SAN JOSE SCALE AND ITS NATURAL ENEMIES: INVESTIGATING NATURAL AND AUGMENTED CONTROLS

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ABSTRACT

The biological control laboratory has studied San Jose scale (SJS) and its natural enemies from 1999 through 2004. This past year, our research focused on (1) completion of *Aphytis vandenboschi* laboratory studies, (2) initiation of *Encarsia perniciosi* laboratory studies, (3) determining seasonal SJS and natural enemy phenology, and (4) investigating a fall-season mass release of *Aphytis vandenboschi*.

The most important SJS parasitoids are the small wasps *Encarsia perniciosi*, *Aphytis aonidiae*, and *Aphytis vandenboschi*. Previous work showed that *Encarsia* was the dominant parasitoid, although the *Aphytis* species are a critical component of biological control, especially when SJS populations reach higher densities. Although both parasitoid species are found in most orchards, their effectiveness is variable and difficult to predict. We therefore conducted laboratory studies of parasitoid biology to better understand their strengths and limitations as SJS biological control agents. We found that *A. vandenboschi* prefers 3rd instar SJS for oviposition (depositing an egg), while 1st instars are more typically used for "host feeding," which often kills more SJS than parasitism. *Aphytis vandenboschi* temperature development was studied at constant temperatures between 13 and 34ºC (54 to 97ºF). Parasitoid development was a significant linear function of temperature between 18 to 32ºC or 64-90ºF (the higher the temperature the faster the parasitoid developed). However, below or above this range, development slowed and, at the extreme temperatures tested (13 and 34ºC or 54 to 97ºF), there was significant parasitoid mortality. The results suggest that *Aphytis vandenboschi* is an excellent SJS natural enemy when it has suitable SJS stages (second and third instars) available and air temperatures range from 55 to 90°F.

Similar studies with *E. perniciosi* show that it has a lower development threshold of 30ºC (86ºF). In addition, comparisons of adult longevity at different temperatures show that *A. vandenboschi* live significantly longer than *E. perniciosi* at 31ºC (88ºF), the highest temperature tested. These results suggest that *E. perniciosi* may decline in the summer at least partly as a result of a lower temperature tolerance compared to *A. vandenboschi*.

Our field research component has been reduced and refocused. In previous studies we sampled many different orchards to provide an overview of SJS and parasitoid densities. This past year

we focused on three orchards, with SJS collected monthly and dissected to determine SJS and parasitoid stage and condition. In fall 2004, we completed the collection and processing of samples but the data have not yet been analyzed.

Over the past three years we have investigated the potential of mass releases of *Aphytis vandenboschi.* These studies were conducted in small cages because we could not produce the needed number of parasitoids for larger-scale trials. This past year, our efforts have been joined by the Foothill Agricultural Research insectary. They have produced 1000s of *A. vandenboschi*, which have been field-released at three different sites (Kearney Agricultural Center and two commercial orchards). Because laboratory trials indicate that mid-summer air temperatures may reduce release effectiveness, we concentrated our release trials in the late summer and fall (20 August 2003 to present). Pre-release samples were collected during the spring and summer months. Post-release samples were collected through harvest 2004. The data have not yet been analyzed.

INTRODUCTION

San Jose scale, *Quadraspidiotus perniciosus* (Comstock), 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 including 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, based largely on insecticide controls. 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, which was typically combined with an organophosphate, or spring and summer applications of organophosphates (Dowing and Logan 1977, Westigard 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®) offered promise for spring and summer suppression of SJS. 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 are often timed to periods of peak crawler emergence. Phenology models of SJS development have been used to improve application timing. Because the crawlers are hard to monitor and count, their emergence patterns are best 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 flies. SJS pheromone traps can also attract one of the primary

SJS parasitoids – a small "aphelinid" wasp named *Encarsia perniciosi* (formerly called *Prospaltella perniciosi* Tower) (McClain et al. 1990, Rice and Jones 1982). Two other SJS parasitoids, *Aphytis aonidiae* (Mercet) and *Aphytis vandenboschi* Rosen are sometimes also found on the SJS pheromone traps.

The combination of good sampling methods, a phenology model to time insecticide applications, and reliable insecticide products has been the standard for most IPM programs. 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. More recently, SJS infestations have been reported in more northern counties and in almonds and walnuts (Nick Mills and Roger Duncan, pers. comm.). This is particularly unusual because most almond and stone fruit growers with farms in Merced, Modesto, and Stanislaus counties have rarely had to apply treatments for SJS (except on nectarine cultivars on which SJS readily settle on the fruit and even small populations can result in noticeable 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 fluctuations have been considered.

OBJECTIVES (1999-2004):

1. To compare potential field and laboratory effectiveness of *Encarsia perniciosi*, *Aphytis aonidiae*, and *Aphytis vandenboschi* – three parasitoids of the San Jose scale.

2. To investigate *A. vandenboschi* and *E. perniciosi* biology and determine host-stage preference, levels of host feeding, temperature tolerances and potential for insectary rearing.

3. To investigate interactions between *E. perniciosi* and *Aphytis* species.

4. To survey parasitoid populations and their relative abundance in northern, central and southern San Joaquin Valley locations.

PLANS AND PROCEDURES

Field studies: Phenology and Augmentation of Parasitoids

To complete our studies of seasonal changes in parasitoid and scale populations, in 2004 we intensively sampled three orchards in Fresno and Kern counties. SJS-infested branch tips were collected monthly, placed in cold storage, and later examined to determine SJS and parasitoid stage and condition. Approximately 30 branch tips were collected at each site on each collection date.

In 1999 we tested the potential of a commercially available parasitoid (*Aphytis melinus*) to attack SJS in the field. Initial laboratory studies show this parasitoid species would host feed and parasitize SJS. However, tests in the open-field found this species did not affect SJS density or parasitism levels. For this reason, we established insectary colonies of SJS parasitoid: *Aphytis*

vandenboschi and *Encarsia perniciosi*. Material from these colonies was used for the field augmentation trials and laboratory studies of parasitoid biology described in this report, as well as, in previous years, for bioassays of commonly used insecticides.

In 2000 and 2002, we tested the effectiveness of mass-releases of *Encarsia perniciosi* and *Aphytis vandenboschi*. The experimental block was located at KAC and had not received insecticides for the previous 10 years. Earlier samples indicated that SJS, *Encarsia perniciosi*, and *Aphytis vandenboschi* were present in the orchard. To begin, branches were checked for SJS that, if found, were removed. The selected branches were then inoculated with ~500 SJS crawlers (from the insectary) in late spring or summer to match the natural SJS population. The inoculated branches were enclosed in large, self-supporting organdy cages (~1 m long × 0.4 m diameter). After the SJS reached an appropriate size, a pre-release count of the number of settled SJS was determined for each branch. *Encarsia perniciosi* or *Aphytis vandenboschi* were then released into the cages at either 1:5 or 1:10 ratios of parasitoid : SJS (2000) or a 1 : 20 ratio (2002), based on pre-release counts. After 4-6 weeks, the cages were removed, the branches were cut from the trees and taken to the laboratory, and SJS were counted and dissected to determine their development stage and condition (live, dead 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 2001, 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 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.

Results from these earlier trials showed the parasitoids had some promise, but could not realistically control an SJS outbreak. For this reason, in 2003 and 2004 we focused on late summer and fall releases of *A. vandenboschi*, for use as an inoculative control of SJS for the following season. Release rates and methods are as described above for the open field release.

Parasitoid Biology

In 2001-2003, laboratory work focused on the biology of *Aphytis vandenboschi*. In 2004, we completed research on *A. vandenboschi*, which had proved the easiest to rear. Because of its relatively low numbers, we have been particularly interested in this species as a candidate for augmentative release, particularly early in the season. Resident *Encarsia* numbers are typically very high in the spring and do not warrant augmentation.

We wished to study the biology of *E. perniciosi* nonetheless in order to determine 1) why it falls off later in the season, and whether augmentation would be of use then; and 2) whether it competes with the *Aphytis* species and thus might lower their effectiveness when both are present. The major obstacle to the completion of this work was the difficulty of establishing an *Encarsia* colony in the Berkeley insectary. In July 2003, however, we managed to rear the

offspring of about 50 *E. perniciosi* originally reared from scales collected from almond, peach, and nectarine trees in Fresno and Kern counties. By January 2004, however, we were consistently rearing several hundred *E. perniciosi* every week, enough to begin a series of biological studies.

Sources of laboratory insects and maintenance of colonies. Laboratory cultures of SJS were originally derived from field samples collected in nectarine and peach orchards in the southern San Joaquin Valley, California. Scales were reared on butternut squash obtained from local grocery stores. The squash were cleaned with dish soap, sprayed with a 10% bleach solution, and allowed to air dry shortly before use. Each squash was inoculated with 300-500 SJS crawlers using a fine-haired paint-blending brush. The colonies were maintained in a temperature-controlled insectary room at 25 ± 1.5°C or 75 ± 35°F (16:8 L:D). They were held in wooden sleeve cages with glass tops and organdy sides in order to prevent contamination and to retain the winged males in the vicinity. To maintain genetic diversity, new scales were added in from field-collected material at least once a year.

The cultures of the two parasitoids were reared in separate rooms to prevent cross-contamination. Adult wasps were placed, along with SJS–infested squash, into one-gallon plastic tubs with mesh-covered lids. The subsequent generation then emerged from the container through a funnel embedded in the organdy and into glass vials for use in the experiments or to be returned to the colony.

Temperature development

Aphytis vandenboschi. The effect of constant rearing temperatures on *A. vandenboschi* development time was determined at 13, 15, 17, 20, 25, 30, 32 and 34°C (55, 59, 63, 68, 77, 86, 90, 93°F). Squash infested mainly with third instar SJS were exposed in 1-gallon plastic tubs to 50-100 *A. vandenboschi* adults. Third instars were used because similar *Aphytis* species are known to prefer older scales for oviposition. After 24 h, the squash were removed from the containers and cleared of all remaining wasps. They were then returned to their containers and randomly assigned to one of the eight temperature treatments. The infested squash were examined every three days for the higher temperature treatments (20°C or 68°F and above) and every five days for the lower temperatures (13, 15, and 17°C or 55, 59, 63°F). The examinations consisted of inspecting individual scales for *A. vandenboschi* eggs by pulling their covers away with forceps beneath a dissecting microscope. Scale stage, condition, and host feeding, as well as parasitoid presence and stage were all recorded for each scale examined. Scales were inspected until 10 *A. vandenboschi* were discovered. These examinations were continued in this regular schedule until all of the *A. vandenboschi* had either died or emerged as adults.

There were four replicates (each inoculated squash was a replicate) for each temperature treatment. Temperatures (T) were maintained at T ± 1°C (34°F), with a 16:8 (L:D) photoperiod. Temperature cabinets were randomly assigned to each temperature treatment.

Encarsia perniciosi. Similar procedures were used for *Encarsia* except that no attempt was made to determine the duration of each larval stage, the difficulty being an internal parasitoid. Instead, only the development time from oviposition to adult emergence was determined. The temperatures tested were 13, 15, 20, 25, 28, 30, and 31°C (55, 59, 68, 77, 82, 86, and 88°F). The plastic tubs were fitted with a glass vial at the top (as for colony maintenance) and the vials were checked daily to count emerging wasps. The scales were exposed to the wasps as 2nd instars and there were four replicates (=a squash in a tub) at each temperature. At 20, 25, and 28°C (68, 77, 82°F), an additional 4 replicates were done using 1st rather than 2nd instars for oviposition.

Adult longevity at different temperatures. Wasps were taken from the colony within 5 h of emergence. Groups of five wasps were placed in 40-mL. glass vials provisioned with a streak of 50% honey-water and plugged with a wad of cotton wrapped in fine cotton cloth. Each replicate consisted of 4 such vials (20 wasps). There were five temperature treatments (15, 19, 23, 26, and 32°C or 59, 66, 73, 79, and 90°F constant day/night temperature; lighting 16:8 L:D). At 23, 26 and 32°C (73, 79, 90°F), the wasps were checked and the honey streak replenished every 24 h; at the two coolest temperatures the wasps were checked and the honey replenished every 48 h. The number of living wasps was recorded until all wasps in the vial had died. Wasps that died from other than apparently natural causes—for example, those that got stuck in the honey or in the folds of the cotton plug—were excluded from the analysis. For both wasp species, there were three replicates at 15, 19, and 23°C (59, 66, and 73°F); and four replicates at 26 and 31°C (79, 88°F).

Host-stage preference for oviposition and (for A. vandenboschi) host feeding

Aphytis. In the temperature-development experiment described above, the squash were examined immediately after the 24-h exposure period. The scale covers were dissected away as described above. We recorded the presence of wasp eggs and also host feeding, the latter indicated by the appearance of shriveled scales or by scar-like, darkened marks on the body of the scale. The stages of the attacked scales were also recorded.

Encarsia. Because *Encarsia* eggs are placed inside the host's body, host-stage preference must be determined by direct observation of female behavior rather than by looking for deposited eggs. Twenty female wasps about 48 h old were individually isolated in small plastic containers (about 11 cm diam × 5 cm deep with ventilation hole about 4 cm^2 was cut in the lid and covered with fine mesh). Each wasp was presented with a piece of squash bearing about 100 scales in roughly equal proportions of 1st, 2nd, and 3rd instars. Each wasp was observed for 30 min, and the stages encountered and used for oviposition were recorded.

Lifetime fecundity

Aphytis. Within 4 h of emergence, wasps were isolated individually in small plastic containers about 11-cm diam × 5-cm deep. A ventilation hole about 4 cm^2 was cut in the lid and covered with fine nylon mesh. A piece of round filter paper covered the bottom of each container, and a streak of 50% honey-water solution was applied to the sides and to the lid of the container . Each wasp was provided with a piece of squash bearing about 100 scales. Every 24 h, the wasp was captured and held for a few minutes in a glass vial while squash was replaced with a fresh piece and the filter paper was replaced too and new streaks of honey applied. This procedure continued until the wasp was found dead in the container. The experiment was conducted in the colony room, 16:8 L:D and 24°C (75°F).

Two experiments were performed, in order to test the hypothesis that the availability of the stage preferred for host-feeding (i.e., 1st instars) would increase fecundity. In the first, the squash pieces had only 3rd instar scale. In the second, the squash pieces had a mixture of instars, dominated by 3rds and 1sts. Each scale was checked for eggs shortly after it was removed from the container. In each experiment 25 wasps were tested. We compared lifetime total fecundity, daily fecundity patterns, and longevity for the two treatments.

RESULTS

Phenology and Augmentation of Parasitoids

The work of previous years (1999-2003), following SJS and parasitoid density in nine orchard blocks, brought one aspect of augmentation into better focus. It appears that mass producing and releasing *Encarsia perniciosi* early in the season, when it occurs naturally in high numbers, would not be beneficial. This parasitoid is in all orchards with SJS, regardless of insecticide use, and early-season release would probably not add significantly to existing parasitoid populations.

In fall 2004, we completed the collection and processing of branch samples for our study of seasonal patterns in SJS and parasitoid populations. The data have not yet been analyzed.

Our initial release studies were conducted with a limited number of available *Aphytis* and *Encarsia* from the colonies to release. Results from previous years show that *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.

In fall 2004, we completed the final augmentation experiments, taking advantage of improved insectary methods to release greater numbers of parasitoids. The data have not yet been analyzed.

Parasitoid Biology

One of the biggest hurdles for the augmentation program has been the development of insectary procedures to mass-rear viable and effective natural enemies. Large colonies of *A. vandenboschi* and *E. perniciosi* have now been established. Our laboratory studies on parasitoid preference for SJS stages and on parasitoid fecundity and host feeding limits are designed to determine when to release the parasitoids and what release rates are necessary to suppress SJS.

Temperature development

As reported last year, at 15ºC (59ºF)*Aphytis* develops but at a very slow pace, and after 58 days most of the parasitoid population has reached the pupal stage. The population remained in this stage and these results suggest that, during the winter, the parasitoid may stay in the pupal stage until temperatures increase in spring. At 13ºC (55ºF), development appeared to be disrupted. Very few adults were obtained. Many wasps did not appear to pupate successfully, either remaining mature larvae or expelling meconial pellets without completing the pupal molt. At the other extreme, we identified a clear upper limit to development at 32ºC (90ºF. At this temperature, development was normal, whereas at 33ºC (91ºF), no wasps made it past pupal stage, and at 34ºC (93ºF), most wasps died as young larvae.

We now have partial comparable information for *Encarsia*. Detailed analyses are still in progress, and we have not yet completed the determination of the lower development threshold (the 20 and 13ºC or 68 and 55ºF experiments take several months to complete and are currently underway). Results at 15ºC (59ºF) are similar to those for *Aphytis*. Of particular interest, however, is our finding that the upper development threshold is lower than that for *Aphytis*. Development was normal at 30ºC (86ºF); however, at 31ºC (88ºF), no adult wasps were obtained. Dissection of the scales revealed a few dead pupae, but it appeared that most did not make it past the egg or younger larval stages, i.e., no visible effect on the condition of the hosts was observed.

In addition, our experiments conclusively show that *E. perniciosi* is a koinobiont parasitoid, delaying development until the host reaches 3rd instar regardless of whether the egg was laid in the 1st or 2nd instar. Pupation occurred only in 3rd instars, and observed development times were significantly longer when the host scales were exposed as 1st instars (at 28ºC or 82ºF, 24.50 ± 0.29 d on 2nd, 29.00 ± 0.41 d on 1st, T-test, $p < 0.001$, $T = 2.447$, $df = 6$; and at 25ºC or 77ºF, 25.75 ± 0.63 d on 2nd, 32.75 ± 0.48 on 1st, $p < 0.001$, $T = 2.447$, $df = 6$). Thus, total egg-to-adult development time simply takes longer if the egg is deposited in the 1st instar, presumably because feeding and larval development do not begin until the host reaches the 3rd instar. These results are different from those reported for *E. perniciosi* attacking red scale: in that system, the wasps tend to emerge from 2nd instars if the egg is laid in the 1st instar (or from older 2nd instars if the egg is laid in a very young 2nd) (Yu et al. 1990).

Adult longevity at different temperatures

Comparison of *Aphytis* and *Encarsia* shows that the wasps have similar longevity at all temperatures tested except the highest one, 31°C or 88ºF (Figure 1), where the *Aphytis* lived significantly longer. This result is consistent with our results for temperature development, above, again indicating that *Encarsia* has a lower tolerance for heat extremes.
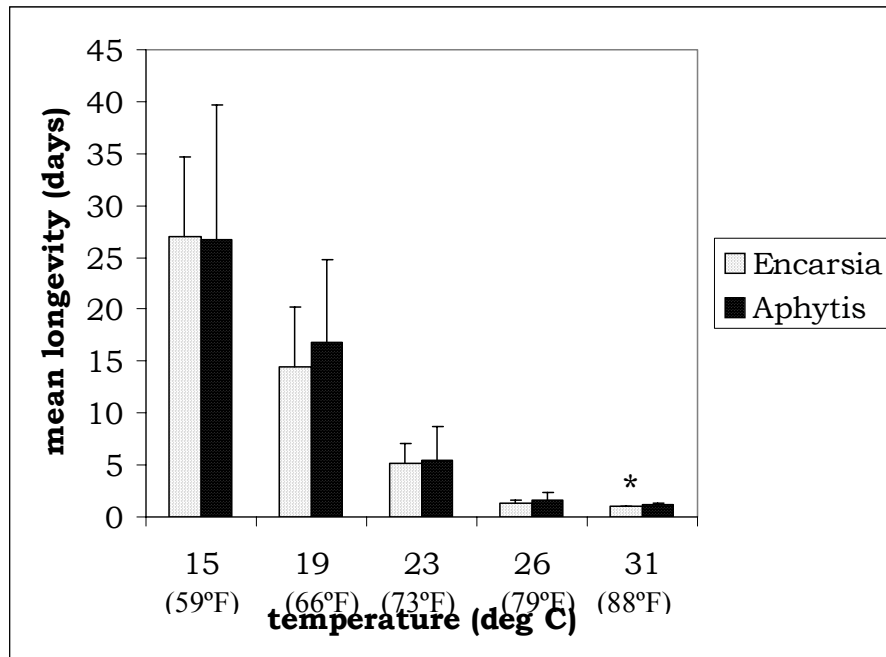

Figure 1. Longevity of adult *E. perniciosi* and *A. vandenboschi* at different temperatures. Differences between the two species are insignificant except at 31ºC (88ºF), where *A. vandenboschi* adults lived longer (1.17 ± 0.06 d *vs.* 1.01 ± 0.01 d, 2-tailed *T*-test, $T = 2.65$, $P = 0.0063$).

Host-stage preference for oviposition and (for A. vandenboschi) host feeding

As reported last year, *A. vandenboschi*, like other *Aphytis* species, prefers 3rd instar scales for oviposition. Younger instars are favored for host-feeding, though all three are apparently usable. Determination of *Encarsia* host-stage preference is still in progress, but preliminary results indicate that it prefers 2nd instar scales, a result that would be consistent with reports for *E. perniciosi* attacking other scale species (e.g., Yu et al. 1990).

Lifetime fecundity

The lifetime fecundity pattern is shown in Figure 2, for fecundity given 3rd instars only, and in Figure 3 for fecundity given a mixture of host stages. We found no significant differences between either treatment, either in the overall pattern, or in total lifetime fecundity (14.68 ± 1.86 (3^{rd}s) and 13.43 ± 1.77 (mixture), $T = 0.4884$, $P = 0.63$, df = 38), or in longevity (6.95 ± 0.74 (3^{rd}s) and 6.19 ± 0.53 (mixture), $T = 0.8380$, $P = 0.407$, df = 39). This result is unexpected, given that the availability of stages used for host-feeding is reported, in other *Aphytis* species, to increase fecundity and longevity and to extend the period during which oviposition occurs. We reported previously, however, that although it *prefers* younger instars, *A. vandenboschi* is capable of host-feeding on 3rd instars. Presumably, therefore, given no choice but 3rd instars, the wasps can feed on them as much as they require, and thus lifetime fecundity patterns are identical to those when younger instars are present.
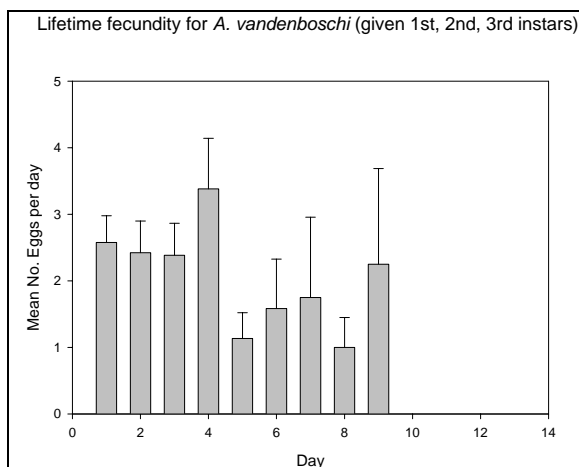

Figure 2. Fecundity of *A. vandenboschi* when a mixture of SJS instars are available.
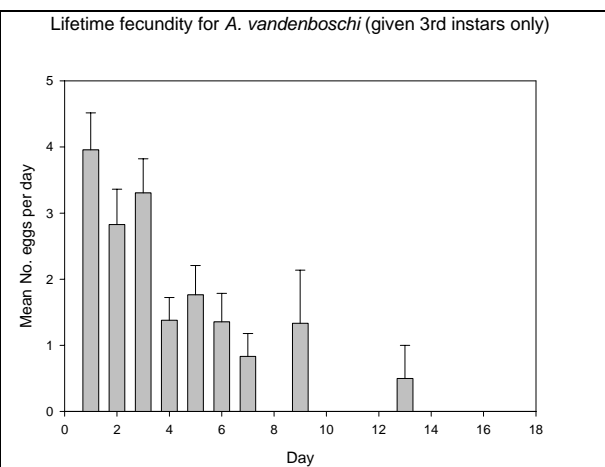
Figure 3. Fecundity of *A. vandenboschi* given only third instar SJS.

The most striking pattern in parasitoid populations observed over five years' of field studies is that *Encarsia* populations peak in the spring, decrease dramatically in the summer, and then increase in the fall as the parasitoids attack the overwintering SJS population. The *Aphytis* species, while never reaching the levels of the spring peak of *Encarsia*, are present in proportionately greater numbers in the summer. We hypothesized that *Encarsia* have slower development and poor survival during the summer months because they cannot tolerate high temperatures.

Our current results from these biology experiments support this hypothesis, permitting now at least a partial explanation for the observed displacement of *E. perniciosi* (which peaks in spring) by *A. vandenboschi* in mid-summer in the San Joaquin Valley. Comparing the responses to temperature of the two species, we find both that the adult longevity of *E. perniciosi* is shorter at high temperatures and that the upper threshold for larval development of this species is lower

than that of *A. vandenboschi*. These results may also help explain why *E. perniciosi* tends to forage in the inner and lower parts of the canopy, which are relatively milder even in the summer heat. Both species fare quite poorly at 31ºC (88ºF), however, which suggests that field releases of either will be more effective if the hottest summer periods are avoided.

The role of temperature probably complements other mechanisms of displacement in explaining how these two species coexist. Our finding that (at least on SJS) *E. perniciosi* must complete its development in the 3rd host instar indicates that it may be directly displaced by *A. vandenboschi* whenever the two species occur together. We are currently starting experiments to determine to what extent this type of competitive displacement may contribute to the observed distribution patterns of the two parasitoid species.

We find that there are several important differences in the biology of our *E. perniciosi* attacking SJS and published reports of this species, which considered its biology when attacking red scale. In the results reported here, we find that on SJS this species appears only to emerge from 3rd instars, regardless of stage attacked, whereas the red-scale population is more flexible. In addition, the two species differ in that the red scale population is entirely female whereas our SJS population contains males and females. These differences suggest that the two populations may in fact be distinct strains and possibly separate species. The level of difference between them certainly warrants further investigation, as it is crucial to determining which species or strains would be most effective in future release programs directed at either pest species.

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