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ORIGINAL PAPER

The effect of sulfur on biological control of the grape leafhopper, *Erythroneura elegantula*, by the egg parasitoid *Anagrus erythroneurae*

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Abstract We examined the toxicity of a fungicide, sulfur, to the egg parasitoid *Anagrus erythroneurae* (Hymenoptera: Mymaridae) Trjapitsyn and Chiapini and the vineyard leafhopper pest *Erythroneura elegantula* Osborn (Homoptera: Cicadellidae) and tested whether or not the use of sulfur in the field affects biological control of *E. elegantula*. Using field cage bioassays, we demonstrated that sulfur is toxic to adult *A. erythroneurae* parasitoids, but not toxic to adult *E. elegantula* leafhoppers. We nonetheless found in a field experiment that sulfur produced no changes in rates of parasitism or *E. elegantula* egg density, and generated only a very small increase in the density of *E. elegantula* nymphs. These results suggest that sulfur, although toxic to *A. erythroneurae*, is not highly disruptive of *E. elegantula* biological control in vineyards. Our results suggest that simple bioassays of acute toxicity may not accurately predict the impact of agricultural chemicals on biological control.

Keywords *Anagrus erythroneurae* · Biocontrol · *Erythroneura elegantula* · Egg parasitoid · Homoptera: Cicadellidae · Hymenoptera: Mymaridae · Natural enemy · Sulfur · *Uncinula necator*

Introduction

It has been a common practice since the 1960s to evaluate the effects of agricultural chemicals on arthropod natural enemies (Croft 1990). Most studies that evaluate the influence of pesticides on beneficial insects are conducted in the laboratory; laboratory based studies make up approximately two-thirds of studies in the SELCTV database, a compilation of studies documenting the impact of pesticides on non-target arthropods (Theiling and Croft 1988). Over 95% of the studies in the SELCTV database simply measure natural enemy mortality (Theiling and Croft 1988), with the goal of understanding how a chemical will effect a beneficial arthropod

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population, and ultimately biological control of a pest arthropod population. This widely accepted approach is based on individual-level effects, rather than population-level effects, and often does not consider the interacting influences of other variables, such as arthropod life stage, temperature, relative humidity, or pesticide persistence, all of which may influence how severely a pesticide interferes with biological control in the field (Robertson and Worner 1990). Additionally, tests that evaluate mortality do not measure the sub-lethal effects of pesticide exposure, such as reduced foraging ability or reproduction (Rosenheim and Hoy 1988; Elzen 1989). Simple laboratory bioassays, although easy to conduct, may therefore underestimate or overestimate the actual impact of a pesticide on a field population of a natural enemy (Campbell et al. 1991; Penman et al. 1986; Robertson and Haverty 1981), and extrapolating laboratory bioassay results to predict how a chemical will influence insect population dynamics in the field may be difficult. Recently, researchers have begun to develop population demographic approaches to the measurement of pesticide impacts on biological control agents, but the application of this methodology is still in the early stages (Stark and Banks 2003).

In this paper, we address the question: are bioassays of acute toxicity accurate predictors of population level effects of a pesticide, sulfur, on a biological control agent, *Anagrus erythroneurae* Trjapitsyn and Chiapini (Homoptera: Cicadellidae). *A. erythroneurae* is a key egg parasitoid of the western grape leafhopper (*Erythroneura elegantula* Osborn, Homoptera: Cicadellidae) in vineyards, and has been intensively studied for conservation biological control. The grape leafhopper feeds on leaf cell contents, which reduces vine vigor and can cause major economic losses (Flaherty et al. 1992; Murphy et al. 1996). *A. erythroneurae* are abundant in California vineyards (Flaherty et al. 1992), and high rates of parasitism are often observed in vineyards by mid season (Doutt and Nakata 1973). Since high rates of parasitism are observed and both the pest and parasitoid have likely coevolved on wild *Vitis* sp., one might expect this system to be a model for successful biological control. However, leafhoppers can still be significant pests (Costello and Daane 1998).

Extensive research on the erratic and often incomplete biological control of *E. elegantula* by *A. erythroneurae* has focused on the hypothesis that *A. erythroneurae* may fail to exert consistent control because it needs an alternate host in order to overwinter successfully (Doutt and Nakata 1965, 1973; Kido et al. 1984; Corbett and Rosenheim 1996a; Murphy et al. 1996, 1998). The grape leafhopper overwinters in the adult stage, whereas the parasitoid overwinters as an immature within its host's egg. This requires that *A. erythroneurae* utilize alternate leafhopper hosts during the winter. When refuges that contain alternate hosts, such as blackberry leafhoppers or prune leafhoppers, are near vineyards, more effective biological control of the grape leafhopper is often observed (Doutt and Nakata 1965, 1973; Kido et al. 1984; Murphy et al. 1996). However, when entomologists have tried to manipulate ecosystems to provide *A. erythroneurae* with an overwintering refuge and thus enhance biological control, their efforts have met with little success (Flaherty et al. 1985, 1992). A considerable amount of work has also focused on the idea that *Anagrus* spp. need nectar resources in order to be more effective biological control agents. Nevertheless, the planting of flowering cover crops in vineyards has not consistently resulted in lower leafhopper densities (Costello and Daane 1998; Nichols et al. 2001; English-Loeb et al. 2003).

Recently, Martinson et al. (2001) have suggested an alternative hypothesis for the failure of biological control in this system. They showed in laboratory bioassays that sulfur, a fungicide commonly used to control the fungal pathogen powdery mildew (*Uncinula necator)*, was persistently toxic to *Anagrus* spp. Sulfur was also found to inhibit the emergence of adult *Anagrus atomus* parasitoids (Williams and Gill 1996). Nearly every grape grower in California uses some formulation of sulfur to control powdery mildew, because sulfur is both inexpensive and effective. Powdery mildew has not shown resistance to sulfur, whereas it has developed resistance to many alternative fungicides (Delye et al. 1997). The nearly universal use of sulfur has left entomologists with little opportunity to compare biological control of leafhoppers in sulfur-treated versus non-sulfur-treated vineyards. Despite the fact that sulfur has been shown to disrupt biological control of other key arthropod pests, perhaps most notably control of *Tetranychus* spp. spider mites by predatory mites in the family Phytoseiidae (Hanna et al. 1997; James et al. 2002; Prischmann et al. 2005; Teodoro et al. 2005), the idea that sulfur is inhibiting the ability of *Anagrus* spp. to control leafhoppers has received little consideration.

Here we explore the influence of sulfur on biological control of *E. elegantula* by *A. erythroneurae* parasitoids. We begin by reporting field-cage bioassays to evaluate the effects of sulfur residues on the survival of *A. erythroneurae* parasitoids and their grape leafhopper hosts. Then, we report a manipulative field experiment, in which we quantified the effects of sulfur and an alternative fungicide (trifloxystrobin) on parasitism of grape leafhopper eggs by *A. erythroneurae* and the resultant densities of leafhopper eggs and nymphs over the growing season.

Materials and methods

Anagrus erythroneurae field cage bioassays

We conducted field cage bioassays to quantify the toxicity of two formulations of sulfur and an alternative fungicide, trifloxystrobin, to *A. erythroneurae*. Bioassays were conducted August–September, 2004 in Davis, CA, USA in a mixed variety vineyard that had not been treated with any pesticides for the previous 2 years. Parasitoids used in the bioassays were reared in the laboratory from leaves collected in an untreated vineyard. Emerging wasps of both sexes were collected between 07:00–09:00 h and then taken immediately to the field, where they were transferred into the experimental bioassay units. The bioassay unit was a clear, plastic cage (~6 cm × 4.5 cm × 2 cm with mesh panels) glued (Elmer's Washable School Glue Gel™) to a single treated grape leaf; each cage was provided with a 0.2 cm × 1 cm strip of filter paper saturated with a mixture of three parts honey and one part water. This cage design produces a relatively low exposure of parasitoids to sulfur residues, because only the floor of the cage (the leaf surface) is treated with a fungicide. Five parasitoids were introduced to each cage. The cages were shaded with a large piece of white paper, which was folded loosely around the entire apparatus.

Leaves were randomly assigned to one of four treatments, each replicated five to seven times: (1) water (control), (2) sulfur dust (80% sulfur, Wilbur Ellis), (3) wettable sulfur (1.08 kg/ ha, Thiolux®, 80% sulfur, Novartis), and (4) trifloxystrobin

(104 g/ha, Flint™; 50% trifloxystrobin, Bayer Crop Sciences, Research triangle park, NC, USA). Sulfur dust was applied with a flour sifter to individual leaves, which were then agitated to leave a thin dusting of sulfur that is typical for commercial applications. Water, wettable sulfur, and trifloxystrobin treatments were applied to runoff with a hand held spray bottle. Bioassays were conducted 1 day after spray treatments were applied. Individual vines were used as blocks: one replicate of each treatment was set up within each vine.

Bioassays were initiated between 09:00 and 11:00 h. All cages were removed 4.4–6.4 h later, returned to the laboratory, and each wasp's status was scored as dead, alive, or missing. We then calculated the proportion of live wasps for each cage. Replicates in which more than half of the wasps escaped from the cages were excluded from the data set. Wasps that were missing or stuck in honey strips were also excluded from the analysis. After excluding five replicate cages in which ≥3 wasps were missing, we retained 23 replicate cages, including: control (5), trifloxystrobin (5), sulfur dust (7), and wettable sulfur (6). In total, 97 individual parasitoids were tested, including: control (22), trifloxystrobin (22), sulfur dust (29), and wettable sulfur (24). Because the duration of the bioassays was variable, we included 'exposure duration' as a factor in our statistical model. We used ANCOVA to examine how wasp survival was influenced by treatment (main effect) and 'exposure duration' (covariate), (JMP Version 4.0.2, SAS Institute 2000). We used an arcsine transformation to satisfy the assumption of normality. Planned contrasts were employed with the sequential Bonferroni method to correct the critical *P*-value for multiple comparisons (Rice 1989).

Erythroneura elegantula field cage bioassays

We tested the toxicity of wettable sulfur and sulfur dust to *E. elegantula* in the field. Protocols were identical to those described above for *A. erythroneurae*, except as noted here. Bioassays were conducted from September 10–28, 2004 in an untreated vineyard in Davis, CA, USA. Adult leafhoppers of unknown sex and age were collected in an untreated vineyard with a sweep net and transferred into bioassay cages. Each cage contained five leafhoppers. The bioassay cages were identical to those used in the *Anagrus* bioassays, with the exception that they were not provisioned with honey. Each treatment was replicated five or six times. Bioassay cages were monitored every other day for 2 weeks to score leafhoppers as dead, alive, or missing. In some cages, ants were observed invading and consuming leafhoppers. When this happened, the leafhoppers were scored as 'missing'. In some cases the glue used to affix the cages to the leaves deteriorated and leafhoppers escaped. In all cases, however, the treated leaf surface that formed the bottom of each cage appeared healthy throughout the experiment. The influence of treatment on leafhopper survival was examined using repeated measures MANOVA performed in JMP Version 4.0.2 (SAS Institute 2000).

Influence of fungicide residues on biological control of *E. elegantula*

A field experiment was conducted May—August 2004 to quantify the influences of fungicide residues on *A. erythroneurae* parasitism of leafhopper eggs and subsequent densities of leafhopper eggs and nymphs. We utilized a randomized complete block

design in a 2.8 ha Chardonnay vineyard in Davis, CA, USA. This vineyard had not been treated with any fungicides, herbicides or pesticides during 2003 or 2004.

The experimental unit was a 0.05 ha plot of grapevines; each plot was seven rows wide by 14 vines long. Treatments, each replicated 10 times, included: (1) no spray (control); (2) wettable sulfur; and (3) trifloxystrobin. Fungicides were applied with a tractor-mounted sprayer, following normal commercial practices. To address the possibility that the control plots might develop heavy infestations of powdery mildew, which could affect vine quality and leafhopper population dynamics, trifloxystrobin was chosen as an additional 'control' treatment in which mildew populations would be suppressed. Sulfur treatments were applied on 4/23, 5/11, 5/26, 6/23, and 7/14, and trifloxystrobin treatments were applied on 4/13, 5/5, 5/24, 6/21, and 7/9, consistent with standard commercial practices.

We evaluated the effect of fungicide residues on biological control of the grape leafhopper using four response variables: current leafhopper egg parasitism, cumulative leafhopper egg parasitism (explained below), leafhopper egg density, and leafhopper nymph density. Response variables were measured every 2 weeks from 29 April to 11 August. Ten leaves were sampled from the middle of each of the 30 plots every sampling period. Leaves were taken from the center of the plot to avoid rows where fungicide treatments may have drifted from adjacent plots. Leafhopper nymphs were counted on each of the leaves in the field. Leaves were then taken to the laboratory and examined under a microscope with transmitted light, and both parasitized and unparasitized leafhopper eggs were counted. Leafhopper eggs that had been parasitized appeared as either orange, reddish, or clear with a white globule inside the egg, whereas unparasitized leafhopper eggs appeared completely clear (Kido et al. 1984; Settle and Wilson 1990). This gave us a measure of current leafhopper parasitism. Leaves also provided a cumulative record of past parasitism of grape leafhopper eggs: leafhopper eggs from which first-instar leafhoppers have successfully emerged can be recognized by a small slit in the leaf tissue located near one egg of the leaf, whereas eggs from which *A. erythroneurae* have emerged can be diagnosed by the perfectly round emergence hole produced by the parasitoid (Murphy et al. 1998). The ratio of parasitoids emerged to leafhoppers + parasitoids emerged gave us an estimate of the cumulative parasitism for each plot. Leaf areas were measured with a portable leaf area-meter (LICOR model LI-3000). We used total leafhopper eggs per cm^2 of leaf tissue as another measure of leafhopper biological control. All data were recorded separately for each leaf and the values for the ten leaves per plot were averaged to obtain a single value for each plot on each sampling date. We used repeated-measures Multivariate Analysis of Covariance (MANCOVA) to compare treatments across the growing season. Planned contrasts were conducted, and the sequential Bonferroni correction was used to adjust *P*-values for multiple comparisons (Rice 1989). Because we were primarily interested in the seasonal means for each of our response variables, we tested these seasonal means for normality. The assumption of normality was satisfied in all cases, so we did not transform the data. Because some of the vines began to decline for an unknown reason during the spring before our experiment was conducted, we also tested whether plot vigor, defined as the number of live vines present in the plot, was a significant explanatory variable for any of our response variables, and we included plot vigor as a covariate in our main analysis to provide statistical control for this source of variation. We included ''block'' as a covariate in our analyses. Means are presented ± 1 SE throughout the text.

Results

Anagrus erythroneurae bioassays

All fungicide treatments produced significant decreases in *A. erythroneurae* survival relative to controls in our short-term field bioassays ($F_{3,22} = 9.37$, $P = 0.0005$, Fig. 1). 'Exposure duration' (i.e., the length of time for which the assay was run, which ranged between 4.4 and 6.4 h) was not significant ($F_{1,22} = 0.001$, $P = 0.96$), so was excluded from the model. Paired contrasts revealed that the fungicide treatments trifloxystrobin and sulfur dust were significantly different from each other ($F_{1,11} = 5.65$, $P = 0.03$), but neither trifloxystrobin nor sulfur dust was significantly different from wettable sulfur (trifloxystrobin and wettable sulfur, $F_{1,10} = 0.90$, $P = 0.36$, sulfur dust and wettable sulfur, $F_{1,12} = 2.39$, $P = 0.15$).

Erythroneura elegantula bioassays

In contrast to the strong acute toxicity of sulfur to *A. erythroneurae* parasitoids, there was no effect of either sulfur dust or wettable sulfur on the longevity of adult grape leafhoppers (repeated measures MANOVA, $F_{1,4} = 0.22$, $P = 0.66$; Fig. 2). Survivorship through day 9 in both fungicide treatments and the control still exceeded 75%. By this date, ants had invaded many cages, and leafhoppers in invaded cages disappeared. Cages in the control treatment were more severely impacted by ants than were cages in either of the sulfur treatments, suggesting that sulfur may repel ants (e.g., Nowbahari and Thibout 1992).

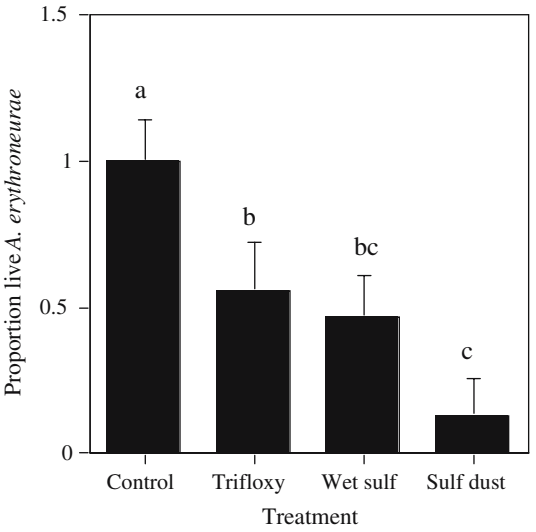

Fig. 1 Survival of *Anagrus erythroneurae* exposed to fungicide treated leaves in short term field bioassays. All fungicide treatments produced significant decreases in parasitoid survival, compared to the control. Pairwise comparisons between the other treatments revealed that trifloxystrobin and sulfur dust were significantly different from each other, but neither trifloxystrobin nor sulfur dust was significantly different from wettable sulfur. Differing letters above columns indicate significant differences between treatments (ANOVA, $P = 0.05$). Abbreviations are as follows: Sulf dust = Sulfur dust, Wet sulf = Wettable sulfur, and Trifloxy = Trifloxystrobin

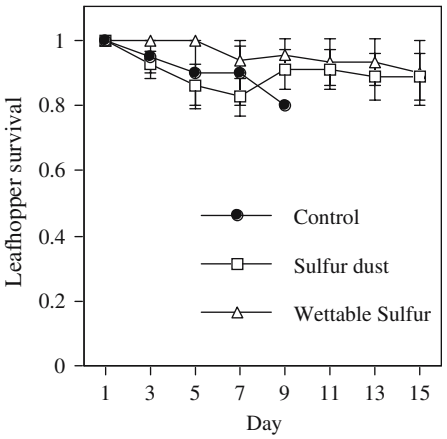

Fig. 2 Survival of *Erythroneura elegantula* exposed to fungicide treated leaves for 15 days. Survivorship values appear to increase for both sulfur treatments, because cages with some dead leafhoppers were removed when ants invaded, thus increasing the overall survivorship at day 9. The number of cages remaining in the field at days 1, 3, 5, 7, 9, 11, 13, and 15 were: 5, 3, 2, 2, 1, 0, 0, and 0 for the control; 6, 6, 6, 6, 5, 5, 4, and 4 for sulfur dust; and 5, 5, 5, 5, 5, 3, 3,and 2 for wettable sulfur

Treated vineyard plots

We did not detect powdery mildew in our experimental plots at any time during the experiment, eliminating the possibility that our fungicide treatments might have changed host plant quality for leafhoppers by producing differential prevalence of mildew infection.

Perhaps surprisingly, our field experiment produced very little evidence that sulfur disrupted biological control of grape leafhoppers by *A. erythroneurae*. After

Table 1 (**a**) Current proportion of leafhopper eggs parasitized, (**b**) Cumulative proportion of leafhopper eggs parasitized, (**c**) Leafhopper egg density and (**d**) Leafhopper nymph density

Source	*df*	*F*	*P*
(a) Current proportion of leafhopper eggs parasitized			
Block	1,25	7.67	0.01**
Plot vigor	1,25	5.31	0.02*
Treatment	2,25	0.49	0.61
(b) Cumulative proportion of leafhopper eggs parasitized			
Block	1,25	11.25	0.002**
Plot vigor	1,25	8.70	0.006*
Treatment	2,25	0.15	0.86
(c) Leafhopper eggs per cm^2 leaf tissue			
Block	1,25	2.15	0.15
Plot vigor	1,25	1.47	0.23
Treatment	2,25	0.30	0.73
(d) Leafhopper nymph density			
Block	1,25	0.66	0.42
Plot vigor	1,25	1.79	0.19
Treatment	2,25	3.46	0.04*

Results of repeated measures MANCOVA comparing the three experimental treatments, plot vigor and block for current proportion of leafhopper eggs parasitized

* $P < 0.05$; ** $P < 0.005$

controlling for effects of block and plot vigor, there were no significant differences among treatments for the current proportion of parasitized leafhopper eggs ($F_{2,25} = 0.49$, $P = 0.61$; Table 1a, Fig. 3a), the cumulative proportion of parasitized leafhopper eggs ($F_{2,25} = 0.15$, $P = 0.86$; Table 1b, Fig. 3b), or leafhopper egg density per cm^2 of leaf tissue ($F_{2,25} = 0.30$, $P = 0.73$; Table 1c, Fig. 3c). There was a small but significant main effect of treatment on leafhopper nymph densities ($F_{2,25} = 3.46$, $P = 0.04$; Table 1d, Fig. 3d), but this difference was not produced by significantly elevated densities of nymphs in the sulfur treatment compared to the control ($F_{1,25} = 0.34$, $P = 0.55$), but rather by a significant difference between the sulfur and trifloxystrobin treatments ($F_{1,25} = 6.39$, $P = 0.01$). When plot vigor was withheld from the model, we obtained the same pattern of results: of the four variables measured, a significant treatment effect was only observed for nymph densities (data not shown). While plot vigor did not affect leafhopper egg or nymph densities, it did

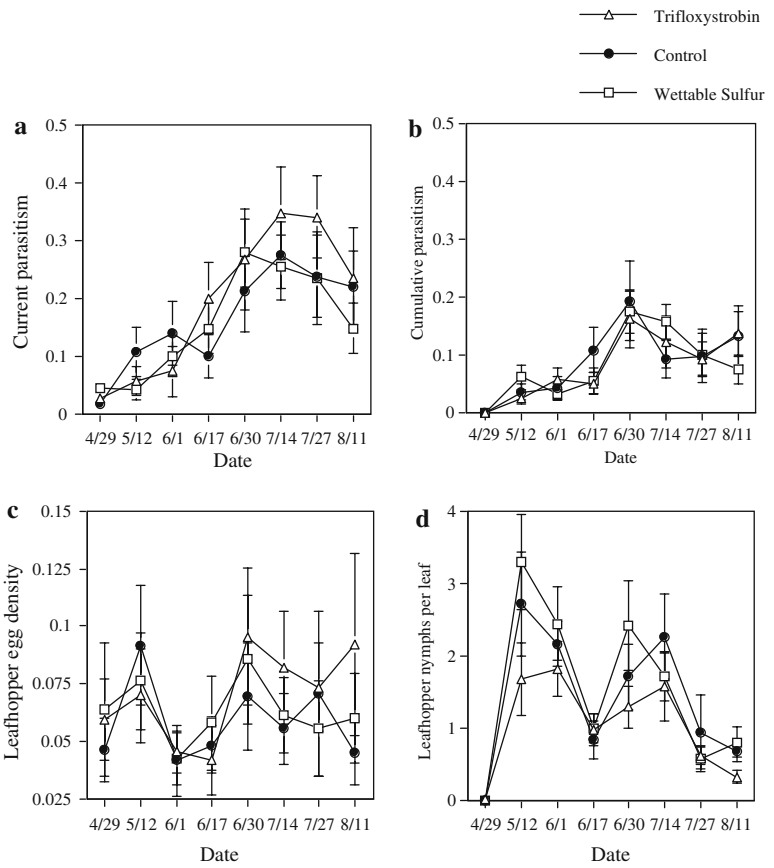

Fig. 3 Results of a field experiment examining the influence of fungicide residues on biological control of *Erythroneura elegantula* by *Anagrus erythroneurae*. Shown are the changes in four measures of parasitoid performance over the course of the growing season: (**a**) the current proportion of leafhopper eggs parasitized; (**b**) the cumulative proportion parasitism (the proportion of *Anagrus* spp. that emerged from leafhopper eggs); (**c**) the density of leafhopper eggs per cm^2 of leaf tissue; and (**d**) the average number of leafhopper nymphs per leaf

have an influence on parasitism rates. Therefore, we included vine vigor as a covariate in our analyses to provide statistical control for this source of variation in parasitism rates. We conclude that our experiment has produced no evidence supporting the hypothesis that sulfur disrupts parasitoid impact on the host (as measured by parasitism) or the resulting density of leafhoppers (eggs or nymphs) in comparison to untreated grapevines.

Discussion

Using field-cage bioassays, we found sulfur to be highly toxic to *A. erythroneurae* but not toxic to the adult stage of *E. elegantula*, a result that is consistent with previous laboratory bioassays that examined sulfur toxicity to *Anagrus* spp. (Martinson et al. 2001; Williams and Gill 1996). Although we did not test sulfur effects on other life stages of *E. elegantula* (eggs, nymphs), and additional bioassays of sulfur impact on young nymphal stages would be useful to rule out toxic impacts, we note that our field experiments did not produce any suggestion that sulfur is toxic to nymphal leafhoppers. Thus, the available evidence suggests that sulfur is highly and selectively toxic to the parasitoid *A. erythroneurae*, creating conditions under which sulfur use might be expected to generate outbreaks of grape leafhopper populations. Nevertheless, in our field experiment we found that neither sulfur nor the alternative fungicide trifloxystrobin influenced levels of *E. elegantula* parasitism or *E. elegantula* egg densities. The only significant treatment effect observed was for densities of leafhopper nymphs, for which neither the sulfur nor the trifloxystrobin treatments generated significant differences relative to the control (instead, the two fungicide treatments were significantly different from each other). Thus, our field experiment has produced very little evidence supporting the hypothesis that sulfur disrupts biological control of *E. elegantula* by *A. erythroneurae*. While the result of our field experiment may seem surprising given that the results of our bioassays, the fact that sulfur was not found to disrupt the impact of *Anagrus* on leafhoppers may be the reason that biocontrol workers were able to conduct extensive research with this parasitoid in sulfur-treated vineyards without ever suspecting a problem.

How can we explain the apparent disconnect between the results of the bioassays and those of the field experiment on season-long biological control? This discrepancy could, in theory, be due to either (a) the field experiment underestimating sulfur's true impact on *A. erythroneurae* relative to the sulfur-free control, or (b) the bioassays overestimating sulfur's true impact on *A. erythroneurae* relative to the sulfur-free control. We now consider these two possibilities in turn.

Why might our field experiment have underestimated the true impact of sulfur? First, we considered the possibility that the patchy decline of our experimental grapevines might have generated enough within-vineyard heterogeneity in vine vigor to overwhelm any potential treatment effect due to sulfur toxicity. To assess this possibility, we included plot vigor as a covariate in our main analysis. We found that indeed, plot vigor did influence our measures of parasitism, as did our blocking factor, whereas neither plot vigor nor block explained a significant amount of variation in leafhopper egg or nymph densities. Nevertheless, after controlling for the effects of plot vigor and block, we still observed no underlying main effect for our fungicide treatment on measures of parasitism. Variable vine vigor therefore does not appear to mask an expected negative effect of sulfur on parasitoid performance.

Second, a perennial concern facing field plot experimentation with mobile (winged) arthropods is the possibility that movement of focal arthropods between plots could erode differences between treatments. Our plots were not as small as in many experiments performed at university farms, but still involved plots that may not have been sufficiently large to prevent between-plot movement by both the adult leafhoppers and the adult parasitoids. Mark-release-recapture experiments performed with *Anagrus* spp. suggest that these parasitoids can move over relatively large distances (Corbett and Rosenheim 1996b; Cronin and Haynes 2004). We point out, however, that between-plot movement would be expected to reduce, but generally not eliminate entirely, differences between treatments, and our experimentation did not reveal even a non-significant trend toward depressed parasitism or elevated leafhopper densities in the presence of sulfur when compared to a sulfur-free control. Furthermore, in a companion study we evaluated the influence of sulfur residues on reproductive success of *Anagrus* spp. observed at a much larger spatial scale: the full spatial extent of commercial vineyards. This study provided an important confirmation of the basic conclusion that emerged from our small-plot field experiment: *Anagrus* reproductive success was not depressed in sulfur-treated vineyards relative to sulfur-free vineyards. Thus, sulfur appears to have no detectable effect on *Anagrus* spp. performance across both small and large spatial scales in the field. In sum, the data seem to suggest that the minimal disruptive influence of sulfur on *Anagrus* performance in the field is real.

Is it possible instead, therefore, that our bioassays have overestimated sulfur's true impact relative to a sulfur-free control treatment? Perhaps the most likely explanation for an exaggeration of the negative impact of a pesticide's impact during an acute toxicity assay is that bioassays conducted in the laboratory are difficult to extend to the field, where the mode of exposure and effective bioavailability of the toxin may be quite different (e.g., Robertson and Haverty 1981; Robertson and Worner 1990; Fauziah 1990; Wright and Verkerk 1995). Photo-degradation of pesticide residues in the field may rapidly reduce the effective toxicity of many pesticides to biological control agents (Caboni et al. 2002). However, our acute toxicity bioassays were performed in the field, not in the laboratory, and Martinson et al. (2001) have demonstrated that that toxicity of sulfur residues to *Anagrus* spp. persists in the field for several weeks. Furthermore, field observations made in 2001 suggest that in a real, commercial setting *Anagrus* parasitoids exposed to naturally weathered sulfur residues were in fact dying within 6–9 h of their emergence (J. A. Rosenheim, unpubl. data). Thus, we see no reason to suspect that our bioassays were erroneously inflating the acute toxicity of sulfur to *A. erythroneurae*. Instead, we suggest that the most likely source of a possible overestimate of sulfur's impact on *Anagrus* performance relative to a sulfur-free control is that our bioassays may have overestimated *A. erythroneurae's* performance in the absence of sulfur. In particular, our field cage bioassays provided parasitoids with an environment in which they were protected from predators and in which they had continuous access to food (honey). As discussed in Jepsen et al. (in press), data from *A. erythroneurae* collected in sulfur-treated and sulfur-free commercial vineyards suggest that parasitoids may be subject to significant predation risk and may often fail to obtain sugar-rich meals. Like most commercial vineyards, the site of our field experiment was a vineyard whose floor was largely devoid of flowering plants (unpublished data), a setting in which starvation conditions are likely to impose greatly reduced longevities on all parasitoids, regardless of their exposure to pesticides. At this particular

site, only 30% of collected female *A. erythroneurae* had consumed a nectar meal (M. E. Bench, unpublished data). Thus, if even in the absence of sulfur, parasitoids are still subject to intense mortality from these other factors (starvation, predation), the added mortality from sulfur may be rendered largely redundant.

We conclude, then, that sulfur is indeed highly toxic to *A. erythroneurae*, but that it may impose mortality that is largely redundant to other mortality factors already present in a typical commercial vineyard setting. This interpretation could be tested in the field by alleviating all of the potential constraints acting on *A. erythroneurae* simultaneously (for example by providing flowering cover crops and withholding sulfur applications), and then looking for significantly enhanced parasitoid performance. Such experimentation might also lead to direct recommendations for how the erratic biological control of *Erythroneura* spp. leafhoppers might be substantially improved.

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