Pheromone-Based Monitoring of *Pseudococcus maritimus* (Hemiptera: Pseudococcidae) Populations in Concord Grape Vineyards

Author(s): B. W. Bahder , R. A. Naidu , K. M. Daane , J. G. Millar , and D. B. Walsh
Source: Journal of Economic Entomology, 106(1):482-490. 2013.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/EC12138

Pheromone-Based Monitoring of *Pseudococcus maritimus* (Hemiptera: Pseudococcidae) Populations in Concord Grape Vineyards

B. W. BAHDER,[1,2] R. A. NAIDU,[3] K. M. DAANE,[4] J. G. MILLAR,[5] AND D. B. WALSH[1]

J. Econ. Entomol. 106(1): 482–490 (2013); DOI: http://dx.doi.org/10.1603/EC12138

ABSTRACT The grape mealybug, *Pseudococcus maritimus* (Ehrhorn), is the dominant mealybug in Washington's Concord grape vineyards (*Vitis labrusca* L.). It is a direct pest of fruit clusters and a vector of grapevine leafroll-associated viruses. Using traps baited with the sex pheromone of *Ps. maritimus*, we determined the optimal trap density for monitoring *Ps. maritimus*, with the goal of providing a more rapid monitoring method for *Ps. maritimus* than visual surveys. Varying densities of pheromone-baited traps (one, four, and eight traps per 12.14 ha) were deployed in Concord vineyards to monitor *Ps. maritimus* seasonal phenology in 2010 and 2011. In both years, flights of adult males were detected in early May and captures peaked twice per season in mid-June and mid-August, indicating two generations each year. Trap data were analyzed using Taylor's Power Law, Iwao's patchiness regression, and the K parameter of the negative binomial model to determine optimal sample size. The formula using the K parameter provided the lowest required sample size, showing that four to eight traps per 12.14 ha were needed to provide 30% sampling precision efficiency throughout the entire season. Fewer traps were needed during flight peaks when trap capture numbers were great. Only one pheromone-baited trap per 12.14 ha was sufficient to provide *Ps. maritimus* flight phenology data to make informed management decisions. Species-specific pheromone-baited traps deployed for *Planococcus ficus* (Signoret), *Pseudococcus longispinus* (Targioni Tozzetti), and *Pseudococcus viburni* (Signoret) did not detect any of these species in the vineyards sampled.

KEY WORDS grape, mealybug, monitoring, pheromone, Washington state vineyard

The grape mealybug, *Pseudococcus maritimus* (Ehrhorn), was first reported as a pest in Washington state in 1950 (Frick 1952). In Washington, *Ps. maritimus* populations have been reported to infest a variety of fruits including apricot (Madsen and McNelly 1960), grape (Frick 1952, Cone 1971), and pear (Doutt and Hagen 1950). Historically, economic losses in vineyards were caused by the mealybugs' honeydew, which provided a substrate for the growth of sooty mold and other food contaminants, or direct infestation of the grape clusters by mealybugs (Daane et al. 2012). However, *Ps. maritimus* is capable of transmitting grapevine leafroll-associated viruses (GLRaVs; Golino et al. 2002, Tsai et al. 2011), which are the causal agents of grapevine leafroll disease (GLRD; Golino et al. 2002, Rayapati et al. 2008), the most important viral disease of grapevines worldwide (Martelli et al. 2002), thus elevating the economic consequences of *Ps. maritimus* infestations.

Ps. maritimus females have three larval instars and an adult stage, whereas males have three larval instars, a pupal stage (fourth instar), and a winged adult stage (Grimes and Cone 1985b). Stages present on the vine and locations and densities of mealybugs vary seasonally (Daane et al. 2012). *Ps. maritimus* populations typically complete two generations per year, overwintering under the bark of the trunk, cordon, or spurs as eggs in the cotton-like ovisac, or as first instar crawlers (Geiger and Daane 2001, Grasswitz and James 2008). With warming spring temperatures, part of the population moves upward to feed on the canes and leaves for several weeks and then moves back to protected locations under the bark to complete development. Gravid females also deposit ovisacs under loose bark. Eggs from the second generation hatch in midsummer, and the immature *Ps. maritimus* repeat the upward movement on the vine, with the mealybugs commonly infesting grape clusters touching the vine trunk or spurs (Geiger et al. 2001). As temperatures cool in the fall, much of the mealybug population moves back to protected locations and mated females from this summer generation oviposit under the bark, establishing the next overwintering generation.

Historically, management of *Ps. maritimus* in Washington's Concord cultivar grapes relied on applica-

[1] Department of Entomology, Washington State University, Irrigated Agriculture Research and Extension Center (IAREC), 24106 N. Bunn Road, Prosser, WA 99350-8694.

[2] Corresponding author, e-mail: bbahder@wsu.edu.

[3] Department of Plant Pathology, Washington State University, Irrigated Agriculture Research and Extension Center (IAREC), 24106 N. Bunn Road, Prosser, WA 99350-8694.

[4] Department of Environmental Science, Policy, and Management, University of California, Berkeley, 137 Mulford Hall, Berkeley, CA 94720-3114.

[5] Department of Entomology, University of California, Riverside, 3401 Watkins Drive, Riverside, CA 92521.

0022-0493/13/0482–0490$04.00/0

tions of broad-spectrum organophosphate insecticides (Grimes and Cone 1985a), many of which have lost their efficacy because of the development of resistance (Flaherty et al. 1982), or are no longer registered for use in vineyards. Currently, the predominant *Ps. maritimus* treatment in Washington wine grapes involves chemigation, whereby imidacloprid is applied through the drip system and the vines take up the material systemically (Daane et al. 2006). However, most of Washington's Concord cultivar vineyards are irrigated by overhead sprinklers, eliminating the option of chemigation, and thereby leaving foliar sprays as the primary control tool. With some foliar materials, application timing is important to target immature mealybugs (crawlers through second instars) that have less of the protective waxy coating than adults, and which are also mobile, allowing them to get more exposure to the applied insecticide(s). The efficacy of foliar insecticides can be further limited because the dense canopy structure in many Concord cultivar vineyards reduces insecticide coverage and because of the seasonal movement of mealybugs from exposed to more protected locations on the vine described above.

For the above reasons, monitoring for *Ps. maritimus* constitutes a crucial component of vineyard integrated pest management (IPM) programs, to detect small populations that could result in the transmission of GLRaVs, and to time foliar insecticide applications to the presence of immature stages. However, developing comprehensive monitoring guidelines for *Ps. maritimus* has been problematic because of the mealybugs' cryptic nature, their clumped distribution within vineyards, and their distribution on different parts of the vine during different times of the year (Geiger and Daane 2001). Sampling techniques for *Ps. maritimus* have included labor-intensive visual counts based on a unit area or time, which targeted both immature mealybugs and adult females, and sampling a number of randomly selected vines within a block (Grimes and Cone 1985b, Geiger and Daane 2001). Traditionally, adult males of *Ps. maritimus* have not been sampled due in part to their small size and ephemeral presence. However, the recent identification of the *Ps. maritimus* sex pheromone (Figadère et al. 2007, Zou et al. 2010) provided the means to develop a sensitive and selective method to sample male mealybugs with pheromone-baited traps.

The primary objectives of this study were to determine the seasonal flight activity of adult males of *Ps. maritimus* in Washington Concord cultivar vineyards, and to determine optimal pheromone trap densities in vineyards to use in a *Ps. maritimus* monitoring program. A secondary objective was to use pheromone traps to monitor for the presence of three mealybug species known to be present in western United States vineyards but not yet recorded from vineyards in Washington. These are *Planococcus ficus* (Signoret), *Pseudococcus longispinus* (Targioni Tozzetti), and *Pseudococcus viburni* (Signoret) (Daane et al. 2008).

Materials and Methods

Field Test Sites. Experiments were conducted during the 2009–2011 growing seasons in commercial Concord cultivar vineyards located near the Washington State University Irrigated Agriculture Research and Extension Center (IAREC) in Prosser, WA. These vineyards were managed with standard commercial practices, with the exception that no insecticides were applied against *Ps. maritimus.* They are referred to by vineyard site or owner as Alguist (Alq), Boast (Boa), Cabbage (Cab), Daves (Dav), Hogue (Hog), Little (Lit), New Vineyard (NV9), Sunridge (Sun), and Vanhinkin (Van).

Pheromone-Baited Traps. In all trials, sex pheromones 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) were synthesized as previously described and loaded on 11 mm gray silicon rubber septa (West Pharmaceutical Services, Lititz, PA) as hexane solutions (25 μg of racemic pheromone in 25 μl of hexane), separately for each species. Pheromone lures were deployed in delta sticky traps (Suterra Inc., Bend, OR) and placed in the upper vine canopy as described by Walton et al. (2004).

Trap Density. In 2009, 10–13 traps baited with *Ps. maritimus* pheromone were placed in each of 12 vineyards to help locate sites with measurable mealybug densities. Each site was large enough to be divided into multiple 12.14 ha plots. Traps were deployed from 23 June to 1 October 2009; traps were collected and adult male mealybugs were counted weekly, with the pheromone lures replaced every 6–8 wk.

From the vineyards sampled in 2009, seven vineyards with measurable *Ps. maritimus* populations were selected for study in 2010. Each vineyard was divided into three 12.14 ha plots. Traps were deployed at densities of one, four, or eight *Ps. maritimus* pheromone-baited traps per 12.14 ha. Traps in the four- and eight-trap density treatments were placed 10 vines apart in a single row. Traps were deployed from 20 May to 27 October 2010. Each vineyard had all three trapping densities set in a randomized block design, with each vineyard serving as a replicate.

Additionally, in 2010, traps baited with the pheromones of *Ps. viburni*, *Ps. longispinus*, *Pl. ficus*, or blank control lures were deployed in each of the seven sampled vineyards, with one trap per species (or control) per vineyard. Any mealybugs captured in these traps were collected, stored at −80°C and later identified using multiplex polymerase chain reaction (PCR) methods, as described by Daane et al. (2011).

In 2011, nine vineyards were selected for study, each with one 12.14 ha plot. Each plot had only one trap density (one, four, or eight), with treatments established in a completely randomized design among the nine vineyards, resulting in three replicate vineyards for each trap density. Traps were deployed from 12 April to 24 October 2011.

Data Collection and Analysis. Trapped male mealybugs were counted using a Leica MZ7.5 dissecting

microscope (Leica, Wetzlar, Germany). Data were analyzed using Systat 12 (Systat Software, Inc. 2007) and STATGRAPHICS Plus Version 5.0 (Statistical Graphics Corp. 2000). Data are presented as means (±SE). Analysis of variance (ANOVA) was used to determine treatment effects on each sample date and differences in average trap captures among vineyard sites, with vineyards modeled as a blocking factor, and treatment means separated using Tukey's pairwise comparisons ($P < 0.05$). Trap data were also analyzed to determine treatment effects on the mean number of adult male *Ps. maritimus* captured per trap among trap densities over time (season-long capture patterns) using Repeated Measures ANOVA.

Sample Size. A number of formulae have been used to express a population's spatial distribution, typically based on the variance to mean relationship from collected samples of the population (Karandinos 1976, Young and Young 1998). Taylor (1961) related the variance and mean as: $s^2 = ax^b$, where s^2 is the sample variance and x is the sample mean, which is solved with the linear regression of the natural logarithms: $\ln(s^2) = \ln(a) + b\log(x)$. The slope b, or Taylor's coefficient, also describes the population's distribution pattern: If $b > 1$, the distribution is aggregated or clumped (the data best fit a negative binomial distribution), if $b < 1$ the distribution is uniform (the data are best fit to a binomial distribution), and if $b = 1$ the population has a random distribution (the data fit a Poisson distribution) (Young and Young 1998). Iwao's patchiness regression (Iwao 1968) is based on Lloyd's mean crowding index ($m = x + s^2/x - 1$) and the regression model is $m = \alpha + \beta x$, where the slope (β) has the same meaning as Taylor's coefficient (Ifoulis and Savopoulou–Soultani 2006). Optimal sample size (N_{opt}), referring to trap density, was then determined using Taylor's coefficient:

$$N_{opt} = (Z_{\alpha/2}/C)^2 \, (ax^{b-2}) \qquad [1]$$

and Iwao's patchiness regression:

$$N_{opt} = (Z_{\alpha/2}/C)^2 \, ((\alpha + 1)/x + (\beta - 1)) \qquad [2]$$

where $Z_{\alpha/2}$ is the upper $\alpha/2$ of the standard normal distribution, α is the set confidence level, and C is the precision error. Here we used a 95% CI so that $Z_{\alpha/2}$ is 1.96, after Mallampalli and Isaacs (2002), and a precision level of 30%. Optimal sample size was also determined using the K parameter of the negative binomial model (Young and Young 1998):

$$N_{opt} = (Z_{\alpha/2}/C)^2 \, (1/x + 1/K_c) \qquad [3]$$

where K_c is the common parameter of the negative binomial model. Individual K values were determined from variance and mean data for each sample date and vineyard, as $K = x^2/(s^2 + x)$ and K_c is the average of the K values. Bacca et al. (2008) used a derivation of equation 3 to determine sample size for a fixed mean population and varying precision levels as:

$$N_{opt} = (1/C^2) \, (1/x + 1/K_c) \qquad [4]$$

For all sample size analyses, mean and variance data were derived from the 2010 collections within each vineyard, which provided 13 trap counts (one, four, and eight traps per 12.14 ha treatments) in each of seven vineyards for each of 23 sample dates. The 2010 data were also analyzed within each treatment, which provided data for treatments of one (seven traps), four (28 traps), and eight (56 traps) traps per 12.14 ha plot per sample date. For this analysis, the mean of means was used to reduce the impact of high variability in mealybug counts among vineyards, and prevent pseudo-replication (resulting in seven replicates for each sample date).

Results

Seasonal Phenology. Over 23 sample dates in 2010, a total of 168,092 adult male *Ps. maritimus* were recorded from 2,090 pheromone trap-weeks, with an average of 80.4 ± 3.6 *Ps. maritimus* per trap per week. Over 28 sample dates in 2011, total and average counts were lower, with 32,435 adult male *Ps. maritimus* recorded from 1,092 trap-weeks, with an average of 29.7 ± 2.2 *Ps. maritimus* per trap per week. There was a significant difference among vineyards in the average trap catch, across all sampling dates, in 2010 and 2011 (Fig. 1). Seasonal phenology was similar in both years of the study (Fig. 2). In 2010, *Ps. maritimus* was found in low numbers during the first week that traps were deployed (20–27 May) in all vineyards sampled. The peak of the first flight occurred during the week of 3–10 June 2010, and the second flight peaked from 5 to 25 August followed by a small decline and a smaller peak in the second week of September (Fig. 2). In 2011, traps were deployed earlier (12 April) to catch the beginning of the first flight, which was detected from 2 to 10 May. The peak of the first flight occurred between 6 and 27 June 2011; the peak of the second flight was between 22 and 29 August, with a smaller peak after in the second week of September (Fig. 2).

Two distinct flight periods were recorded in both years, with a relatively similar seasonal phenology, although peak flights occurred earlier in 2010 than in 2011. Individual data loggers were not placed in each block, but degree-day (DD) accumulation, based on Washington State University's AgWeatherNet (http://www.weather.wsu.edu) for grape development, showed the accumulated DD (base 10°C) at IAREC were low (2,325 DD) in the 2010 season as compared with the long-term average of 2,526 DD. DD accumulation was even lower in 2011 (2,312 DD).

Trap Density Comparisons. In 2010, there was no season-long difference in the pattern of adult male captures among the three trapping density treatments over the 23 wk sample period (Repeated Measures ANOVA: $F = 0.72$; df = 2, 18; $P = 0.50$; Fig. 3a). When data were analyzed on a weekly basis, using the mean per vineyard for each trapping density (seven replicates per treatment), there were no significant treatment effects among the one, four, or eight traps per 12.14 ha treatments on any of the 23 sampling dates.

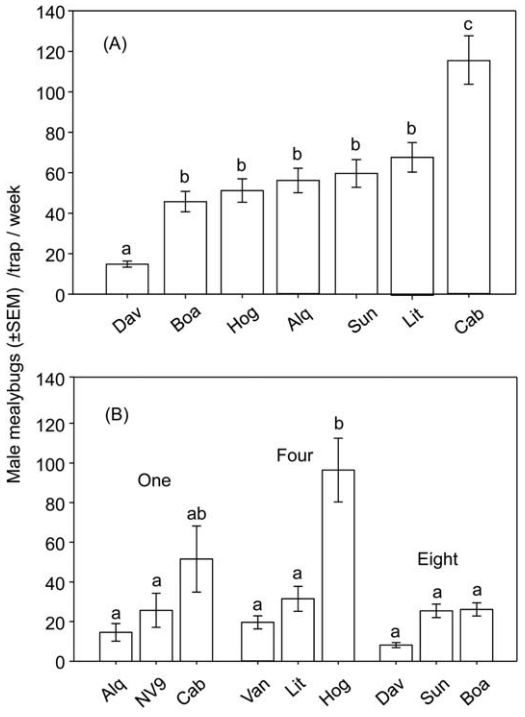

Fig. 1. Mean numbers (±SE) of adult male *Pseudococcus maritimus* recorded per trap per week in (A) 2010 (23 sample dates) and (B) 2011 (28 sample dates) show a significant difference among 12.14 ha vineyard blocks sampled (ANOVA for 2010: $F = 18.63$, df = 6, 2885, $P < 0.001$; 2011: $F = 16.32$, df = 8, 1083, $P < 0.001$). The vineyards corresponding to the abbreviations shown on the x-axis are listed in the methods section.

In 2011, no season-long difference in the pattern of adult male captures was found among trap density treatments (Repeated Measures ANOVA: $F = 0.91$; df = 2, 6; $P = 0.45$; Fig. 3b). When data were analyzed on a weekly basis, using the mean per vineyard for each trapping density (three replicates per treatment), there were significant treatment effects in week 6, when more adult male *Ps. maritimus* were recorded from plots with four traps per 12.14 ha over the other treatments (ANOVA: $F = 28.00$; df = 2, 6; $P = 0.001$), and week 12 when more males were recorded from plots with four traps per 12.14 ha than the plots with eight traps per 12.14 ha (ANOVA: $F = 5.77$; df = 2, 6; $P = 0.040$). During the 2011 season, when treatments were in different vineyards rather than in each vineyard, there was greater variation in male mealybug counts among treatments (Fig. 3b). When all trap data were used (3, 12, and 24 traps per sample date for trap densities of one, four, and eight traps per 12.14 ha, respectively), traps at a density of eight per 12.14 ha consistently had lower trap catches than traps at densities of one and four per 12.14 ha (Fisher least significant difference [LSD], $P < 0.05$).

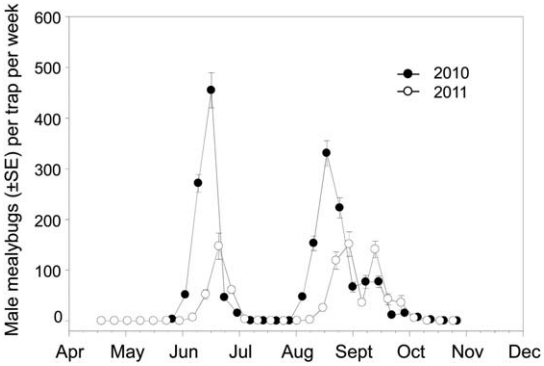

Fig. 2. Mean numbers (±SE) of adult male *Pseudococcus maritimus* recorded per trap per week in 2010 and 2011 show two distinct flight periods in each year.

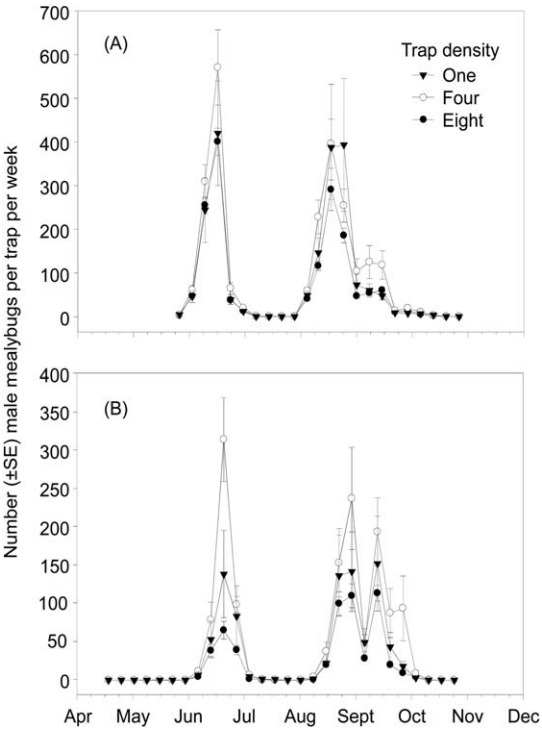

Fig. 3. Mean numbers (±SE) of adult male *Pseudococcus maritimus* recorded per trap per week for trapping densities of one, four, and eight traps per 12.14 ha in (A) 2010 and (B) 2011.

Sample Size. The relationship between ln variance and ln mean was significant for adult male *Ps. maritimus* captures (Fig. 4). The slope (b), Taylor's coefficient, was significantly >1 for male captures when data were analyzed by vineyard (Student's t-test; $b = 1.53 \pm 0.537$; $t = 61.62$; $P < 0.001$) or by treatment (Student's t-test; $b = 1.783 \pm 0.032$; $t = 55.57$; $P < 0.001$). These data indicate that pheromone trap captures of adult male *Ps. maritimus* are well described by the negative binomial distribution, and that the population is in an aggregated pattern. The mean to variance data analyzed by

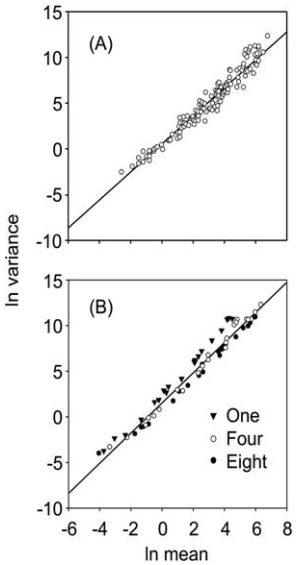

Fig. 4. Regression curve between the natural logarithm (ln) of the variance and the ln of the mean (x) number of adult male *Pseudococcus maritimus* captured to obtain the coefficients of Taylor's power law for data collected in 2010 showing positive relationship between ln variance and ln mean when data were analyzed by (A) vineyard ($y = 1.53x + 0.537$; $F = 3797$, df = 1, 146; $P < 0.001$; $r^2 = 0.963$) or (B) treatment ($y = 1.783x + 0.444$; $F = 3087$; df = 1, 65; $P < 0.001$; r^2 0.979).

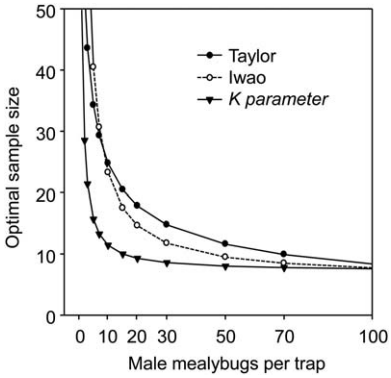

Fig. 5. Relationship between optimal sample size (pheromone traps per 12.14 ha vineyard) and adult male *Pseudococcus maritimus* per trap per week for sample size models based on Taylor's Power Law (equation 1 where $a = 1.71$ and $b = 1.53$), Iwao's regression parameters (equation 2 where $\alpha = 2.16$ and $\beta = 1.19$), and the K parameter of the negative binomial (equation 3 where $K_c = 5.97$). Data were from 2010 trap captures, with mean–variance relationships determined from each 13 trap data set collected in each of seven sampled vineyards on 23 sample dates.

vineyards provided a lower slope and was therefore used to determine optimal sample size for all equations used.

Relationships between the optimum number of samples (pheromone traps) per 12.14 ha sample block and mean number of captured adult male *Ps. maritimus* per trap per week (pest density), are shown for Taylor's coefficient (equation 1), Iwao's regression parameters (equation 2), and the K_c parameter of the negative binomial (equation 3), using a 30% precision level (Fig. 5). The 30% precision lines began to level off at 20 adult males captured per trap using the K parameter of the negative binomial model, but not until 70 adult males were captured using Taylor's and Iwao's parameters, the latter two of which also required more traps for the same level of precision (Fig. 5). At 50 mealybugs per trap, the required sample size began to level off, and was 11.6, 9.5 and 8.0 traps per 12.14 ha for Taylor's coefficient, Iwao's regression parameters, and the K_c parameter, respectively. The complete data set included sample dates between flights when trap captures averaged <5 *Ps. maritimus* per trap per week (Fig. 2). Using the complete trap data set, the K_c parameter was 5.97; sample dates with >50 or 100 adult male *Ps. maritimus* per trap per week better reflected trap efficiency during active flight periods, resulting in K_c parameters of 8.92 and 10.50, respectively, and required sample sizes of 5.9 and 4.6 traps per 12.14 ha, respectively (Fig. 6a). All models suggested that more than eight traps per 12.14 ha were needed for a 30% precision level, which would be more than vineyard pest managers would consider economical. For this reason, we further analyzed the data to determine if the optimal sampling number could be lowered, still using the existing mean to variance relationship. The required number of traps for varying precision levels (equation 4; Bacca et al. 2008) stabilized at the 40% precision error at <1 trap per 12.14 ha, for K_c parameter values from trap data with >50 and 100 captured *Ps. maritimus* per trap (Fig. 6b). Increasing precision to the levels of 30% and 20% required 1.2–2.5 and 2.7–5.7 traps per 12.14 ha, respectively, for varying these same K_c values (Fig. 6b). Our initial analyses (Fig. 5) used season-long trap captures, including periods before, between, and after peak flight periods, when trap captures typically ranged from 0 to three males per trap per week (Fig. 2). At lower trap capture densities, more traps were needed to assess the population density, using any of the optimal sampling models (Fig. 5). This was similarly shown for visual samples of *Ps. maritimus* on the vine (Geiger and Daane 2001). Most sampling programs show fewer samples are needed with increasing density of the sampled population (e.g., Mallampalli and Isaacs 2002, Ifoulis and Savopoulou-Soultani 2006) when the population fits the negative binomial model (Young and Young 1998). For this reason, optimal sample size was also determined using the K_c parameter for trap captures with >50 and >100 *Ps. maritimus* per trap. These were reasonable capture rates during the flight period; in traps with adult males (excluding trap captures with zero males), average trap capture was 107.4 ± 4.5 and 59.4 ± 4.0 males per trap per week in 2010 and 2011. With this analysis, required sample size was reduced to five traps per 12.14 ha, with little difference between traps with >50 or >100 male captures (Fig. 6a). Bacca et al. (2008) also used the K parameter to determine optimal sample size of pher-

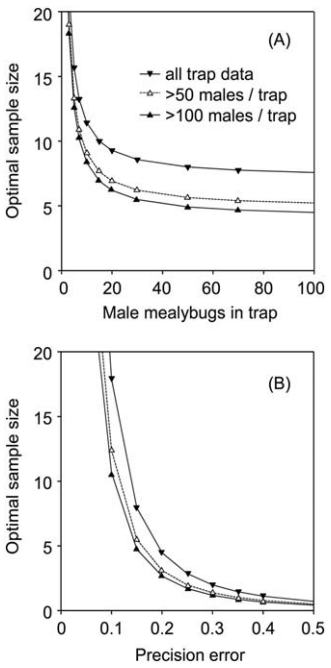

Fig. 6. (A) Relationship between optimal sample size (pheromone traps per 12.14 ha vineyard) and adult male *Pseudococcus maritimus* per trap per week using the K parameter of the negative binomial (equation 3 where K_c = 5.97; all 2010 trap data, as in Fig. 5), Kc = 8.92 (pheromone traps with >50 males per trap), and Kc = 10.50 (pheromone traps with >100 males per trap) and Kc = 10.50; and (B) number of pheromone traps (samples) per 12.14 ha vineyard needed under different precision errors from 5 to 40% as determined using Kc (equation 4 determined from all 2010 trap data), traps with >50 males, and traps with >100 males per trap per sample date.

omone traps with varying precision levels and a fixed population size of the coffee leaf miner, *Leucoptera coffeella* (Guérin–Méneville & Perrottet). Using this more simplified equation ($Z_{\alpha/2}$ is replaced by 1), *Ps. maritimus* sample size was reduced to about one and two pheromone traps per 12.14 ha for precision levels of 30 and 25%, respectively (Fig. 6b).

Mealybug Species. In 2010, there were 12, 19, 12, and 24 male mealybugs captured in traps baited with pheromones of *Ps. viburni*, *Ps. longispinus*, *Pl. ficus*, or a solvent control (no pheromone), respectively. Multiplex PCR analysis determined that all of these collected male mealybugs were *Ps. maritimus.*

Discussion

Ps. maritimus was previously reported to have two generations per year in Washington vineyards, based on visual samples of life stages found on the vine (Grimes and Cone 1985b, Geiger and Daane 2001). Here, we provide evidence of two distinct flight periods (Fig. 1). In 2011, the first male *Ps. maritimus* were caught on 10 May; based on the assumption of a 10°C lower temperature developmental threshold for *Ps. maritimus* (Geiger and Daane 2001), this corresponded to a 107 DD accumulation from 1 January 2011 to first capture. The beginning of the first flight was likely missed in 2010 because the first traps were deployed on 10 May (152 DD accumulation) and these traps captured several adult male *Ps. maritimus*, suggesting that the flight had begun before traps were deployed. The unusually low spring temperatures also may have been a factor in the reduced *Ps. maritimus* trap captures in 2011 compared with 2010, either as a result of reduced mealybug survival, or reduced flight activity by males. The two peaks in trap captures were in early June and mid-August, which match the seasonal periods when adult females are producing ovisacs (Grimes and Cone 1985b, Geiger and Daane 2001). The first flight peak in 2011 occurred 1 wk later than in 2010, and the second flight peak in 2011 occurred 2 wk later than the second peak in 2010. This slight delay was most likely a result of below average temperatures in the spring of 2011 (WSU AgWeatherNet 2011) that also caused delays in the development of many fruit crops, including juice grapes, in central Washington.

Pheromones have long been used in insect pest management programs, primarily to monitor pest densities (Burkholder 1985, Suckling 2000, Way and van Emden 2000). However, the use of semiochemicals for monitoring mealybugs is relatively new and/or rarely practiced. The pheromone of the citrus mealybug, *Planococcus citri* (Risso), has been known for >30 yr (Bierl–Leonhardt et al. 1981), and whereas it has been used in some Mediterranean countries for monitoring (Franco et al. 2004), this practice has not been widely adopted and we could find no guidelines for its use in control decisions. In vineyards, pheromone trapping for *Pl. ficus* in South Africa (Walton et al. 2006) and California (Millar et al. 2002) has been tested and pheromone lures are commercially available; however, *Pl. ficus* trapping in California has not yet seen widespread adoption because most managers apply insecticides as soon as any *Pl. ficus* are found in vineyards. In nursery systems with ornamental plants, Waterworth et al. (2011a) recently tested pheromone-baited traps for monitoring *Ps. viburni*, *Ps. longispinus*, and *Pl. citri* and showed that trap counts of male mealybugs were correlated with mealybug species and densities on nearby plants, suggesting considerable potential for pheromone-based monitoring of mealybugs in nursery systems.

In this study, we provide baseline data to develop a pheromone-based monitoring program for *Ps. maritimus* in vineyards. We showed that trap catches (Fig. 2) match the known seasonal phenology for *Ps. maritimus.* The number of traps needed per vineyard block was determined using field trials with different trapping densities, and through analysis of 2010 trap capture data using Taylor's Power Law, Iwao's regression parameters, and the K parameter from the negative binomial model. In 2010, seasonal average trap captures were different among individual vineyards (Fig. 1a), but male *Ps. maritimus* captures per trap were similar across all treatments (Fig. 3a) and on individ-

ual sample dates. These data indicate that a single trap per 12.14 ha block (averaged across seven replicates) matches the results of four and eight traps per block densities. In 2011, trap density treatments were each placed in separate vineyards, and for this reason, seasonal average trap captures were different among individual vineyards (Fig. 1b), creating differences in average trap captures per sample date and density treatment (Fig. 3b). The more important measure in 2011 was seasonal phenology of male flight periods as determined from the trap catch data. Here, the traps performed well, with the first seasonal captures of adult males being similar across all treatments (four of nine blocks on 18–25 May), as were peak counts in the first flight (nine of nine blocks on either 14–21 May or 21–28 May) (Figs. 1 and 3).

Peak counts in the second flight were less synchronized as compared with those in the first flight, and there was a double peak in both study years. The double peak could result from a late developing second generation or a partial third generation of adult males emerging. Another possible explanation for the double peak is related to the changing age structure of the female population. The late August to early September decline in captures may result from the presence of sexually mature females producing pheromone that competed with the trap lures. Once these females were mated, there might be less competition from them, resulting in an increase in trap captures. However, Waterworth et al. (2011b) showed that mated *Ps. viburni* and *Ps. longispinus* females, both close relatives of *Ps. maritimus*, remated multiple times, suggesting that mature females might still be producing pheromone. Thus, it seems less likely that the double peak in the pheromone trap catches was related to competition of lures with pheromone-producing females.

Three commonly used regression models were used to estimate optimal sample size: Taylor's Power Law (TPL), Iwao's patchiness regression (IPR), and the negative binomial model. For pest management, sampling precision levels between 20–35% are desirable, whereas for research, precision levels between 10 and 20% are often required. While precision was set to the same value for each method, one cannot assume that this desired level of precision would have been equally well achieved by the three models. In addition, the sample sizes tested in this study were observed trap densities that had been used in previous IPM programs. The three models used to estimate sample size were used to determine if the current practices met a fixed level of precision in monitoring populations of *Ps. maritimus.* While the nominal level of precision for research purpose is attainable at higher sample sizes, especially when the adult male population is low, for growers seeking a faster, more economical means for detecting *Ps. maritimus* to guide their IPM programs, a lower sample size (one trap per 12.14 ha.) is adequate. The purpose of the pheromone-based monitoring programs for *Ps. maritimus* is first to determine presence or absence of the pest and secondly to be able to determine what stage of development the mealybugs are in and at what time of the growing season so as to guide application of insecticide treatments so they are most effective (i.e., targeting first instar crawlers). Whereas TPL and IPR may yield more precise estimates more frequently, the less precise K model is adequate for IPM programs that seek to use insecticide treatments, when efficacy is independent of population estimates, regardless of the level of precision.

It is also important to emphasize the usefulness of pheromone traps in detecting invasive mealybug species. In the Washington vineyards monitored, no *Ps. viburni*, *Ps. longispinus*, or *Pl. ficus* were found in any of the vineyards. The numbers of males caught in traps baited with pheromones of those three species were less than those caught in control traps, and conclusive identification by DNA analysis confirmed that all specimens caught were *Ps. maritimus* that had randomly flown into the traps.

Pheromone traps are commonly used to monitor first flight periods to set a biofix of population development or to determine peak population flight activity, which is often correlated to subsequent economic damage (Suckling 2000, Way and van Emden 2000). The use of pheromone traps at either one or two per 12.14 ha vineyard block is far less time consuming than visual searches of vines, which required 18–25 vines sampled for 5 min each to provide similar precision levels (Geiger and Daane 2001). Whereas trap captures of adult male *Ps. maritimus* were clumped in distribution ($b > 1$), the winged adult males disperse over far greater distances than the sessile and highly clumped immature and female populations, thus requiring fewer samples. The determination of an optimal trap density that provides reliable information on pest populations while minimizing the costs of monitoring is an important aspect in the development of a vineyard IPM program. For example, to use insect growth regulating (insect growth regulator [IGR]) insecticides effectively for *Ps. maritimus* control, growers need to know when crawlers, the targeted life stage, are most likely to be present. Extrapolating forward from peaks of flight activity by mate-seeking adult males, and using the number of DD required for eggs to be laid and to hatch, should allow accurate estimation of when to spray IGRs for maximum efficacy. For this type of monitoring, our results show that one trap per 12.14 ha of vineyard may be an effective tool to accurately detect adult male *Ps. maritimus* flight activity. Our results also demonstrate that pheromone-baited traps provide a sensitive method of detecting *Ps. maritimus* infestations. Future studies must be conducted to correlate trap densities to mealybug damage levels in vineyards (Walton et al. 2004, Ifoulis and Savopoulou–Soultani 2006), and to determine optimal trap placement (Bacca et al. 2006). All of these factors will be critically important for better management and control of *Ps. maritimus* and the leafroll viral pathogens that they transmit.

Acknowledgments

Funding for this project was provided by the United States Department of Agriculture—National Institute of Food and Agriculture Specialty Crop Research Initiative 2009-02486, Concord Research Council, WA Wine Advisory Committee, and the Washington State Commission on Pesticide Registration. Vineyards sampled were provided by Hogue Ranches (Prosser, WA) and National Grape. We thank John Hutchins and Brian Hogg for helpful discussion on optimal sample size models. We also thank Holly Ferguson for review of the manuscript.

References Cited

Bacca, T., E. R. Lima, M. C. Picanço, R.N.C. Guedes, and J.H.M. Viana. 2006. Optimum spacing of pheromone traps for monitoring the coffee leaf miner *Leucoptera coffeella.* Entomol. Exp. Appl. 119: 39–45.

Bacca, T., E. R. Lima, M. C. Picanço, R.N.C. Guedes, and J.H.M. Viana. 2008. Sampling plan for the coffee leaf miner *Leucoptera coffeella* with sex pheromone traps. J. Appl. Entomol. 132: 430–438.

Bierl–Leonhardt, B. A., D. S. Moreno, M. Schwarz, J. Fargerlund, and J. R. Plimmer. 1981. Isolation, identification and synthesis of the sex pheromone of the citrus mealybug, *Planococcus citri* (Risso). Tetrahedron Lett. 22: 389–392.

Burkholder, W. E. 1985. Pheromones for monitoring and control of stored-product insects. Annu. Rev. Entomol. 30: 257–272.

Cone, W. C. 1971. Grape mealybug control in Concord grape field trials in central Washington. J. Econ. Entomol. 64: 1552–1553.

Daane, K. M., W. J. Bentley, V. M. Walton, R. Malakar–Kuenen, J. G. Millar, C. A. Ingels, E. A. Weber, and C. Gispert. 2006. New controls investigated for vine mealybug. Calif. Agric. 60: 31–38.

Daane, K. M., M. L. Cooper, S. V. Triapitsyn, V. M. Walton, G. Y. Yokota, D. R. Haviland, W. J. Bentley, K. E. Godfrey, and L. R. Wunderlich. 2008. California vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. Calif. Agric. 62: 167–176.

Daane, K. M., M. C. Middleton, R. Sfroza, M. L. Cooper, V. M. Walton, D. B. Walsh, T. Zaveizo, and R.P.P. Almeida. 2011. Development of a multiplex to distinguish mealybug species found in North American vineyards. Environ. Entomol. 40: 1595–1603.

Daane, K. M., R.P.P. Almeida, V. A. Bell, M. Botton, M. Fallahzadeh, M. Mani, J. L. Miano, R. Sforza, V. M. Walton, and T. Zaveizo. 2012. Biology and management of mealybugs in vineyards, pp. 271–308. *In* N. J. Bostanian, R. Isaacs, and C. Vincent (eds.) Arthropod Management in Vineyards. Springer, The Netherlands.

Doutt, R. L., and K. S. Hagen. 1950. Biological control measures applied against *Pseudococcus maritimus* on pears. J. Econ. Entomol. 43: 94–96.

Figadère, B. A., J. S. McElfresh, D. Borchardt, K. M. Daane, W. Bentley, and J. G. Millar. 2007. *trans*-α-Necrodyl isobutyrate, the sex pheromone of the grape mealybug, *Pseudococcus maritimus.* Tetrahedron Lett. 48: 8434–8437.

Flaherty, D. L., W. L. Peacock, L. Bettiga, and G. M. Leavitt. 1982. Chemicals losing effect against grape mealybug. Calif. Agric. 36: 15–16.

Franco, J. C., P. Suma, E. B. da Silva, D. Blumberg, and Z. Mendel. 2004. Management strategies of mealybug pests of citrus in Mediterranean countries. Phytoparasitica 32: 507–522.

Frick, K. E. 1952. The value of some organic phosphate insecticides in control of grape mealybug. J. Econ. Entomol. 45: 340–341.

Geiger, C. A., and K. M. Daane. 2001. Seasonal movement and distribution of the grape mealybug (Homoptera: Pseudococcidae); developing a sampling program for San Joaquin Valley vineyards. J. Econ. Entomol. 94: 291–301.

Geiger, C. A., K. M. Daane, W. J. Bentley, G. Y. Yokota, and L. A. Martin. 2001. Sampling program for grape mealybugs improves pest management. Calif. Agric. 55: 19–27.

Golino, D. A., S. T. Sim, R. Gill, and A. Rowhani. 2002. California Mealybugs can spread grapevine leafroll disease. Calif. Agric. 56: 196–201.

Grasswitz, T. R., and D. G. James. 2008. Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. Entomol. Exp. Appl. 129: 268–275.

Grimes, E. W., and W. W. Cone. 1985a. Control of the grape mealybug, *Pseudococcus maritimus*, (Hom.: Pseudococcidae), on Concord grape in Washington. J. Entomol. Soc. B. C. 82: 3–6.

Grimes, E. W., and W. W. Cone. 1985b. Life history, sex attraction, mating, and natural enemies of the grape mealybug, *Pseudococcus maritimus* (Homoptera: Pseudococcidae). Ann. Entomol. Soc. Am. 78: 554–558.

Hinkens, D. M., J. S. McElfresh, and J. G. Millar. 2001. Identification and synthesis of the sex attractant pheromone of the vine mealybug, *Planococcus ficus.* Tetrahedron Lett. 42: 1619–1621.

Ifoulis, A. A., and M. Savopoulou–Soultani. 2006. Developing optimum sample size and multistage sampling plans for *Lobesia botrana* (Lepidoptera: Tortricidae) larval infestation and injury in northern Greece. J. Econ. Entomol. 99: 1890–1898.

Iwao, S. 1968. A new regression method for analyzing the aggregation of animal populations. Res. Pop. Ecol. 10: 1–20.

Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22: 417–421.

Madsen, H. F., and L. B. McNelly. 1960. Control of the grape mealybug on apricots. J. Econ. Entomol. 53: 354–357.

Mallampalli, N., and R. Isaacs. 2002. Distribution of egg and larval populations of cranberry fruitworm (Lepidoptera: Pyralidae) and cherry fruitworm (Lepidoptera: Tortricidae) in highbush blueberries. Environ. Entomol. 31: 852–858.

Martelli, G. P., A. A. Agranovsky, M. Bar–Joseph, D. Boscia, T. Candresse, R.H.A. Coutts, V. V. Dolja, B. W. Falk, D. Gonsalves, W. Jelkmann, et al. 2002. The family Closteroviridae revised. Arch. Virol. 147: 2039–2044.

Millar, J. G., and S. L. Midland. 2007. Synthesis of the sex pheromone of the obscure mealybug, the first example of a new class of monoterpenoids. Tetrahedron Lett. 48: 6377–6379.

Millar, J. G., K. M. Daane, J. S. McElfresh, J. Moreira, R. Malakar–Kuenen, M. Guillen, and W. J. Bentley. 2002. Development and optimization of methods for using sex pheromone for monitoring the mealybug *Planococcus ficus* (Homoptera: Pseudococcidae) in California vineyards. J. Econ. Entomol. 95: 706–714.

Millar, J. G., J. A. Moreira, J. S. McElfresh, K. M. Daane, and A. S. Freund. 2009. Sex pheromone of the longtailed mealybug: a new class of monoterpene structure. Org. Lett. 11: 2683–2685.

Rayapati, N. A., S. O'Neal, and D. Walsh. 2008. Grapevine leafroll disease. Washington State University Extension Bulletin EB2027E.

Statistical Graphics Corp. 2000. STATSGRAPHICS Plus Version 5.0. Statistical Graphics Corp. Princeton, NJ.

Suckling, D. M. 2000. Issues affecting the use of pheromones and other semiochemicals in orchards. Crop Prot. 19: 677–683.

Systat Software, Inc. 2007. Systat 12. Systat Software, Inc. San Jose, CA.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189: 732–735.

Tsai, C., A. Rowhani, D. A. Golino, K. M. Daane, and R.P.P. Almeida. 2010. Mealybug transmission of grapevine leafroll viruses: an analysis of virus-vector specificity. Phytopathology 100: 830–834.

Walton, V. M., K. M. Daane, and K. L. Pringle. 2004. Utilizing the sex pheromone of *Planococcus ficus* to improve pest management in South African vineyards. Crop Prot. 23: 1089–1096.

Walton, V. M., K. M. Daane, W. J. Bentley, J. G. Millar, T. E. Larsen, and R. Malakar-Kuenen. 2006. Pheromone-based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in California vineyards. J. Econ. Entomol. 99: 1280–1290.

Waterworth, R. A., R. A. Redak, and J. G. Millar. 2011a. Pheromone-baited traps for assessment of seasonal activity and population densities of mealybug species (Hemiptera: Pseudococcidae) in nurseries producing ornamental plants. J. Econ. Entomol. 104: 555–565.

Waterworth, R. A., I. M., Wright, and J. G. Millar. 2011b. Reproductive biology of three cosmopolitan mealybug (Hemiptera: Pseudococcidae) species, *Pseudococcus longispinus*, *Pseudococcus viburni*, and *Planococcus ficus*. Ann. Entomol. Soc. Am. 104: 249–260.

Way, M. J., and H. F. van Emden. 2000. Integrated pest management in practice: pathways towards successful application. Crop Prot. 19: 81–103.

WSU AgWeatherNet. 2011. The Washington Agricultural Weather Network Version 2.0, Washington State University, Prosser, WA. (http://weather.wsu.edu/).

Young, L. J., and J. H. Young. 1998. Statistical ecology: a population perspective. Kluwer Academic Publishers, Norwell, MA.

Zou, Y., K. M. Daane, W. J. Bentley, and J. G. Millar. 2010. Synthesis and bioassay of racemic and chiral *trans*-α-necrodyl isobutyrate, the sex pheromone of the grape mealybug *Pseudococcus maritimus*. J. Agric. Food Chem. 58: 4977–4982.

Received 5 April 2012; accepted 15 October 2012.