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# Development of Biological Control of *Tetranychus urticae* (Acari: Tetranychidae) and *Phorodon humuli* (Homoptera: Pemphigidae) in Oregon Hop Yards

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**ABSTRACT** The temporal development of biological control of arthropod pests in perennial cropping systems is largely unreported. In this study, the development of biological control of twospotted spider mite, *Tetranychus urticae* Koch, and hop aphid, *Phorodon humuli* (Schrank), in a new planting of hop in Oregon is described over a period of 9 yr (2005–2013). Both the abundance and diversity of natural enemies increased over time. Known predators of hop aphid (Coccinellidae and Anthrenidae) were present in all years; however, stable biological control of hop aphid was not achieved in most years and aphicides were required to suppress populations at commercially acceptable levels in 5 of 9 yr. Populations of aphidophagous coccinellids developed synchronously with hop aphid populations, and temporal correlations indicated these are the primary predatory insect associated with hop aphid regulation. However, sampling methods did not assess levels of aphid parasitoids and hyperparasitoids and their contribution to biological control was unquantified. Spider mite biological control was associated primarily with predatory mites (Phytoseiidae) and *Stethorus* spp. (Coccinellidae). The magnitude of temporal correlations of abundance of these predators with spider mites was found to be greatest on the same sampling dates and at lags of 7–14 d. Stable biological control of spider mites occurred after four field seasons, suppressing spider mites to levels similar to those commonly achieved with chemical control. A survey of 11 commercial hop yards in Oregon documented pest and natural enemy densities under commercial management practices over a period of 4 yr (2008–2011). Natural enemy abundance in commercial hop yards was similar to that of a 2- to 3-yr-old hop yard with limited disturbance. Whereas total reliance on biological control for hop aphid is unlikely to be successful, there appears to be unrealized potential for biological control of spider mites in commercial production. Dynamic action thresholds that consider the value of natural enemies are needed for both pests.

**KEY WORDS** conservation biological control, cross-correlation, integrated pest management

Enhancing the environment through practices that conserve natural enemy abundance and diversity has been termed conservation biological control (CBC; Ehler 1998). In the broadest sense, CBC seeks to attract and maintain natural enemies and enhance the

longevity and efficacy of resident natural enemy populations to suppress pests (Barbosa 1998). The primary effort of CBC is directed toward encouraging the resident natural enemy population to thrive through strategies such as use of selective pesticides, provision of refugia, and careful selection of cultural practices (Eilenberg et al. 2001).

The hop plant, *Humulus lupulus* L., is a dioecious perennial plant with annual shoots that can climb to heights >4–5 m in a single growing season (Neve 1991). Female strobiles, termed cones, are produced on lateral branches and contain the economically valuable bittering acids and oils that act as a preservative and flavoring of beer (Neve 1991). This crop offers a unique opportunity for assessing development of CBC. The rapid growth habit of the plant, at times up to 15 cm per day, and production of copious amounts of succulent leaf tissue are thought to make hop a preferred host of several arthropod pests and diseases (Mahaffee et al. 2009). The two primary arthropod pests of hop in the northern hemisphere are

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the hop aphid, *Phorodon humuli* (Schrank), and the twospotted spider mite, *Tetranychus urticae* Koch. Management of arthropod pests typically involves annual applications of miticides and aphicides. Numerous applications of foliar fungicides are also made for disease management (Gent et al. 2009, Mahaffee et al. 2009).

The potential for biological control of aphid pests and twospotted spider mite is well supported in several systems, including hop (Aveling 1981, Neve 1991, James and Barbour 2009, Weihrauch 2009). Aphids are preyed on by numerous predator, parasitoid, and pathogen species (Frazer et al. 1981, Obrycki et al. 2009). For example, an assemblage of aphidophagous organisms preying on pea aphid (*Acyrtosiphon pisum* Harris) in alfalfa generally includes coccinellids, spiders, anystid mites, lacewings, syrphids, anthocorids, phalangids, and aphidiids (Frazer et al. 1981). Hop aphids are commonly preyed on by members of coccinellids and anthocorids, although populations of at least 50 aphids per leaf may be needed for the establishment of these predators. There is currently little evidence to suggest stable and effective control of hop aphid can be achieved solely by parasitoids and pathogens (Neve 1991, Trouve et al. 1997, Hartfield et al. 2001, Weihrauch 2009).

Predators of twospotted spider mite consist of many generalist arthropods, including members of the Acarina (e.g., Anystidae) and the insect orders Coleoptera, Neuroptera, Hemiptera, Thysanoptera, and Diptera. Specialist predators include certain phyto-seiid mites as well as mite-feeding lady beetles, *Stethorus* spp. (Pruszyński and Cone 1972, Strong and Croft 1993, Biddinger et al. 2009). In natural conditions, twospotted spider mite generally is regulated by predatory arthropods (James et al. 2001, Gardiner et al. 2003), suggesting that this pest could be effectively suppressed by CBC in managed agroecosystems. However, no studies in hop have actually quantified the temporal development of biological control or attempted to identify the natural enemies most critical to its success.

Our objectives in this study were to describe the temporal development of biological control in hop, identify the association of predatory arthropods with hop aphid and twospotted spider mite, and assess the potential for biological control to suppress these pests. We draw on 9 yr of observations of pest and predatory arthropod abundance from nonmiticide-treated plots in western Oregon. Predatory arthropod abundance and diversity in nonmiticide-treated experimental plots are contrasted with those found in commercial hop yards to assess the degree of biological control potential.

## Materials and Methods

**Experimental Plots and Data Collection.** Data were collected from nonmiticide-treated plots each year from 2005 to 2013. The plots were established in an experimental hop yard near Corvallis, OR, that was planted in April 2005 to the 'Willamette.' The total area

of the yard was  $\approx 0.75$  ha, and the yard was surrounded by mowed grass and annual cereal or vegetable crops. Hop plants were arranged on a 2.1-m grid pattern and under a 5-m trellis. Several studies occurred during the 9 yr of data collection that evaluated the impact of sulfur and other fungicides on twospotted spider mite, hop aphid, and their natural enemies. Details of two of these studies are provided in Gent et al. (2009) and Woods et al. (2012), but only data from plots receiving no miticides or sulfur fungicides are reported herein. In a given experiment, plots were arranged in a randomized complete block design with four or five replicates. An individual plot consisted of 16 plants during 2005 and 2006 and other 8 plants in other years. In all experiments, the nontreated plots were separated by at least one row of plants not treated with insecticides, miticides, or fungicides. Standard production practices for western Oregon were followed in all years. In 2005, 2006, and 2007, irrigation was supplied by sprinklers every 7–14 d as needed for crop development, whereas in subsequent years irrigation was supplied daily by a surface drip system. In the planting year 2005, 46-0-0 fertilizer was applied to each plant by hand ( $\approx 14$  g per plant). Granular nitrogen, phosphorous, and potassium were broadcast applied during 2006–2013 in April, May, and June according to standard commercial recommendations (Gingrich et al. 2000), with total kilograms of nitrogen per hectare totaling 101, 54, 135, 135, 131, 137, 119, and 148, respectively. Basal foliage and weeds were controlled during 2006–2013 with applications of herbicides, as described in detail in Supp Table 1 (online only).

Throughout the duration of these studies, selective insecticides were applied in certain years to reduce confounding effects from other pests. During 2007–2009, *Bacillus thuringiensis* (0.15 kg a.i./ha, Javelin WG, Certis USA, LLC, Columbia, MD) was applied in July for the control of lepidopteran pests. During 2007–2009, 2011, and 2012, pymetrozine (0.034 kg a.i./ha, Fulfill, Syngenta Crop Protection, Greensboro, NC) was applied for the control of hop aphid when populations exceeded 90 aphids per leaf. An additional application of imidacloprid was injected into the drip irrigation system (0.02 liters a.i./ha, Provado 1.6 F, Bayer CropScience LP, Research Triangle Park, NC) in July 2009. No aphicide was applied during 2005, 2006, 2010, or 2013. Foliar insecticides were applied using an airblast orchard sprayer. Application volume varied with plant growth in each year, but ranged from 468 to 749 liters/ha. No other miticides or insecticides were applied to the sampled plots or directly neighboring plants.

For comparative purposes, a survey of pest and predator abundance was conducted in 11 commercial hop yards during 2008–2011. The yards were located in Marion County in western Oregon. Three hop yards were surveyed in each of 2008 and 2009, four hop yards were surveyed in 2010, and one hop yard was surveyed in 2011. Although the number of hop yards sampled in a given year varied, the objective of this sampling was to obtain a representative estimate of arthropod abundance under commercial management practices. This

objective was accomplished despite variation in the number of yards sampled in each year. Crop management practices and the application of pesticides were at the discretion of individual growers and varied among yards according to the cooperating growers' standard production practices.

**Arthropod Sampling in Experimental Plots.** In the experimental plots during 2005–2011 and 2013, leaf samples were collected every 7–14 d beginning in mid-April to early May, continuing until cone harvest during mid to late August. In 2012, only four biweekly samples were collected beginning in mid-July simply owing to time constraints. On each sampling date, 10–40 leaves were collected from each plot and motile spider mite stages, spider mite eggs, apterous hop aphids, predatory mites (Phytoseiidae), mite-eating ladybeetles (*Stethorus* spp.), and minute pirate bugs (*Orius* spp.) were identified and enumerated. Non-carine, winged, or mobile natural enemies are referred to herein as macropredators. The number of leaves sampled varied depending on time of year, sampling protocols in the original experiments, and the practical limitations of field research. For instance, sample sizes were necessarily reduced at certain times of year because removing 40 leaves each week would defoliate plants over the course of a season. Nonetheless, a representative sample was ensured at every sampling point by collecting a minimum of 10 leaves from each plot. More intensive sampling at other times likely led to greater precision in population estimates on certain dates, but these differences are of little importance for the analyses described below.

*Neoseiulus fallacis* (Garman) was the only species recovered. Samples of adult predatory mites were identified under low magnification (60 $\times$ ) with the aid of a stereomicroscope, with a subset of females mounted on slides and identified by G. W. Krantz (Oregon State University, Corvallis, OR) or F. Beaulieu (Agriculture and Agri-Food Canada, Ottawa, Ontario). Other life stages were simply categorized as phytoseiidae and were not identified to species.

Leaves were collected from the lower canopy (<2 m) when plants were <2 m tall and from both the lower and upper canopy (>2 m) when plants were taller than 2 m. Leaves were collected from only the four plants in the middle of each plot to reduce plot-to-plot interference from other treated plots in the yard. Four to five replicate plots were sampled in a given year. 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 organisms either directly on the leaves or after transferring to a corn syrup-coated glass plate using a mite brushing machine (Leedom Engineering, Twain Harte, CA). A comparison of spider mite enumeration directly from leaves versus after processing with a mite brushing machine has been presented in detail by Macmillan (2005). The correlation of the methods was deemed adequate for the analyses described herein.

Certain arthropods may not be estimated with precision from leaf samples. To capture and estimate

macropredators of spider mites and aphids, canopy shake samples also were collected during 2006–2009, 2012, and 2013, with sampling occurring every 7–14 d from mid-June to mid-August. In 2012, only four shake samples were collected during July and August owing to resource limitations. To avoid potential confounding effects from highly dissimilar sampling dates, data from 2012 were excluded from certain analyses, as described below. Natural enemy assessments were made from the four plants in the middle of each plot. Canopy shake samples were collected by placing a 1-square meter white cloth under a hop bine and vigorously shaking the bine for 3 s. Dislodged arthropods were identified and counted on the cloth or collected into vials holding 70% ethanol for later confirmation or identification in a laboratory. One sample was collected from each of the four to five replicate plots on each sampling day.

**Arthropod Sampling in Commercial Yards.** In the 11 commercial hop yards, leaf samples were collected biweekly beginning in late April to early May, continuing until cone harvest during mid- to late August. In 2008 and 2009, on each sampling date, 30 leaves were collected arbitrarily at a height of  $\approx$ 1.5 m from the ground in a 0.5-ha section of the yard designated for sampling. In 2010 and 2011, 30 leaves were collected from the lower canopy when plants were less than  $\approx$ 2 m tall. When plant growth exceeded 2 m, 15 leaves were collected from both the upper (>1.5 m) and lower (<1.5 m) canopy. Samples were collected biweekly and processed as described above.

Canopy shake samples also were collected to capture macropredators that may be poorly represented on leaf samples. In 2008 and 2009, beginning in mid-June, biweekly arthropod samples were collected by canopy shake samples. Hop bines were shaken for  $\approx$ 3 s over a 1-square meter funnel, and natural enemies that fell into the funnel were brushed into a collection vial holding 70% ethanol. From each yard, arthropods collected from each of three hop bines were combined for identification and enumeration. At least three samples were collected from each hop yard, thus at least nine bines were sampled from each yard. Macropredators were identified and enumerated with the aid of a stereomicroscope. In 2010 and 2011, shake samples were conducted as described above for experimental plots but using a 1-square meter white cloth instead of a funnel of the same area. Other aspects of the sampling were as described for 2008 and 2009.

**Data Analysis.** The number of spider mite, hop aphid, *Stethorus* spp., and predatory mites on leaves, and number of Anystidae, Coccinellidae, predatory Hemiptera, *Stethorus* spp., and total predators collected in shake samples on each assessment date were plotted over time. Arthropod days for each organism were calculated using a macro in SigmaPlot version 11.0 (Systat Software, Inc., San Jose, CA). To determine differences in arthropod abundance among years, arthropod days for each arthropod group were analyzed using generalized linear mixed models (GLIMMIX procedure) in SAS version 9.2 (SAS Institute 2008, Cary, NC). Data from shake samples col-

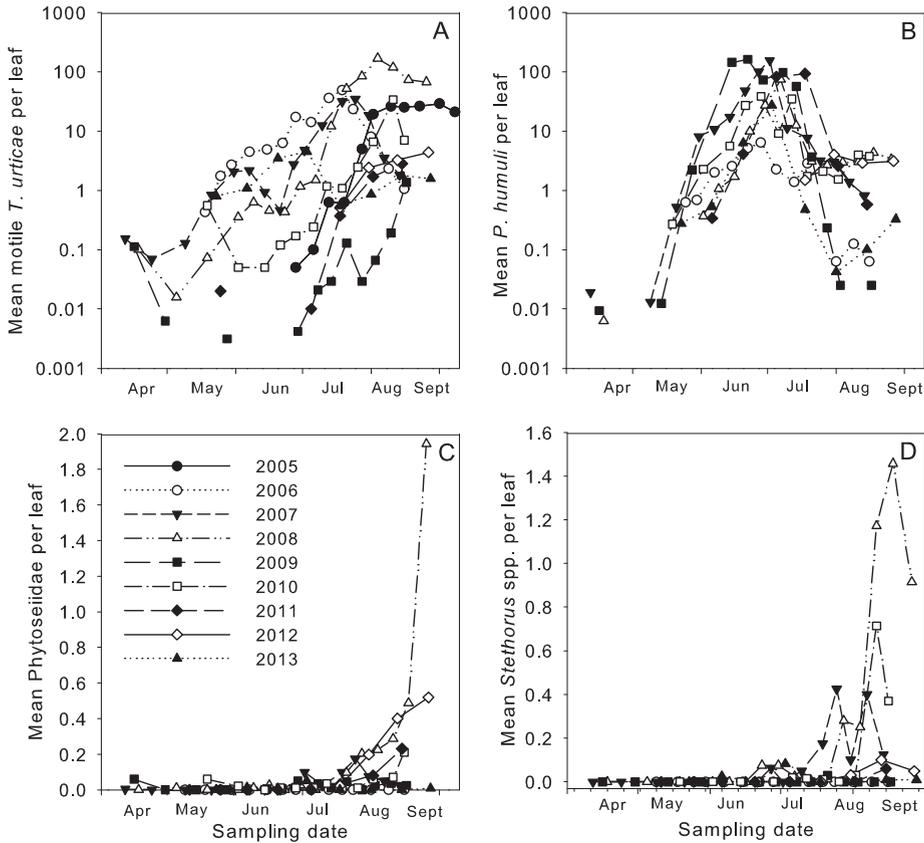


Fig. 1. Abundance of *T. urticae*, *P. humuli*, Phytoseiidae, and *Stethorus* spp. (A, B, C, and D, respectively) per leaf in nonmiticide-treated plots during 2005–2013. Errors bars are omitted to improve legibility of the figures.

lected in 2012 were excluded from these analyses owing to the shorter duration of sampling that year. Abundance of hop aphid, spider mites, or both, was included in the analysis as a covariate for each predator group to control for differences in prey abundance. None of the covariates was significant in the analysis.

The data set allowed for an analysis of the temporal relatedness of pest and predators groups at varying points in time (lag periods) based on cross-correlation (Scutareanu et al. 1999). Cross-correlation is a time series statistic used to measure the similarity of two series. The cross-correlation coefficient is standardized so that the cross-correlation coefficient ranges from  $-1$  to  $1$ , with the sign of the statistic indicating the nature of the relationship (positive or negative correlation) and the magnitude of the statistic proportional to the strength of the relationship. Because the cross-correlation coefficient quantifies the temporal relatedness of two series, when applied to predator and prey, this statistic can be used to make inferences about the potential for predation (Scutareanu et al. 1999). In this study, the data collected were observational and therefore identification of a direct causal relationship of predation is not possible. Nonetheless, the analysis can provide evidence

for which organisms develop most synchronously with a pest and thus are most likely to be important in CBC. Data for the analysis were log transformed to achieve reasonable variance homogeneity, and missing values were interpolated as described in Lingeman and van de Klashorst (1992). Cross-correlation analyses for each organism pair were performed using PROC ARIMA in SAS version 9.2 (Yoo and O’Neil 2009). Cross-correlations of predator–prey pairs were conducted across years for each predator group identified in shake samples (Anystidae, aphidophagous Coccinellidae, predatory Hemiptera, *Stethorus* spp., and total predators) and on leaves (Phytoseiidae) with prey species identified on leaves (*T. urticae* and *P. humuli*).

**Results**

**Abundance of Arthropods in Experimental Plots.** In all years, spider mites and hop aphid were detected in the yard early in the growing season, with the exception of the initial planting year (2005) when spider mites were not detected on leaves until late June and hop aphid was absent (Fig. 1A and B). The earliest spider mites were first detected on 12 April and the latest on 25 May (Fig. 1A), excluding 2012 in which

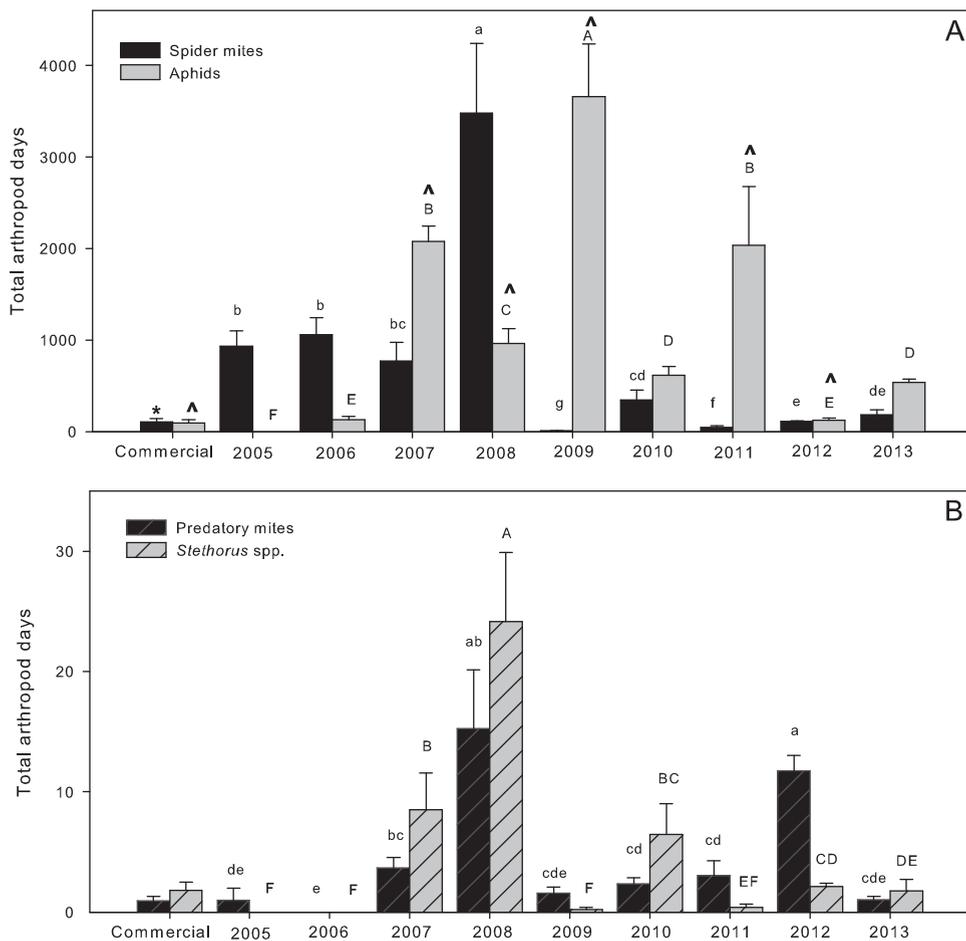


Fig. 2. Total arthropod days accumulated for *T. urticae* (mean  $\pm$  SEM) and *P. humuli* (mean  $\pm$  SEM) (A); Phytoseiidae (mean  $\pm$  SEM) and *Stethorus* spp. (mean  $\pm$  SEM) (B) in nonmiticide-treated plots during 2005–2013 and commercial hop yards (2008–2011). Data collected from commercial hop yards consisted of 11 total hop yards, 3 in each of 2008 and 2009, 4 in 2010, and 1 in 2011. Aphicides were applied in certain years, as indicated by “\*.” An asterisk indicates miticides were applied in commercial yards.

sampling did not begin until 18 July. The earliest hop aphid was first detected on 16 April and the latest on 28 June, exclusive of 2012 (Fig. 1B).

The severity of spider mite outbreaks varied among seasons ( $F = 51.28$ ;  $df = 8, 29$ ;  $P < 0.0001$ ; Fig. 2A). The most severe outbreaks occurred during the first four seasons after planting, with mean cumulative spider mite days ranging from 774 to 3478. Thereafter, the severity of outbreaks diminished and ranged from 8 to 344 spider mite days.

Hop aphid abundance varied over years, and increased after the first two seasons ( $F = 250.03$ ;  $df = 8, 29$ ;  $P < 0.0001$ ; Fig. 2A). Seasonal trends in hop aphid abundance after the first two seasons were not apparent, due in part to aphicide applications made in four of the seven years during 2007–2013. The most severe outbreaks occurred during 2007–2009 and 2011, with mean cumulative aphid days ranging from 964 to 3665; aphicide applications were made in each of these years. Outbreaks in 2005, 2006, 2010, 2012, and 2013

were less severe, with mean cumulative aphid days ranging from 0 to 615. During these years, an aphicide application was made only during 2012.

The occurrence of arthropod species was diverse, with >20 different organisms present and identified to family, genus, or species (Table 1). Some level of predatory arthropods were present on all leaf and shake samples collected during 2006–2009, 2012, and 2013 (Figs. 1C, D, and 3). The abundance and diversity of predators generally increased through time (Table 1; Figs. 2B, 3, and 4). For instance, macropredators were absent on leaf samples in 2005, while in 2010 and 2011 two or more predator groups were present on at least 13% of leaves sampled.

Predatory mites were present in all years with the exception of 2006 and were first detected on 23 August in 2005, 12 April in 2007, 5 May in 2008, 16 April in 2009, 19 May in 2010, 18 July in 2011, 18 July in 2012, and 6 June in 2013 (Fig. 1C). Although predatory mites were nearly undetectable for the first two seasons, their

**Table 1. Predatory arthropod colonization and abundance on hop plants in Corvallis, OR, during 2005–2013**

	Year first detected	Proportion of sampling years detected
<b>Coccinellidae</b>		
<i>Harmonia axyridis</i>	2006	0.67
<i>Cycloneda polita</i>	2007	0.56
<i>Coccinella septempunctata</i>	2007	0.56
<i>Coccinella transversoguttata</i>	2008	0.33
<i>Adalia bipunctata</i>	2007	0.22
<i>Psyllobora</i> spp.	2006	0.67
Coccinellidae larvae <sup>a</sup>	2006	0.89
<i>Stethorus</i> spp. A&L <sup>b</sup>	2006	0.29
<b>Other predatory Coleoptera</b>		
Cantharidae (soldier beetle)	2008	0.11
Staphylinidae (rove beetle)	2007	0.44
<b>Predatory Hemiptera</b>		
<i>Deraeocoris brevis</i> A&N <sup>b</sup>	2006	0.67
<i>Heterotoma</i> spp.	2007	0.44
Nabidae	2007	0.44
<i>Orius</i> spp. A&N <sup>b</sup>	2006	0.89
Predatory Miridae	2006	0.44
<b>Neuroptera</b>		
Chrysopidae & Hemerobiidae (green & brown lacewing)	2006	0.33
Neuroptera larvae <sup>a</sup>	2006	0.67
<b>Other</b>		
Hymenoptera	2006	0.67
Aeolothripidae (predatory thrips)	2007	0.56
Syrphidae A&L <sup>b</sup>	2006	0.33
Anystidae	2007	0.56
Spiders	2006	0.67
Forficulidae (earwigs)	2006	0.56

<sup>a</sup> Coccinellidae larvae include all aphid-feeding ladybeetle larvae. Neuroptera larvae include Chrysopidae and Hemerobiidae larvae.

<sup>b</sup> A, adult; L, larval stage; N, nymphs.

abundance increased over time and was maximum in 2008 during a severe spider mite outbreak. The mean cumulative predatory mite days varied significantly among years, ranging from 0 to 15.24 ( $F = 7.38$ ;  $df = 8, 29$ ;  $P = <0.0001$ ; Fig. 2B). Predatory mites were found more often and in greater abundance in leaf samples following the severe spider mite outbreak in 2008, with mean cumulative predatory mite days ranging from 1.04 to 11.75 during 2009–2013 (Fig. 2A and B).

*Stethorus* spp. were detected on both leaf and shake samples. Larval and adult stages were present in leaf samples in all years with the exception of 2005 and 2006, and were detected first on 27 June in 2007, 23 June in 2008, 21 July in 2009, 6 July in 2010, 15 August in 2011, 18 July in 2012, and 6 June in 2013 (Figs. 1D and 3B). However, abundance of *Stethorus* spp. varied among years ( $F = 19.64$ ;  $df = 8, 29$ ;  $P = <0.0001$ ; Fig. 2B). On leaves, *Stethorus* spp. abundance was relatively low during the first two seasons, as well as 2009 and 2011, with mean cumulative *Stethorus* spp. days ranging from 0 to 0.39. *Stethorus* spp. abundance peaked in 2008 during a severe spider mite outbreak, but temporal trends in other years were not apparent. After 2008, mean cumulative *Stethorus* spp. days ranged from 1.79 to 24.16.

The most common predators found in shake samples collected during 2006–2009 and 2013 were

grouped by arthropod (Anystidae, Coccinellidae, predatory Hemiptera, and *Stethorus* spp.), with all non-Hymenopteran arthropods included in a “total predator” group (Figs. 3 and 4). The shake data from 2012 are not included here owing to the shorter duration of sampling that year, which would confound analysis of means among years. With the exception of Anystidae and predatory Hemiptera in 2006, all predatory arthropods were found on the date of the first shake sample of the season (Fig. 3). The abundance of Anystidae and total predators increased over time ( $F = 36.56$ ;  $df = 4, 15.49$ ;  $P = <0.0001$ ;  $F = 118.32$ ;  $df = 4, 14.63$ ;  $P = <0.0001$ , respectively; Fig. 4). The abundance of Coccinellidae, predatory Hemiptera, and *Stethorus* spp. was more variable, but there was a general trend for increasing abundance over time ( $F = 79.04$ ;  $df = 4, 13.66$ ;  $P = <0.0001$ ;  $F = 24.28$ ;  $df = 4, 14.5$ ;  $P = <0.0001$ ;  $F = 4.47$ ;  $df = 4, 30$ ;  $P = 0.0059$ , respectively; Fig. 4).

**Cross-Correlation Analysis.** The overall pattern of cross-correlation varied between hop aphid and the predator groups, with significant cross-correlations found for at least one lag period between hop aphid and Anystidae, Coccinellidae, predatory Hemiptera, *Stethorus* spp., and total predators (Fig. 5A). For hop aphid and Anystidae, significant cross-correlations occurred at lags of 1, 2, and 3. This indicates a general asynchrony in the maximal populations of these organisms. For hop aphid and Coccinellidae, significant cross-correlations occurred at lags of -1, 0, 1, 2, and 3. The strongest correlation between hop aphid and Coccinellidae was centered on a lag of 1, indicative of a high degree of temporal synchrony between these organisms. For hop aphid and predatory Hemiptera, a positive cross-correlation occurred at a lag 3, and negative cross-correlations were detected at lags of -1, -2, and -3. Similar to hop aphid and Anystidae, the linear trend in the cross-correlations is indicative of asynchronous populations of hop aphid and predatory Hemiptera. For hop aphid and total predators, significant cross-correlations were found at lags of 0, 1, 2, and 3, which were heavily influenced by the number of aphidophagous Coccinellidae relative to other predators.

The overall pattern of cross-correlation between spider mite and the major predator groups was variable and primarily negative, with the exception of a strong positive correlation between spider mites and *Stethorus* spp. (Fig. 5B). Significant cross-correlations were observed between spider mites and *Stethorus* spp. at lags of 0, 1, and 2. The pattern of correlation centered on a lag of 1 is indicative of a high degree of synchrony between these two arthropods. There was a significant negative association between spider mites and Anystidae, Coccinellidae, predatory Hemiptera, and total predators at lags of -3 to 3 (Fig. 5B).

On leaves, a significantly positive cross-correlation was observed between spider mites and Phytoseiidae, with a cross-correlation coefficient at lags of -1, 0, 1, 2, and 3 (Fig. 6). The strongest correlation was at a lag of 2, and the pattern of the relationship indicated that

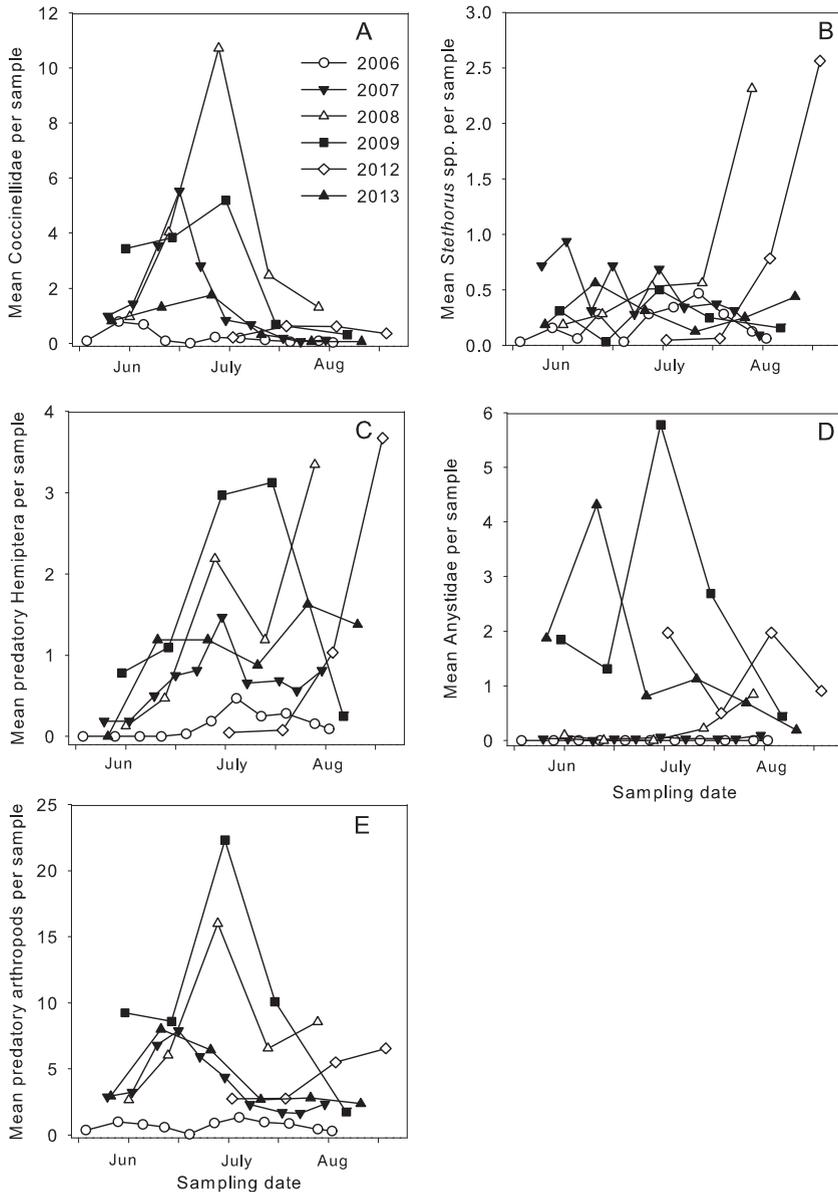
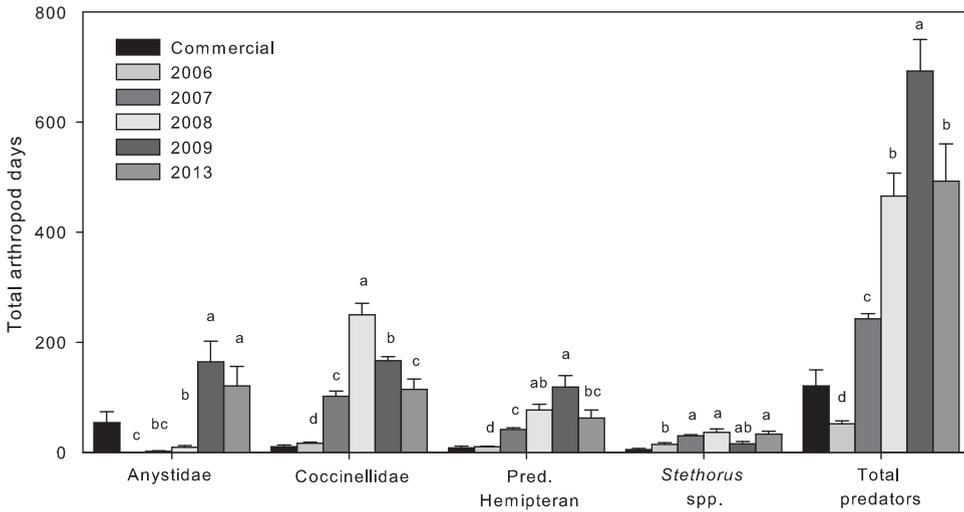


Fig. 3. Abundance of aphidophagous Coccinellidae, *Stethorus* spp., predatory Hemiptera, Anystidae, and total predators (A, B, C, D, and E, respectively) per shake sample in nonmiticide-treated plots during 2006–2009, 2012, and 2013. Errors bars are omitted to improve legibility of the figures.

predatory mites were present during and after peak abundance of spider mites.

**Commercial Hop Yards.** Spider mites and hop aphid were detected in all commercial hop yards sampled with 106.4 mean cumulative spider mite days and 93.9 mean cumulative aphid days for all sampling years (Fig. 2A). Predatory mites were found in only 5 of the 11 hop yards sampled, with 0.95 mean cumulative predatory mite days (Fig. 2B). On leaves, mean cumulative *Stethorus* spp. days were 1.8 for all sampling years. For predatory arthropods identified in shake samples, mean cumulative arthropod days were 53.6,

10.6, 8.7, 5.4, and 120.3 for Anystidae, Coccinellidae, predatory Hemiptera, *Stethorus* spp., and total predators, respectively (Fig. 4). These levels were comparable with the abundance of these predators in the experiment plots 2–3 yr after planting, but substantially less than the levels that developed in subsequent years. Miticides were applied in 10 of the 11 commercial hop yards, and aphicides were applied in every yard. The number of spider mites observed in commercial hop yards was similar to that of the experimental plots once biological control was established during and after 2009.



**Fig. 4.** Total arthropod days accumulated for Anystidae (mean ± SEM), Coccinellidae (mean ± SEM), predatory Hemiptera (mean ± SEM), *Stethorus* spp. (mean ± SEM), and total predators (mean ± SEM) in nonmiticide-treated plots during 2006–2009, and 2013. Data collected from commercial hop yards consisted of 11 total hop yards, 3 in each of 2008 and 2009, 4 in 2010, and 1 in 2011.

**Discussion**

Hop is a unique system that can be considered a highly disturbed perennial crop because although the plant is perennial, the shoots are annual and little of the plant remains to provide overwintering refugia (Neve 1991). Disease and weed management practices can further disturb the system through the removal of basal foliage, herbicide application, and tillage (Mahaffee et al. 2009). It has been unclear whether natural enemies will become resident, how long this will take, and to what degree they can be relied on for pest suppression in hop. The research presented herein begins to address these uncertainties and provides a description of the potential development of biological control within a new planting.

During the course of 9 yr, biological control developed over time, which was reflected in both greater diversity and abundance of natural enemies. This was associated with suppression of spider mites 4 yr after planting. Comparable biological control of hop aphid was not observed, and selective aphicides were applied in most years when populations far exceeded action thresholds. Aphid predators were present in greatest abundance when hop aphid abundance was greatest, but generally were unable to exert the level of control needed to maintain populations below commonly accepted action thresholds of 5–10 aphids per leaf (Dreves 2010).

Coccinellids and generalist predators (e.g., *Orius* spp.) known to prey on aphids (Aveling 1981, Frazer et al. 1981, Obrycki et al. 2009) were detected in every year of the study in experimental plots. However, sampling methods did not allow for collection and enumeration of aphid parasitoids and hyperparasitoids; thus, their prevalence and contribution to aphid biological control is not reflected in these analyses. A

variety of hymenopteran parasitoids have been recorded from hop aphids in Washington during spring before they colonize hops (Wright and James 2002). Similarly, several fungi also are known to attack hop aphid (Dorschner et al. 1991, Hartfield et al. 2001), but abundance of entomophagous fungi was not quantified. Despite the lack of sampling for these natural enemies, the recurrent outbreaks of hop aphid suggest that in most years, the cumulative effect of measured and unmeasured natural enemies of hop aphid was insufficient to provide population regulation at levels generally accepted in commercial production, a requisite of biological control.

It is possible that in certain circumstances hop aphid could be tolerated at greater levels than occurred in this study without economic damage to the host. Precise economic thresholds for hop aphid are undetermined, although a study conducted in Germany suggested that up to 50 aphids per hop cone did not cause a reduction in alpha-acids, but may lead to cosmetic defects that renders a crop unmarketable (Weihrauch 2009, Lorenzana et al. 2010, Weihrauch et al. 2012). The variability of biological control of hop aphid, coupled with the potential for yield loss, crop rejection, and pricing penalty owing to contaminated cones suggests that aphid-resistant cultivars, floral resource provisioning, and other strategies are imperative to enable effective CBC of this pest. The observations in this study support the general conclusion that biological control of hop aphid is largely inadequate (Weihrauch 2009). Therefore, a practical approach to hop aphid management seems to be one that places an emphasis on arthropod sampling and avoidance of conditions known to exacerbate outbreaks to delay, or in certain years, eliminate the need for chemical intervention.

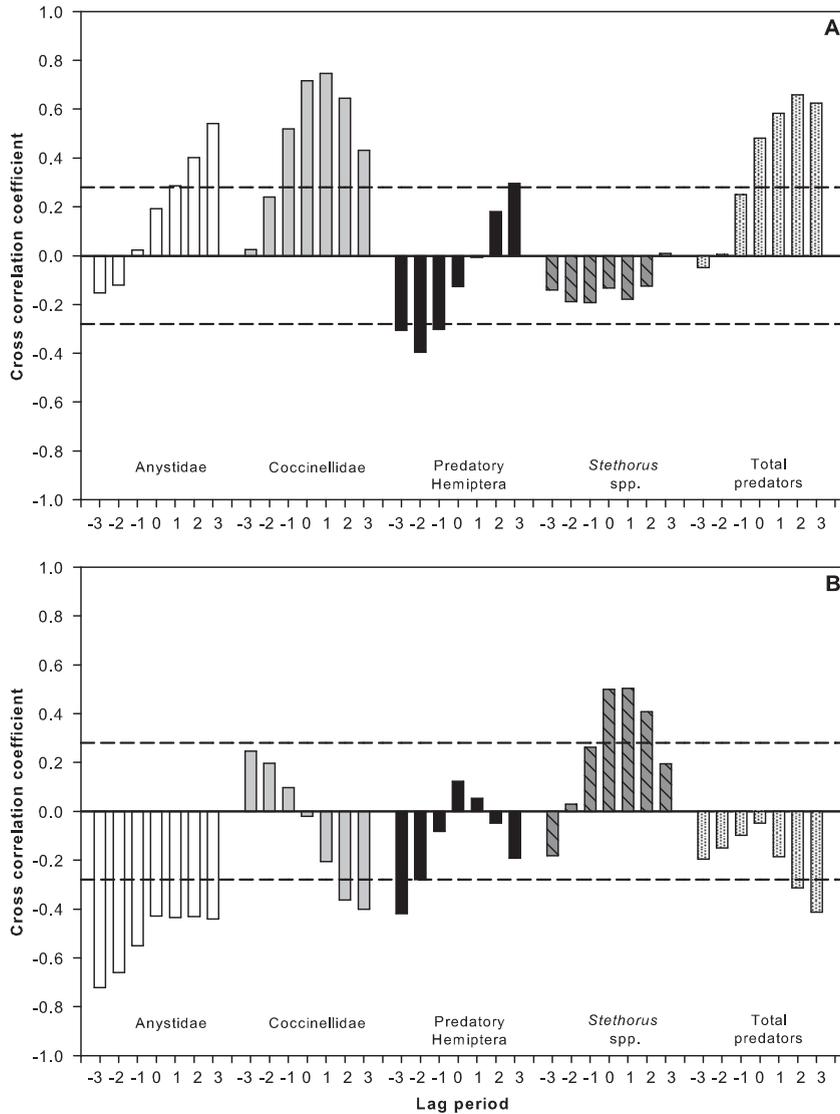


Fig. 5. Cross-correlation of *P. humuli* (A) and *T. urticae* (B) with Anystidae, Coccinellidae, predatory Hemiptera, *Stethorus* spp., and total predators. Data collected from mean leaf samples (*P. humuli* and *T. urticae*) and mean shake samples (all predator groups) from 2006 to 2009, and 2013. Dashed lines indicate critical values for significance ( $\alpha = 0.05$ ).

Action thresholds for spider mites on hop vary by growing region, time of year, environmental conditions, and control measures available (Strong and Croft 1993, Weihrauch 2005). Strong and Croft (1993) suggested an action threshold of 10 spider mites per leaf during the growing season in the Willamette Valley, OR; however, the authors stated that the true economic impact on the crop needs further study. During 2009–2013 in this study, spider mites exceeded 10 per leaf on only two sampling dates, and populations were suppressed below this level within 14 d. It is noteworthy that consistent suppression of spider mites was achieved after the severe outbreak of spider mites in 2008, even though there was a conversion from sprinkler irrigation to drip irrigation in 2007 and

use of imidacloprid in 2009. Spider mite outbreaks in hop yards tend to be favored by dry, dusty conditions (James and Barbour 2009), and such conditions may be more likely to occur with drip irrigation versus sprinkler irrigation. James and Price (2002) also reported stimulation of fecundity in *T. urticae* when exposed to residues of imidacloprid. Development of biological control despite these factors suggests there is good potential for CBC to provide effective management of spider mites in hop yards, as suggested by James et al. (2001, 2003).

Initial efforts toward biological control in hop placed an emphasis on predatory mites (Phytoseiidae) through inoculative releases for control of spider mites (Strong and Croft 1993, 1995, 1996). To control weeds

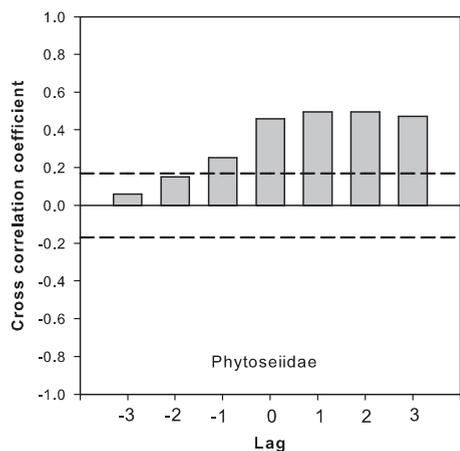


Fig. 6. Cross-correlation of *T. urticae* and Phytoseiidae. Data collected from mean leaf samples from 2005 to 2011, and 2013. Dashed line indicates critical values for significance ( $\alpha = 0.05$ ).

and foliar diseases, tillage and the removal of lower foliage commonly is used in hop production (Neve 1991, Mahaffee et al. 2009). Strong and Croft (1995) attributed the lack of success of phytoseiid release to the combination of rapid growth rate of the hop plant and common practices for disease management, such as chemically removing the lower foliage. Although the precise mechanisms controlling the success of augmentative releases of phytoseiids are unknown, augmentative releases of phytoseiids generally have not been successful or adopted in commercial hop production. More recent work on biological control of spider mites in hop has postulated that an assemblage of natural enemies beyond phytoseiids is vital to suppressing spider mites (James et al. 2001, 2003; Gent et al. 2009; Woods et al. 2011, 2012).

The greatest levels of biological control of spider mites occurred following a severe outbreak of the pest in 2008, coincident with establishment of predatory mites but also increasing diversity and abundance of macropredators. Phytoseiid mites are often considered critical for biological control of spider mites in perennial crops, and other predators often are considered to be of secondary importance (McMurtry and Croft 1997, Nyrop et al. 1998). Research on the biological control of spider mites in apple, grape, walnut, almond, and raspberry consider phytoseiid mites to be the primary biological control agents (Hoy et al. 1979, Whalon and Croft 1984, Prischmann et al. 2002, Roy et al. 2005, Steinmann et al. 2011). In walnut, almond, and raspberry, *Stethorus* spp. is reported as a secondary predator (Hoy et al. 1979, Roy et al. 2005, Steinmann et al. 2011). The minute pirate bug, *Orius tristicolor* (White), and the six-spotted thrips, *Scolothrips sexmaculatus* (Pergande), are regarded as secondary predators in almond and walnut (Hoy et al. 1979, Steinmann et al. 2011). The data reported on these perennial crops are similar to our observations in hop in that phytoseiids and *Stethorus* spp. appear to

be the primary predators regulating spider mites. Cross-correlation analyses indicated predatory mites and *Stethorus* spp. abundance were strongly correlated with spider mite abundance. These organisms appear to be the predators most likely to be essential for regulation of spider mites in hop, although their relative importance is not clear from these analyses. Both phytoseiids and *Stethorus* spp. tend to specialize on spider mites, but may consume alternative foods when spider mites are scarce (Overmeer 1985, Biddinger et al. 2009). The evidence presented here suggests spider mite abundance is the primary determinate of the population density of these predators.

There does appear to be an assemblage of predators present and consuming spider mites at some level (James et al. 2001, 2003; Gent et al. 2009; Woods et al. 2012), although generalist predators (Anystidae and predatory Hemiptera) were not strongly correlated with spider mites and their role in CBC of spider mites on hop appears secondary or minor. This analysis suggests a collection of at least three natural enemies is involved in successful biological control of spider mites. Of the arthropods identified as foundational for biological control, predatory mites tend to be the most sensitive to broad-spectrum pesticides and other disturbance (James and Coyle 2001). Pragmatically, then, practices that conserve predatory mites are likely to enhance the environment for all predators likely to be important in biological control of spider mites.

The commercial hop yard data indicated that the abundance and diversity of natural enemies in these yards was similar to that of the experimental plots in the second and third years after planting before biological control was fully developed. However, the putatively key predator groups identified in the experimental plots were present in many of the commercial yards, albeit at low levels, indicating an unrealized potential for CBC of spider mites in commercial production.

The data presented in this article address the first three postulates presented by Naranjo and Ellsworth (2009) for successful biological control: 1) biological control agents must be present and abundant in the untreated system; 2) biological control agents must be able to survive, at some level, the application of selective controls; and 3) some assessment of the functionality of conservation biological control must be demonstrated. An abundance of natural enemies resulted in stable biological control of spider mites in hop after an establishment period of 4 yr. The data collected from commercial hop yards provide evidence for the presence of natural enemies that are able to withstand, at some level, the application of pesticides and cultural disturbances that often are nonselective. We also provide evidence for stable biological control of spider mites in hop in a minimally disturbed production system. The analyses are limited by the inability to determine the individual contribution of a given organism to CBC and the lack of explicit identification of all organisms (e.g., parasitoids) that may provide population regulation. Nonetheless, future re-

search in these areas may be accelerated, as efforts are focused on putatively important predators.

Within the limitations (field size, cultivar, and environment) of the experimental plots evaluated, biological control of spider mites occurred after the fourth season, but it is unknown if this can be scaled up to a commercial setting. It is possible that the stability of spider mite biological control observed in an experimental setting may be an artifact of a small planting, and more research is needed to effectively develop CBC in commercial hop production. It is also important to consider that the data from the experimental plots were collected from a single hop yard in Oregon, and it is difficult to extrapolate these results to other hop-growing regions (e.g., Washington and Idaho). Nonetheless, we propose that this body of data supports the functionality of CBC of spider mites in hop in Oregon and suggests potential for less reliance on chemical control in commercial production.

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