 1.6 F, Bayer CropScience)	0.31
C	Control	30 June	bifenazate (Acramite® 50WS)	0.22	8 June	imidacloprid (Provado® 1.6 F)	0.31

* A dashed line indicates that no sprays were applied.

Table 1.2. Mean seasonal density \pm SEM (prior to first miticide application) of *Phorodon humuli*, Phytoseiidae, and *Tetranychus urticae* on hop leaves in relation to methyl salicylate (MeSA) deployment in hop yards in Oregon

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

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

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

Table 1.3. Mean seasonal density \pm SEM (prior to first miticide application) of arthropods collected in shake samples in relation to methyl salicylate (MeSA) deployment in hop yards in Oregon

Grower	Treatment	Arthropod (mean \pm SEM per shake) ^{a,c}					
		Coccinellidae				Parasitic Hymenoptera	Macropredators ^b
		<i>Hypena</i> spp.	<i>Anystis</i> spp.	Aphido- phagous lady beetles	<i>Stethorus</i> spp.		
2008							
A	MeSA	1.17 \pm 0.44	3.67 \pm 0.83	0	0.33 \pm 0.33	0.67 \pm 0.33	4.83 \pm 0.5
A	Control	0.33 \pm 0.19	3 \pm 1.21	0	0.11 \pm 0.11	0.33 \pm 0.19	4.22 \pm 1.21
B	MeSA	28.27 \pm 6.74	7.73 \pm 2.17	0.02 \pm 0.02	0.20 \pm 0.13*	1.53 \pm 0.40	10.73 \pm 2.91
B	Control	31.67 \pm 6.14	1.93 \pm 0.60	0.02 \pm 0.02	0.07 \pm 0.07	2.40 \pm 0.61	5.80 \pm 1.31
C	MeSA	0.35 \pm 1.14	2.78 \pm 1.06	0	0.11 \pm 0.11	2.22 \pm 0.73	5.67 \pm 1.201
C	Control	-	-	-	-	-	-
2009							
A	MeSA	0 \pm 0	0 \pm 0	0.06 \pm 0.06	0 \pm 0	1.33 \pm 0.88	1.5 \pm 0.77
A	Control	0 \pm 0	0.83 \pm 0.44	0.06 \pm 0.06	0.50 \pm 0.50	0.17 \pm 0.17	2.33 \pm 1.18
B	MeSA	1.00 \pm 0.33	7.67 \pm 2.92	0 \pm 0	0.50 \pm 0.50	0.33 \pm 0.33	9.00 \pm 3.22
B	Control	0.17 \pm 0.17	10.17 \pm 0.83	0 \pm 0	0.17 \pm 0.17	0.17 \pm 0.17	10.67 \pm 0.78
C	MeSA	0 \pm 0	0.17 \pm 0.17	0 \pm 0	0.17 \pm 0.17	0.83 \pm 0.33	1.337 \pm 0.46
C	Control	0 \pm 0	0 \pm 0	0.06 \pm 0.06	0 \pm 0	1.83 \pm 0.93	2.33 \pm 1.20

^a Data reported are from sampling dates after MeSA was deployed, but excludes data collected after the first miticide/insecticide application made by the cooperating grower. In 2008, Grower C applied a non-selective miticide to the control plot prior to the first shake sample of the season on 26 June.

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

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

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

Table 1.4. Mean seasonal density (prior to first miticide application) ± SEM of arthropods on sticky traps in relation to methyl salicylate (MeSA) deployment in hop yards in Oregon

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

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

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

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

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

Table 1.5. Effect of methyl salicylate (MeSA) deployment on seasonal abundance of *Phorodon humuli*, *Stethorus* spp., and *Tetranychus urticae* on hop

		Area under the population development curve ^{a,b}		
Year	Treatment	<i>Phorodon humuli</i>	<i>Stethorus</i> spp.	<i>Tetranychus urticae</i>
2008	MeSA	13.8	1038.9a	78.2a
	Control	7.6	441.5b	204.1b
2009	MeSA	201.6	804.7	68.6
	Control	176.1	468.6	52.0

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

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

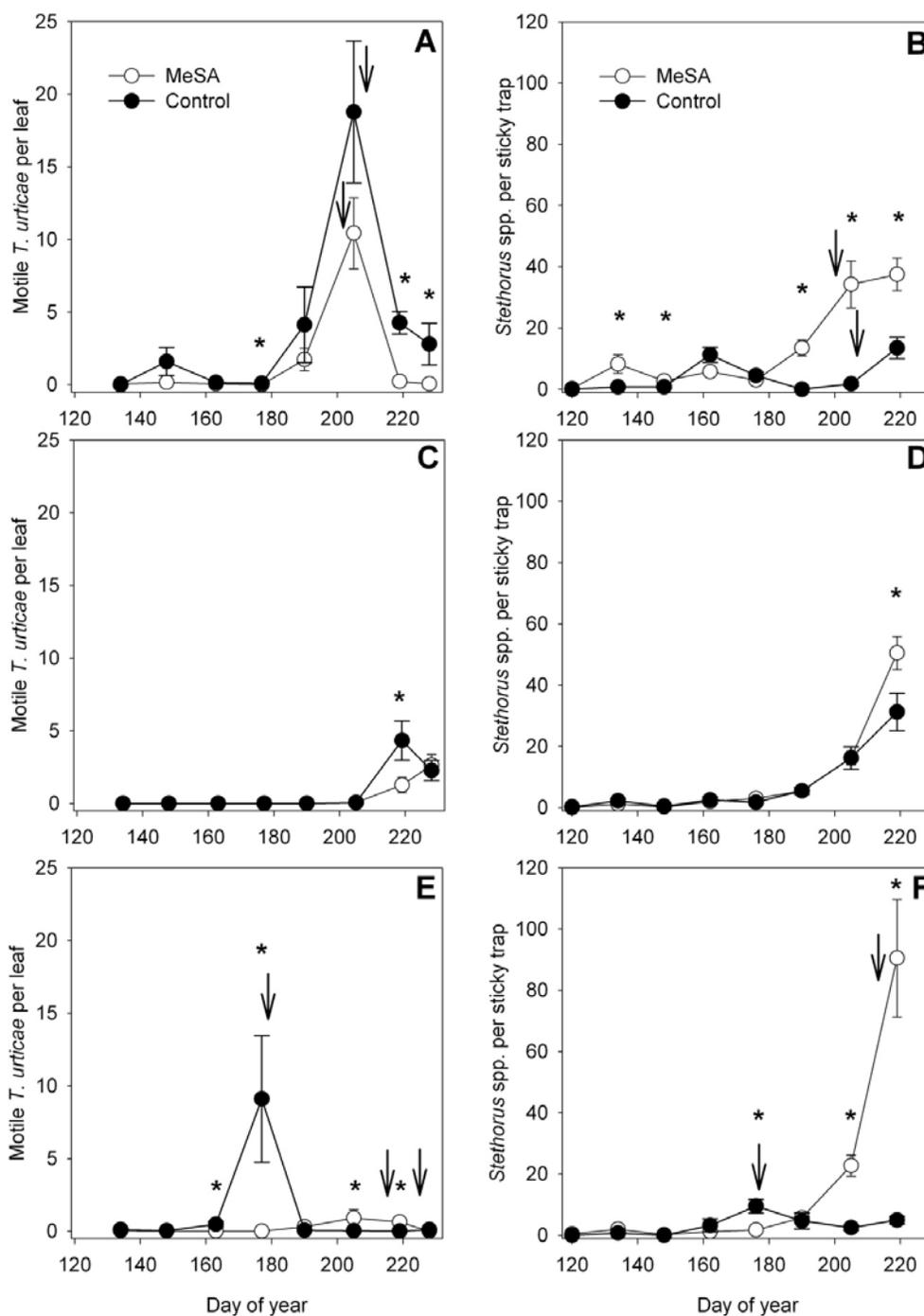


Fig. 1.1 Density of *Tetranychus urticae* (mean \pm SEM) (A, C, E) per leaf and *Stethorus* spp. (mean \pm SEM) per sticky trap (B, D, F) in MeSA-baited and control plots in 2008. Grower A, B, and C are represented in (A, B), (C, D) and (E, F), respectively. *T. urticae* data are means from 30 leaves per plot. *Stethorus* spp. data are

means from four traps per plot. Arrows indicate the application of a miticide by the cooperating grower. Significantly different means on specific dates are based on a Mann-Whitney rank sum test ($\alpha = 0.10$) and are noted by an asterisk. Across farms, area under the population development curve for *T. urticae* and *Stethorus* spp. in the MeSA-baited plots was significantly different than the control plots (*T. urticae*: $F = 14.74$; $df = 1,2$; one-sided test $P = 0.031$; *Stethorus* spp.: $F = 12.52$; $df = 1,2$; one-sided test $P = 0.035$).

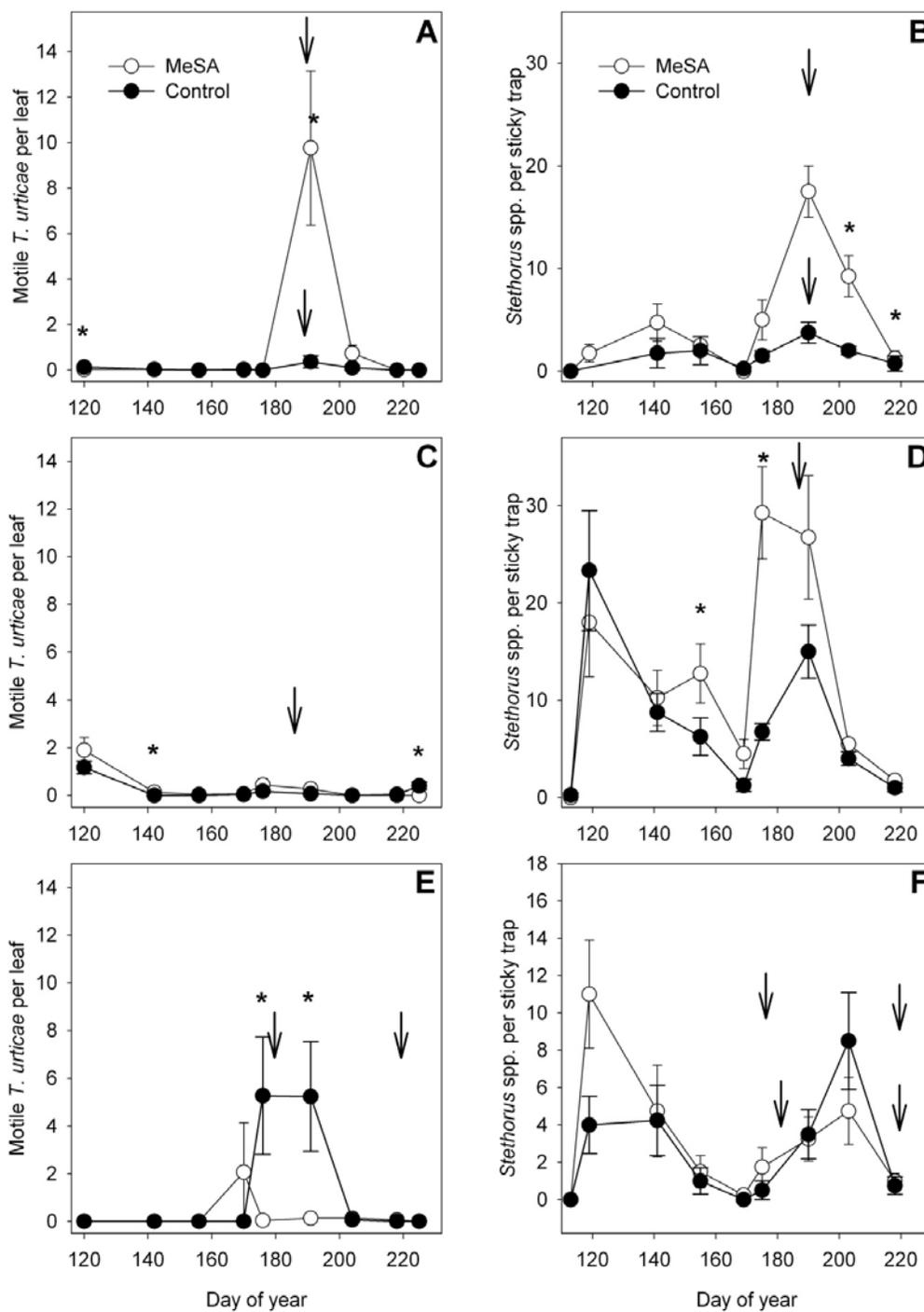


Fig. 1.2. Density of *Tetranychus urticae* (mean \pm SEM) (A, C, E) per leaf and *Stethorus* spp. (mean \pm SEM) (B, D, F) in MeSA-baited and control plots in 2009. Grower A, B, and C are represented in (A, B), (C, D) and (E, F),

respectively. *T. urticae* data are means from 30 leaves per plot. *Stethorus* spp. data are means from four traps per plot. Arrows indicate the application of a miticide by the cooperating grower. Significantly different means on specific dates are based on a Mann-Whitney rank sum test ($\alpha = 0.10$) and are noted by an asterisk. Across farms, the area under the population development curve for *T. urticae* and *Stethorus* spp. were similar among MeSA-baited and control plots (*T. urticae*: $F = 0.18$; $df = 1,2$; one-sided test $P = 0.358$; *Stethorus* spp.: $F = 3.63$; $df = 1,2$; one-sided test $P = 0.099$).

CHAPTER 3:
POPULATION DENSITY AND PHENOLOGY OF *Tetranychus urticae* KOCH
(ACARI: TETRANYCHIDAE) IS LINKED TO SULFUR-INDUCED
OUTBREAKS OF THIS PEST

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Abstract

The twospotted spider mite, *Tetranychus urticae* Koch is a worldwide pest of numerous agronomic and horticultural plants. Sulfur fungicides are known to induce outbreaks of this pest on several crops, although mechanisms associated with sulfur-induced mite outbreaks are largely unknown. Studies were conducted during 2007 to 2009 in Oregon and Washington hop yards to evaluate the effect of timing of sulfur applications on *T. urticae* and key predators. In both regions, applications of sulfur made relatively late in the growing season (mid-June to mid-July) were associated with the greatest exacerbation of spider mite outbreaks, particularly in the upper canopy of the crop. The severity of mite outbreaks was closely associated with sulfur applications made during a relatively narrow time period coincident with the early exponential phase of spider mite increase and rapid host growth. A nonlinear model relating mean cumulative mite days during the time of sulfur sprays to the percent increase in cumulative mite days (standardized to a non-treated plot) explained 58% of the variability observed in increased mite severity related to sulfur spray timing. Spatial analysis of spider mites counts and their eggs in the Oregon plots indicated patterns of motile stages of spider mites were similar among leaves treated with sulfur versus nontreated leaves; however, in two of three years eggs were less aggregated on leaves of sulfur-treated plants, pointing to enhanced dispersal. Apart from one experiment in Washington, relatively few predatory mites were observed during the course of these studies and sulfur-induced mite outbreaks generally occurred irrespective of predatory mite abundance. Collectively, these studies indicate sulfur

induces mite outbreaks through direct or indirect effects on *T. urticae*, mostly independent of predatory mite abundance or toxicity to these predators. Avoidance of negative side effects of sulfur sprays was achieved by carefully timing applications to periods of low spider mite abundance and slower host development, which is generally early to mid-spring for hop.

Introduction

The application of pesticides can result in unforeseen consequences, particularly negative impacts on non-target organisms, leading to disturbance of natural enemy populations and outbreaks of secondary pests (Boudreaux 1963, Hardin et al. 1995). The twospotted spider mite, *Tetranychus urticae* Koch, is a common secondary pest (van de Vrie et al. 1972) and its resurgence was associated with the advent of the use of synthetic pesticides developed during and after World War II (Boudreaux 1963, Huffaker et al. 1969). While spider mites occur on wild and feral plants, populations are generally regulated by an assemblage of natural enemies (Huffaker et al. 1970, James et al. 2001). The indiscriminate use of pesticides has been implicated as a main cause of resurgence of spider mites in many cropping systems (Hardin et al. 1995).

Pest resurgence associated with the use of pesticides may be because of toxicity to natural enemies, a direct effect on the pest organism (e.g., increased fecundity), an impact on the host plant, or a combination of these factors (Bartlett 1968, van de Vrie et al. 1972, Hardin et al. 1995). Certain pesticides are well-known to induce spider mite outbreaks (McMurtry et al. 1970, James and Price 2002), although dusty conditions (Walsh 2002), drought stress (Rodriguez and Rodriguez 1987, English-Loeb 1990), and plant nutrition (Kielkiewicz 1990, Nachman and Zemek 2002) can also be associated with outbreaks.

The use of sulfur as both a fungicide and insecticide is documented to have occurred since the days of Homer (1000 B.C.) (Williams and Cooper 2004). Sulfur is

commonly utilized in several crops for the control of powdery mildew diseases, for example grape (Hanna et al. 1997, Costello 2007), hop (Mahaffee et al. 2003, Gent et al. 2008), and apple (Beers et al. 2009). Although sulfur is primarily used as a fungicide, the insecticidal and in particular the acaricidal properties of sulfur are well documented (McMurtry et al. 1970, Auger et al. 2003, Price and James 2006, Beers et al. 2009). The broad use of sulfur has been an impetus for research on its impacts on non-target organisms and natural enemy disturbance, and has been reported in a diversity of cropping systems, including apple (Childers and Enns 1975), pecan (Ball 1982), grape (Hanna et al. 1997, Prischmann et al. 2005), and hop (James and Coyle 2001, James and Prischmann 2010), among others.

In the case of hop (*Humulus lupulus* L.), powdery mildew (caused by *Podosphaera macularis* (Wallr.:Fr.) U. Braun & S. Takamatsu), and twospotted spider mite are two important pests in most production areas in the Northern Hemisphere (Mahaffee et al. 2009). Hop plants have tremendous growth potential. Bines grow as much as 15 to 25 cm per day, and may reach 5 m or more by early summer (Neve 1991). The rapid growth habit of the plant provides abundant succulent leaf tissue which can be favorable for both powdery mildew and spider mites (Mahaffee et al. 2009). As many as six to 10 annual applications of sulfur-based products, horticultural oils (e.g., paraffinic oil), and synthetic fungicides (e.g., myclobutanil, quinoxifen, spiromamine, and trifloxystrobin) are applied to manage hop powdery mildew (Royle 1978, Mahaffee et al. 2003, Gent et al. 2008). Multiple miticide applications also may be applied to suppress spider mites (James and Barbour

2009). Management tactics for powdery mildew may affect spider mites (and vice versa), and previous studies have indicated that powdery mildew management tactics, in particular the use of sulfur fungicides, may negatively impact pest and predatory arthropod populations (Strong and Croft 1996, James and Prischmann 2010, Gent et al. 2009).

The complexity of the pest-predator-plant system can make the determination of a causal agent of a pest outbreak challenging, and relatively few studies have investigated mechanisms associated with sulfur-induced mite outbreaks other than pesticide toxicity to natural enemies (Hardin et al. 1995). In previous research (Gent et al. 2009), we evaluated various fungicide programs and their impact on the pest and predatory arthropods in the hop system. Intensive use of sulfur, common in hop production (Gent et al. 2008), resulted in the highest spider mite abundance in both the cool, maritime climate of western Oregon and the semi-arid climate of central Washington. In these studies, inhibitory effects of sulfur on natural enemies generally were negligible, except late-season suppression of predatory mites in Washington. This study raised questions of the mechanism at play, given that sulfur still induced spider mite outbreaks in Oregon when predatory mites were essentially absent from the experimental plots.

Sulfur fungicides are inexpensive, efficacious, and useful for resistance management, and consequently are a key component of commercial spray programs (Gent et al. 2008, Mahaffee et al. 2009). Given the necessity of its use for disease management in many cropping systems, the question remains whether sulfur can be

integrated into a powdery mildew management program without inciting spider mite outbreaks. This question was the impetus for this study. We sought to investigate the impact of sulfur fungicide application timings on pest and predatory arthropods in hop, and identify factors associated with sulfur-induced mite outbreaks.

Materials and Methods

Experimental Design and Treatment Application

Experiments were conducted in 2007, 2008, and 2009 in experimental plots near Corvallis, Oregon, and at the Washington State University Irrigated Agricultural Research and Extension Center near Prosser, Washington, to determine the impact of sulfur fungicide timing on the population densities of spider mites and predatory mites. In Oregon, plots were established in a hop yard planted in 2005 to the aroma cultivar Willamette with plants on a 2.1 m grid and under a 5 m trellis. Each experiment was arranged in a randomized complete block design with each treatment replicated four times. In 2007 and 2008, a plot consisted of 8 hop plants in a 2 x 4 rectangular array. In 2009, plots consisted of 16 plants in a 4 x 4 arrangement. Each plot was separated by at least one row of untreated plants. In 2007, irrigation was supplied by sprinklers every 7 to 14 days as needed for crop development, whereas in 2008 and 2009 irrigation was supplied daily by a surface drip system. In Washington, plots were established in a hop yard planted in 1991 to cultivar Willamette with plants spaced on 2.1 m grid and under a 5 m trellis as in Oregon. Each plot consisted of six consecutive plants in a row arranged in a completely randomized design with four

replications. Plots were separated by at least one row of untreated plants. Irrigation was supplied daily by a drip system. Oregon, granular nitrogen, phosphorous, and potassium were soil applied in April, May, and June according to standard commercial recommendations (Gingrich et al. 2000). In Washington, nitrogen fertilizer was injected into the drip irrigation system in April, May, and June. No other nutrients were applied.

Various timings of sulfur applications as part of an overall fungicide program for powdery mildew were evaluated to identify particular periods of the year when sulfur sprays would affect the severity of subsequent spider mite outbreaks. In both Oregon and Washington, we evaluated fungicide programs with seven or eight total fungicide applications where sulfur was applied only three times at various periods during the season as described below. On the remaining application dates, the plots received a rotation of three synthetic fungicides that are known to have minimal impacts on spider mites and their key natural enemies: trifloxystrobin (0.14 kg a.i./ha, Flint 50WG, Bayer CropScience, Research Triangle Park, NC), spiroxamine (0.36 kg a.i./ha, Accrue, Bayer CropScience) and quinoxyfen (0.10 kg a.i./ha, Quintec, Dow AgroSciences, Indianapolis, IN). In Oregon three sulfur timings were evaluated: early season (sprays beginning near 15 April), mid-season (beginning near 15 May) and late season (beginning near 15 June). In each of these treatments, three sequential sprays of sulfur (as described below) were made at biweekly intervals. These treatments were compared to a rotation of only the synthetic fungicides and a non-treated control. In Washington, four sulfur timings were investigated, with each application made at

weekly rather than biweekly intervals. These timings were: early season (sprays beginning in early May), mid-season (beginning late May), late season (beginning mid June), and very late season (beginning early July). This difference between states reflects general grower use patterns for sulfur in each state due to more severe powdery mildew pressure in Washington. In Washington, the alternating application of synthetic fungicides occurred biweekly, as in Oregon. In both states, sulfur was applied as Microthiol Disperss (Cerexagri, Inc. North America, King of Prussia, PA) at 5.38 kg a.i./ha. To avoid confounding effects from other arthropod pests, in all years of the trial in Oregon, *Bacillus thuringiensis* (0.15kg a.i./ha, Javelin WG, Certis USA, LLC, Columbia, MD) and pymetrozine (0.034kg a.i./ha, Fulfill, Syngenta) were applied for the control of lepidopteran pests and hop aphid (*Phorodon humuli* Shrank), respectively. These applications were made on day of year 183, 194, and 169 in 2007, 2008, and 2009, respectively. This was adequate to suppress these pests in 2007 and 2008, although in 2009 the application of pymetrozine was inadequate to control the severe outbreak of hop aphid and imidacloprid (0.02 liters a.i./ha, Provado 1.6F, Bayer) was injected into the surface drip irrigation system on day of year 190. No other pesticides were applied to the plots or neighboring plants.

Applications were made with an Eagle BP40 backpack sprayer (Eagle-1 Manufacturing, Monroe, WA) in Oregon or a Stihl Model SR420 backpack sprayer (STIHL, Virginia Beach, VA) in Washington. Application volume increased with plant development during the season, and ranged between 374 liters/ha in early to

mid-spring to 1515 liters/ha during and after flowering (early to mid-July in both states).

Arthropod Sampling

Leaf samples were collected at weekly to biweekly intervals beginning with a pre-treatment assessment in mid-April to early May and continuing until cone harvest during mid to late August. On each sampling date, 10 to 20 leaves were collected from each plot and motile spider mite stages, spider mite eggs, hop aphid nymphs, predatory mites (Phytoseiidae), mite-eating ladybeetles, *Stethorus* spp., and minute pirate bugs *Orius tristicolor* Say were enumerated. Due to space limitations, only motile spider mite and predatory mite data are presented in this paper.

Predatory mite populations in Washington were comprised of two species: *Galendromus occidentalis* Nesbitt and *Neoseiulus fallacis* Garman, generally with *G. occidentalis* as the predominant species. In Oregon, when predatory mites were observed *Neoseiulus fallacis* was the dominant species. Most adult predatory mites were identified under low magnification (60×) with the aid of a stereomicroscope, with a subset slide mounted and identified based on morphological characters. Nymphs were simply categorized as predatory mites, and were not identified to species.

In Oregon, leaves were collected from the four plants in the middle of each plot to reduce plot-to-plot interference. When plant growth exceeded approximately 2 meters, samples were collected from lower (< 2 m) and upper (> 2 m) positions in the canopy. Samples were collected from only one height in Washington, approximately 2

m. Leaves were collected into paper bags, stored on ice in a cooler, and promptly transported to a laboratory. Enumeration of arthropods was conducted under a stereomicroscope, observing them either on the leaves directly or after transferring them to a corn syrup-coated glass plate using a mite brushing machine (Leedom Engineering, Twain Harte, CA).

Direct or indirect exposure of spider mites to sulfur potentially could increase dispersion as mites are repelled and repulsed by sulfur residues (Walsh and Grove 2005). An indirect measure of dispersion is the degree of spatial aggregation, which can be quantified using various spatial statistics (Binns et al. 2000). In Oregon, to assess spatial patterns of spider mites and their eggs in response to sulfur treatment, we collected additional leaf samples in each year from the non-treated plots and the early sulfur treatment (2007) or late sulfur treatment (2008 and 2009). In these samples, 20 to 40 leaves were collected from the upper and lower canopy (2008, upper canopy only) and were scanned individually under low magnification to obtain counts of predatory mites, motile spider mites, and their eggs per leaf for later spatial analyses as described below. There were a total of 22 such intensive samples collected in 2007, 24 in 2008, and 41 in 2009.

Cone Quality Assessments

At harvest, the incidence of spider mite damage was assessed on approximately 100 cone sub-samples from each treatment replicate. Cones were harvested on day 238, 233, and 236 in Oregon in 2007, 2008, and 2009, respectively,

and day 242, 241, and 247 in Washington in 2007, 2008, and 2009, respectively. In 2007, mite damage was rated using an ordinal scale where 1 = no damage, 2 = slight discoloration or damage on a single or few bracts, 3 = moderate levels of discoloration or damage (greater damage than '2' but less than 25% of cone area exhibiting discoloration or damage), and 4 = severe cone discoloration (damage on greater than 25% of the cone or cone abortion). In 2008 and 2009, cone visual quality was rated by a commercial merchant using their standard hop rating scale, where 1 = "excellent", 2 = "excellent (-)", 3 = "good (+)", 4 = "good", 5 = "good (-)", 6 = "poor", and 7 = "poor (-)". These evaluations were conducted in a blind manner where the cone samples were coded so that the merchant was unaware of the treatment each sample received.

Data Analysis

Spider mite and predatory mite populations on each assessment date were plotted over time to develop population curves. The area under this curve was calculated for each plot to develop a single composite value to express arthropod abundance over the entire season. Area calculations were performed using a macro available in SigmaPlot version 11.0 (Systat Software, Inc., San Jose, CA). The area values were log-transformed to achieve normally distributed residuals with a common variance and then analyzed using a linear mixed-model as described below and in Gent et al. (2009). When spatial location of the plots indicated non-independence of the residuals (as determined by plotting a variogram of the residuals), a linear mixed-

model repeated in space (the coordinate location of each plot) was used to account for spatial aggregation of mites (Littell et al. 2006). Several spatial covariance structures were investigated and the best fitting model was selected by minimizing Akaike's Information Criterion (AIC). The analysis was conducted in PROC MIXED or PROC GLIMMIX in SAS with denominator degrees of freedom determined using a general Kenward-Roger approximation, which is appropriate for correlated residual structures (Littell et al. 2006). Block was considered a random effect in the analyses. If a significant treatment effect was found, individual treatments were compared using an LSD test.

The ordinal rating scale used for mite damage assessment on cones was analyzed using a nonparametric ANOVA-type statistic (Shah and Madden 2004), as described previously (Gent et al. 2009). In this analysis, a relative treatment effect ranging from 0 to 1 is calculated for each treatment. To obtain a single measurement for each experimental unit, the data including sub-samples were ranked and a mean rank was calculated to obtain a single value for each experimental unit. Differences between treatments were considered statistically significant when 95% confidence intervals for the relative treatment effects did not overlap. Analyses were conducted in PROC MIXED in SAS using macros developed by Brunner et al. (2002).

Data was also compiled across years and states for a combined analysis of the effect of sulfur timing on spider mite abundance. To do this, the severity of the spider mite outbreak within a given experiment was standardized relative to the non-treated control for that experiment. The mean area under the pest development curve for each

treatment was expressed as a percentage of the non-treated control by subtracting the AUPDC for a given fungicide treatment by the AUDPC for the non-treated control and then dividing the difference by the AUDPC of the non-treated. This was done for each experiment in Oregon and Washington, and then each year was considered an experimental unit (replication). When the data were averaged over 2007, 2008, and 2009 and plotted as a bar graph, two groups were apparent: (i) the synthetic fungicide treatment, early sulfur, and mid-sulfur timings and (ii) the later sulfur timings. Given these two groups, classification variables ('0' and '1') were assigned to each group and the relative severity of the spider mite outbreak across all years of the experiment for each state was analyzed in PROC MIXED in SAS. Year was considered a random effect in the analysis.

To quantify the abundance of spider mites between specific time points during the season (e.g., during sulfur application), cumulative mite days (CMD) were calculated using the formula:

$$\text{CMD} = \sum[(\text{mean mites}_t + \text{mean mites}_{t+1})/2] \times (t_1 - t)$$

where t is the day at sample time t and t_1 is the day of the next sampling. Scatterplots were constructed to relate the severity of the entire mite outbreak (CMD, standardized to the nontreated for a given experiment as described above) as the dependent variable versus CMD only accumulated during the sulfur applications as the independent variable. In Oregon, these calculations were made for both the lower and upper canopy sampling heights. Plots of these data indicated a curvilinear relationship. Nonlinear,

least-squares curve fitting was conducted using several models implemented in SigmaPlot version 11.0 that describe an exponential rise to a maximum relationship between these variables. The best fitting model that provided a reasonable description of the data was selected based on goodness-of-fit statistics and visual inspection of residual plots.

Spatial Analysis

Data from the intensive sampling of the non-treated and selected sulfur treatments were used to fit Taylor's power law. Taylor's power law (Taylor 1984) describes the relationship between the variance and mean for count data with no upper limit as:

$$S^2 = am^b$$

where S^2 is the sample variance, m is the mean, and a and b are parameters. To solve for these parameters, the equation was linearized by log transformation, yielding a simple linear regression with intercept $\log(a)$ and slope b . The mean and variance among the individual leaf samples were calculated and a linear regression model was fit using the REG procedure in SAS. A slope greater than 1 indicates an aggregated pattern with the degree of aggregation directly proportional to the slope; a slope of 1 indicates a random spatial pattern. To test if the degree of aggregation of spider mite motiles and eggs was similar between the treatments, the slopes of the line for the nontreated and sulfur-treated plots were compared using an F -test (i.e., hypothesis test) in the REG procedure.

Results

Oregon 2007

Spider mites were detected at low levels on the first sampling date in Oregon on 12 April (day 102) (Figs. 2.1A and B; Table 2.1). The area under the pest development curve was similar among treatments in the lower canopy ($P = 0.2667$), but differed in the upper canopy when spatial influences were accounted for in the analysis ($F = 4.28$; $df = 4, 14.9$; $P = 0.0167$) (Table 2.2). Pairwise contrast indicated that the area under the curve was greater in the late season sulfur treatment than all other treatments ($F = 13.79$; $df = 1, 14.9$, $P = 0.0021$) (Table 2.2). The differences in spider mite abundance were not associated with increased cone damage. Median cone damage due to spider mites was rated as the same in all treatments, and the relative effect was statistically equivalent among all treatments as indicated by overlapping 95% confidence intervals (Table 2.3). Few predatory mites (less than 0.10 ± 0.05 per leaf) were found throughout the season (Table 2.1; Fig 2.2A and B). Seasonal abundance of predatory mites among treatments, as measured by the area under the development curve, was similar in both canopy heights ($P \geq 0.2083$) (Table 2.2).

Oregon 2008

Again in 2008, spider mites were detected at low levels on the first sampling date in Oregon on 18 April (day 109) (Fig. 2.1C), and a severe outbreak of spider mites later developed in all treatments (Fig. 2.1C and D). The area under the pest

development curve for spider mites in the lower canopy varied by 1.9-fold among treatments but was statistically similar among fungicide treatments ($P = 0.3192$) (Table 2.2). There were differences in mite abundance in the upper canopy among fungicide treatments when spatial influences were accounted for in the analysis ($F = 3.15$; $df = 4, 15$; $P = 0.0455$). The area under the curve was 2.1 and 2.8-fold greater in the mid-season and late season sulfur treated than the nontreated plots, respectively (pairwise contrast $F = 9$; $df = 1, 15$; $P = 0.009$, and $F = 7.66$; $df = 1, 15$; $P = 0.0144$, respectively) (Table 2.2). Fungicide treatments were associated with differences in cone quality. Plots that received the late sulfur treatment had significantly poorer cone quality when compared to the other treatments, with the exception of the early sulfur treatment ($F = 3.9$; $df = 3.01, 10.4$; $P = 0.0423$) (Table 2.3).

Synchronized with the large spider mite outbreak in 2008, predatory mites peaked late in the season in both the upper and lower canopy (Fig. 2.2C and D; Table 2.1). Although the late-sulfur treatment appeared to substantially reduce abundance of predatory mites (Fig. 2.2D), treatment differences were not detected at either canopy height ($P \geq 0.3841$) (Table 2.2).

Oregon 2009

Spider mites were detected at low levels on the first sampling date in Oregon on 16 April (day 106) and remained low for the duration of the season (Fig. 2.1E and F). Due to the low abundance of spider mites in both the lower and upper canopy, area under the pest development curve was low for all treatments and treatment effects

were not detected ($P \geq 0.2857$) (Tables 2.1 and 2.2). The low number of spider mites in all treatments in 2009 did not lead to differences in cone quality (Table 2.3).

Predatory mites were not found in all treatments at either height, and densities for 2009 were low (Fig. 2.2E and F; Tables 2.1 and 2.2), and similar among treatments in both canopy heights ($P \geq 0.2512$).

Washington 2007

Spider mites were observed on the first sampling date on 2 May (day 122) (Fig. 2.3A; Table 2.4). The analysis for the area under the pest development curve for spider mites indicated a significant treatment effect ($F = 3.27$; $df = 5, 15$; $P = 0.0339$), with spider mite abundance 2.2-fold greater in the late season sulfur treatments as compared to the nontreated (pairwise contrast $F = 11.22$; $df = 1, 12.42$; $P = 0.0055$) (Table 2.2). These differences in spider mite abundance on leaves among treatments did not impact cone quality significantly (Table 2.3).

Predatory mite abundance was relatively low throughout the season, however there was a significant fungicide treatment effect at $\alpha = 0.1$ ($F = 2.23$; $df = 5, 17$; $P = 0.0981$) for the area under the development curve for predatory mites (Fig. 2.3B; Table 2.2). The very late sulfur treatment reduced predatory mite abundance 6.4-fold as compared to the nontreated plots (pairwise contrast $F = 8.53$; $df = 5, 17$; $P = 0.0095$) (Table 2.2).

Washington 2008

Spider mites were observed during the first sampling on 21 May (day 142) (Fig. 2.3C; Table 2.4), and fungicide treatment had a small effect on seasonal spider mite abundance ($F = 2.56$; $df = 5, 13.1$; $P = 0.0794$) when spatial effects were accounted for in the analysis. The most severe outbreak of spider mites was observed in the very late sulfur treatment, which was 2.3-fold greater than that of the nontreated plots (pairwise contrast $F = 12.61$; $df = 1, 12.9$; $P = 0.0036$) (Table 2.2). As before, the differences in spider mite levels on leaves between treatments did not translate into differences in mite damage to cones (Table 2.3).

Predatory mite abundance peaked relatively late in the season, and similar numbers of predatory mites were observed among treatments ($F = 0.66$; $df = 5, 18$; $P = 0.6561$) (Fig. 2.3D; Tables 2.2 and 2.4).

Washington 2009

As in all other experiments, spider mites were observed early in the season on the first sample date, 6 May (day 126) (Fig. 2.3E; Table 2.4). There was a significant treatment effect on the area under the pest development curve for spider mites when spatial effects were included in the model ($F = 3.14$; $df = 5, 13.9$; $P = 0.0421$) (Table 2.2). There was evidence ($F = 9.2$; $df = 1, 13.7$; $P = 0.0091$) for greater numbers of spider mites in the late season sulfur treatment versus the nontreated plots (1.4-fold greater area under the curve; Table 2.2). Cone quality again was similar among the fungicide treatments (Table 2.3). Predatory mite densities generally tracked spider

mite populations, although the area under the development curve for predatory mites was not affected significantly by fungicide treatment ($F = 0.71$; $df = 5, 13.9$; $P = 0.6250$) (Fig. 2.3F; Table 2.2).

In the summary analysis considering each year as a replication, spider mite outbreaks (standardized to the nontreated plot in a given experiment) were significantly more severe when sulfur was applied later in the season (Fig. 2.5). This was true for both low and high canopy heights sampled in Oregon ($F = 4.83$; $df = 1, 10$; $P = 0.0269$, and $F = 4.83$; $df = 1, 11$; $P = 0.0296$, respectively) and Washington ($F = 25.47$; $df = 1, 11$; $P = 0.0002$). For the treatments receiving sulfur sprays, CMD was correlated with the severity of the subsequent spider mite outbreak during the time when sulfur was applied (Fig. 2.6). Standardized severity of the mite outbreak was modeled to be dependent on the CMD during the sulfur sprays through the equation, $y = -18.499 + 132.516 * (1 - \exp(-0.019 * \text{CMD during sulfur sprays}))$, which described 58% of the observed variability in mite outbreak severity.

Spatial analysis

Dispersion of both spider mite motiles and eggs was measured on individual leaves in Oregon in 2007, 2008, and 2009 in sulfur-treated and nontreated plots. Spider mite motiles in sulfur-treated and nontreated plots in Oregon were similarly aggregated among all years, as indicated by a comparison of the slopes of the Taylor's power law regressions ($P \geq 0.2949$). Whereas aggregation of motile stages were similar in this analysis, for spider mite eggs the slope of regression was significantly

shallower for sulfur-treated leaves versus nontreated leaves in both 2007 and 2009 ($F = 40.51$; $df = 1, 9$; $P = 0.0001$; $F = 14.56$; $df = 1, 21$; $P = 0.001$, respectively) (Figs. 2.4A and C). This indicates that eggs were more dispersed on sulfur-treated leaves than on nontreated leaves. In 2008, a year with a very severe outbreak of spider mites in the late sulfur treatment, an opposite pattern was observed and eggs were slightly more dispersed on the nontreated leaves than the sulfur-treated leaves ($F = 7.13$; $df = 1, 10$; $P = 0.0235$) (Fig. 2.4B). On the leaves used for the spatial analysis, predatory mites occurred at a frequency of less than 4% on all of the leaves sampled. Predatory mites occurred on 2.5 and 2.7 percent of the leaves sampled in the sulfur-treated and nontreated plots respectively, in 2007, 3.7 percent of the leaves sampled in both treatments in 2008, and 1.3 percent of the leaves sampled in both treatments in 2009.

Discussion

Sulfur applications made later in the season induced spider mite outbreaks in five of six location-years across Oregon and Washington. In both regions there was a generally similar response to late sulfur treatments, resulting in mite outbreaks 1.1 to 2.8-fold more severe than the outbreaks in the nontreated control plots in each experiment. Consistent with previous field studies on several host plants (James et al. 2002, Prischmann et al. 2005, Costello 2007, Gent et al. 2009), sulfur tended to suppress spider mites while applications were being made but populations later resurged when applications ceased. This resurgence was primarily associated with sulfur applications made in the later portions of the season. Early to mid-season application timings generally did not exacerbate mite outbreaks. Several potential

mechanisms could be associated with and/or interacting to incite sulfur-induced spider mite outbreaks including: (i) natural enemy destruction; (ii) a direct or indirect effect on the mites themselves; and/or (iii) an alteration (reduced or enhanced) of the nutritive quality of the host plant (Hardin et al. 1995).

Addressing the first potential mechanism of natural enemy destruction, with only one exception (Washington 2007), predatory mite abundance was statistically unaffected by sulfur treatment in both states and all years. In that experiment there was weak evidence ($\alpha = 0.1$) for reduced densities of predatory mites in the very late season sulfur treatment. The negative impact of sulfur on predatory mites, key predators of twospotted spider mites (McMurtry and Croft 1997), has been documented in laboratory bioassays (James and Coyle 2001, Beers et al. 2009) and in the field in multiple systems (Childers and Enns 1975, Ball 1982, Prischmann et al. 2005). However, there have been variable results reported on the impact of sulfur to predatory mites on individual plants (Stavrinides and Mills 2009) and in larger field studies (Costello 2007, Gent et al. 2009). Some of the variability reported in previous studies on the impact of sulfur on predatory mites may be influenced by species (James and Rayner 1995), formulation (Beers et al. 2009), developmental stage, or environmental conditions, such as increasing temperature and humidity which influence the acaricidal activity of sulfur (Auger et al. 2003). In a study conducted on individual grape plants, micronized sulfur reduced twospotted spider mite abundance, but did not affect a common predatory mite species, *G. occidentalis* (Stavrinides and

Mills 2009), while dry flowable sulfur was nontoxic to both *G. occidentalis* and spider mites in bioassays conducted by Beers et al. (2009).

While these differences could partially explain the variable response of sulfur on predatory mites, and in turn spider mite outbreaks, sulfur sprays may induce spider mite outbreaks in the absence of predatory mites. We previously reported that micronized sulfur induced spider mite outbreaks on hop when predatory mites were essentially absent in a newly planted yard (Gent et al. 2009). Similarly in the current study, a statistically significant impact of sulfur on predatory mites was not found in five of the six location-years of this trial. Costello (2007) also found predatory mite abundance and the ratio of spider mites to predatory mites were similar on grape treated with sulfur versus other fungicides, although spider mite outbreaks were observed when sulfur was applied before bloom. These results point to a mechanism other than or in addition to predatory mite disturbance that must be involved in sulfur-induced mite outbreaks in the hop system.

A direct and/or indirect behavioral or physiological effect of sulfur on spider mites is a second possible mechanism involved in sulfur-induced spider mite outbreaks. Hormoligosis, increased fecundity as a result of a stimulatory effect from a sub-lethal dose of a pesticide, has been reported for spider mites with numerous compounds (Huffaker et al. 1969) and could be a potential factor in sulfur-induced spider mite outbreaks. In laboratory bioassays, however, Beers et al. (2009) found three sulfur-containing products had no effect on twospotted spider mite fecundity or total eggs produced. Price and James (2006) also found no evidence for increased

daily or lifetime egg production of *T. urticae* exposed to sulfur residues. In contrast, exposed spider mites produced less total eggs as compared to non-exposed spider mites due to reduced longevity (approximately 55% less). Neither Beers et al. (2009) nor Price and James (2006) found evidence to support a hormoligotic effect from sulfur. The collective evidence points to an indirect effect of sulfur in inducing spider mite outbreaks.

Enhanced dispersion of spider mites is a potential indirect effect from sulfur use. In hop, the common cultural practice of removing basal foliage and the rapid growth rate of the plant has been suggested as contributing to the success of spider mites on this host by ‘escaping’ predation through superior dispersal (Strong et al. 1997, Strong et al. 1999). Dispersion is most common in pre-reproductive female spider mites and once a suitable host is reached, feeding and oviposition are uninhibited and populations grow rapidly (Boudreaux 1963, Kennedy and Smitley 1985). Dispersal behavior is often a response to host plant quality (Kennedy and Smitley 1985), although the use of pesticides may stimulate dispersal (Rodriguez and Rodriguez 1987). Certain pesticides, including sulfur, are well known to be repulsive and/or repellent to spider mites and can incite an “irritable behavior” in spider mites (e.g., Walsh and Grove 2005). Such irritable behavior could enhance dispersion of spider mites to new leaves to avoid contact with the irritant, thereby indirectly increasing egg laying because of the pseudo-colonial nature of spider mites (van de Vrie et al. 1972). Indeed, greater aggregation of spider mite eggs on nontreated versus sulfur-treated leaves was observed in two of the three years of study in Oregon. While

the differences in aggregation from the effect of sulfur were relatively modest, these differences were statistically significant and provide indirect evidence for sulfur acting to enhance dispersion among leaves. Spatial patterns are of course the result of many factors (Taylor 1984), and during the severe mite outbreak in Oregon in 2008 no evidence of increased dispersal among leaves was found using Taylor's power law.

For the third potential explanation, the association of mite outbreak severity to the timing of sulfur applications could involve a mechanism operating directly on spider mites but interacting with host growth dynamics. In this work, late and very late (in Washington) season sulfur applications made during the early exponential phase of spider mite population increase were associated with the most severe outbreaks of mites (Fig. 2.7). Plant phase, nutrition, and photosynthetic rates have been correlated with spider mite population growth (Karban and Thaler 1999). In strawberry, reproductive plant growth was associated with spider mite population increase (Poe 1971, Shanks and Doss 1989). In the current study, the late (and very late) sulfur applications began during mid-June to early July, corresponding with the period of most rapid plant growth and the onset of flowering (Neve 1991). The month before flowering is the time of maximum leaf biomass accumulation in hop because lateral branches develop during this period (Neve 1991). In comparison to all vegetative organs, leaves on lateral branches contain the highest level of nitrogenous substances and reducing sugars (Rybáček 1991). These leaves are likely the most nutritionally suitable for *T. urticae* based on studies on other plants (Karban and Thaler 1999). In the current study, sulfur applied during this time period, coincident with the

exponential phase of mite population development, were closely linked to the severity of the subsequent outbreak. Disentangling the nutritional quality of the newly produced leaves from the physiological changes associated with flowering was not attempted in this research, although both potentially could contribute to the severity of mite outbreaks, particularly if sulfur applications enhance dispersion or alter resource assimilation.

There appears to be a density-dependent relationship between the number of spider mites present when sulfur is applied and the severity of the ensuing mite outbreaks. This point is illustrated by the lack of a spider mite outbreak in Oregon 2009 following the late season sulfur sprays (Fig. 2.1E and F), which indicates that a 'critical mass' of spider mites must be present during sulfur sprays to incite an outbreak. It appears that the acaricidal effect of sulfur when applied at low spider mite densities during periods of limited host growth can inhibit an outbreak, shown by the cluster of points in lower left corner of Figure 2.6, but this effect is not apparent at higher spider mite densities. While the relationship shown in Figure 2.6 implies a direct impact of sulfur on the spider mites, the response variable CMD is also influenced by temperature, host plant physiology, and mite population dynamics, and this correlation may be a simple proxy for a much more complex interaction of other factors involved in sulfur-induced mite outbreaks. More study is needed to elucidate these potential interrelationships.

It is interesting to contrast the findings of Costello (2007) on grape with the current study on hop. Costello (2007) found that sulfur sprays made early in the

growing season (bud break to early bloom) were responsible for perturbation of mite outbreaks rather than the sprays made after bloom as reported here for hop. Although apparently contradictory, the development of leaf tissue on grape is nearly exponential during shoot elongation preceding bloom, and these leaves contain the highest level of nitrogen during this period of growth (Mullins et al. 1992). The pre-bloom sulfur spray timings investigated by Costello (2007) also correspond to the period when the mite outbreak would reach its early exponential phase. Thus, sulfur applications made during the period of rapid host development coincident with increasing mite abundance appear linked to mite outbreaks in both hop and grape and warrant further investigation.

Although the precise mechanisms involved with sulfur-induced mite outbreaks remain unclear, the results of this study clearly point to several practical strategies to integrate sulfur use for powdery mildew management with conservation biological control of spider mites. Avoidance of negative side effects of sulfur sprays was achieved by carefully timing applications to periods of low spider mite abundance and/or slow host development, which is generally early to mid-spring for hop. Use of sulfur after mid-June in Oregon and Washington hop yards generally will tend to disrupt conservation biological control. Based on now 10 site-years of observations in the current study and previous work (Gent et al. 2009), this statement seems to be valid irrespective of predatory mite abundance. While the potential mechanisms surrounding sulfur-induced spider mite outbreaks remain unresolved, this work

provides evidence for a more complex explanation than simply one of natural enemy disturbance.

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Table 2.1. Mean seasonal density \pm SEM of arthropods per hop leaf in relation to fungicide treatment and height in canopy, Corvallis, Oregon, 2007-2009

Arthropod/Year	Fungicide treatment (mean \pm SEM per leaf) ^a									
	Lower canopy: 0-2 m					Upper canopy: 2-6 m				
	Nontreated	Synthetic	Early sulfur	Mid-sulfur	Late sulfur	Nontreated	Synthetic	Early sulfur	Mid-sulfur	Late sulfur
2007:										
Phytoseiidae	0.02 \pm 0.01	0.02 \pm 0.01	0	0.02 \pm 0.01	0.01 \pm 0.01	0.03 \pm 0.02	0.10 \pm 0.05	0.01 \pm 0.01	0.01 \pm 0.01	0.04 \pm 0.02
<i>Tetranychus urticae</i>	1.33 \pm 0.22	0.98 \pm 0.16	1.48 \pm 0.36	1.17 \pm 0.23	0.89 \pm 0.18	20.52 \pm 5.79	16.04 \pm 4.95	10.52 \pm 2.43	15.81 \pm 6.37	23.64 \pm 7.97
2008:										
Phytoseiidae	0.32 \pm 0.15	0.12 \pm 0.07	0.26 \pm 0.10	0.30 \pm 0.12	0.37 \pm 0.15	0.31 \pm 0.18	0.22 \pm 0.10	0.17 \pm 0.08	0.20 \pm 0.08	0.09 \pm 0.04
<i>Tetranychus urticae</i>	10.42 \pm 2.46	14.27 \pm 3.55	17.69 \pm 5.15	13.22 \pm 2.53	20.16 \pm 4.48	83.71 \pm 23.63	101.98 \pm 22.70	97.72 \pm 19.91	143.22 \pm 30.23	187.83 \pm 54.99
2009:										
Phytoseiidae	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.001 \pm 0.001	0.02 \pm 0.01	0.01 \pm 0.004	0	0.01 \pm 0.01	0	0.01 \pm 0.004
<i>Tetranychus urticae</i>	0.11 \pm 0.05	0.04 \pm 0.03	0.05 \pm 0.03	0.10 \pm 0.10	0.37 \pm 0.16	0.28 \pm 0.16	0.16 \pm 0.11	0.02 \pm 0.02	0.02 \pm 0.02	0.03 \pm 0.03

^a Plots were treated every 7-14d with a rotation of synthetic fungicides (as described in text) or micronized sulfur (Microthiol Disperss) during the sulfur spray timing. All sprays ceased on the following dates: 11 July 2007; 11 July 2008; and 10 July 2009.

Table 2.2. Effect of fungicide treatment on area under the development curve for spider mites (*Tetranychus urticae*) and phytoseiids on hop plants in Oregon and Washington, 2007-2009

Year ^a	Treatment	Oregon				Washington	
		<i>T. urticae</i>		Phytoseiidae		<i>T. urticae</i>	Phytoseiidae
		Lower canopy ^{bc}	Upper canopy	Lower canopy	Upper canopy	Lower canopy	Lower canopy
2007	Nontreated	98.8	1225.1a	1.7	2.1	1857.0a	47.6a
	Synthetic	109.9	947.2a	2.2	5.7	1961.1a	24.2a
	Early sulfur	73.9	607.6a	0	0.7	2044.1a	20.1a
	Mid sulfur	89.8	939.8a	2.1	0.6	2283.7a	26.1a
	Late sulfur	83.3	1378.9b ^c	0.7	2.3	4007.7b	31.3a
	Very late sulfur	-	-	-	-	1824.2ab	7.4b
2008	Nontreated	1009.5	4582.4a	24.7	14.2	1088.4a	110.2
	Synthetic	1352.9	6971.8a	10.6	11.7	1833.6b ^c	75.3
	Early sulfur	1573.8	6689.4a	19.8	9.3	1613.8ab	85.4
	Mid sulfur	1332.5	9684.5b	27.4	11.1	1765.8ab	104.0
	Late sulfur	1943.3	13042.5b	31.9	6.0	2277.0ab	70.2
	Very late sulfur	-	-	-	-	2515.4b	63.4
2009	Nontreated	8.6	10.7	2.3	0.6	4891.2a	125.5
	Synthetic	2.2	6.2	1.3	0	4698.3ab	144.7
	Early sulfur	3.4	0.7	1.4	0.7	4175.2a	188.9
	Mid sulfur	7.9	0.7	0.1	0	3759.9a	175.5
	Late sulfur	34.9	2.2	2.2	0.8	7049.0b	78.4
	Very late sulfur	-	-	-	-	5903.6a	154.9

^a The area under the development curve was calculated by plotting mean arthropod population over time and calculating the area under the curve by integration. Treatment means were analyzed using a linear mixed-model repeated in space

(coordinate location of plots) to account for spatial aggregation of mites among plots, and treatments were compared using an *F*-protected LSD test. Treatments within a given location and year followed by the same letter are not significantly different at $\alpha = 0.05$, except where noted.

^b Ten to 20 leaves were collected per plot on each assessment date. In Oregon, as plants grew taller than approximately 2 meters samples were taken from at two levels, lower canopy (<2 m) and upper canopy (> 2 m). Samples were collected from one height (approximately 2 m) in Washington. The very late sulfur treatment was not evaluated in Oregon. See text for an explanation of the treatments.

^c The synthetic treatment in Washington 2008 for *T. urticae* was significantly greater than the nontreated at $\alpha = 0.056$. All other treatment differences were significant at $\alpha = 0.05$.

Table 2.3. Effect of fungicide treatment on cone color, Oregon and Washington, 2007-2009

Year	Fungicide treatment	Cone visual appearance ^a					
		Oregon			Washington		
		Median	Mean rank	Relative effect ^b	Median	Mean rank	Relative effect ^b
2007	Nontreated	1	20.5	0.50 (0.50-0.50)	2	10	0.40 (0.30-0.51)
	Synthetic	1	20.5	0.50 (0.50-0.50)	2	10	0.40 (0.30-0.51)
	Early sulfur	1	20.5	0.50 (0.50-0.50)	2	12.75	0.51 (0.32-0.70)
	Mid-sulfur	1	20.5	0.50 (0.50-0.50)	2.5	15.5	0.63 (0.38-0.80)
	Late sulfur	1	20.5	0.50 (0.50-0.50)	2	12.75	0.51 (0.32-0.70)
	Very late sulfur	-	-	-	2.5	14	0.56 (0.23-0.83)
2008	Nontreated	3.5	6.0	0.28 (0.17-0.47)	5.5	12	0.48 (0.27-0.70)
	Synthetic	3.5	7.63	0.36 (0.18-0.63)	5.5	12	0.48 (0.27-0.70)
	Early sulfur	4.5	10.75	0.51 (0.29-0.73)	5.5	12	0.48 (0.27-0.70)
	Mid-sulfur	4	10.63	0.51 (0.35-0.66)	5.5	12	0.48 (0.27-0.70)
	Late sulfur	5.5	17.5	0.85 (0.71-0.89)	5.5	12	0.48 (0.27-0.70)
	Very late sulfur	-	-	-	6	15	0.60 (0.26-0.84)
2009	Nontreated	4.5	11.13	0.53 (0.33-0.71)	4.5	14.75	0.59 (0.36-0.78)
	Synthetic	4.25	10.5	0.50 (0.21-0.79)	4	7.63	0.30 (0.18-0.48)
	Early sulfur	4.0	9.75	0.46 (0.24-0.71)	4	17.38	0.70 (0.46-0.84)
	Mid-sulfur	4.5	11.5	0.55 (0.29-0.77)	4	10.25	0.41 (0.20-0.67)
	Late sulfur	4.25	9.63	0.46 (0.21-0.74)	4	12.13	0.48 (0.30-0.68)
	Very late sulfur	-	-	-	4.5	12.88	0.52 (0.24-0.78)

^a In 2007, cones were rated a using a four-step scale, where 1 = no damage and 4 = severe cone discoloration or damage on greater than 25% of the cone or cone abortion. In 2008 and 2009, cones were rated using a seven-step ordinal scale where 1 = excellent cone quality and 7 = very poor; see text for details.

^bData was analyzed using a nonparametric ANOVA-type statistic. Relative effect ranges from 0 to 1, where 1 indicates the greatest cone damage. Relative effect is significantly different if the 95% confidence intervals do not overlap. The very late sulfur treatment was not evaluated in Oregon. See text for an explanation of the treatments.

Table 2.4. Mean seasonal density \pm SEM of arthropods per hop leaf in relation to fungicide treatment, Prosser, Washington, 2007-2009

Arthropod/Year	Fungicide treatment (mean \pm SEM per leaf) ^a					
	Nontreated	Synthetic	Early sulfur	Mid-sulfur	Late sulfur	Very late sulfur
2007						
Phytoseiidae	0.41 \pm 0.08	0.25 \pm 0.05	0.19 \pm 0.04	0.22 \pm 0.06	0.26 \pm 0.05	0.06 \pm 0.02
<i>Tetranychus urticae</i>	15.45 \pm 1.41	16.14 \pm 1.66	17.11 \pm 2.03	31.13 \pm 4.98	32.56 \pm 3.47	18.28 \pm 1.64
2008						
Phytoseiidae	1.04 \pm 0.12	0.83 \pm 0.10	0.94 \pm 0.10	1.02 \pm 0.12	0.72 \pm 0.09	0.68 \pm 0.09
<i>Tetranychus urticae</i>	10.19 \pm 1.42	17.32 \pm 2.43	15.03 \pm 2.43	16.55 \pm 1.74	21.29 \pm 2.04	23.34 \pm 2.50
2009						
Phytoseiidae	1.21 \pm 0.19	1.36 \pm 0.28	1.78 \pm 0.33	1.64 \pm 0.27	0.79 \pm 0.13	1.48 \pm 0.28
<i>Tetranychus urticae</i>	46.13 \pm 5.68	43.83 \pm 4.98	38.62 \pm 4.60	34.87 \pm 3.57	64.19 \pm 8.48	54.79 \pm 5.84

^a Plots were treated every 7 to 14 days with a rotation of synthetic fungicides or micronized sulfur (Microthiol Disperss) during the sulfur spray timing. The very late sulfur treatment was not evaluated in Oregon. See text for an explanation of the treatments. All sprays ceased on the following dates: 25 July 2007; 6 August 2008; 27 July 2009.

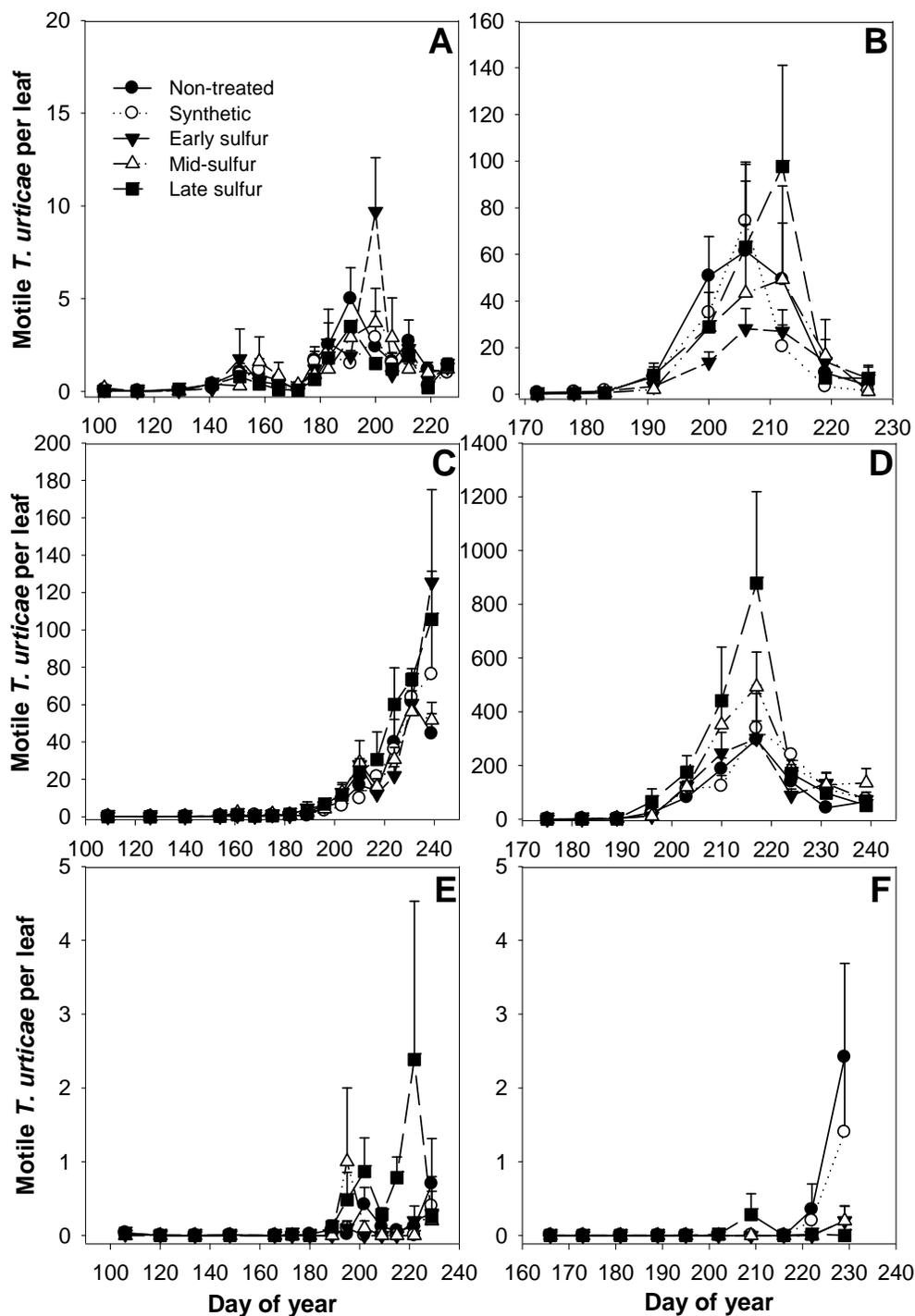


Fig. 2.1. Abundance of *Tetranychus urticae* (mean \pm SEM) on hop leaves in relation to fungicide treatment in Oregon in the lower (A, C, E) and upper canopy (B, D, F); in

2007 (A, B), 2008 (C, D), and 2009 (E, F), respectively. Lower canopy data was collected from 10-20 leaves per plot sampled at a height of $< 2\text{m}$. Upper canopy data was collected from 10-20 leaves per plot sampled at a height of $> 2\text{m}$. Data reported is a mean of 4 replications per treatment.

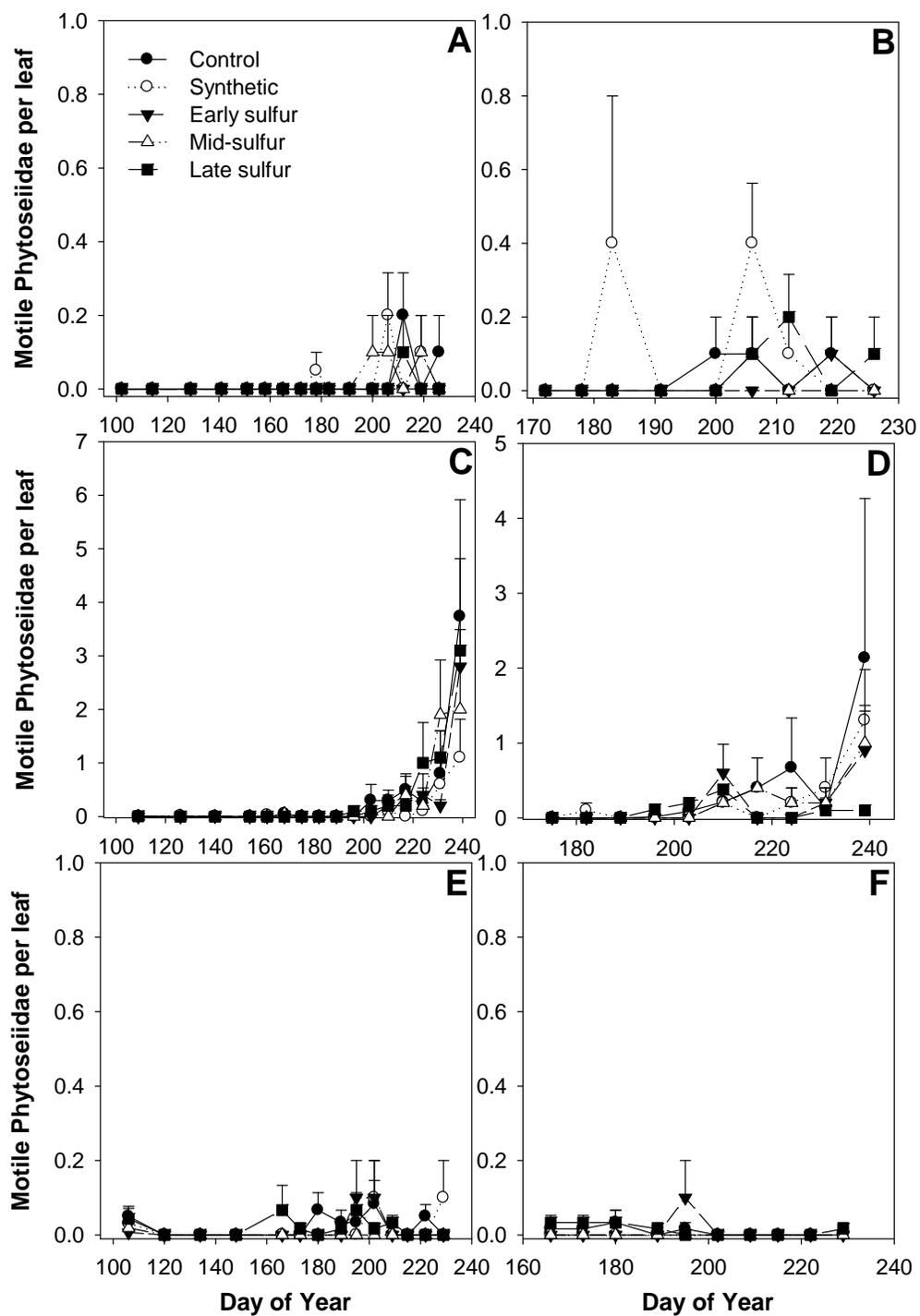


Fig. 2.2. Abundance of Phytoseiidae (mean \pm SEM) on hop leaves in relation to fungicide treatment in Oregon in the lower (A, C, E) and upper canopy (B, D, F) in

2007 (A, B), 2008 (C, D), and 2009 (E, F), respectively. Lower canopy data was collected from 10-20 leaves per plot sampled at a height of $< 2\text{m}$. Upper canopy data was collected from 10-20 leaves per plot sampled at a height of $> 2\text{m}$. Data reported is a mean of 4 replications per treatment.

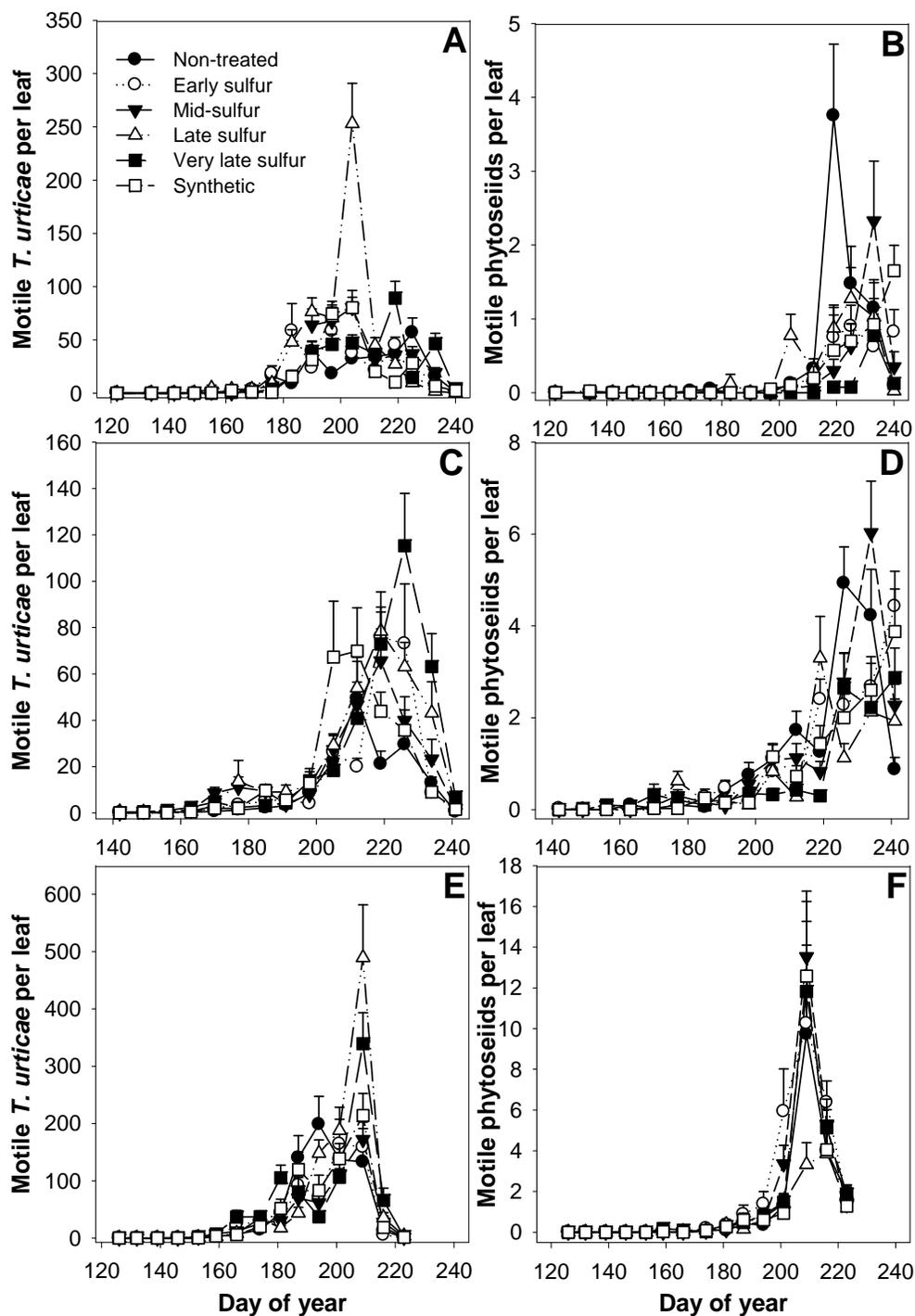


Fig. 2.3. Abundance of *Tetranychus urticae* (mean \pm SEM) and Phytoseiidae (mean \pm SEM) on hop leaves in relation to fungicide treatment in Washington in 2007 (A, B),

2008 (C, D), and 2009 (E, F), respectively. Data was collected from 10 leaves per plot sampled at a height of approximately 2m. Data reported is a mean of 4 replications per treatment.

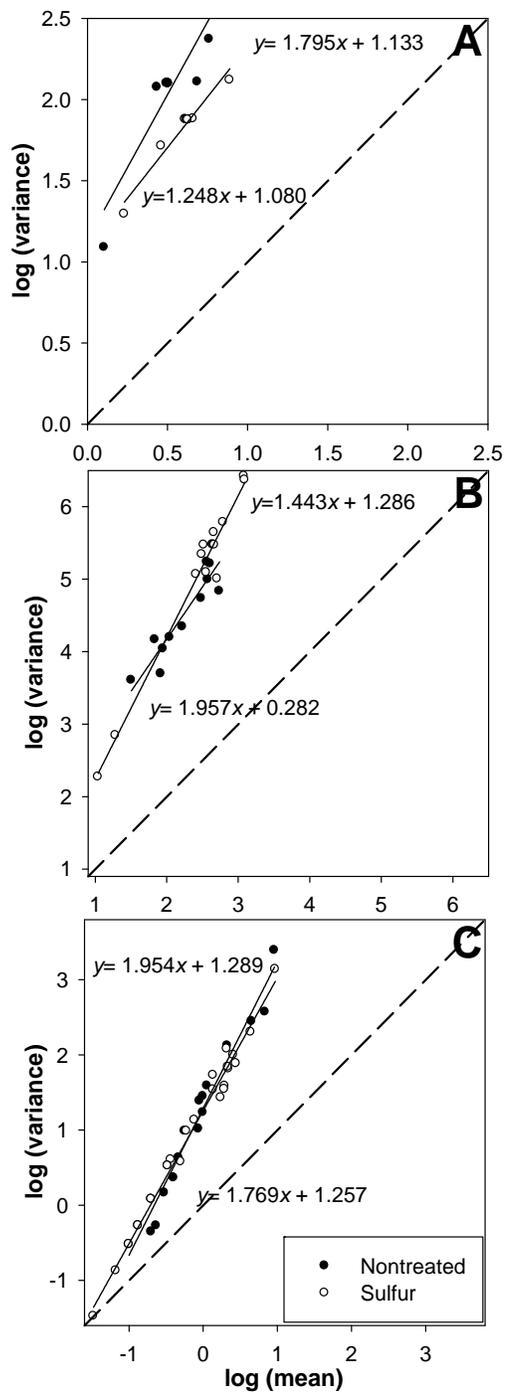


Fig. 2.4. Taylor's power law relationships for spider mite eggs on individual leaves in nontreated (closed circles) and sulfur treated plots (open circles) in Oregon in 2007-2009 (A, B, and C respectively).

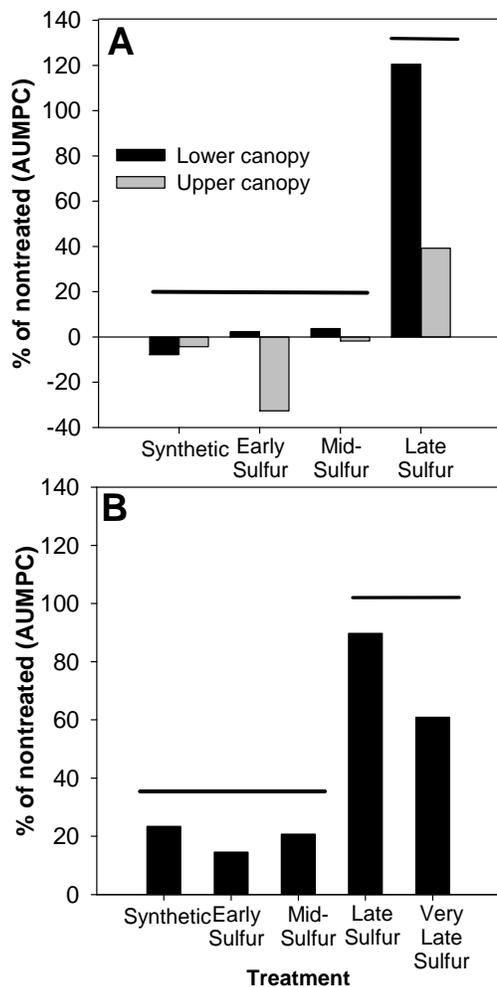


Fig. 2.5. Mean severity of spider mite outbreaks (measured by area under the pest development curve) for each treatment relative to the nontreated control in a given year in Oregon for both the lower and upper canopy (A) and one canopy height in Washington (B). The bars indicate significantly different groups for Oregon in the lower and upper canopy ($P = 0.0269$ and $P = 0.0296$, respectively) and Washington ($P = 0.0002$). Data includes the means from three experiments conducted in each state during 2007-2009. See text for further details on the analysis conducted.

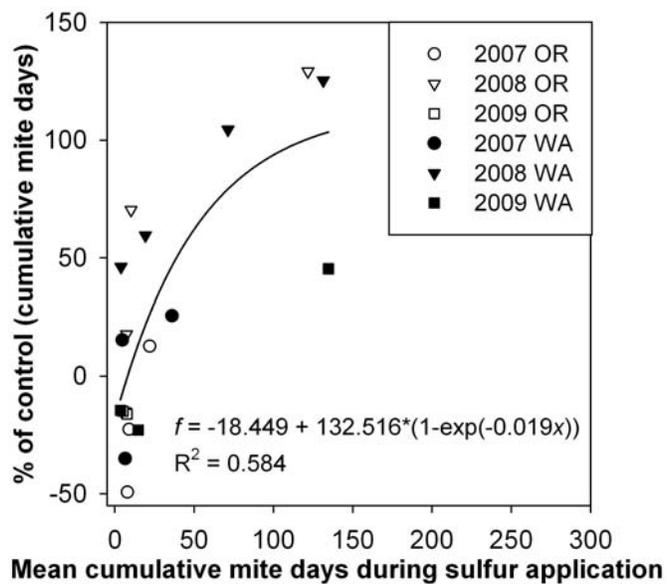


Fig. 2.6. Spider mite outbreak severity in sulfur-treated plots (standardized to the corresponding nontreated plot) in relation to the mean cumulative mite days during the time period of when sulfur was applied in each plot. All treatments represented in the figure received three sequential sulfur sprays at varying times during the season.

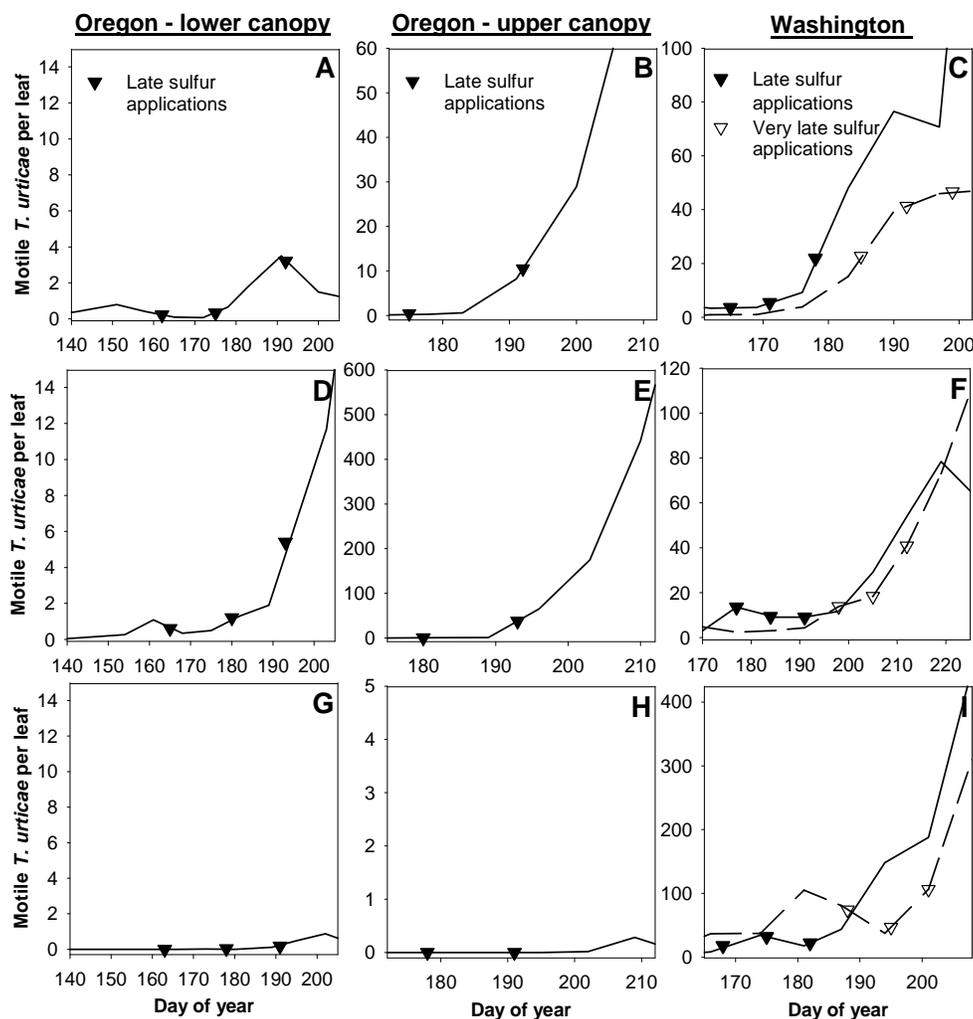


Fig. 2.7. Abundance of motile *Tetranychus urticae* when sulfur fungicides were applied in the late season sulfur treatments in Oregon in the lower canopy in 2007-2009 (A, D, G), and the upper canopy in 2007-2009 (B, E, H), respectively. Abundance of motile *T. urticae* when sulfur fungicide applications were applied in the late (solid line) and very late season sulfur treatments (dashed line) in Washington in 2007-2009 (C, F, I), respectively. Solid triangles indicate late season sulfur treatment applications and open triangles indicate very late season (Washington only) sulfur applications. The ordinate axis scale has been adjusted in each figure to better visualize the point of inflection in the spider mite population development curve.

GENERAL CONCLUSIONS

Given the continued damaged caused by twospotted spider mites in hop and other agricultural systems, and the widespread reliance on chemical control measures for management, means to improve the reliability of conservation biological control (CBC) are needed. The purpose of this thesis was to evaluate approaches and tactics to enhance CBC of this pest. To this end, means to (i) enhance early season recruitment and retention of natural enemies to hop yards via deployment of synthetic methyl salicylate (MeSA) and (ii) minimize disruptions to biological control induced by sulfur fungicide applications were investigated.

Deployment of synthetic MeSA in Oregon hop yards was found to have a modest but positive influence on recruitment of certain natural enemies and suppression of spider mites. There was a trend for higher densities of *Stethorus* spp., a key predator of spider mites, in hop yards where MeSA was deployed, and a corresponding reduction in the number of spider mites (40-91% depending on the hop yard). However, these reductions of spider mites were only statistically significant in one year of the study (2008). Unlike previous studies in Washington hop yards (James and Price 2004), other major groups of predators (e.g., Chrysopidae, *Orius tristicolor*) were not found in higher in abundance in yards baited with MeSA. The number of hop aphids found in baited versus unbaited yards also was similar. To adequately determine the efficacy of a semiochemical, it is valuable to consider the influence of the local agro-ecology of each site, as well as the application method, timing, and rate. When these factors are considered, it seems possible that certain hop yards could

benefit from the deployment of synthetic MeSA; however the optimal application rate, timing, and method may be site-specific and needs to be optimized through further research before being recommended for practical use.

The use of sulfur to manage hop powdery mildew can exacerbate twospotted spider mite outbreaks (Gent et al., 2009) and this thesis research found that the degree of perturbation of twospotted spider mite outbreaks was related to the timing of sulfur applications in a density-dependent fashion based on the number of spider mites present at the time of the sulfur application. In both Oregon and Washington, later season timings of sulfur applications (mid June or later) resulted in the highest abundance of twospotted spider mites compared to nontreated plots, while sulfur applications made prior did not cause mite outbreaks more severe than those of nontreated plots. Sulfur toxicity to phytoseiid mites is often implicated as a main cause of sulfur-induced outbreaks of spider mites (e.g., Beers et al. 2009). Reduced abundance of phytoseiid mites due to sulfur applications made in the later portions of the season were noted in only one of the six location-years of this study. While phytoseiid mites are sensitive to sulfur applications, it is also clear that there are other mechanisms at play, given that spider mite outbreaks also occurred in the near absence of phytoseiid mites in both grape and hop (Costello 2007, Gent et al. 2009) and evidence of altered dispersion of spider mite eggs was found in two of three years of these studies. More research is needed to determine the mechanism(s) involved with sulfur-induced spider mite outbreaks, however this work implicates a mechanism other than, or in conjunction with, natural enemy disturbance.

A common theme throughout these studies is that CBC is most efficacious when the entire agroecosystem and production factors are considered. Early season recruitment of natural enemies through the use of semiochemicals will require prior knowledge of the agroecosystem and it is possible that use of MeSA will be most successful where the conservation of natural enemies and the long-term development of habitat is an established practice, potentially implicated by the site specificity of the data. Clearly, MeSA deployment does not provide a therapeutic function as a pesticide application does, as many CBC tactics rely on a series of conservation practices to be efficacious (Barbosa 1998, Jonsson et al. 2008). An example of such is the consideration of the timing of sulfur fungicides. CBC was aided when sulfur was applied only when the number of twospotted spider mites present on leaves was very low and/or before periods of rapid host growth and flowering. On hop, these conditions tend to occur during the early to mid portions of the season before about mid June. When sulfur was applied after this time when spider mites were present, the number of twospotted spider mites increased beyond levels that could be controlled by natural enemies without economic damage to the crop. Therefore, careful selection of fungicides to manage hop powdery mildew late in the season could reduce the risk of needing to apply additional miticides.

Economically viable IPM approaches increase the likelihood of their adoption by producers (Wearing 1988). A strategy of assessing spider mite abundance before each sulfur application should be an economical and practical means to minimize sulfur-induced mite outbreaks and manage powdery mildew on hop and perhaps in

other systems. However, more research is needed to develop a cost-effective means of deploying MeSA or other semiochemicals for this approach to be cost-effective and reliable in commercial production. With these considerations, this research provides additional strategies to further enhance the CBC of twospotted spider mite in hop yards.

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APPENDIX

Appendix 1: Effect of Sulfur Fungicide Timing on Hop Aphid

The damson-hop aphid, *Phorodon humuli* (Schrank) (Homoptera: Aphididae) is a common pest of hop and feeding by large numbers of aphids can reduce crop yield and/or quality. Crop damage is incurred mainly when affected cones are contaminated by secondary organisms that colonize aphid honeydew (Weirauch 2009). Commercial hop farmers in the U.S. generally apply at least one aphicide annually to control this pest (Mahaffee et al. 2009). The impact of sulfur fungicides used for the management of powdery mildew on hop aphid is not well known, however a slight reduction from sulfur fungicides was observed in experimental plots in Washington (Gent et al. 2009). The effect of sulfur application timing on hop aphid numbers prior to this study was unknown.

Experimental design, treatment administration and hop aphid nymph enumeration were as described in Chapter 3. Count data collected over time were analyzed using a repeated measures analysis. Statistical analyses were conducted using a mixed model in the GLIMMIX procedure in SAS version 9.2. Discrete data were log transformed to achieve normally distributed residuals with a common variance. Multiple covariance structures were investigated, and the simplest covariance consistent with the data were selected on Akaike's Information Criterion (AIC). Degrees of freedom were estimated by the Kenward-Roger method.

Results and Discussion

Fungicide treatment had no effect on hop aphid abundance in repeated measures analysis in any of the years of the experiments in Oregon (Fig. Appendix 1.1., Table Appendix 1.1. and 1.2.). Conversely, sulfur application timing significantly affected hop aphid abundance in 2007 and 2009 in Washington (Fig. 1.2., Table Appendix 1.3. and 1.4.). In these two years, the early season sulfur treatment reduced hop aphid abundance in these plots 48 to 66% compared to nontreated plots, while hop aphid abundance were unaffected by sulfur fungicide applications in 2008. In 2009, there was a significant treatment x time interaction, indicating that treatment differences were influenced by sampling date (Table Appendix 1.4). There was a significant treatment effect beginning at day of year 159 and ending at day of year 201, essentially capturing the period of aphid population growth during late spring and early summer (Fig. Appendix 1.2C.). Treatment effects were non-significant on all other sampling dates.

Gent et al. (2009) also reported a slight reduction in hop aphid abundance from sulfur application in Washington, as was found in this study. The current study identified early season (mid-May to June) applications as having the largest effect on hop aphid abundance; however this was only significant in two years of the experiments (2007 and 2009) (Table Appendix 1.3). Similar to the findings of Gent et al. (2009), sulfur fungicides did not have an effect on hop aphid abundance in Oregon. Under certain environmental conditions the use of sulfur early in the season appears to

reduce hop aphid abundance. However, early season sulfur fungicide applications appear to be mostly innocuous to hop aphid and their natural enemies in Oregon and have only a relatively small effect in Washington.

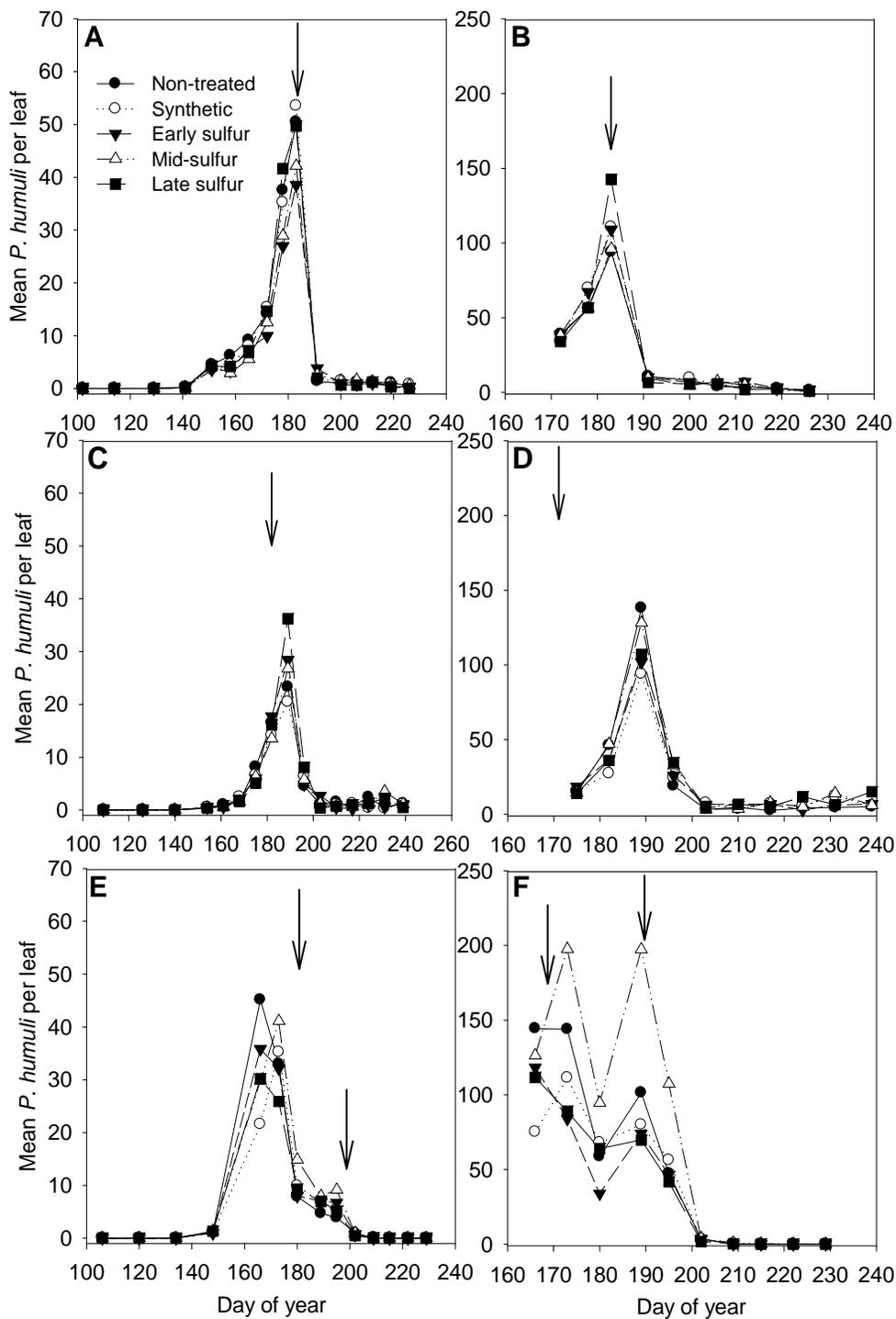


Figure Appendix 1.1. Mean number of *Phorodon humuli* on hop leaves in relation to fungicide treatment in Oregon in the lower (A, C, E) and upper canopy (B, D, F) in

2007 (A, B), 2008 (C, D), and 2009 (E, F), respectively. Lower canopy data was collected from 10-20 leaves per plot sampled at a height of < 2m. Upper canopy data was collected from 10-20 leaves per plot sampled at a height of > 2m. Data reported is means of 4 replications per treatment. Arrows indicate the application of an aphicide as described in Chapter 3. Error bars are not presented to improve legibility.

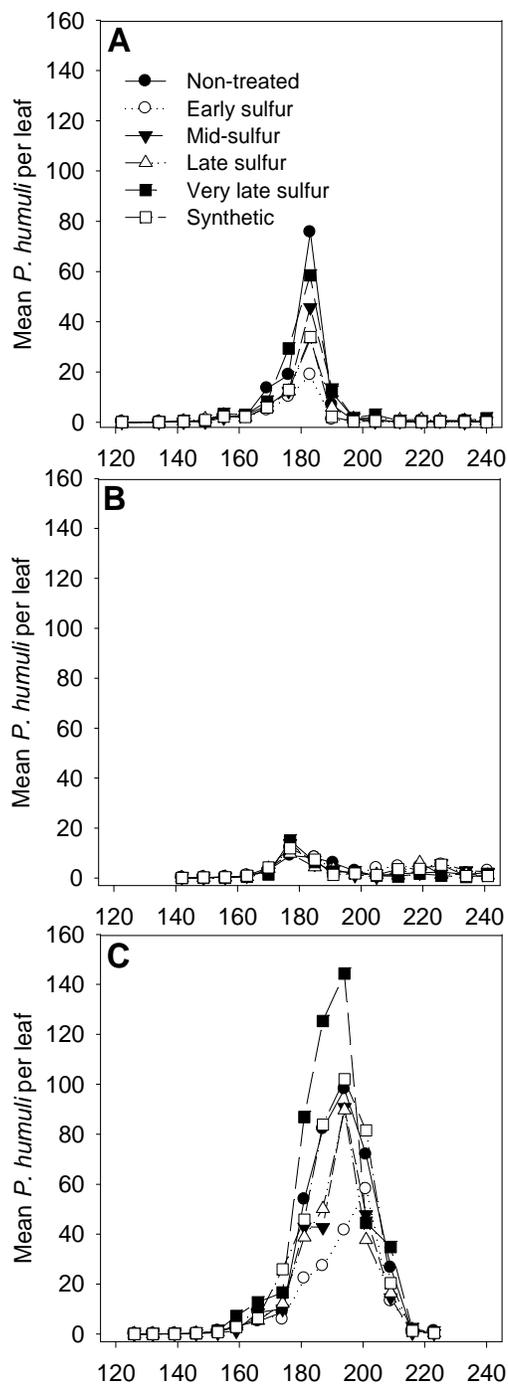


Figure Appendix 1.2. Mean number of *Phorodon humuli* on hop leaves in relation to fungicide treatment in Washington in 2007 (A), 2008 (B), and 2009 (C). Data reported

is means of 4 replications per treatment. No aphicides were applied during these experiments Error bars are not presented to improve legibility.

Table Appendix 1.1. Mean seasonal abundance \pm SEM of hop aphid (*Phorodon humuli*) per leaf in relation to fungicide treatment and height in canopy, Corvallis, Oregon 2007-2009.

Treatment	Fungicide treatment (mean \pm SEM per leaf) ^a					
	Lower canopy ^b			Upper canopy ^b		
	2007	2008	2009	2007	2008	2009
Control	8.04 \pm 1.47	3.83 \pm 0.83	6.92 \pm 2.00	24.23 \pm 3.91	25.42 \pm 7.75	49.92 \pm 10.25
Synthetic	7.87 \pm 1.34	3.65 \pm 0.84	5.74 \pm 1.49	27.21 \pm 4.70	21.56 \pm 5.29	39.37 \pm 7.77
Early Sulfur	6.07 \pm 0.99	4.18 \pm 1.07	6.52 \pm 1.77	27.55 \pm 4.45	19.52 \pm 4.20	36.06 \pm 7.52
Mid-Sulfur	6.49 \pm 1.11	3.86 \pm 0.91	7.51 \pm 1.89	24.41 \pm 3.96	23.98 \pm 6.61	72.76 \pm 16.34
Late Sulfur	7.85 \pm 1.38	4.94 \pm 1.25	5.67 \pm 1.43	28.38 \pm 5.77	23.94 \pm 5.13	37.82 \pm 7.71

^a Plots were treated every 7-14 days with a rotation of synthetic fungicides or micronized sulfur during the sulfur spray timing (as described in Chapter 3). All fungicide sprays ceased on the following dates: 11 July 2007; 11 July 2008; and 10 July 2009.

^b Lower canopy data was collected from 10-20 leaves per plot sampled at a height of < 2m. Upper canopy data was collected from 10-20 leaves per plot sampled at a height of > 2m.

Table Appendix 1.2. Significance of factors in repeated measures analysis for hop aphid (*Phorodon humuli*) in Oregon in both the lower and upper canopy.

		Mixed model Type 3 fixed effects							
		Lower canopy ^a				Upper canopy ^b			
Year	Effect	Num DF	Den DF	<i>F</i>	<i>P > F</i>	Num DF	Den DF	<i>F</i>	<i>P > F</i>
2007	Fungicide	4	72.4	1.68	0.1647	4	38.3	1.71	0.1671
	Time	15	208	331.61	< 0.0001	8	43.1	298.53	< 0.0001
	Time*Fungicide	60	199	1.18	0.1956	32	63.3	1.57	0.0635
2008	Fungicide	4	3.51	3.11	0.1663	4	13	1.10	0.2986
	Time	9	45	1.16	0.3460	9	7.1	50.54	< 0.0001
	Time*Fungicide	36	45	0.96	0.5473	36	9.6	1.18	0.4134
2009	Fungicide	4	7.93	0.65	0.6457	4	3.51	3.11	0.1663
	Time	13	55	0.91	0.5439	9	45	1.16	0.3460
	Time*Fungicide	52	41.4	1.13	0.3410	36	45	0.96	0.5473

^aLower canopy data was collected from 10-20 leaves per plot sampled at a height of < 2m.

^bUpper canopy data was collected from 10-20 leaves per plot sampled at a height of > 2m.

Table Appendix 1.3. Mean seasonal abundance \pm SEM of hop aphid (*Phorodon humuli*) per leaf in relation to fungicide, Prosser, Washington, 2007-2009.

Treatment ^a	Mean \pm SEM per leaf ^b		
	2007	2008	2009
Control	7.30 \pm 4.56ab	2.76 \pm 0.72	23.52 \pm 8.98abc
Synthetic	7.27 \pm 3.65a	2.86 \pm 0.85	24.74 \pm 9.29ab
Early Sulfur	2.46 \pm 1.20c	3.46 \pm 0.85	12.23 \pm 4.57c
Mid-Sulfur	4.12 \pm 1.96ab	2.96 \pm 0.90	17.06 \pm 6.75bc
Late Sulfur	5.16 \pm 2.74bc	2.39 \pm 0.70	17.34 \pm 6.93c
Very Late Sulfur	3.60 \pm 2.05c	2.29 \pm 1.00	31.77 \pm 12.47a

^a Plots were treated every 7-14 days with a rotation of synthetic fungicides or micronized sulfur during the sulfur spray timing. See text in Chapter 3 for an explanation of the treatments. All sprays ceased on the following dates: 25 July 2007; 6 August 2008; 27 July 2009.

^b Means within a year and column followed by a different letter are significantly different ($\alpha = 0.05$), as determined by repeated measures analysis, see Table Appendix 1.4.

Table Appendix 1.4. Significance of factors in repeated measures analysis for hop aphid (*Phorodon humuli*) in Washington.

Mixed model Type 3 fixed effects					
Year	Effect	Num DF	Den DF	<i>F</i>	<i>P</i> > <i>F</i>
2007	Fungicide	5	68.7	6.01	0.0001
	Time	16	262	84.99	< 0.0001
	Time*Fungicide	80	247	1.29	0.0697
2008	Fungicide	5	68.8	1.64	0.1611
	Time	15	254	29.37	< 0.0001
	Time*Fungicide	75	242	1.00	0.4796
2009	Fungicide	5	53.5	3.71	0.0059
	Time	14	241	151.53	< 0.0001
	Time*Fungicide	70	228	1.51	0.0127