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ORIGINAL PAPER

The impact of sulfur on the reproductive success of *Anagrus* spp. parasitoids in the field

Sarina J. Jepsen · Jay A. Rosenheim · Chris E. Matthews

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Abstract Pesticides targeted at pest species have often been demonstrated to have strong adverse effects on the survival of biological control agents in short-term laboratory bioassays; however, studies examining the influence of pesticides on the actual reproductive success of biological control agents in the field are rare. Because natural enemy reproduction is often directly tied to biological control success, effects of pesticides on reproduction are of central importance. Here we use a new technique to examine the influence of sulfur, a fungicide widely used in grape production, on the reproductive success of *Anagrus erythroneurae* (Hymenoptera: Mymaridae) and *Anagrus daanei* (Hymenoptera: Mymaridae), egg parasitoids of the grape leafhopper, *Erythroneura elegantula* (Homoptera: Cicadellidae). Sulfur has previously been shown to be highly toxic to *Anagrus* spp. in short-term laboratory and field bioassays, creating the expectation that sulfur should also reduce *Anagrus* reproductive success in the field. Surprisingly, in two studies, the first comparing the oviposition success of *Anagrus* collected live in paired sulfur-treated versus untreated vineyards and the second comparing the lifetime reproductive success of *Anagrus* collected at the end of their lives in unpaired sulfur-treated versus untreated vineyards, we found no effect of sulfur on parasitoid reproductive success. In this system, traditional short-term assays of laboratory toxicity do not appear to predict effects on parasitoid reproductive success, suggesting that demographic approaches to assessing the disruptive effects of pesticides may have an important role in designing IPM programs.

Keywords *Anagrus daanei* · *Anagrus erythroneurae* · *Erythroneura elegantula* · Homoptera · Cicadellidae · Hymenoptera: Mymaridae · Pest resurgence · Side effect · Sulfur

S. J. Jepsen (✉) · J. A. Rosenheim · C. E. Matthews
Department of Entomology, University of California, Davis, USA
e-mail: sarinajepsen@gmail.com

Introduction

A central challenge to the successful integration of biological and chemical control practices in a program of integrated pest management is the identification of pesticides that have few deleterious effects on biological control agents (van Emden and Peakall 1996). To this end, researchers have invested a great deal of effort in screening pesticides for their effects on key biological control agents. The vast majority of this effort has employed simple laboratory assays of acute toxicity of pesticide residues to parasitic and predatory insects (Croft 1990). Short-term assays are standardized and simple to conduct, and thus are the obvious first method of choice, given the vast number of potential pesticide × biocontrol agent combinations that must be screened.

It has long been appreciated, however, that acute lethal effects of exposure to pesticide residues do not necessarily provide a full understanding of potential pesticide impact in nature. Several studies have suggested that acute toxicity tests may underestimate the negative effects of a pesticide on a biocontrol agent, because a parasitoid that survives exposure to a pesticide residue may still not forage or reproduce normally (Croft 1990). It is, of course, also possible that an acute toxicity test may overestimate the disruptive effects of pesticide exposure for a biocontrol agent. Simple laboratory bioassays are generally designed as 'worst case scenarios', which may be followed by semi-field tests, and sometimes field tests when negative side-effects are observed (i.e., the principle of sequential testing recommended by the IOBC; see Candolfi et al. 2000). Furthermore, laboratory bioassays are generally conducted in an environment that is benign to the biocontrol agent, with the goal of achieving low levels of mortality in the control treatment. In nature, however, even an agroecosystem that is free of pesticide residues may still be relatively hostile to a biological control agent if it is devoid of key food resources (reviewed in Jervis et al. 1993; Heimpel et al. 1997), occupied by higher-order predators (Rosenheim 1998), or presents harsh abiotic conditions (Suh et al. 2002). These factors could, in theory, reduce survivorship in the absence of pesticide residues to levels that approach those seen in the presence of the pesticide residue. Under such a scenario, the mortality imposed by the pesticide may become effectively redundant to other mortality factors already present.

Demographic approaches to assessments of pesticide effects have been proposed that provide measures of pesticide effects on multiple fitness components of a biocontrol agent, including survival and reproduction (reviewed in Stark and Banks 2003). However, these methods are just beginning to be utilized; the vast majority of studies conducted to evaluate the impact of a pesticide on a biocontrol agent have been based on mortality, including median lethal dose studies (Theiling and Croft 1988). Here we introduce a new technique that allows us to provide the first direct measure of how pesticide exposure affects the reproductive success of individual parasitoids in the field. Our primary goal is to determine whether the expectation of strong negative effects of pesticide residues on the performance of the adult life stage of a biocontrol agent, derived from acute toxicity bioassays, is realized in the observed disruptive effects of pesticide use in the field. To answer this question, we used the model system: *Anagrus* spp. parasitoids of the grape leafhopper. In California vineyard ecosystems, the endemic egg parasitoids *Anagrus erythroneurae* Trjapitsyn (Hymenoptera: Mymaridae) and Chiapini and *Anagrus daanei* Triapitsyn

(Hymenoptera: Mymaridae) parasitize the eggs of the western grape leafhopper, *Erythroneura elegantula* Osborn (Homoptera: Cicadellidae). However, these parasitoids produce only erratic and unpredictable suppression of leafhopper populations, and growers frequently use insecticides to control the leafhoppers (Flaherty et al. 1992).

The efficacy of *Anagrus* spp. in biological control of the grape leafhopper has been linked to the presence of an overwintering refuge, as the parasitoids require an alternate host to overwinter (Doutt and Nakata 1965, 1973; Kido et al. 1984; Corbett and Rosenheim 1996; Murphy et al. 1996, 1998). The grape leafhopper overwinters in the adult stage, whereas the parasitoid spends the winter as an immature within a host egg. After completing nine to ten generations during the growing season (Flaherty et al. 1992), *Anagrus* spp. adults emigrate from vineyards to find alternate hosts in which their eggs can overwinter. When refuges that contain alternate hosts are near vineyards, more effective biological control of the grape leafhopper is often observed (Doutt and Nakata 1965, 1973; Kido et al. 1984; Corbett and Rosenheim 1996; Murphy et al. 1996, 1998), as the parasitoid is able to reinvade the vineyard in late March or early April, when *E. elegantula* begin to lay eggs (Flaherty et al. 1992). Entomologists have tried to manipulate grape ecosystems to provide *Anagrus* spp. with overwintering refuges and thus enhance biological control, but their efforts have met with little success (Flaherty et al. 1985, 1992). Decades after this research began, the grape leafhopper remains a highly damaging herbivore of California grapes (Triapitsyn 1998).

An alternate explanation for the frequent failure of *Anagrus* spp. to suppress leafhopper populations below the economic threshold is that the ubiquitous use of sulfur in vineyards may be interfering with parasitoid reproduction. Sulfur, a fungicide used against a foliar pathogen of grapes, powdery mildew (*Uncinula necator* Schwein.), has been shown to be toxic to *Anagrus* spp. in laboratory and field cage bioassays (Williams and Gill 1996; Martinson et al. 2001; Jepsen et al. in press). Martinson et al. (2001) showed that sulfur residues are highly toxic to *Anagrus* spp. for 2 weeks, and their data suggest that the sulfur residues probably remained toxic to the parasitoids for the entire 6-week period of their trial. Grape growers in California continue to use sulfur because it is inexpensive and effective, despite its ability to disrupt biological control of *Tetranychus* spp. spider mites by predatory mites (Hanna et al. 1997; James et al. 2002; Prischmann et al. 2005; Teodoro et al. 2005) and cause dermatitis among field workers (U.S. Environmental Protection Agency 1991). Powdery mildew has developed resistance to many alternative fungicides (Delye et al. 1997), but has not shown resistance to sulfur.

Although sulfur residues are demonstrably toxic to adult *Anagrus* spp., it is unknown what influence this toxicity has on parasitoid reproduction in the field. *Anagrus* spp. are short lived, even under the most benign laboratory conditions (English-Loeb et al. 2003), and nothing is known about their longevity under field conditions. We therefore formulated the question: does sulfur toxicity interfere with *Anagrus* spp. reproduction in the field? To answer this question, we conducted an observational study using paired vineyard sites (each sulfur treated vineyard paired with an untreated vineyard) and used a novel technique to study the oviposition success of live, field caught wasps. We also measured the lifetime reproductive success of parasitoids that we caught in the field, at sulfur-treated and untreated sites, at the time of their natural death. We did not test the possibility that sulfur residues may negatively impact the immature stages of *Anagrus* spp. However,

immature *Anagrus* spp. are protected inside the leafhopper egg within the grape leaf, and a study by Martinson et al. (2001) showed relatively low mortality of immature *Anagrus* spp. even when exposed to organophosphate insecticides.

Materials and methods

Development of a technique to estimate parasitoid reproductive success

The unusual biology of *Anagrus* spp. provided us with an opportunity to study the reproductive success of individual female parasitoids caught in vineyards. *Anagrus* spp. have been reported to be strictly proovigenic parasitoids; females have their full lifetime complement of eggs already mature when they emerge as adults (Cronin and Strong 1990; Jervis et al. 2001, this paper). Furthermore, as we demonstrate here, *A. erythroneurae* also does not resorb any eggs. Therefore, at any given time, the number of eggs that a female *Anagrus* has in her ovaries is equal to the number of eggs she contained upon emergence minus the number of eggs she has laid up to that point in her life. Because during the course of ovarian dissections of *A. daanei* we never observed immature oocytes or oocytes that were being resorbed, we adopt the working hypothesis that this species, like its congeners, is also proovigenic and does not resorb eggs. While *A. erythroneurae* is the more common parasitoid of the grape leafhopper in the sites where we worked, *A. daanei* is also present (Tables 1 and 2). Therefore, we include both species in our analyses.

We conducted an experiment to test whether *A. erythroneurae*, like other members of the genus, is proovigenic, and also to provide the first test of whether an *Anagrus* sp. is able to resorb eggs. Many parasitoids, when food is scarce, will resorb some of their eggs to avert starvation (Rosenheim et al. 2000; Jervis et al. 2001). If *Anagrus* spp. were able to resorb eggs, we would be unable to determine the oviposition success of parasitoids simply by counting their residual inventory of eggs.

Wasps were reared in the laboratory from *Vitis vinifera* leaves harboring parasitized leafhopper eggs collected in an untreated vineyard in Davis, California during August 2003. Leaves were held for parasitoid emergence in a darkened box that had a single illuminated funnel and a collecting vial on its lid. Emerging wasps were collected each morning. Newly emerged wasps were assigned to one of three treatments: (1) initial egg load treatment, (2) honey treatment, and (3) starvation treatment. For the initial egg load treatment, female wasps ($n = 42$) were dissected immediately upon emergence. The wasps in the honey and starvation treatments were housed individually in small glass vials. Vials were checked daily and dead parasitoids were collected and dissected. For the honey treatment ($n = 24$), parasitoids were provisioned with (a) a small strip of filter paper that was saturated in a solution of 1 part honey to 3 parts water, and (b) a source of water (a moist cotton wick connected to a water reservoir). Comparisons between the initial egg load treatment and the honey treatment enabled us to test whether *A. erythroneurae* would continue to mature eggs over its lifetime if provided unlimited access to a sugar source. For the starvation treatment ($n = 26$), parasitoids were provided only with the moist cotton wick. The starvation treatment subjected the wasps to nutritional stress and the absence of hosts; if *A. erythroneurae* were able to resorb eggs, we expected them to do so under these conditions. Therefore, comparisons between the initial egg load treatment and the starvation treatment allowed us to determine if *A. erythroneurae* would resorb eggs.

Table 1 Date, pair number, vine treatment, location, sample size, parasitism rate and host density (parasitized eggs/cm^2 of leaf tissue) for paired sites study of the oviposition success of *A. erythroneurae* and *A. daanei*

Date of collection	Pair	Vine treatment	California site & location	Number of *A. erythroneurae*	Number of *A. daanei*	Parasitism rate	Parasitized eggs/cm^2
7/9/2003	1	Sulfur	Viticulture West Davis	30	0	0.48	1.08
6/25/2003	1	Untreated	Plant pathology West Davis	30	0	0.45	0.3156
7/10/2003	2	Sulfur	Cypress West Pope Valley	27	4	0.36	0.23
7/11/2003	2	Untreated	Kimsey's Pope Valley	6	23	0.55	0.66
7/15/2003	3	Sulfur	Plant pathology East Davis	29	0	0.08	0.21
7/7/2003	3	Untreated	Rand's Davis	29	0	0.14	0.52
7/29/2003	4	Sulfur	Viticulture East Davis	31	0	0.25	0.35
7/28/2003	4	Untreated	Village Homes Davis	34	0	0.33	0.19
7/30/2003	5	Sulfur	Niebaum- coppola Napa Valley	29	0	0.16	0.55
7/16/2003	5	Untreated	Blair Napa Valley	27	0	0.33	0.44
8/6/2003	6	Sulfur	Cypress East Pope Valley	18	18	0.76	3.3
7/31/2003	6	Untreated	Wilm's Pope Valley	0	26	0.49	1.01

Table 2 Month and year of collection, vine treatment, location, sample size, parasitism rate, and host density (parasitized eggs/cm^2 of leaf tissue) for the study on lifetime reproductive success of *A. erythroneurae* and *A. daanei*

Month and Year of collection	Vine treatment	California site & location	Number of dead female *A. erythroneurae*	Number of dead female *A. daanei*	Parasitism rate	Parasitized eggs/cm^2
8/2000	Sulfur	Liberty Galt	0	8	0.16	0.43
8,9/2000	Sulfur	Niebaum-coppola Napa Valley	8	0	0.91	3.45
7/2000	Sulfur	Viticulture East Davis	13	0	0.36	3.68
7,8/2001	Sulfur	Hafner Alexander Valley	69	8	0.71	2.72
8,9/2001	Sulfur	Niebaum-coppola Napa Valley	9	0	0.59	0.74
7,9/2002	Untreated	Wilm's Pope Valley	8	10	0.26	0.5
7,8,9/2002	Untreated	Kimsey's Pope Valley	11	0	0.2	0.24
7/2004	Untreated	Kimsey's Pope Valley	0	20	0.63	1.15

Dissections were conducted in depression slides under a dissection microscope with transmitted light. Jeweler's forceps were used remove the wasp's ovaries. The eggs were teased from the ovaries and counted. Parasitoids were then slide mounted in Hoyer's solution, identified to species (Triapitsyn 1998), and the length of each hind tibia was measured using an ocular micrometer as an index of parasitoid size.

The tibia:egg load relationships were compared across treatments using ANCOVA. In cases where the hind tibia lengths of a single parasitoid differed, the larger tibia length measurement was used in the analysis.

To establish the relationship between parasitoid hind tibia length and initial egg load, and to assess the possibility that sulfur residues might change initial egg loads, we reared *A. erythroneurae* during August 2003 from leaves collected at two sulfur treated and two untreated vineyards in Davis, USA. *Anagrus daanei* were reared only from untreated leaves collected in one vineyard in Pope Valley. Parasitoids were reared as described above during the first 2 days following leaf collection. Dissections, tibia measurements, and species identifications were made as described above. We used linear regression equations developed for *A. erythroneurae* and *A. daanei* to predict the initial egg loads of parasitoids caught in the field. ANCOVA and pairwise contrasts were used to test whether or not the relationship between *A. erythroneurae* hind tibia length and egg load was influenced by site or by sulfur application. Throughout the text, means are presented ±1 standard error.

Observational field studies

To determine if *Anagrus* spp. reproduction differed between sulfur treated and untreated vineyards, we compared parasitoid reproduction at paired vineyard sites from June to August, 2003. We collected approximately 30 female parasitoids of mixed and unknown ages from each of six sulfur-treated and untreated vineyards. Sulfur treated vineyards generally received sulfur applications every 7–21 days, from May to August. Untreated vineyards did not receive any sulfur applications throughout the entire growing season. Both the sulfur treated vineyards and the untreated vineyards were otherwise free from insecticides. The untreated vineyards were paired both spatially and temporally with the sulfur treated vineyards (Table 1).

Wasps were collected by beating grape foliage over white plastic boards between 09:00 a.m. and 11:00 a.m. Parasitoids that were clearly alive (standing on their legs and walking) were collected off the boards, held singly in vials on ice, and brought to the laboratory. Female wasps were dissected, egg loads counted, and wasps slide mounted, identified to species, and tibia lengths measured as described above. To estimate the initial egg load for each parasitoid, we used our previously obtained relationships between tibia length and initial egg load for *A. erythroneurae* and *A. daanei*. We subtracted the number of eggs observed during the dissection from the estimated initial egg load of each parasitoid to estimate the number of eggs each wasp laid prior to collection.

To estimate host availability at each site, we examined leaves for leafhopper eggs. Thirty leaves were collected from each site, and each leaf's area was measured using a portable leaf area-meter (LICOR model LI-3000). *E. elegantula* lay their eggs in the open blade of grape leaves; thus, the eggs are readily detectable when the leaves are viewed under transmitted light (Flaherty et al. 1992). Each of the 30 leaves

collected was viewed under a microscope using transmitted light, and all live eggs categorized as unparasitized or parasitized. Eggs categorized as unparasitized were clear, whereas parasitized eggs either had a whitish mass at one end or were orange or red (Kido et al. 1984; Settle and Wilson 1990). Estimates of host availability included only unparasitized eggs, and host density is presented as hosts per cm^2 of leaf tissue. We compared oviposition success at sulfur treated and untreated vineyards using an ANCOVA with host density as a covariate (using a 1-tailed test) and vineyard treatment (sulfur treated versus untreated) and wasp species (*A. erythroneurae* versus *A. daanei)* as main effects. The data satisfied the assumption of normality, and thus no transformations were used.

To compare the lifetime reproductive success of *Anagrus* spp. in sulfur treated and untreated vineyards, we collected parasitoids at the time of their natural death in vineyards from June to August 2000–2004 (Table 2). Two methods were used to collect wasps at the time of their death. From 2000 to 2002 we placed white, plastic pan traps (35 cm × 45 cm, NSF, Huntington Beach, CA, USA) on the ground below grape vines early in the morning, and then checked them over the course of the day to collect dead wasps as they fell out of the canopy. The pans were not filled with any liquid; to reduce the loss of parasitoids from wind blowing them out of the trays, we affixed plastic and metal screening material to the interior surface of each tray. These screens acted as wind baffles. Wasps that were not standing on their legs and that were completely immobile were considered dead and collected with fine paintbrushes. Dead wasps were put in vials, stored on ice, and brought to the laboratory for dissection to quantify the number of eggs remaining in the ovaries. In 2004, dead wasps were collected on beat trays. Vines were shaken over the pans to dislodge dead wasps from the surfaces of grape leaves in the canopy. Wasps were dissected as described earlier.

Working with dead wasps raises the possibility that we might collect a female that had been dead for too long to still contain intact eggs, and that this would produce a spurious record of zero eggs remaining. To reduce the likelihood of this error, we adopted a rating scheme for the internal condition of each wasp collected, and only included in our final data set those wasps whose conditions were sufficiently good to be readily dissectible. We rated the internal conditions of each wasp as follows: 1 = soft internal tissues are present; 2 = soft internal tissues absent. Wasps that received a rating of 2 for the internal condition were excluded from the data analysis. Host availability was measured as in the paired vineyard sites study. The data satisfied the assumption of normality, and thus were not transformed.

We compared mean lifetime reproductive success in sulfur treated vineyards and untreated vineyards using ANCOVA with unparasitized hosts per cm^2 of leaf tissue as the covariate (1-tailed test) and species and vineyard treatment as the main effects. Unlike the 'paired sites' study described above, our study of lifetime reproductive success lacked any temporal or spatial blocking. To provide some sense of the magnitude of the potential problem created by the lack of blocking, we used data from the 'paired sites' study to test for effects of sampling time (seasonality) or location. To test for an effect of sampling time, we used 'week sampled' as a continuous variable in our model. Since our untreated sites were only in Pope Valley, whereas our sulfur treated sites were in many different locations (including: Galt, Rutherford, Alexander Valley and Davis), we tested for a difference in our paired sites data set between Pope Valley and all of the other sites sampled to see if the Pope Valley site was atypical. We examined the number of eggs laid by parasitoids using an ANCOVA with hosts/cm^2 of

leaf tissue as a covariate and 'week sampled' and 'location' as main effects. All statistical tests were performed with JMP version 4.0.2. (SAS Institute 2000).

Results

Development of a technique to estimate parasitoid reproductive success

We found no differences in egg loads when we subjected *A. erythroneurae* to three experimental treatments (initial egg load; honey; starvation; $F_{2,76} = 136.8$, $P = 0.17$). The observed relationships between hind tibia length and initial egg load for each treatment were as follows: treatment 1, wasps dissected at emergence: $y = 545.9x - 66.5$ ($r^2 = 0.45$, $n = 82$, $P < 0.0001$); treatment 2, wasps provisioned with honey and water and dissected at the time of their death: $y = 464.8x - 49.1$ ($r^2 = 0.37$, $n = 16$, $P = 0.01$); treatment 3, wasps provisioned only with water and dissected at the time of their death: $y = 149x + 3.6$ ($r^2 = 0.08$, $n = 22$, $P = 0.18$). We conclude therefore that *A. erythroneurae* adults do not mature additional eggs and furthermore do not resorb eggs, even under conditions of starvation and in the absence of hosts.

For each of the four *Anagrus* populations sampled, including two from sulfur-treated vineyards and two from untreated vineyards, we obtained a strong positive relationship between hind tibia length and egg load (Fig. 1). We detected no significant differences between sites ($F_{3,155} = 0.8$, $P = 0.44$) or between the two sulfur-treated and the two untreated vineyards ($F_{1,155} = 2.6$, $P = 0.10$). We therefore combined the data across all four sites to obtain a regression equation that we used in all subsequent studies to estimate initial egg load for *A. erythroneurae* ($y = 432.1x - 46.3$, $r^2 = 0.38$, $n = 162$, $P < 0.0001$).

We reared only a small sample of *A. daanei*, and observed a noisy and non-significant relationship between female size and initial egg load ($y = 306.1x - 30.1$, $r^2 = 0.06$, $n = 23$, $P = 0.22$). Although we could simply use the observed overall mean initial egg load as an estimate of starting egg load for *A. daanei*, we reasoned that the regression equation that we obtained ($y = 306.1x - 30.1$, $r^2 = 0.06$, $n = 23$, $P = 0.22$) is still likely to produce a somewhat improved estimate of starting egg load. We therefore used this equation to estimate the initial egg load for all field-collected *A. daanei*.

The mean initial egg loads observed were 28.4 ± 0.5 for *A. erythroneurae*, and 19.1 ± 1.4 for *A. daanei*.

Observational field studies

For *A. erythroneurae* and *A. daanei* parasitoids collected live in the field, we found no differences between the mean number of eggs laid in sulfur-treated versus untreated vineyards (Table 3, Fig. 2). The effect of 'species' was non-significant. The effect of block (pair number) was also non-significant ($F_{5,13} = 0.9$, $P = 0.53$), and therefore block was removed from the model. We did find evidence of increased reproductive success in vineyards harboring higher density host populations (Table 3). We observed a difference in the number of parasitoids that succeeded in laying ≥75% of their full complement of eggs at sulfur-treated versus untreated sites: only 13 ± 6% of the *Anagrus* spp. collected in sulfur-treated sites had laid ≥75% of their lifetime complement of eggs, compared to 30 ± 8% for *Anagrus* spp. collected from untreated

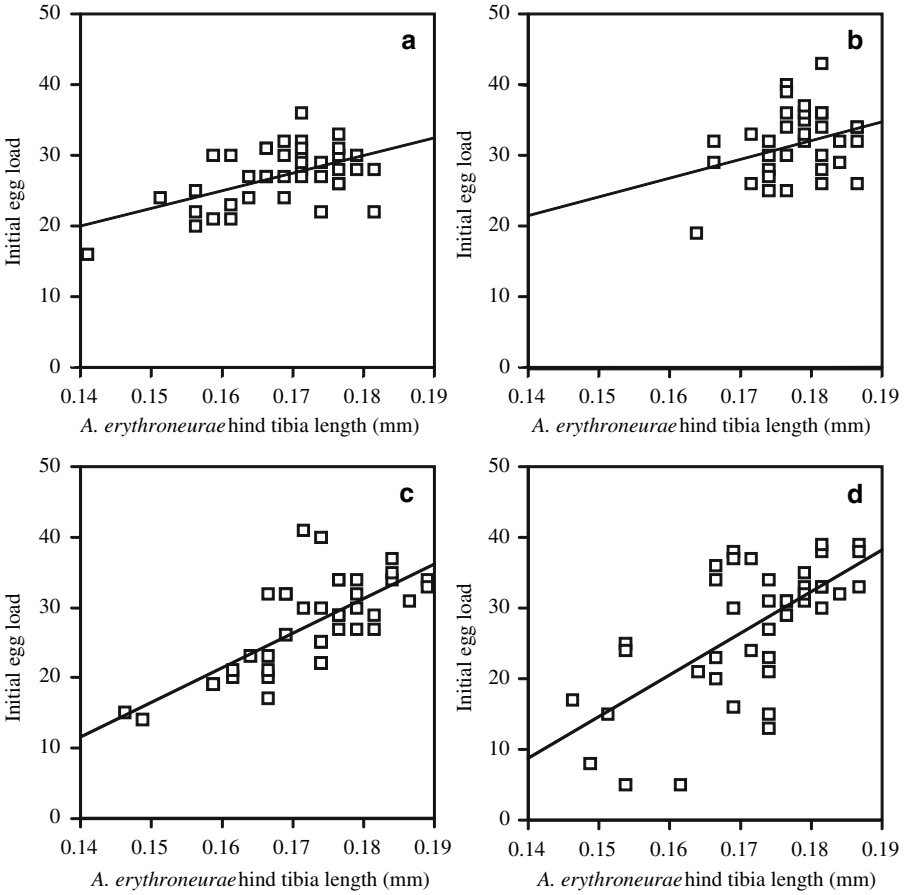

Fig. 1 *A. erythroneurae* rearing experiment. (**a**) Parasitoids reared from sulfur treated site #1 (Viticulture and Enology West vineyard, UC Davis, $y = 248.7x - 14.7$, $r^2 = 0.27$, $n = 40$, $P = 0.0005$). (**b**) Parasitoids reared from sulfur treated site #2 (Viticulture and Enology East vineyard, UC Davis, $y = 263.4x - 15.2$, $r^2 = 0.13$, $n = 38$, $P = 0.02$). (**c**) Parasitoids reared from untreated site #1 (Plant Pathology vineyard, UC Davis, $y = 494.7x - 57.7$, $r^2 = 0.52$, $n = 40$, $P = 0.0001$). (**d**) Parasitoids reared from untreated site #2 (Rand's vineyard, Davis, $y = 591.6x - 74.2$, $r^2 = 0.43$, $n = 42$, $P = 0.0001$)

Table 3 ANCOVA comparing oviposition success of *A. erythroneurae* and *A. daanei* in sulfur treated and untreated vineyards in the paired vineyard sites study

Effect	df	*F* ratio	*P* value
Sulfur	1,13	0.67	0.43
Species	1,13	0.23	0.64
Host density	1,13	5.19	0.02

vineyards ($F_{1,9} = 5.7$, $P = 0.04$; effect of species was non-significant). Although we observed that fewer *Anagrus* spp. are able to lay nearly their full complement of eggs in sulfur-treated vineyards than in untreated vineyards, the results of this experiment suggest that sulfur has only a very minor impact on *Anagrus* reproduction.

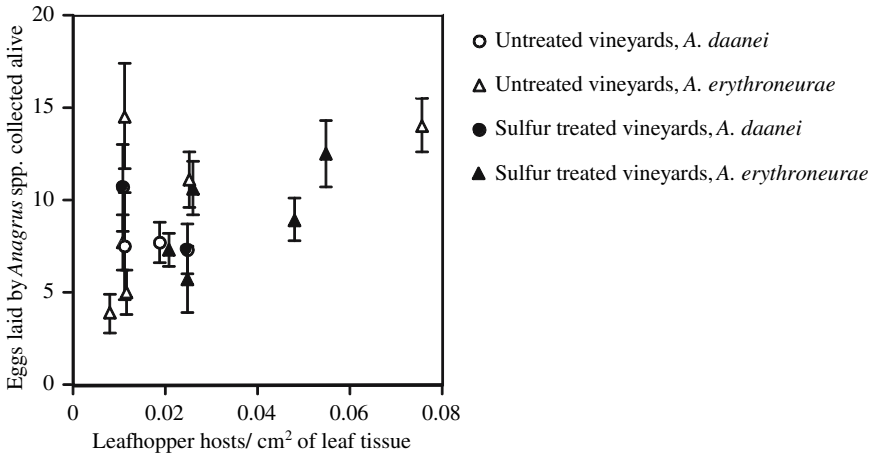

Fig. 2 Paired sites study: comparison of eggs laid by *A. erythroneurae* and *A. daanei* captured live in sulfur treated and untreated vineyards

Table 4 ANCOVA comparing lifetime reproductive success of *A. erythroneurae* and *A. daanei* in sulfur treated and untreated vineyards

Effect	df	*F* ratio	*P* value
Sulfur	1,9	2.08	0.19
Species	1,9	1.77	0.23
Host density	1,9	0.4	0.27

We found no difference in the mean lifetime reproductive success of wasps collected at the end of their lives in sulfur-treated versus untreated vineyards (Table 4, Fig. 3). All of our estimates of lifetime reproductive success for wasps collected in sulfur-free sites were derived from collections in the Pope Valley. To address the

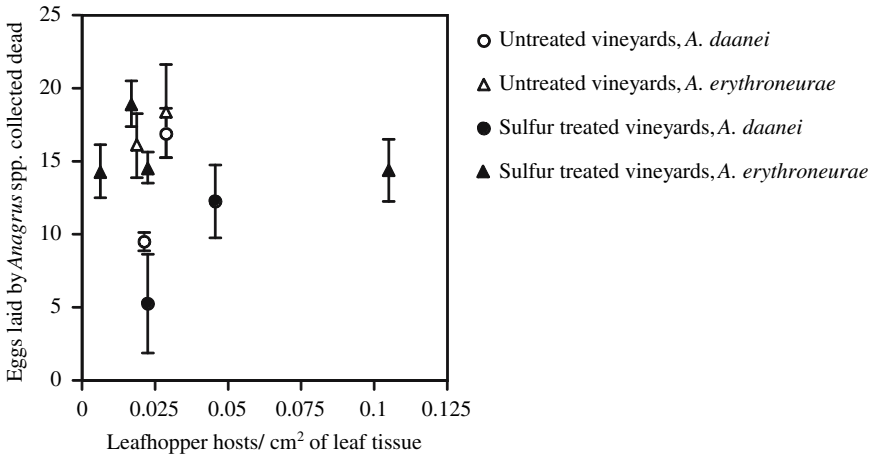

Fig. 3 Lifetime reproductive success study: comparison of eggs laid by *A. erythroneurae* and *A. daanei* caught at the time of their natural death at sulfur treated sites and untreated sites

possibility that the Pope Valley differed from other locations in opportunities for parasitoid reproduction, we examined data from live-collected parasitoids, and found no significant difference between the Pope Valley sites and all other sites combined ($F_{1,14} = 0.1$, $P = 0.71$). We also found no suggestion that the seasonal timing of the collection (week sampled) influenced our measure of reproduction among live wasps ($F_{1,21} = 1.0$, $P = 0.32$). These results suggest that the lack of a difference between the lifetime reproductive success of parasitoids collected in sulfur treated vineyards and untreated vineyards is real, and not an artifact of the shortcomings of our sampling design. Neither the parasitoid species effect nor the host density covariate was significant (Table 4).

Discussion

We have demonstrated that *A. erythroneurae* is strictly proovigenic and does not resorb eggs. In fact, all mymarid species studied so far are proovigenic (Jervis et al. 2001). This allowed us to develop a new technique for quantifying the reproductive success of individual parasitoids collected in the field. Previous studies employing short-term bioassays had demonstrated that sulfur residues are acutely toxic to *Anagrus* spp. (Williams and Gill 1996; Martinson et al. 2001; Jepsen et al. 2007). Nevertheless, our studies suggest that the reproductive success of *Anagrus* spp. parasitoids is not depressed in sulfur-treated vineyards compared to untreated vineyards. This conclusion was supported both by collections of live wasps from paired sulfur-treated and untreated vineyards and by collections of dead wasps, which provided estimates of lifetime reproductive success. Thus, our study suggests that short-term bioassays overestimate the disruptive potential of sulfur use in California vineyards.

We suggest that the most likely explanation for this surprising result is that even a sulfur-free vineyard may be a relatively hostile environment for *Anagrus* spp. parasitoids, such that the expected longevity of an *Anagrus* parasitoid may be very short, irrespective of the presence of sulfur residues. *Anagrus* spp. longevity is very short in the laboratory: *Anagrus* spp. live an average of 6–7 days when provisioned with a sugar source and water, but live less than one day when provisioned with only water or air ($n = 40$) (English-Loeb et al. 2003). Field longevity for *Anagrus* spp. is unknown. Factors that could shorten the longevity of an *Anagrus* parasitoid in the field are: a shortage of sugar rich foods, predation events, and high temperatures.

Studies of nectar feeding by *A. erythroneurae* in untreated northern California vineyards suggest that the majority of wasps are not successfully obtaining sugar meals. Only 35.8 ± 8.6% of *Anagrus* scored positive in anthrone tests ($n = 354$) (M.E. Bench unpublished data), indicating that they had consumed a sugar meal.

We do not have a good estimate of predation risk to *Anagrus* in the field. However, fragmentary observations suggest that the risk of predation might be high: in just 9.1 h of focal observations of *Anagrus* spp. in the field, two instances of predator attack were recorded; in one instance, the parasitoid escaped from an immature crab spider, and in the other instance the parasitoid was killed and eaten by a late-instar nypmhal *Orius tristicolor* (J.A. Rosenheim unpublished data). If these preliminary observations are confirmed in a larger data set, they will suggest that most parasitoids are killed by predators within the first day of their adult lives, as has been observed for many other parasitoid species (Rosenheim 1998).

High temperatures may also reduce *Anagrus* lifespan in the field. Many of the vineyards in our studies routinely reach temperatures that exceed 37°C in the summer; field longevity under these circumstances may be much shorter than in laboratory assays that are run at room temperature. However, we do not favor this explanation for the discrepancy between our bioassay results (Jepsen et al. 2007) and our studies of *Anagrus* reproduction, because our bioassays were also conducted in the field during the hottest months of the summer.

If it is generally true that sulfur-induced mortality is primarily redundant to these additional sources of mortality, then we might expect to see a characteristic signature in the distribution of reproductive success values across parasitoids. In particular, we might expect sulfur poisoning to prevent any parasitoids (even those who manage to find food and who are lucky enough to escape predators) from living long enough to reach very high levels of reproductive success, where they lay all or nearly all of their eggs. We did indeed see such a deficit of parasitoids with very high levels of reproductive success among our parasitoid collections from sulfur-treated sites. Thus, although sulfur may have a minimal influence on mean reproductive success of *Anagrus* spp. parasitoids in vineyards, it may nevertheless be killing the oldest individuals within the parasitoid population, thus acting to reduce the likelihood that parasitoids approach their maximum potential for reproductive success. Our data also raise the question of whether sulfur might have a greater impact on *A. daanei* than *A. erythroneurae*, since we observed high ratios of *A. daanei* to *A. erythroneurae* only in untreated vineyards. Further work is needed to explore this possibility.

There has been a considerable effort to improve biological control of grape leafhoppers and variegated leafhoppers (*Erythroneura variabilis* Beaver, Homoptera: Cicadellidae) by planting flowering cover crops to provide *Anagrus* spp. with sugar-rich foods (Costello and Daane 1998; Nicholls et al. 2001; English-Loeb et al. 2003). These studies have not consistently demonstrated that cover crops enhance *Anagrus* performance. Different plant species can differ considerably in their attractiveness and nectar accessibility to parasitoids (Wäckers 2004), and this may contribute to variability in cover crop usefulness for parasitoids. However, the nearly ubiquitous use of sulfur may have prevented an optimal test of the value of cover crops: even if the wasps were protected from starvation, they would not have been protected from sulfur poisoning. In our studies, *Anagrus* spp. were protected from sulfur poisoning, but may not have been protected from starvation. A potential avenue for future study in this system would be to examine *Anagrus* spp. reproduction in sulfur-free vineyards that also are provisioned with flowering cover crops.

In a companion study, we conducted a manipulative field experiment to test whether or not the removal of sulfur or the replacement of sulfur with a less toxic fungicide would improve season-long grape leafhopper biological control. Levels of parasitism generated by *Anagrus* and densities of grape leafhoppers were largely unchanged by removing sulfur from the system (Jepsen et al. 2007), a result that is consistent with the results of the current study. Thus, we have multiple forms of evidence suggesting that the acute toxicity of sulfur to *Anagrus* spp. does not translate into a disruption of biocontrol: we see virtually no effect on parasitoid reproduction, parasitism rates, or host densities. We conclude that demographic approaches to evaluating the disruptive effects of pesticides on biological control agents may in some cases be critical extensions to the more traditional use of short-term assays of parasitoid mortality.

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