Seasonal Biology of *Ferrisia gilli* (Hemiptera: Pseudococcidae) in California Sierra Foothill Vineyards

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ABSTRACT The mealybug *Ferrisia gilli* Gullan is an emerging pest of wine grapes grown in California's Sierra foothills. A relatively new species, it had previously been recorded as a pest of pistachio, almond, and ornamentals. It was first reported on grape in El Dorado County in 2003 and has since established and spread. Nondestructive monitoring of grape vine sections was conducted in untreated vineyard plots and compared with destructive sampling conducted in grower-treated plots in 2008 and 2009 to determine *F. gilli* life stage seasonal presence, number of generations, location on the vine during the season, and damage potential to fruit clusters. Two generations were observed to be completed during the season. *F. gilli* overwintered under the bark at the base of the trunk, trunk, and cordon as second and third instars. Adults were found at the base of emerging shoots (spring) or on and under bark of old and new spurs. Live crawlers were born in June (first generation) and late August to September (second generation), and migrated to leaves to feed before moving to protected locations under bark or into fruit clusters. Lower mealybug densities and fruit damage were recorded on vines with than without insecticide treatment(s). Parasitized mealybugs were collected in low numbers and an *Acerophagus* sp. was the dominant parasitoid.

KEY WORDS *Ferrisia gilli*, mealybug, *Vitis*, grapes, *Acerophagus*

The mealybug, *Ferrisia gilli* Gullan, is a recently described pest on pistachios and almonds in California's San Joaquin Valley (Gullan et al. 2003, Haviland et al. 2006) and an emerging pest on wine grapes in California's Sierra foothill (El Dorado County) and one North Coast (Lake County) appellation (California Department of Food and Agriculture [CDFA] 2013). Because this mealybug is new to science (Gullan et al. 2003), relatively little is known about its biology, how to monitor its densities on grapevines, or its potential damage to the grape crop. On pistachios, *F. gilli*'s seasonal phenology was recently described (Haviland et al. 2012), and some information has been presented on the efficacy of pesticides applied to pistachios (Haviland 2006). However, we found no published information for *F. gilli* populations in vineyards and the ensuing fruit damage.

F. gilli is probably native to southeastern United States and has likely been in California for quite some time; the earliest definite record is from Shasta County on *Catalpa* sp. in 1968 (Gullan et al. 2003). However, during the early period of this pest's presence in California, *F. gilli* samples were identified as the striped mealybug, *Ferrisia virgata* (Cockerell). Gullan et al. (2003) distinguished *F. gilli* as a new species, and Kaydan and Gullan (2012) recently presented a taxonomic revision of *Ferrisia*, confirming *F. gilli* as a separate species while noting the problems in distinguishing among species within this group. As a crop pest, *F. gilli* first appeared in pistachio and almond orchards in the San Joaquin Valley (Gullan et al. 2003). By 2006 it had spread to at least 3,000 acres of pistachios in 11 California counties, causing damage by staining shells and reducing yields (Haviland et al. 2006, 2012). *F. gilli* did not reach economically important densities in vineyards until it was found in El Dorado County in 2003. Located east of California's Sacramento Valley and spanning to the Sierra Nevada range, El Dorado is one of seven counties that comprise California wine grape crush district 10 and form the Sierra foothill appellation. *F. gilli* in El Dorado County vineyards quickly spread and, from 2005 to 2007, was the target of a joint eradication effort managed by the CDFA and the El Dorado County Agricultural Commissioner's office.

Mealybugs have become increasingly important vineyard pests owing to the damage that results from their feeding, honeydew and sooty mold accumulation, and their ability to transmit grapevine leafroll-associated viruses (GLRaV), particularly GLRaV-3 (Daane et al. 2012). Known vectors of GLRaV include the grape mealybug, *Pseudococcus maritimus* (Ehrhorn), the longtailed mealybug, *Pseudococcus longispinus* (Targioni–Tozzetti), the obscure mealybug, *Pseudococcus viburni* (Signoret), the citrophilus

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mealybug, *Pseudococcus calceolariae* (Maskell), the Comstock mealybug, *Pseudococcus comstocki* (Kuwana), *Planococcus ficus* (Signoret), the citrus mealybug, *Planococcus citri*, the apple mealybug, *Phenacoccus aceris* (Signoret), the Bohemian mealybug, *Heliococcus bohemicus* Sulc (Daane et al. 2012), and most recently, *F. gilli* (C.M.W., unpublished data). Each species has a unique life history, geographic origin and distribution, and set of biological traits that impact their pest status and the level of management needed. To aid in identification of vineyard mealybugs in North America, polymerase chain reaction (PCR) multiplex, which includes *F. gilli*, was recently developed (Daane et al. 2011) and is currently being used by vineyard managers in Oregon and Washington, where only *Ps. maritimus* has been reported as attacking grapes.

Current research in wine grapes has focused on the role of mealybugs with the incidence of grapevine leafroll disease (Rayapati et al. 2008; Tsai et al. 2008, 2010; Charles et al. 2009; Almeida et al. 2013), caused by a complex of virus genera within the family Closteroviridae, whose phylogeny of species and their variants are under proposed revision (Martelli et al. 2012, Sharma et al. 2011). Grape leafroll disease found in Sierra foothill vineyards is predominantly caused by GLRaV-2 and -3 (R.P.P.A., unpublished data), and the common mealybug species found in vineyards are *Ps. maritimus* and *F. gilli* (L.R.W., unpublished data).

The major aim of this research was to describe *F. gilli* seasonal phenology and damage in vineyards in California's Sierra foothills. Earlier studies reported *F. gilli* completed three generations per year and overwintered as immatures on pistachios in San Joaquin Valley orchards (Haviland et al. 2012). In pistachio, even small *F. gilli* populations are relatively easily seen on the leaves, fruit clusters, trunk, and scaffolding branches, alerting pest managers to the need for treatment. We understood that *F. gilli* vineyard infestations were not as easily sampled and presented a greater challenge for vineyard managers making treatment decisions. Therefore, we also conducted an initial assessment of *F. gilli* damage on untreated versus treated vines. The collected information is used to improve insecticide treatments based on local *F. gilli* biology in Sierra foothill vineyards, and has implications for vineyard managers worldwide because *F. gilli* has the potential to spread to new regions.

Materials and Methods

Sample Sites. Five commercial wine grape vineyards located in El Dorado County and known to be infested by *F. gilli* were studied. All vineyards were spur pruned on either a bilateral (four vineyards) or a vertical (one vineyard) cordon system and used standard fertilization, powdery mildew control, and irrigation practices for the Sierra foothill region. The five vineyards are referred to hereafter as A to E; vineyard cultivars and elevations differed, and were A: 'Merlot' at 425 m; B: 'Pinot Gris'at 1035 m; C: 'Mourvedre' and 'Grenache' at 730 m; D: 'Zinfandel' at 885 m; and E: 'Pinot Gris' and 'Pinot Noir' at 845 m. The difference in cultivar and elevation resulted in different harvest dates: late August for the Merlot, early September for the Pinot Gris and Pinot Noir, and late September to early October for the Zinfandel, Mourvedre, and Grenache. Insecticides applied for *F. gilli* also differed among the studied vineyards (Table 1) and were left to the vineyard managers' discretion. In vineyards A and B, samples were taken in 2008 only; in vineyards C to E, samples were taken in both 2008 and 2009. The vineyards also differed in rootstock, which is mentioned here as a possible contributing, but unknown, factor.

Table 1. Sierra foothill vineyards monitored

Vineyard	Insecticides applied against *F. gilli*	
	2008 season	2009 season
A-lower	Chlorpyrifos,[a] buprofezin,[b] and stylet oil[c]	—[d]
A-upper	Buprofezin[b] and stylet oil[c]	—[d]
B	Buprofezin[e]	—[d]
C	Acetamiprid and buprofezin[f]	Buprofezin[g]
D	Acetamiprid and buprofezin[f]	Buprofezin[g]
E	Buprofezin[h]	No insecticide

[a] Chlorpyrifos (Lorsban 4E, Dow AgroSciences LLC, Indianapolis, IN) was applied at 4.7 liters/ha between 18–19 Mar. in 561 liters water per hectare.

[b] Buprofezin (Applaud 70DF, Nichino America Inc., Wilmington, DE) was applied at 651.5 g/ha on 25 and 30 June in 561 liters water per hectare.

[c] Stylet oil (JMS Flower Farms Inc., Vero Beach, FL) was applied at 1.25% by vol, in 561 liters water per hectare between 8–16 June.

[d] Not sampled in the 2009 season.

[e] Buprofezin was applied at 840.6 g/ha in 935 liters water per hectare on 28 June.

[f] Mix of buprofezein at 840.6 g/ha and acetamiprid (Assail 70WP, Cerexagri-Nisso LLC, King of Prussia, PA) at 70.05 g/ha was applied in 1,122 liters water per hectare on 30 June (C) and in 1,178 liters water per hectare on 10 July (D).

[g] Buprofezin was applied at 840.6 g/ha in 1,122 liters water per hectare on 25 June (C) and at 888.2 g/ha in 1,178 liters water per hectare on 26 June (D).

[h] Buprofezin was applied at 840.6 g/ha in 701.25 liters water per hectare on 29 June.

In each site, a 0.4-ha plot (500–700 vines) was designated as the sampled population for monitoring using a destructive sampling method. In addition, grower cooperators agreed to have a smaller (20–90 vines) plot in each vineyard that was not treated with a pesticide(s) targeting *F. gilli.* Because *F. gilli* was a new pest with no known management program, only one plot in each vineyard was left untreated to minimize the risk to cooperating vineyard managers. At least two unsprayed rows of vines buffered the untreated plot from the insecticide-treated vines. The untreated plots were sampled using a nondestructive sampling method to better monitor *F. gilli* seasonal phenology. Monitoring was biweekly beginning in May and continuing through harvest, whereas postharvest monitoring was conducted monthly.

Destructive Sampling of Insecticide-Treated Vines. Vines were randomly selected for a timed destructive sampling, based on a methodology described by Geiger and Daane (2001). Each sampled vine was

searched for a total of 3 min, with experienced samplers looking for *F. gilli* anywhere on the vine that the mealybug was predominately found. Bark was peeled away; leaves were removed and inspected; and fruit clusters were destroyed to find and measure mealybug populations. On each sample date, 20 vines were randomly selected. The number of mealybugs found was recorded, and categorized by mealybug developmental groupings: immatures (second or third instars), adults, and adults with crawlers; condition: live or parasitized; and location on the vine: hidden under the bark or exposed on the canes, leaves, or fruit clusters. At the beginning of the season, each vineyard was mapped and a randomized list of sample vines was generated so that no vine was sampled twice.

Nondestructive Sampling of Untreated Vines. For the nondestructive sampling, 10 untreated vines with moderate *F. gilli* populations were selected in each of the five (2008) untreated plots. The vines did not receive any insecticides but did receive fungicides to control powdery mildew, *Uncinula necator* (Schwein.) Burrill. The vines were divided into seven sections—trunk base (5 cm below ground level to 30 cm above), trunk, armpit (between the spurs on the vertical cordon trained vines and under the cordon on the bilateral cordon trained vines), old spur positions (or old canes), new spur positions (new shoots or canes), leaves, and grape clusters (when present). On each sample date, each section was nondestructively searched for 3 min, with bark on the trunk and spurs carefully peeled back and replaced, and leaves and clusters searched in place, moving leaves and berries as best as possible to find mealybugs without destroying the habitat. For each vine section, mealybug density was recorded by development stage category, as described previously.

Fruit Cluster Damage. Mealybug damage to the fruit was assessed, on both insecticide-treated and untreated vines, using a 0–3 cluster rating scale based on previous studies with *Ps. maritimus* (Geiger and Daane 2001) and *Ps. viburni* (Daane et al. 2006). The rating scale varied according to mealybug density. In 2008, "0" represented no mealybugs; "1" represented honeydew, 10 mealybugs or less, or both; "2" indicated clusters with >10 mealybugs, sooty molds, or honeydew; and "3" was assigned to heavily infested unmarketable clusters. In 2009, the ratings were similar in vineyard E, but were adjusted in the two heavily infested vineyards (C and D), where "0" represents no mealybugs, "1" represents 1–5 mealybugs, "2" indicates 6–15 mealybugs, and "3" was assigned to heavily infested unmarketable clusters (>15 mealybugs).

The number of vines and clusters sampled per plot also varied, depending on plot size and mealybug density. In 2008, there were 200 clusters rated in the larger treated plots at one cluster per vine, and 200 clusters rated in the smaller (60–90 vines) untreated plots by sampling more than one cluster per vine. In 2009, cooperating growers reduced the size of the untreated plots in vineyard C and D, and this changed the available number of vines that could be sampled. To reduce variance, we sampled the same number of clusters per vine in both treated and untreated plots, but still had to vary the number of vines sampled. In vineyard C, we sampled five clusters from each of the 40 untreated vines and 40 randomly selected treated vines (200 clusters per plot). In vineyard D, we sampled 10 clusters from each of the 20 untreated vines and 20 randomly selected treated vines (200 clusters per plot). Vineyard E was not treated for mealybugs in 2009, and we sampled only 100 clusters per plot, with 10 clusters from each of the 10 untreated "monitored" vines.

Because *F. gilli* damage has never before been rated with this system, we also counted the total number of mealybugs per cluster in a subsample (50 clusters) of the rated clusters.

Natural Enemies. During all timed nondestructive and destructive counts, mummified mealybugs were also recorded as live or empty (i.e., emerged adult parasitoid) and in exposed or hidden locations. To increase the amount of available information on parasitoid species in Sierra foothill vineyards, *F. gilli* nymphs and apparently live mummies (i.e., mummies with no exit hole) were sporadically collected, individually placed into gelatin capsules, and held at room temperature to rear out adult parasitoids. When live mummies were found during routine sampling, they were collected. At harvest when greater numbers of mealybugs were present in clusters, both apparently live mummies and healthy nymphs were collected with the expectation that some healthy nymphs were parasitized. After at least 2 mo, the collected nymphs and mummies were checked and the number of dead or mummified mealybugs, and emerged adult parasitoids were recorded. The emerged parasitoid species were placed in 70% alcohol and later identified to family or genus.

During each biweekly sampling, the number of larval and adult lady beetles, lacewings, spiders, and other generalist predators were also recorded.

Statistics. In-season monitoring results are presented as mealybug population density (log [mean + 1]) for adult females and crawlers, and immature (second and third instars) stages. Comparisons of seasonal phenology and density were made by visually assessing graphed *F. gilli* counts among sampled vineyards. Location of *F. gilli* on the seven different vine sections was also summarized across all five (2008) and three (2009) vineyards by averaging across the nearest sample date, within a 1-wk period.

When comparing untreated and insecticide-treated vines sampled within each vineyard, timed counts were transformed to total mealybugs per 3-min count, which may have underestimated the numbers of mealybug on the untreated vines where whole vine samples required 3-min counts on each vine section on each sample date (21 min per vine). Within each of the vineyards monitored, there was no plot replication of insecticide-treated and untreated vines, and the insecticides used varied among sites (Table 1). Therefore, there was no replication in each site. To measure any impact of insecticides within each of the sampled vineyards, data of the season-long treatment effects

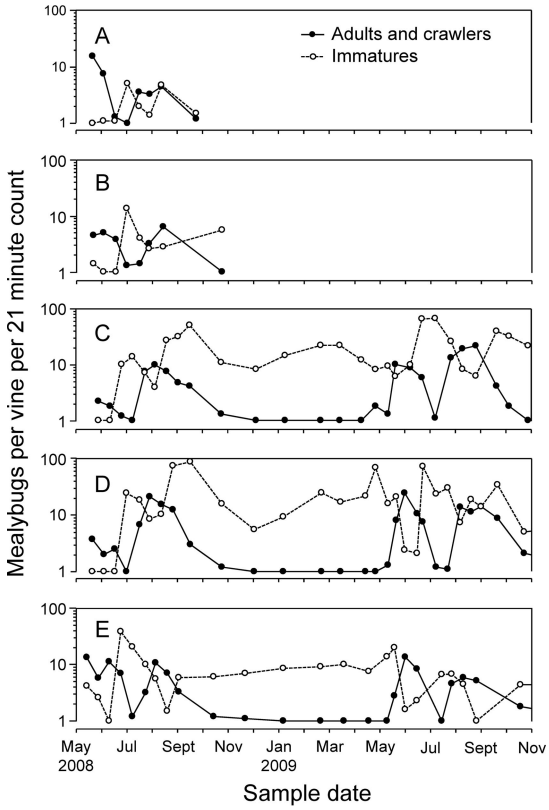

Fig. 1. *F. gilli* population density (log [mean + 1]) for adult females and crawlers, and immature (second and third instars) stages in five Sierra foothill vineyards (A–E).

(untreated vines versus insecticide treatments) on mealybug density were analyzed using a general linear model for each paired comparison for each of the five vineyards surveyed in 2008. The model used the total mealybugs per 3-min search per vine section as a function of treatment, sample date, and treatment × sample date interaction. Sample date was set as a categorical variable to exclude its impact on treatment. If the treatment × sample date interaction term is not significant ($P > 0.05$), this analysis is equivalent to an analysis of covariance (ANCOVA) with sample date as the covariate.

For fruit damage ratings, the treatment effects were compared using contingency tables, with treatments separated in each vineyard using Pearson's χ^2 analysis.

Results

Nondestructive Sampling in Untreated Vines. The seasonal presence and density of *F. gilli* mature adult females and gravid adults with crawlers (these categories were combined for better graphic presentation), and immatures show two broods of crawlers per year, with one complete and one overwintering generation per year (Fig. 1). The population overwinters as immatures, with no mature gravid adult females found during the winter months, which was most clearly seen in those vineyards sampled for two consecutive years (Fig. 1C–E). In each vineyard monitored, there were two peak periods of immatures present on the vine in the summer and fall, although the timing of those peaks varied among vineyards. Generally, the overwintered *F. gilli* immatures developed to mature adults and then gravid adults with crawlers by mid-June to early July (first generation). These nymphs matured to gravid adults by late July to early August and produced crawlers by late August to mid-September. The crawlers typically developed to second or third instars to form the overwintering generation (Fig. 1).

Among the five vineyards monitored in 2008, there was variation in *F. gilli* population density and seasonal development. Vineyards A and B had the lowest populations, peaking in the first generation with a mean of 4.1 and 12.6 nymphs per sample, respectively, and then remaining quite low thereafter, averaging less than five mealybugs per vine for the rest of the 2008 season (Fig. 1A and B). In 2008, the other three vineyards had much higher *F. gilli* population densities, with second-generation peak counts at 48.9 and 85.1 nymphs per sample on 16 September (Fig. 1C and D, respectively) and a first-generation peak count at 37.8 nymphs per sample on 24 June (Fig. 1E).

The three vineyards with higher mealybug densities were sampled over the winter and throughout the 2009 season. During the winter of 2008–2009, the numbers of immatures remained relatively constant, with the counts in late February 2009 at 20.8, 23.5, and 8.2 nymphs per sample (Fig. 1C–E, respectively). Overwintering immatures molted into adults by May, with peak counts on 22 May at 8.8 and 23.2 adults per sample (Fig. 1C and D, respectively) and on 2 June at 11.7 adults per sample (Fig. 1E). The 2009 population density and age structure also showed two broods per year, similar to 2008 data. Gravid adults were first observed on 9, 23, and 16 June, although recorded at low densities (Figs. 1C–E, respectively). Counts of nymphs peaked ≈1 mo later on 9 July at 64.9 nymphs per sample (Fig. 1C), on 23 July at 70.3 nymphs per sample (Fig. 1D), and on 16 July at 5.7 nymphs per sample (Fig. 1E). By early August, gravid adults were found, and the second peak of nymphs was most clearly observed on 21 September at 37.9 and 32.8 nymphs per sample in vineyards C and D, respectively (Fig. 1C and D).

Results from the vine-section monitoring revealed that the *F. gilli* populations moved among vine sections throughout the season, from cryptic positions on the trunk and spurs during the winter to more exposed locations on the shoots and leaves in spring and summer. *F. gilli* overwinters as immatures (second and third instars) primarily under bark at the trunk base, trunk, and, to a lesser extent, under the cordon armpit (Fig. 2). In May 2008, when monitoring in the vineyards began, immatures and adults were found under the bark of the cordon and old spurs, at the base of the spurs, and on new shoots. As the population matured in spring, the percentage of mealybugs found on new spurs and new shoots on 21 May ranged from 25%

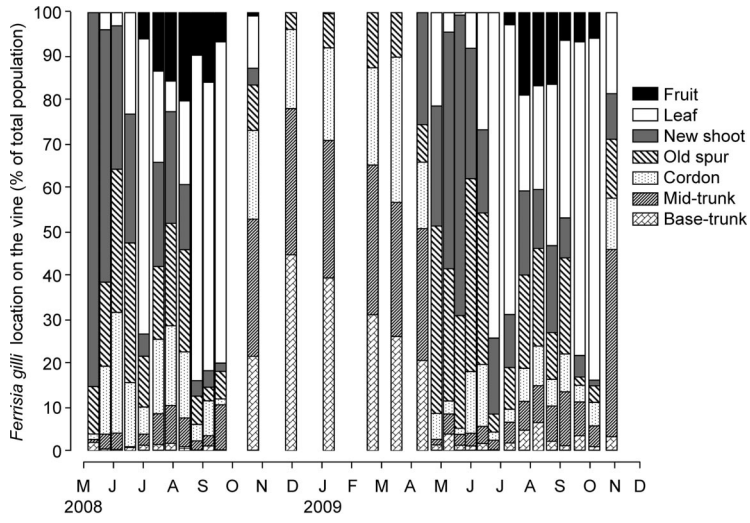

Fig. 2. Seasonal changes in the location of *F. gilli* recorded on untreated vines that were searched in seven sections—trunk base (5 cm below ground level to 30 cm above), trunk, armpit (between the spurs on the vertical cordon trained vines and under the cordon on the bilateral cordon trained vines), old spur positions (or old canes), new spur positions (new shoots or canes), leaves, and grape cluster (when present). Data were collected in 2008 (five vineyards sampled) and 2009 (three vineyards sampled), El Dorado Co., CA.

(vineyard C) to 85.4% (vineyard E), whereas the percentage of mealybugs found at the old spur position ranged from 10.8% (vineyard E) to 33% (vineyard C), and there was still a large population on the ventral side of the cordon (or "armpit") at some sites (42% at vineyard C).

In early to midsummer 2008, the first summer generation had begun (Fig. 1) and the majority of mealybugs were crawlers (first instars) and more actively moving onto the leaves (Fig. 2). In late June and early July, for example, 46.3, 86, 77.2, and 87.7% of the mealybugs were found on the leaves in vineyards A, B, C, and D, respectively. As these immature stages developed to mature and gravid adults from late June through mid-July, there was some movement back to the protected locations under the bark on cordon, old spurs, and new shoots, and a portion of the population moved into grape clusters beginning in July (Fig. 2). In late July 2008, for example, within individual vineyards, we recorded 29–45% of the mealybugs on new spurs, 15–34.9% on old spurs, 12–20.4% on the armpit of the cordon, and only 3.2–5.3% were on leaves and 1.6–7% in clusters. In late July 2009 (vineyards C to E), there was a similar movement to the more protected sections as the females matured and prepared to produce crawlers. Within individual vineyards, a greater percentage of the population was found on the leaves and in the clusters than in 2008: 16.8–22.6% of the mealybug population on new spurs, 17–24% on old spurs, 3.2–11.4% on the cordon, 19–26.6% on leaves, and 14.7–22.3% in fruit clusters. The populations' seasonal movement from protected locations to leaves was repeated during production of crawlers that began the second generation, observed from late August into October (Fig. 2). By late September in vineyards C and D, for example, 78–84% and 66–77% of the population were observed on leaves in 2008 and 2009, respectively, whereas only 7–13.5% were on fruit clusters.

By harvest time, the percentage of the population found in fruit clusters ranged from 3% (vineyard D, 2009) to 19.4% (vineyard E, 2008). The leaves appeared to remain a preferred feeding site at this time (Fig. 2). Postharvest monitoring (late October) showed most mealybugs moving to cryptic positions under bark of the trunk, old spurs, and trunk base (Fig. 2). For example, in vineyard C, we recorded 51.5 and 89.8% of the mealybugs under and on trunk bark on the late October samples in 2008 and 2009, respectively. The preferred overwintering location and movement to these sites did vary among vineyards and season. In vineyard D, for example, 28.4% of the mealybugs were at the base of the trunk, 24.3% were under or on the trunk, 19.6% were under the armpit, and 9.5% were on the old spurs in late October 2008, whereas 51% of the mealybugs were on leaves, 25.5% were on the old spur positions, 9.8% were at the new spur positions, and only 9.8% were on or under trunk bark in late October 2009. By November, the *F. gilli* populations were located primarily on old wood under the bark of the base of the trunk, on mid-trunk, or on the cordon (primarily on the armpit) (Fig. 2). For example, in vineyards C and D, only 0–2.4 and 2.2–14.9% of the recorded mealybugs were found on the old spurs (2008 and 2009, respectively); the rest of the population was under the bark of older wood sections.

Destructive Sampling. In 2008, the season-long counts of mealybugs were lower on the insecticide-treated vines, as compared with the no insecticide vines, for each of the five vineyards (Fig. 3).

The 2008 fruit cluster ratings showed a pattern similar to the season long counts, with less mealybug

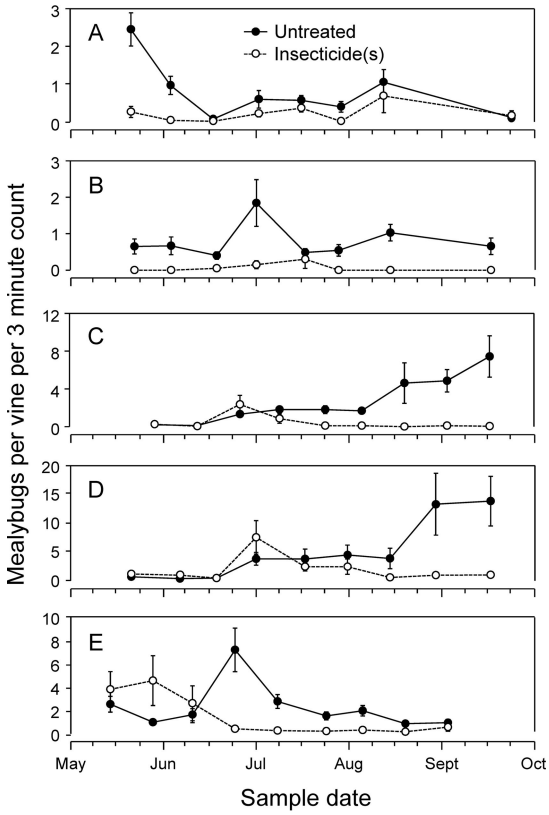

Fig. 3. *F. gilli* population density (mean ± SE) was lower on vines treated with insecticides in each of five sampled vineyards (A–E, see Table 1 for the insecticide applications in 2008, letters A to E correspond to the described vineyards); season-long mealybug density was compared for each vineyard using a general linear model and setting sample date as a categorical value (vineyard A: $F = 3.789$; df = 16, 363; $P < 0.001$; vineyard B: $F = 6.852$; df = 17, 177; $P < 0.001$; vineyard C: $F = 3.068$; df = 17, 252; $P < 0.001$; vineyard D: $F = 5.332$; df = 17, 252; $P < 0.001$; and vineyard E: $F = 8.735$; df = 17, 252; $P < 0.001$).

damage in insecticide-treated vines in four of five vineyards (Fig. 4A). Vineyards C and D had the highest mean number of mealybugs in untreated plots (Fig. 3A) and also had the highest fruit cluster damage in untreated plots (Fig. 4A). The percentage of clusters that were rated a 0 (no mealybugs present) ranged from 84.5 to 96.4% in the insecticide-treated blocks, compared with 58.5–90% in the untreated blocks; data of 0 ratings are not shown (in Fig. 4A) to provide a better visual representation of mealybug damage. Relatively few clusters were rated a 2 (>10 mealybugs): 0–1.75 and 1.0–8.5% in the treated and untreated plots, respectively. Only a few clusters (<3%) were rated a 3 (unacceptable damage). In addition, in 2008 the rated fruit clusters were also dissected to determine the total mealybug counts per cluster, which was 2.46 ± 0.68 mealybugs per cluster (range: 0.04–5.06) in the treated plots and 9.33 ± 2.77 mealybugs per cluster (range: 2.10–15.94) in the untreated plots (n = 50 clusters per plot per vineyard).

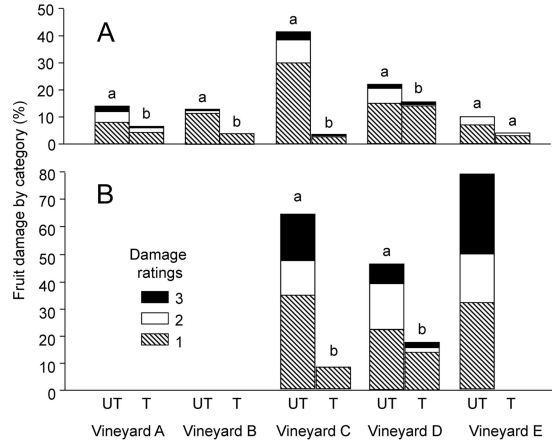

Fig. 4. *F. gilli* fruit damage ratings in five (2008) (A) and three (2009) (B) Sierra foothill vineyards (A to E) with vines either treated (T) or untreated (UT) with insecticides (see Table 1 for the insecticide applications, letters A to E correspond to the described vineyards) show lower fruit damage in insecticide-treated vines in 2008 and 2009 seasons (2008: vineyard A: $\chi^2 = 9.93$; df = 3; $P = 0.019$; B: $\chi^2 = 11.14$, $P < 0.001$; vineyard C: $\chi^2 = 90.76$, $P = 0.004$; vineyard D: $\chi^2 = 9.12$, $P = 0.028$; and vineyard E: $\chi^2 = 5.587$, $P = 0.061$; 2009: vineyard C: $\chi^2 = 142.9$, $P < 0.001$ and vineyard D: $\chi^2 = 47.85$, $P < 0.001$). In 2008 (all vineyards), 0 = no mealybugs, 1 ≤ 10 mealybugs, 2 ≥ 10 mealybugs, and 3 = heavily infested unmarketable clusters; in 2009, the ratings were similar in vineyard E, but were adjusted in vineyards C and D, where 0 = no mealybugs, 1 = 1–5 mealybugs, 2 = 6–15 mealybugs, and 3 = heavily infested unmarketable clusters (>15 mealybugs).

In 2009, there was significantly less fruit damage in the insecticide-treated plots compared with the untreated plots in vineyards C and D (Fig. 4B). The figure shows a dramatic increase in fruit damage in the untreated plots from 2008 to 2009 in each of the three vineyards sampled. In 2008, in vineyard C, 58.5% of the clusters were clean, but only 35.5% of the clusters were clean in 2009. Likewise, 78% of the clusters in vineyard D were clean in 2008, whereas only 53.5% of the clusters measured were rated a 0 in 2009. The percentage of clusters rated in each damage category increased from 2008 to 2009, including the number of unmarketable fruit. In vineyard C, clusters rated as unmarketable increased from 3 to 17% and in vineyard D the unmarketable fruit increased from 1.5 to 7%. Vineyard E did not receive any insecticides for mealybugs in 2009 (Table 1), and there 79% of the clusters had some level of mealybug infestation (ratings of 1–3) and 29% of these were rated as unmarketable (Fig. 4B).

Natural Enemies. In 2008, a total of 409 live nymphs and mummified mealybugs (apparently live, without exit holes) were collected from untreated and treated vines. The majority of the 2008 collection, 365 of the 409, was live nymphs (no evidence of parasitism) collected in summer and fall from untreated vines; 73 nymphs were collected in July and August, and 292 nymphs were collected from clusters before or after

harvest in September and October. Nymphs were collected in 2008 regardless of whether they appeared parasitized. There were 187 mummified (apparently live, without exit holes) mealybugs collected from untreated vines in 2009; most of these (115) were collected from untreated clusters during the fruit cluster evaluation before harvest in vineyard C on 9 October 2009. From the 2008 collection, only five parasitoids emerged, whereas adult parasitoids emerged from 18 of the 187 (9.6%) mummified mealybugs collected in 2009, and an additional 19 of the 187 mummies collected in 2009 had a parasitoid present inside the mummy that did not successfully emerge. When we include both emergence records, the total parasitism of the 2009 collection was 37 of 187 (19.8%). The collection and location that yielded the greatest number of mummies was before harvest in the fruit clusters in vineyard C, where of the 200 untreated clusters we measured, 38 (19%) had at least one mummy, apparently live or exited, present. Of the 115 apparently live mummies collected from untreated clusters in vineyard C, 27 (23.5%) had parasitoids present inside the mummy and adult parasitoids emerged from 9 of the 27.

More than 95% of the emerged parasitoids were *Acerophagus* species. The number of adult *Acerophagus* that emerged from each mummy ranged from 1 to 12. Adult *Acerophagus* were also observed during field-monitoring studies of stinging and parasitizing mealybug nymphs. A hyperparasitoid (Signiphoridae) and an unidentified encyrtid parasitoid also emerged.

Generalist predators observed in the monitored vineyards included lady beetles (Coccinellidae), lacewings (Chrysopidae), preying mantids (Mantidae), damsel bugs (Nabidae), snakeflies (Raphidiidae), and spiders. Although present, numbers of generalist predators were generally low.

Discussion

Our studies of *F. gilli* seasonal biology in California's Sierra foothill vineyards revealed several key points that impact management decisions. First, we show *F. gilli* produced two broods of crawlers per year, two generations, in Sierra foothills vineyards. We describe the general seasonal phenology of the population (Fig. 1): *F. gilli* give birth to crawlers (first instars) rather than producing an ovisac (Gullan et al. 2003), the periods of first instar presence were mid-June to early July and late August to mid-September. Haviland et al. (2012) reported three generations (two and a partial overwintering third) per year for *F. gilli* on pistachios in the San Joaquin Valley. The difference is not surprising, not only were the populations on different hosts, but the average monthly maximum temperatures from May to August in Tulare County were 2.6–4.6°C warmer in 2008 and 3.5–6.6°C warmer in 2009 than those in El Dorado County (California Irrigation Management Information System [CIMIS] 2012), where the sampled vineyards were from 425 to 1035 m in elevation. Depending on vineyard location and seasonal temperature, a partial third generation may occur for *F. gilli* in grapes as well. The *F. gilli* seasonal phenology is similar to that of *Ps. maritimus*, which has been reported to have two generations per year in wine grape vineyards in Washington (Grimes and Cone 1985, Bahder et al. 2013) and California's coast regions (Bentley et al. 2013), and two and a partial third generation on table and raisin grapes in the San Joaquin Valley (Geiger and Daane 2001). We found that crawlers from the second brood typically developed to second or third instars to form the overwintering generation (Fig. 1). Similarly, Haviland et al. (2012) reported that the immature stages, particularly the first instar, were the overwintering stages in pistachios, whereas in this study more second and third instars were found during the winter months than first instars (Fig. 1). This may be in part because of the sample design (timed counts) and the difficulty in finding the small (≈1 mm) first instars in the cracks and crevices under the vine bark during the winter samples. Moreover, the timed-count sampling program used would overweigh mealybug density on those vine sections where mealybugs were easier to find (e.g., leaves).

Second, we provide the first description of *F. gilli* populations' seasonal movement on the vine (Fig. 2). The population overwinters primarily under the bark of the trunk base, mid-trunk, and cordon. Similarly, the *F. gilli* population on pistachio overwintered on the trunk and main scaffolds of the pistachio tree (Haviland et al. 2012), and *Ps. maritimus* and *Ps. viburni* were reported to overwinter on the vine's trunk, cordon, and spurs in the San Joaquin Valley (Geiger and Daane 2001) and Central Coast region (Daane et al. 2007). As the immatures develop to adults in spring, there is a movement to the spurs and new shoots (Fig. 2), where the production of crawlers for the first generation primarily takes place and the resulting crawlers and immatures then move to the leaves. This is an important time for monitoring to determine the size and location of *F. gilli* populations.

Monitoring for vineyard mealybugs can be labor intensive (Geiger and Daane 2001). Although pheromones have been characterized and synthesized for *Ps. maritimus* (Zou et al. 2010), *Ps. viburni* (Millar and Midland 2007), *Ps. longispinus* (Millar et al. 2009), and *Pl. ficus* (Hinkens et al. 2001) and can be used to monitor population densities (Millar et al. 2002, Walton et al. 2004, Bahder et al. 2013), the *F. gilli* sex pheromone has not been identified. However, canopy shoot thinning typically occurs in May to early June, and field crews can be trained to identify and flag vines with *F. gilli*, which at this stage is fairly large (3–4 mm as an adult) and visible. We have successfully led training sessions with farm workers on *F. gilli* identification. Several of our grower cooperators report that well-trained field crews have helped locate new infestations during this late spring period.

Combining seasonal density (Fig. 1) and phenology (Fig. 2), we show that at the beginning of the first and the second generation, the immature *F. gilli* were found primarily on the leaves. On pistachios, the *F. gilli* population also fed on the leaves in the first genera-

tion, but only a small percentage of the population, with most of the second and third generation found in the pistachio fruit cluster (Haviland et al. 2012). Mealybug feeding location in pistachio corresponded with carbohydrate allocation in the pistachio tree as shown in Spann et al. (2008). In Sierra foothill vineyards, a much smaller portion of the *F. gilli* population was in the fruit clusters. This may, in part, be because of the late maturation of the fruit clusters, which were harvested from late August to early October, or the food quality (e.g., carbohydrate levels) of grape versus pistachio leaves for immature *F. gilli.*

The exposed positioning of a large portion of the immature *F. gilli* population on the leaves in June and September (Fig. 2) has implications for insecticide-based control programs. Our comparison of insecticide-treated and untreated plots within each vineyard (A to E) show the various insecticide applications (Table 1) reduced mealybug season-long density (Fig. 3) and crop damage (Fig. 4). The two in-season insecticide materials used were an insect growth regulator (IGR) (buprofezin) and a contact neonicotinoid (acetamiprid). Application of either material in June, when the mealybugs are exposed, has been widely adopted by pistachio growers with *F. gilli* infestations in the San Joaquin Valley (Haviland 2006, Bentley et al. 2009). Before our study, vineyard growers were applying treatments for *F. gilli* on 4 July, based on a rough extrapolation of phenology in the San Joaquin Valley. We found first brood *F. gilli* crawlers on leaves in late June to early July, a key window for applying insecticides while crawlers are exposed in the canopy and before they move into clusters. During our study, we were able to communicate with grower cooperators about the presence of *F. gilli* crawlers to more accurately time treatments, sometimes a week or more earlier than the previous 4 July benchmark. As a result, most vineyard managers used a late-June application (Table 1) to target the exposed immature stages as they were moving onto the vine leaves. The need for an insecticide treatment was readily apparent by the amount of damage in the untreated plots in 2009 (Fig. 4). Additional studies are ongoing to compare insecticides and application timing in replicated trials (L.R.W. et al. unpublished data).

Worldwide there are several parasitoid species that attack *Ferrisia* sp. (Noyes and Hayat 1994), but most published records cite the importance of predators as control agents (e.g., Mani and Krishnamoorthy 2008). Little is known, however, about natural enemies that attack *F. gilli*, due primarily to its recent designation as a new species (Kaydan and Gullan 2012, Gullan et al. 2003). California surveys of *F. gilli* in alternate hosts such as almond, grape, and persimmon have found parasitism by wasps in the genera *Acerophagus* (=*Pseudaphycus*), *Chysoplatycerus*, and *Anagyrus* (Haviland et al. 2006, 2012; L.R.W. and K.M.D., unpublished data). In our 2008 collections of live mealybugs, we reared only a few parasitoids (including *Acerophagus*). This may be because of the fact we collected mostly mealybug nymphs instead of visible mummies, so there was a greater chance they were not parasitized. In 2009, we focused on collecting apparently live mummies from untreated vines before harvest, and we were more successful. A number of the 2009 collected mummies had adult parasitoids that were very visible upon dissection, but did not emerge. We identified *Acerophagus* sp. as the main parasitoid that emerged, which account for a reasonable season-long parasitism level of ≈10%, based on a comparison of recorded mummies to recorded live mealybugs in untreated plots. The most likely *Acerophagus* species reared from the collected *F. gilli* would be *Acerophagus meracus* Gahan, *Acerophagus mundus* Gahan, or *Acerophagus meritorus* Gahan, which have all been reported from *F. virgata* (Noyes and Hayat 1994). Although we have tentatively identified the specimens as either *A. meritorus* or *A. mundus*, the original species descriptions provided by Gahan do not provide clear separation of these species (Daane et al. 2008). To date, parasitism of *F. gilli* in pistachios has been negligible, most likely owing to applications of broad-spectrum insecticides routinely applied to control bugs (Miridae, Rhopalidae, Pentatomidae, and Coreidae) and other pistachio insect pests (Haviland et al. 2012). In contrast, *Acerophagus* and other parasitoids attacking *F. gilli* found in almonds in the San Joaquin Valley has provided effective control (K.W. Daane, personal observation). Future work in Sierra foothill vineyards will determine whether this difference results from the parasitoids temperature limits in the relatively cooler wine grape region, or the additional annual generation of *F. gilli* provides a better source of available host material for *Acerophagus.*

Our aim was to improve pest management of *F. gilli* in Sierra foothill vineyards. Aided by prior work in pistachios (Gullan et al. 2003, Haviland et al. 2012), we improved upon insecticide programs by describing the seasonal phenology and feeding locations of *F. gilli* on the vine. The population moves to the new shoots in April and May, providing a window to visually monitor for mealybug presence and then schedule treatments for when the first instars move to the leaves, where they would be easier to target with an IGR or neonicotinoid. Although there is not a commercial pheromone to assist in monitoring efforts, we estimate that as of 2012, *F. gilli* is in ≈350 acres of wine grapes grown in El Dorado County, and continues to spread in geographic distribution. In 2009, it was found in Lake County, CA (CDFA 2013), and has apparently spread there as well. It is anticipated that information contained in this manuscript will provide beneficial information on biology and management of *F. gilli* in vineyards, should this pest spread to other regions where grapes are produced.

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