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SUMMARY OF ALMOND RESEARCH 1999

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Title: Part I: Testing chemical controls for navel orangeworm and peach twig borer Part II: San Jose Scale Natural Enemies: Investigating the Potential for Natural or Augmented Control

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Part I - Testing chemical controls for navel orangeworm and peach twig borer

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Project Justification

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Moth larvae feeding in fruit and growing shoots remain a primary concern for stone fruit and almond growers. The oriental fruit moth (OFM) and peach twig borer (PTB) are two of the more costly pests in peaches, nectarines and plums and PTB is a primary pest of almonds. University of California researchers have developed excellent information on OFM and PTB biology, which has been used to develop monitoring programs, and cultural and chemical control practices. Currently, OFM can be controlled with in-season applications of an organophosphate (e.g., Guthion), pyrethroid (e.g. Asana) or carbamate (e.g., Lannate) insecticide. For PTB, a dormant season application of an combined with an organophosphate (e.g., Diazinon) can reduce numbers of overwintering PTB larvae and, when needed, a well-timed spring-summer application(s) of an organophosphate (e.g., Lorsban) or carbamate (e.g., Lannate) can be used to suppress rising PTB densities and protect the crop.

Even with these proven "standard" control practices, OFM and/or PTB populations may require additional control measures; after which there can still be, in some orchards/seasons, significant economic damage. More important is the possibility that future legislative restrictions will remove from use some of the more effective insecticide products. For this reason, research has been ongoing to develop new, or improve current, control programs. To this goal, this past decade has seen the development and widespread use of two "least-toxic" control alternatives for these moth pests. OFM pheromone confusion has a confirmed success record in

stone fruit (see Rice & Kirsch, *in* Ridgway et al., 1990, *Behavior-modifying Chemicals for Insect Management:* 193-211). Similarly, a highly successful "soft" PTB control program uses *Bacillus thuringiensis* (Bt) applications near bloom in stone fruit (see Barnett *et al., 1993, California Agriculture,* 47[5]: 4-6).

Although successful "standard" and "least-toxic" control practices exist, continued research on pest management alternatives is warranted. For example, the effectiveness ofOFM pheromone confusion can breakdown if dispensers are incorrectly placed in the field. A rarer, but potential, situation is when the initial density of overwintering OFM can be so great as to overwhelm the confusion program. The current "least-toxic" insecticide program for PTB is *Bacillus thuringiensis* applications in spring and/or summer (see Figure 1). However, while this program has been effective in early-harvested peach and nectarine cultivars, its use in late harvested stone fruit and almonds is limited because PTB populations have the chance to recover during the season (residual activity of *B. thuringiensis* is shorter during the hot summer temperatures and require multiple applications to control PTB). This is especially troublesome in almonds, where PTB density and problems can increase throughout the season. For these reasons, continued research of alternative controls for moth pests is still warranted.

Objective.

To test alternative chemicals for their efficiency against the peach twig borer and to screen this same material for secondary effects on beneficial insects common to peach and almond orchards

Procedures

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In the first project, we screened Dimilin, Confirm, and Success for efficacy against peach twig borer (PTB). Our original goal was to help with registration of alternative materials for use on almond (Dimilin is not currently registered on almonds). Tests with Confirm and Success were added as a comparison. Even though the above information indicates natural regulation of PTB is still some distance away, there are alternative PTB controls that utilize "soft" insecticides. These materials include bacterial by products (e.g., Success® - which is currently registered for use in almonds) and insect growth regulators (IGRs), which are larvicides that interfere with an insects' chitin deposition (the outside shell) and this prevents the insect from molting (e.g., Dimilin® and Confirm®, which are seeking registration).

In 1998 and 99, we used a "diet-incorporated" assay to develop LD50s and LD90s for three insect growth regulators (IGRs): Dimilin, Confirm, and Success. These IGRs are larvicides (which kill the moth larva) that interferes with an insects' "chitin deposition" and this prevents the insect from molting. It is considered a "soft" insecticide that is safe compound to use (e.g., mammalian toxicity: Dimilin $LD_{50} = 4640.0$ mg/kg; in comparison, Guthion $LD_{50} = 4.4$ mg/kg). All three products were found effective against PTB (Figures 1 & 2).

In 1999, we continued tests on the effectiveness of these products in the field. Almond trees were treated with four times the label rates of the three different IGRs (Dimilin, Confirm, and Success, and a control treatment; randomized block design; 3 replicates). Nuts from those trees were collected at 1, 6, 12, and 22 days after spray application and placed, individually, in plastic rearing cells. To each cell, a PTB (larva) was added. Because any insecticide application has the potential to affect other pest and beneficial insects in the orchard, we also screened the tested products against some of the more common beneficial insects: green lacewing (larva). Goniozus legneri (adult), or Aphytis spp (adult) was added (20 replicates each). The condition of the insects (alive or dead) was checked at 5, 7, 10, 12, 14 and 17 days (until all insects had died).

Note here that initial funding for this work was provided by Uniroyal (\$5,000 in 1996 and 1997) to complete laboratory and field bioassays of their product (Dimilin) against PTB. The results showed Dimilin (like the other IGRs tested) to be a very effective PTB insecticide. As a result, Uniroyal is in the process of registering Dimilin for use on almonds.

PTB LD50s: 1999 Lab Studies PTB LD50s: 1998 Lab Studies 99 99 Dimilin Mortality (%) Mortality (%) 90 90 70
50
30 70
50 thuringiensis 30 **O Dimilin (IGR, ingested)** ó Confirm (IGR, ingested) 10 10 Success (Bacteria hyproduct. contact) 0.01 0.1 $\ddot{\mathbf{1}}$ 10 100 0.01 0.1 $\overline{1}$ 10 Dose (µl/l diet) Dose (µl/l diet) Figure 1. Dimilin (and other products) were shown to Figure 2. Pesticide screening was continued in 1999. have good activity against PTB (a lower dose and higher Same results, the IGRs and Success all proved effective kill, note however that costs and application rates are not at controlling PTB in laboratory diet-incorporated provided) studies.

Results

Results in both 1998 and 1999 have shown all products tested are quite effective against PTB. The 1999 study in almonds best represents a field-scenario. Here, Dimilin and Success provided >80% control for a 3-week period post applications (Figure 4). Confirm also controlled PTB during this period, although at a somewhat reduced effectiveness in on the latter sampling dates. Comparison between products are difficult because we used different 3-4 time the label rate in this first trial.

One problem with thee products, as compared with Bt, is their potential negative effects on beneficial insects in the orchard. While there is a need to repeat work with the IGRs on adult parasitoid mortality, results in 1999 showed some mortality of *Aphytis* and *Goniozus* adults to some of the products tested, while none of the products had a significant effect on green lacewings. However, valuable conversations with product company representatives have led us to question our methodology. In 2000, we will conduct some simple laboratory tests to confirm or refute the effect of IGRs and Success on common natural enemies found in stone fruit and almond orchards. A discussion of this work, especially the effect on beneficial insects, will be presented in next year's full report.

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Part I: San Jose Scale Natural Enemies: Investigating Natural or Augmented Controls Part II

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Project Justification

In almonds, San Jose scale (SJS) has been, typically, a secondary pest. Typically, SJS pest status is highest on stone fruit, especially nectarine cultivars, where scale readily settle on the fruit and even small popUlations can result in serious cosmetic damage and crop loss. Further, SJS outbreaks were often attributed to insecticide treatments applied for moth pests (which may disrupt SJS natural enemies) or seasonal fluctuations. However, there have been recently been SJS infestations reported in Fresno, Tulare and Kern county almond and stone fruit orchards, where SJS reached primary pest status and resulted in the infested branches dying back and a yield reduction (culled stone fruit).

In response to grower concerns, the California Tree Fruit Agreement (CTFA) organized a SJS research team to investigate possible reasons for increased SJS pest status and to develop better control practices. Research areas have been prioritized and include studies of chemical controls (Walt Bentley and Rich Coviello), sampling methods (Walt Bentley), SJS insecticide resistance (Beth Grafton-Cardwell), SJS field biology studies (Walt Bentley and Kent Daane), and biological controls. I am a member of the SJS research team and in 1999 conducted research on the natural regulation of SJS scale. This work was supported by joint funding from CTFA, California Cling Peach Growers Advisory Board (CPGAB) and the Almond Board of California.

Grower observations and 1999 research suggest that SJS can be (and is most commonly) controlled by the action of three small parasitic wasps. Highlights from the 1999 research

include: (i) three parasitoid species dominate the natural enemy complex *(Encarsia perniciosus, Aphytis aonidiae,* and *Aphytis vandenboschi),* (ii) *E. pernicious* was found in every orchard sampled, while *Aphytis* species and lady bird beetles were less common in orchards treated with an organophosphate, (iii) parasitoid collections on "live" SJS traps indicate all three parasitoid species can be in equal abundance (in some orchards in late season), but catches in SJS pheromone traps commonly show *Encarsia* is the dominant parasitoid, suggesting that *Encarsia* adults are attracted, more than *Aphytis,* to the SJS pheromone lure, (iv) throughout the year on "squash" traps and pheromone traps, *E. perniciosus* was by far the dominant parasitoid, (v) total SJS abundance is higher on "hangers" (stone fruit fruiting wood) and new "sucker" wood than on older scaffolding branches, and the highest density of SJS (per sample area) was typically on second-year wood (the hangers in stone fruit orchards) and (vi) the three parasitoid species were not evenly distributed, with *Encarsia* more common on wood deeper inside the tree while *Aphytis* spp. were more evenly distributed throughout the tree canopy.

From these results, we hypothesize that the more hidden location of *Encarsia* may provide some protection from insecticide applications as compared to the more exposed location of *Aphytis.* We also hypothesize that in "outbreak" seasons, both *Encarsia* and *Aphytis* species may be needed for natural control of SJS because different species may forage preferentially on different parts of the tree canopy.

Objectives:

1. Describe the natural enemy complex attacking SJS in stone fruits and determine the field effectiveness of common parasitoid species.

(a) Survey stone fruit orchards with and without SJS scale infestations to determine if resident natural enemies have the potential to naturally regulate SJS densities.

(b) Compare natural enemy densities with management practices and orchard condition.

2. Establish insectary colonies of selected SJS natural enemies for laboratory and field experiments.

- (a) Determine the effect of commonly used insecticides on selected natural enemies.
- (b) Investigate the biology and potential effectiveness of common SJS parasitoids.
- 3. Test selected SJS parasitoids for use in augmentative release programs.

(a) Release selected SJS parasitoids in small cage trials to determine release rates and timing that provide SJS control.

(b) Test SJS parasitoids in open-field release trials in commercial vineyards (this subobjective is dependent on results from Objective 3a and will, therefore, not start in 1999).

Procedures

Objective 1.

An initial objective is to determine the efficacy of resident natural enemies and whether their species composition (what kinds of natural enemies) or abundance (how many) vary

between different crops (e.g., almonds, stone fruit) or grower cultural practices (e.g., insecticide use). SJS and natural enemy populations were studied in detail in **11** stone fruit blocks (Fresno and Tulare County), which were sampled weekly during the growing season. These orchards represented blocks with as without insecticide treatment for SJS (Table 1). Additionally, we surveyed 5 almond orchards (Fresno and Kern Co.), fresh market stone fruit (Fresno and Kings Co.) and cling peach orchards (Butte and Glenn Co.).

Table 1. Sampled fields, 1999 season.

Sampling methods included SJS pheromone traps and double-sided sticky tape. In stone fruit orchards, five SJS pheromone traps were placed in each stone fruit orchard to sample for male SJS and, on the same five sampled trees, sticky-tape was placed around two limbs to sample for crawlers. Traps and tape were changed weekly from May until December.

In all the stone fruit orchards and almond orchards we also made periodic field collections of scale for SJS parasitoids. Another sampling methods for parasitoids was placement of squash infested with SJS in the orchards. The purpose of the infested squash was to create a "controlled infestation" of SJS at different times of the year. The squash were infested in the insectary with 100s of SJS crawlers, and were held until the scale settled. The squash were then hung in the orchard for 2-3 weeks, removed before the scale produced another generation of crawlers, and brought back to the laboratory and placed in emergence containers. Live parasitoid were collected, identified and used for insectary colonies. The number of live and parasitized SJS on the squash was counted.

At harvest, 1,000 fruit were collected from the monitored stone fruit orchards and number of infested fruit (SJS, worms, katydid, and thrips) and the number of scale per fruit were recorded.

To determine parasitoid distribution in the canopy, we conducted two intensive sampling procedures. First, in a 15-year old stone fruit orchard with high SJS densities, all three parasitoid species and no insecticide sprays, we completed "whole" limb sampling. On each of three trees, one scaffolding branch was cut at the base, near ground level. The entire scaffolding and its offshoots were divided in upper and lower and inner and outer sections of scaffolding, fruiting wood (or "hangers"), new growth, sucker wood and leaves. This material was bagged and stored at 34°F until dissected. In the laboratory, all SJS were recorded by stage and condition (live, dead, parasitized). From the parasite's exit whole, parasitoid species could be identified as either *Encarsia* or *Aphytis.* In a second experiment, yellow or white sticky cards (3 X 5 inch) were hung in trees at combinations high or low; inner or outer; and north, east, south or west directions. Cards were placed in the trees, timed to parasitoid emergence during the third generation. After two weeks, the cards were removed and the number of SJS and different parasitoid species were recorded.

Objective 2.

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What is the effect of common insecticides on SJS natural enemies? To determine if the disruption of biological control (by chemicals) is responsible for some of the SJS outbreaks, bioassays will be performed on *Encarsia pernicious, Aphytis vandenboschi* and *A. aonidiae.* This work is needed to determine if small amounts of residual insecticides can kill parasitoids, which may explain the poor natural regulation of SJS after orchards receive only 1 to 2 insecticide applications (often for moth or mite pests).

Our goal in 1999, to complete this work, was the establishment of parasitoid colonies. Live parasitoid reared from SJS infested squash placed in orchards. This material was placed on clean squash in the insectary. By trial and error, procedures for parasitoid production were developed.

Objective 3.

One goal of the field surveys was to determine the importance of individual parasitoid species in the regulation of SJS. Because there has been a great deal of success with the augmentation of parasitoids against "diaspid scale" (e.g., releases of *Aphytis melinus* for control of red scale on citrus trees), in 1999, we worked towards the development of an augmentation program for SJS.

One of the biggest hurdles for an augmentation program is the development of insectary procedures to mass-rear viable and effective natural enemies. As mentioned above, colonies of *A. vandenboschi* and *E. perniciosus* have been established. We our currently conducting laboratory studies on parasitoid preference for SJS stages – to better determine when to release

the parasitoids $-$ and parasitoid fecundity and host feeding limits $-$ to better determine the release rates needed to suppress SJS.

Initial release studies were conducted with a parasitoid of red scale. Laboratory studies with *A. melinus* (which is commercially available) indicated that this species could attack SJS (both host feeding and parasitism). For this reason, small scale field studies followed laboratory studies. Over 50,000 *A. melinus* were release in three trees. Hung in each tree were squash with 100s of SJS scale. After 7 days the squash were taken to the laboratory and the number of scale counted.

Currently, colonies of *A. vandenboschi* and *E. perniciosus* have been established from fieldcollected material. During the dormant season and spring, laboratory studies will be conducted to determine their potential in augmentation programs, which will be tested in summer 2000.

Results

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Objective 1.

Results show three parasitoid species dominate the natural enemy complex: *Encarsia perniciosus, Aphytis aonidiae,* and *Aphytis vandenboschi.(Figure* 5) Highlights of this work are: *(1) E. pernicious* was found in every orchard sampled, (2) *Aphytis* species were less common, (2) on SJS pheromone traps, the ratio of *Aphytis* : *Encarsia* species is lower than from "live" SJS squash traps (this result indicates that *Aphytis* species densities are not well-represented by collections on SJS pheromone traps), (3) in most orchards, SJS was not an economic problem, in large part due to the action of natural enemies or insecticide sprays.

Of the stone fruit blocks sampled weekly there was a wide spread of SJS damage. Orchards using a dormant treatment of oil and insecticide (Supracide or Sevin) or an in-season spray (pencap for OFM, Lorsban for moths, Carzol for thrips) had SJS densities below 2% fruit infestations (Figure 6). More important for this work is the variation between blocks without insecticide treatments, where, SJS fruit infestation (at harvest, ranged from three blocks near 15% damage to 2 blocks with no SJS damage (or <0.2%).

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If parasitoids are truly controlling the scale, when not disrupted by insecticides, then why this difference? We have further divided the blocks into three categories: (A) – dormant oil, (B) - dormant oil and an in-season insecticide (only two blocks were in this category, one received Carzol and the other Lannate), and (3) a dormant oil & OP and one or more in-season insecticide(s). The seasonal numbers of SIS males in pheromone traps (Figure 7) and the number of SIS crawlers on sticky tape (Figure 8) provide some indication of differences between these orchard categories. Clearly, there are more SIS in orchards without synthetic insecticides (other than oil) (Figure 7A, 8A). Therefore, if oil alone is to be used as a SJS control, improvements to spray application should be considered. Surprising is that category $B -$ summer insecticides used for thrips and worms – blocks had relatively good SJS numbers in pheromone traps (Figure 7B) and low crawler counts on sticky tape (Figure 8B). Note that in these same blocks there was an increase in the number of parasitoids collected (pheromone traps) throughout the season (Figure 16B). Counts of adult male and crawler SIS were low in category B blocks (Figure 7C and 8C), which received a dormant OP treatment.

Note that these are not replicated blocks and, for this reason, there can be many betweenorchard factors that influence SIS numbers. Nevertheless, from this initial data analysis, a number of questions are raised.

One area that must be improved to better understand scale and parasitoid densities is the sampling methodology. For example, in Figure 9, the ratio of crawlers caught on tape did not

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correlation well to the numbers of adult males caught in pheromone traps. One obvious reason for the "L" shape formed by the data points is the category "C" orchard blocks that received summer insecticide applications (e.g. insecticide sprays reduced crawler numbers after high pheromone catches of adult males). Still, it is surprising to that find high crawler counts associated with low adult male counts. While not strong, there was a correlation between the number of SJS and the number of parasitoids caught in pheromone traps. Here, the obvious explanation is that more parasitoids were found in blocks with more scale.

We conclude that pheromone traps and sticky tape can be used to indicate when adult flight occurs and the period of crawler hatch. But, until better analysis of a larger data set is available, trap information can not be correlated to SJS or parasitoid densities. A good indication of the sampling difficulties is seen in a comparison of crawler counts on the two pieces of sticky tape placed in the same tree (Figure 11). The poor correlation from branch to branch (and tree to tree, data not shown) implies that many samples must be taken in order to get an accurate count. Clearly, the sole purpose of sticky tape is to determine egg hatch and crawler movement, not to indicate SJS density.

In all three orchard categories, there was a distinct, but often different, pattern of parasitism (Figures 12-14). In comparison to scale density, the ratio of adult parasitoid to SJS caught on pheromone traps shows a clear differentiation between orchard categories only in the overwinter generation (Figure 12). At that time, there were far more parasitoids coming out of blocks in Category A (dormant oil) than either block that received insecticides (again, there were only two blocks in Category B and this is not a replicated study). Note that the overwintered generation has a much higher parasitoid : SJS ratio $(50-125:1)$, in part, because of the small SJS male flight at this time of year. During the season, the parasitoid : SJS ratio dropped, but in every Category there were always more parasitoids caught than SJS in each generation.

Use of infested squash to compare parasitoid effectiveness and species composition was not effective. In stone fruit orchards that had squash place out each month, the number of parasitoids recovered was very low, with average recovery <4 live parasitoids per squash. In

total, there were 316 Encarsia perniciosus, 23 Aphytis aonidiae and 30 A. vandenboschi recovered. While there was an overabundance of scale on each squash, female SJS were preferred to male 6.367:1 (most likely because of their size). Distribution of the parasitoids varies both in space and time, providing wide differences in parasitoid recovery. For example, average live parasitoid recovery was 3.84 per squash and parasitized scale (e.g. live and emergence hole) was only 6.14 per squash ($n=98$ squash). However, if only squash with some parasitoid activity are counted (e.g., remove the zeros), than the average is 12.8 per squash $(n=45)$.

The number of live parasitoids reared from infested squash suggests more parasitoids are available in July, August, and September. However, this may be an artifact of the number of SJS on the trees that are available for the parasitoids – and not an indication that percentage parasitism is greatest in the summer.

We conclude that the parasitoid has a difficult time finding the scale – probably dependent on the selected tree and the short time the squash were hung in the orchard. To compare species composition over the season, all squash from stone fruit and orchards were combined (including squash placed in field to collect parasitoids) and the collection dates were rounded to the nearest month. Results show that a steady increase in the average number of parasitoids reared from SJS infested squash. *Encarsia perniciosus* was the most common parasitoid and was found throughout the season (Figure 13). Both *Aphytis* species were low in abundance. The results bring to question the method parasitoids (all species) search for the scale. Once on the tree, it is likely that parasitoids walk on almond or stone branches - rather than fly to host cues.

The number of parasitoid adults caught in pheromone traps was quite interesting because of the distinct differences between treatments (Figure 14). First, most parasitoids were *Encarsia*, (

which was found in every orchard monitored – regardless of the insecticide treatments applied. Category C is the easiest to explain: parasitoids were not caught (in any numbers) during the summer because there were few SJS and insecticides were applied. Category B is also relatively straight forward: as the number of SJS increased during the season (Figure 7B), the number of parasitoids increased (Figure 14B). Category A is, however, more difficult to explain because the parasitoid catches began quite high and then decreased to a relatively steady level throughout the growing season (Figure 14A), even though scale density increased (Figure 7A). This pattern might be explained by trap inefficiency during some periods (for example, parasitoids are more attracted to available SJS than pheromone traps) or to a natural reduction of parasitoids in June and July.

To determine where on the tree the SJS are most common and to compare SJS distribution to parasitoid species composition and abundance, whole scaffolding branches were removed from a stone fruit orchard that was moderately infested with SJS. Results show that most "visible" SJS located on old scaffolding wood is dead, while the live population resides on new scaffolding wood, and first and second year growth. SJS was especially prominent on sucker growth (Figures 15-18).

This distribution becomes important because the three parasitoid species were not evenly distributed. *Encarsia* was more common on the older scaffolding wood, deeper inside the tree, while *Aphytis* spp. were more common on the outer or smaller branches. The difference in parasitoid distribution in this unsprayed orchard may explain between orchard differences in

parasitoid species composition: *Encarsia* was present in all orchards sampled, including those receiving insecticides, while *Aphytis* were more common in fields with summer insecticide treatments. We hypothesize that the more hidden location of *Encarsia* may provide some protection from insecticide applications as compared to the more exposed location of *Aphytis*.

Objective 2.

Tests of chemical effects on beneficial insects have not yet begun. To accomplish this goal, we have established a strong colony of A. vandenboschi; we have weaker colonies of E. *perniciosus* and A. *aonidiae*. Bioassays will begin with A. *vandenboschi* this spring/summer.

Objective 3.

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Tests of parasitoid augmentation began with studies of *Aphytis melinus*, a parasitoid already commercially available as a natural enemy of red scale in citrus. Initial laboratory studies indicatedA. *melinus* would attack SJS. In cages, levels of parasitism ranged between 25- 40%. Further, almost all remaining scale were killed by host feeding. This study had the disadvantage of enclosing parasitoids and SJS in a box, and in the laboratory, a very artificial situation.

The first field tests with *A. melinus* suggested that these parasitoids, which are close relatives to the *Aphytis* species attacking SJS, may have some benefit in an augmentative release. SJS on squash were parasitized by *A. melinus,* released in the same tree at a rate of 10,000 per tree. In this study, we also found parasitized SJS -17% of the scale on squash had been attacked. Of course this rate and methodology is economically infeasible. This work was followed by a larger field experiment, testing releases of 50,000 *A. melinus* per acre under field conditions. In a commercial orchard, squash were placed every other tree, moving in all directions, from a center release tree. At that center tree, 50,000 *A. melinus* were released. There were three replicates. Results of this open-field, commercial release were disappointing. *A. melinus* releases showed no reduction of SJS densities and little or no *A. melinus* activity (on squash that were placed throughout the release site). Initial conclusions are that *A. melinus* does not attack SJS under field conditions, it simply keeps searching for red scale and moves out of the release arena.

The first tests using *Aphytis* from the insectary colonies began in March 2000. *Aphytis vandenboschi* was released at a rate of 1,000 per tree in each of five trees. Samples are being taken from the release tree outward by collecting resident SJS on infested branches. Results from these studies will be available in summer 2000.

Currently, colonies of *A. vandenboschi* and *E. perniciosus* have been established from field-collected material. During the dormant season and spring, field and laboratory studies will be conducted to determine their potential in augmentation programs, which will be tested in summer 2000.