Population Density and Phenology of *Tetranychus urticae* (Acari: Tetranychidae) in Hop is Linked to the Timing of Sulfur Applications

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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 2007–2009 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 total cumulative mite days (standardized to a nontreated plot) explained 58% of the variability observed in increased spider mite severity related to sulfur spray timing. Spatial patterns of spider mites in the Oregon plots indicated similar dispersal of motile stages of spider mites 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 exacerbation of spider mite outbreaks by 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.

KEY WORDS conservation biological control, *Humulus lupulus*, pest resurgence, *Podosphaera macularis*

The application of pesticides can result in unforeseen consequences, particularly, negative impacts on nontarget 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 and 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 certain 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 (e.g., some pyrethroid, organochlorine, and organophosphate compounds) are well-known to induce spider mite outbreaks (McMurtry et al. 1970, James and Price 2002, James and Barbour 2009), 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.

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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 used 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 negative impacts on phytoseiids, 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 15–25 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 that can be favorable for both powdery mildew and spider mites (Mahaffee et al. 2009). As many as 6 to 10 annual applications of sulfur-based products, horticultural oils (e.g., paraffinic oil), and synthetic fungicides (e.g., myclobutanil, quinoxyfen, spiroxamine, 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 previous studies have indicated that powdery mildew management tactics, in particular the use of sulfur fungicides, may 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 semiarid 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. The study raised questions of the mechanism at play, given that sulfur 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 sulfur 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, OR, and at the Washington State University Irrigated Agricultural Research and Extension Center near Prosser, WA, 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 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 eight hop plants in a 2 × 4 rectangular array. In 2009, plots consisted of 16 plants in a 4 × 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 d 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. In 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 (Gent et al. 2009, James and Coyle 2001). The rotation was (in order): trifloxystrobin (0.14 kg active ingredient [AI]/ha, Flint 50 WG, Bayer CropScience, Research Triangle Park, NC), spiroxamine (0.36 kg AI/ha, Accrue, Bayer CropScience) and quinoxyfen (0.10 kg AI/ha, Quintec, Dow AgroSciences, Indianapolis, IN). In Oregon three sulfur timings were evaluated in each of 2007–2009: early season (sprays beginning day of year 107, 110, and 107, respectively), mid-season (sprays beginning 133, 138, and 135, respectively), and late season (sprays beginning 162, 164, and 162, respectively). In each of these treatments, three sequential sprays of sulfur (as described below) were made at biweekly intervals. These treatments were compared with a rotation of only the synthetic fungicides and a nontreated control. In Washington, four sulfur timings were investigated, with each application made at weekly rather than biweekly intervals. In 2007–2009, these timings were: early season (sprays beginning 123, 135, and 127, respectively), mid-season (beginning 143, 156, and 147), late season (beginning 165, 174, and 168, respectively), and very late season (beginning 185, 197, and 188, respectively). This difference between regions reflects general grower use patterns for sulfur in each state because of more severe powdery mildew pressure in Washington. In Washington, the alternating application of synthetic fungicides occurred biweekly, with the same products and sequence as in Oregon. In both Oregon and Washington, sulfur was applied as Microthiol Disperss (Cerexagri, Inc. North America, King of Prussia, PA) at 5.38 kg AI/ha. To avoid confounding effects from other arthropod pests, in all years of the trial in Oregon, *Bacillus thuringiensis* (0.15 kg AI/ha, Javelin WG, Certis USA, LLC, Columbia, MD) and pymetrozine (0.034 kg AI/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 AI/ha, Provado 1.6 F, 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 pretreatment assessment in mid-April to early May and continuing until cone harvest during mid to late August. On each sampling date, 10–20 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. In Oregon, canopy shake samples also were collected from each plot biweekly as described previously (Gent et al. 2009) to enumerate other predatory insects associated with the plants and treatments. Canopy shake sample data are not presented because of space constraints. However, cumulative arthropod-days (explained below) for all nonacarine predators recovered in shake samples were similar among treatments in all location-years ($P \geq 0.0931$).

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 *N. 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 ≈2 m, samples were collected from lower (<2 m) and upper (>2 m) positions in the canopy. Samples were collected from only one height in Washington, ≈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 weekly in each year from the nontreated plots and the early sulfur treatment (2007) or late sulfur treatment (2008 and 2009) beginning after the first sulfur spray was applied. In these samples, 20–40 leaves were collected from the upper and lower canopy (2008, upper canopy only) and were scanned individually with the aid of a stereomicroscope 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 ≈100 cone 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 <25% of cone area exhibiting discoloration or damage), and 4 = severe cone discoloration (damage on >25% of the cone or cone abortion). In 2008 and 2009, cone visual quality was rated by a commercial hop merchant (Brewers Supply Group, Yakima, WA) using their standard hop rating scale, where 1 = "excellent", 2 = "excellent (-)", 3 = "good (+)", 4 = "good", 5 = "good (-)", 6 = "poor", and 7 = "poor (-)." Two sub-samples per plot were rated separately in 2009. In all years, evaluations were conducted in a blind manner where the cone samples were coded so that the rater was unaware of the treatment each sample received.

Data Analysis. Spider mite, predatory mite, and predacious insect populations on each assessment date were plotted over time to calculate arthropod-days (cumulative mite-days [CMD] or insect-days) using a macro available in SigmaPlot version 11.0 (Systat Software, Inc., San Jose, CA). These values were log-transformed when needed 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 nonindependence 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). This is analogous to accounting for serial correlation of residuals in a repeated measures analysis. In this context, however, the correlation of residuals is in space not time. When evidence of spatial correlation is detected, the correlation can be explicitly modeled by specifying an appropriate covariance structure for the residuals. In essence, this removes the effect of spatial correlation to obtain more accurate estimates of treatments means. 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 least significant difference test.

The ordinal rating scale used for mite damage assessment on cones was analyzed using a nonparametric analysis of variance (ANOVA)-type statistic as described by Shah and Madden (2004) and Gent et al. (2009). In this analysis, a relative treatment effect ranging from 0 to 1 is calculated for each treatment, based on an empirical distribution function of ranks of the medians (explained in Shah and Madden 2004). Relative treatment effects represent probabilities that one random variable is larger than another. Calculation of relative treatment effects are tedious, but can be obtained easily using macros developed by Brunner et al. (2002). 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 were 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 nontreated control for that experiment. Mean mite-days for each treatment was expressed as a percentage of the nontreated control by subtracting the mite-days for a given fungicide treatment by the mite-days for the nontreated control and then dividing the difference by the mite-days of the nontreated. 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: 1) the synthetic fungicide treatment, early sulfur, and mid-sulfur timings; and 2) the later sulfur timings. Given these two groups, a linear contrast was conducted to test whether means between the two groups varied significantly. Analyses were conducted using PROC MIXED in SAS, with year 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), CMD were calculated using the formula:

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

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) 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 were selected based on the pseudo-R^2, standard error of parameter estimates, and visual inspection of residual plots.

Spatial Analysis. Data from the intensive sampling of the nontreated and selected sulfur treatments were

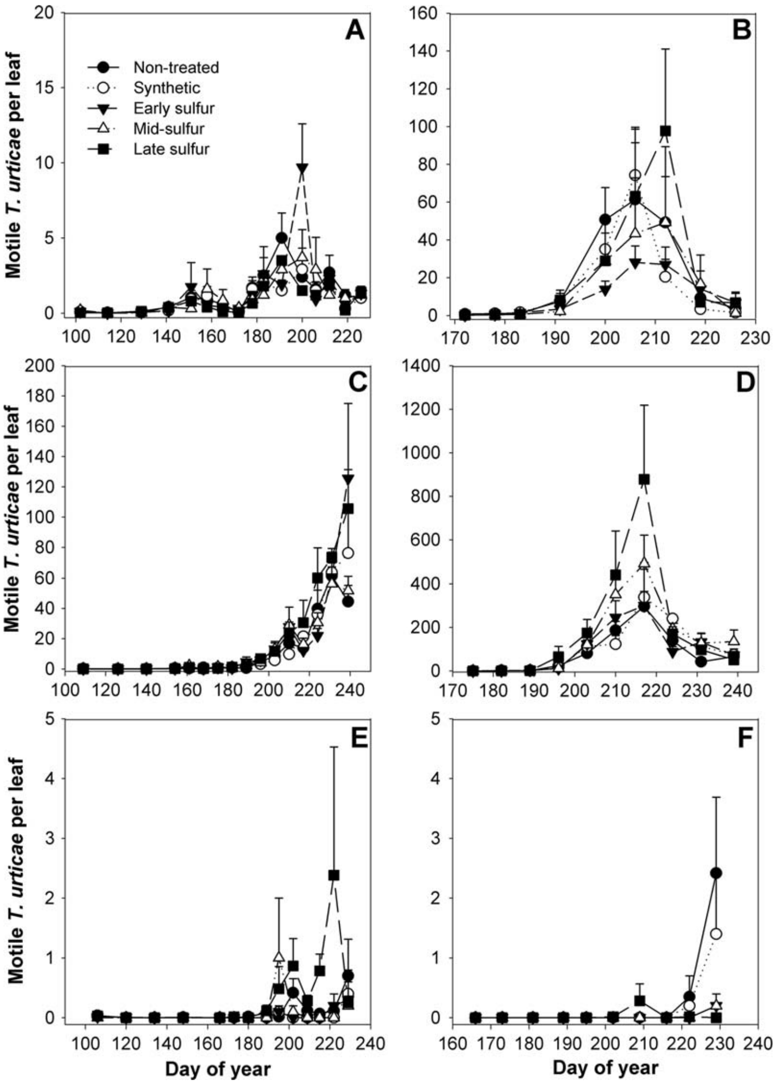

Fig. 1. Abundance of *T. urticae* (mean ± SEM) on hop leaves in relation to fungicide treatment in Oregon in the lower (A, C, E) and upper canopy (B, D, F); in 2007 (A, B), 2008 (C, D), and 2009 (E, F), respectively. Lower canopy data were collected from 10 to 20 leaves per plot sampled at a height of <2 m. Upper canopy data were collected from 10 to 20 leaves per plot sampled at a height of >2 m. Data reported are means of four replications per treatment.

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 >1 indicates an aggregated pattern with the degree of aggregation directly proportional to the slope; a slope of one 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) (Fig. 1A,B; Table 1). CMD were similar among treatments in the lower canopy ($F = 1.49$; df = 4, 12; $P = 0.2667$), but differed in the upper canopy ($F = 4.28$; df = 4, 14.9; $P = 0.0167$) (Table 2). Pairwise

Table 1. Mean seasonal density ± SEM of arthropods per hop leaf in relation to fungicide treatment and height in canopy, Corvallis, Oregon 2007–2009

Arthropod/yr	Fungicide treatment (mean ± 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 ± 0.01	0.02 ± 0.01	0	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.10 ± 0.05	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02
T. urticae	1.33 ± 0.22	0.98 ± 0.16	1.48 ± 0.36	1.17 ± 0.23	0.89 ± 0.18	20.52 ± 5.79	16.04 ± 4.95	10.52 ± 2.43	15.81 ± 6.37	23.64 ± 7.97
Stethorus spp.	0.03 ± 0.02	0.36 ± 0.09	0.02 ± 0.01	0.08 ± 0.04	0.03 ± 0.02	0.16 ± 0.07	0.06 ± 0.02	0.17 ± 0.06	0.06 ± 0.03	0.15 ± 0.06
O. tristicolor	0	0	0	0	0	0	0	0	0	0
2008										
Phytoseiidae	0.32 ± 0.15	0.12 ± 0.07	0.26 ± 0.10	0.30 ± 0.12	0.37 ± 0.15	0.31 ± 0.18	0.22 ± 0.10	0.17 ± 0.08	0.20 ± 0.08	0.09 ± 0.04
T. urticae	10.42 ± 2.46	14.27 ± 3.55	17.69 ± 5.15	13.22 ± 2.53	20.16 ± 4.48	83.71 ± 23.63	101.98 ± 22.70	97.72 ± 19.91	143.22 ± 30.23	187.83 ± 54.99
Stethorus spp.	0.12 ± 0.05	0.16 ± 0.07	0.37 ± 0.11	0.20 ± 0.09	0.23 ± 0.09	0.43 ± 0.11	0.82 ± 0.23	0.75 ± 0.25	1.50 ± 0.47	1.31 ± 0.33
O. tristicolor	0	0	0	0	0	0.02 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0
2009										
Phytoseiidae	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.001 ± 0.001	0.02 ± 0.01	0.01 ± 0.004	0	0.01 ± 0.01	0	0.01 ± 0.004
T. urticae	0.11 ± 0.05	0.04 ± 0.03	0.05 ± 0.03	0.10 ± 0.10	0.37 ± 0.16	0.28 ± 0.16	0.16 ± 0.11	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.03
Stethorus spp.	0.001 ± 0.001	0.005 ± 0.005	0	0.005 ± 0.005	0.003 ± 0.002	0	0	0	0	0
O. tristicolor	0	0	0	0	0	0.01 ± 0.01	0	0.03 ± 0.02	0.01 ± 0.01	0

[a] Plots were treated every 7–14 d 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.

contrast indicated CMD were greater in the late season sulfur treatment than all other treatments (F = 13.79; df = 1, 14.9; P = 0.0021) (Table 2). The differences in spider mite abundance were not associated with increased cone damage. Median cone damage because of 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 3).

Few predatory mites (<0.10 ± 0.05 per leaf) were found throughout the season (Table 1; Fig. 2A,B). Seasonal abundance of predatory mites among treatments, as measured by mite-days, was similar in both canopy heights ($P \geq 0.2083$ in all analyses) (Table 2). Abundance of *Stethorous* spp. and *O. tristicolor* were unaffected by fungicide treatment at either canopy height ($P \geq 0.1064$).

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. 1C), and a severe outbreak of spider mites later developed in all treatments (Fig. 1C,D). CMD in the lower canopy varied by 1.9-fold among treatments but was statistically similar among fungicide treatments (F = 1.99; df = 4, 15; P = 0.1485) (Table 2). There were differences in mite abundance in the upper canopy among fungicide treatments (F = 3.15; df = 4, 15; P = 0.0455). CMD were 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). Fungicide treatments were associated with differences in cone quality. Plots that received the late sulfur treatment had significantly poorer cone quality when compared with the other treatments, with the exception of the early sulfur treatment (F = 3.9; df = 3.01, 10.4; P = 0.0423) (Table 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. 2C,D; Table 1). Although the late-sulfur treatment appeared to substantially reduce abundance of predatory mites (Fig. 2D), treatment differences were not detected at either canopy height ($P \geq 0.3841$) (Table 2). Abundance of *Stethorous* spp. in the upper canopy also was significantly greater in the mid- and late-sulfur treated plots compared with the nontreated (F = 5.48; df = 4, 15; P = 0.0064) (Table 1). However, no effect of fungicide treatment was apparent on *Stethorous* spp. in the lower canopy (F = 1.53; df = 4, 15; P = 0.2451) or *O. tristicolor* (F = 0.48; df = 4, 15; P = 0.752).

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. 1E,F). Because of the low abundance of spider mites in both the lower and upper canopy, mite-days were low for all treatments and treatment effects were not detected ($P \geq 0.2857$) (Tables 1 and 2). The low number of spider mites in all treatments in 2009 did not lead to differences in cone quality (Table 3). Predatory mites were not found in all treat-

Table 2. Effect of fungicide treatment on CMD for spider mites and phytoseiids on hop plants, Oregon and Washington, 2007–2009

Year[a]	Treatment	Oregon				Washington	
		T. urticae		Phytoseiidae		*T. urticae*	Phytoseiidae
		Lower canopy[b]	Upper canopy	Lower canopy	Upper canopy	Lower canopy[c]	Lower canopy
2007	Nontreated	98.8	1,225.1ab	1.7	2.1	1,857.0a	47.6a
	Synthetic	109.9	947.2a	2.2	5.7	1,961.1a	24.2a
	Early sulfur	73.9	607.6a	0	0.7	2,044.1a	20.1a
	Mid sulfur	89.8	939.8a	2.1	0.6	2,283.7a	26.1a
	Late sulfur	83.3	1,378.9b	0.7	2.3	4,007.7b	31.3a
	Very late sulfur	—	—	—	—	1,824.2ab	7.4b
2008	Nontreated	1,009.5	4,582.4a	24.7	14.2	1,088.4a	110.2
	Synthetic	1,352.9	6,971.8a	10.6	11.7	1,833.6b[c]	75.3
	Early sulfur	1,573.8	6,689.4a	19.8	9.3	1,613.8ab	85.4
	Mid sulfur	1,332.5	9,684.5b	27.4	11.1	1,765.8ab	104.0
	Late sulfur	1,943.3	1,3042.5b	31.9	6.0	2,277.0ab	70.2
	Very late sulfur	—	—	—	—	2,515.4b	63.4
2009	Nontreated	8.6	10.7	2.3	0.6	4,891.2a	125.5
	Synthetic	2.2	6.2	1.3	0	4,698.3ab	144.7
	Early sulfur	3.4	0.7	1.4	0.7	4,175.2a	188.9
	Mid sulfur	7.9	0.7	0.1	0	3,759.9a	175.5
	Late sulfur	34.9	2.2	2.2	0.8	7,049.0b	78.4
	Very late sulfur	—	—			5,903.6a	154.9

[a] CMD were calculated by plotting mean arthropod pop 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 least significant difference 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 ≈2 m samples were taken from at two levels, lower canopy (<2 m) and upper canopy (>2 m). Samples were collected from one ht (≈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 $\alpha = 0.05$.

ments at either height, and densities for 2009 were low (Fig. 2E,F; Tables 1 and 2), and similar among treatments in both canopy heights ($P \geq 0.2512$). When *Stethorous* spp. and *O. tristicolor* were found, their abundance was unaffected by fungicide treatment ($P \geq 0.449$ in all analyses) (Table 1).

Washington 2007. Spider mites were observed on the first sampling date on 2 May (day 122) (Fig. 3A;

Table 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 >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. Two sub-samples per plot were collected and rated separately in 2009.

[b] Data was analyzed using a nonparametric ANOVA-type statistic. Relative effect ranges from 0 to 1, where 1 equals the greatest cone damage. Relative effect is significantly different if the 95% confidence intervals do not overlap. Within a given year and state, significant differences from the nontreated plots are noted by an asterisk. The very late sulfur treatment was not carried out in Oregon. See text for an explanation of the treatments.

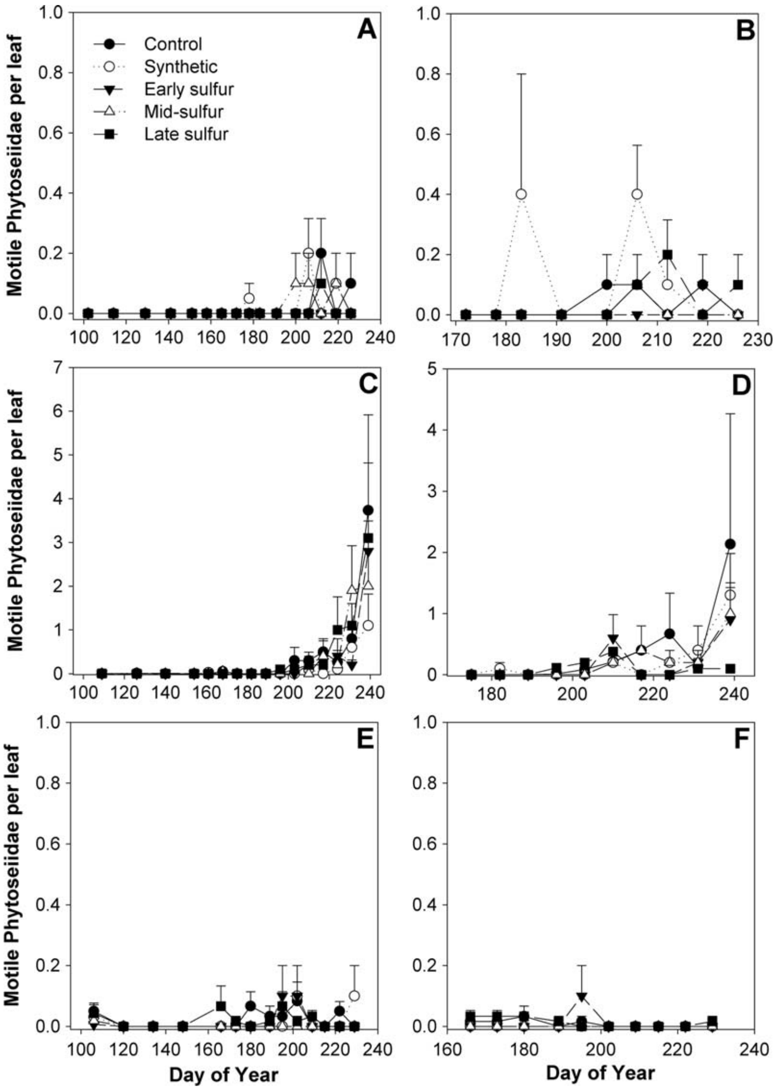

Fig. 2. Abundance of Phytoseiidae (mean ± SEM) on hop leaves in relation to fungicide treatment in Oregon in the lower (A, C, E) and upper canopy (B, D, F) in 2007 (A, B), 2008 (C, D), and 2009 (E, F), respectively. Lower canopy data were collected from 10 to 20 leaves per plot sampled at a height of <2 m. Upper canopy data were collected from 10 to 20 leaves per plot sampled at a height of >2 m. Data reported are means of four replications per treatment.

Table 4). Fungicide treatment significantly affected CMD ($F = 3.27$; df = 5, 15; $P = 0.0339$), with spider mite abundance 2.2-fold greater in the late season sulfur treatments as compared with the nontreated plots (pairwise contrast $F = 11.22$; df = 1, 12.42; $P = 0.0055$) (Table 2). These differences in spider mite abundance on leaves among treatments did not impact cone quality significantly (Table 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 CMD for predatory mites (Fig. 3B; Table 2). The very late sulfur treatment reduced predatory mite abundance 6.4-fold as compared with the nontreated plots (pairwise contrast $F = 8.53$; df = 5, 17; $P = 0.0095$) (Table 2). Abundance of *Stethorous* spp. and *O. tristicolor* were unaffected by fungicide treatment ($P \geq 0.1502$) (Table 4).

Washington 2008. Spider mites were observed during the first sampling on 21 May (day 142) (Fig. 3C; Table 4), and fungicide treatment had an effect on seasonal spider mite abundance at $\alpha = 0.1$ ($F = 2.56$; df = 5, 13.1; $P = 0.0794$). 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). As before, the differences in spider mite levels on leaves between treatments did not translate into differences in mite damage to cones (Table 3).

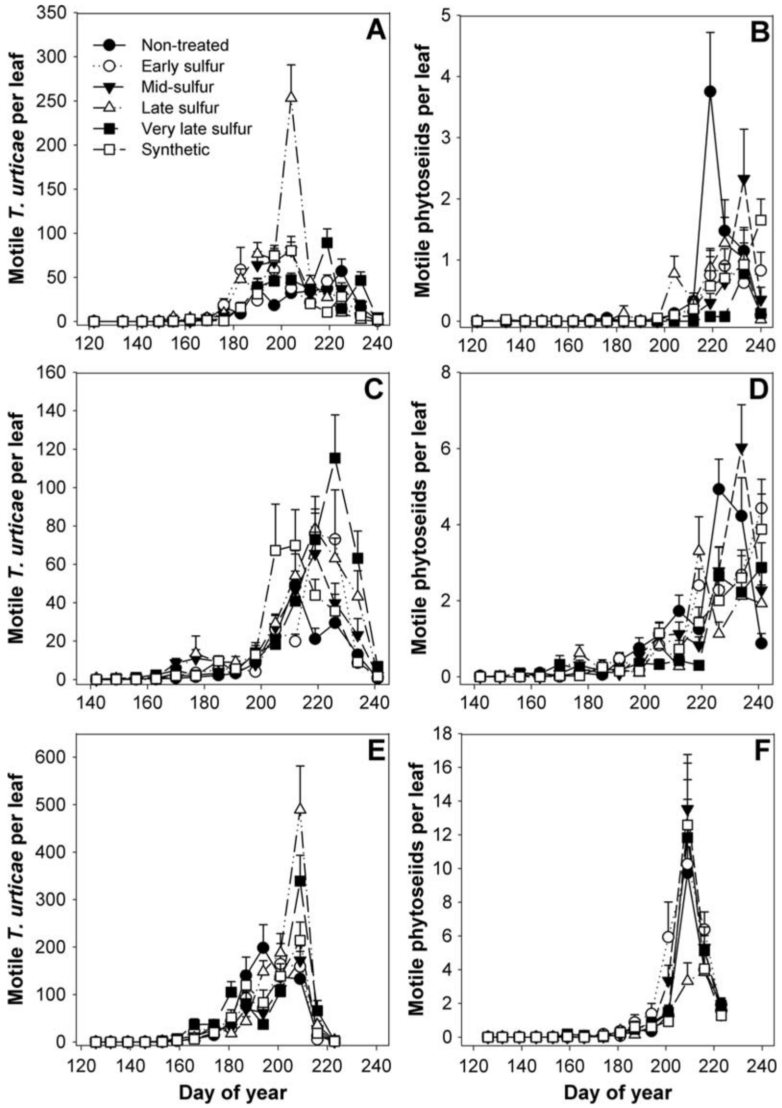

Fig. 3. Abundance of *T. urticae* (mean ± SEM) and Phytoseiidae (mean ± SEM) on hop leaves in relation to fungicide treatment in Washington in 2007 (A, B), 2008 (C, D), and 2009 (E, F), respectively. Data were collected from 10 leaves per plot sampled at a height of ≈2 m. Data reported are means of four replications per treatment.

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. 2D; Tables 2 and 4). Abundance of *Stethorous* spp. and *O. tristicolor* were unaffected by fungicide treatment ($P \geq 0.0826$) (Table 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. 3E; Table 4). There was a significant treatment effect on CMD ($F = 3.14$; df = 5, 13.9; $P = 0.0421$) (Table 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 mite-days; Table 2). Cone quality again was similar among the fungicide treatments (Table 3).

Predatory mite densities generally tracked spider mite populations, although CMD for predatory mites was not affected significantly by fungicide treatment ($F = 0.71$; df = 5, 13.9; $P = 0.6250$) (Fig. 3 F; Table 2). Abundance of *Stethorous* spp. was similar among fungicide treatments ($F = 2.2$; df = 5, 18; $P = 0.0995$). There were significantly more *O. tristicolor* on plants that received the sulfur at the mid timing compared with plants that were nontreated, treated with synthetic fungicide, or received the very late sulfur treatment ($F = 4.73$; df = 5, 15; $P = 0.0086$) (Table 4).

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. 4). This was true for both low and high

Table 4. Mean seasonal density ± SEM of arthropods per hop leaf in relation to fungicide treatment, Prosser, WA, 2007–2009

Arthropod/yr	Fungicide treatment (mean ± SEM per leaf)[a]					
	Nontreated	Synthetic	Early sulfur	Mid-sulfur	Late sulfur	Very late sulfur
2007						
Phytoseiidae	0.41 ± 0.08	0.25 ± 0.05	0.19 ± 0.04	0.22 ± 0.06	0.26 ± 0.05	0.06 ± 0.02
T. urticae	15.45 ± 1.41	16.14 ± 1.66	17.11 ± 2.03	31.13 ± 4.98	32.56 ± 3.47	18.28 ± 1.64
Stethorus spp.	0.09 ± 0.01	0.08 ± 0.01	0.15 ± 0.03	0.15 ± 0.03	0.09 ± 0.02	0.13 ± 0.02
O. tristicolor	0.04 ± 0.01	0.02 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
2008						
Phytoseiidae	1.04 ± 0.12	0.83 ± 0.10	0.94 ± 0.10	1.02 ± 0.12	0.72 ± 0.09	0.68 ± 0.09
T. urticae	10.19 ± 1.42	17.32 ± 2.43	15.03 ± 2.43	16.55 ± 1.74	21.29 ± 2.04	23.34 ± 2.50
Stethorus spp.	0.18 ± 0.03	0.13 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.17 ± 0.02	0.21 ± 0.03
O. tristicolor	0.07 ± 0.01	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.03	0.08 ± 0.01
2009						
Phytoseiidae	1.21 ± 0.19	1.36 ± 0.28	1.78 ± 0.33	1.64 ± 0.27	0.79 ± 0.13	1.48 ± 0.28
T. urticae	46.13 ± 5.68	43.83 ± 4.98	38.62 ± 4.60	34.87 ± 3.57	64.19 ± 8.48	54.79 ± 5.84
Stethorus spp.	0.08 ± 0.01	0.0.16 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.11 ± 0.02	0.22 ± 0.04
O. tristicolor	0.08 ± 0.01	0.10 ± 0.01	0.12 ± 0.02	0.18 ± 0.02	0.10 ± 0.02	0.15 ± 0.02

[a] Plots were treated every 7–14 d 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 Aug. 2008; 27 July 2009.

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 during the time when sulfur was applied was correlated with the severity of the subsequent spider mite outbreak (Fig. 5). Standardized severity of the spider 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. 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

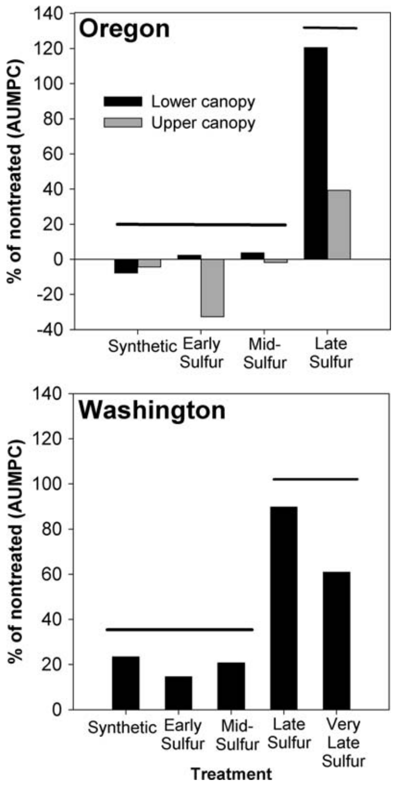

Fig. 4. Mean severity of spider mite outbreaks (measured by CMD for each treatment relative to the nontreated in Oregon for both the lower and upper canopy (A) and one canopy height in Washington (B). The bars indicate significantly different groups for Oregon in the lower and upper canopy ($P = 0.0269$ 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 details of the analysis.

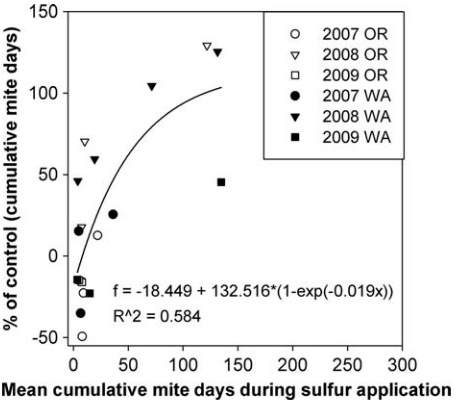

Fig. 5. Spider mite outbreak severity in sulfur-treated plots (standardized to the corresponding nontreated plot) in relation to the mean CMD during the time period of when sulfur was being applied in each plot. All treatments represented in the figure received three sequential sulfur sprays at varying times during the season.

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; $P = 0.0001$ and $F = 14.56$; df = 1, 21; $P = 0.001$, respectively). The regression equations for sulfur-treated and nontreated leaves in 2007 were log(variance) = 1.248(log[mean]) + 1.080 ($n = 11$; $R^2 = 0.96$) and log(variance) = 1.795(log[mean]) + 1.133 ($n = 11$; $R^2 = 0.81$), respectively. In 2009, the regression equations for sulfur-treated and nontreated leaves were log(variance) = 1.769(log[mean]) + 1.257 ($n = 18$; $R^2 = 0.98$) and log(variance) = 1.954 (log[mean]) + 1.289 ($n = 23$; $R^2 = 0.97$). 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; $P = 0.0235$). The regression equations for sulfur-treated and nontreated leaves were log(variance) = 1.443(log[mean]) + 1.286 ($n = 24$; $R^2 = 0.97$) and log(variance) = 1.957 (log[mean]) + 0.282 ($n = 24$; $R^2 = 0.85$), respectively. On the leaves used for the spatial analysis, predatory mites occurred at a frequency of <4% on all of the leaves sampled. Predatory mites occurred on 2.5 and 2.7% of the leaves sampled in the sulfur-treated and nontreated plots respectively, in 2007, 3.7% of the leaves sampled in both treatments in 2008, and 1.3% 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 1.1–2.8-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: 1) natural enemy destruction; 2) a direct or indirect effect on the mites themselves; and/or 3) an alteration (reduced or enhanced) of the nutritive quality or defense responses of the host plant (Hardin et al. 1995).

Previous bioassays (James and Coyle 2001) and field studies in hop (Gent et al. 2009) have demonstrated that sulfur and other fungicides are innocuous to predatory insects. Again in this study, no evidence of suppressed populations of predacious insects was found. In contrast, a significant differences in predatory insect abundance was because of greater levels of *Stethorous* spp. on leaves that received a sulfur treatment compared with nontreated leaves. Presumably the positive effect of sulfur on predatory insects in these instances was associated with greater prey abundance induced by sulfur treatment or type I errors because of stochastic effects.

Importantly though, with only one exception (Washington 2007), predatory mite abundance was similarly unaffected significantly 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). Tolerance of local populations of phytoseiids to sulfur also may be possible (Hoy 1985).

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). Negative impacts on predatory insects from sulfur also were not found. In the current study, a 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 sublethal 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 fewer total eggs as compared with nonexposed spider mites because of reduced longevity (≈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, 1999). Dispersion is most common in prereproductive 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 2 of the 3 yr 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 plant 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. 6). 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, was 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 after the late season sulfur sprays (Fig. 1E,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 Fig. 5 but this effect is not apparent at higher spider mite densities. This tendency was most prominent in the upper canopy in Oregon (as shown in early sulfur treatment in Figs. 1B and Fig. 4), potentially because of the very limited host growth during sulfur applications. While the relationship shown in Fig. 5 implies a direct impact of sulfur on the spider mites, the response variable CMD is also influenced by temperature, host plant physiology, and spider 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 prebloom sulfur spray timings investigated by Costello (2007) also correspond to the period when the mite outbreak would reach its early exponential phase. Thus, sulfur

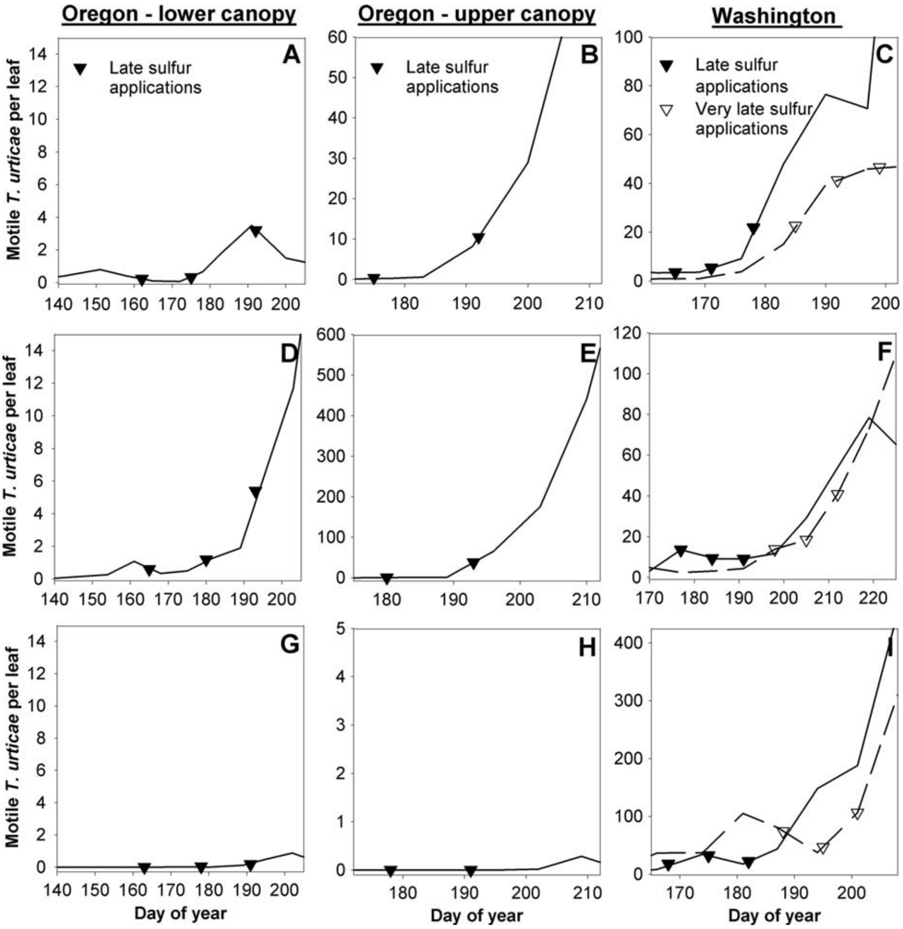

Fig. 6. Abundance of motile *T. urticae* when sulfur fungicides were applied in the late season sulfur treatments in Oregon in the lower canopy in 2007–2009 (A, D, G), and the upper canopy in 2007–2009 (B, E, H), respectively. Density of motile *T. urticae* when sulfur fungicide applications were applied 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 scale has been adjusted in each figure to represent the point of inflection in the spider mite population curve.

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. Altered physiological or defense responses in response to sulfur (the so-called sulfur-induced resistance; Haneklaus et al. 2007) potentially could be involved in perturbation of spider mite outbreaks because of negative regulation of defense pathway involved in protection from herbivory (Kunkel and Brooks 2002).

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 can be 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 increase the severity of spider mite outbreaks. 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 phytoseiid disturbance.

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