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Inoculative Releases of *Goniozus legneri* for NOW Control in Almonds: 1995 Report

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The almond IPM program for navel orangeworm (NOW), *Amyelois transitella* (Walker), was developed from solid research on NOW biology (e.g. Wade 1961, Caltagirone et al. 1968, Curtis & Barnes 1977, Tzanakakis & Barnes 1988, Sanderson et al. 1989) and has become one of the more successful IPM programs in California. This "conventional" NOW control program begins in the dormant season with a cultural control - orchard sanitation. After harvest, almond nuts left on the trees serve as the only host site for overwintering NOW, orchard sanitation removes these nuts, or "mummies," thereby reducing NOW (Engle & Barnes 1983, Zalom et al. 1984). If needed, NOW populations can be reduced in numbers by applying an in-season insecticide spray, typically used in May or July (Barnes & Curtis 1979). The program is completed with another cultural practice - an early almond harvest. Removal of the almond crop before the third NOW generation greatly reduces nut infestation levels (Zalom et al. 1984, Connell et al. 1989). Using these IPM practices, growers should expect <2% NOW nut infestation levels.

Still, almond growers lose millions of dollars to NOW each season and have been experimenting with different control strategies. One such alternative is the release of a bethylid parasitoid, *Goniozus legneri* Gordh, which attacks, develops upon, and kills NOW larvae. The potential for NOW control without synthetic insecticide treatments has been documented by the Biologically Integrated Orchard Systems (BIOS) program (CAFF 1995), and many of these growers are using *G. legneri* releases as a part of their IPM program. Moreover, the almond industry is as close as any agricultural commodity at completing a "least-toxic" or "sustainable" insect control program and *G. legneri* release, in lieu of insecticides, complements previously developed IPM programs for mites (Hoy et al. 1978) and peach twig borer (PTB), *Anarsia lineatella* (Barnett et al. 1993). Augmentation is a viable alternative to insecticides and in this past decade there has been increased use of insectary-reared natural enemies for the control of insect pests (Ridgway et al. *submitted*). Nevertheless, there are no scientifically documented guidelines for the release of *G. legneri* in almond orchards. These guidelines are needed; first, to ensure that the releases do indeed work and, second, to improve upon existing methods. Most successful augmentation programs have, in common, research that clearly outlines target pest and natural enemy biology; biotic and abiotic interactions at the release site; and release rates, timing, and methods. Here, we report on the first 9 months of a 3-4 year study that will document the effectiveness of current *G. legneri* release programs and suggest improvements to release methodologies.

Materials and Methods

I. Release Methodology.

An essential part of an augmentative program is the release method. It's a question of cost and effectiveness, *G. legneri* cost money and growers want to release the least number of *G. legneri* in the most efficient way to provide effective control. Further, researchers (e.g. Dreistadt et al. 1986) have shown that resident predators, such as ants, can cause significant mortality of released beneficial insects. An informal survey of PCAs and orchard managers found that *G. legneri* were released in many different ways (e.g. as pupae adults, in gelatin capsules or in dixie cups). Typically, ca. 1,000 *G. legneri* are released per acre, spread over 3-4 release periods - ca. 200-300 in March-April, ca. 300-500 in May-June, ca. 200-300 July-August, and ca. 300-500 in September-October. In most programs, 7-12 *G. legneri* pupae are enclosed in a single gelatin capsule (ca. 1 inch in length), these capsules are opened and spread throughout the orchard, placed in tree crotches.

Initial tests were conducted to determine if there were differences in the survival of released *G. legneri* pupae. *G. legneri* pupae were purchased from an insectary and divided into two categories: (1) 1-3 day old pupae, hereafter referred to as "white-capped", and (2) 4-5 day old, well-developed pupae, hereafter referred to as "black-capped". Gelatin capsules were placed individually in the lower scaffolding of almond trees at 10 AM, 20 capsules each of white-capped and black-capped pupae were used. During the next 5 days, the condition of the pupae and observations on foraging predators was checked each afternoon. Of primary interest is the possible predation by ant and earwig species foraging on the almond trunk. This experiment was conducted in August in an orchard with noticeable ant populations.

II. Release Effectiveness.

On-Farm Studies.

There are a number of obstacles in the evaluation *G. legneri* field effectiveness, such as the movement of *G. legneri* and varying NOW densities. In 1995, we developed a methodology to determine field parasitism rates using "sentinel nuts." First, NOW are produced in the KAC insectary (using methods described by Finney & Brinkman 1967). When the NOW larvae reach the second instar they are used to inoculate unshelled almond nuts. The inoculated nuts are stored singly in 20 ml rearing containers and held at 23-25°C. After ca. 7 days, those nuts that have been successfully inoculated (indicated by frass accumulation) are removed and stored at 13-15°C to slow NOW development. Just prior to field use, the sentinel nuts are placed individually in wire cages (ca. 5 cm x 5 cm) that can be stapled to the almond tree.

Our first field evaluations were made in commercial orchards, utilizing a paired-orchard design with 1 orchard receiving *G. legneri*-release and another orchard used as a no-release control. In all orchards, 4 sentinel nuts were attached to 25 almond trees, which were spaced evenly throughout a 250-tree section (25 trees x 10 rows). In the release orchards, *G. legneri* were released at rates between 350-700 *G. legneri* per acre (typical release rates are between 250-350 *G. legneri* per acre per release). After 14-21 days, sentinel nuts were removed and the number of NOW parasitized was recorded. These field tests were conducted in Butte and Madera Counties.

Data from the paired-orchard studies quickly indicated that there were greater between-orchard differences in the background levels of *G. legneri* activity than was accounted for by the *G. legneri* release. Further, *G. legneri* parasitism was often higher in orchards that had either a (1) long-term *G. legneri* release program or (2) a high density of NOW. For these reasons, we decided to continue field evaluations using a replicated split-plot design in orchards that had low NOW population densities. In each tested orchard, 4 "split-plot" blocks were established with 2 treatments, (1) *G. legneri* release or (2) a no-release control. Treatment plots were 10 trees x 10 rows, with a 20 tree x 10 row buffer zone between each treatment plot. Blocks were separated by 10 rows. In each plot, 4 sentinel nuts were attached to 25 almond trees, which were spaced evenly throughout each 100-tree section (nuts were placed on every-other tree on every-other row). *G. legneri* were then released at rates between 350-700 *G. legneri* per acre. After 14-21 days, sentinel nuts were removed and the number of NOW parasitized was recorded. These field tests were conducted in Butte, Merced, and Madera Counties.

NOW Density Study.

L. E.. Caltagirone observed that *G. legneri* populations would most commonly build to numbers (e.g., .50% NOW parasitism rates) only in orchards that had high NOW nut infestation rates. For this reason, we tested whether or not NOW density affects rates of *G. legneri* parasitism. In a commercial almond block, small plots (9 trees, 3 trees by 3 rows) were used to establish 4 different NOW densities; treatments were 1, 5, 10, and 20 sentinel nuts per tree. A randomized block design with 5 replicates was used. Blocks were separated by 3 rows and plots, within each block, were separated by 3 trees in each row. *G. legneri* were then released evenly throughout the experimental site at a rate of 700 *G. legneri* per acre. After 21 days, sentinel nuts were removed and the number of NOW parasitized was recorded. This field test was conducted in Madera County.

G. legneri Overwintering Studies.

A more controversial practice is the incomplete removal of overwintered almond mummies, which serve as the only winter host site for NOW and may also be the primary host site for *G. legneri*. For background information, we counted the numbers of mummies per tree in 5 almond orchards. In each orchard, 100-500 nuts were collected and the number of NOW and *G. legneri* recorded. This investigation will be used to provide a rough correlation between numbers of overwintering mummies and levels of *G. legneri* parasitism in different almond growing regions.

Sampling different almond field in winter does not take into account the inherent differences found among almond orchards and regions. For a more scientific evaluation, we are working with a cooperative grower that uses a NOW control program that includes winter sanitation and year-round *G. legneri* releases. In 3 - 80 acre blocks, the grower has left a single 8-acre section unsanitized, e.g., all overwintering nuts were left on all trees. The average number of mummies per tree were determined. Populations levels of NOW and *G. legneri* in the overwintered nuts were determined by collecting almond mummies in each 8-acre section and in 3 transects moving away from the unsanitized portion. More importantly, the number of NOW and *G. legneri* moving out from the unsanitized portion of each orchard is being sampled to determine whether almond sticktights (combined with *G. legneri* release) provide a source of more NOW or *G. legneri*. To sample, 3 transects were laid through each orchard and NOW and *G. legneri* populations are being monitored in rows 1-10, 11-20, 21-30, and 31-40 away from the unsanitized section.

Results

I. Release methodology.

Results show that the release of "black-capped stage" pupae or recently hatched adults was the best way to guarantee the effective release of insectary material. Most problems occurred when "white-capped" pupae were released. White-capped pupae are *G. legneri* that have only recently developed to the pupae stage and this material required >3 days for adult *G. legneri* to emerge (at a constant 78°F). During this period the *G. legneri* were subject to numerous forms of mortality. In the experimental orchard used, ca. 20% of the released white-capped pupae survived; mortality factors observed included foraging southern fire ants, *Solenopsis xyloni*, and native gray ants, *Formica aerata*, and physical factors, such as irrigation water (dissolving the capsule) and high temperatures (killing the pupae). In contrast, the black-capped pupae were ready to emerge on the release date; in fact, in most gelatin capsules there were 1-3 adults (of the 10 *G. legneri* per capsule) that had already emerged by the 10 AM release period. It is estimated that ca. 95% of the black-capped pupae survived to the adult stage. These preliminary observations on the importance of release methodology were confirmed through problems we encountered in our on-farm release trials. For example, in 2 on-farm release trials, ca. 80% of white-capped pupae released in gelatin capsules were destroyed by earwigs.

At this time, we suggest that insectary material be stored until some adult *Goniozus* begin to emerge, this will help to estimate the developmental stage of the remaining insectary material. A goal would be to have >75% of the released material hatch within 1 day of release. Of course, release of adult *Goniozus* provides an even better assurance that release material will escape predation, however, there are disadvantages to adult releases as well. For example, a common problem is the transport and storage of insectary material. We held black-capped pupae and adults, in closed gelatin capsules, at 94°F, a common summer temperature. Results showed 100% of the adults were dead within 3 hours, while the none of the pupae were damaged. Future studies will more completely investigate release methodologies, including release rates and release periods.

II. Release effectiveness.

A. On-Farm Studies.

Results from on-farm studies were very inconsistent, nevertheless, in each trial there was a greater level of *G. legneri* parasitism in the release orchards and plots. In the paired-orchard study we found great differences between release and no release orchards (Figure 1).

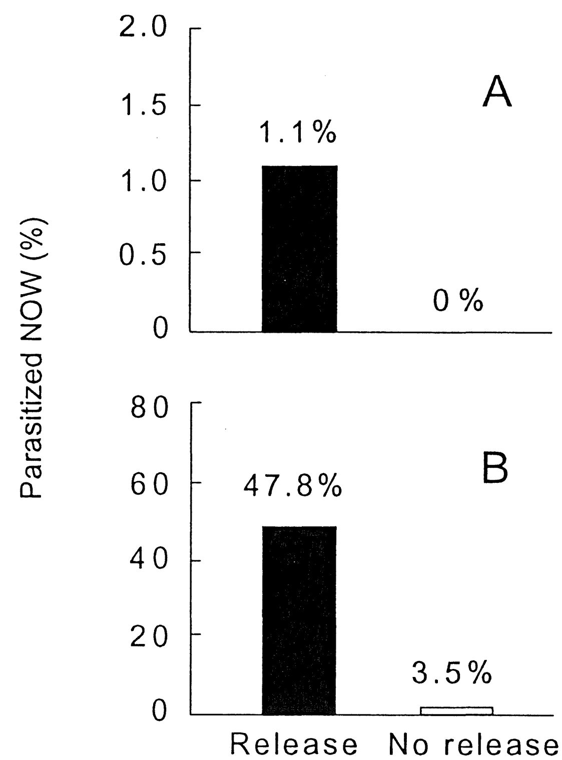

Figure 1. The percent NOW parasitism in paired orchard plots was higher in release orchards. Data were collected using 100 sentinel nuts per orchard.

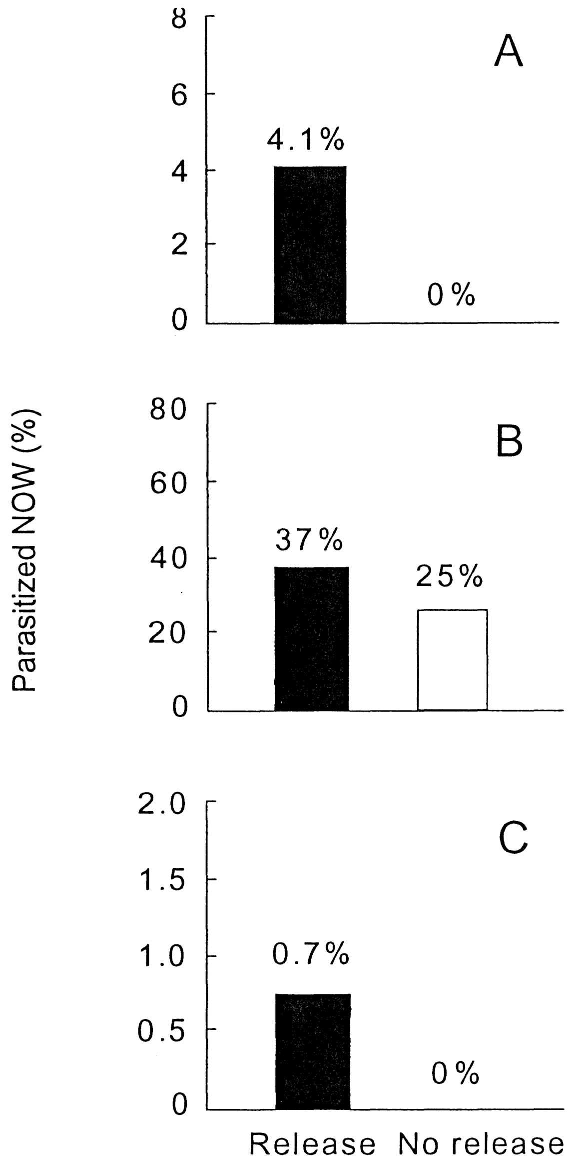

Figure 2. The percent NOW parasitism in replicated release and no-release plots was significantly higher in release plots. Data were collected using 100 sentinel nuts per orchard.

In the Arbuckle trial, both orchards had little natural *G. legneri* activity, low NOW densities (insecticides were used if needed), and winter sanitation. A small increase in percent parasitism was found (Figure 1A). In the Madera trial, the release orchard had a history of high NOW nut infestation rates, previous years with *G. legneri* release (ca. 1,000 parasites per acre per year), and incomplete winter sanitation; the paired no-release site was managed by the same grower but had distinctly lower natural levels of NOW nut infestation rates. There was a dramatic difference in percent parasitism between these blocks (Figure 1B), nevertheless, the release site had high nut infestation rates again at the 1995 harvest. In replicated plots, the variation between orchards was reduced considerably; thereby better comparing the affect of *G. legneri* release within orchards with either low and high natural levels of NOW or *G. legneri* (Figure 2). In each case, percent parasitism was increased in release orchards (significantly so in Figure A & B).

The high rates of parasitism in 1 of 2 non replicated trials and 1 of 3 replicated trials is encouraging, especially since the ineffectiveness of 2 trials could be attributed to mistakes in release methods used. In 2 of the release trials, the *G. legneri* material released was partially (ca. 80%) destroyed by foraging earwigs or ants; in each case, NOW parasitism was <2%. We attribute the field-mortality of *G. legneri* pupae to our use of white-capped pupae which lead to a >3 day period before adult *G. legneri* emerged.

B. NOW Density.

One discouraging aspect of the 2 "successful" field trials was an apparent correlation between high percent parasitism and high nut infestation levels. In other words, the percent parasitism was highest in fields that also had a high incidence of infested nuts (which probably led to higher numbers of *G. legneri*). However, the NOW density trial did not show the expected correlation between NOW density and percent parasitism (Figure 3). We believe the experimental methodology was to blame. The use of sentinel nuts to monitor a single *G. legneri* release does not take into account the natural increase of *G. legneri* throughout the season. More encouraging was the relatively high levels of parasitism found at each NOW density tested. This same field was used as the no-release site in the paired orchard study in June (Figure 1B), at which time only 3.5% parasitism was recorded and suggesting that with proper release methodology the *G. legneri* levels can be increased.

C. Overwintering *G. legneri* Studies

The overwintering experiments are based on these facts: overwintering almonds serve as a host for NOW (Caltagirone et al. 1968), orchard sanitation reduces NOW infestation rates (Zalom et al. 1984), and there is

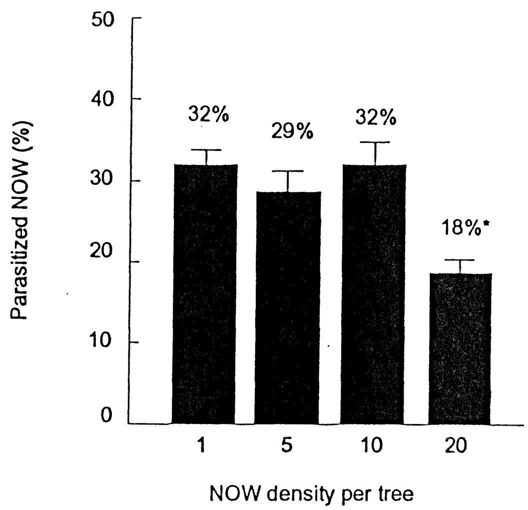

Figure 3. The percent NOW parasitism in plots with different NOW densities showed no difference at lower NOW densities and a significantly lower percent parasitism at the highest NOW density.

a positive correlation between the numbers of overwintering nuts and *G. legneri* (Legner & Warkentin 1988). Therefore, incomplete orchard sanitation will lead to higher numbers of NOW and *G. legneri*; however, the resulting levels of NOW percent parasitism or NOW nut infestation at harvest are not know.

To get background information, almond orchards were sampled by (1) the use of sentinel nuts to determine *G. legneri* activity and (2) the collection of almond mummies to determine levels of overwintering NOW infestation and *G. legneri* parasitism. Because some growers release *G. legneri* in October-November to knock down overwintering NOW populations, we were interested in whether or not adult *G. legneri* were active during this period. Results from sentinel nuts placed in orchards in November indicate there was little *G. legneri* adult activity during the colder months. Parasitism rates of NOW in sentinel nuts were 0, 0, 2.1, and 3.3% in 4 tested Madera County orchards (there were also 2 adult *G. legneri* found).

G. legneri adults were not expected to be very active during the winter months. Further, the sentinel nuts do not account for the number of *G. legneri* in the orchard. For this assessment collections of almond mummies on the ground (just after shaking for sanitation) or in the tree were made in February, March, and April. In sampled Merced and Madera County orchards, NOW nut infestation rates were high and parasitism rates by *G. legneri* were low (Table 1).

Table 1. Overwintering almond nut infestation by NOW and NOW parasitism by *G. legneri*

County / sample location	Nuts infested by NOW(%)	NOW per infested nut	NOW parasitized by *G. legneri*	Adult *G. legneri*	Nuts sampled
Madera/ground	32.7	1.4	0.6	3	458
Madera/ground	43.4	1.8	0.4	4	348
Madera/ground	27.6	1.2	0	0	423
Madera/ground	41.1	1.7	0	2	319
Madera/tree	19.2	1.7	0	0	104
Madera/tree	35.2	1.6	1.4	1	125
Madera/tree	30.9	2.2	1.2	0	123
Merced/ground	42.1	-	0.9	32	510
Merced/ground	20.5	1.7	0	0	170
Merced/ground	19.2	1.6	0	0	244
Merced/ground	18.9	1.8	0	0	179
Merced/tree	58.2	-	0	13	432
Merced/tree	15.7	1.5	4.1	5	204
Merced/tree	1.4	1.0	0	0	65
Merced/tree	16.7	1.3	3.1	2	292

In some of the sampled orchards, observations of NOW and *G. legneri* in October (post-harvest) indicated very high levels of NOW parasitism by *G. legneri*. The sharp decline in *G. legneri* suggests a poor overwintering survival of the parasite, which, unfortunately was not duplicated by NOW larvae. A number of possible explanations exists, including: (1) sampling error (we are making these observation on very few sampled nuts and sample locations and from only 1 winter season), (2) *G. legneri* larvae observed in September-October develop to the adult stage and the adults, while not actively attacking NOW, do survive but do not overwinter in the almond nut, and (3) some of the *G. legneri* development stages (e.g., first instars) can not survive winter conditions in the Central valley. The latter hypothesis would be supported by the native range of *G. legneri* and the low NOW parasitism rates in spring.

The overwintering trial in which the number of almond mummies are to be experimentally manipulated has begun. In 3 almond blocks (ca. 80 acres each) sections (ca. 10 acres each) were not winter sanitized. Average almond mummy counts in unsanitized sections of the 3 blocks were 165.9, 149.2, and 119.1 almond sticktights per tree; and average almond mummy counts in sanitized sections were 8.5, 9.8, and 28.8. While there is a considerable difference between unsanitized and sanitized sections, we had hoped for even lower nut counts in unsanitized sections. Trapping for adult NOW and *G. legneri* number in and moving out of these sections has begun.

Summary

Biological control has been a significant component of almond IPM research and resulted in the importation of *G. legneri* and *Copidosoma plethorica* (formerly *Pentalitomastix plethorica* Caltagirone) (Caltagirone et al. 1964, Legner & Silveira-Guido 1983). Legner & Warkentin (1988) present evidence that *G. legneri* responds in a density dependent manner to NOW numbers. Unfortunately, *G. legneri* can not naturally regulate NOW below acceptable damage levels and, for this reason, growers that are transitioning their farms away from in-season insecticide applications have been releasing *G. legneri* to improve biological control (CAFF 1995).

Nevertheless, there are no guidelines for *G. legneri* release that have been tested in replicated field experiments. To date, we have shown that poor release methodology can significantly reduce *G. legneri* effectiveness. We will work with the 3 major *G. legneri*-producing insectaries to develop standard release-guidelines. We also showed that *G. legneri* effectiveness varied greatly between trials - the exact reasons for this variation are not yet clear. Finally, very low overwinter NOW parasitism levels lead us to question the survival *G. legneri* during the colder months. Future experiments will more closely follow (1) regional differences in *G. legneri* activity, release methodology, *G. legneri* biology (as it relates to release effectiveness), and overwintering *G. legneri* survival.

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