Effects of Powdery Mildew Fungicide Programs on Twospotted Spider Mite (Acari: Tetranychidae), Hop Aphid (Hemiptera: Aphididae), and Their Natural Enemies in Hop Yards

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ABSTRACT  Twospotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae), and hop aphid, Phorodon humuli (Schrank) (Hemiptera: Aphididae), are the most important arthropod pests of hop (Humulus lupulus L.) in the Northern Hemisphere. A potential barrier for greater adoption of conservation biological control strategies for spider mites and hop aphid is the extensive use of fungicides for management of hop powdery mildew, Podosphaera macularis (Wallr.:Fr.) U. Braun & S. Takamatsu. Field studies conducted in experimental plots in Oregon and Washington in 2005 and 2006 quantified the effects of powdery mildew fungicide programs (i.e., sulfur, paraffinic oil, and synthetic fungicides) on arthropod pests and natural enemies on hop. Fungicide treatment significantly affected spider mite populations in all four studies. Multiple applications of sulfur fungicides applied before burr development resulted in 1.4–3.3-fold greater spider mite populations during summer. Near the cessation of the sulfur applications, or after a lag of 20–30 d, spider mite populations increased significantly faster on sulfur treated plants compared with water-treated plants in three of four experiments. The effect of paraffinic oil on spider mites was varied, leading to exacerbation of spider mites in Oregon and Washington in 2005, suppression of mites in Oregon in 2006, and no significant effect compared with water in Washington in 2006. Significant relative treatment effects for cone damage due to spider mite feeding were detected in Oregon in 2005 in plots treated with sulfur and paraffinic oil compared with water and synthetic fungicides. Mean populations of hop aphids were similar among treatments in Oregon, although sulfur treatment suppressed hop aphid populations in Washington in 2005 and 2006. Populations of individual predacious insect species and cumulative abundance of macropredators were not consistently suppressed or stimulated by treatments in all trials. However, predatory mite abundance in Washington was affected by fungicide treatments, with plots treated with sulfur consistently having 10-fold fewer phytoseiids per leaf compared with the other treatments. Based on the results of these studies, powdery mildew fungicide programs that minimize or eliminate applications of sulfur and paraffinic oil would tend to conserve predatory mites and minimize the severity of spider mite outbreaks. However, mechanisms other than direct or indirect toxicity to phytoseiid mites likely are associated with exacerbation of spider mite outbreaks on hop.

KEY WORDS  conservation biological control, Humulus lupulus, Phorodon humuli, predaceous mites, Podosphaera macularis

Hop (Humulus lupulus L.) is a dioecious climbing perennial bine grown for its female strobiles, commonly referred to as “cones.” Shoots begin to emerge from hills (a single or multiple plants planted together) in the early spring. The bines grow rapidly, as much as 15–25 cm per day, and they may reach 5 m or more by early summer (Neve 1991). About this time, the cone-bearing lateral branches begin to develop in response to photoperiod. The commercial value of hop cones results from the soft resins (α and β acids) and essential oils produced in the lupulin glands, which impart flavor and aroma to beer and aid in

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preservation due to their antimicrobial properties (Barth et al. 1994). Nearly all commercial hop production in the United States occurs in the Pacific Northwest states of Idaho, Oregon, and Washington. The climatic range of the hop growing regions in western Oregon and northern Idaho generally is cool and rainy, whereas production regions in southern Idaho and central Washington are warm to hot and semiarid (Barth et al. 1994).

Twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and hop aphid, *Phorodon humuli* (Schrank) (Hemiptera: Aphididae), are the key arthropod pests of hop in the Northern Hemisphere (Neve 1991). Management of these pests is achieved largely by annual applications of pesticides (Strong and Croft 1995, Mahaffee et al. 2009), which has resulted in resistance development to multiple compounds (Campbell 1978, Weichel and Nauen 2003). Augmentative biological control of spider mites focusing on phytoseiid mites (Strong and Croft 1995, Lilley and Campbell 1999) has not been successful in commercial production. However, a complex of natural enemies occurs on cultivated and feral hop plants and can provide significant suppression of spider mite and hop aphid populations if not disrupted by use of broad-spectrum pesticides or cultural practices such as spring pruning (Aveling 1981; Strong and Croft 1993; Campbell and Cone 1994; Strong and Croft 1996; James et al. 2001, 2003; Gardiner et al. 2003; James and Price 2004). Recent research has focused on greater reliance on conservation and attraction of endemic natural enemies to reduce chemical inputs for management of arthropod pests on hops (James et al. 2003, James and Price 2004).

A potential barrier for greater adoption of conservation biological control strategies for spider mites and hop aphid is the extensive use of fungicides for management of hop powdery mildew *Podosphaera macularis* (Wallah. Fr.) U. Braun & S. Takamatsu. Powdery mildew was first observed in commercial hop yards in the Pacific Northwestern United States in 1997, and the disease now becomes epidemic annually in most years planted to susceptible cultivars (Mahaffee et al. 2003, Gent et al. 2008). The disease is managed chiefly by regular and season-long applications of sulfur-based products, horticultural oils (e.g., parafinic oil), and synthetic fungicides (e.g., myclobutanil, quinoxylfen, spiroxamine, and trifloxystrobin) (Boyle 1978, Mahaffee et al. 2003, Gent et al. 2008). As many as six to 10 fungicide applications might be made annually to suppress the disease (Turecek et al. 2001, Gent et al. 2008).

Preliminary in vitro assays indicated that certain sulfur-based fungicides can be toxic to natural enemies of key hop pests, including the phytoseiid mites *Galendromus occidentalis* Nesbitt, *Neoseiulus fallacis* Garman, and *Amblyseius andersoni* L., as well as the predacious lady beetle *Harmonia axyridis* (Pallas) (James and Coyle 2001). These studies and others suggest that indiscriminate use of sulfur, and possibly other fungicides, may disrupt equilibria between predator and prey required for successful conservation biological control (Rabbinge 1985, Strong and Croft 1995). Sulfur applications may provide temporary suppression of spider mites and certain natural enemies, but spider mite populations often resurge when sulfur applications cease, possibly as a result of more negative impacts of sulfur on spider mite natural enemies (McMurtry et al. 1970; Croft 1990; Thomson et al. 2000; James and Coyle 2001; James et al. 2002; Prischmann et al. 2005a, 2005b; Costello 2007) and/or sublethal and behavioral effects of sulfur on spider mites (Ayyappath et al. 1997, James and Price 2002, Walsh and Grove 2005).

A critical need exists to integrate insect, mite, and disease management strategies into a complete integrated pest management (IPM) system for hop. An important step toward this goal is to determine the effect of inorganic and synthetic fungicides on arthropod pest and natural enemy assemblage dynamics, and powdery mildew control under field conditions. Information on direct effects of fungicides on arthropod pests and natural enemies is available from laboratory studies and from cropping systems other than hops; little field-based research has been conducted on the effects of fungicide programs on conservation biological control of spider mites and hop aphid in hop yards. Results of studies from other crops are not directly transferrable to hops because of the unique growth habit of the crop resulting from rapid vegetative development that produces a dynamic three-dimensional canopy architecture for spider mites, hop aphids, and their natural enemies. The objective of this study was to quantify the effects of powdery mildew fungicide programs (i.e., sulfur, parafinic oil, and synthetic fungicides) on arthropod pests and natural enemies under field conditions in the contrasting climates of western Oregon and central Washington.

**Materials and Methods**

**Experimental Design and Treatment Application.** Experiments were conducted in 2005 and 2006 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 different fungicide programs on population densities of spider mites, hop aphids, and key natural enemies. In Oregon, plots were established in a hop yard planted in April 2005 (day of year 104) to the aroma ‘Willamette’ with hills on a 2.1-m grid (narrow spacing) and a 5-m trellis. A plot consisted of 12 plants in a 3-hill × 4-hill rectangular array, and it was arranged in a randomized complete block design with four replications. Each plot was separated by one or two rows of untreated plants. Irrigation was supplied by sprinklers every 7–14 d as needed for crop development. In Washington, plots were established in a hop yard planted in 1991 to Willamette with hills on a 2.1-m grid and a 5-m trellis. Plots consisted of six consecutive hills in a row arranged in completely randomized design with four replications. Plots were separated by at least one row of nontreated plants. Irrigation was supplied daily by...
a subsurface drip system. In Oregon and Washington, granular nitrogen, phosphorous, potassium, and sulfur fertilizers were applied in May, June, and July according to standard commercial recommendations (Gingrich et al. 2000).

Three powdery mildew fungicide programs were evaluated at both locations in 2005 and 2006. Treatments consisted of micronized sulfur (Microthiol Dispers, Cerexagr, Inc., North America, King of Prussia, PA) applied at 5.38 kg (AI)/ha, paraffinic oil (JMS Stylet Oil, JMS Flower Farms, Inc., Vero Beach, FL) at 1% vol/vol (range, 3.64–11.54 liters [AI]/ha depending on application volume), a rotation of synthetic fungicides and a water control. In Oregon, the synthetic fungicide rotation included trifloxystrobin (Flint 50 WG, Bayer CropScience, Research Triangle Park, NC) applied at 0.14 kg (AI)/ha, spiroxamine (Accrue, Bayer CropScience) applied at 0.36 kg (AI)/ha, and quinoxyfen (Quintec, Dow AgroSciences, Indianapolis, IN) applied at 0.10 kg (AI)/ha. In Washington, the synthetic fungicide rotation included trifloxystrobin at 0.14 kg (AI)/ha, myclobutanil (Rally 40W, Dow AgroSciences) applied at 0.18 kg (AI)/ha, and quinoxyfen at 0.10 kg (AI)/ha. No other pesticides were applied to the plots or neighboring plants.

Treatments were applied in mid-May (day 131) to early June (day 154) and continued until late July (day 203) to mid-August (day 225). To simulate grower interference. As plants grew taller than ~2 m, samples were collected from low (<2-m) and high (>2-m) positions in the canopy. Samples were collected at an approximate height of 2 m in Washington. 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 stereo microscope either directly on the leaves or after transferring to a liquid-detergent-coated glass plate with a mite brushing machine (Leedom Engineering, Twain Harte, CA).

Nonacarines, winged or mobile natural enemies (macropredators) of spider mites and aphids were enumerated from vacuum samples in Oregon in 2005 and canopy shake samples in Washington in 2005 and Oregon in 2006 at 7–14-d intervals. Shake samples were not collected in Washington in 2006 due to labor and resource limitations. Families or species counted included Coccinellidae [aphid-feeding lady beetle species, including H. axyridis, Coccinella septempunctata L., Coccinella transversoguttata (Falderman), Cycloneda polita Casey, and the mite-eating lady beetles, S. p. picipes and S. p. punctum, O. tristicolor, and green lacewings (Chrysopa spp.). In Oregon, natural enemy assessments were made from the four plants in the middle of the plots to reduce plot-to-plot interference. Vacuum samples were obtained using a modified leaf blower (model PB-1010, Echo, Lake Zurich, IL) to collect arthropods into a vial (Gardiner et al. 2003, Prischmann et al. 2005b). Each of the four plants in the middle of the plot was vacuumed for 5 s, for a total of 20 s per plot. Vials of recovered arthropods were placed in a cooler and transported to a laboratory for identification and enumeration. Canopy shake samples were collected by placing a funnel with a 1-m² bowl or 1-m² white cloth under a hop bine and vigorously shaking the bine. Dislodged arthropods were
identified and counted on the cloth or collected into vials for later identification in a laboratory. One sample was collected from each replicate plot on each sampling day.

At harvest, the incidence of spider mite damage was assessed on 100 cone subsamples from each plot (400 per treatment). Cones were harvested on day 288 and 255 in Oregon in 2005 and 2006, respectively, and day 255 and 248 in Washington in 2005 and 2006, respectively. Although plants at the Oregon location were planted in April 2005, it is common for hop plants to produce a partial crop during the first year of growth. In all experiments, the plants had reached the top wire of the trellis (~6 m) at harvest. Mite damage on each of the cones was rated using an ordinal scale, where 1 is no damage; 2 is slight discoloration or damage on a single or few bracts; 3 is moderate levels of discoloration or damage, but <25% of cone area exhibiting discoloration or damage; and 4 is severe cone discoloration or damage on >25% of the cone or cone abor
tion. Cones also were inspected for honeydew and sooty mold associated with hop aphid feeding.

Statistical Analysis. Differences in numbers of pest and beneficial arthropod species on leaves and canopy samples were determined by a linear mixed effects repeated measures analysis by using the MIXED procedure in SAS version 9.1 (SAS Institute 2002). Discrete data were log-transformed to achieve normally distributed residuals with a common variance. Multiple covariance structures were investigated, and the simplest covariance structure consistent with the data were selected based on Akaike’s Information Crite
rion (AIC) (SAS Institute 2002). Degrees of freedom were estimated by the Kenward-Roger method.

Spider mite and hop aphid populations on each assessment date were plotted over time to develop population curves. The “area under the pest development curve” was calculated in SigmaPlot version 9.0 (Systat Software, Inc., San Jose, CA) for each plot to develop a composite value for each treatment. Area under the pest development curve was analyzed using a linear mixed-model repeated in space (coordinate location of plots) to account for spatial aggregation of mites and aphids. Several spatial covariance structures were investigated and the best fitting model was selected by minimizing AIC. The analysis was conducted in PROC MIXED in SAS with denominator degrees of freedom determined using a general Satterthwaite approximation (SAS Institute 2002). Block was consid
ered a random effect in the analysis.

The rate of spider mite population development during the exponential phase of the mite outbreak was calculated to determine the effect of fungicide treat
tment on developmental rate. A detailed explanation of the method can be found in Madden et al. (2007). Briefly, to calculate the rate of mite population develop
tment, a linear mixed model analysis of covari
ance (ANCOVA) was conducted in PROC MIXED in SAS. This involved fitting a linear mixed-model to log-transformed mite population estimates with time as a continuous fixed effect with an appropriate co
variance structure as determined by AIC (Madden et al. 2007). The resultant slope parameter of the lines corresponded to the rate of mite increase. Treatment effects were compared by pairwise contrasts.

The ordinal rating scale used for mite damage assessment was analyzed using a nonparametric ANOVA-type statistic appropriate for ordinal data in designed experiments (Shah and Madden 2004). In this analysis, a relative treatment effect ranging from 0 to one 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 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 by using macros developed by Brunner et al. (2002).

Results

Oregon 2005. Spider mites were first detected in plots in Oregon on 19 June 2005 (day 170), and popula
tions increased rapidly in the lower and upper canopy beginning in mid- to late July (Fig. 1A and B). Fungicide treatment significantly affected spider mite populations in the lower canopy \((F = 4.62; df = 3, 55.6; P = 0.0057)\) and upper canopy samples \((F = 5.35; df = 3, 93; P = 0.0019)\). Plots treated with sulfur or paraffinic oil had the largest populations of spider mites in the lower \((\text{mean seasonal populations } = \text{SEM}, 23.19 \pm 6.75 \text{ and } 20.59 \pm 4.26 \text{ per leaf, respectively})\) and upper canopy \((62.00 \pm 16.17 \text{ and } 46.72 \pm 12.31, \text{ respectively})\) \((P = 0.9102)\) among treatments in the lower and upper population curves. The resultant slope parameter of the lines differed significantly among fungicide treatments \((P = 0.0019)\) (Fig. 1A and B; Table 1). Populations of spider mites on plants treated with synthetic fungicides were greater numerically than populations on plants treated with water in the lower \((12.86 \pm 2.74 \text{ versus } 8.94 \pm 2.36)\) and upper canopy \((29.28 \pm 6.64 \text{ versus } 14.25 \pm 3.54)\), although statistical differences between the synth
etic and water treatments on individual days were not detected on any individual sampling dates. In the upper canopy, spider mite abundance in the sulfur and paraffinic oil treatments were similar to the water and synthetic treatments until 30 d after the last sulfur or paraffinic application were made \((day 191)\). Thereaf
er, spider mite abundance was greater in the sulfur and paraffinic oil treatments compared with the water and synthetic treatments \((P = 0.0019)\) (Fig. 1). Trends in spider mite eggs generally mirrored that of motile stages and these data are not presented.

The area under the pest development curve varied 2.5-fold among treatments in the lower canopy, but statistical differences were not detected \((F = 0.75; df = 3, 12; P = 0.5431)\). Spider mite populations in the upper canopy differed significantly among fungicide treatments at \(\alpha = 0.10 \ (F = 2.70; df = 3, 12; P = 0.0929)\) \((Table 2)\). Pairwise contrast indicated that the area under the curve was 2.5-fold greater for sulfur than for water-treated plots \((F = 7.89; df = 1, 12; P = 0.0138)\).

The rate of mite increase was similar \((F = 0.18; df = 3, 35.6; P = 0.9102)\) among treatments in the lower canopy, ranging between 1.05 \((\text{water and paraffinic oil})\) and 1.08 mites per leaf per day \((\text{sulfur})\). In the
upper canopy, the rate of mite population development ranged from 1.10 (synthetic treatment) to 1.18 (sulfur) mites per leaf per day. Sulfur and paraffinic oil increased the rate of mite population development 0.06–0.08 mites per leaf per day in the upper canopy as compared with water or synthetic treatments (F = 3.43; df = 3, 21.4; P = 0.0354).

Median cone damage due to spider mites was rated as “none” in all treatments. However, relative treatment effects for cone damage were 0.28–0.44 greater for sulfur and paraffinic oil treatments compared with synthetic or water treatments. Mite damage on cones treated with sulfur or paraffinic oil was significantly more severe than for cones treated with synthetic fungicides or water as determined by lack of overlap of the 95% confidence intervals for relative treatment effects. Cone damage associated with hop aphid was not observed.

Seasonal mean populations of hop aphids were very low in the lower and upper canopy (0.01 ± 0.01–0 per leaf, respectfully) and did not differ significantly among treatments (F = 0.41; df = 3, 37.8; P = 0.7452) (Table 1). The area under the pest development curve for hop aphid was <1 for all treatments (F = 0.93; df = 3, 9; P = 0.4660).

Similarly, predatory mites were nearly absent in all plots (Table 1). Populations of combined macropredators were similar among treatments (F = 1.30; df = 3, 35.3; P = 0.2909), as well for each individual macropredator group or species analyzed (P > 0.05) (Fig. 1C; Table 3). Oregon 2006. Spider mites were first detected in plots on 18 May (day 138) (Fig. 2A). Mean mite populations in the lower canopy ranged from 4.23 ± 0.62 (synthetic) to 7.38 ± 1.42 (sulfur) mites per leaf and were similar among treatments (F = 1.88; df = 3, 43; P = 0.1471) (Table 1). In the upper canopy, paraffinic oil suppressed mite populations (6.58 ± 2.20) compared with other treatments on all sampling dates except day 173 (F = 21.96; df = 3, 46.8; P < 0.0001).

Spider mite abundance in the sulfur treatment was similar to that in the water and synthetic treatments until day 173; thereafter, mite abundance was greater in the sulfur treatment on days 179, 186, 200, and 205 (P ≤ 0.0359) (Fig. 2B). Trends in spider mite eggs generally mirrored that of motile stages, and these data are not presented.

Area under the pest development curve in the lower canopy varied by 1.7-fold among treatments but was statistically similar among fungicide treatments (F = 1.73; df = 3, 12; P = 0.2130). Pairwise contrasts indicated significantly higher spider mite populations on sulfur-treated plants compared with synthetic-fungicide treated plots at α = 0.1 (F = 4.59; df = 1, 12; P = 0.0535). The area under the pest development curve in the upper canopy was 5.2-fold greater in sulfur-treated plots compared with paraffinic oil-treated plots (F = 6.92; df = 3, 7.75; P = 0.0138) (Table 2).

The rate of mite increase was reduced by 0.05–0.10 mites per leaf per day by paraffinic oil in the lower canopy as compared with the other treatments (F = 3.41; df = 3, 21.7; P = 0.0356). In the upper canopy, sulfur increased the rate of mite population development 0.17–0.29 mites per leaf per day compared with water, paraffinic oil, or synthetic fungicide treatments (F = 9.95; df = 3, 26.2; P < 0.0001). Median cone damage due to spider mites was rated as none for all treatments, and treatment effects were not detected as indicated by the overlapping 95% confidence intervals for the relative treatment effects.
At two levels, lower canopy (2005: 0–2 m; 2006: 0–2 m) and upper canopy (2005: 2–6 m; 2006: 2–6 m) in Oregon, as plants grew taller than 2 m, significant differences were noted in the development curve for spider mites on hop plants in Oregon and Washington, 2005 and 2006.

Trends in hop aphid and spider mite populations were mirrored in trends in populations of generalist-feeding lady beetles and Stethorus spp., respectively (Fig. 2E). Populations of macropredators were similar among treatments when analyzed cumulatively (F = 1.66; df = 3, 156; P = 0.1782) (Table 3; Fig. 2F), or by predator genus or family (P > 0.05). As in 2005, predatory mites were nearly absent in all plots.

In 2005, spider mites were first observed in plots on 6 June (day 157). Populations increased rapidly, particularly in the sulfur and paraffinic oil treatments, reaching maximum population densities ranging between 92.9 and 334.6 mites per leaf by 15 August (day 227) (Fig. 3A). Fungicide treatment significantly affected spider mite populations (F = 6.14; df = 13, 1224; P = 0.0004). Mean populations of spider mites were greatest in plots treated with sulfur (95.20 ± 13.57) or paraffinic oil (120.01 ± 17.75) and lowest in plots receiving synthetic fungicides (56.00 ± 8.94) or water (35.50 ± 4.24) (Table 4). Spider mite abundance in the sulfur and paraffinic oil treatments was similar to the water treatment until day 200, which was 20 d after the last sulfur or oil applications were made on day 180. Thereafter, significantly less spider mites were detected on water-treated plants compared with sulfur or paraffinic oil-treated plants on days 200, 213, and 227 (P = 0.0437) (Fig. 3A). Statistical differences between the synthetic and water treatments on individual days were detected only on day 186 (P = 0.0060).

The area under the pest development curve for spider mites was 3.1- and 3.3-fold greater for sulfur and paraffinic oil treatments as compared with water (F = 9.40; df = 3, 10.8; P < 0.0024) (Table 2). The area under the curves for synthetic fungicides and water were similar (P = 0.2123).

Sulfur and paraffinic oil significantly increased the rate of spider mite population development 0.11–0.19 mites per leaf per day compared with water, respectively (F = 6.53; df = 3, 45.2; P < 0.0009). Spider mite population development was similar in synthetic fungicide and water-treated plots (P = 0.4839). Median cone damage due to spider mites was rated as “slight” for all treatments, and it did not differ significantly among treatments.

Mean populations of hop aphid ranged between 6.29 ± 0.97 and 13.96 ± 2.05 per leaf over the season (Fig. 3B; Table 4), and populations were significantly affected by fungicide treatment (F = 6.53; df = 3, 45.2;
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Table 3. Mean seasonal density ± SEM of beneficial arthropods per hop plant by suction or shake sampling in relation to fungicide treatment, Oregon 2005 and 2006, and Washington 2005

<table>
<thead>
<tr>
<th>Yr and location</th>
<th>Arthropod*</th>
<th>Fungicide treatment (mean ± SEM per plant)</th>
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<tr>
<td></td>
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<td>Water</td>
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<td>Sulfur</td>
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<td>Parafinic oil</td>
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<td>Synthetic</td>
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<td>Oregon 2005</td>
<td>Anthocoridae</td>
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<tr>
<td></td>
<td>Orinus spp.</td>
<td>0.01 ± 0.01</td>
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<td></td>
<td>Coccinellidae</td>
<td>0.01 ± 0.00</td>
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<td></td>
<td>Lady beetles</td>
<td>0.01 ± 0.00</td>
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<tr>
<td></td>
<td>Stethorus spp.</td>
<td>0.01 ± 0.00</td>
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<tr>
<td></td>
<td>Macropredators</td>
<td>0.14 ± 0.03</td>
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<tr>
<td>Oregon 2006</td>
<td>Anthocoridae</td>
<td>0.38 ± 0.15</td>
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<tr>
<td></td>
<td>Orinus spp.</td>
<td>0.38 ± 0.14</td>
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<tr>
<td></td>
<td>Coccinellidae</td>
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<tr>
<td></td>
<td>Lady beetles</td>
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<td></td>
<td>Stethorus spp.</td>
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<td>Macropredators</td>
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<tr>
<td>Washington 2005</td>
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<td>O. tristicolor</td>
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<td></td>
<td>Coccinellidae</td>
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<td></td>
<td>Lady beetles</td>
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<td>Stethorus spp.</td>
<td>0.10 ± 1.06</td>
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<td></td>
<td>Macropredators</td>
<td>1.54 ± 6.40</td>
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*Lady beetle species included C. polita, H. axyridis, C. septempunctata, and C. transversoguttata. Orinus spp. in Oregon were not identified to the species level. Macropredators encompass all nonacarine, winged, or mobile natural enemies of spider mites and/or aphids observed in the leaf samples.

Plots were treated every 7–14 d with water, micronized sulfur (Microthiol Dispers), parafinic oil (JMS Stylet Oil), or a rotation of the synthetic fungicides described in the text. Applications of micronized sulfur and parafinic oil ceased at burr stage, corresponding to 10 July 2005 and 1 July 2006 in Oregon and 29 June 2005 in Washington. Thereafter, plots where treated with synthetic fungicides to simulate grower practices.

P = <0.0009. Fungicide treatment suppressed hop aphid populations on days 157, 171, 186, and 200 compared with treating with water (P = 0.0391). Populations of hop aphid on plants treated with parafinic oil were significantly different than the water treatment on days 157 and 171 (P ≤ 0.0431) (Fig. 3B). However, the area under the pest development curve for hop aphid did not differ among treatments (F = 0.78; df = 3, 9.21; P = 0.5356). Cone damage associated with hop aphid was not observed.

Predatory mite abundance was affected by fungicide treatments (F = 20.40; df = 3, 1245; P < 0.0001) (Fig. 3C). Plots treated with sulfur consistently had the lowest density of predatory mites, with a seasonal mean population of 0.03 ± 0.02, which was 0.66, 0.67, or 1.32 predatory mites per leaf less than plots treated with parafinic oil, synthetic fungicides, or water, respectively (Table 4). Differences in predatory mite abundance were particularly evident during the final evaluation, where sulfur treated plots had 16.8–34.9-fold fewer phytoseiids per leaf compared with the other treatments (Fig. 3C).

Abundance of Stethorus spp. and O. tristicolor in leaf samples varied from 0.16 ± 0.03–0.48 ± 0.08 (Table 4), and it was significantly affected by fungicide treatment (Stethorus spp.: F = 4.96; df = 3, 1245; P = 0.0020 and O. tristicolor: F = 2.70; df = 3, 1245; P = 0.0443). However, no treatment consistently suppressed or increased Stethorus spp. or O. tristicolor. Abundance of combined macropredators on leaves was similar among treatments (F = 1.97; df = 3, 1245; P = 0.1170) (Table 4).

Fungicide treatment also affected abundance of O. tristicolor in canopy shake samples (F = 4.03; df = 3, 69; P = 0.0107), with 0.27–0.29 per shake sample fewer O. tristicolor in synthetic fungicide treated plots than in plots treated with sulfur, parafinic oil or water. However, consistent trends in abundance of O. tristicolor over all sampling periods were not observed. Populations of other macropredators recovered in shake samples, individually or combined, were similar among treatments (combined macropredators: F = 1.07; df = 3, 69; P = 0.3683) (Table 3).

Washington 2006. Spider mites were observed during the first sampling on 23 May (day 143). Spider mite populations generally increased in all treatments until 1 August (day 213) or 8 August (day 220), reaching maximum population densities of 120.8–231.0 mites per leaf (Fig. 4A). Spider mite populations were significantly affected by fungicide treatment (F = 23.85; df = 3, 522; P < 0.0001). Seasonal mean populations of spider mites were greatest in plots treated with sulfur (mean 71.58 ± 4.32) and lowest in plots treated with water (48.45 ± 4.84) (Table 4). Statistically significant differences among treatments were found on days 171, and 192–234 (P ≤ 0.0192) (Fig. 4A), although no treatment consistently had more or less spider mites compared with the water treatment on all sampling dates. Spider mite abundance in the sulfur treatment was similar to the water treatment until 27 d after the last sulfur application was made (day 220); thereafter, spider mite abundance was greater than in the water treatment.

Correspondingly, the area under the pest development curve for spider mites was 1.3, 1.4, or 1.6 times greater for sulfur treated plots compared with plots treated with synthetic fungicides, water, or parafinic oil (Table 2) (F = 8.33; df = 3, 7.16; P = 0.0098). The
The area under the curves for synthetic fungicide, parafinic oil, and water-treated plots were similar ($P > 0.05$). The rate of mite population development among treatments did not differ significantly ($F = 0.51; df = 3, 24.4; P = 0.7071$). Median cone damage due to spider mites was rated as "moderate" for all treatments, and fungicide treatment did not significantly affect cone quality ratings.

Mean populations of hop aphid ranged between $3.83 \pm 0.45$ and $8.80 \pm 1.37$ per leaf over the season (Fig. 4B; Table 4), and they were affected by fungicide treatment ($F = 10.61; df = 3, 465; P < 0.0001$). Differences among treatments were significant on sampling days 164–192, with a tendency for sulfur-treated plots to have lower abundance of hop aphid compared with water or synthetic fungicide treatments (Fig. 4B). However, the area under the pest development curve for hop aphid was similar among treatments ($F = 0.94; df = 3, 11.8; P = 0.4543$). Cone damage associated with hop aphid was not observed.

Mean abundance of predatory mites ranged from $0.52 \pm 0.10$ in the sulfur treatment to $1.74 \pm 0.19$ mites per leaf in the synthetic fungicide treatment (Table 4), and it was significantly affected by fungicide treatment ($F = 45.84; df = 3, 498; P < 0.0001$). Fewer predatory mites were observed in plots treated with sulfur or parafinic oil than in plots treated with water on all sampling dates after day 199, and these differences were most pronounced at later sampling dates (Fig. 4C). Abundance of predatory mites in the synthetic treatment was less than the water treatment only on day 234 ($P = 0.0002$), when predatory mite...
abundance was 3.73 mites per leaf greater in the water treatment.

*Stethorus* spp. and *O. tristicolor* were the dominant macropredators recovered from leaf samples, and the abundance of both was significantly affected by fungicide treatment (*Stethorus* spp.: $F = 5.58; df = 3, 851; P = 0.0009$ and *O. tristicolor*: $F = 3.36; df = 3, 2139; P = 0.0181$). Mean number of *Stethorus* spp. and *O. tristicolor* adults and larvae per leaf ranged from 0.10 to 0.02–0.03 among treatments (Table 4).

Table 4. Mean seasonal density ± SEM of arthropods on hop leaf samples in relation to fungicide treatment, Prosser, WA

<table>
<thead>
<tr>
<th>Location and yr</th>
<th>Arthropod a,b</th>
<th>Fungicide treatment (mean ± SEM per leaf) c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Sulfur</td>
</tr>
<tr>
<td>Washington 2005</td>
<td>Anthocoridae</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td></td>
<td><em>O. tristicolor</em></td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Coccinellidae</td>
<td>1.35 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Lady beetles*</td>
<td>10.30 ± 1.31</td>
</tr>
<tr>
<td></td>
<td><em>P. humuli</em></td>
<td>35.50 ± 4.24</td>
</tr>
<tr>
<td></td>
<td><em>T. urticae</em></td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Coccinellidae</td>
<td>1.62 ± 0.18</td>
</tr>
<tr>
<td></td>
<td><em>Tetranychus urticae</em></td>
<td>48.45 ± 4.84</td>
</tr>
<tr>
<td></td>
<td>Macropredators</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>Washington 2006</td>
<td>Anthocoridae</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td></td>
<td><em>O. tristicolor</em></td>
<td>0.73 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Lady beetles*</td>
<td>1.62 ± 0.18</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Macropredators</td>
<td>0.12 ± 0.02</td>
</tr>
</tbody>
</table>

a Phytoseiidae species were *G. occidentalis* and *N. fallacis*.
bLady beetle species included *H. axyridis*, *C. septempunctata*, and *C. transversoguttata*. Macropredators encompass all nonacarine, winged, or mobile natural enemies of spider mites and/or aphids observed in the leaf samples.
cPlots were treated every 7–14 d with water, micronized sulfur (Microthiol Dispers), paraffinic oil (JMS Stylet Oil), or a rotation of synthetic fungicides described in the text. Applications of micronized sulfur and paraffinic oil ceased on 29 June in 2005 and applications of micronized sulfur ceased on 28 June in 2006 (burr stage). Thereafter these plots were treated with synthetic fungicides to simulate grower practices.

Fig. 3. Density of *T. urticae* (mean ± SEM) (A), *P. humuli* (B), phytoseiidae (C), and total macropredators (D) on hop leaves in relation to fungicide programs in Washington in 2005. Data are means of 10 leaves per plot from each of four replications per treatment. Macropredators encompass all nonacarine, winged or mobile natural enemies of spider mites and/or aphids observed in the samples.

![Graphs showing density of arthropods](image-url)
lations varied significantly among treatments on individual sampling dates although there were no consistent trends for any treatment to suppress or increase populations on all sampling dates. Abundance of total macropredators ranged from 0.30 ± 0.04 per leaf with water to 0.43 ± 0.04 with synthetic fungicides, and the repeated measures analysis detected a significant treatment effect ($F = 5.07; df = 3, 1,121; P = 0.0017$): macropredator populations varied significantly ($P = 0.0022$) among treatments on several sampling dates (days 199–220 and day 234), although there were no consistent trends for any treatment to suppress or increase populations on all sampling dates (Fig. 4D).

**Discussion**

In 2 yr of field studies in the contrasting climates of western Oregon and central Washington, multiple applications of sulfur fungicides applied before burr development resulted in 1.4–3.3-fold greater spider mite populations during summer. During the period of weekly sulfur applications relatively few mites were present in both Oregon and Washington. Near the end of the sulfur applications, or after a lag of 20–30 d from the end of sulfur applications, spider mite populations increased significantly faster in the sulfur-treated plots in three of the four experiments. These results are consistent with previous research that showed spider mites tend to be suppressed by multiple applications of sulfur, but their populations recover and buildup after the cessation of sulfur applications (James et al. 2001, James and Prischmann 2006, Costello 2007). Laboratory bioassays showed field rate applications of sulfur to a Washington population of *T. urticae* were not toxic but did reduce adult mite longevity and therefore fecundity by $\approx 50\%$ (Price and James 2008). Multiple applications of sulfur is predicted to have a progressively suppressive effect on *T. urticae* populations, which is consistent with the reports cited above. In Oregon, differences in the rate of spider mite population development and cumulative populations determined from the area under the pest development curve were most pronounced in the upper canopy where most cones are formed. Consequently, there was a tendency for a higher level of spider mite damage to cones, although differences in cone damage due to fungicide treatments were noted in only one of the four trials.

The effect of paraffinic oil applications on spider mite outbreaks varied among experiments. This treatment increased the area under the pest development curve compared with water or synthetic fungicide treatment in Washington in 2005 but suppressed mite populations compared with sulfur or synthetic fungicide treatments in Oregon in 2006. In Oregon in 2005, paraffinic oil increased the rate of mite population development in the upper canopy and also led to an increase in mite damage to cones.

Populations of most predatory insects (macropredators), individually or combined, were not affected consistently by fungicide treatments in both years. However, suppression of predatory mites was
many fungicides were repellent and repulsive to temperature. Walsh and Grove (2005) found that due to resource depletion, overpopulation, and/or exacerbation of spider mite outbreaks on hop (McMurtry et al. 1970, Costello 2007). The lack of substantial populations of predatory mites in the experiments in Oregon, which are key predators of spider mites (McMurtry and Croft 1997), precludes conclusions about the effect of these fungicides on these key natural enemies in this cool, maritime climate. Although predatory mites have been documented in association with hops in western Oregon (Strong and Croft 1993), predatory mites were nearly absent in the hop yard in Corvallis. Phytoseiid mites often overwinter in hop yards in association with crowns of hop plants, and cultural practices such as spring crowning and basal defoliation (stripping) for management of powdery mildew and downy mildew can reduce survival of *N. fallacies* (Strong and Croft 1996). However, crowning and stripping were not conducted in these experiments. Presumably, a resident population of predatory mites was not established in this yard since it was planted in 2005.

Nonetheless, the exacerbation of the spider mite outbreak in Oregon was similar to that observed in Washington. The reason for the increase in spider mite populations in response to sulfur and paraffinic oil in the absence of predatory mites is unclear. Mechanisms other than direct or indirect toxicity to phytoseiid mites likely are associated with exacerbation of spider mite outbreaks on hop (McMurtry et al. 1970, Costello 2007). Spider mites under natural conditions are pseudocolonial, tending to occur in a group near a foundress female (Van de Vrie et al. 1972). Dispersal typically occurs after conditions become unfavorable due to resource depletion, overpopulation, and/or environmental factors (e.g., changing photoperiod or temperature). Walsh and Grove (2005) found that many fungicides were repellent and repulsive to *T. urticae* and resulted in nonlethal "irritable behavior." Based on the results of laboratory bioassays, they suggested that mites irritated by fungicide exposure will tend to migrate to areas of the crop canopy where chemical contact is reduced, and they also speculated that fungicides could have other direct and indirect effects on natural enemies. Such a dispersion effect could be significant on hop due to the rapid development of the crop canopy in three dimensions during the period when sulfur and paraffinic oil would be applied intensively for powdery mildew (Gent et al. 2008). Enhanced dispersion of spider mites to newly emerged hop leaves in the upper canopy, where residues of sulfur and paraffinic oil would be reduced, may explain why differences in spider mites populations were most pronounced among treatments in the upper canopy. Because the younger leaves likely had a lower carbon-to-nitrogen ratio, potentially, the greater rate of spider mites population development in the upper canopy could be related to the nutritional suitability of these leaves for spider mite development (Hoffland et al. 2000, Wermeling er et al. 1991, Wilson 1994). However, mechanisms associated with exacerbation of spider mite outbreaks was beyond the scope of the current studies. Future research will investigate the effect of sulfur and paraffinic oil on repulsion and dispersion of spider mites on hop plants under controlled and field conditions.

Synthetic fungicides significantly reduced predatory mite populations in Washington in 2005, but not in 2006. Importantly, the reduction in predatory mites documented in 2005 due to synthetic fungicides did not seem to disrupt their biological control activity compared with sulfur and paraffinic oil. Consistent effects on other natural enemies were not detected. In Washington, however, the synthetic fungicide and water treatments tended to have more hop aphids compared with sulfur treatments. Toxicity of sulfur to other aphid species has been reported previously (Mishra 1996), although more complex mechanisms associated with host defense responses or nutritional suitability also may be involved (Yusuf and Collins 1998). Nonetheless, sulfur seemed to provide some suppression of *P. humuli* in Washington in both 2005 and 2006.

Together, these studies clearly indicate that the aggregate effect of applications of sulfur and, to a less extent paraffinic oil, are to disrupt biological control of *T. urticae* whether populations of phytoseiid mites are present or not. However, direct negative effects on predacious insects were not consistently observed, suggesting that the fungicides evaluated are relatively nontoxic to the key natural enemies of *P. humuli*, which is supported by previous laboratory bioassays (James and Coyle 2001). Based on the results of these studies, powdery mildew fungicide programs that minimize or eliminate applications of sulfur and paraffinic oil would tend to conserve predatory mites and minimize the severity of spider mite outbreaks.

Synthetic fungicide alternatives to sulfur and paraffinic oil can provide highly efficacious powdery mildew control (Gent et al. 2008). However, potential barriers to greater adoption of synthetic fungicide alternatives to sulfur and paraffinic oil are their expense ($50–$80/ha or more) and their specific modes of action that are prone to development of pathogen resistance (McGrath 2001). Resistance to strobilurin and demethylation inhibitor classes of fungicides has been reported extensively for other powdery mildew fungi (Ypema et al. 1997, McGrath 2001). Therefore, management of powdery mildew without sulfur or paraffinic oil likely is not predicted to be economically or strategically sustainable. Research is needed to identify the effect of a limited number of carefully timed applications of sulfur or paraffinic oil on conservation biological control of spider mites on hop. Based on the results in Oregon where predatory mites essentially were absent, a strategy of applying sulfur or paraffinic oil only when predatory mites are not present may not prevent exacerbation of spider mite populations. Additional studies are underway to develop cost-effective fungicide programs that are compatible with conservation biological control and that minimize exacerbation of spider mite outbreaks.
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