SUMMARY OF ALMOND RESEARCH 1996 and 1998

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Title: Augmentative Release of *Goniozus legneri* to Control Navel Orangeworn in Almonds

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Abstract

The release of a parasitic wasp, *Goniozus legneri* Gordh, for control of the navel orangeworm (NOW), *Amyelois transitella* (Walker), was evaluated. In 1996, research investigated (i) the effectiveness of commercial release programs in both NOW percentage parasitism and nut infestation, (ii) how well standard release practices place viable G. legneri into the orchard, and (iii) the effect of orchard sanitation of overwintering nuts on the density of *G. legneri* and NOW. In 1997 (funded by UC Statewide IPM Project), we focused efforts on *G. legneri* release methods and parasitoid biology, especially during the overwintering period, to determine methods to consistently produce higher parasitism rates (e.g., >30%). In 1998, research was concluded with studies to (i) establish release guidelines that place viable *G. legneri* in the orchard at the most effective time and (ii) better understand *G. legneri* movement within the orchard, and (iii) determine *G. legneri* biology with respect to reproduction and overwintering survival. Highlights of this work are as follows:

Commercial release operations had variable results, from 0-45% parasitism. Release methods, orchard management practices, NOW densities, and seasonal variation are suspected factors causing this variation in *G. legneri* effectiveness. *G. legneri* released as pupae in gelatin capsules (the standard methodology) had >80% mortality, mostly resulting from foraging activity of ants (*Formica aerata*, *Solenopsis xyloni*, and *Solenopsis* sp.). We recommend adult *G. legneri* releases. Adult *G. legneri* were marked with "mouse IgG" to follow their movement throughout the orchard. Results showed *G. legneri* dispersed from the release site throughout the tree canopy and to distances up to 12 trees from the release site (although there was a reduction in percentage parasitism at distances >10 trees from the release site). We conclude that *G. legneri* can be released at only 2-3 locations per acre, thereby, reducing labor costs. Important work was completed on the effect of incomplete winter sanitation of "nut mummies" on NOW and *G. legneri* numbers. Results show that almond "stick-tights" provide refuge for NOW but do not consistently increase percentage parasitism by *G. legneri*. Laboratory studies support these conclusions. *G. legneri* adults do not oviposit at temperatures <65°F, suggesting post-harvest releases have little effect. Further, *G. legneri* larvae did not ambient winter temperatures in Fresno County (1997-1998). Together, laboratory and field data suggest that parasitoid numbers, especially immature stages, will drop dramatically during the Central Valley winters. We strongly recommend sanitation be part of all control programs.

Introduction

The almond IPM program for navel orangeworm (NOW), *Amyelois transitella* (Walker), 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 and Barnes 1983, Zalom et al. 1984). If needed, NOW can be controlled by applying an in-season insecticide spray, typically used in May or July (Barnes and 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 $<3\%$ NOW nut infestation levels.

Still, almond growers lose millions of dollars to NOW each season. In orchards with unusually high pest pressure, nut losses $>3\%$ are not uncommon, even with insecticide applications. Many growers have been experimenting with different control strategies, which is especially timely given the potential loss of some of the standard chemical treatments through FQPA restrictions. One alternative is the release of a bethylid parasitoid, *Goniozus legneri* Gordh, which attacks, develops upon, and kills NOW larvae. In the 1970s and 1980s, the California Almond Board supported work to import NOW natural enemies for biological control. About 15 NOW parasitoid species were imported into California, 2 of these species, *G. legneri* and *Copidosoma plethorica* (formerly *Pentalitomastix plethorica* Caltagirone), were established and have become important in the natural regulation of NOW (Caltagirone et al. 1964, Legner and Silveira-Guido 1983). Gordh (1982) and Gordh et al. (1983) provided a great deal of information on *G. legneri* biology. 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, some growers purchase insectary produced parasitoids to release in their orchards and imrove biological control.

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 BIOS growers are using *G. legneri* releases as a part of their IPM program. Moreover, the almond industry is as close as any agricultural commodity to completing a "least-toxic" or "sustainable" insect control program. *G. legneri* releases, in lieu of insecticides, complement previously developed IPM programs for mites (Hoy et al. 1978) and peach twig borer (PTB), *Anarsia lineatella* (Zeller) (Barnett et al. 1993). Augmentation is a viable alternative to insecticides and in this past decade there has been an increase in the use of insectary-reared natural enemies for the control of insect pests. Nevertheless, there are no scientifically documented guidelines for the release of *G. legneri* in almond orchards. These guidelines are needed both to ensure that the releases do indeed work and to improve upon existing release methods. Therefore, successful augmentation programs need research that clearly outlines target pest and natural enemy biology; an understanding of biotic and abiotic interactions at the release site; and guidelines for effective release rates, timing, and methods. Here, we present a final report on different aspects of *G. legneri* research, which included: field effectiveness, release methodology, *G. legneri* movement, overwintering sanitation, *G. legneri* biology.

Procedures

Release Effectiveness. A "paired-orchard" evaluation of commercial *G. legneri* releases was made in 1996 in 3 almond growing regions: Colusa County (northern region), Merced County (central region), and Kern County (southern region). Six commercial orchards (3 pairs) release were used in each region. Orchards pairs were most often two nearby blocks of the same age and variety and managed by the same grower. In each pair, one orchard received *G. legneri* using commercial methods and the other was a no-release control (treatments selected randomly). No insecticidal sprays were planned during the release season, although most of the orchards had received a dormant oil and OP application. To monitor parasitoid levels, sentinel nuts were placed in the center 5-acre sections of each plot (100 nuts, 25 trees). To produce sentinel nuts, NOW were reared in the insectary using methods described by Finney & Brinkman (1967). Prior to field tests, 1st and 2nd instar NOW were collected and placed in unshelled almond nuts. The inoculated nuts were stored at 23-25°C until the NOW developed to the 3rd – 4th stage. Just prior to field use, the sentinel nuts were placed individually in wire cages (5 cm × 5 cm) that could be stapled to the almond tree. Additionally, 1,000 nuts were collected at harvest and dissected in the laboratory, with the number of NOW and parasitoids recorded for each orchard. These same procedures were used to monitor 4 pairs of BIOS orchards in 1997 in Madera and Merced counties.

Replicated evaluations of *G. legneri* releases were made in large commercial orchards (100 acre blocks, S&J Ranch and Hopton Ranch, Madera County) in 1996 and 1997. *G. legneri*-release and no-release treatments were set in a randomized block design (split-plot with four 25-acre blocks). In the *G. legneri*-release plots, parasitoids were released as adults, NOT PUPAE, at 700 *G. legneri* per acre. To monitor parasitoid levels, sentinel nuts were placed in the center 5-acre sections of each plot (100 nuts, 25 trees). After ~20 days, the sentinel nuts were removed and the percent parasitism was recorded. Also, at harvest, 500 nuts were collected from each center section and the number of NOW and parasitoids were recorded.

Release Methodology. An essential part of an augmentation program is the release method. At issue is 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 have shown that release methodology effects natural enemy survival (Daane and Yokota 1997) and that resident predators can cause significant mortality of released beneficial insects (Dreistadt et al. 1986, Rosenheim and Wilhoit 1993). An informal survey of PCAs and orchard managers found that *G. legneri* were released in many different ways (e.g. as pupae or adults, in gelatin capsules or in Dixie cups). Typically, ~1,000 *G. legneri* are released per acre, spread over 3 or 4 release periods: 200–300 in March-April, 300–500 in May-June, 200–300 in July-August, and 300–500 in September-October. In most programs, 7–12 *G. legneri* pupae are enclosed in a single gelatin capsule (~1 inch in length), these capsules are opened and placed in tree crotches throughout the orchard.

Release method. Tests were conducted to determine the survival of *G. legneri* released as pupae in gelatin capsules. Commercially produced *G. legneri* were divided into two categories: 1–3 day old pupae, which will take 3 or more days before adults emerge and are

hereafter referred to as "white-capped," and 5–6 day old pupae, which will produce adults in 1–2 days and are hereafter referred to as "black-capped." Capsules each of white-capped and black-capped pupae were placed individually in the lower scaffolding of 20 randomly selected almond trees (from a block of 100 trees). During the next 5 days, the condition of the pupae and the presence of foraging predators were recorded. Of primary interest was predation by ant and earwig species foraging on the almond trunk. These experiments were conducted in June 1996 and July and August 1997 at the Kearney Agricultural Center.

Parasitoid Movement. After a release, within and between-tree movement of adult *G. legneri* will determine where *G. legneri* must be released to provide even coverage. To study within-tree movement, sentinel nuts were placed in individual almond trees at lower and upper canopy heights in each cardinal direction (4 nuts at each position). Ten or 50 (depending on the trial) *G. legneri* females were released at the center-base of the scaffolding. After ~18 days, the sentinel nuts were removed and dissected in the laboratory, recording NOW and *G. legneri* presence and percentage parasitism. The study was conducted in a 2 acre almond block at the Kearney Agricultural Center. There were 7 replicates (trees) for each of 4 trials, conducted on 26 March, 11 April, 24 May and 3 July 1997.

To study between-tree movement, a 102 acre commercial orchard (Madera County) was divided into four 25-acre blocks (each block: 27 rows × 60 trees). A center release point was established in the middle of each block and sentinel nuts and 4 sentinel nuts were placed on every row and on every other tree from that point to monitor 8 rows and 14 trees away from the release point. *G. legneri* females were released at a center point in each of 4 replicates. Additionally, at harvest, ~150 nuts were collected from trees at 1-10, 11-20, 21-30, 31-40, and 41-50 trees away from each release points. The nut damage and number of NOW and parasitoids were recorded.

An important consideration was the background level of parasitism in the orchard, "Were *G. legneri* recovered from the release or from a resident population?". In 1998, we tested methods to mark released parasitoids. For short-term marking, we tested colored paints and florescent dyes (1997) and mouse immunoglobulin (IgG) solution (1998). The IgG was applied externally (sprayed) or internally (honey) with a solution strength of 1.0 mg/ml mouse IgG with 25 ml water. Individuals were assayed with a direct enzyme-linked immunosorbent assay (ELISA). For long-term marking (e.g. overwintering), we tested enriched ^{15}N food (1998-99). Green beans were grown in a 0.35 M, ^{15}N -enriched potassium nitrate (KNO_3 – $^{15}NO_3$) fertilized soil, harvested, dried, and mixed with the NOW artificial diet. NOW were reared on the diet and then exposed to *G. legneri*. The labeled NOW and F_1 and F_2 *G. legneri* progeny were dried and ground to a powder and then analyzed using a Tracermass ANCA-MS (Automated $^{15}N^{13}C$ Analyzer-Mass Spectrometry).

Release rates. Release rates were studied in 1996 and 1998. In 1996, a 100-acre commercial orchard (Hopton Ranch, Madera County) was divided into 5 blocks (each block, 27 rows × 60 trees) and each block was divided into 5 plots. The center 25 trees (5 rows × 5 trees) in each plot were assigned 1 of 5 treatments (using a randomized block design): (1) a no-release control and *G. legneri* release at rates equivalent to (2) 100, (3) 500, (4) 1,000, and (5) 2,000 *G. legneri* per acre. Parasitoids were released as pupae in gelatin capsules placed in

individual trees (using the standard release practice). To monitor *G. legneri* effectiveness, 4 sentinel nuts were placed in each tree in each plot (100 sentinel nuts per plot). After 21 days, the sentinel nuts were removed and percentage parasitism determined.

The proper release rate is a function of both number of natural enemies placed in the field and the pest density (more pests will require more natural enemies for control). In earlier studies, researchers observed that high densities of *G. legneri* and high levels of NOW parasitism rates were found in orchards that had high NOW nut infestation rates (L. E. Caltagirone, personal communication). For this reason, the effect of varying NOW densities on percentage parasitism was tested. A large orchard (Madera County, 1997) was divided into 5 blocks (5 rows × 5 trees), each block was divided into 4 plots with the center 9 trees (3 trees × 3 rows) constituting each plot. Using a randomized block design, 4 different NOW treatment densities were established in each block: 1, 5, 10, and 20 sentinel nuts per tree. *G. legneri* were 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.

Overwintering Sanitation. A more controversial practice is to retain overwintered almond mummies to increase the numbers of *G. legneri* (CAFF 1995) Almond mummies, or "stick-tights," serve as the only winter host site for NOW (Caltagirone et al. 1968) and have been suggested as be the primary overwintering site for *G. legneri* (Legner and Warkentin 1988). Earlier work showed that orchard sanitation reduces NOW infestation rates (Zalom et al. 1984) and this has been incorporated into standard almond management practices. However, it has been suggested that because there is a positive correlation between the numbers of overwintering nuts and the numbers of *G. legneri* – it may follow that leaving overwintering nuts on the trees might lead to higher numbers of *G. legneri* in the spring which would, in turn, reduce NOW throughout the season. A series of experiments were conducted to determine the effect of winter sanitation or the lack thereof on the densities of *G. legneri* and NOW, percentage parasitism of NOW, and infested nuts at harvest.

For background information, 100-500 nuts were collected throughout the overwintering period, from different almond orchards, and the number of NOW and *G. legneri* were recorded. For a more scientific evaluation, we undertook an ambitious project with the cooperation of S&J Ranch. In three 80-acre blocks, a single 8-acre section was left unsanitized (i.e., all overwintering nuts were left on all trees). The average number of mummies per tree was determined. Population 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 was 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. NOW and *G. legneri* populations were monitored in trees that at 1-10, 11-20, 21-30, and 31-40, 41-50, and 51-60 rows away from the unsanitized section.

In winter 1997/98, NOW and *G. legneri* were placed in almond nuts, protected from rain in a weather-station shelter, and held in ambient temperatures until March 1998. Replicates of different development stages were tested for both NOW (3rd, 4th, and 5th instars)

and G. legneri (eggs, 2nd – 3rd instars, pupae and adults). Condition (alive or dead) and development were checked every 2 weeks. In 1998-99 (work is ongoing), laboratory studies of *G. legneri* development and biology were studied at different temperatures to determine the potential overwintering survival of *G. legneri*. The laboratory data will also be used to model *G. legneri* population development, determine the best NOW stage for *G. legneri*, and help to establish release rates.

Results and Discussion

Release effectiveness. In Colusa, Merced and Kern counties, there was a trend of higher percentage NOW parasitism in sentinel nuts and harvested nuts (Fig. 1); however, parasitism levels were only different between release and non-release pairs in Colusa County. The level of parasitoid activity varied greatly, from 0% (Kern county) to 38% (Merced County). The level of NOW infestation in harvested nuts also varied greatly (0-24%) (Fig. 2). We believe that sentinel nuts can detect parasitoid activity and that the low percent parasitism recorded in some fields is representative of parasitoid activity in these orchards (probably due to poor release methodology and orchard cultural practices). The variability in NOW numbers and *G. legneri* activity between orchards regions brought to question the following: (1) the effectiveness of paired-orchard comparisons, (2) commercial release methods, (2) prior insecticide use on *G. legneri* (all orchards had dormant insecticide sprays), and the importance of repeated *G. legneri* release.

In replicated studies (Madera County 1997) we found a non-significant trend of higher parasitism in release than non-release plots in sentinel nuts ($18.2 \pm 4.4\%$ vs. $12.3 \pm 6.1\%$, respectively) and at harvest ($35.7 \pm 5.4\%$ vs. $26.3 \pm 7.7\%$, respectively). Data from harvested nuts show a very low infestation level in both the release and non-release plots ($1.4\% \pm 0.5$ vs. $2.3 \pm 0.8\%$, respectively).

Release Methodology. Release Methods. Results were consistent for each trial (1996 and 1997). Earwigs and ants (*Formica aerata, Solenopsis xyloni* and *Solenopsis sp.*) quickly found the capsules and killed both "white-capped" (3-4 days until emergence) and "black-capped" (1 day until emergence) pupae (Fig. 3). In the experimental orchard, ~20% of the released white-capped pupae survived.

At this time, we suggest that insectary material should 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 none of the pupae were damaged.

Parasitoid Movement. Results show adult *G. legneri* moved throughout the tree, with the number of *G. legneri* per sentinel nut similar in all cardinal directions and low and high canopy positions (Fig. 4). This is important because some almond trees reach 20–30 ft heights, yet parasitoids are released at ground level. Low parasitism rates (1996 and 1997 trials) suggest an influence of dormant sprays (see IB).

Both 1997 and 1998 results indicate that released *G. legneri* spread up to 10 trees or 5 rows from the release point (Fig. 5). The 1997 results showed an effect of direction with significantly greater percentage parasitism north ($17.0 \pm 3.6\%$) of the release point than south ($6.2 \pm 2.0\%$) (Fig. 5A). We believe this difference was the result of resident *G. legneri* from an almond block north of the tested block. The experiment was repeated (1998) with parasitoids marked with mouse IgG (see below) to separate resident from released parasitoids. The 1998 trial again showed good movement, with 42% of recovered *G. legneri* marked (indicated by ELISA). Percentage parasitism was very low, probably because sentinel nuts were pulled down so quickly (IgG marker lasted <8 days) (Fig. 5B,C).

Marking adult *G. legneri* with paint was not effective because many *G. legneri* were not marked and others were impeded by too much paint. Similarly, florescent dyes had inconsistent results with a $<10\%$ of *G. legneri* still marked after 3 days and none >5 days. Mouse IgG marked *G. legneri* evenly across the population and lasted for ~10 days. Best results were obtained when IgG was mixed with honey and parasitoids fed and walked on the marker (Fig. 6). Long-term marking with ^{15}N produced excellent results, with F_1 marked throughout their lifetime; however, the F_2 progeny was not marked.

From these experiments, we conclude that *G. legneri* disperses from the release site and therefore can be released as adults at only 2–4 sites per acre, thereby reducing costs of placing parasitoids in the field.

Release Rates. There was higher percent parasitism at the higher release rates (Fig. 7). We believe that low percentage parasitism levels recorded were due to use of pupae in gelatin capsules (1996) and the short duration that sentinel nuts were left in the field (1998). This work did produce a definitive set of conclusions. While a density effect was observed, percentage parasitism was extremely low in both studies. The results may best suggest that single *G. legneri* releases, regardless of the release rate, can not be used like insecticide applications to lower NOW densities. Rather, a program of good IPM practices that includes annual releases of *G. legneri* may help sustain parasitoid populations and suppress NOW densities.

Overwintering Sanitation. We did not expect *G. legneri* adults to be very active during the winter months and the data confirms these suspicions. For example, almond mummies collected on the ground (just after shaking for sanitation) or in the tree were made in showed NOW nut infestation rates were high and parasitism rates by *G. legneri* were low during the winter period (Table 1). 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*.

Results from the "unsanitized" experiment show the number of NOW overwintering in mummies was quite high, while the number *G. legneri* was low. Results from 1 of 3 blocks

show that at 50 and 60 rows from the unsanitized sections there was a drop in NOW eggs (Fig. 8A), no change in percentage parasitism (Fig. 8B), and a drop in damaged nuts (Fig. 8C). We believe that the great distance the NOW adult can fly, combined with high levels of overwintered mummies in the "sanitized portion" resulted in NOW movement far out from the unsanitized portion.

Laboratory experiments with *G. legneri* show a strong preference for larger NOW. While eggs are laid on later instar NOW (Fig. 9), *G. legneri* will oviposit on 3rd instar NOW. Results imply that release should begin as soon as 3rd instar adults are available. This is, in part, due to the long life span of the parasitoid (Fig. 10.) and because more of the smaller stage NOW are killed (Fig. 10). Overall egg production and NOW mortality implied ~20 NOW killed per *G. legneri* and nearly a 100 fold increase in parasitoid numbers (Figs. 10 & 11). These results have never been seen in field studies, probably because of poor searching ability or increased parasitoid mortality in the field.

All overwintering experiments (1997 and 1998) indicate a considerable drop in parasitoid numbers during the winter and that only pupae and adult *G. legneri* survive (Figs. 13). In the open and enclosed (e.g. sentinel nuts) parasitized NOW, all egg and larvae died and most pupae died. Most important was survival of most adult *G. legneri* and that when these adults were brought back into the laboratory (and warmer temperature), after a 2 to 3 month overwintering period, about 60% of the specimens oviposited. Temperature cabinet work is ongoing, initial results show adult *G. legneri* survive at sustained temperatures >45°F but will not parasitize nearby NOW until temperature are >52°F (Fig. 13).

From this work, we conclude that while overwintered nut may indeed increase the density of *G. legneri* in the orchard, it also favors the increase of NOW in the orchard. Both laboratory and field studies show that NOW survives overwintering temperatures easily, while *G. legneri* eggs and larvae do not. This information suggests that all parasitoids that have not developed to the adult stage by November will most likely not survive through the winter. It also implies that post-harvest releases may not increase *G. legneri* numbers the following season if the off-spring of released adults do not develop through a complete generation. Combined, the results strongly suggest overwintering sanitation practices be continued as part of an overall IPM program for NOW. Releases of G. legneri in the spring and summer can be use to reduce the numbers of NOW in sticktights missed by sanitation.

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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

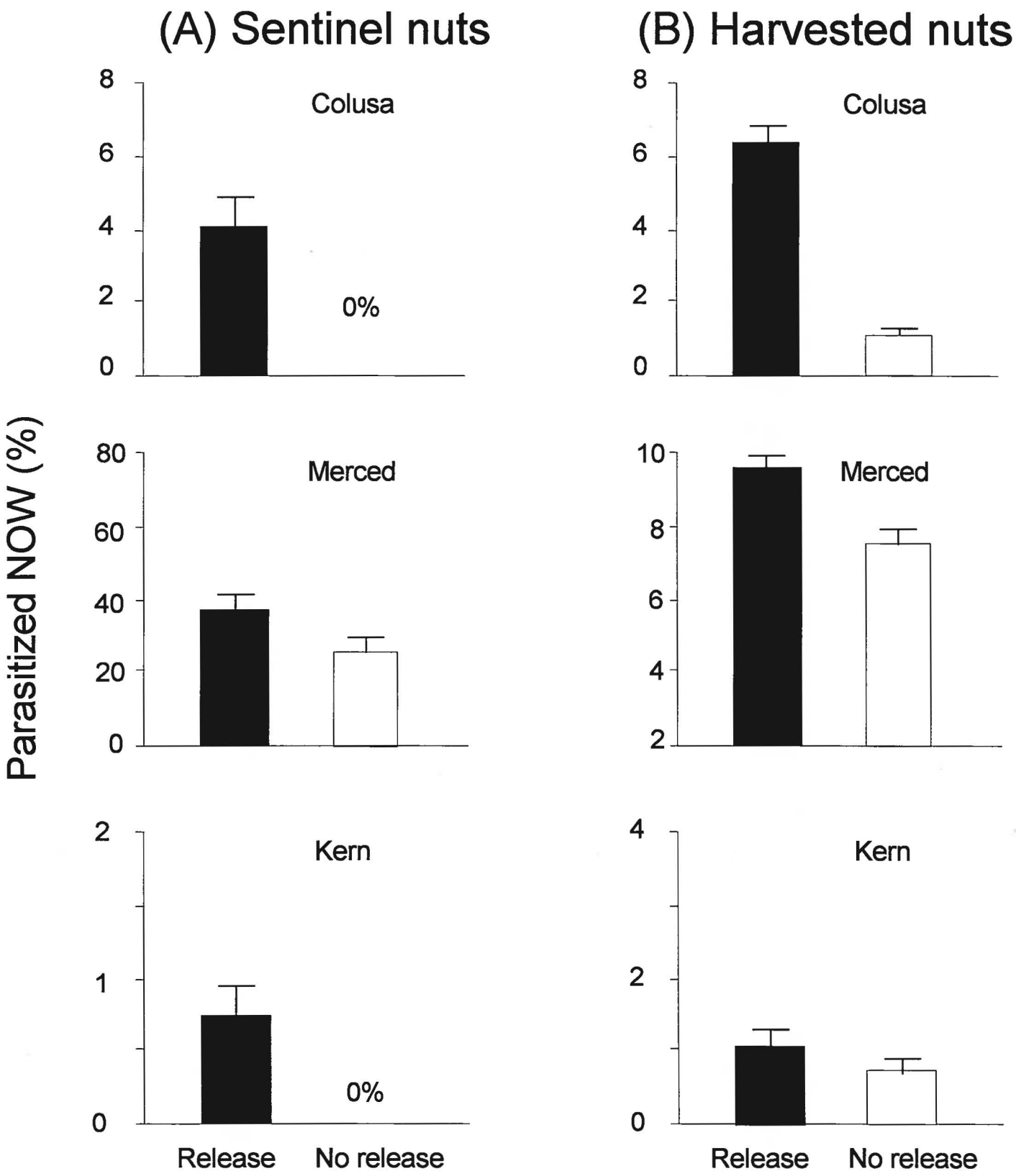

Fig. 1. Paired orchard release studies in 1996 in Colusa, Merced, and Kern counties (average) show a trend of greater percentage NOW parasitism in (A) sentinel nuts in June/ July samples and (B) harvested nuts. Nevertheless, there was between orchard variation in NOW parasitism (in the same region) and damage at harvest was high in block 3 (>10%) in both release and non-release sites.

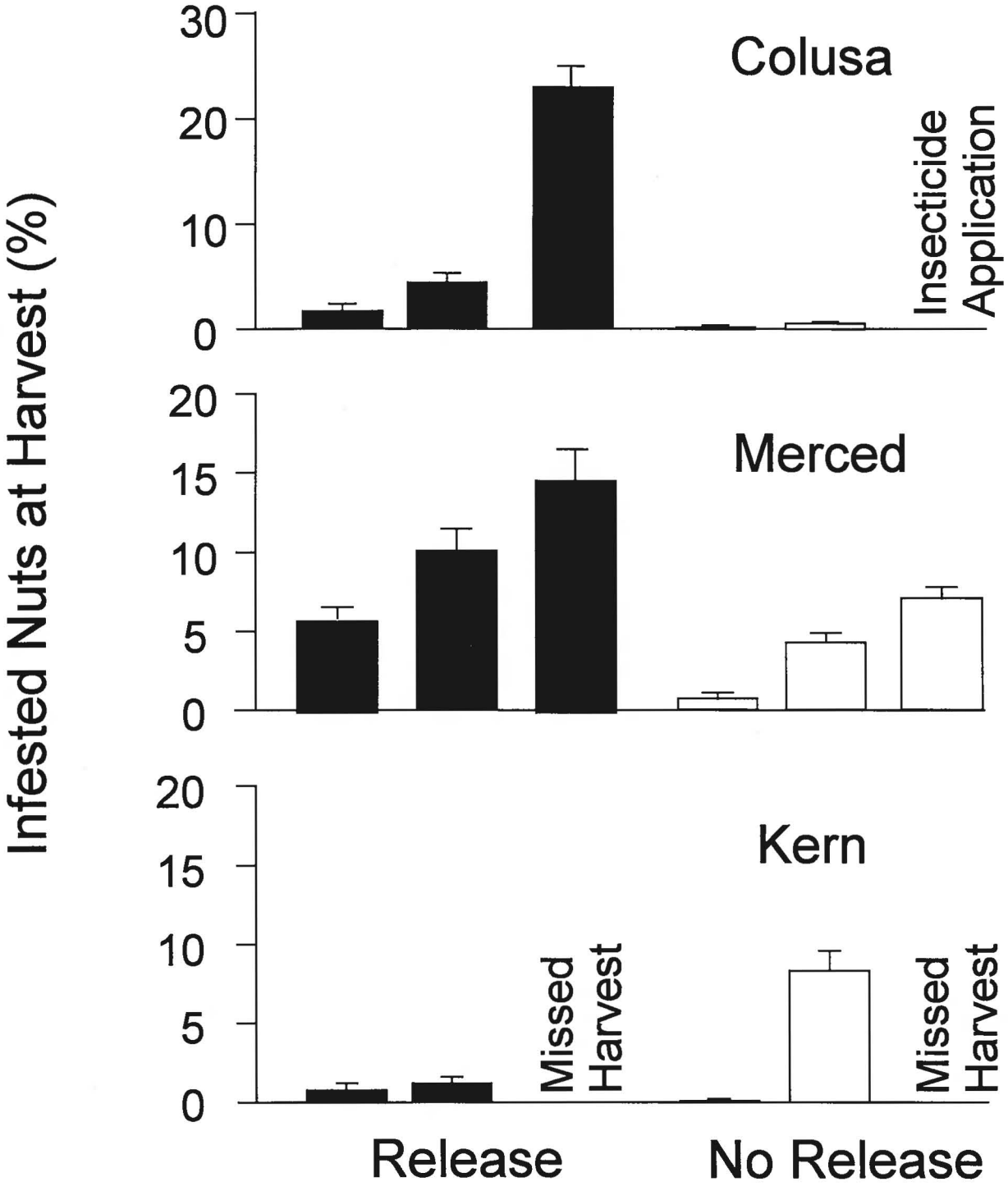

Fig. 2. Paired orchard studies in 1996 in Colusa, Merced and Kern counties (individual orchards) show a trend of greater NOW damage in harvested nuts. There was great between region and between orchard variation in NOW damage at harvest. SEMs were derived by splitting 1,000 harvested nuts into 100 nut blocks within each orchard.

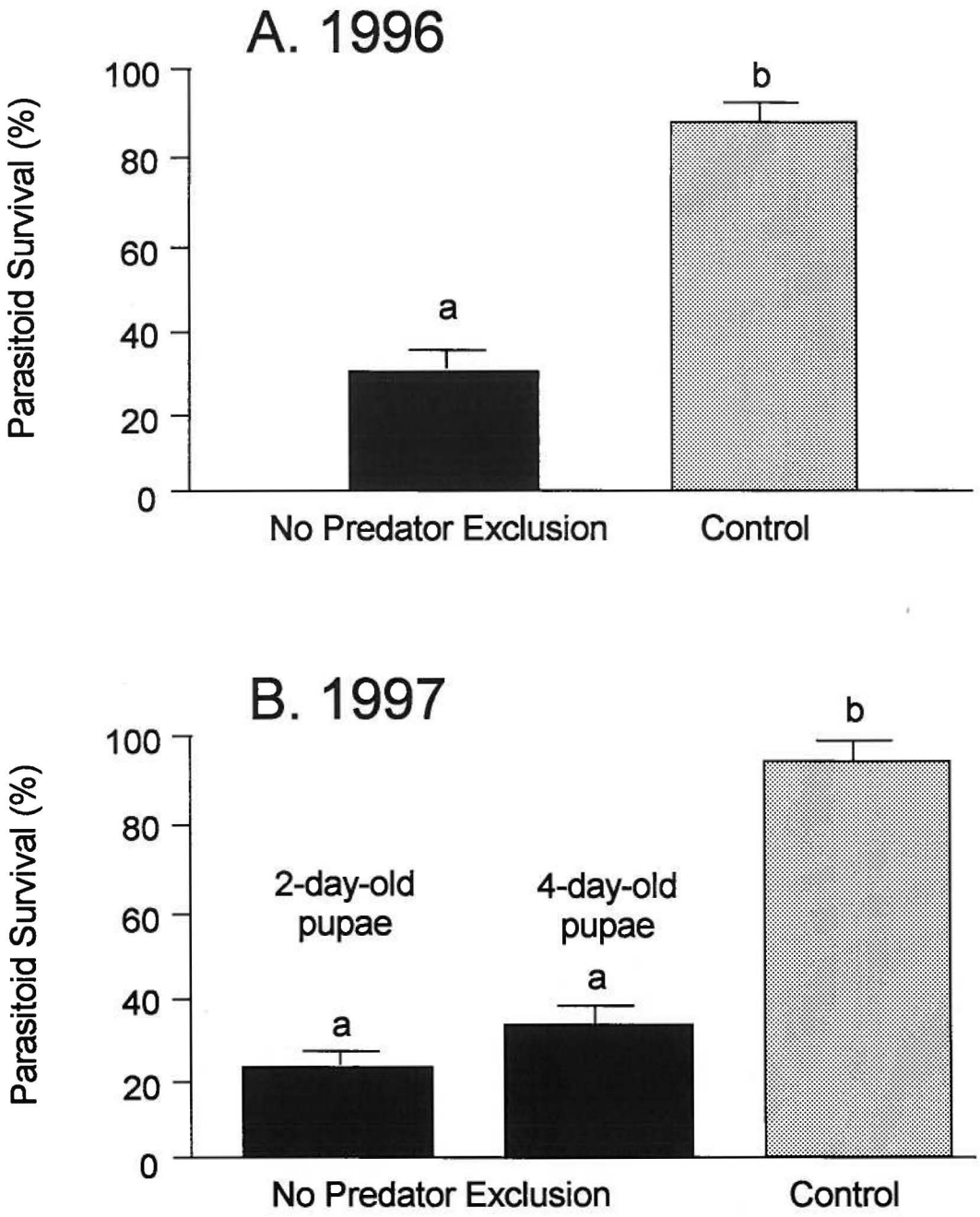

Fig. 3. *G. legneri* released as pupae in gelatin capsules resulted in significant mortality in (A) 1996 and (B) 1997 trials. In 1997, pupae were separated into two groups, depending on develoment. Results show even well developed pupae (and adults) were killed by foraging ants.

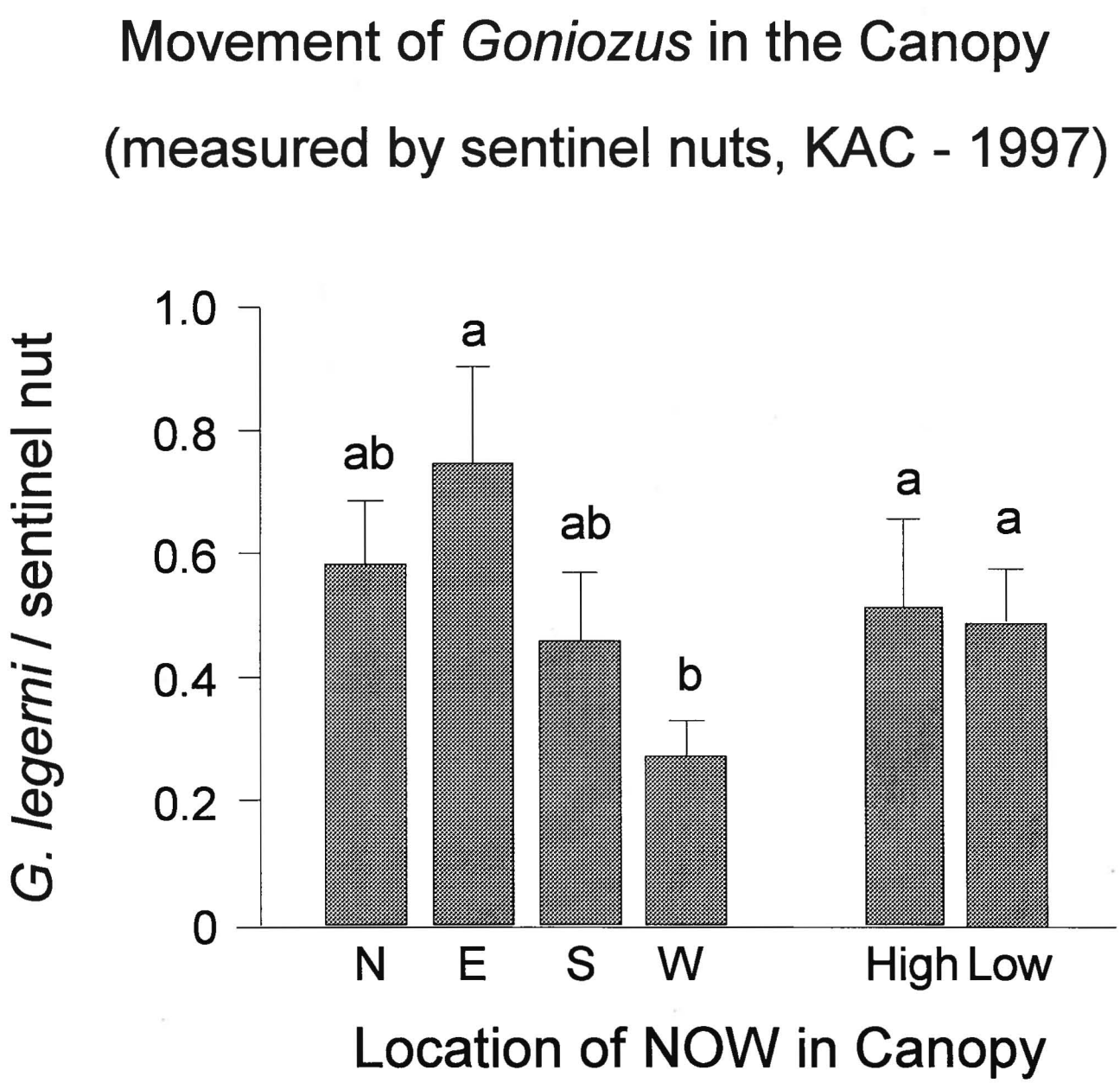

Fig. 4. *G. legneri* adults moved throughout the canopy. Data presented are from 1997 trials. Studies conducted in 1996 showed very low percentage parasitism (<2%). Results indicate that parasites released at ground level will move to the upper canopy. Different letters abve each mean (direction and high vs. low positions) indicate a significant difference, $P<0.05$, Tukeys HSD comparison.

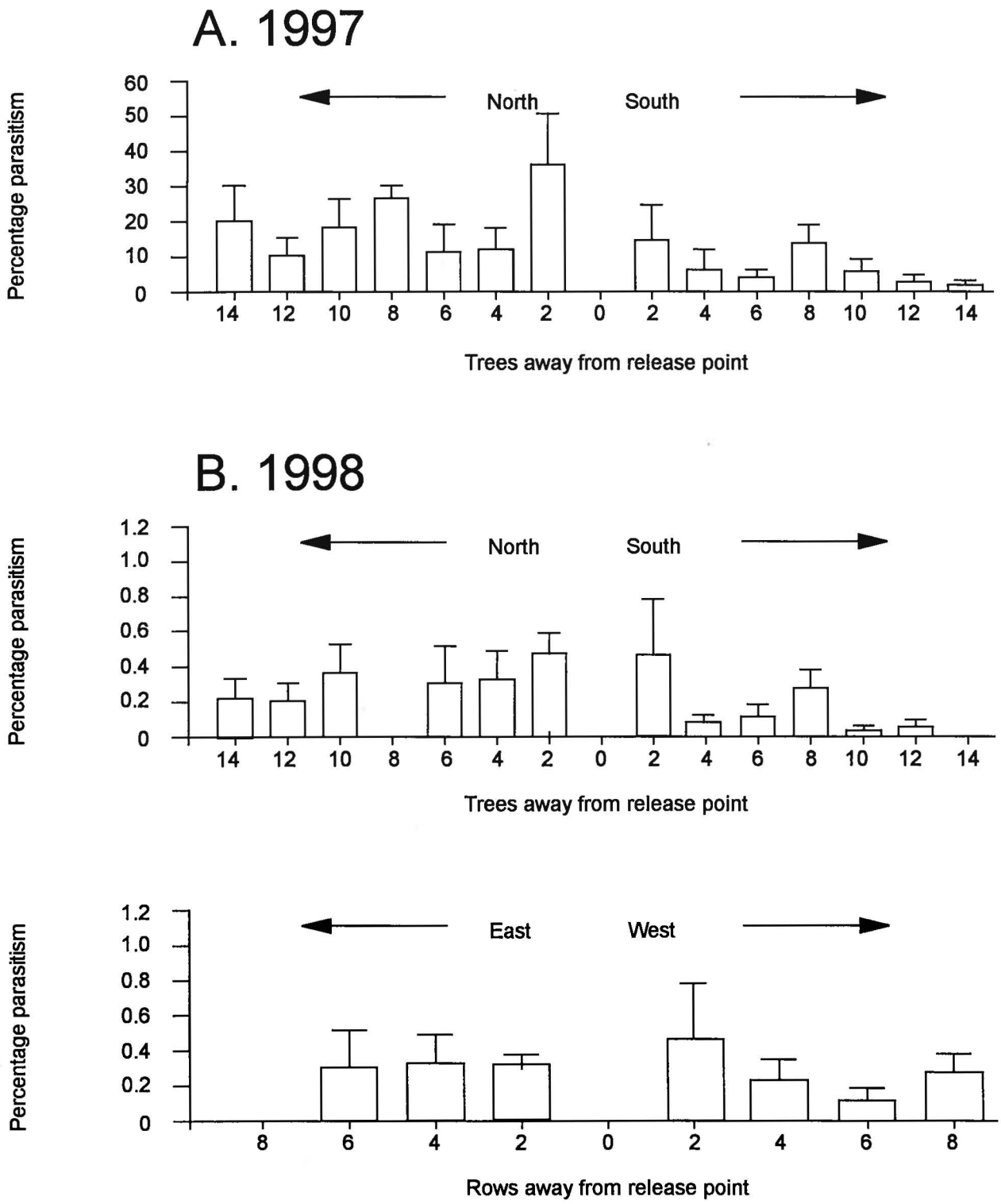

Fig. 5. *G. legneri* adults moved throughout the orchard, from a center release point, as measured by sentinel nuts (harvest data not shown). (A) 1997 data show more parasite activity north of the release point, probably resulting from parasite movement from a neighboring orchard. (B) 1998 data show similar parasite activity in all directions, these parasitoids were marked with a mouse IgG which showed positive at all collection points.

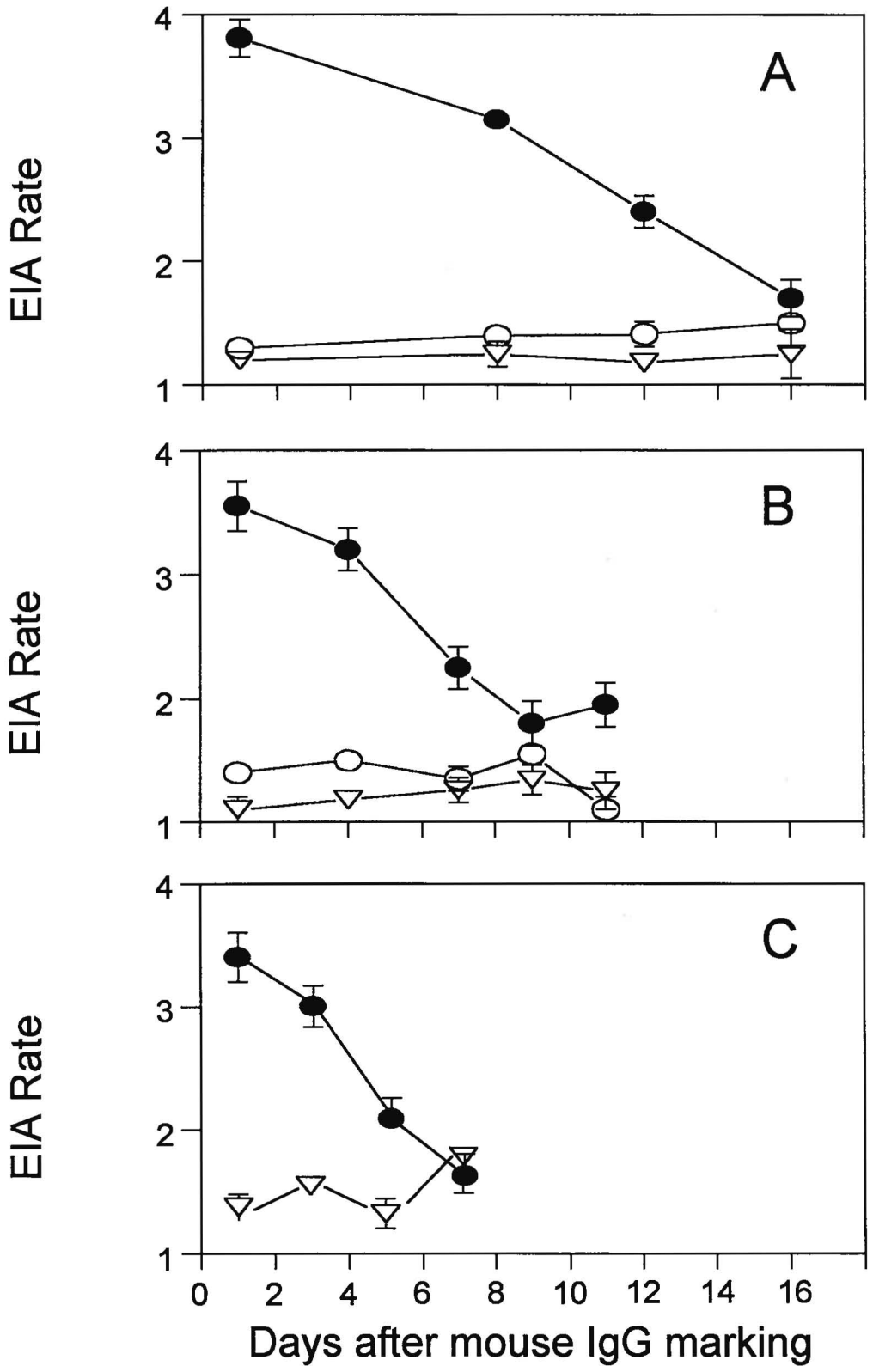

Fig. 6. *G. legneri* adults were marked with mouse IgG and then analyzed using ELISA until marked and control parasitoids showed similar EIA levels. Trial 1 (A) and 2 (B) show good retention of IgG mixed with honey (●) compared with no IgG honey (○) and no IgG (▽) controls. Trial 3 (C) tested the same mouse IgG sprayed externally on parasites (●) vs control (▽), but retention was not as good as when miced w/ honey.

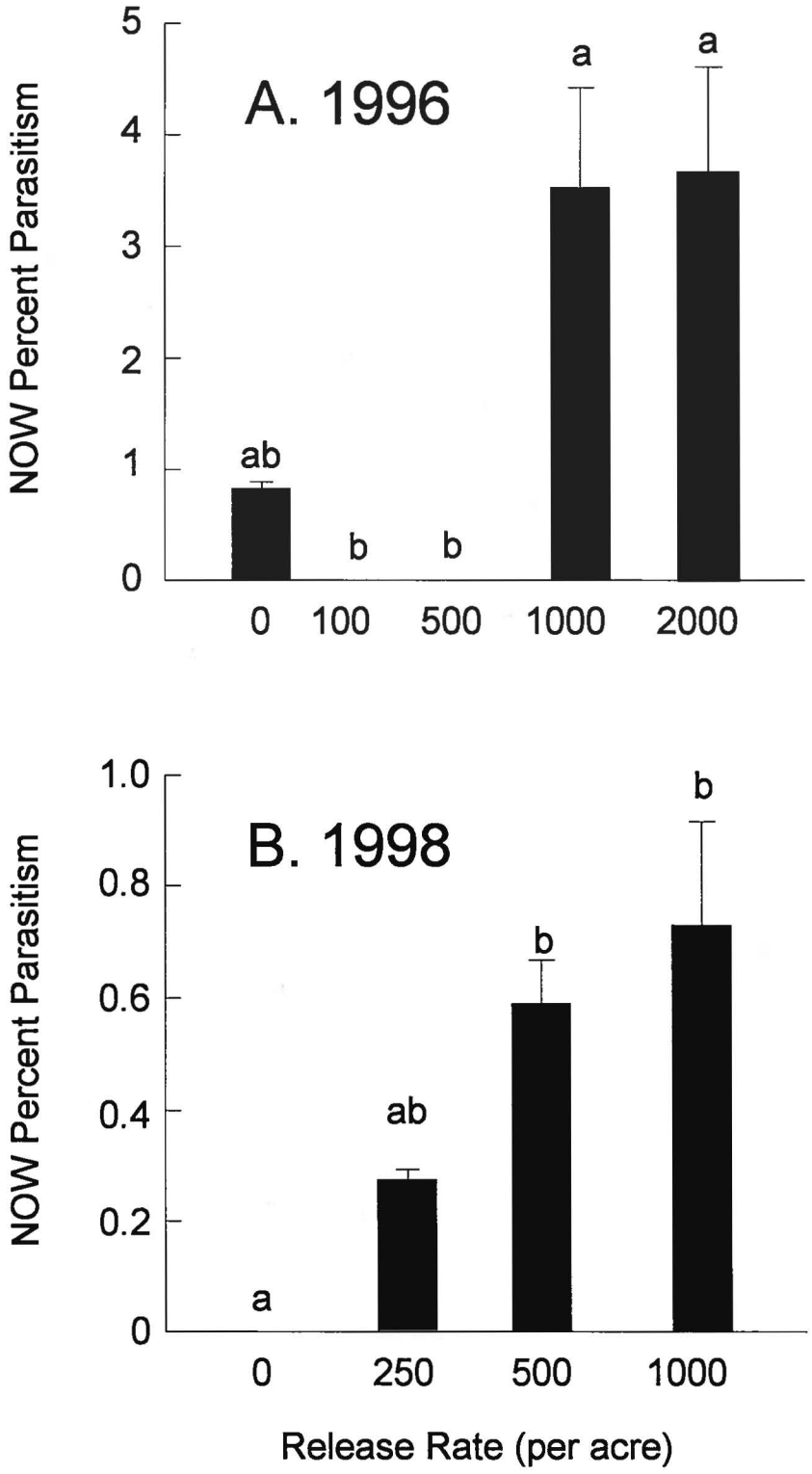

Fig. 7. The release rate of *G. legneri* affected the percentage parasitism in sentinel nuts. In 1996 (A) parasites were released in gelatin capsules and the low parasitism (%) is attributed to high parasite mortality inside the capsules. In 1998 (B) the release rate trial was part of the mark/release trial and sentinel nuts were placed out for only 7 days, resulting in low parasitism (%). In each graph, different letters abve each mean indicate a significant difference, $P<0.05$, Tukeys HSD comparison.

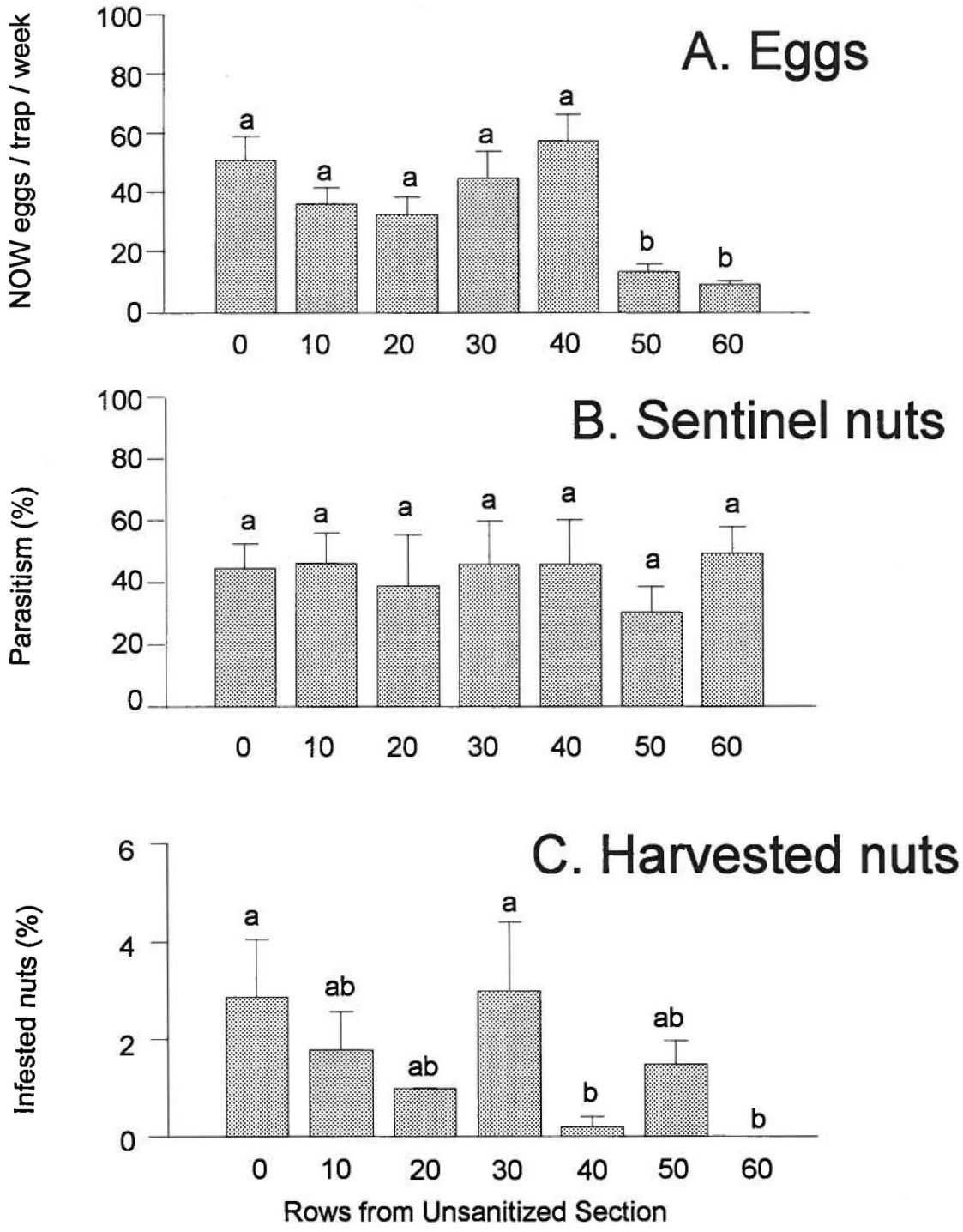

Fig. 8. In 2 of 3 blocks testing overwintering sanitation, there was little difference between treatments. In those blocks transects ran only 40 rows from unsanitized sections. In 3, there were significantly differences at rows 50 and 60 from unsanitized sections. There were fewer NOW eggs (A), while no difference in parasite numbers (B) and more damage (C) at rows 50 and 60. In each graph, different letters abve each mean indicate a significant difference, $P<0.05$, Tukeys HSD comparison.

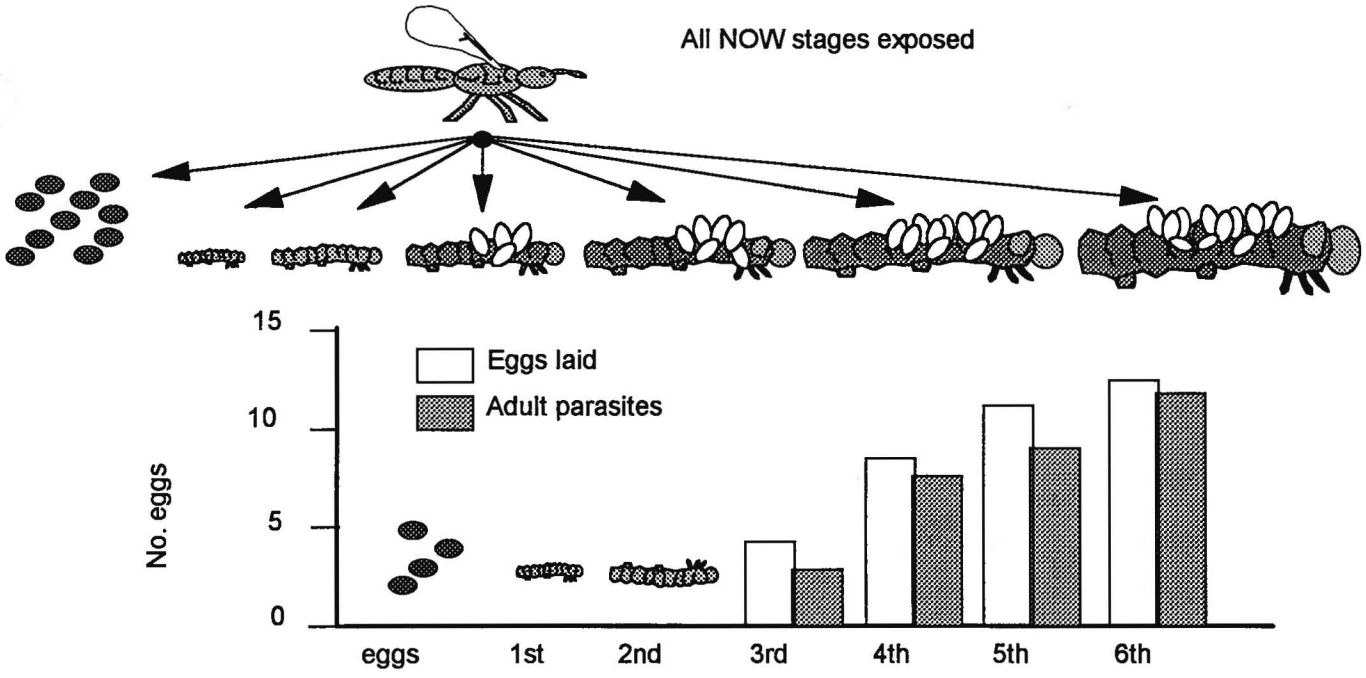

Fig.9. Laboratory studies with *G. legneri* show the parasite will not oviposit on eggs (as expected), 1st, or 2nd instar NOW. It will kill 2nd instar NOW. The number of eggs laid and the resultant number of adults reared is directly related to NOW stage (or size). This implies, for release timing, that releases should begin as soon as 3rd instar NOW become available, because the longevity of the *G. legneri* will carry it to the later stages.

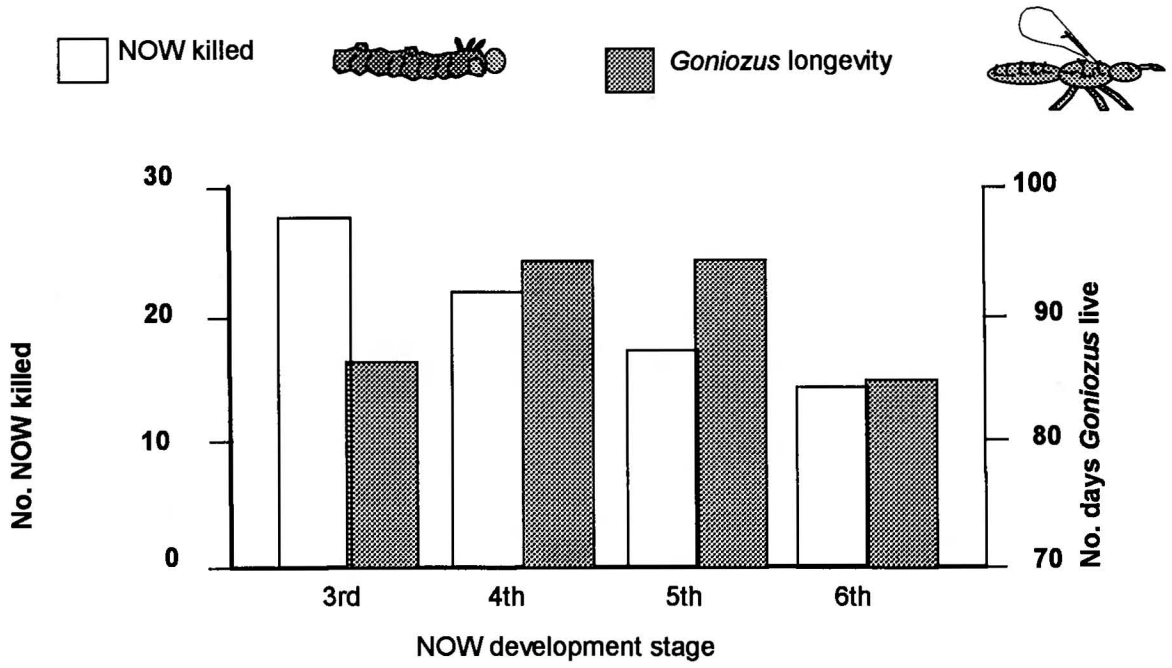

Fig.10. Laboratory studies with *G. legneri* show the parasite will kill (oviposition or stinging without oviposit on) more 3rd instar (smaller) than 6th instar (larger) NOW. This is because the parasite will lay fewer eggs on the smaller hosts, therefore, it must kill more hosts to lay its full compiment of eggs)and some of the killed 3rd instars are rejected for oviposition).

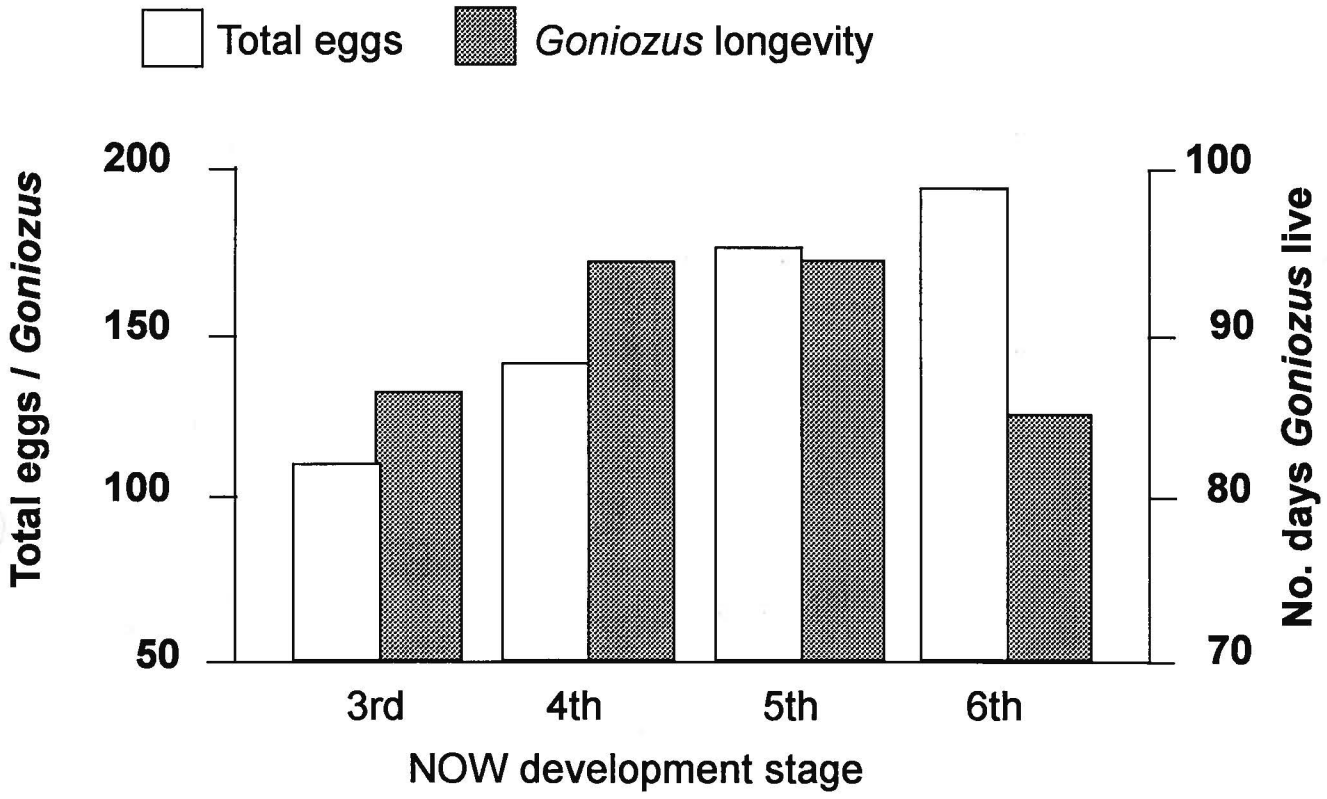

Fig.11. Laboratory studies with *G. legneri* show the parasite will lay more eggs on larger hosts, measured throughout its lifetime. The results imply a single parasite can have a great increase (over 100 fold) in the field, however, such increases are rarely or never attained. Other studies indicate the parasitoid is a poor host searcher and does not survive winter temperatures in the Central Valley.

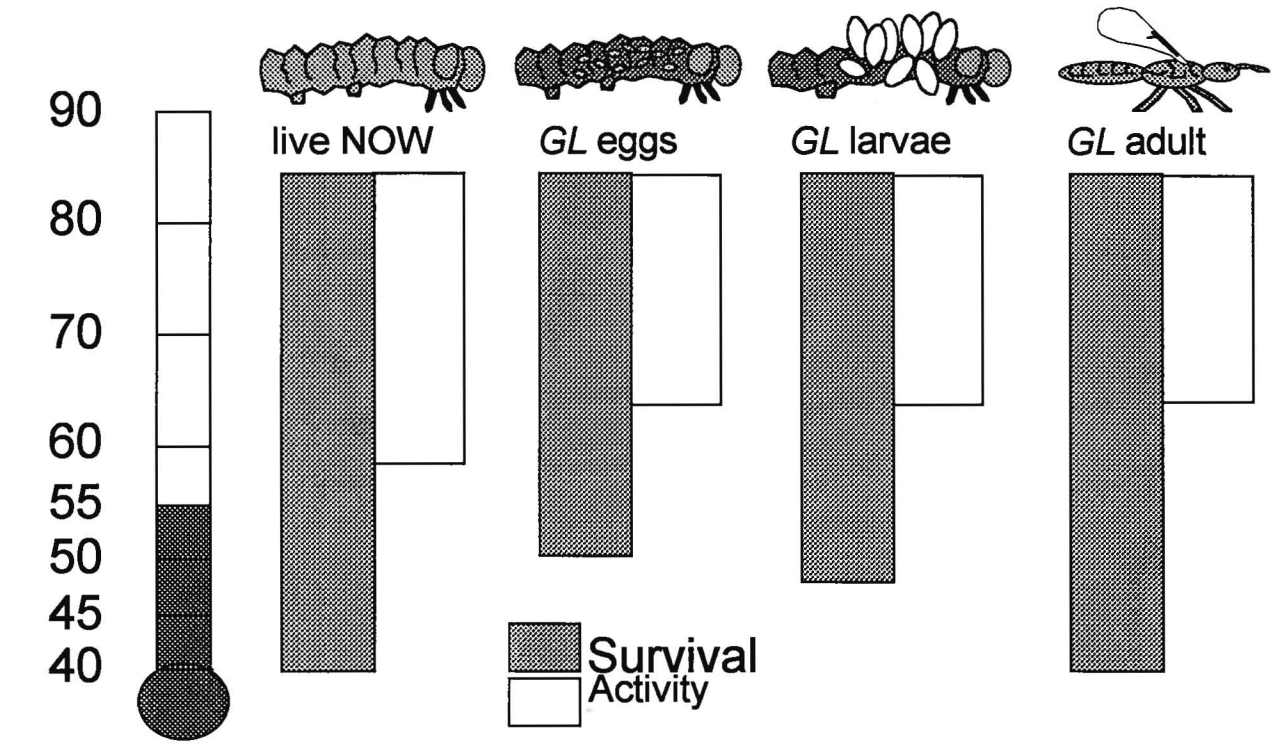

Fig.12. Laboratory studies NOW and *G. legneri* show the moth survives lower temperature than the parasite. More importantly, G. legneri eggs and larvae do not survive sustained temperatures <50F and will probably not overwinter in California's Central Valley. *G. legneri* adults will not oviposit at temperatures <65F.

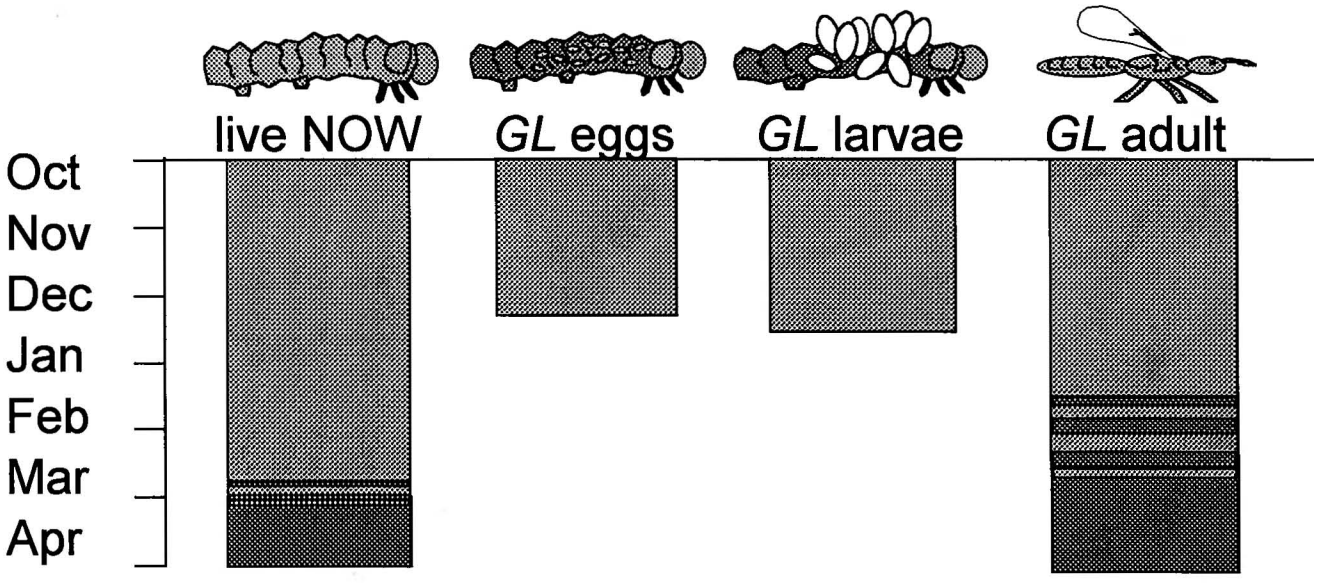

Fig.13. Field studies NOW and *G. legneri* show the moth survived winter temperature (1997 to 1998, Fresno County) with G. legneri eggs and larvae did not. G. legneri adults survived, but would not oviposit onto NOW (present in the peitri dish) until temperature were >65F in late February. Adult G. legneri held for 4 month were able to oviposit within hours of begin brought into the laboratory and held at warmer teperatures.