

**Almond Board of California Project Number: 00-KD-o0**

## **SUMMARY OF ALMOND RESEARCH 2000**

**Kent M. Daane<sup>1</sup>, Glenn Y. Yokota<sup>1</sup>, Brent A. Holtz<sup>2</sup>, Walt J. Bentley<sup>3</sup>**

<sup>1</sup> Center for Biological Control, Division of Insect Biology (ESPM), UC Berkeley; <sup>2</sup> UCCE, Farm Advisor, Madera County, <sup>3</sup> UC Statewide IPM Program

**Project Title: Part I: Evaluation of “soft” insecticides on moth pests and their natural enemies**

**Part II: Population dynamics of San Jose scale and its natural enemies: investigating the potential for natural or augmented control**

Almond growers lose millions of dollars to insect pests each season. In the first project, least-toxic chemical controls for the peach twig borer (PTB) were investigated. In a second project, a control program for the San Jose scale (SJS) was investigated under a cooperative project sponsored by Almond Board of California research combined with funding from CDFA’s Department of Pesticide Regulation and commodity boards of the California Tree Fruit Agreement and California Cling Peach Advisory Board.

### **Part I: Evaluation of “soft” insecticides on moth pests and their natural enemies**

In 2000, we screened Dimilin®, Confirm®, and Success® for efficacy against peach twig borer (PTB). Dimilin® and Confirm® are insect growth regulators (IGRs), which are larvicides that interfere with the insects' chitin deposition (the outside shell) and this prevents the insect from molting. Currently, Dimilin® and Confirm® are not registered for use in almonds, but both have been pursuing registration.. Success® is a bacterial by product and is currently registered for use in almonds. Our goal was to provide information that might better usher these “softer” products into widespread use. Because any insecticide application has the potential to affect other pest and beneficial insects in the orchard, we also screened the tested IGRs against some of the more common beneficial insects in almond orchards.

In 1998, and 1999, we used "diet-incorporated" bioassays to develop LD50s and LD90s for Dimilin®, Confirm®, and Success®. All three products were found effective against PTB. In 1999, we began field tests of these products on PTB and some commonly found beneficial insects. Almond trees were treated, using commercial methodologies, with 4\_ the label rate for each product and compared with a no-spray control (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), green lacewing (larva), *Goniozus legneri* (adult), or *Aphytis* spp (adult) was added (20 replicates each). The insects were checked at daily intervals and their condition (alive or dead) recorded. Results from this study show all products had better than 95% kill of PTB when exposed to nuts collected 1 day after

spray application. Dimilin® and Success® remained active at the 6 day period, while Confirm® efficacy dropped. This was especially evident at the 12 and 22 day periods. At this high rate (4\_ the label rate), we also detected some affect on beneficial insects, particularly parasitoids.

For this reason, this study was repeated using label rates, more replicates, and San Jose scale (SJS) parasitoid common to almond orchards (*Encarsia perniciosus* and *Aphytis vandenboschi*).

Results again showed that all materials were quite effective against PTB, with Success® providing the best control for the greatest period of time. Success® and Confirm® also showed activity against NOW. Of the natural enemies tested, green lacewings and *Goniozus legneri* (a parasitoid of NOW) were unaffected by any of the tested insecticides (in the 1999 trial, there was some insecticidal activity of Confirm® on *Goniozus*). In the 2000 trial, there was no discernable effect of the IGRs on the small SJS parasitoids. Preliminary results suggest mortality of *Encarsia perniciosus* and *Aphytis vandenboschi* was greater on nuts recently sprayed with Success®. Because the mode of action o Success® would suggest that this product should not kill adult parasitoids, these results are held in question. For this reason, we are currently conducting more controlled nut-dip bioassays and results from these trials are not yet available

Title: Part II: San Jose Scale Natural Enemies: Investigating the Potential for Natural or Augmented Control

## **Part II: Population dynamics of San Jose scale and its natural enemies: investigating the potential for natural or augmented control**

In the second project, we investigated population outbreaks of San Jose scale, looking specifically at the impact of natural enemies and methods to manipulate natural enemy numbers. San Jose scale (SJS) has recently moved from a secondary pest problem to a primary concern in stone fruits; economic damage in almonds and cling peaches, while less common, has also occurred. The importance of SJS may increase in the near future because possible legislative restrictions, guided by FQPA and directed against organophosphates and dormant-season applications, may remove some of the more effective chemical controls. In response to grower concerns, a SJS research team was organized to investigate possible reasons for increased SJS pest status and to determine control alternatives. Research areas have been prioritized and include alternate chemical controls (to provide immediate solutions), SJS insecticide resistance (to determine reasons for failure of current insecticides and to predict appropriate resistant management programs to maintain effectiveness of registered insecticides), SJS field biology studies (to determine if recent SJS outbreaks were induced by changes in management practices), and biological controls (this study).

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 7 stone fruit orchards (Fresno County), which were sampled biweekly during the growing season. Additionally, we surveyed almond orchards (Fresno and Kern Co.) and fresh market stone fruit (Fresno and Kings Co.). Sampling methods included, SJS pheromone traps, field collections of scale, and temporary placement of squash that were infested with SJS in the orchards (the squash were brought back to

the laboratory and the number of dead or parasitized SJS (and parasitoid species composition) were determined.

Results show three parasitoid species dominate the natural enemy complex: *Encarsia perniciosus*, *Aphytis aonidiae*, and *Aphytis vandenboschi*. Highlights of this work are: (1) *E. perniciosus* was found in every orchard sampled, (2) *Aphytis* species were less common, (2) *Aphytis* species were less common on SJS pheromone traps, as compared with *Encarsia*, than the recovery of parasitoids from “live” SJS 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.

Results of orchard surveys clearly indicate that sampling methods for parasitoids (and SJS) need improvement. 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. 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*.

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. In 2000, successfully established laboratory colonies of *A. vandenboschi* and *E. perniciosus*. However, numbers of parasitoids produced remained disappointingly low. In September 2000, we conducted our first field release trials of *A. vandenboschi* and *E. perniciosus*. From this work we can not yet determine the potential for commercial augmentation programs. However, two artifacts of this work are important. First, there is significant crawler mortality simply because the small, delicate insects can not find a suitable feeding sites. This indicates that, as many growers have suspected, tree age, cultivar, and orchard vigor may play a role in SJS densities. Second, mortality of the larger, settled SJS was higher on the uncaged plots than the caged plots. Visual observation revealed that green lacewing larvae were very adept at feeding on SJS and accounted for considerable mortality of the settled scale.

## **Project Justification**

San Jose scale (SJS), *Quadraspidiotus perniciosus*, is a “hard” or “diaspid” scale. It is so small that its first development stage is often hard to see on fruit or branches. It has a large range of susceptible host plants that includes stone fruit, pears, apples and many nut crops (Gentile and Summers 1958, see UC IPM website). It is most likely of Asian origin, brought into California in the 1870s on peach trees shipped from China (Gentile and Summers 1958). Because it has wide geographic and host ranges, it quickly became a key pest of most deciduous fruit orchards in North America and remained so until an Integrated Pest Management (IPM) program was developed. Much of the development work towards a SJS program was completed in California.

From the 1950s-90s, when SJS populations flared up, control was often easily achieved through a well-timed insecticide application of a dormant oil (typically combined with an organophosphate) or spring and summer applications of organophosphates (Dowing and Logan 1977, Westgard 1977, 1979, Rice et al. 1979). The dormant season oil and organophosphate application targeting peach twig borer, *Anarsia lineatella*, also provided SJS control. More recently, the use of higher grade oils or bacterial-by-products (Success®) provides promise for less-toxic pesticide use to be incorporated into the almond and stone fruit IPM programs. Walt Bentley and Rich Coviello are working on improved controls with oils and other products.

Because insecticides work best against the smaller scale, insecticide applications should be timed to peak crawler emergence. Because the crawlers are hard to monitor and count, their emergence patterns are most easily determined based on “phenology” or development models that use the adult male flights to fix important periods in the SJS development patterns (Jorgenson et al. 1981). Sampling for SJS utilizes pheromone traps that attract adult male SJS – the only development stage and sex that fly. SJS pheromone traps can also attract one of the primary SJS parasitoids – a small “aphelinid” wasp named *Encarsia perniciosi* (formerly called *Prospaltella perniciosi*) (McClain et al. 1990, Rice and Jones 1982).

The combination of good sampling methods, a phenology model to time insecticide applications, and reliable insecticide products has resulted in a good IPM program for SJS. For the above reasons, it was unusual when high densities of SJS were reported in the 1990s on stone fruit and almonds throughout the Central Valley and in particular in Fresno, Tulare and Kern counties. SJS pest status is especially high on nectarine cultivars, where scale readily settle on the fruit and even small populations can result in serious cosmetic damage. The exact causes of these outbreaks are not known but insecticide resistance, insecticide disruption of SJS natural enemies, poor insecticide application methods (e.g., poor coverage), and natural between-season fluctuations have been questioned.

In response to grower concerns, a SJS research team was organized 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, sampling methods, SJS insecticide resistance, SJS field biology studies and biological controls. Work presented here investigated the population dynamics (or the change in density over time) of SJS and its natural enemies. In this year’s report we focus on seasonal changes in SJS and parasitoid densities between orchards with different management strategies. We also report on within tree distribution of SJS and its parasitoids. Three parasitoid species dominate the natural enemy complex: *Encarsia perniciosi*, *Aphytis aonidiae*, and *Aphytis* nr. sp. *vandenboschi*. Note here that we list the third listed species as *Aphytis* nr. sp. *vandenboschi*, with “nr. sp.” meaning “near species.” This second “*Aphytis*” species may include more than one species, which we believe are near *A. vandenboschi*. In a second series of experiments, we investigated the potential of augmentative release of SJS parasitoids and the effect of common “soft” insecticides, targeted

against the oriental fruit moth (OFM) and peach twig borer (PTB), against SJS natural enemies. This work was supported by joint funding from CTFA, California Cling Peach Growers Advisory Board and the Almond Board of California.

**Objective (1999-01):**

1. To describe the natural enemy complex attacking SJS and determine its field effectiveness against SJS:
  - (a) survey almond and stone fruit orchards to determine the natural regulation of SJS,
  - (b) compare natural enemy densities in orchards with different management practices,
  - (c) investigate the biology and potential effectiveness of common SJS parasitoids,
  - (c) establish insectary colonies of selected SJS parasitoids,
  - (d) determine the effect of common insecticides on selected SJS natural enemies, and
  - (f) test selected SJS parasitoids for use in augmentative release programs.
2. To assess the efficacy of “soft” insecticides (e.g., Dimilin, Success, Confirm) against moth pests in laboratory and field trials and determine their potential effects on non-target insects.

**Procedures:**

*Part I.* The densities of SJS and resident natural enemies were followed in nine stone fruit blocks (Fresno and Tulare County). These orchards represented blocks with and without insecticide treatment for SJS or other insect pests, that we have loosely categorized as conventional, sustainable and organic (our term organic does not necessarily signify a CCOF orchard) (Table 1).

Table 1. Sampled fields, 2000 season.

Orchard/ Block	Cultivar	Dormant Spray	In-Season Spray
Conventional			
R- M- RGN	Royal Glo Nectarine	Supracide/Oil	Lannate
Ri – M- RDN	Rose Diamond Nectarine	Supracide/Oil	Lannate
Y – SBN	Spring Bright Nectarine	Lorsban/Oil	Carzol
Sustainable			
W – ASN	Arctic Snow Nectarine	Asana/Oil	Success
Br – FSN	Favorite Sun Nectarine	Oil	Bt/Carzol
Br – SKN	Honey Kist Nectarine	Oil	Bt/Lannate/Omite
Organic			
M – ELP	Elegant Lady Peach	Oil	Bt
N – FP	Friar Plums	Oil	none
N – EHP	Elephant Heart Plums	Oil	none
B – LP	Laroda Plums	Oil	none
B – MGN	May Grand Nectarine	Oil	none

SJS were sampled throughout the growing season. In each stone fruit orchard, five SJS pheromone traps were used to sample for male SJS and, on the same five sampled trees, double-sided sticky-tape was placed around two limbs to sample for crawlers. Traps and tape were changed weekly from March until December.

We also made periodic field collections of SJS and its natural enemies in the nine stone fruit orchards and five “supplemental” almond orchards. Different sampling methods were utilized. First, because SJS pheromone traps do not provide a clear picture SJS parasitoid species composition or abundance, we placed squash infested with SJS (from the insectary) in each orchard. To accomplish this, squash were infested with ~1000 SJS, after the scale settled (once settled the scale will not move) and developed to proper “host stages” the squash were placed into orchards. In 2000, we held the squash in the tree canopy in an open-topped plastic container, supported from a limb by metal hangers. Therefore, the squash did not contact the tree limbs such that parasitoids had to walk or fly onto the squash (this probably lowered parasitism rates). The squash were left in the orchard for about 3 weeks, removing them before the scale produced another generation of crawlers that would move off the squash. In the insectary, the squash were placed in emergence containers. In 2000, we placed a single squash in each field in June and again in September. Live parasitoid were collected and identified to species. In this manner, we hoped to create a “controlled infestation” of SJS at different times of the year.

A second sampling method was the periodic collection of infested wood, which was brought to the laboratory and placed in emergence containers to rear out live parasitoids. Collection of infested wood could only be economically accomplished in those fields with moderate to heavy SJS infestations, because of the time needed to collect SJS from a lightly infested field.

A third experiment investigated parasitoid distribution in the canopy by using 3 × 5 inch sticky cards – without a SJS pheromone lure. In three stone fruit blocks (one plum and two nectarine) white sticky cards (3 × 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 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> SJS peak density periods. After ~30 days, the cards were removed and the number of SJS and parasitoids were recorded. During these same periods, beating-tray samples were made at each site to determine the kinds and numbers of SJS predators. We also used a modification of this same design to test the effect of yellow vs. white sticky cards and height position of sticky cards baited with SJS pheromone lures.

The canopy position of SJS and parasitoids has important implications for trap placement, insecticide control and canopy management (more important for plums). We continued to study SJS and parasitoid distribution by destructively sampling scaffolding branches in 15-year old May Grand nectarine block with high SJS densities. On each of three trees, one scaffolding branch was cut at the base, near ground level. The entire scaffolding and its off-shoots 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 hole, parasitoid species could be identified as either *Encarsia* or *Aphytis*. These samples were collected in spring and summer 2000 and winter 2000-01. These data will be compared to data collected with SJS pheromone traps, yellow sticky cards, and double-sided tape placed in or near sampled trees.

Most importantly, at harvest we collected 1000 fruit from ten trees in each of the nine orchards. The number of infested fruit (SJS, worms, katydid, and thrips damage) and the number of scale per fruit were recorded.

*Part II.* In 1999, we established insectary colonies of two parasitoid species: *Encarsia perniciosus* and *Aphytis* species (near species *A. vandenboschi*). Parasitoids from these colonies were used for (i) augmentation experiments, (ii) bioassays of commonly used insecticides and (iii) laboratory studies of parasitoid biology.

*Augmentation trials.* In 1999 we tested the potential of a commercially available parasitoid (*Aphytis melinus*) to attack SJS in the field. Initial studies showed this parasitoid species would host feed upon and parasitize SJS under laboratory conditions. However, tests in the open-field found this species did not affect SJS density or parasitism levels.

For the above reasons, in 2000 we tested the effectiveness of mass-releases of *Encarsia perniciosi* and *Aphytis* nr. sp. *vandenboschi* – both parasitoids were reared at the KAC Insectary. The site used was an experimental almond block located at KAC. On 27 and 28 July, four branches on each of eight trees were inoculated with ~200 SJS crawlers from the insectary. The almond block had not received insecticides – other than a dormant oil treatment for the previous 10 years. Earlier samples indicated that SJS, *Encarsia perniciosi*, *Aphytis* nr. sp. *vandenboschi* and *Aphytis aonidiaie* were present in the orchard. For this reason, the branches were checked for SJS that, if found, were removed. On 31 July, the inoculated branches were enclosed in large, self-supporting organdy cages (~1 m long × 0.4 m dia.). On 14 August, the number of settled

SJS was recorded for each branch. and one of five treatments was randomly assigned: release of *Encarsia perniciosi* or *Aphytis* nr sp. *vandenboschi* at 1:5 and 1:10 ratios of parasitoid : SJS (based on pre-release estimates) and a no-release control. There were five replicates for each treatment. On 18 August the *Encarsia perniciosi* treatments were established, when SJS reached the 1<sup>st</sup> and 2<sup>nd</sup> instar stages (the preferred host stages for *Encarsia perniciosi*). On 25 August the *Aphytis* nr. sp. *vandenboschi* were released into the cages when the SJS had developed to the 2<sup>nd</sup> and 3<sup>rd</sup> instar stages (*Aphytis* nr. sp. *vandenboschi* prefers larger SJS than *Encarsia perniciosi*). On 15 September, all branches were cut from the trees, taken to the laboratory and dissected for SJS, which were recorded by density and condition (live, dead [host fed] or parasitized).

In a second augmentation trial, the same orchard block at KAC was used for an "open-field" release. From 31 July to 3 August four branches on each of six trees were inoculated with ~200 SJS crawlers. On 25 August a pre-release count was made and on 1 and 6 September, 50 *Encarsia perniciosi* and *Aphytis* nr. sp. *vandenboschi* were released in three randomly selected trees. On 21 September the branches were removed and the SJS condition on each branch was recorded as before.

*Insecticide Bioassays.* The effect of insecticide applications (targeting moth pests) on SJS natural enemies was evaluated in an almond orchard at KAC. During the summer, trees were sprayed with common insecticides at label rates. Thereafter, nuts or leaves were removed from these trees and unsprayed trees on different days after insecticide application dates. These "field-sprayed" samples were used to bioassay SJS parasitoids, which determined how many days after field application that insecticides can kill parasitoids. The added value of doing this experiment is that it provided a more realistic determination of a pesticide effect after a number of days after application -- with insecticide degeneration progressing under normal conditions. 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 1-2 insecticide applications (often for moth or mite pests).

This winter/spring (2001) we will complete a simple leaf-dip bioassay of the effects of common insecticides on SJS parasitoids. Leaves will be dipped in different insecticides at different percentages of a field-rate dilution. Leaf material will be allowed to dry and then placed in open-ended glass tubes. Parasitoids will be added to the tubes, which will be sealed with organdy cloth. Each day thereafter the condition (live or dead) of the parasitoids will be determined.

*Parasitoid Biology.* This winter/spring (2001) we will begin evaluation of the potential of mass rearing *Encarsia perniciosi*, *Aphytis aonidiae*, and/or *Aphytis* nr. sp. *vandenboschi* for commercial field release, aspects of their biology will be studied in the laboratory. Because a great deal of information has already been collected on *E. perniciosus* and *A. aonidiae*, by other researchers, our research will focus on *A. nr. sp. vandenboschi*. Utilizing SJS laboratory colonies (reared on acorn or butternut squash), data will be collected on parasitoid longevity, fecundity, preferred host stage attacked, host feeding and potential for mass rearing. These sets of data will be compared to previous studies with other parasitoid species.



This work will build on studies already conducted (e.g., Flanders 1960, Gulmahamad, H., and P. DeBach. 1978a, 1978b, McClain et al. 1990a, 1990b, Rice and Jones 1982). One of our primary interests is the potential control of SJS with *Encarsia perniciosi*, *Aphytis aonidiae*, and/or *Aphytis* nr. sp. *vandenboschi* released singly or in combinations of 2 or 3 species. In brief, do *Aphytis* spp. interfere with *Encarsia* or visa versa. We are also interested in determining why (as our data indicate) *Aphytis* starts to build up later in the season.

## **Objective 2 – Insecticide Screening for Moth Pests (20% research time)**

During summer, almond trees at KAC were sprayed with selected insecticides at label and 2X label rates (randomized block design, 3 replicates). Almond nuts (~20 per tree) from treated and control trees were collected at day 1 and every 5 days thereafter (for 30 days) and brought to the laboratory. There, second or third instar PTB were taken from an insectary colony and placed with nuts in small, rearing containers (1 nut per container). The condition (live or dead) of PTB was checked 3 days after larvae were added to the container. Containers with live PTB were re-sealed and re-checked 4-6 days later. PTB mortality was compared to controls. In a similar manner, other moth pests (e.g., NOW and OFM) and beneficial insects (e.g., *Aphytis* sp., and green lacewings) were exposed.

This research will determine field-efficacy of tested insecticides against moth pests and the potential disruption of biological control. The work will also provide information on the residual activity of each insecticide on different insect species, which may prove valuable with respect to insecticide timing and potential secondary pest outbreaks.

Another method used to compare insecticides is to determine LD50s or the dose of insecticide at which 50% of the targeted animal dies. For many lepidopteran pests, laboratory evaluation of insecticide toxicity uses the leaf-dip method; however, because NOW, PTB, and OFM all feed on the nut, we will use a diet-incorporated bioassay for the IGR products (or other products that kill the insect after they are ingested) and topical tests for other insecticides. PTB and OFM will be reared on a diet containing a mixture of lima beans, distilled water, wheat germ, yeast and agar as main ingredients. The amount of insecticide added will be measured with micropipettes for liquid formulations or on an analytical balance for solid formulations. As the diet cools it solidifies.

Initial rates tested were to get a dose response that ranged between 10 – 90% kill. After which, for each dose tested there will be 3-5 replicates of 20 diet cups. To each diet cup, second to third instar PTB, OFM or NOW will be added (we currently have colonies of PTB and NOW). All diet cups will searched after 7 days and moth larvae condition (live or dead) and instar determined. The larvae that are alive will be kept for a 14 day check.

For each replicate, the percentage mortality will be computed. Dose response to determine LD50s and LD90s will be calculated using Polo (LeOra Software, Berkeley, CA). Data will be adjusted to mortality in corresponding control replicates using Abbott's formula.

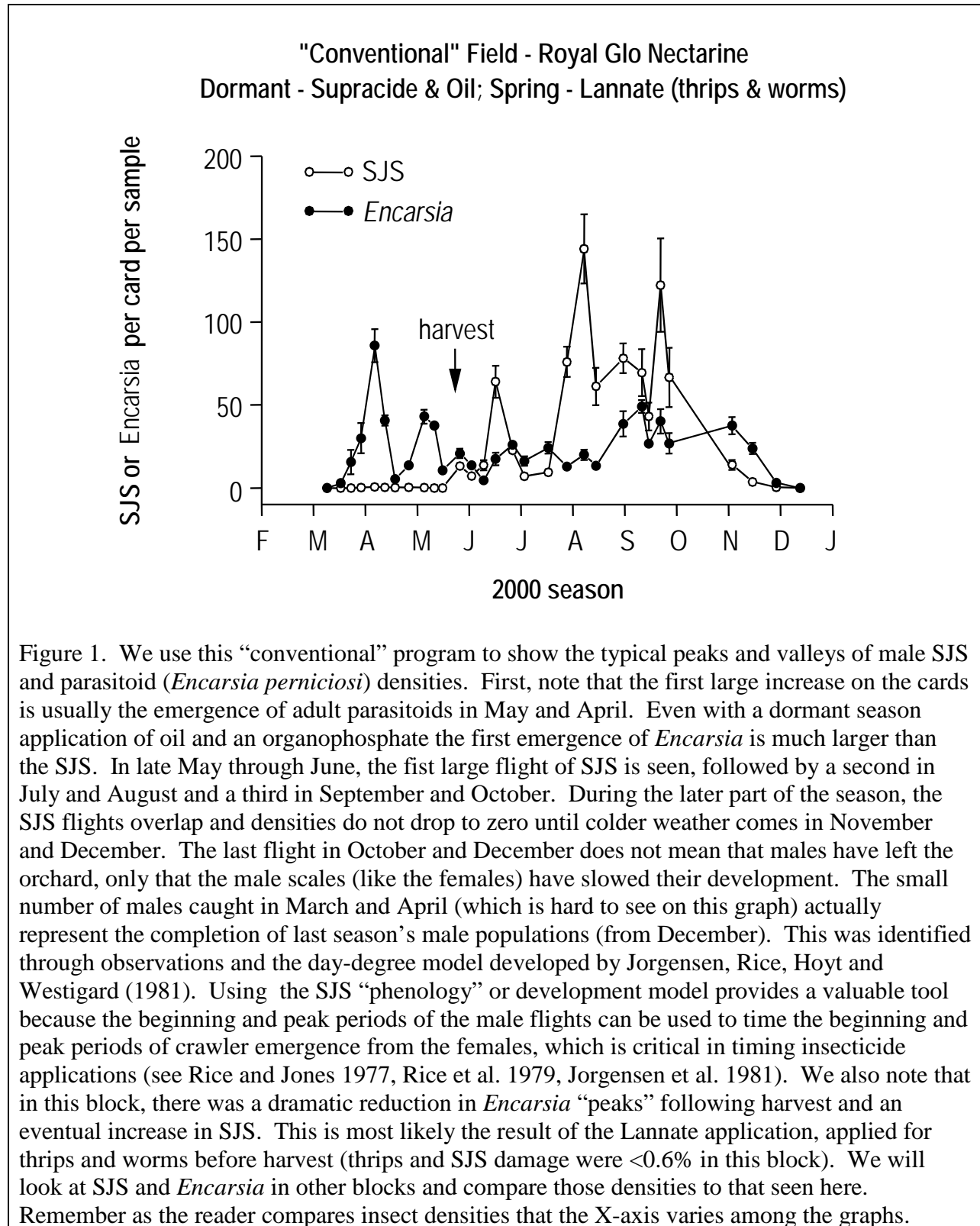
## Results

Of the 9 orchard blocks sampled there was a wide range of SJS damage (Table 2). We also recorded damage from worms, katydid and birds – all of which were low ( we selected orchards based on their history of SJS density, not other pest species). Conventional 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 low SJS infestations (0-0.6% and 0-2.4%, respectively) (Table 2). More important for this work is the variation in SJS infestation in those blocks without insecticide treatments, which ranged from 0-22% (Table 2). Only a single block had no SJS damage (M – ELP); however, the three blocks that had 7.4-12.1% SJS infestation had, on average < 5.5 SJS per infested fruit. On the two plum cultivars (N – EHP and B – LP) this is not enough SJS to cull the fruit; on the nectarine cultivar (B – MGN) the average 3.5 SJS per infested fruit might also not have caused the grower too much concern.

Table 2. Fruit damage.

Orchard	Damaged Fruit (%) – 2000 Season				
	SJS	SJS / fruit	Worms	Katydid	Bird
R- M- RGN	0.6 ± 0.4	2.3 ± 1.1	0	0	0
Ri – M- RDN	0	0	0.1 ± 0.1	0.3 ± 0.2	0
Y – SBN	0	0	0	0	0
W – ASN	0	0	0	0	0
Br – FSN	2.4 ± 1.4	8.1 ± 2.7	0	0	0
Br SKN	0.1 ± 0.1	0.1 ± 0.1	0	0.2 ± 0.1	0
N – FP	22.2 ± 6.9	17.8 ± 1.6	0	0	0
N – EHP	12.1 ± 1.2	5.5 ± 0.8	0	0	0
M – ELP	0	0	0	0.1 ± 0.1	0
B – MGN	8.6 ± 0.7	3.5 ± 0.4	0	0	0
B – LP	7.4 ± 0.8	5.1 ± 0.7	2.2 ± 0.6	0	0.2 ± 0.1

A sticky card baited with a SJS pheromone lure is still one of the best methods to monitor the change in SJS density during the season. The cards provide information on SJS males (indicating peak flight periods and can be used to time insecticide sprays) and levels of *Encarsia perniciosi* – the most common SJS parasitoid. From the 2000 data we found wide variation in SJS male flight densities and here we attempt to pick patterns out of this data set. We will start with a discussion of the SJS and *Encarsia* seasonal patterns. Remember that the sticky cards pick up *Encarsia* but may not record comparable levels of other natural enemies.



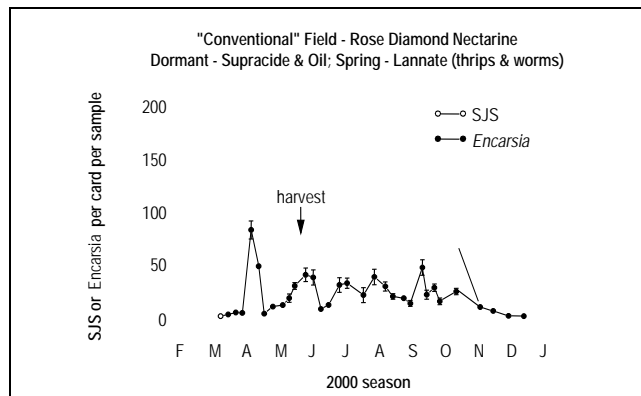


Figure 2. This graph should look similar to Figure 1 because similar insect management practices were used in the block were similar. Again, we see a strong first flight of *Encarsia*, indicating that this parasitoid species is in the orchard early, in greater numbers than SJS, and has apparently been little affected by the dormant OP application. Parasitoid populations decrease and SJS increases shortly after the Lannate application. However, this increase in SJS came long after harvest and no SJS fruit infestation was measured.

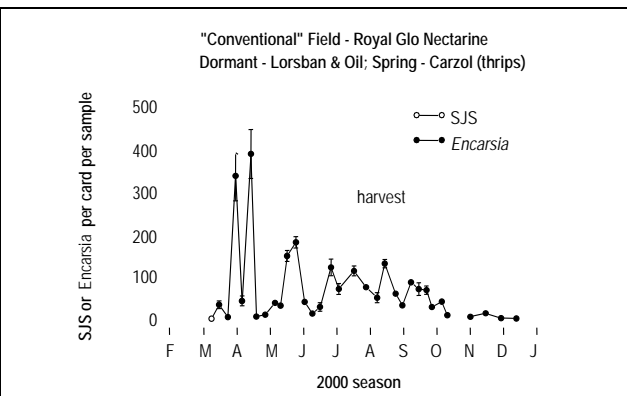


Figure 3. This block represents a movement towards more sustainable practices. The dormant oil and OP treatment remained – but Carzol was used for thrips control. A large double spike of *Encarsia* was recorded in March and April. These parasitoid peaks were seen again in March, June, July, August and September – most likely representing different generations – *Encarsia* and *Aphytis* will have 2-3 generations for every SJS generation. Note that SJS numbers never were higher than parasitoid numbers – not surprisingly there were no SJS infested fruit.

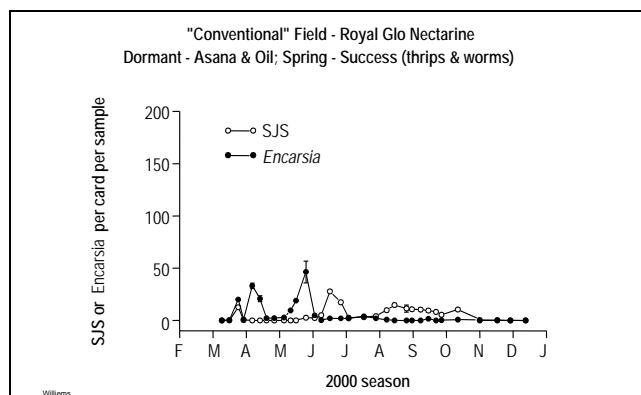


Figure 4. While considered “conventional,” management of this block has moved away from dormant oil and OP applications, replacing this practice with Asana – a strong pyrethroid. In previous years the grower used a dormant oil & Supracide combination, followed by an in-season application(s) of Pencap; not surprisingly, this block has a history of low SJS densities. The only surprise here is the mid and late season reduction of *Encarsia*, which does question the effect of Success on parasitoid survival. The grower should follow parasitoid and SJS densities next season.

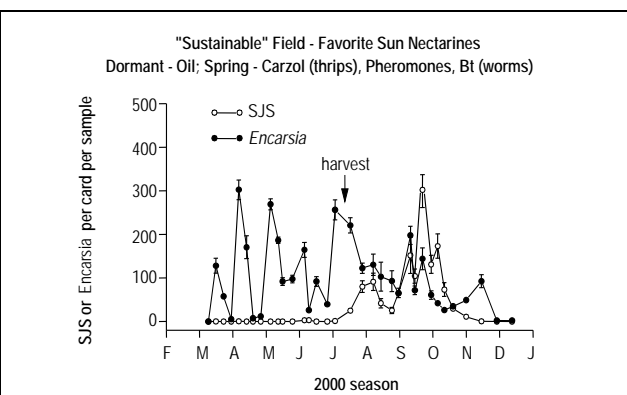


Figure 5. This grower moved away from OP application(s) about eight years ago. Note how strong the initial *Encarsia* population is. We can clearly see two peaks in parasitoid density coming from the overwintered population (in March and April). This is probably the same generation, first emerging from overwintered pupae and then larvae. Other strong flights of the parasitoids are seen in May, June and July. Parasitoid flight decreases for in August – perhaps because of temperature tolerances – followed by an “escape” of SJS. This SJS increase occurred long after harvest and had no effect on fruit damage (2.4%).

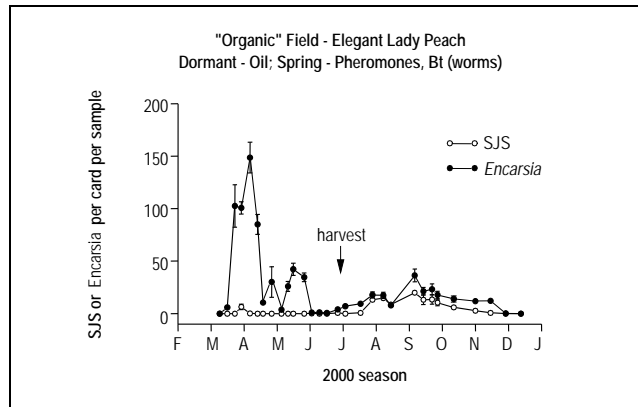


Figure 6. After removal of a dormant OP, the next step towards a “less-toxic” program is removal of broad-spectrum summer treatments. In this CCOF orchard the transition has been flawless – dormant oil for scale, Bt for peach twig borer and pheromone confusion for oriental fruit moth. Harvest samples found no or little SJS (0%), worms (0%) or katydid (0.1%) damage. As before, note how strong the *Encarsia* density is in spring. SJS is barely recorded by pheromone traps before harvest and the slight increase in August and September is met by a larger increase of the parasitoids.

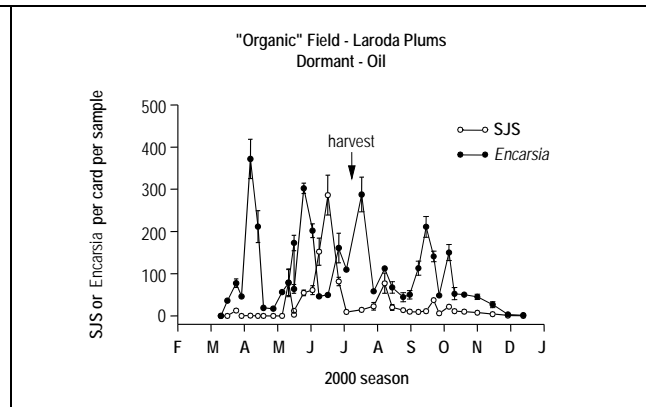


Figure 7. With the exception of oil and Bt, this plum block has not received any insecticide application for over 5 years. The *Encarsia* population looks strong and the individual peaks, representing different generations, are easily seen. Fruit infestation in this block was high (7.4%) but infested fruit had, on average, only 5.1 scale per fruit. For plum growers this may not represent an economic loss because SJS are harder to see on the dark fruit and do not cause the same discoloration. Damage resulted from a June escape of SJS that was followed by an increase in parasitoid density and an August-October reduction in SJS.

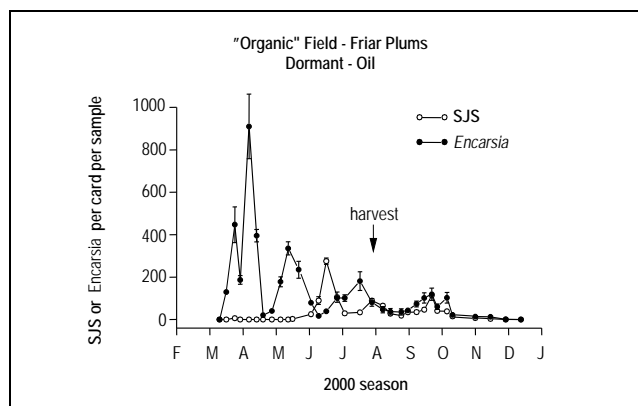


Figure 8. This block has a history of SJS presence. At the start of the season the *Encarsia* density is very high (note the X-axis). But if a high parasitoid density is the good news, the bad news is that this implies high densities of SJS may be present. Until June, there is not much SJS on the pheromone traps. A single SJS spike is seen in mid-June, after which pest densities drop to low levels. Fruit infestation (12.1%) was a concern. This graphs shows the importance of harvest date for stone fruit growers.

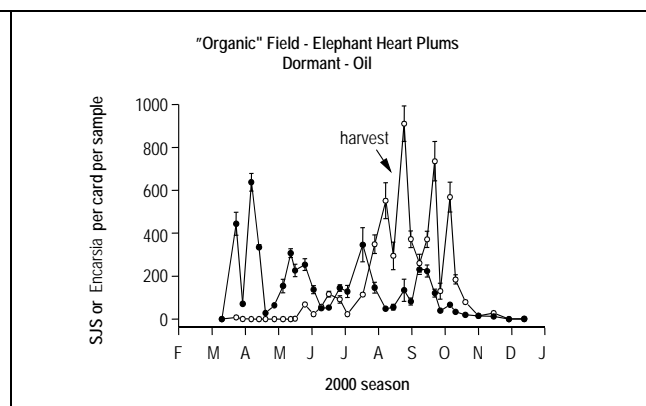


Figure 9. The SJS population escaped parasitoid control. Fruit infestation was high (22.2%) although the damage to plums is reduced because fruit with only a few SJS would not be culled. No in-season sprays were applied so it is unclear why the SJS population rose so quickly in August or why the parasitoid population did not respond. Fruit damage was increased because the harvest date was at the end of August.

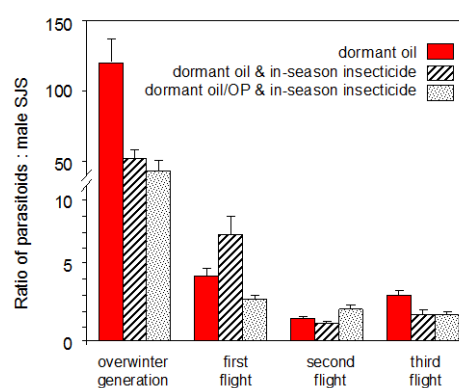
The above description of pheromone trap data for SJS and *Encarsia perniciosi* provides a foundation to better discuss natural regulation of SJS. In the 6 fields without OP applications in dormant or growing seasons, the SJS fruit infestation levels were 0, 0, 0.1, 2.4, 7.4, 8.6, 12.1 and 22.1% (Table 2). Fruit infestation levels >7% were mostly in plum cultivars with harvest dates near or after July 1<sup>st</sup> – all of which may make infestations more likely. Also, in all but one field (Fig. 8), SJS populations were brought to relatively low densities after a mid-season “escape.” Clearly, SJS in stone fruit can be managed with a dormant OP application. Nevertheless, management systems can be improved by better understanding natural enemy effectiveness.

In all three orchard categories (Conventional, Sustainable and Organic), there was a distinct pattern of parasitism, with *Encarsia perniciosi* populations stronger at the beginning of the season and *Aphytis* spp. more common towards the end of the season (Table 2). In 1999 and 2000, we used squash infested with SJS to compare parasitoid effectiveness and species composition in June and September. Recovery of both parasitoid species was poor in June and September collection periods. In June, from 10 squash (infested with ~1000 SJS each) we recovered 100 parasitoids. Initial emergence (40 days) was 74 *Encarsia perniciosi*, 1 *Aphytis aonidiae* and 3 *Aphytis* nr. sp. *vandenboschi*; secondary emergence (90 days – which may include a second generation inside the emergence containers) was 65 *Encarsia perniciosi*, 0 *Aphytis aonidiae* and 35 *Aphytis* nr. sp. *vandenboschi*. In September, initial emergence (40 days) was 18 *Encarsia perniciosi*, 2 *Aphytis aonidiae* and 10 *Aphytis* nr. sp. *vandenboschi*; secondary emergence (90 days) was 5 *Encarsia perniciosi*, 1 *Aphytis aonidiae* and 52 *Aphytis* nr. sp. *vandenboschi*. So this pattern of *Encarsia perniciosi* densities higher at the beginning and *Aphytis* spp. stronger in August and September held true in 2000 as well. There was also a difference between orchards with or without insecticides (Table 3) –but this is a poor comparison because the difference in SJS density (and hence the number of parasitoids in the field) is not taken into consideration.

Table 3. Parasitoids reared from infested squash.

	<i>Encarsia perniciosi</i>		<i>Aphytis aonidiae</i>		<i>A. nr. sp. vandenboschi</i>	
	Insecticides	No Sprays	Insecticides	No Sprays	Insecticides	No Sprays
June	0 a	27.8 ± 10.1 b	0 a	0.2 ± 0.2 a	0 a	7.6 ± 7.1 a
September	1.0 ± 0.8 a	3.5 ± 0.9 b	0.2 ± 0.2 a	0.3 ± 0.3 a	8.7 ± 8.7 a	2.5 ± 2.5 a

Figure 12. A better indication of the presence and importance of SJS parasitoids in the different management systems was provided in 1999. Here the ratio of parasitoids to male SJS (caught in pheromone traps) is presented. Data clearly show that SJS parasitoids were present in all three management categories. These data do not, however, show the difference in parasitoid species composition, which seems to indicate that while *Aphytis* is much higher and more important than pheromone traps would indicate – its density is lower in orchards that receive summer insecticide treatments.



Work in 1999 suggest that sampling methods for parasitoids need to be improved; continuing this research in 2000, we found that trap placement can also affect recorded densities of SJS. In 1999, to determine where on the tree the SJS and its parasitoids are most common we removed whole scaffolding branches and recorded the presence of SJS and its parasitoids on leaves, limbs, fruit, hangers. During this sample, levels of parasitoid activity on high and low or inner and outer tree positions were also recorded. In 2000, we placed stick cards throughout the tree canopy (some with and others without pheromone lures). Here, we sought to determine which parasitoids were most common, where are they on the tree, and is there any seasonal or among orchard variation.

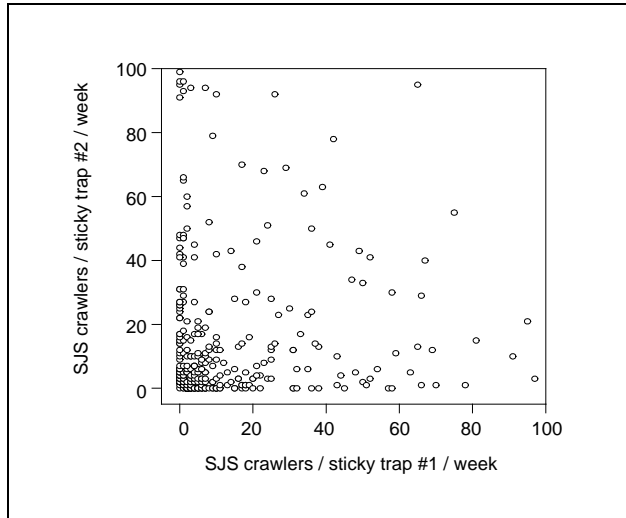
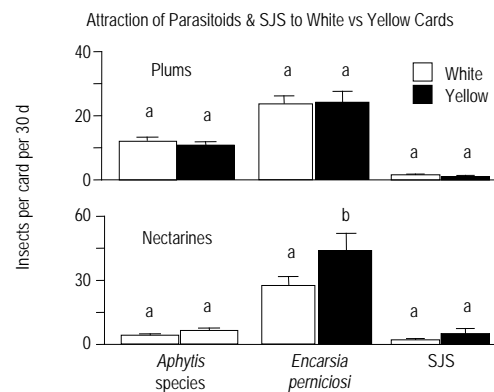


Figure. 11. A good indication of the sampling difficulties is seen in a comparison of crawler counts on the two pieces of sticky tape placed in the same tree. The poor correlation from branch to branch (and tree to tree, data not shown) implies that many samples must be taken in order to get an accurate count. For example, in orchards with low SJS the PCA would have to place sticky tape on too many trees to get an indication of SJS that would be comparable to the efficiency of pheromone traps. Clearly, the sole purpose of sticky tape is to determine egg hatch and crawler movement, not to indicate SJS density.

Figure 12. One initial concern was the color (white) of the standard SJS pheromone trap with respect to SJS and parasitoid collection. Earlier work in North Carolina suggested that trap color (white, blue, black, yellow or red) influenced parasitoid catch (McClain et al. 1990). We tested the two common trap colors (yellow and white) and found few important difference in parasitoid or SJS collection. Note that the data presented are from cards WITHOUT pheromone lure – which dramatically drops the number of SJS males and *Encarsia perniciosi* that are caught (*E. perniciosi* is also attracted to the SJS pheromone [Rice and Jones 1982]).



Different letters above each pair indicate a significant difference between means ( $\pm$ SEM) ( $t$ -test,  $P < 0.05$ )

Data collected in 1999 suggested that distribution of SJS and parasitoids varied in the stone fruit canopy – with *Encarsia perniciosi* more common on the interior portions of the canopy and *Aphytis* spp. distributed throughout. Other researchers have suggested SJS is not evenly distributed – with SJS more common on the bark than on the leaves or fruit and on the smaller limbs on the tops of the tree than on the larger interior sections (Morgan and Angle

1969). However, this work was completed in British Columbia, Canada, where cultivars, seasonal temperatures, and natural enemies would be very different. Can SJS and parasitoid distribution affect IPM programs? Yes! For example, insecticide application that provides better coverage to the outer portion of the tree – compared to the inner regions – might not provide the best SJS control and could negatively affect one parasitoid species (*Aphytis*) more than another (*Encarsia*). Below, we examine SJS and parasitoid distribution with 3×5 inch sticky cards (with no pheromone lure).

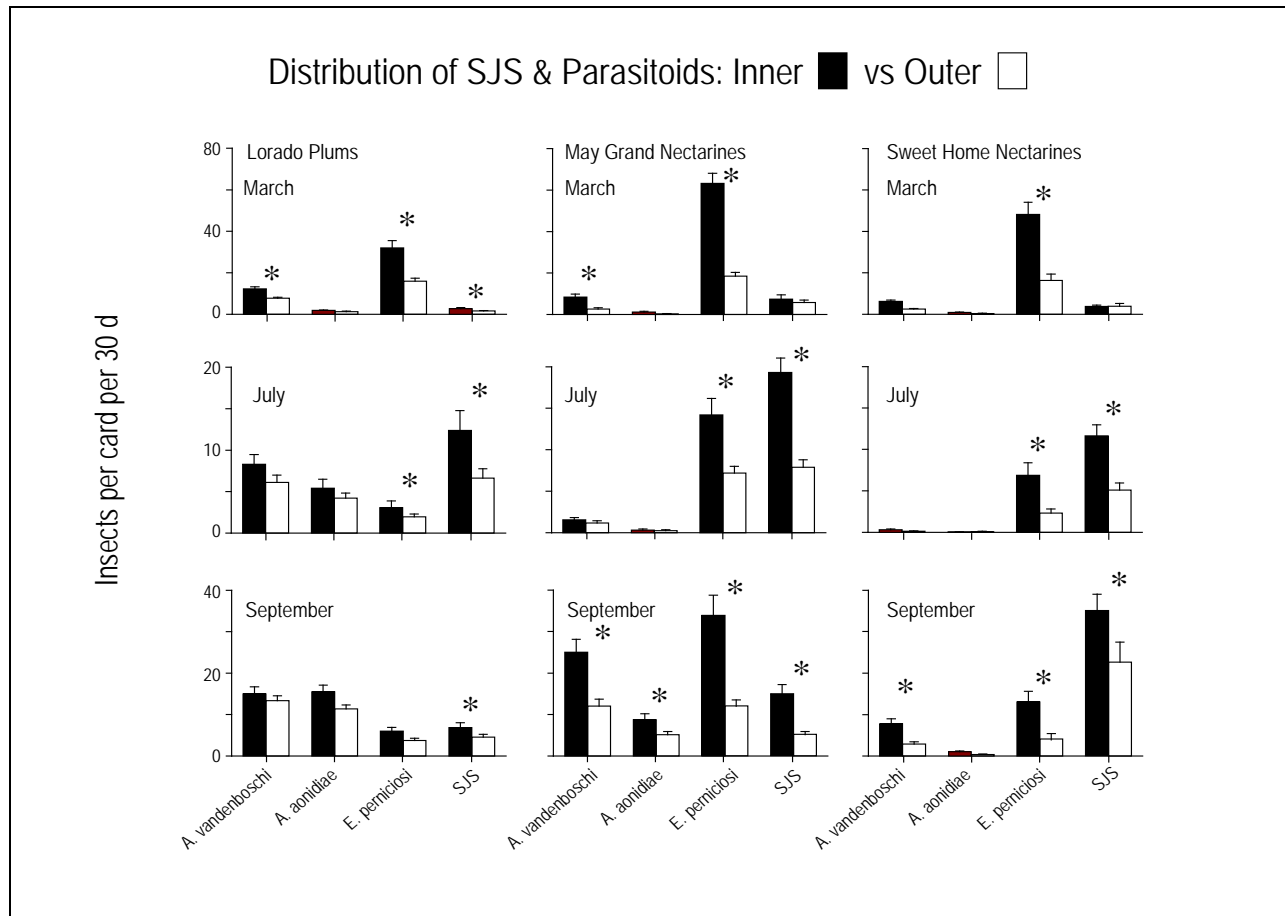
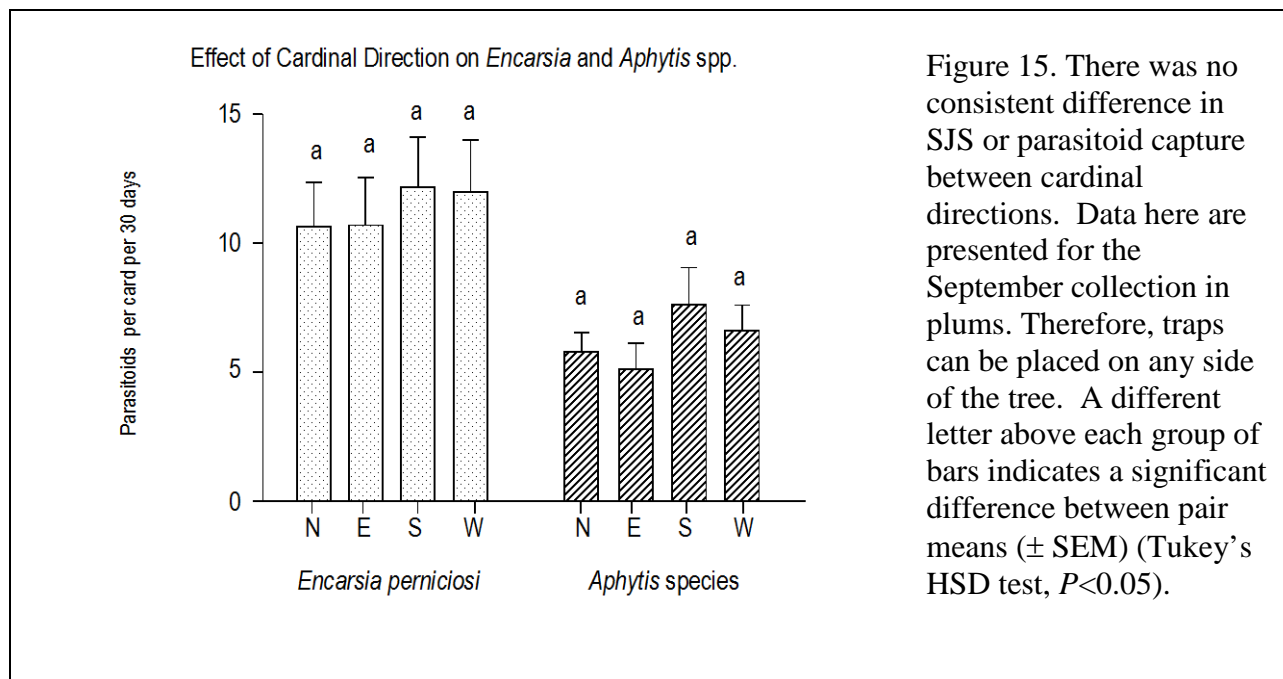
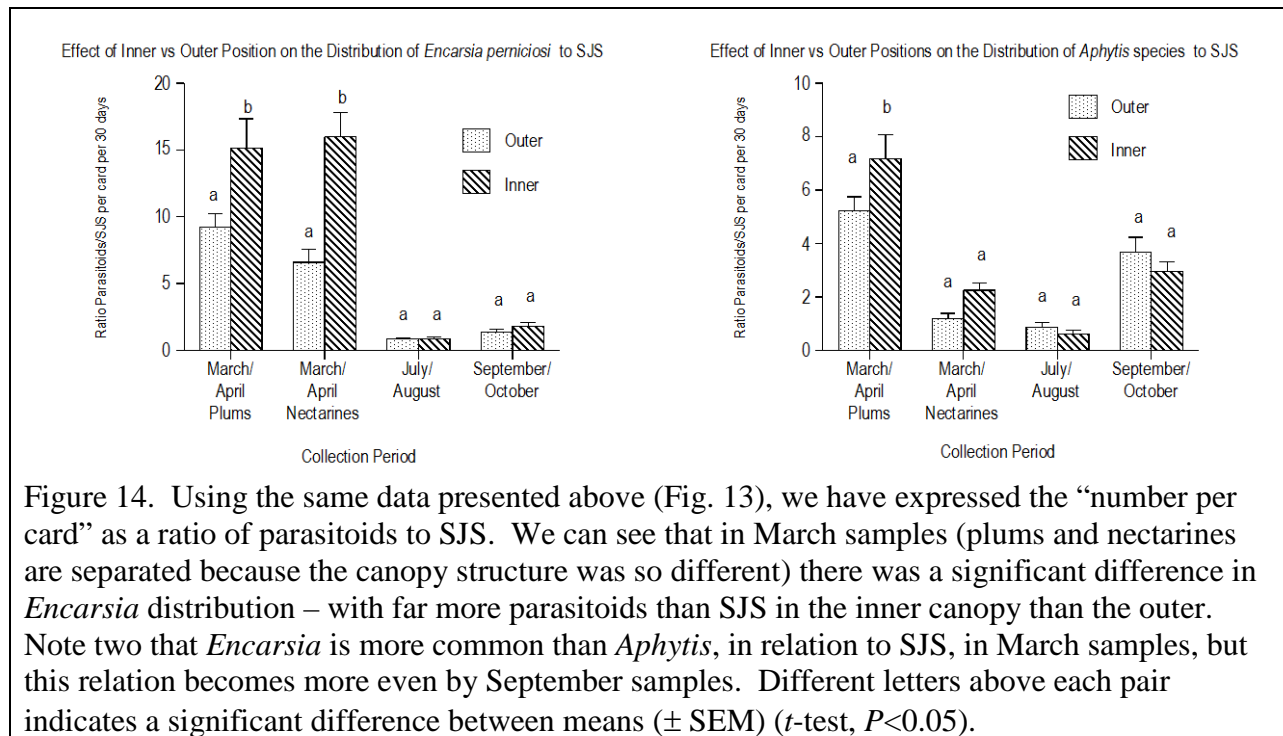


Figure 13. The within tree distribution of SJS and *Encarsia* and *Aphytis* spp. was examined using small sticky cards (without pheromones). We use data that compared inner vs. outer sections of the canopy to show the overall design – samples were collected in three orchards (Laroda plums, May Grand nectarines and Sweet Home nectarines) and at three different periods of the season (March, July and September). In this graph a very clear pattern is present. *Encarsia perniciosi* and male SJS were caught in higher densities in the inner portions of the canopy. Below, we will further explore this trend and discuss how this might relate to parasitoid effectiveness and SJS monitoring programs. The next graph shows parasitoid distribution in relation to SJS density. Note also that in March and, to some extent in July samples, *Encarsia perniciosi* is the dominant parasitoid, while in September the *Aphytis* spp. are more common. Also note that without a SJS pheromone lure added, the *Aphytis*, *Encarsia* and SJS densities are not that much different. An asterisk above each pair indicates a significant difference between pair means ( $\pm$  SEM) (*t*-test,  $P < 0.05$ ).





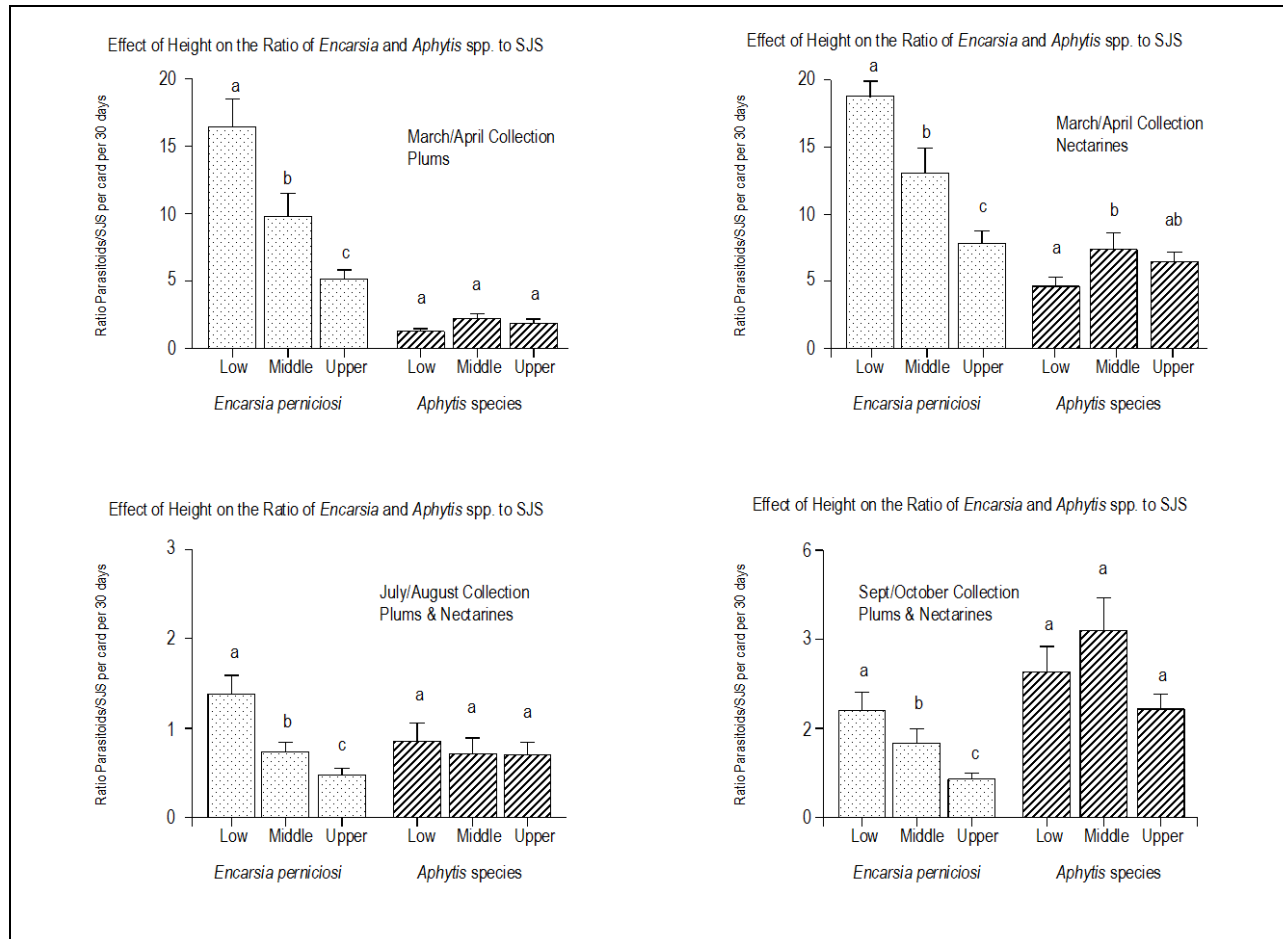


Figure 15. Differences in the ratio of parasitoids to SJS location are very clear with respect to trap height placement. *Encarsia perniciosi* is more common (with respect to SJS) on the lower canopy sections. In contrast, there is no clear preference for *Aphytis* spp. These data imply that an orchard with only *Encarsia* present will have a lower percentage parasitism in the upper canopy.

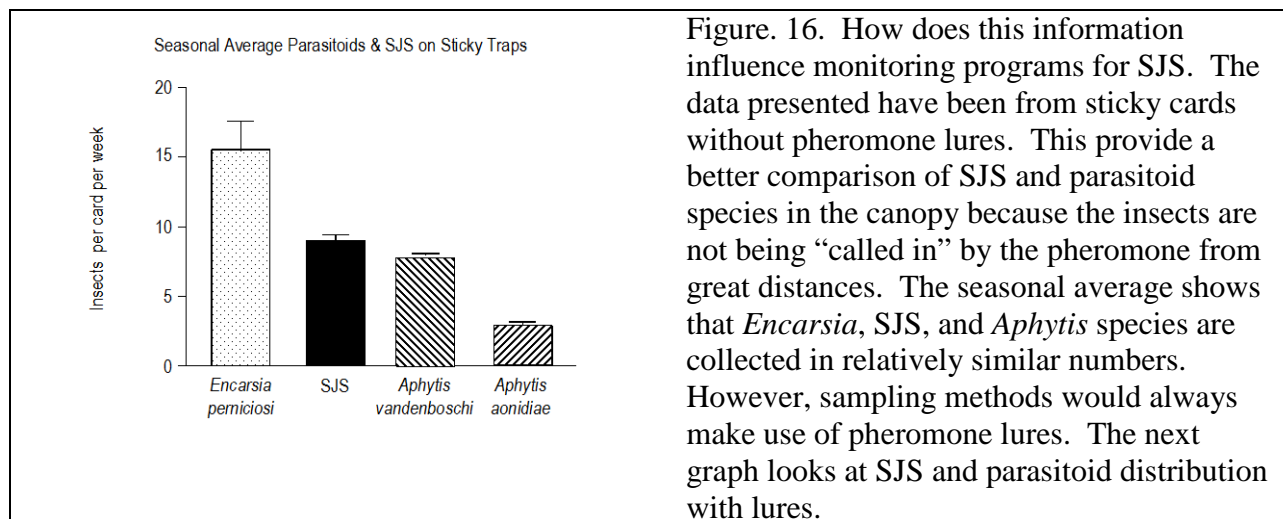


Figure. 16. How does this information influence monitoring programs for SJS. The data presented have been from sticky cards without pheromone lures. This provide a better comparison of SJS and parasitoid species in the canopy because the insects are not being “called in” by the pheromone from great distances. The seasonal average shows that *Encarsia*, SJS, and *Aphytis* species are collected in relatively similar numbers. However, sampling methods would always make use of pheromone lures. The next graph looks at SJS and parasitoid distribution with lures.

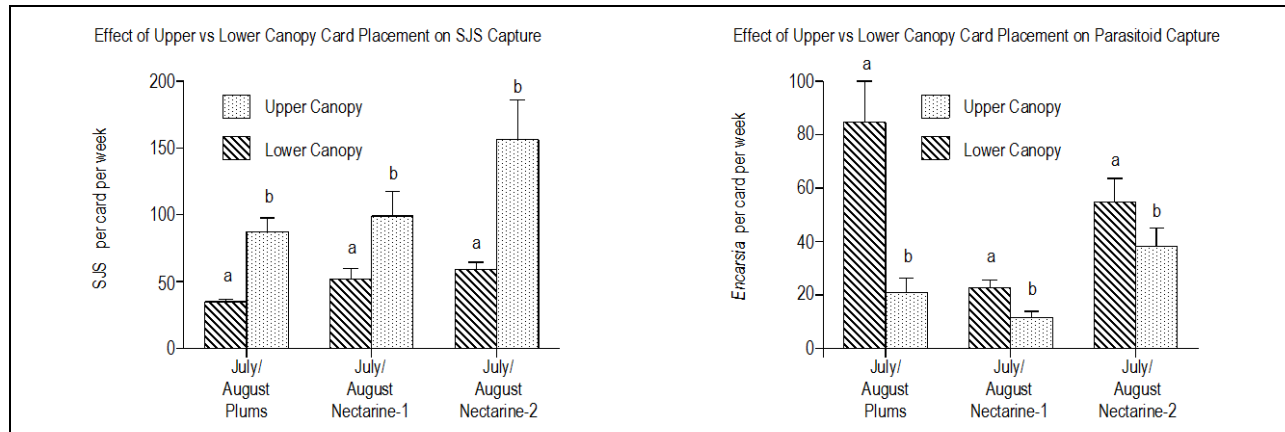


Figure 17. The relationship between SJS and trap height held true even when SJS pheromone lures were added to the sticky traps. Significantly more SJS were collected in upper canopy sections than the lower canopy. In contrast, significantly more *Encarsia perniciosi* (the only parasitoid species attracted to the SJS pheromone lure) were collected in the lower canopy.

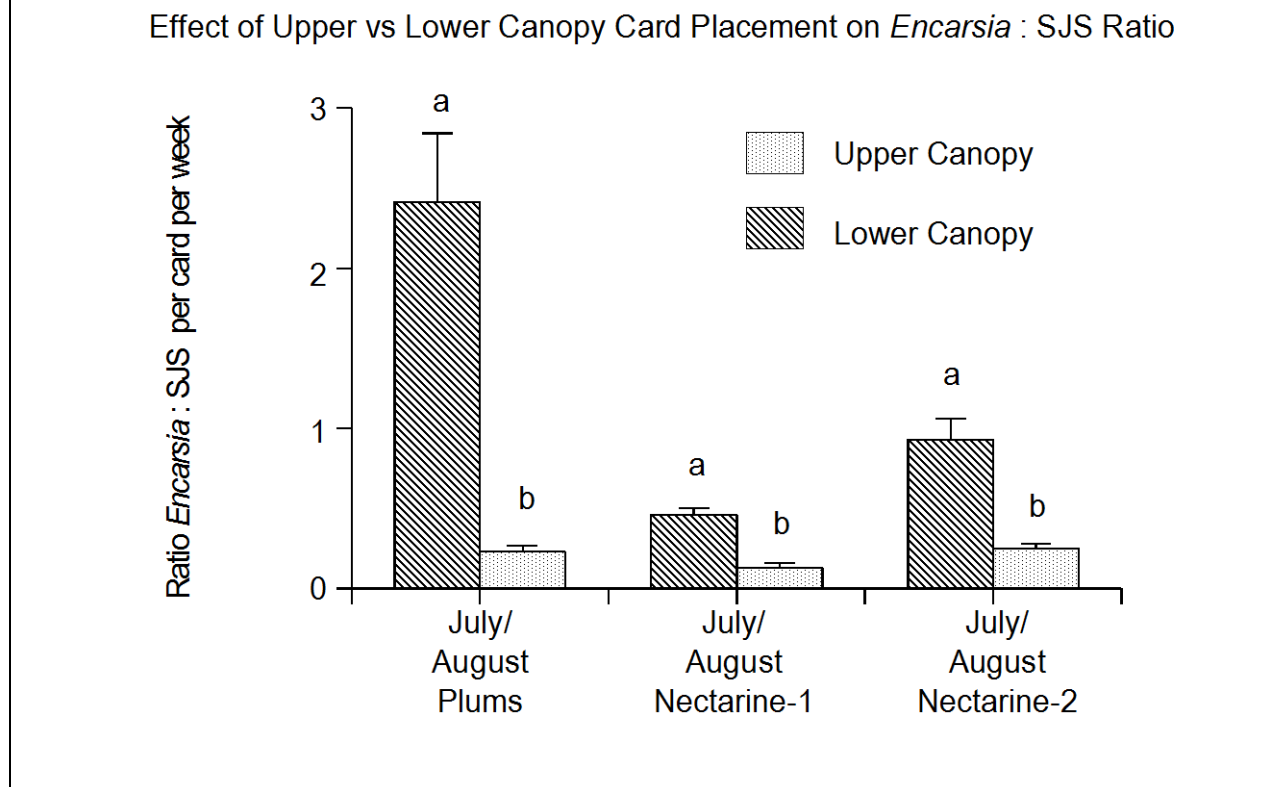


Figure 18. This has important implications for sampling. Low card placement will describe a SJS and parasitoid complex with far more parasitoids than SJS. A high card placement will do just the opposite –leaving the PCA with the impression that there are far more SJS. And in either case, the SJS pheromone-baited traps will not monitor for *Aphytis* species, which may be the more important parasitoid later in the season.

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. It has become apparent that the importance of *Aphytis* spp. has been sorely underestimated – probably due to the conspicuous presence of *Encarsia* on the pheromone traps. At the project’s conclusion, collected data will provide a better description of management practices that disrupt either SJS or its parasitoids and which may result in SJS outbreaks.

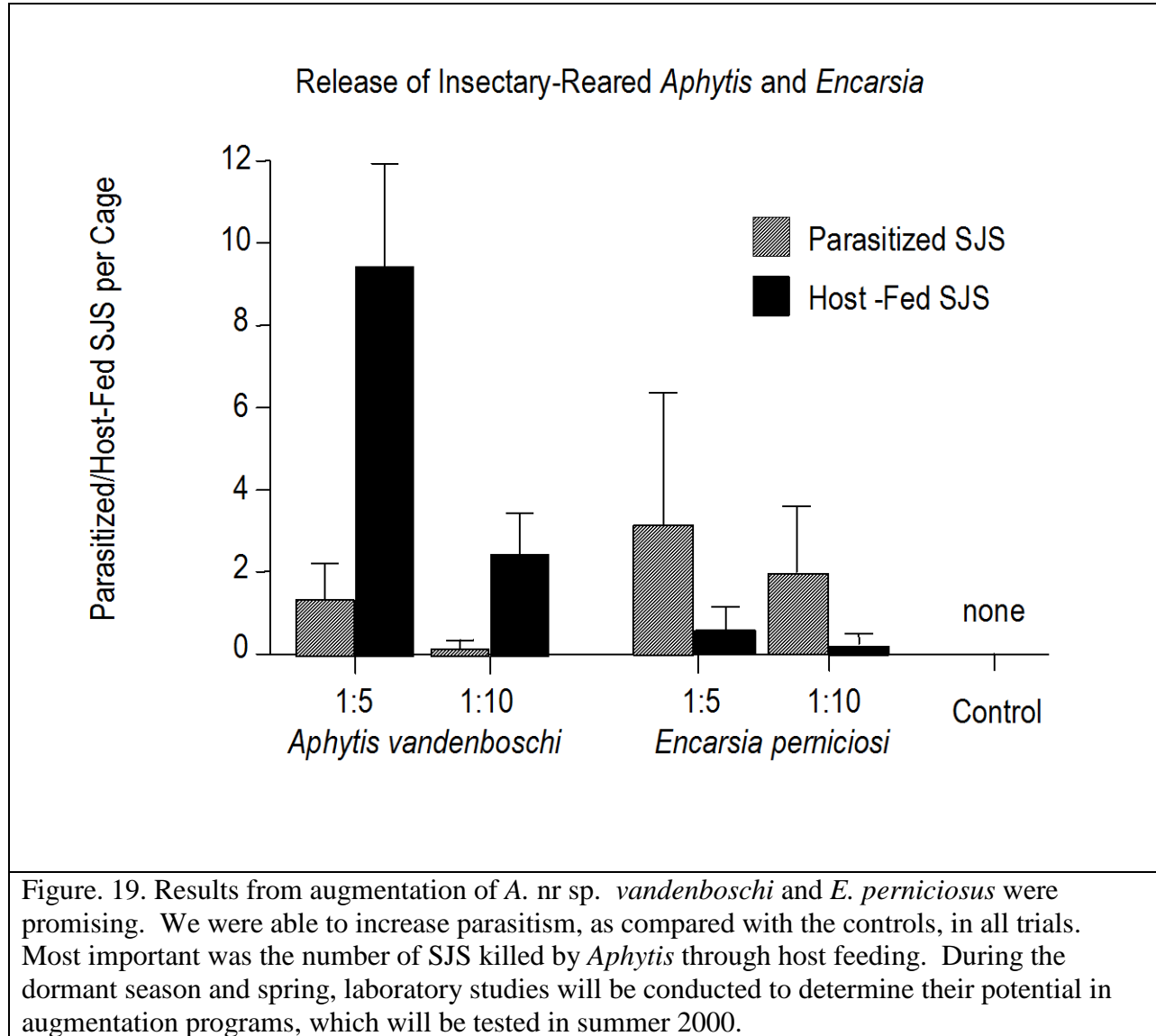
One of the more difficult projects has been the investigation of an augmentation program. The work described above - following SJS and parasitoid density in nine orchard blocks - brought one aspect of augmentation into better focus. It appears that mass producing and release *Encarsia perniciosi* early in the season would not be beneficial. This parasitoid is in all orchards with SJS, regardless of insecticide use, and early season release would probably not be able to add significantly to parasitoid population.

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 are 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). Levels of parasitism in closed containers 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, a very artificial situation. For this reason, small scale field studies followed laboratory studies. Over 50,000 *A. melinus* were released in three trees. Hung in each tree were squash with 100s of SJS scale. In this study, we also found parasitized SJS – 17% of the scale on squash had been attacked. 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. 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.

For this reason, work in 2000 focused on *Encarsia* and *Aphytis* nr sp. *vandenboschi*. The data presented below show how confusing the difference between the two parasitoid genera can be. While *Encarsia* is far more common on the traps, is clearly more common early in the season, and may parasitize more SJS..... it may not be the most important parasitoid from June through August. Our initial studies with augmentation suggest this hypothesis to have some validity. *Aphytis*, while it may not parasitize as many SJS throughout the season, may kill considerably more due to the process of “host feeding,” which means the parasitoid sticks the

SJS with its ovipositor to cause “bleeding.” The parasitoid feeds on the SJS juices to help develop eggs and the “poked” SJS eventually dies. Host feeding is far more common on small SJS, while larger SJS are used for egg deposition. Furthermore, initial collections of overwintered SJS indicate a greater presence of *Aphytis* than previously recorded.



## Objective 2.

In 2000, we screened Dimilin®, Confirm®, and Success® for efficacy against peach twig borer (PTB). Dimilin® and Confirm® are insect growth regulators (IGRs), which are larvicides that interfere with the insects' chitin deposition (the outside shell) and this prevents the insect from molting. Currently, Dimilin® and Confirm® are not registered for use in stone fruit or almonds, but both have been pursuing registration.. Success® is a bacterial by product and is currently registered for use in almonds. Our goal was to provide information that might better

usher these “softer” products into widespread use. Because any insecticide application has the potential to affect other pest and beneficial insects in the orchard, we also screened the tested IGRs against some of the more common beneficial insects in almond orchards.

In 1998, and 1999, we used "diet-incorporated" bioassays to develop LD50s and LD90s for Dimilin®, Confirm®, and Success®. All three products were found effective against PTB. In 1999, we began field tests of these products on PTB and some commonly found beneficial insects. Almond trees were treated, using commercial methodologies, with 4× the label rate for each product and compared with a no-spray control (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), green lacewing (larva), *Goniozus legneri* (adult), or *Aphytis* spp (adult) was added (20 replicates each). The insects were checked at daily intervals and their condition (alive or dead) recorded. Results from this study show all products had better than 95% kill of PTB when exposed to nuts collected 1 day after spray application. Dimilin® and Success® remained active at the 6 day period, while Confirm® efficacy dropped. This was especially evident at the 12 and 22 day periods. At this high rate (4× the label rate), we also detected some affect on beneficial insects, particularly parasitoids. For this reason, this study was repeated using label rates, more replicates, and San Jose scale (SJS) parasitoid common to almond orchards (*Encarsia perniciosi* and *Aphytis vandenboschi*).

Results again showed that all materials were quite effective against PTB, with Success® providing the best control for the greatest period of time. Success® and Confirm® also showed activity against NOW. Of the natural enemies tested, green lacewings and *Goniozus legneri* (a parasitoid of NOW) were unaffected by any of the tested insecticides (in the 1999 trial, there was some insecticidal activity of Confirm® on *Goniozus*). In the 2000 trial, there was no discernable effect of the IGRs on the small SJS parasitoids. Preliminary results suggest mortality of *Encarsia perniciosi* and *Aphytis vandenboschi* was greater on nuts recently sprayed with Success®. Because the mode of action of Success® would suggest that this product should not kill adult parasitoids, these results are held in question. For this reason, we are currently conducting more controlled nut-dip bioassays and results from these trials are not yet available

Tests of chemical effects on beneficial insects are nearly completed

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