INVESTIGATION OF GRAPE MEALYBUG POPULATION DYNAMICS TO FORECAST AND PREVENT OUTBREAKS AND IMPROVE CONTROL

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III. SUMMARY:

Vineyard blocks in which we released *Pseudaphycus angelicus*, an important grape mealybug parasitoid, in 2000 were monitored in the 2001 season. In the Tulare County table grape block, lowered levels of mealybug damage and elevated levels of parasitism (compared to 2000) were found throughout the release and control plots, suggesting that overwintered parasitoid had moved from the initial release location. Releases planned for the 2001 season were less successful because of low insectary production of *P. angelicus.* Mid-season releases with a small number of parasitoids did not show any affect on mealybug density or damage ratings. Similarly, releases of green lacewing eggs did not show a reduction of mealybugs or economic damage. Studies testing alternative bait-control for the Argentine ant were tested. A 25% sugar solution and small amounts (0.001-0.0001%) of either imidacloprid, fipronil or thiomethoxam were placed in bait traps throughout a heavily infested Napa Valley wine grape vineyard. Results show a significant late-season reduction of ant activity at the fipronil and thiomethoxam treatments. However, there was no reduction in mealybug density or increase in parasitoid activity. While results showed no economic reduction of Argentine ants, we are encouraged by new information gained and will make the needed adjustments for study in the 2002 season. Finally, we tested the effect of nitrogen fertilization (0, 25, 50, 100, 200 and 400 lbs/ac) and girdling and gibberellic acid practices on mealybug populations. Results from field trials showed no difference between N fertilization treatments or berry sizing practices in mealybug density or egg deposition. We believe these field results were influenced by resident natural enemies, which lowered mealybug densities. In controlled greenhouse trials, mealybug densities and egg deposition on potted plants were greater in higher N fertilization treatments. We conclude that more vigorously growing vines can increase mealybug pest status. There is less evidence that berry-sizing practices have any influence on mealybug pest densities.

IV. OBJECTIVES and EXPERIMENTS CONDUCTED TO MEET OBJECTIVES:

1. Investigate the potential of rearing and mass-releasing *Acerophagus notativentris* and *Pseudaphycus angelicus* for control of grape and longtailed mealybugs.
2. Conduct studies on the effect of ants on mealybug and natural enemy populations and alternative control methods to reduce ant problems in vineyards.
3. Complete studies on the effects of selected management practices on mealybug biology.
4. Mass produce two parasitoid species (*Pseudaphycus flavidulus* and *Leptomastix epona*) of obscure mealybug and release these parasitoids in Central Coast vineyards.

Objective 1: Mass-production and field release of *P. angelicus*.

Between 1998-2000, we developed methodologies to mass produce *Pseudaphycus angelicus*, a parasitoid of the grape and longtailed mealybug. In 2001, we attempted to increase production and test mass-release of *Pseudaphycus angelicus* in commercial table and raisin grape vineyards (the results have a direct bearing on programs for longtailed mealybug in the Central Coast).

Squash were inoculated with 500-1,500 longtailed mealybug crawlers from a "mother colony" of infested squash. The mealybug colony was isolated in a "rearing-room" and held until the mealybugs reach the 2^{nd} - 3^{rd} development stage. At that time, the infested squash are placed 3-5 per cage (ca. 1.5 × 1 × 0.6 ft), moved to the "parasite-room" and ~100 *P. angelicus* are added. It is estimated that the *P. angelicus* go through 2-3 generations before the cage is ready for parasitoid harvest. Parasitoid recovery from each cage varied from ca. 500-5,000 parasitoids per cage. In 2001, our target is the production of 100,000 *P. angelicus* for field studies (this is a small amount compared to a commercial operation like FAR Insectaries production of vine mealybug parasitoids). In a similar manner as the *P. angelicus* production methods were developed, we intended to investigate production methods of the grape mealybug parasitoid *Acerophagus notativentris* – which has proved difficult to rear.

To determine if inoculative releases in the previous year would have a beneficial carry over effect into 2001, we continued to monitor previous release sites. In 2000, we release ca. 13,000 *Pseudaphycus angelicus* in a table grape vineyard near Delano, CA and ca. 7,000 *Pseudaphycus angelicus* in a wine grape vineyard near Napa, CA. Both vineyards had a history of grape mealybug damage in the 2000 season and in both we made recoveries of *Pseudaphycus angelicus* at higher levels in the release sections as compared to the no release sections. In 2001, no releases were made in these blocks, but we monitored the mealybug and parasitoid activities across both release and no release plots (the hypothesis was that the released parasitoids would overwinter and spread throughout the blocks). At both sites, mealybug density was estimated by searching the spurs for crawlers (11 April 2001) and 3-min. counts on 160 vines (4 vines X 5 rows X 8 plots = 180 vines total) on 17 July 2001. The 3-minute, destructive search for live and mummified mealybugs was developed in the 1998-99 season (see Geiger et al. 2000, 2001, Geiger & Daane 2001 for more details). In brief, the crew will search anywhere on the vine, using cues to mealybug presence (such as foraging ants) to determine where to sample. All mealybugs found during the 3-minute period were recorded, noting development stage. All mummies found were marked during the search (colored push-pins) and collected after the 3-minute count is complete.

At harvest time (16 August 2001), economic damage to grape bunches was rated using a 0 – 3 scale, based on table grape standards: 0 means no mealybug damage, 1 means honeydew present but the bunch is salvageable (e.g., a water dip could be used), 2 means honeydew and mealybugs are present but at least part of the bunch is salvageable (e.g., a water dip and hand removal of infested stems and berries), and 3 means a total loss (Geiger and Daane, 2001). From each vine, nine grape bunches were sampled: three each from the mid- (e.g., just above the main trunk), right- and left-sides of the vine, with a total of 50 vines per plot (10 vines X 5 rows) and there were 8 plots; altogether we checked 3600 bunches. An additional 450 bunches from 40 vines located outside the plot (450 bunches X 8 plots = 2880 bunches total) were searched. During the same period, all the mealybugs present on the searched bunches were collected. The collected

mealybugs/mummies were taken to the laboratory where their development stage was recorded and they were placed individually into gelatin capsules and held for parasitoid emergence.

In 2001, we continued to conduct the open-field release studies of the mass-produced *Pseudaphycus angelicus* – however, low parasitoid production limited our release efforts. A new site for *P. angelicus* release was established in three commercial vineyard blocks, near London, California (Tulare County). In March 2001, three ~16 acre blocks of spur pruned, Exotic (Block #1 and 2) and Flame Seedless (Block #3) table grapes were surveyed. The Exotic blocks had 75 vines per row, and Flames block had 117 vines per row. Each block was used as a replicate in a split plot design, with either a *P. angelicus* release and a no-release control treatment randomly assigned to each plot (3 replicates total). Within each split plot, 60-vine (6 rows × 10 vines) quadrants were assigned for stratified randomly sampling. Our initial survey of mealybug crawlers was made with a spur count 6 March 2001.

Prior to releasing *P. angelicus*, we evaluated grape mealybug crawlers levels on grape bunches and leaves that touched old wood or bark. A presence/absence method was used for these crawler counts, surveying 3 bunches and 3 leaves per vine, 4 vines per plot (24 bunches and leaves total per quadrant). After which, *P. angelicus* were first released on 6 July 2001, at a rate of ~350 per plot, and a second release on 17 July 2001 at a rate of ~600 *P. angelicus* per plot.

Because we had problems with *P. angelicus* production, we also tested lacewing (*Chrysoperla rufilabris* (Neuroptera: Chrysopidae)) release in adjacent plots (using the same design, with lacewing release plots at least 20 vines from the *P. angelicus* release. Each plot consists of 10 vines X 6 rows (60 vines total). On 22 June and 13 July 2001 lacewings were released at a rate of 100 eggs per vine per date. To estimate release numbers, 100 lacewing eggs were counted in the laboratory and deposited on an index card with a sticky surface, and this card was used as a reference to estimate the number of lacewing eggs in the field (egg hatch in the laboratory was 80%). The lacewing eggs were released directly on the cordon, often near grape bunches touching the wood. During first release we observed some native gray ants (*Formica aerata*) attacking the released eggs. Therefore, during the second release, we sprayed the vine with water to disrupt the ant foraging. Prior to releasing the lacewing eggs, we estimated the presence/absence of crawlers and other stages of the mealybugs (21 June 2001) by searching 3 bunches and 3 leaves per vine, 4 vines within the block, and 4 vines on each on the head and the tail end of the plot not exceeding 10 vines away from the plot border (altogether 12 vines per row), and 3 alternate rows per plot.

In both *P. angelicus* and lacewing release plots, the overall effectiveness of released treatments was determined using the fruit damage ratings. To estimate economic damage, on August 2 (during the harvest time) each plot was rated for mealybug infestation on 40 vines. A 0—3 scale was used, as described earlier.

Work on release of green lacewing larvae and adults was also conducted in two wine grape vineyards near Napa, California. Plot design and sampling was similar to work completed near London, California. However, releases of lacewing larvae rather than eggs were made. The Napa Valley lacewing release plots received the following treatments: a no-release control or 5, 10, 25 or 50 lacewing larvae per vine. Two trials were conducted, one each in the spring and

summer. These data have not yet been completely entered into the computer and will be presented in the 2002 report.

Objective 2: Ant controls and the effect on parasitoid activity.

Part I. Our previous research showed that ants interfere with the biological control of mealybugs (Daane et al. 2000; other researchers have completed similar studies). Unfortunately, ant controls are rarely complete and the insecticide materials used for ants are often more toxic (to humans and the environment) than materials used to control mealybugs. In 2000, we conducted field trials in Sonoma and San Luis Obispo counties with "bait traps" filled with either boric acid or imidacloprid as the toxicant (Daane et al. 2001, AVF Report; Bianchi et al. 2000, Annual Report to the Central Coast Growers Association). These "alternative" controls were compare with a "standard" ground application of chlorpyrifos (Lorsban®) and a no-spray control. In 2001, we sought the assistance of true ant experts. Our bait-insecticide efforts were joined by Drs. Mike Rust and John Klotz of the University of California, Riverside. Their expertise greatly improved the efficiency of our ant research. We report here on the results from Sonoma County only, which had grape mealybug tended by the Argentine ant, *Linepithema humile*.

A 20 acre, block in a Sonoma County wine grape vineyard was used to test ant controls. The block was an ~11-year-old, spur-pruned Chardonnay cv. for still wine that had a history of very high Argentine ant and mealybug densities. Six 10-row × 100-vine blocks were established. Each block was divided into three 10-row × 25-vine treatment plots (18 plots total). Treatments were sugar bait traps with very low (<0.001-0.0001%) additions of either imidacloprid (0.0001%), fipronil (0.0001%), or thiomethoxam (0.001%). Bait traps were made by out 2.5 foot sections of PVC pipe (~2 in diameter). The traps were filled with shredded wood (swamp cooler pads) to provide structure for the ants to walk on and then capped at both ends. Bait trap toxicants were mixed in a 25% sugar solution that acted as an ant-attractant. Three half-inch holes were drilled into one top-side of the PVC pipe to provide an entrance for ants to get to the bait (the smaller the hole the less the evaporation).

Ant density was measured using two methods. First, small (50 ml) vials were fitted with caps with a large hole drilled through the center and a mesh covering between the cap and the tube. When inverted, the mesh held the sugar-water (25%) in the tube, but an Argentine ant could feed through the mesh – pulling the sugar water out. By measuring how much fluid was used during a 1 or 2 day period, the number of ant visits could be estimated. Eighteen monitoring tubes were placed in each plot, with the amount of liquid removed adjusted for evaporation (determined by 2 tubes per plot with ant exclusion) and compared between treatments. The monitoring tubes were left in place for 1-2 days, depending on temperature and ant activity, and were placed in the field every 1-3 weeks, depending on the seasonal period and ant activity. A second, less technical method, to determine ant activity was to count the number of ants on 6 vines randomly selected in each plot (using the 3 inner rows of each block). One half of each vine was randomly selected and a visual count of ants was made of all ants moving up or down the inner cordon for a 30-second period. Samples were taken biweekly during the growing season for ant densities and monthly for mealybug densities.

To estimate mealybug density, we used the 3-minute timed counts described previously. Four vines per plot were sampled monthly during the growing season. Economic damage was rated using the 0—3 scale, as described previously.

Part II. We collaborated with Walt Bentley (KAC – UC Areawide IPM Advisor) on trials investigating the use of a 80% common vetch, 20% Merced rye mix of ground cover that was shown to lower ant density in small plot trials. The working hypothesis is that in early spring ants forage on the ground cover and not the vine. This ant-free "window" provides an important period for overwintering parasitoids to emerge from their mealybug hosts and find and kill fresh mealybugs. To study this on a larger scale, the 80% common vetch, 20% Merced rye mix was seeded in fall 2000 in a commercial raisin vineyard near Del Rey, CA and a commercial wine grape vineyard near Napa, CA. The two treatments tested were (1) a winter-spring ground cover and (2) clean-cultivated. In October 2000, the cover crop plots were seeded to a 4:1 mixture of Merced rye (*Secale cereale* L) and common vetch (*Vicia sativa* L.). The control plots did not receive any seeds, and were kept free of ground covers by cultivating the middles between the rows. In both treatments, the berms directly under the vines were kept free of ground covers with applications of glyphosate. In June, the cover crop was plowed down. The experimental design was a randomized complete block, with five replicates of each block. Each treatment plot was 0.6 acre (5 rows wide by 55 vines long).

Mealybugs, ants and parasitoid levels were estimated using timed counts and collections of mealybugs to rear out parasitoids, as described above and in greater detail in the Malakar-Kuenen et al, 2001 report (for vine mealybug). At harvest-time, economic damage will be rated on a minimum of 10 vines per plot, using the 0—3 scale described previously.

Objective 3: Effects of vineyard cultural practices on mealybug densities.

Of many cultural practices used in vineyard management, we identified three that may affect mealybug densities: N fertilization, girdling practices, and the level and schedule of gibberellic acid applications. In 2000, most of the mealybugs in our experimental plots were parasitized by resident parasitoid populations of *Acerophagus notativentris* and *Pseudaphycus angelicus* - this created difficulties in assessing the effects of cultural practices on the grape mealybug population. Therefore, this year (2001), we inoculated the vines with the grape mealybug crawlers on the cut spurs, at a rate of one spur per vine and two vines per plot in Nitrogen study block (4 vines total per plot) and one vine per plot in the *Girdling & Gibberellic Acid* study block (3 vines total per plot). Before placing on the test vines, cut spurs from an infested vineyard were examined and the number of mealybugs estimated. After which, the infested spur was tied against a 1-year-old cane with rough bark on the treatment vine (6 and 7 March 2001). The spurs used contained at least 20 crawlers per spur, if there were fewer than 20 crawlers then a second spur was added. These spurs were collected from a local vineyard, which we checked before hand to assure only grape mealybug was present.

Nitrogen Studies. The effects of N fertilization on grape mealybug and its parasitoids were studied in an experimental vineyard at the Kearney Agricultural Center (KAC). Six levels of nitrogen fertilization were applied in a Thompson seedless block established by Pete Christensen (UCCE viticulturist) in 1992. N fertilization treatments were: 1) 0 lbs N/acre, 2) 25 lbs N/acre

(28.1 kg/ha), 3) 50 lbs N/acre (56.2 kg/ha), 4) 100 lbs N/acre (112.3 kg/ha), 5) 200 lbs N/acre (124.6 kg/ha), and 6) 400 lbs N/acre (449.3 kg/ha) same as in last year's study. To attain these different levels, ammonium nitrate, $(NH_4)_2NO_3$ was applied at pre-bloom stage (19 April 2001), with all plots irrigated one week before (13 April 2001) and after (27 April 2001) N application. Treatments were set in a randomized complete block design with 6 replicates (36 plots total). A single plot consisted of 4 vines (0.011 acre), with each plot separated by a no-treatment vine. These treatments have been maintained for >7 years.

To measure the N level on the leaf samples, 60 leaves were sampled from each plot (selected leaves were opposite to the cluster) when the vines were 80-100% bloom (10 May 2001) and near the harvest time (23 August 2001). Leaf petioles were removed from each leaf, soaked in soap water for 2 minutes, triple rinsed with tap water, and then given a final rinse with de-ionized water. Washed petioles were air-dried, placed in an oven (at 21.2°C) for 24 hours, and then crushed into a powder. The powdered samples were sent to DANR Analytical Laboratory (Davis, CA) for NO_3 analysis.

The effects of N on vine physiology that might influence mealybug population dynamics might best be recorded during the growing season by measuring vine vigor. For this, we recorded cane growth (total primary cane length) at early season (12 June 2001) on 4 canes per plot (1^{st}, 3^{rd}, 5^{th} and 8^{th} nodes on the fruit cane). Because more vine growth might provide a larger canopy, resulting in more shade and micro-climatic differences in temperature, vine canopy temperatures were recorded with data loggers (HOBO®, Onset Computer Corporation, Bourn, MA) placed near the canopy. Data loggers were placed in a subsample of the plots on mid March and temperature was recorded at 1-hour intervals throughout the study. This year we did not measure leaf water turgor.

Mealybug densities were measured using 3-minute counts per vine during the mid- (4 June 2001) and late-seasons (1 August 2001). Since mealybugs were seeded as the crawlers in March, we skipped the early-season count (in spring). Near harvest time (5 September 2001), we rated the fruit damage using a scale of 0-3 (see above for details) on 9 bunches per vine, 2 vines per plot. We collected all ovipositing and gravid females found on the bunches. These adult females were brought to the laboratory and placed individually in gelatin capsules. Specimens were held at room temperature for the next 4 weeks to provide sufficient time to complete oviposition or parasite development if any were present. After which, eggs were counted and the combined metafemur and metatibia length was measured using an ocular micrometer (fitted in a light microscope).

Girdling & Gibberellic Acid Studies. In 1999, an experimental plot was established at KAC to test the effects of girdling and gibberellic acid on mealybug densities. All vines were cane-pruned Thompson Seedless cv., with no pesticides used except dusting sulfur. Treatments were a 1) control, 2)girdling only (mid-June), 3) gibberellic acid (GA) only (2 applications, one at 75% bloom, one after berry set), 4) girdling and GA. For GA treatments, a berry "thinning spray" was applied at 70% bloom (10 May 2001) at 12 g a.i. per acre (Pro Gibb®, Abbott Laboratories, Chicago, IL), followed by two berry "sizing sprays" when berries were 4-5 mm in diameter (21 and 24 May 2001) at 60 g a.i. per acre. For girdled treatments, vines were ringed when berries were at 4-5 mm (22 May 2001). A complete factorial, randomized complete block

experimental design was used with 12 replicates (3 vines per replicate, 48 plots total) same as in year 2000.

Mealybug densities were estimated using the 3-minute count, described previously, on one vine per plot on 4 June and 1 August 2001. Just before harvest, 9 grape bunches per vine per plot were collected and all mealybugs were counted and the grape bunches were rated for economic damage, using the 0–3 scale.

Greenhouse studies. The effects of N fertilization on mealybug population dynamics were examined more closely in the greenhouse using similar procedures to those in 2000. The only difference is that, this year, we inoculated the potted vines with the crawlers on the cut spurs on 7 March 2001. These potted vines were planted from grape cuttings (Thompson Seedless cv.) in 1998 in 15 cm pots in a sand : perlite : peat moss mix (1:1:1 ratio). In February 2001, all leaves and canes longer than 30 cm were pruned to give a uniform height, shape and size to each plant and all the plants were treated with MiracleGro®. On 19 April 2001, N fertilizer was applied in the form of granular ammonium nitrate $(NH_4)_2NO_3$, at 6 different target rates: 0, 25, 50, 100, 200, and 400 lbs N per acre with 10 replicates per treatment. The amount of fertilizer used was based on pot size (15 cm diameter) and the percentage of available N element in ammonium nitrate (33.5%). N applied was 0, 0.15, 0.31, 0.61, 1.22, and 2.44 gm $(NH_4)_2NO_3$ per pot to make an equivalent of 0, 25, 50, 100, 200, and 400 lbs N per acre treatments in the field, respectively. Plants were watered thoroughly before N fertilization and lightly just afterwards (about 50 ml per pot) to settle fertilizer into each pot. During the course of the experiment, plants were watered as needed with enough water applied for proper growth but not in excess that would cause leaching of N (applied water was caught in a dish beneath each plant). Fertilization treatments were reapplied in early October when all plants showed signs of N depletion (leaf yellowing and drop).

To exclude other grape pests and mealybug parasitoids and predators, each potted vine was caged individually. Plants were examined each month and mealybug density and life stages were recorded. In October and November, gravid females from the second generation were collected and placed individually in gelatin capsule for ~4 weeks while they completed oviposition. The number of eggs deposited by each mealybug was counted. Length of metafemur and metatibia of the mealybugs were measured using an ocular micrometer housed in a Nikon microscope.

At the end of the trial, all leaves were collected from the vines and the leaf petioles were washed, dried and crushed to a powder, as described previously. NO_3 analysis was performed at the DANR Analytical Laboratory (Davis, CA).

V. SUMMARY OF MAJOR RESEARCH ACCOMPLISHMENTS and RESULTS:

Objective 1: *Pseudaphycus angelicus* production and release.
Production of *Pseudaphycus angelicus* in the insectary was very poor. The new methodologies employed worked – too well. In winter 2000/01 we began to build up parasitoid colonies from 1,000s to 10,000s. In January, there were enough parasitoid for release but the mealybug colony began to decline, possibly from the host quality of the older butternut squash (there is a period

between the fall and spring production of squash). In brief, our parasitoid multiplied too quickly and, because we underestimated our mealybug production, the parasitoids "crashed" the mealybug colony eventually dropping off in numbers as fewer large hosts were available. For this reason, parasitoid release did not occur until too late in the 2001 season.

In our old study 2000 release blocks we observed a positive response to the *Pseudaphycus angelicus* release with overall low mealybug density in all the release and control blocks and similarly the economic damage rate was low among all the blocks (Table 1A and 1B). It appears that *P. angelicus* has spread between the release and control plots uniformly and there was no difference in parasitism levels among these plots ($t = 0.64$; $df = 6$; $P = 0.55$) (Table 2). Further, the *P. angelicus* percent parasitism on the control blocks was higher than the released blocks indicating that *P. angelicus* had moved into the newer areas.

Table 1 A. Proportion of grape mealybug crawlers present on the spurs in April 2001 in a vineyard, where we released *Pseudaphycus angelicus* in year 2000 and mean number of mealybugs present on the vines using 3-min counts on July 2001, Delano, California, 2001.

Plot	Treatment	Total no. of spurs checked	% of spur with crawlers (mean ± SEM)*	Number of GMB per vine
1N	Control	306	4.57±1.65	0.2
2N	Control	306	9.15±4.07	0
3N	Control	378	8.55±3.12	0
4S	Control	90	4.45±2.22	0
1S	*P. angelicus* released	90	0.00±0.00	0
2S	*P. angelicus* released	90	4.45±3.30	0.35
3S	*P. angelicus* released	90	6.67+3.44	0
4N	*P. angelicus* released	417	5.29±1.90	0.70

*1 way-ANOVA, $F = 1.085$; $df = 7, 40$; $P = 0.39$ for the % of crawlers present on the spurs.

Table 1 B. Economic damage ratings in the previously *Pseudaphycus angelicus* released (in year 2000) experimental blocks in Delano, California, 2001.

Damage rating	Control	*P. angelicus* released*
0	91.45	92.41
1	5.99	5.15
2	2.47	2.41
3	0.09	0.03

*Paired t-test between Control and released shows no difference between the treatments.

Table 2. Level of Parasitism in the previously *Pseudaphycus angelicus* released (in year 2000) experimental blocks in Delano, California, 2001.

Treatment	% with *Pseudaphycus angelicus* (Mean ±SE)	Mean % with *Acerophagus notativentris* (Mean ±SE)
P. angelicus release	4.61±3.08	0.81±0.81
Control	7.35±3.03	2.69±2.30

This year, we had a very low production of *P. angelicus* for parasitoid release. However, we established a new site with a moderate grape mealybug infestation and combined *P. angelicus* with *Chrysoperla rufilabris* (lacewing) releases. Early estimation of crawlers using the presence/absence method showed a presence of 29.63%, 11.11% and 25.93% crawlers on the leaves and bunches on blocks 1, 2 (Exotic cv.) and 3 (Flames cv.) respectively. There was no significant difference in the level of crawlers infestation among these blocks ($F = 0.40$; df = 2,6; $P = 0.69$). Later, a late-spring/early summer count prior to *P. angelicus* release showed a slightly higher mealybug population on the release plots than on the randomly selected control blocks (F=6.02; df = 1, 12; P = 0.02) (Fig 1).

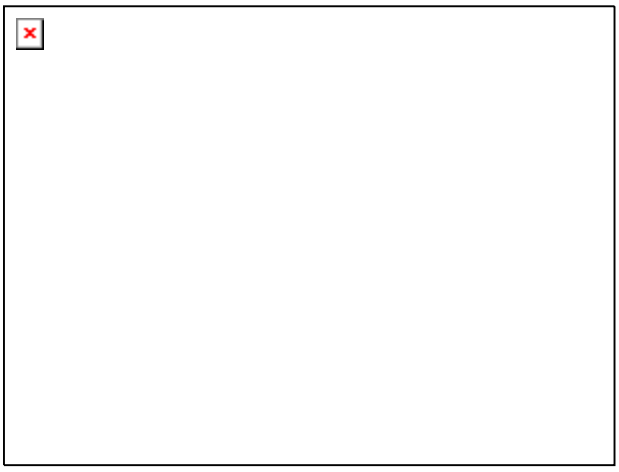

Figure 1. Pre-*Pseudaphycus angelicus* release counts of leaves and bunches with crawlers (6 July 2001), London, CA.

Figure 2. Pre-*Chrysoperla rufilabris* release counts of leaves and bunches with crawlers (21 June 2001), London, CA..

Because *P. angelicus* production was low, we released *C. rufilabris* ahead of *P. angelicus* and in adjacent plots. There was no difference in the number of crawlers on leaves and grape bunches before *C. rufilabris* release (Figure 2). An ANOVA test shows that there is no difference in crawlers presences among the blocks ($F = 0.114$; df = 2,14; $P = 0.89$) and between treatment plots within the blocks ($F = 0.09$; df = 1,14; $P = 0.77$).

Damage ratings (Table 4), indicate that the majority of grape bunches were clean. Very few bunches were categorized as a total loss (category rating = 3). *C. rufilabris* released plots had the highest number of clean fruits (87.13±3.71). When we combined the category 2 and 3 rated fruits and did the ANOVA, there was no significant difference among the three treatment groups ($F = 0.314$; df, 2, 15; $P = 0.74$). The *P. angelicus* released and the control groups had similar percentage of fruits with mealybugs (6.2 ± 4.5% and 5.1 ± 2.7%, respectively). *C. rufilabris* certainly made a difference in lowering mealybug damage. Our *P. angelicus* release numbers

were not high enough to give an adequate control to the current population of grape mealybugs in our experimental plots.

Table 4. A comparison of proportion of clean fruits on the *Chrysoperla rufilabris*, *Pseudaphycus angelicus* released plots and the control plots, London, CA. Damage ratings were conducted on 360 grape bunches per plot (total; 3280 bunches). 2 August 2001.

Block	Treatment	Clean bunches (%)	Some honeydew (%)	Honeydew / Mealybugs (%)	Honeydew and Mealybugs, total loss (%)
1	*P. angelicus*	93.89	3.33	2.78	0
1	*C. rufilabris*	93.89	3.61	2.5	0
1	Control	91.39	2.22	6.39	0
2	*P. angelicus*	89.72	5.00	5.00	0.28
2	*C. rufilabris*	86.39	8.33	5.28	0
2	Control	85.83	7.22	6.67	0.28
3	*P. angelicus*	44.17	26.94	28.61	0.56
3	*C. rufilabris*	81.11	10.28	8.06	0.56
3	Control	65.28	17.50	17.22	0

Objective 2: Ant controls

Results from ant control trials in San Luis Obispo County (working with the obscure mealybug) and Sonoma County (working with the grape mealybug) were somewhat similar. First, research conducted in 2000 with boric acid in sugar bait traps showed a slight reduction in the number of ants foraging on grape vines, but no reduction in the level of mealybugs and no increase in parasitoid activity.(data from Bianchi et al., Central Coast trial). For this reason, we decided to try bait traps with more toxic materials (e.g., imidacloprid, fipronil and thiomethoxam). For this, we received help and collaboration from Dr. Rust and Klotz. The 2001 trial was considerably larger and we also acknowledge the considerable help we received from the Domaine Chandon winery, near Napa California. While data from the 2001 season are still being processed, we can make some preliminary observations.

Mealybug and ant densities were not similar across blocks before treatments were applied. For both, there was an effect caused by the vineyard slope, with fewer mealybugs and ants on the top of the hillside as compared to the lower, flatter regions of the 20 acre block. The abbreviated analysis here does not take into account blocking across the gradient, which we will do in a future, more complete analysis.

Results from monitoring tubes showed a great amount of ant activity in June and July. Each gram of sugar bait material removed represent 1000s of ant visits. Treatments were applied in early June and the amount of ant feeding (from monitoring tubes) dropped dramatically (Figure. However, this reduction was observed uniformly across all treatments, including the control.

After harvest (September 7-12), the amount of sugar bait removed increased sharply. We similarly observed a reduction of summer ant visits to the bait traps containing the insecticides. We believe this mid-summer reduction does not reflect the ant density, rather the ants preferred to tend those mealybugs in the grape bunches rather than visit either the monitoring tubes or bait stations. There were no differences amoung treatments in the number of mealybugs of parasitoids.

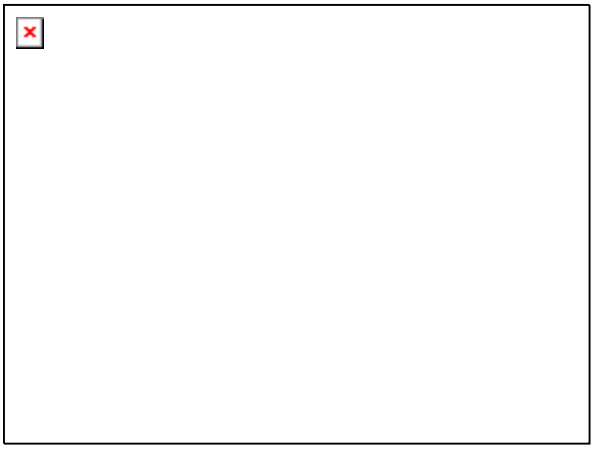

Figure 3. The amount of sugar-solution removed from monitoring tubes (all treatments combined) shows the pattern of ant activity – greater at both the beginning and end of the season. Sonoma County.

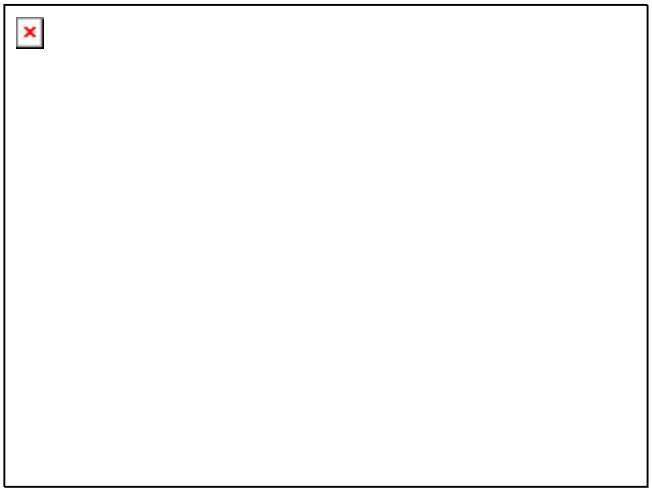

Figure 4. The amount of sugar solution removed from monitoring tubes shows a significant reduction of ant activity in treatments with fipronil and thiomethoxam baited traps. Sonoma County

The results were a bit disappointing but do provide valuable information for next season. First, the bait stations were filled with a shavings of aspen wood to provide a structure for the ants to walk over while feeding on the sugar water / insecticide. We found that the aspen wood rotted in the tubes and produced a rancid smell that resulted in the ants avoiding the tubes. In the 2002 season, we will work with other filler material for the bait traps, and we will test other bait trap designs. Second, the poor ant control of all treatments may also have been a result of late treatment application (June), especially because the ants appeared to prefer to tend the mealybugs rather than feed from the sugar stations. Finally, we will adjust plot size and reduce the treatments tested to compensate for the movement of ants among treatment plots.

Objective 3: Vineyard Cultural Practices

There was a positive correlation between the amount of Nitrogen applied on April 2001 and the vine shoot length measured in June 2001 (Fig. 5). Nitrogen treatments and the NO_3 content of the petioles from early season (10 May 2001) were significantly correlated ($F=27.81$; $df=5, 29$; $P<0.001$). Petiole samples from the late season (near harvest time) are currently being completed by DANR laboratory. Winter whole-vine cane prunings are currently being taken (initial observations show a difference, with more wood produced with higher N treatments). Because of high rate of parasitism in our Nitrogen block last year, we had few overwintering mealybugs.

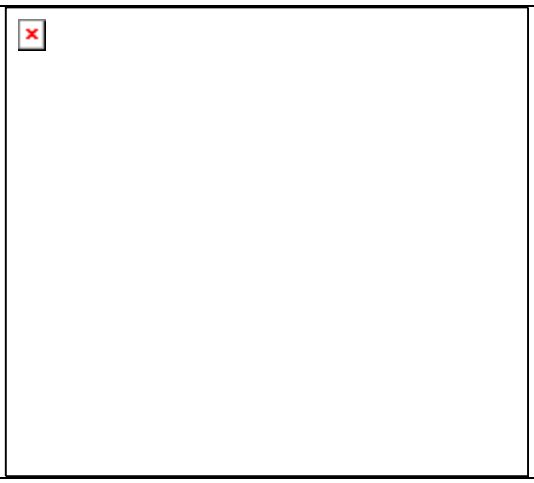

Figure 3. The amount of Nitrogen (Ammonium Nitrate granules) applied in April 2001 and its effect on the vine shoot growth (mean of primary shoots #1, 3, 5, and 8).

We seeded the vines with grape mealybug crawlers in March 2001. The 3-min counts in mid-season (June 2001) and late-season (August 2001) are shown in Fig. 4a and 4b. We are unable to show any direct effect of N on the density of mealybugs from these data.

Although, there was no relationship between the amount of nitrogen applied on the vines and the density of the mealybugs on those vines, there was a slight correlation between the number of the eggs produced by the summer generation mealybugs and the amount of N applied in the field (Fig. 6). We converted the actual length of metatibia and metafemur of females into 4 categories (meta-tibia and meta-femur length, 0.49-0.54 mm = 1; 0.55-0.60 mm = 2; 0.61-0.66 mm = 3; 0.67-0.73 mm = 4). There is a slight effect (but not statistically significant) of the length of the meta-tibia and meta-femur on the number of the eggs produced by the females (Fig 7) disregarding the amount of N applied ($F = 0.85$; $df = 3,52$; $P = 0.48$) in the field. Parasitism accounted for 34% of the mortality of mealybugs collected late season in the season, after fruit damage ratings (5 September 2001), among these 7% died from *A. notativentris* and 14% from *P. angelicus* (Table 5). The fruit damage rating showed very little effect of the grape mealybugs, 92-99% of the fruits were clean, there were no grape bunches with category 3 damage.

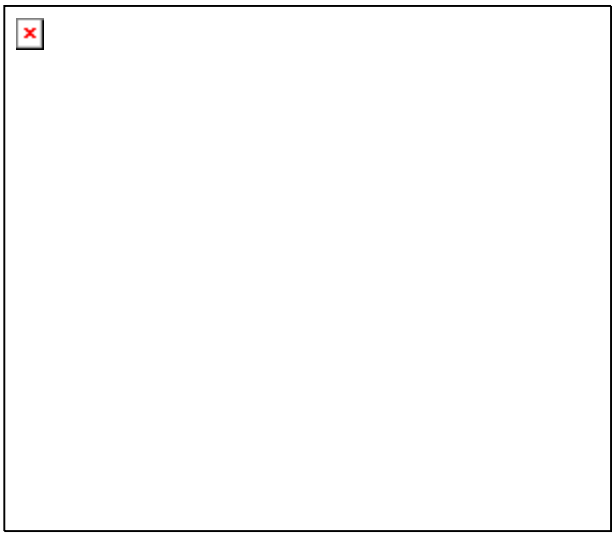

Figure 4a. Three-min counts of grape mealybugs in the mid-season in the Nitrogen block, Kearney Agricultural Center, Parlier, CA, June 2001.

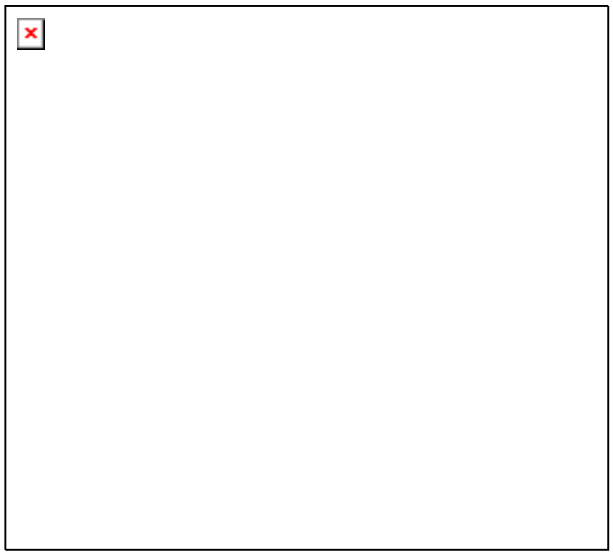

Figure 4b. Three-min counts of grape mealybugs in the late-season in the Nitrogen block, Kearney Agricultural Center, Parlier, CA, August 2001.

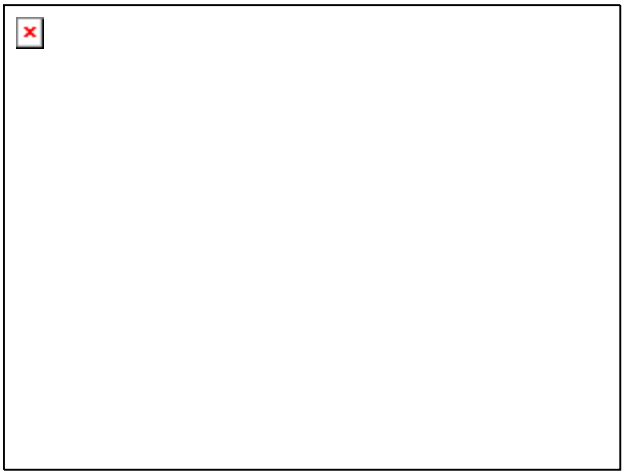

Figure 7. The effect of nitrogen application on the number of the eggs produced by the female grape mealybug.

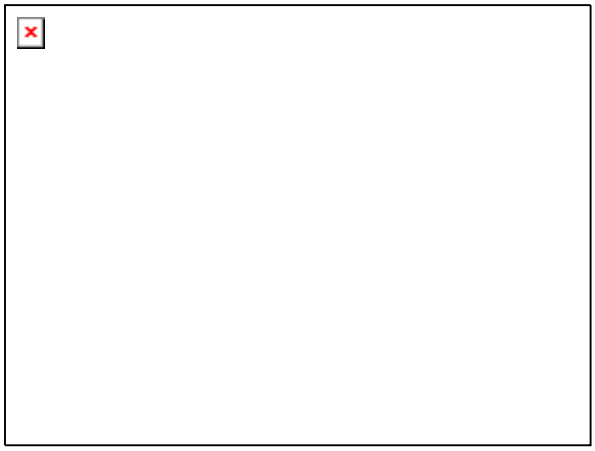

Figure 8. The number of the eggs deposited and the length of metatibia and metafemur of the female grape mealybugs from the Nitrogen Block at KAC, Parlier, CA 2001.

Results from the girdle and GA treatments show no significant difference in mealybug density (overwintered generation and first summer generation) (Fig. 9). These results are similar to work completed in the same block in 1999 and 2000. However, research in a nearby block showed mealybug densities were higher on girdled than non-girdled vines in 1 of 2 years (W Bentley, personal communication). We conclude that any effect of berry sizing practices on mealybug density is slight.

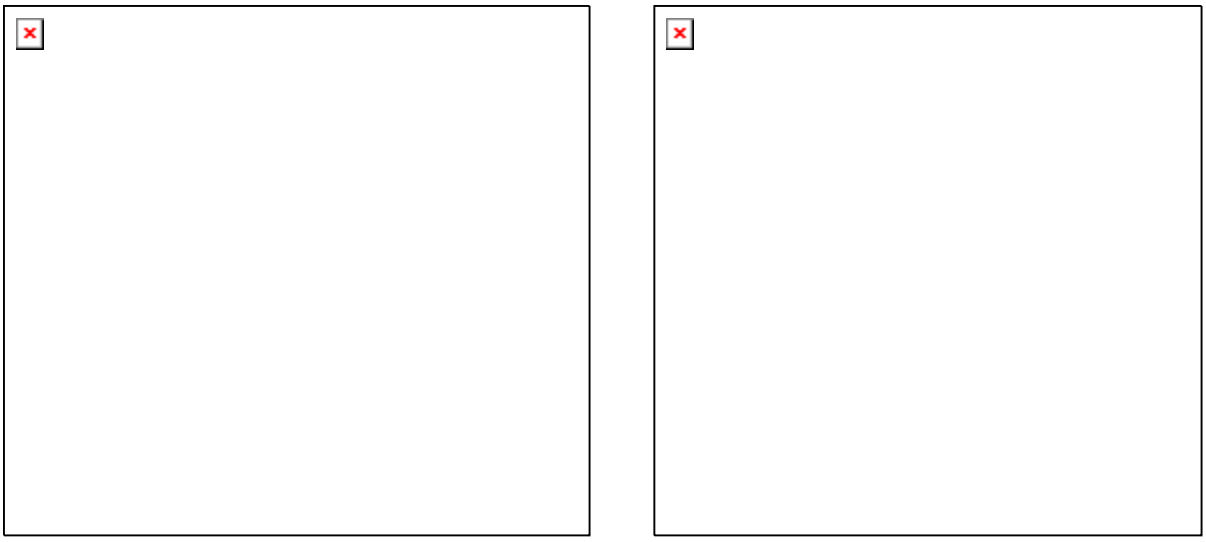

Figure 9. (A) June and (B) August number of grape mealybugs (mean ± SE) in control, girdled, gibberellic acid (GA), and "girdled + GA" treatments show no significant difference between treatments except in one case (with Fishers' PLSD test at 5% level of significance), G&G Block, KAC, Parlier, CA 2001. One –way ANOVA tests for (A), F = 0.12; df = 3, 44; P = 0.95; and for (B), F = 1.96; df = 3, 44; P = 0.13).

Work conducted in the greenhouse provided conditions to better control N levels and natural enemies that influence mealybug densities. In these more controlled studies, N-fertilization treatments had a significant and positive correlation with mealybug egg deposition ($F = 4.38$; df = 5,242; $P = 0.0008$) (Fig. 10) and is similar to previous year's results. The number of the eggs deposited by the females from this year (highest average no. = 60 eggs/female) were fewer than those from last year (highest average no. = 180 eggs/female). In the field trials also we observed the similar trend, the average number of the eggs deposited by the females from this year were fewer than those from the last year. Perhaps the mealybugs we inoculated into the vines early in March 2001 have lower fecundity rates than those we inoculated in 1998 for 1999 and 2000 studies. There was a significant relation between the length of the meta-tibia and meta-femur (combined) of the female grape mealybug and the number of the eggs deposited ($F = 3.12$; df = 3,215; $P = 0.03$) (Fig. 11).

Mealybug census taken in the greenhouse did not show any faster or slower development of mealybugs due to nitrogen levels (Fig. 12). This indicates that the higher level of N does not promote the faster development of the mealybugs, but it does increase the mealybug size or fecundity. Levels of N in the petioles are being analyzed at DANR laboratory.

We conclude that more vigorously growing vines can increase mealybug pest status. There is less evidence that berry sizing practices have any influence on mealybug pest densities.

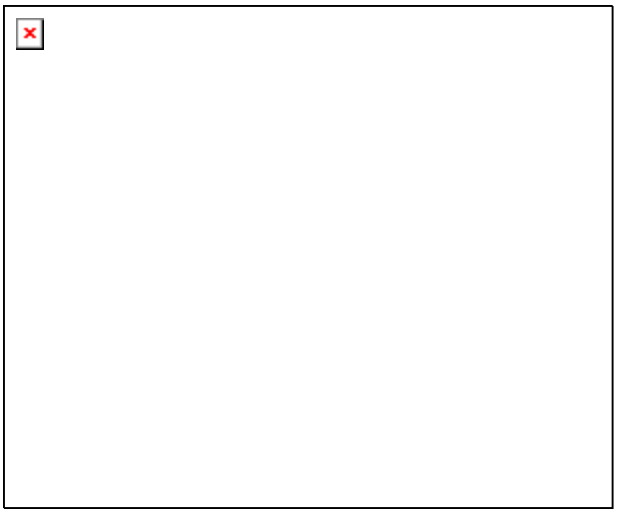

Figure 10. Average number (±SE) number of eggs per female grape mealybug reared on potted vines grown in the greenhouse under six N fertilization treatments, KAC, Parlier, CA, 2001.

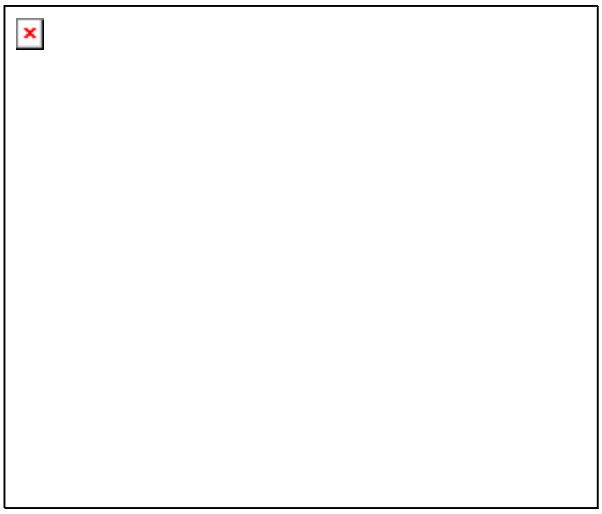

Figure 11. Relationship between the number of the eggs produced by grape mealybugs and the length of their meta-tibia and meta-femur. These mealybugs were reared in the greenhouse grown potted vines treated with six N treatment levels, KAC, Parlier, CA, 2001. Note that length of meta-tibia and meta-femur is categorical – category 1 = 0.30-0.39 mm; 2 = 0.40-0.49 mm; 3 = 0.50-0.59; and 4 = 0.60-0.69 mm long.

Figure 12. Distribution of proportion of the grape mealybug instars reared on the vines treated with six different levels of N in the greenhouse, KAC, Parlier, CA 2001.

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Geiger, C. A. and K. M. Daane. 2001. Seasonal movement and sampling of the grape mealybug, *Pseudococcus maritimus* (Ehrhorn) (Homoptera: Pseudococcidae) in San Joaquin Valley vineyards. J. Econ. Entomol. 94; 291-301.

Geiger, C. A., K. M. Daane, and W. J. Bentley. 2001. Sampling program for grape mealybugs improves pest management. Cal. Ag. May-June 55: 19-27.

VI. OUTSIDE PRESENTATIONS OF RESEARCH:

List of Journals and Articles – I do not list reports to AVF, CTGC, CRMB, VC or CCGPRVE. I list manuscripts for refereed journals that are in preparation, but I do not list planned articles that are not yet near submission.

Geiger, C. A., and Daane, K. M. 2000. Agronomic practices and mealybug infestations among California table grape growers: Results of a 1999 survey. San Joaquin Valley Table Grape Seminar, 23 Feb 2000, Visalia, California. 23 pp.

Peacock, B., Daane, K., Beede, B., and Haines, D. 2000. Vine mealybug: A serious new pest in the San Joaquin Valley. University of California, Division of Agriculture and Natural Resources. Publication IPM6-00, Oakland, CA

Geiger, C. A. and Daane, K. M. 2000. 1999 table grape survey, Part I: mealybugs and agronomic factors. *Grape Grower* 32(8): 16-21, 26-27.

Geiger, C. A. and Daane, K. M. 2000. 1999 table grape survey, Part II: grower information sources pest monitoring, and adoption of IPM methods. *Grape Grower* 32(9): 21-28.

Daane, K. M. 2000. Developing an Integrated Pest Management program in California vineyards: hitting a moving target, pp. 364-369. In. *Proceedings of the American Society of Enology and Viticulture*, Seattle, Washington, June 19-23.

Malakar-Kuenen, R., Daane, K. M., Bentley, W. J., Yokota, G. Y., and Martin, L. 2001. Population dynamics of the vine mealybug and its natural enemies in the Coachella and San Joaquin Valleys. University of California Plant Protection Quarterly 11(2): 1-3.

Geiger, C. A., Daane, K. M., and Bentley, W. J. 2001. Development of a sampling program for improved management of the grape mealybug. *California Agriculture*. 55(3):19-27.

Geiger, C. A., and Daane, K. M. 2001. Seasonal movement and sampling of the grape mealybug, *Pseudococcus maritimus* (Ehrhorn) (Homoptera: Pseudococcidae) in San Joaquin Valley vineyards. *Journal of Economic Entomology* 94: 291-301.

Godfrey, K., Daane, K. M., Bentley, W. J., Gill, R., and Malakar-Kuenen, R. 2002. Mealybug found in California vineyards. University of California Division of Agriculture and Natural Resources Leaflet. (Accepted)

Millar, J. G., Daane, K. M., McElfresh, J. S., Moreira, J., Malakar-Kuenen, R., Guillen, M., and Bentley, W. J. 2002. Development and optimization of methods for using sex pheromone for monitoring vine mealybug in California vineyards. *Journal of Economic Entomology* (Submitted)

Daane, K.M., Malakar-Kuenen, R., Geiger, C.G., and Yokota, G.Y. Influence of vineyard management practices on the abundance and development of the grape mealybug, *Pseudococcus maritimus*. (in preparation for *Entomologia Experimentalis et Applicata*)

Malakar-Kuenen, R., Daane, K. M., Guillen, M., Yokota, G. Y., and Bentley, W. J. Seasonal distribution of the vine mealybug on the vine in Coachella and San Joaquin Valley vineyards. (in preparation for *Journal Economic Entomology*).

Malakar-Kuenen, R., K. M. Daane. Seasonal development of the vine mealybug, *Planococcus ficus*, in California's Central Valley. (in preparation for *Environmental Entomology*).

Daane, K. M., Jani, A., and Bianchi, M. Establishment of *Pseudaphycus flavidulus* and *Leptomastix epona* in California vineyards for control of obscure mealybug. (in preparation for *Pan Pacific Entomologist*).

Daane, K. M., Jani, A., M. Bianchi, and Geiger, C.G.. The importance of controlling ants to help natural enemies control vineyards mealybugs. (in preparation for *BioControl*).

Oral Presentations -- Grape and vine mealybug reports have been combined because information from both is almost always presented.

Inoculative release of beneficial insect arthropods in perennial systems – the transition from theory and experimental to commercial use. *2nd Annual California Conference on Biological Control*. Riverside, CA. Jul 2000. *35 min presentation, ~120 researchers and PCAs in attendance.*

Grape: Integrating biological control into a constantly changing environment of new pests and new pesticides. *2nd Annual California Conference on Biological Control*. Riverside, CA. Jul 2000. *35 min presentation, ~120 researchers and PCAs in attendance.*

Biological control for new pests invasions in California vineyards: obscure mealybug as a case example. *XXI International Congress of Entomology* (and *XVII Brazilian Congress of Entomology*). Foz do Iquassu, Brazil. Aug 2000. POSTER Presentation.

The role of cover cropping in vineyard IPM. *XXI International Congress of Entomology* (and *XVII Brazilian Congress of Entomology*). Foz do Iquassu, Brazil. Aug 2000. POSTER Presentation.

Mealybug Field Tours. California Table Grape Commission and the University of California Field Day. Parlier and Del Rey, CA. Aug 2000. *4 hr field day, ~40 growers and PCAs in attendance.*

Grape Pests, Diseases, and Disorders (Viticulture & Enology118), UC Davis (Fall Quarter, 2000). A 1.5 hr lecture on the chemical, cultural and biological control programs developed to suppress leafhopper populations in California vineyards. Course instructor: Dr. L Williams

Mealybugs in vineyards: is the vine mealybug going to move further north in the Central Valley? Merced College Pest Management Update Class. Merced, CA. Oct 2000. *1hr presentation, ~50 growers and PCAs in attendance.*

Leafhoppers and mealybugs. Grape Pest Management (Fruit Science 414). California Polytechnic State University, San Luis Obispo, CA. May 2001. *1hr lecture to, ~50 students.*

Augmentative biological control and the insectary industry: navel orangeworm and vineyard mealybugs as case studies. Western Regional 185 Meeting (Biological Control Conference). South Lake Tahoe, CA. Oct 2001. *30 min presentation, ~30 researchers and USDA administrators in attendance.*

Is biological control of the obscure and grape mealybugs dependent on ant control? *11th Annual Research Workshop on Grape Pest Management*. Parlier, CA. Nov 2001. *20 min presentation, ~40 researchers/cooperative extension and grape industry personnel in attendance.*

Vine mealybug management. Reedley College's PCA Continuing Education Seminars. Reedley, CA. Nov 2001. (second author with R. Malakar-Kuenen). *30 min presentation, ~30 growers and PCAs in attendance.*

Vine mealybug age structure and distribution on the vine influences parasitoid effectiveness. *11th Annual Research Workshop on Grape Pest Management.* Parlier, CA. Nov 2001. (second author with R. Malakar-Kuenen). *20 min presentation, ~40 researchers, cooperative extension and grape industry personnel in attendance.*

Vine mealybug, a new pest in the San Joaquin Valley. *Annual Meeting of the Entomological Society of America*. San Diego, CA. Dec 2001. (junior author with R. Malakar-Kuenen). *10 min presentation, ~40 researchers and students in attendance.*

Population dynamics of the vine mealybug in the Coachella Valley. *Annual Meeting of the Entomological Society of America.* San Diego, CA. Dec 2001. (junior author with M. Guillen). POSTER presentation.

Vine mealybug and its parasitoids compared between the San Joaquin Valley and Coachella Valley vineyards. Madera Lunch and Vineyard Management Series (Dr. G. Leavitt organizer). Jan 2002. Madera, CA. *30 min presentation, ~25 PCAs and growers in attendance.*

The increasing spread of the vine mealybug, a new pest in the San Joaquin Valley. *Sun Maid Best Pest Management Seminar.* Selma, CA. Jan 2002. *30 min presentation, ~50 PCAs and growers in attendance.*

ACKNOWLEDGMENTS:

We thank Jocelyn Millar (UC Riverside) for discussions on mealybug biology; Zaninovich Vineyards, Taylor Farms, Domain Chandon Vineyards, Rutherfurd Vineyards, Colinas Farming Company, Tudor Ranch, Edna Valley Vineyards, Paragon Ranch for providing field sites; Nicole Vjada, Kristi Baggs, Jeff Esajian, Mariana Krugner, Jose Tinoco, Nathan Vik, and Rodney Yokota, for help with field and laboratory samples.

VIII. RESEARCH SUCCESS STATEMENTS:

The development of least toxic controls for mealybugs in the grape mealybug complex were not fully addressed until this research. We began work with a description of seasonal mealybug age structure and seasonal development, which has resulted in the production of guidelines for more rapid sampling. During the past two years we switched our focus to control programs, investigating both biological and cultural control. Our work clearly showed the importance of ant control in the establishment of effective biological controls. This led to ongoing collaborative projects with Dr. Mike Rust and Dr. John Klotz to develop better ant control programs for vineyards. A 25% sugar solution and small amounts (0.001-0.0001%) of either imidacloprid, fipronil or thiomethoxam were placed in bait traps throughout a heavily infested Napa Valley wine grape vineyard. Results show a significant late-season reduction of ant activity at the fipronil and thiomethoxam treatments. However, there was no reduction in mealybug density or increase in parasitoid activity. While results showed no economic reduction of Argentine ants, we are encouraged by new information gained and will make the needed adjustments for study in the 2002 season. We also have developed insectary rearing methods for some of the more important mealybug parasitoids. Currently we are testing the augmentative release of one of those parasitoids: Pseudaphycus angelicus. Finally, we have carefully studied the effects nitrogen fertilization (0, 25, 50, 100, 200 and 400 lbs/ac) and girdling and gibberellic acid practices on mealybug populations. Results from field trials showed no difference between N fertilization treatments or berry sizing practices in mealybug density or egg deposition. We believe these field results were influenced by resident natural enemies, which lowered mealybug densities. In controlled greenhouse trials, mealybug densities and egg deposition on potted plants were greater in higher N fertilization treatments. We conclude that more vigorously growing vines can increase mealybug pest status. The effect of cultural practices have always been questioned by growers and now some of these questions have been answered.

VIII. FUNDS STATUS:

Salary positions include a post-doctoral step II (Raksha Malakar-Kuenen) appointment at 100% that was divided between research on the grape mealybug complex (this project) and one on the vine mealybug, an SRA III (Glenn Yokota), and seasonal Laboratory Assistants. Travel costs include five trips to the San Luis Obispo, shared car rental for a “lab vehicle at UC Berkeley and KAC, and shared travel cost reimbursement for KMD (Berkeley to Fresno). Supplies included minor field equipment (pruning shears, paper and plastic bags, etc.) and insectary supplies (squash, potatoes and cages) (ca. $3,000). Funding was requested from the California Table Grape Commission, California Raisin Marketing Board, Viticulture Consortium, and American Vineyard Foundation through a joint grant.