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Effects of fungicides on a mycophagous coccinellid may represent integration failure in disease management

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ABSTRACT

The adults and larvae of halyziine coccinellids (Coleoptera: Coccinellidae: Halyziini) are obligate mycophages on hyphae and conidia of powdery mildew (PM) (Erysiphales) fungi, that are plant pathogens warranting chemical control in many managed systems. These insects have been observed to reduce PM severity through consumption. Fungicide applications, however, may interfere with this ecological service. Five commercial fungicides were topically applied to the mycophagous coccinellid, *Psyllobora vigintimaculata*, in the laboratory to gauge contact toxicity. In order to detect interference in the field, population density of naturally occurring *P. vigintimaculata* was assessed weekly in a northern California vineyard (*Vitis vinifera*, cultivar "Chardonnay") over 3 years in relation to PM (*Erysiphe necator*) severity and in the presence of various fungicides. Wettable sulfur was toxic to adults in the laboratory, resulting in complete cohort mortality 24 h after application. Topical applications of a strobilurin fungicide (trifloxystrobin) and a demethylation inhibitor fungicide (myclobutanil) also resulted in significant adult mortality. Rapid and complete larval mortality was observed in the laboratory after contact with wettable sulfur and myclobutanil. There was no effect on survival after contact with the PM-antagonistic bacterium, *Bacillus subtilis*. Vineyard density of *P. vigintimaculata* was reduced in vines receiving applications of sulfur and myclobutanil, even when considering the covariate PM severity. The microbial antagonist, *Streptomyces lydicus*, did not significantly affect insect density. Our study questions the integration of chemical disease management with naturally occurring mycophagous agents in some agricultural systems.

1. Introduction

Integrated pest management (IPM) has been described as a process involving compatible use of multiple tactics for simultaneous control of all classes of pests while considering economics and the environment (Prokopy, 2003). This description suggests that pest management tactics should be used in an integrated sense, and as such, should not interfere with each other. Integration and integration failure can be vertical (within one class of pests) or horizontal (among classes of pests) (Ehler, 2006). Applications of insecticides have been shown to disrupt the efficacy of predatory natural enemies (Hattingh and Tate, 1995; Grafton-Cardwell and Gu, 2003), exhibiting vertical integration failure, and fungicide applications have been observed to reduce local abundance of predators and parasitoids of arthropod pests (Martinson et al., 2001; Michaud, 2001), exemplifying horizontal integration failure. These concepts can be applied to disease management as well. Indeed, it has been demonstrated that fungicide applications can be detrimental to disease biocontrol programs involving fungal antagonists (Fravel et al., 2005; Tobin et al., 2008). Discussions of IPM disruption, however, have yet to address instances of vertical integration failure with respect to mycophagous arthropods.

The obligate powdery mildews (PM) (Erysiphales) are collectively considered one of the most important plant-pathogenic fungi in the world in terms of host range, crop damage, and management expense (Amano, 1986; Takamatsu, 2004; Glawe, 2008). These largely ectotrophic fungi can affect all aboveground portions of plants, leading to yield losses (Miller et al., 2003), decreased aesthetic value and plant death due to utilization of plant nutrients and the subsequent disruption of respiration and photosynthesis (Yorinori et al., 2004). Typical chemically based disease management programs have historically led to the resistance of PM to several important fungicides (Gubler et al., 1996; McGrath, 2001).

Biological control of PM may offer solutions to resistance and other fungicide-related problems such as residues in food crops, worker health and safety, and negative effects to nontarget organisms. Control of PM equivalent to that obtained through fungicide applications using microbial agents such as the spore-form-

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ing PM-antagonistic bacterium *Bacillus subtilis* (Ehrenberg) Cohn has been recorded in greenhouse trials (Chase, 2004). Other microbial agents, such as the related *B. pumilis* Meyer and Gottheil and the fungal hyperparasite *Ampelomyces quisqualis* Cesati may also be effective PM control options (Falk et al., 1995). Arthropods have been considered as consumers of PM in novel approaches to biological control of plant pathogens. For instance, tydeid mites (Acari: Tydeidae) associated with grape vines were observed to reduce severity of PM on wine grapes (English-Loeb et al., 1999). All members of the beetle tribe Halyziini (= Psylloborini) (Coleoptera: Coccinellidae) are obligate consumers of various PM conidia and hyphae in all mobile life stages (Gordon, 1985; Sutherland and Parrella, 2009a). The cosmopolitan genus *Psyllobora* is a representative of this tribe in natural and managed systems worldwide, and therefore may be utilized as a native biological control agent of PM (Cruz et al., 1989; Almeida and Milleo, 1998). Soylu and Yigit (2002) observed a measurable reduction in PM conidia on leaves occupied by *P. bisoctonotata* Mulsant larvae when compared to unexposed leaves in the laboratory, and Sutherland and Parrella (2006) quantified removal of visible PM by *P. vigintimaculata* (Say) using a linear modeling approach. The western North American native *P. vigintimaculata* is common in California ecosystems and has been recorded feeding on PM-infected agricultural crops such as cucurbits and wine grapes (Sutherland and Parrella, 2009b). It is reasonable to assume that *P. vigintimaculata* contributes to PM management through its feeding activity, and so therefore may serve as a biological control agent and as a component of overall IPM.

Grapes are the second most valuable agricultural commodity in California. According to the California Association of Winegrape Growers (MFK Research, 2007), farm receipts exceeded $2.1 billion in 2006. Additionally, this crop was indirectly responsible for $2 billion in tourist expenditures and $16.5 billion in wine sales. Grape PM, *Erysiphe necator* Schwein [= *Uncinula necator* (Schwein) Burrill], is the most serious and widespread grape pathogen in California, and considerable resources are spent to prevent and combat infection (Gubler and Hirschfelt, 1991). Applications of sulfur, an elemental fungicide, insecticide, and acaricide, are widely used for PM control in grapes. This use of sulfur makes wine grape vineyards the largest pesticide user by site for the state (California Department of Pesticide Regulation, 2008). Sulfur has been shown to have direct and indirect negative effects on local beneficial arthropods. High toxicity of sulfur residues to predatory mites (Acari: Phytoseiidae) has been documented in vineyards (Kreiter et al., 1998) and in strawberry fields (Coop and Croft, 1995), often resulting in a secondary pest outbreak of spider mites (Acari: Tetranychidae). Contact toxicity of sulfur to egg parasitoids (Hymenoptera: Mymaridae) was observed in wine grapes by Martinson et al. (2001). Strobilurins, strong natural antibiotics that inhibit fungal respiration, and demethylation inhibitors (DMI), also known as sterol biosynthesis inhibitors Class1 (SBI1), are also commonly used as effective commercial powdery mildew fungicides (California Department of Pesticide Regulation, 2008). There are questions regarding the effects these chemicals may have on natural enemies present in the crop as well. Investigation has shown a negative, though nonsignificant, effect on larvae of aphidophagous coccinellids after topical applications of strobilurins (Michaud, 2001), and DMIs were documented as toxic to phytoseiid mites on apple trees (Raudonis et al., 2004). Given that *P. vigintimaculata* is present feeding on the PM of grape vines, the question arises as to what effect fungicide applications may have on its survival and population density, and the resulting ecological service of its PM consumption.

The objectives of our study were first to determine the effects of direct contact of commonly used PM fungicides on the survival of *P. vigintimaculata* and then to document possible vertical integration failure in the fungicide-intensive agroecosystem of a California commercial vineyard. We hypothesized that topical applications of fungicides will increase mortality when compared to applications of water and that grape vines treated with these fungicides in the field will harbor lower densities of this native mycophage than will untreated grape vines.

2. Materials and methods

2.1. Laboratory bioassay

A colony of *P. vigintimaculata* was maintained using *Golovinomyces cichoracearum* (DC.) Heluta [= *Erysiphe chicoracearum* DC., see Belanger et al. (2002)], a generalist PM affecting Compositaceae and Cucurbitaceae, as a food source on either *Gerbera jamesonii* Adlam or *Zinnia elegans* Jacquin. The insect colony was held at 25 ± 5 °C under high-pressure sodium lighting (1000 W) in four insect cages containing discrete life stages of uniformly aged individuals. Each cage consisted of a wood frame (46 × 76 × 42 cm) enclosed with insect exclusion screening (No-Thrips, Green-Tek, Inc., Janesville, WI, 150 × 150 μm). Newly emerged adults, freshly laid eggs and actively foraging larvae of uniform age could be taken from this colony as needed. A vacuum aspirator constructed of a 30-ml plastic vial (DiluVial, Fisherbrand, Inc., Hampton, NH), vinyl tubing (180 PVC 3/16″, Nalgene, Rochester, NY) and a screen filter was used to remove adults. Second instar larvae were removed using a fine paintbrush (#20/0, Royal, White Plains, MD). Insects were stored for ~24 h in a refrigerator at 13 °C with excised PM-infected leaves and filter paper moistened with deionized water prior to treatment.

Individuals were placed in a petri dish (55 × 15 mm, Fisherbrand, Inc., Hampton, NH) arena containing moistened filter paper (55 mm, #1, Whatman International Ltd., Maidstone, UK) and were then sprayed directly with one of the fungicidal treatments while in these arenas (see below). After treatment each insect was immediately moved to a new petri dish arena and given an excised PM-infected *G. jamesonii* leaf disc (10 mm dia) as a food source. New leaf discs and deionized water (0.3 ml) were added at 24-h increments. Arenas were held in an incubator (Percival Scientific, 1-30 BL, Perry, IA) at 25 °C and 50% relative humidity under fluorescent lights with a 14-h photoperiod. The number of survivors was recorded 1 h after treatment and at every 24-h increment after treatment for 96 h for adults and until pupation for larvae. Pupae were kept in the incubator to assess emergence success.

In total, there were seven treatments (Table 1a). Five different fungicidal materials were applied: three commercial standard fungicides; trifloxystrobin (QoI-strobilurin:oximino-acetate; Compass 50WDG, Olympic Horticultural Products, Mainland, PA), piperalin (amine:piperidine; Pipron, SePro Corporation, Carmel, IN), and myclobutanil (DMI:triazole; Systhane WSP, Dow AgroSciences, Indianapolis, IN); elemental sulfur in the form of wettable powder (Safer Garden Sulfur, Saferbrand, Lititz, PA); and the microbial antagonist *B. subtilis* strain QST 713 (Rhapsody AS, Agraquest, Davis, CA). Treated insects were compared to control groups consisting of untreated and water-treated insects. A total of 22 adults and 15 second instar larvae of *P. vigintimaculata* were assigned to each treatment, with each individual representing an experimental replicate. An airbrush spray tower (3000S Airbrush Kit, Aztec, Inc., Rockford, IL) was used to administer 0.5 ml of fungicide solution directly to the dorsum of each individual. Deionized water (15 ml) was used to flush the spray apparatus between treatments. In order to minimize dilution and contamination, 0.5 ml of each treatment material was used to flush the sprayer prior to its initial application.

Survival of treated *P. vigintimaculata* individuals was analyzed using both the log-rank and Wilcoxon χ^2 Kaplan–Meier survival

Table 1
(a) Trade name, active ingredient, and concentration applied of topical fungicidal treatments tested on adults and larvae of *Psyllobora vigintimaculata* in order to gauge contact toxicity. (b) Trade name, active ingredient, and application rate of fungicidal treatments applied to a commercial vineyard in the presence of naturally occurring *P. vigintimaculata* in order to detect decreases in insect density and disruption of aggregative numerical response.

No.	Product	Active ingredient (A.I.)	Concentration (mg A.I. per liter)
(a)			
1	Untreated	N/A	N/A
2	Water	N/A	N/A
3	Rhapsody AS	*Bacillus subtilis* spores	135
4	Safer® garden Sulfur	Elemental sulfur	3800
5	Compass 050 WDG	Trifloxystrobin	70
6	Systhane WSP	Myclobutanil	116
7	Pipron	Piperalin	532
			Application rate, frequency (amount formulated product/volume water/application, frequency in days)
(b)			
1	Untreated	N/A	N/A
2	Water	N/A	N/A/10.2 L, 14 days
3	Actinovate AG	*Stretomyces lydicus*	7.2 g/12.1 L, 10 days
4	JMS Stylet Oil	White mineral oil	102 ml/10.2 L, 10 days
5	Kumulus DF	Elemental sulfur	16 g/10.2 L, 14 days
6	Thioben 90	Elemental sulfur	90%, 10 kg/ha, 7 days
7	Rally	Myclobutanil	2.0 g/10.2 L, 21 days
8	Abound	Azoxystrobin	10.5 ml/15.1 L, 14 days
9	Flint 50WG	Trifloxystrobin	1.0 g/10.2 L, 21 days
10	Quintec 2.08SC	Quinoxyfen	2.1 ml/10.2 L, 14 days

analyses (SAS Institute, Inc., 2005). Peto's log-rank test is generally the most appropriate method, but the Prentice-modified Wilcoxon test is more sensitive when the ratio of hazards is higher at early survival times than at late ones (Peto and Peto, 1972; Kalbfleisch and Prentice, 1980). Insects still alive at the end of observation were assigned a right censorship code since information on their survival beyond this censoring time was unavailable (Motulsky, 1995). Mean comparison for percent mortality and percent successful pupation was performed via Student's *t* tests utilizing the sequential Bonferroni Procedure for alpha manipulation, therefore reducing the probability of a Type I error (Holm, 1979; Morikawa et al., 1996).

2.2. Commercial vineyard sampling

A large commercial vineyard (Chardonnay) in Courtland, California (Grape Crush District 17) was the site of our study. Considerable work has been done here in the past examining the efficacy of various PM fungicides (see Janousek et al., 2006; Janousek and Gubler, 2008). Treatment materials (Table 1b) included wettable sulfur (Kumulus DF, MicroFlo Products, Memphis, TN), sulfur dust (Thioben 90, Martin Operating Partnership, LP, Kilgore, TX), strobilurins (azoxystrobin: Abound, Syngenta, Greensboro, NC; and trifloxystrobin: Flint 50WG, Bayer CropScience, Research Triangle Park, NC), a quinoline fungicide (quinoxyfen: Quintec 2.08SC, Dow AgroSciences), a DMI (myclobutanil: Rally, Dow AgroSciences), a mineral oil (Stylet-Oil, JMS Flower Farms, Inc., Vero Beach, FL), and the antagonistic bacterium *Streptomyces lydicus* strain WYEC108 (Actinovate AG, Natural Industries, Inc., Houston, TX). Treatments were applied via high pressure handgun application wands attached to 25-gallon (94.6 L) or 50-gallon (189 L) sprayers (Nifty Fifty, Oesco, Inc., Conway, MA). Total application volumes, frequencies and rates varied with treatment (Table 1b). Vines receiving fungicidal treatments were observed alongside and compared to vines treated with water and vines left untreated. Sampling began just prior to the projected release of PM ascospores, the primary spring inoculum, and the subsequent onset of disease in this region. In 2006, sampling began June 1, and in 2007 and 2008 sampling began in April (April 26 and April 16, respectively). Experimental units were 50 equally spaced groups of three (2006) or two (2007, 2008) adjacent vines. According to previous observations, adult *P. vigintimaculata* are attracted to yellow sticky cards used for insect monitoring (Sutherland and Parrella, unpublished data). Therefore, double-sided yellow sticky cards (10×16 cm) (Seabright Laboratories, Inc., Emeryville, CA) were hung from the vine cordon within the south-facing canopy and employed as a weekly measure of insect density, expressed as adult beetles per card (both sides). These traps were intended to catch adult *P. vigintimaculata* in flight as they responded to PM infection in the immediate locale. In order to measure concurrent fluctuations in disease severity, a sampling unit of five (2006) or three (2007, 2008) randomly selected fully-expanded leaves within the lower canopy were examined weekly until just prior to harvest (July 20, August 15, and July 31, for 2006, 2007, and 2008, respectively) for presence and severity of PM. Severity of PM infection was expressed as an ordinal severity index from zero (no infection visible: 0% infection) to five (entire leaf surface covered by visible PM hyphae: 100% infection) for each sampled leaf. Therefore, according to addition of the indices from each leaf in the sampling unit, the overall severity index ranged from 0 to 25 for each experimental unit in 2006, and from 0 to 15 in 2007 and 2008. This severity index was then expressed as a percentage to facilitate data pooling and analysis. A similar ordinal PM severity scale has been standardized and validated through correlation involving digital photography coupled with image analysis software and measurements of conidia density using a hemocytometer (Sutherland and Parrella, 2009b).

Adult *P. vigintimaculata* are known to exhibit a positive aggregative numerical response to increasing PM severity on many host plants, including wine grapes (Sutherland and Parrella, 2009b). Therefore, PM severity was generally expected to covary with insect density. In order to detect fungicide treatment effects on insect density, fungicide effects on PM severity and subsequent PM severity effects on insect density had to be taken into consideration. Accordingly, PM severity was treated as a continuous covariate for analysis of covariance (ANCOVA) (SAS Institute, Inc., 2005). Multiple measurements over time were simplified by designating experimental units as random variables nested within treatments, allowing for an unbalanced mixed model repeated measures analysis (Davis, 2002). Treatment effects were further elucidated when

considering individual data points by comparing slopes and *x*-intercepts from linear regressions of insect density by PM severity for different fungicidal materials (Zar, 1996). The overall effect of treatment on PM severity (fungicide efficacy) and the overall effect of PM severity on insect density (measure of numerical response) were determined through one-way ANOVA on mean responses of experimental units (Davis, 2002). Treatment means for PM severity and insect density were compared using the Tukey–Kramer highly significant difference (HSD) test for comparisons with all means (SAS Institute, Inc., 2005).

3. Results

3.1. Laboratory bioassay

Sulfur was toxic to *P. vigintimaculata* adults, resulting in 91% mortality within 1 h of treatment and 100% mortality within 24 h of treatment. There was no significant difference in response between the untreated and the water treated groups, so these treatments were pooled to form a control group containing 44 individuals. Survival analysis revealed that treatments of trifloxystrobin and myclobutanil produced greater mortality in adults than observed in the control, but less than that seen in the sulfur-treated group (Fig. 1a and Table 2). There were no differences between the control and *B. subtilis* or piperalin in terms of adult mortality.

All sulfur-treated larvae died within 1 h of treatment. All treatments except piperalin and the untreated control produced some mortality in larvae at the first observation event (Fig. 1b and Table 2). No significant differences in response were found between untreated and water treated larvae ($P = 0.035$ with $\alpha' = 0.01$), although 20% mortality was observed in the water treatment 1 h after topical application. Therefore, untreated and water treated larval groups were not combined in the analysis, and tested materials were compared to the water treatment when evaluating toxicity. Application of myclobutanil produced 80% total mortality, and was the only treatment, other than sulfur, which significantly impacted larval survival and development as compared to water treatment. Pupation commenced 96 h after treatment applications, and by 120 h all surviving individuals successfully pupated. There was no measurable effect of tested materials on emergence, since all insects pupating successfully emerged as adults in 5–8 days.

3.2. Commercial vineyard sampling

Considering all three sampling years, there were fewer adult *P. vigintimaculata* trapped weekly in the immediate vicinity of grapevines treated with fungicidal materials than in vines left untreated ($F_{9,\ 554} = 2.46$, $P = 0.014$) (Table 3). Average weekly insect density was highest in untreated vines (5.8 ± 0.4 adult *P. vigintimaculata*/sticky card/week) and lowest in vines treated with strobilurins (0.4 ± 0.9/card/week for trifloxystrobin and 0.5 ± 1.0/card/week for azoxystrobin). As expected, however, fungicide treatments had a significant effect on PM severity ($F_{9,\ 616} = 24.82$, $P < 0.0001$), exhibiting varying degrees of fungicidal efficacy. Trifloxystrobin, the mineral oil and quinoxyfen were the most effective suppressive materials, all maintaining PM severity at or below 15%, while sulfur dust, no treatment and water treatments were the least effective, all harboring mean PM severity above 30% (Table 3). As in previous observations (Sutherland and Parrella, 2009b), PM severity had a significant positive effect on insect density ($F_{1,\ 554} = 244.9$, $P < 0.0001$). Regarding the effect of fungicide treatment on insect density, more than twice the variation in insect density was explained by fungicide treatment when PM severity was included as a covariate in ANCOVA (R2258 = 0.41) as when fungicide was used as the only factor in a one-way ANOVA ($R^2 = 0.37$) (Table 4).

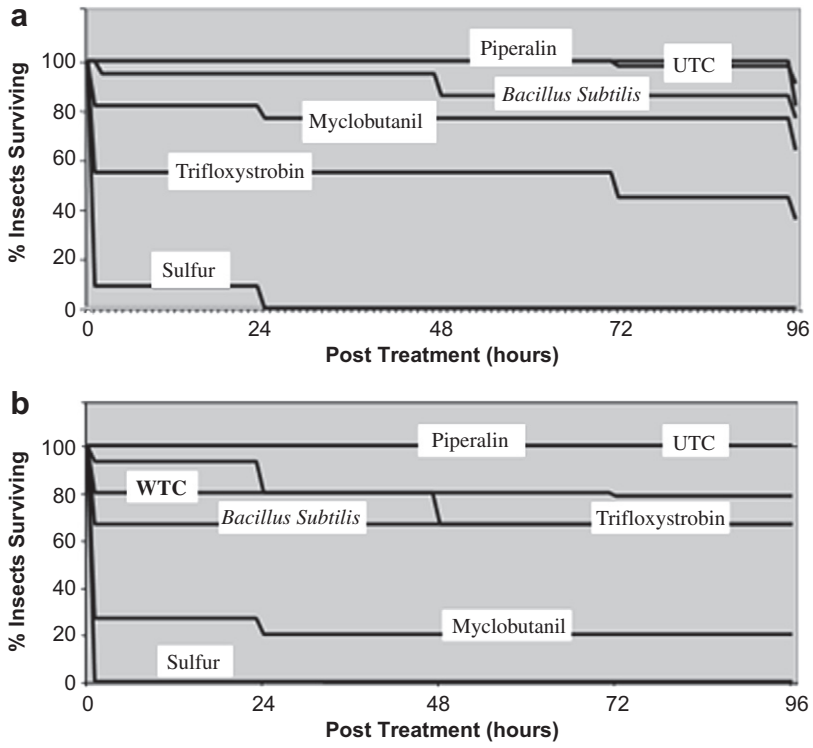

Fig. 1. (a) Survival analysis of *Psyllobora vigintimaculata* adults directly treated with selected fungicidal materials (UTC = untreated control). Insects were observed for 96 h. Log-rank $\chi^2 = 111.05$, $p < 0.0001$; Wilcoxon $\chi^2 = 108.69$, $p < 0.0001$, df = 5, $n = 154$. (b) Survival analysis of *Psyllobora vigintimaculata* second instar larvae directly treated with selected fungicidal materials (UTC = untreated control, WTC = water treated control). Insects were observed for 120 h. Pupation began at 96 h post treatment. All pupating insects emerged successfully. Log-rank $\chi^2 = 63.93$, $p < 0.0001$; Wilcoxon $\chi^2 = 63.35$, $p < 0.0001$, df = 6, $n = 105$.

Table 2
Survival of adults and second instar larvae of *Psyllobora vigintimaculata* after direct contact (topical application in laboratory) with selected fungicidal materials. Means in each column followed by the same letter are not significantly different according to comparisons for each pair of means using Student's t and Bonferroni α adjustment procedure so that $\alpha' = \alpha/n - 1$ where n is the number of comparisons and the desired α is 0.05.

Treatment	Mean (± SEM) duration of adult survival[a] (h)	Mean (± SEM) duration of larval survival[b] (h)
Untreated control	95.5 ± 0.5a	120.0 ± 0.0a
Water treated control	–	93.0 ± 13ab
Bacillus subtilis	88.4 ± 4.3ab	80.3 ± 15b
Elemental sulfur	3.1 ± 1.4d	1.0 ± 0.0c
Myclobutanil	75.5 ± 8.3b	26.3 ± 13c
Trifloxystrobin	50.6 ± 10c	89.7 ± 12ab
Piperalin	96.0 ± 0.0a	120.0 ± 0.0a
t-distribution value	2.61	2.69
α'	0.01	0.0083

[a] Adults were given fresh water and food daily, and observed for 96 h total.
[b] Larvae were given fresh water and food daily, and observed for 120 h total.

Table 3
Mean powdery mildew (PM) severity and mean density of mycophagous coccinellid *Psyllobora vigintimaculata* in a commercial vineyard following applications of selected fungicidal materials. Means in each column followed by the same letter are not significantly different according to analysis of covariance on response means from each experimental unit and the Tukey–Kramer HSD test.

Treatment	Average PM severity (mean ± SEM, as % infection)	Average insect density (mean ± SEM, as No. of adults/week/card)
Untreated	39.1 ± 1.3e	5.4 ± 0.5a
Water treated	31.3 ± 3.2de	1.8 ± 1.0ab
Streptomyces lydicus	24.2 ± 2.6bcd	2.5 ± 0.9ab
Mineral oil	10.8 ± 3.9ab	0.6 ± 1.4ab
Wettable sulfur	29.3 ± 3.9cde	2.4 ± 1.3ab
Sulfur dust	51.8 ± 3.0f	0.9 ± 1.2b
Myclobutanil	24.9 ± 2.8bcd	0.9 ± 0.9b
Azoxystrobin	20.6 ± 3.2abcd	0.6 ± 1.1b
Trifloxystrobin	10.7 ± 2.8a	0.4 ± 1.1ab
Quinoxyfen	15.3 ± 2.8abc	0.6 ± 1.0ab

When comparing the bivariate relationship between PM severity and insect density among treatments, fungicide applications generally resulted in lower slopes and higher x-intercepts (Fig. 2 and Table 5). In addition, the correlative nature of the relationship was generally much weaker when fungicidal materials were present.

4. Discussion

Trifloxystrobin, a strobilurin fungicide, was toxic to both larvae and adults of *P. vigintimaculata* when applied topically in the laboratory. Furthermore, density of this mycophagous insect was depressed in the vineyard following applications of the strobilurin fungicides azoxystrobin and trifloxystrobin despite the presence of PM. Disruption of biological control due to strobilurin applications has been documented several times when using antagonistic fungi as biological control agents (Fravel et al., 2005; Tobin et al., 2008; Bruck, 2009). This disruption is somewhat foreseeable since strobilurin fungicides disrupt fungal respiration in general (Clough and Godfrey, 1998), and so should be expected to be toxic to fungal plant pathogens and beneficial fungal antagonists alike. Strobilurin toxicity to arthropods, however, has not been specifically addressed, though Michaud (2001) did measure some larval mortality when treating *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) with azoxystrobin.

Topical laboratory applications of two closely related fungicidal materials, myclobutanil and piperalin, had very different effects on *P. vigintimaculata*. Application of myclobutanil (DMI, SBI1) significantly increased mortality of adults and larvae, while no differences were observed between individuals treated with piperalin (Amine, SBI2) and individuals left untreated or treated with deionized water (Fig. 1). The active ingredients of both materials act on sterol biosynthesis in fungi, albeit different target sites, and therefore might be expected to elicit similar biological responses. It is possible, however, that differences in the fungicides' specific formulations were responsible for these observed differences in toxicity. Myclobutanil applications in the vineyard resulted in significantly lower densities of *P. vigintimaculata* as compared to no treatment at all. Since piperalin was not included in the vineyard efficacy trial, its potential compatibility with mycophagous coccinellids in the field could not be evaluated.

Sulfur was clearly toxic to this insect when directly applied to either adults or larvae. The sulfur used in these laboratory experiments was formulated for ease of application and consisted of wettable powder pre-emulsified in a concentrated solution. Many commercial greenhouse operations utilize elemental sulfur as a

Table 4
Statistical comparison of ANOVA and ANCOVA with respect to the effect of fungicidal material treatments on density of native mycophage *Psyllobora vigintimaculata*, measured weekly as the number of adults caught on a yellow sticky card in the immediate vicinity of treated vines, with each experimental unit designated as a random variable nested within treatment. Powdery mildew (PM) severity, expressed as % infection, is treated as a continuous covariate in ANCOVA.

Source	df	Sum of squares	Mean square	F ratio	Prob > F
ANOVA (insect density = treatment; insect density is directly affected by fungicide treatment)					
Model	96	7877.52	82.058	3.483	<0.0001
Error	571	13453.79	23.562		
Total	667	21331.31			
Unit (treatment)	87	4070.17	46.784	1.986	<0.0001
Treatment	9	2669.35	296.594	6.991	<0.0001
		$R^2 = 0.369$			
*ANCOVA (insect density = treatment, PM severity, treatment * PM severity; insect density is affected by fungicide treatment, considering effect of treatment on PM severity)*					
Model	97	11683.329	120.447	7.180	<0.0001
Error	554	9294.088	16.776		
Total	651	20977.417			
Unit (treatment)	87	3581.66	41.169	2.454	<0.0001
treatment	9	790.828	87.870	2.457	0.0138
PM severity	1	4108.82	4108.82	244.918	<0.0001
		$R^2 = 0.557$			

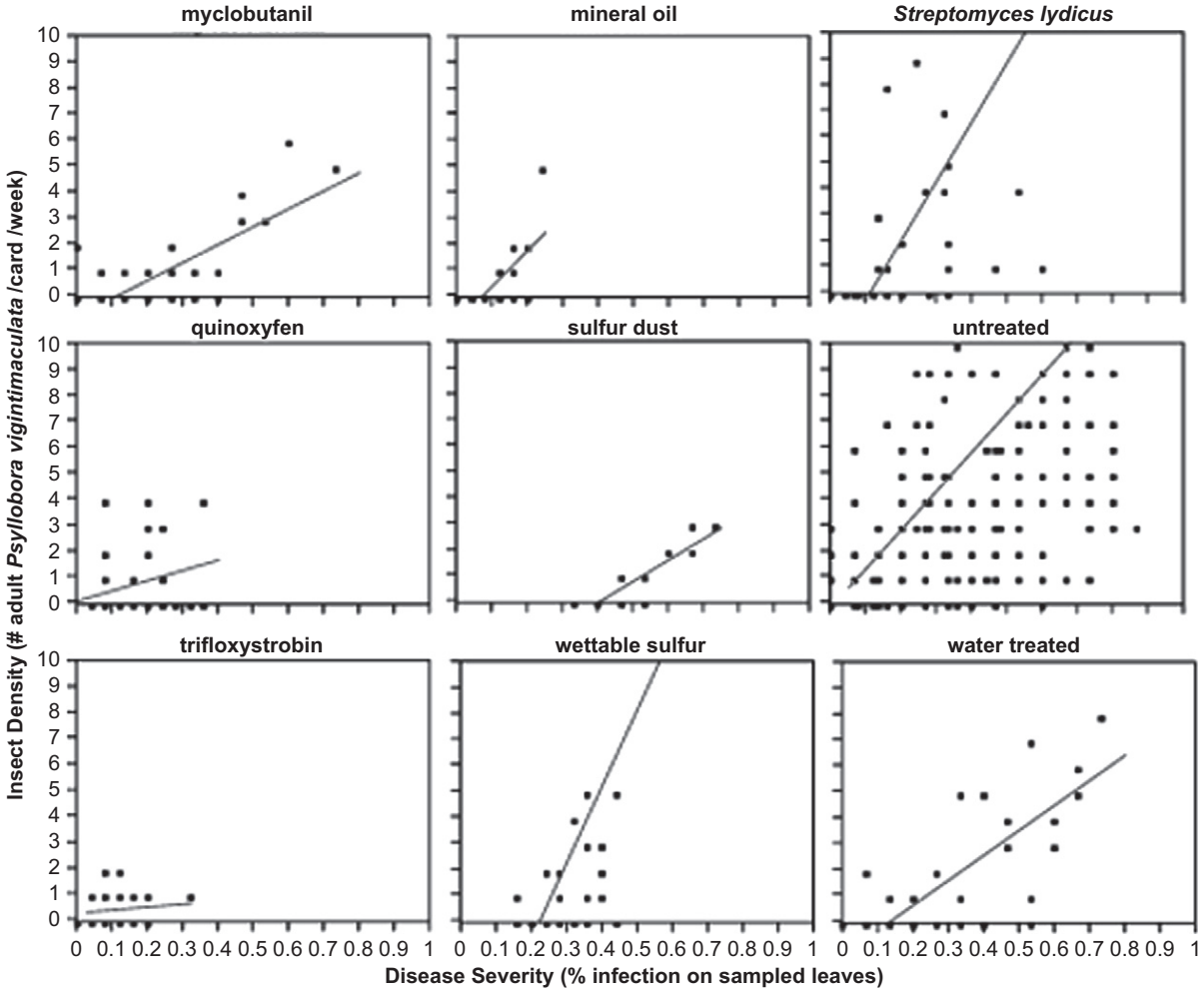

Fig. 2. Nine different fungicidal treatments are graphically compared in terms of the bivariate relationship between powdery mildew (PM) disease severity, as expressed in % infection, and density of the mycophagous beetle *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae) in the immediate locale. Treatments are grouped into columns based on broad fungicidal class and expected toxicity to the insect; inorganic chemical fungicides in column one, organic/elemental fungicidal materials in column two, and treatments expected to have no toxic effect in column three. Application of azoxystrobin resulted in a relationship (R^2 = 0.23, slope = 2.6, *x*-intercept = 0.0034, n = 41) similar to that of another strobilurin, trifloxystrobin (R^2 = 0.01, slope = 1.0, *x*-intercept = −0.303, n = 55), and therefore is not shown for ease of presentation.

Table 5
Comparison of the bivariate linear relationship between powdery mildew (PM) severity, expressed as % infection, and density of the mycophagous coccinellid *Psyllobora vigintimaculata*, expressed as number of insects locally trapped per week, for different fungicide treatments in a commercial vineyard. Values correspond to the analysis of the effect of PM severity on insect density (insect density by PM severity; *y* by *x*) (refer to Fig. 2).

Treatment	n	R^2	Slope	*x*-intercept
Untreated	307	0.266	14.92	0.011
Water treated	48	0.510	9.67	0.137
Streptomyces lydicus	37	0.306	22.95	0.115
Mineral oil	20	0.454	13.90	0.074
Wettable sulfur	27	0.398	29.42	0.224
Sulfur dust	15	0.783	8.26	0.409
Myclobutanil	52	0.603	6.97	0.119
Azoxystrobin	41	0.235	2.60	0.003
Trifloxystrobin	55	0.007	0.97	−0.303
Quinoxyfen	50	0.075	4.05	−0.010

powder that is volatilized through burning. This may produce a different concentration and settling pattern than observed with sprayed materials. Whether volatilization would increase or decrease sulfur's topical toxicity to *P. vigintimaculata* is unknown. Sulfur dust applications in the vineyard resulted in significantly fewer *P. vigintimaculata* adults caught locally during flight, indicating possible toxic effects in the field. The bivariate relationship of PM severity to insect density in sulfur treatments, however, suggests more of a repellent effect. The slope of the relationship is positive and quite high, but the *x*-intercepts, an estimate of the PM severity level at which the insects are initially encountered, are much higher than in other treatments (22.4% infection for wettable sulfur and 40.9% infection for sulfur dust). Thus, *P. vigintimaculata* may not visit grape vines treated with sulfur until PM is already quite severe. This could result from either direct repellency of the sulfur material or its masking of olfactory cues used by adult beetles to locate PM infection.

Quinoxyfen and mineral oil were both highly effective PM control materials during the three years of observation (mean PM severity 15.3% and 10.8%, respectively), and predictably harbored low mean densities of *P. vigintimaculata* (0.6/card/week and 0.6/card/week, respectively). A key difference, however, lies in the slope and correlative degree of the linear relationship between PM severity and insect density (see Fig. 2). Applications of mineral oil resulted in a more strongly correlated relationship (R^2 = 0.45) and a higher slope (13.9) than did applications of quinoxyfen (R^2 = 0.07, slope = 4.05). This suggests that the chemical fungicide quinoxyfen disrupts the numerical response of this coccinellid to a much larger degree than mineral oil, even though both are quite effective as fungicidal materials.

The bacterial antagonists included in this investigation exhibited no measurable toxic effect on *P. vigintimaculata*. Topical applications of *B. subtilis* were not significantly different in terms of adult mortality as compared to no treatment or in terms of larval mortality as compared to treatment with deionized water (Fig. 1). In the commercial vineyard, applications of *S. lydicus* did not significantly reduce the density of locally caught adults, but did significantly reduce PM severity as compared to untreated vines. The relationship between PM severity and insect density on vines treated with *S. lydicus* remained strongly positive ($R^2 = 0.31$, slope = 23.0), suggesting that any decrease in adults was largely due to a decrease in local PM rather than to insect-detrimental effects of the applied material.

Larvae treated directly with deionized water exhibited 20% mortality 1 h after application while adults were unaffected by the same treatment. This may be a result of the small size (~2 mm) and soft-bodied nature of second instar *P. vigintimaculata* larvae. Michaud (2001) recorded negative effects of several fungicides on coccinellid larvae, but none on adults. This apparent "drowning" effect, where gas-exchange and mobility of soft-bodied larvae is impaired due to a film of water, may have been illustrated in the vineyard in this study: mean insect density (as adults/card/week) was less when near water treated vines as compared to when near untreated vines. This difference may reflect a reduction in local larvae successfully emerging as adults and emigrating away from those water treated vines. Rainfall is not common during the typical grape growing season in California.

Harvest quality of wine grapes and the eventual quality of the wine rendered from them are, needless to say, of utmost importance to the high-value industry and have been negatively affected by PM infection in viticultural systems around the world (Pool et al., 1984; Gadoury et al., 2001; Calonnec et al., 2004; Stummer et al., 2005). There is some debate, however, over how severe PM infection must be before wine quality is affected. In France, Calonnec et al. (2004) determined that perceived quality of wines made from cultivars Cabernet Sauvignon and Sauvignon Blanc was not affected until greater than 25% and 50% of grape berries, respectively, were infected. This differs considerably with an Australian study (Stummer et al., 2005), in which total soluble solids were standardized, where negative compositional and sensory effects were detected in Chardonnay wine containing as little as 1–5% infected berries. Wineries in New York state have long adhered to a standard that purchased fruit should not contain more than 3% (by weight) infected berries (Pool et al., 1984). It is even more unclear how leaf infection correlates with berry cluster infection. This relationship has been measured and described for Rosette (*Vitis* interspecific hybrid) grapevines (Pool et al., 1984) and for Concord grapes, *Vitis labruscana*, (Gadoury et al., 2001), but not for cultivars of *Vitis vinifera* such as Chardonnay. Data from these studies suggest that leaf infection at harvest time could be as high as 20.4% in Rosette grapevines (Pool et al., 1984) and 34.9% in Concord grapevines (Gadoury et al., 2001) before the New York state quality standard of 3% infected berries is reached. In our vineyard observations, applications of several fungicidal materials; trifloxystrobin, mineral oil, quinoxyfen, and azoxystrobin, resulted in mean PM severity (as percentage leaf infection) at or below 20%. These most effective treatment materials also harbored low mean densities of *P. vigintimaculata*, but were statistically similar to no treatment at all in terms of insect density, when accounting for the covariate PM severity (Table 3). This is in contrast to observations of plants treated with sulfur or Myclobutanil where, due to factors other than decreased PM severity (possibly lethal and/or sub-lethal effects of the fungicidal materials themselves), insect density was significantly lower than on untreated plants.

In reduced risk pest and disease management there is an emphasis on the retention and conservation of native and established beneficial arthropods. Also, successful incorporation of biological control into IPM programs will increasingly involve combinations of natural enemies and compatible pesticides (Hattingh and Tate, 1995). If *P. vigintimaculata* can be viewed as a valuable PM antagonist, then following the principles of IPM for disease management would mandate true integration of chemical tactics for PM management with various life stages of the native *P. vigintimaculata*. Our data suggest that topical applications of trifloxystrobin, myclobutanil, and sulfur are toxic to *P. vigintimaculata*, while piperalin has no observable effect. Furthermore, field applications of myclobutanil and sulfur, in the fungicide-intensive agroecosystem of a commercial vineyard, significantly decreased the local density of *P. vigintimaculata* and disrupted its response to increasing PM severity. Sub-lethal effects of fungicide application on *P. vigintimaculata*, such as decreases in fecundity or longevity of adults, or changes in development time, were not measured in this study. If the mycophagous coccinellid *P. vigintimaculata* contributes a measurable ecological service in the form of PM removal, and if the system is treated with certain fungicides, then it is possible that lethal and/or sub-lethal effects may mask the perceived benefits imparted.

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