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Phytoseiids in Washington commercial apple orchards: biodiversity and factors affecting abundance

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Abstract *Galendromus occidentalis* (Nesbitt) is an important biological control agent of spider mites (Acari: Tetranychidae) in Washington apple orchards. It was thought to be essentially the sole phytoseiid existing in this system, due in part to its resistance to commonly used orchard pesticides, and organophosphates in particular. To test this assumption, we conducted a survey of 102 commercial apple blocks in Washington to characterize the community of phytoseiid species. Seven phytoseiid species were found in our samples; *G. occidentalis* and *Amblydromella caudiglans* (Schuster) were found in the greatest abundance. We hypothesized that the gradual shift away from the use of organophosphates in recent decades may have caused the change in phytoseiid community structure. The survey data and information regarding the management, location, and surrounding habitat of each block were used to determine what factors affect phytoseiid abundances. *Galendromus occidentalis* abundance was positively affected by the use of conventional (vs. organic) spray programs, and the use of the acaricide bifenazate. *Amblydromella caudiglans* abundance was negatively affected by bifenazate use and positively affected by herbicide strip weediness; it was also less prevalent in 'Golden Delicious' blocks compared to other cultivars. These results indicate that *A. caudiglans* reaches higher abundances in orchards that lack certain agricultural disturbances, whereas *G. occidentalis* can survive in more disturbed environments. Surveys of this nature can provide valuable insight to potential drivers of community structure, allowing for the improvement of integrated pest management programs that incorporate conservation of newly recognized biological control agents such as *A. caudiglans*.

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Introduction

Spider mites (Acari: Tetranychidae) are economically important secondary pests of apple (*Malus domestica* Borkhausen). Tetranychids feed on the cellular contents of leaves, including chlorophyll, compromising the canopy's photosynthetic function, and in severe cases, causing premature leaf abscission. Loss of photosynthetic ability can lead to poor fruit set or size, and leaf abscission also leads to sunburn (Croft 1982; Walter and Proctor 2004). Outbreaks of spider mites are often severe in commercial apple orchards with intensive pesticide use; however, high populations of spider mites are rarely detected in unmanaged apple trees. Like most secondary pests, flare ups of mite populations are attributed to a disruption of biological control (Madsen 1964; Tanigoshi et al. 1983) caused by intensive agricultural inputs and pesticides in particular (Beers et al. 2005; Prischmann et al. 2005; Martinez-Rocha et al. 2008; Duso et al. 2014).

The most extensively studied biological control agent of mite pests in Washington apple orchards is the phytoseiid *Galendromus occidentalis* (Nesbitt). Integrated mite management (IMM), which includes the conservation of *G. occidentalis*, began in Washington in the 1960s and was highly successful (Hoyt 1969; Hoy 2011). This program was based on the discovery that *G. occidentalis* had developed resistance to broad-spectrum organophosphates. This resistance allowed *G. occidentalis* to persist in orchards that used organophosphates to control codling moth (*Cydia pomonella* L.), the most damaging pest of Washington apple, while maintaining *G. occidentalis* populations to provide biological control of spider mites.

Unfortunately, pest mite outbreaks attributed to the breakdown of IMM have been noted in increasing frequency and severity since the early 2000s (Beers et al. 2005). This deterioration is thought to have been caused by reduced organophosphate (OP) use coupled with increased use of "reduced-risk" and OP-replacement insecticides (Environmental Protection Agency 2014) for codling moth control (Beers et al. 2005; Beers and Schmidt 2014). While many phytoseiid species, including *G. occidentalis*, developed resistance to OPs (Hoyt 1969; Motoyama et al. 1970; Croft 1990), many reduced-risk/OP alternative pesticides appear to have toxic effects on predatory mites (Villanueva and Walgenbach 2005; Bostanian et al. 2009, 2010; Gadino et al. 2011; Lefebvre et al. 2011, 2012; Beers and Schmidt 2014).

Apple trees that are unsprayed or minimally sprayed have a diverse phytoseiid fauna (Thistlewood 1991; Horton et al. 2002; Croft and Luh 2004). Pesticide use (especially OPs) in commercial orchards of Washington in the 1960s apparently reduced this fauna to a single resistant species, *G. occidentalis* (Hoyt 1991). The assumption that this was the sole phytoseiid species of any importance in commercial apple orchards went unchallenged during the next 40 years. However, substantial changes in the pesticide program occurred during that time. The number of OPs used declined due to loss of efficacy or loss of registration, and currently only a few remain with limited usage.

The purpose of this study was to determine the species composition of phytoseiid mites in commercial apple orchards in Washington. Our goal was to describe the phytoseiid biodiversity of these orchards and identify factors that affect their abundance.

Characterization of the species composition will allow for a more informed approach to conserving populations of important spider mite predators.

Materials and methods

We surveyed phytoseiid mites from 102 different commercial apple orchard blocks in fruit-growing districts east of the Cascade Mountains in Washington, USA (Fig. 1). From each orchard, we collected a random sample of leaves ($n = 100$), collecting 1–2 leaves per tree. Samples were taken from late May to early September in 2011–2013. The date of collection and GPS coordinates were recorded for each block. All phytoseiid mites were removed from the leaf samples using a paintbrush and preserved in 70 % ethanol until slide-mounting; when possible, specimens were slide-mounted immediately. The presence or absence of the following potential prey species from the leaf samples was also recorded: *Panonychus ulmi* (Koch), *Tetranychus urticae* Koch, *Tetranychus mcdanieli* McGregor, *Aculus schlechtendali* (Nalepa), as well as the stigmaeid predator *Zetzellia mali* (Ewing). Phytoseiids were mounted on a slide in modified Berlese's solution and all adult females were identified to species as per Denmark and Evans (2011). Identifications were confirmed by Dr. James McMurtry, Professor Emeritus of Entomology, University of California, Riverside, CA, USA.

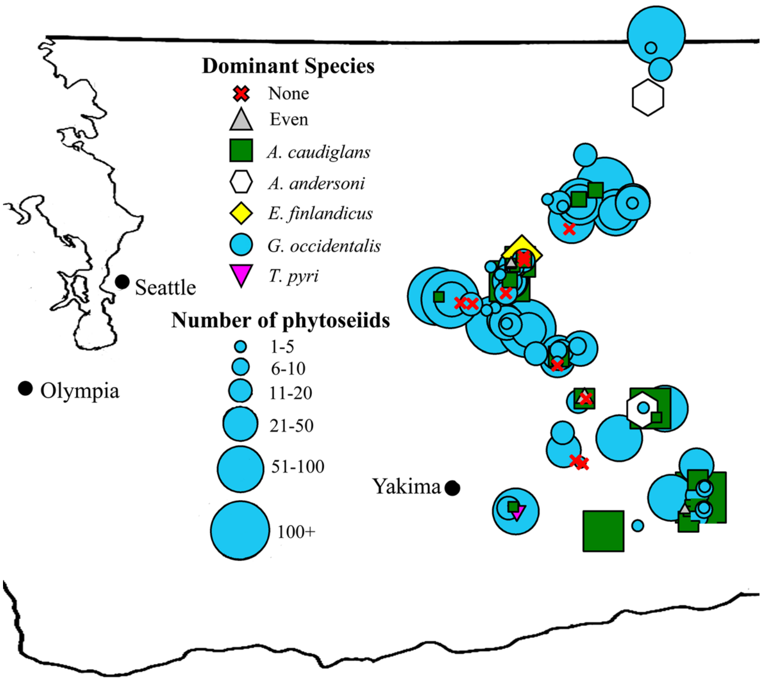


Fig. 1 Locations of sampled commercial apple blocks. *Shape and color of the bubbles* indicate which species was dominant; if none were found an "×" is used. *Diameter of bubbles* indicates total number of phytoseiids found (on a relative scale). (Color figure online)

From each apple orchard where mite samples were collected we also collected data on a suite of factors that might influence predatory mite populations. These factors were examined for their impacts on predatory mites (see "Data analysis" section). To obtain data on cultural practices, pesticide-use history, and mite problems we distributed a questionnaire to pest consultants in charge of management of each block sampled in our surveys. All of the questions asked, and the possible answers, are shown in Online Resource 1. The questions pertained to practices used in the 3 years prior to sampling. To assess the impacts of landscapes surrounding each orchard (which might impact mite movement to and from apple orchards), we gathered landscape data from Cropland Data Layer maps (US Department of Agriculture 2015); these maps provide remotely sensed data on land-use in the United States at a 30 m resolution. The maps for each sample year were imported into ArcGIS (ESRI 2010) and the area for each of four landscape types (orchards, pasture/shrubland, developed, and other) was extracted from a 50 m radius buffer around each sampled block to reflect edge habitat surrounding orchards. To assess the impacts of temperature on predatory mite populations we collected temperature data from the Washington State University AgWeatherNet station (http://weather.wsu.edu/awn.php) nearest to each site from 1 January to the date of sample collection. These data were used to calculate cumulative degree days (DD) at the time of sampling (Jones and Brunner 1993). The horizontal temperature cutoffs for degree day calculations were set at 10 and 37 °C, as per Stavrinides et al. (2010). The complete list of variables collected from each block where mites were sampled can be found in Online Resource 1 (Tables 1–3).

Data analysis

We used logistic regression or general linear models (see below) to examine the impact of multiple factors related to our surveyed orchards on the presence/absence and abundance of the two most common species (*Amblydromella caudiglans* (Schuster) and *G. occidentalis*). Both presence/absence and abundance were assessed separately as they reflect different aspects of predatory mite populations. In each analysis, our full model included all variables collected from each orchard block including cultural practices, orchard management practices, application of specific miticides, the presence of key prey species, climatic conditions, and landscapes. The full initial model used in each case, which contained all possible explanatory variables, is shown in Online Resource 2. However, we used a model selection approach to reduce the number of explanatory variables in all analyses. This approach was used, rather than testing the impacts of over 20 variables in our full model, to develop a "best-fit" model that only included non-correlated explanatory variables that significantly impacted mite populations. Prior to all analyses, categorical survey responses with values less than ~ 10 were collapsed to fewer categories where possible, or omitted from the subsequent analyses (Table 3 in Online Resource 1). Orchard blocks with missing data for a particular factor were excluded from the analysis for that factor, but were included elsewhere.

Presence/absence

We analyzed factors affecting the presence/absence of both *A. caudiglans* and *G. occidentalis* using stepwise logistic regression, where the presence of mites (present or absent) served as the binary response data. We used stepwise regression with an information theoretic approach, where variables were added or removed to minimize Akaike's Information Criterion (AIC), to select a best-fit model that included parameters influencing the presence/absence of each predatory mite species. This model selection approach was used because many variables in our full model (see Online Resource 2) were correlated and/or did not significantly impact mite populations. For factors that were significant in these analyses, we used contrasts to determine differences between categories within factors. We used JMP for these analyses (SAS Institute 2014a).

Abundance

We used general linear models to assess the impacts of our various orchard characteristics (see Online Resource 2) on the abundance of our two predatory mite species. For these models we used a negative binomial distribution (PROC GENMOD, SAS Institute 2014b) based on the distribution of predatory mite abundances. We then used mixed stepwise regression to select a subset of model parameters that minimized AIC. As with the presence/absence models, this model selection approach was used to reduce the number of parameters in our full model down to a subset that best explained variation in predatory mite abundance. For all the variables included in our best-fit model, we used contrasts to determine differences between categories.

Correlation

Factors included within each of the final models were checked for correlation by performing contingency table analysis using Pearson's Chi square tests (SAS Institute 2014a).

Results

Seven species of phytoseiids were found in the survey: *A. caudiglans*, *Amblyseius andersoni* (Chant), *Euseius finlandicus* (Oudemans), *Galendromus flumenis* (Chant), *G. occidentalis*, *Neoseiulus fallacis* (Garman), and *Typhlodromus pyri* Scheuten (Table 1, Online Resource 3). *Galendromus occidentalis* and *A. caudiglans* were the two most common species, both in terms of the number of sites where they were found and overall abundance (Fig. 1; Table 1). The other species were rare (<5 % of individuals) and were only dominant at four of the 102 sites. *Amblydromella caudiglans* was the dominant

Table 1 Species composition and dominance of phytoseiids at 102 sampled apple blocks

	Percent individuals found	Percent sites dominant	Percent sites present
Amblydromella caudiglans	22.35	19.61	50.00
Amblyseius andersoni	3.12	1.96	5.88
Euseius finlandicus	1.17	0.98	0.98
Galendromus flumenis	0.03	0.00	0.98
Galendromus occidentalis	72.95	64.71	75.49
Neoseiulus fallacis	0.03	0.00	0.98
Typhlodromus pyri	0.34	0.98	1.96
Total (*n*)	2917	102	102

species at ~20 % of the sites and reached densities of up to 1.29 mites/leaf in one sample. In 9.8 % of sites surveyed, no phytoseiids were found in the sample.

Presence/absence

The only orchard characteristic that was found to influence the presence of *G. occidentalis* was pesticide intensity (as defined in Table 3 of Online Resource 1) (Fig. 2); this mite was more likely to be present in conventional orchards than in organic orchards, regardless of the "intensity" of the conventional spray program. Herbicide strip weediness and cultivar (Fig. 3) were the only orchard characteristics included in the best-fit model for the presence of *A. caudiglans*, although herbicide strip weediness was only marginally significant ($\chi^2 = 5.78$, $P = 0.056$). *Amblydromella caudiglans* was significantly more likely to be present in 'Gala' blocks and less likely to be present in 'Golden Delicious' blocks compared to other cultivars. No other factors were retained in our final presence/absence models for either species, indicating that other orchard characteristics did not significantly influence the presence of these two predatory mite species.

Abundance

The abundance of *G. occidentalis* was significantly greater in orchards that had used bifenazate (Acramite 50WS, Chemtura, Middlebury, CT, USA) compared to orchards that did not (Fig. 4a). Moreover, the abundance of *G. occidentalis* was significantly higher in orchards with greater pesticide intensity (Fig. 4b). In contrast, the abundance of *A. caudiglans* was significantly lower in orchards that used bifenazate (Fig. 5a). This species was also significantly more abundant in orchards with high herbicide strip weediness (Fig. 5b), but it was significantly less abundant in 'Golden Delicious' blocks compared to other

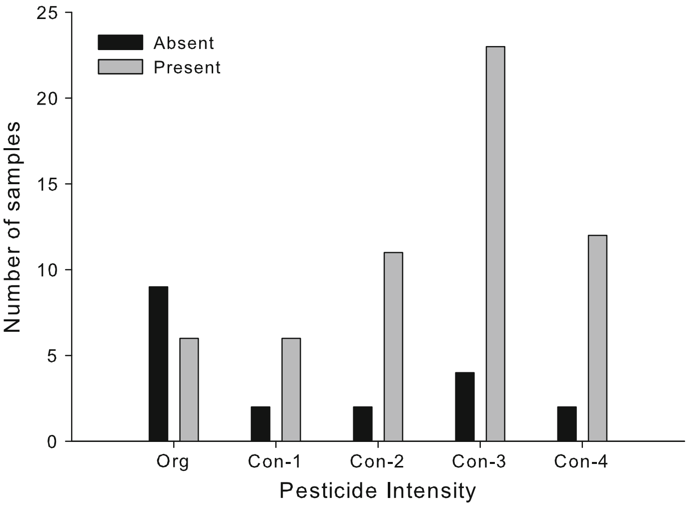


Fig. 2 Number of sites with *Galendromus occidentalis* present/absent and pesticide intensity; $\chi^2 = 11.56$, $df = 1$; $P = 0.02$. "Org" indicates organic orchard, "Con" indicates conventional orchard, the *number* following indicates the pesticide intensity (higher intensities correspond to larger numbers)

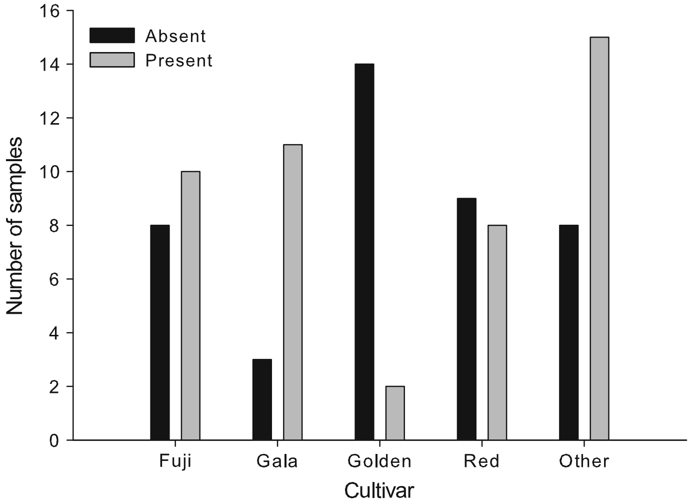


Fig. 3 Number of sites with *Amblydromella caudiglans* present/absent and cultivar; $\chi^2 = 11.62$, df = 4, $P = 0.02$

cultivars (Fig. 5c). No other factors were selected by the procedure to be included in the abundance models for either species.

Correlation

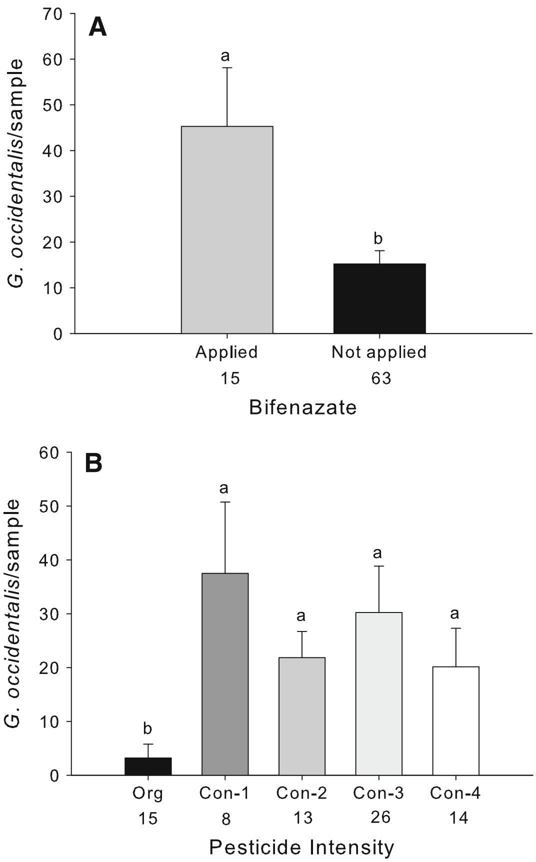
None of the factors included in the four final models were significantly correlated (Table 2).

Discussion

Our study shows that a diverse community of phytoseiid mites exists in commercial apple orchards of Washington State. Contrary to our assumptions, *G. occidentalis* was not the sole phytoseiid in commercial apple orchards. In addition to this well-characterized species, we found *A. caudiglans* in many orchards in high abundance, and collected five other species occasionally at low densities. Similar to our findings that *A. caudiglans* was most common in less disturbed orchards, previous studies (mostly from eastern North America) showed that *A. caudiglans* was only abundant in minimally sprayed orchards or unsprayed feral trees (Oatman 1976; Berkett and Forsythe 1980; Strickler et al. 1987; Thistlewood 1991; Croft and Luh 2004; Bostanian et al. 2006). Few studies in Washington apple orchards have recorded this mite species, however, and those that did were conducted in research orchards or unsprayed trees (Hoyt 1991; Horton et al. 2002; Croft and Luh 2004).

The most likely reason for the high abundances of *A. caudiglans* in our samples is the shift away from the use of OPs for codling moth control in Washington orchards since 1990. Downing and Moilliet (1972) documented this scenario experimentally in British Columbia; *G. occidentalis* replaced *A. caudiglans* following the use of azinphosmethyl, but *A. caudiglans* re-established when those sprays were discontinued. In a New Zealand study, the proportion of *G. occidentalis* in a predatory mite community was higher in an

Fig. 4 Mean *Galendromus occidentalis* abundance and **a** bifenazate use; $df = 1$, $\chi^2 = 3.89$, $P = 0.05$, **b** pesticide intensity; $df = 4$, $\chi^2 = 11.57$, $P = 0.02$. "Org" indicates organic orchard, "Con" indicates conventional orchard, the *number* following indicates the pesticide intensity (higher intensities correspond to larger numbers)



orchard with an OP based pesticide regime, compared to orchards where OPs were not used (Wearing et al. 2014). At the time our samples were taken (2011–2013), azinphos-methyl was under regulatory phase-out (Environmental Protection Agency 2012); however, this phase-out was preceded by decades of declining OP use in Washington apples. While no studies were done in the intervening years, at some point this reduction in use created an ecological opportunity for *A. caudiglans* to establish in a significant number of orchards. In contrast, *G. occidentalis* appeared to benefit from disturbances in orchards given its higher abundance in conventional orchards. An interesting parallel can be found in studies of the shift to reduced-risk/OP alternative pesticides in tart cherries in Michigan (Whalon and Korson 2008). These authors noted that growers using the newer alternative pesticides applied acaricides more frequently than did those using azinphosmethyl.

The use of bifenazate was the only factor found to affect both *G. occidentalis* and *A. caudiglans*. However, by having positive effects on *G. occidentalis* and negative effects on *A. caudiglans*, use of bifenazate led to phytoseiid communities dominated by *G. occidentalis*. These results are in agreement with laboratory tests which show that bifenazate causes higher mortality in *A. caudiglans* than *G. occidentalis* (Schmidt-Jeffris and Beers unpublished). This acaricide is labeled as safe for several mite predators including *G. occidentalis*, however, *A. caudiglans* (because of its relative obscurity) is not listed, and

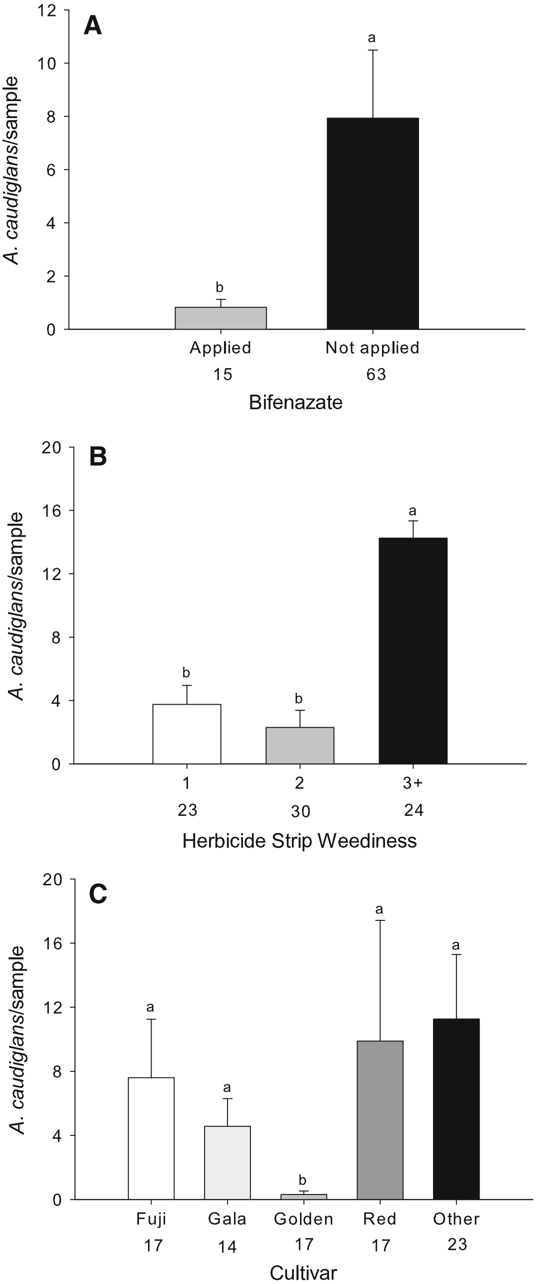


Fig. 5 Mean *Amblydromella caudiglans* abundance and **a** bifenazate use; $df = 1$, $\chi^2 = 13.50$, $P < 0.01$, **b** herbicide strip weediness ranking; $df = 3$, $\chi^2 = 18.21$, $P < 0.01$, **c** cultivar; $df = 4$, $\chi^2 = 10.96$, $P = 0.03$. "1" indicates a virtually weed free herbicide strip, "3+" were all orchards with herbicide strips ranked moderately weedy or higher, with "2" intermediate between weed-free and moderately weedy. Numbers under each category name indicate *n* responses

presumably not tested. Our results, however, suggest this species is highly susceptible to bifenazate or that bifenazate usage shifts competition between predatory mites in favor of *G. occidentalis*.

Amblydromella caudiglans was also affected by other factors beyond bifenazate use. Previous research suggests *A. caudiglans* prefers more pubescent cultivars of apple

Table 2 Contingency table analysis for factors included in the final models of *Galendromus occidentalis* or *Amblydromella caudiglans* presence and abundance

Factor 1	Factor 2	χ^2	*P*
Bifenazate use	Pesticide intensity	6.50	0.16
Bifenazate use	Herbicide strip weediness	2.01	0.14
Bifenazate use	Cultivar	6.75	0.15
Cultivar	Herbicide strip weediness	3.12	0.93

(Downing and Moilliet 1967), and in fact, our survey indicated low abundances of this species it the less pubescent, 'Golden Delicious' blocks (Duso et al. 2009). Trichomes on pubescent varieties can provide phytoseiids shelter from harsh climatic conditions and predators, and can trap pollen, which can be used as a secondary food source (Schmidt 2014). Unlike *G. occidentalis*, *A. caudiglans* is capable of surviving and reproducing on pollen (Putman 1962; McMurtry and Croft 1997). Additionally, some research (McMurtry and Croft 1997; McMurtry et al. 2013) has suggested that generalist predators such as *A. caudiglans* are more affected (either positively or negatively) by host plant characteristics than specialists. These studies potentially explain why cultivar influences the abundance of *A. caudiglans*, but not *G. occidentalis*.

Amblydromella caudiglans were also more abundant in orchards with weedy herbicide strips. Ground cover has been found to increase phytoseiid abundances (Alston 1994; Kawashima and Jung 2010; Mailloux et al. 2010). It has even been suggested that the provision of pollen from ground cover crops may shift community structure in favor of generalist phytoseiids (Aguilar-Fenollosa et al. 2011). Many of the weeds that are common in orchards are flowering plants (e.g., *Taraxacum officinale* Wigg, *Convolvulus arvensis* L., *Trifolium repens* L.) and provide supplementary pollen as food for phytoseiids (Gerson et al. 2003). Increased ground cover, in the form of weedier herbicide strips (as opposed to bare earth), may thus increase abundance of *A. caudiglans*. Ground cover can also provide a reservoir of spider mite prey, allowing predator populations to build up before spider mites become a problem in the canopy (Waite 1988; Takahashi et al. 1998). Weeds can also modify the microhabitat of the orchard floor, making it more suitable for phytoseiids (Croft and McGroarty 1977; Huang et al. 1983). Other authors (Whalon and Korson 2008) have considered the mite predator/prey system, especially as regards diversity, a bioindicator of system health. Our results show that the composition of phytoseiid communities in Washington apples has shifted towards more species-rich communities that are less frequently dominated by *G. occidentalis*. These shifts could affect biological control of mites, given the large body of literature suggesting that increasing predator species richness generally strengthens biological control (Straub et al. 2008; Griffin et al. 2013). However, studies examining predatory mite diversity and biological control have shown mixed results. In experiments conducted in Oregon, biological control of European red mite and twospotted spider mite improved when two species of phytoseiids were present compared to either single species alone (Croft and MacRae 1992a, b). In this system, the phytoseiids *T. pyri* and *G. occidentalis* were most abundant at different points of the growing season, and thus proved temporal complementarity in terms of mite control. Other studies conducted in the greenhouse, laboratory, and field (Schausberger and Walzer 2001; Barber et al. 2003; Rhodes et al. 2006) have similarly found a relationship between increasing phytoseiid richness and mite suppression. However, in these cases the diverse community did not provide more effective control than the most impactful single species; this suggests that the positive effects of biodiversity observed were simply due to the fact that diverse communities contained the most voracious predator species. In contrast, some studies have shown that more diverse communities of phytoseiids are less effective at providing biological control due to intraguild predation (Abad-Moyano et al. 2010; Pina et al. 2012). In apple orchards, *Z. mali* is known to have the potential to disrupt or enhance biological control by phytoseiids because it consumes phytoseiid eggs, but is also an effective predator of pest mites (Clements and Harmsen 1990; Croft and MacRae 1992b, 1993; Villanueva and Harmsen 1998). More work is needed, however, to understand the impacts of shifts in phytoseiid abundance and community structure on biological control in our system.

Even though the single, well-adapted predator model was very successful for many decades in Washington apple, it was very reliant on a single class of insecticides and a specific type of prey (*Tetranychus* spp.). However, the success of this model was not necessarily an indicator of ecosystem stability; its fragility became apparent with regulatory changes of the insecticides used. Large scale sampling provides an opportunity to investigate how natural variation across farms in management practices, landscapes, and abiotic conditions affect community structure and abundances of particular species. Analysis of these surveys can highlight pesticides that should be screened for non-target effects in laboratory assays and controlled field experiments, or identify conservation biological control strategies for particular species. Finally, extensive surveys of agroecosystems may reveal previously overlooked natural enemy biodiversity available for biological control. Our surveys in Washington apple will allow for the re-tooling of IMM through conservation of *A. caudiglans* via the use of selective spray programs in the post-azinphosmethyl era.

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