

## Egg Parasitism by *Trissolcus basalis* (Hymenoptera: Scelionidae) in Architecturally Varied Habitats, and Observations on Parasitism in Macadamia Nut Orchards and Other Habitats Following Augmentative Release

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**Abstract.** The ability of *T. basalis* to locate and parasitize *Nezara viridula* eggs was investigated in habitats with different architectures (monocot, broad leaf shrubs, mixed weeds). *Trissolcus basalis* demonstrated a significant preference for searching in weedy habitats dominated by *Crotalaria pallida*. Dispersal and host utilization efficiency of *T. basalis* was quantified in macadamia orchards on trees and weeds, and in weed-infested pastures. Parasitism of *N. viridula* eggs in macadamia nut orchards on trees and weeds was low. In weedy pasture the effects of predation (42% of eggs destroyed) and parasitism (~16% of eggs destroyed) appeared to be greater than in orchards. Quantification of the level of indispensable mortality contributed by *T. basalis* and predatory insects is needed. While augmentative release of *T. basalis* in macadamia nut orchards shows little potential for suppression of *N. viridula*, releases in weedy habitats adjacent to orchards may contribute to reducing numbers of *N. viridula* moving into orchards from surrounding habitat by increasing mortality at the egg stage. The economics of implementing augmentative releases of *T. basalis* in macadamia nut production will likely render augmentative release strategies impractical.

### Introduction

Three primary insect pests attack macadamia nuts (*Macadamia integrifolia* [Maiden and Betche]) in Hawaii, of which the southern green stinkbug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) is often the most severe (Jones 2002, Jones and Caprio 1992). *Nezara viridula* can inflict high damage levels to macadamia nut kernels, in excess of 70% damage in severe (albeit rare) seasons, but typically less than 3% (Jones 1995, Jones and Caprio 1992, Wright et al. 2007). In many cropping systems, *N. viridula* has been considered to be effectively suppressed by natural enemies (primarily the egg parasitoid, *Trissolcus basalis* (Wollaston), Scelionidae) in classical biological control programs (Caltagirone 1981). However, the periodic outbreaks and mean annual damage levels in macadamia nuts attributable to *N. viridula* in Hawaii (Wright et al. 2007) and previous work on its parasitoids in Hawaii macadamia nut orchards, suggest that *T. basalis*, is not as universally effective as commonly believed (Jones 1995). There is evidence that *T. basalis* is in fact responsible for minimal mortality of *N. viridula* eggs in Hawaii (Jones 1995) and Australia (Clarke 1990). Jones (1995) showed that *N. viridula* eggs in weedy habitats suffered higher parasitism by *T. basalis* than eggs in trees in macadamia orchards, and that even these levels were low in terms of achieving effective biological control of the pest. Predation (primarily by ants) contributed egg mortality orders of magnitude higher than *T. basalis*, reaching almost 50% apparent mortality in some years (Jones 1995). It may be true that *T. basalis* is most effective in habitats dominated by broad-leaf, ephemeral plants: Elher (2002) showed that 100% of

*N. viridula* eggs in egg masses attacked by *T. basalis* were parasitized in tomato and green bean fields, and he attributed suppression of *N. viridula* populations to egg parasitism plus other mortality factors. The data reported by Jones (1995) suggest that *T. basalis* has better host-location capabilities in weeds than in orchards.

Augmentative releases of *T. basalis* may offer an effective alternative means for suppressing *N. viridula*, in the absence of effective classical biological control. Augmentative releases of *T. basalis* are used in Brazil to control *N. viridula* in soybeans (Van Lenteren and Bueno 2003). *Trissolcus basalis* effectively disperses and locates egg masses in tomato fields following augmentative release, parasitizing up to 90% of eggs following releases of only 8,000 wasps per hectare (Justo et al. 1997). Jones (1995) reports anecdotally that macadamia nut growers developed commercial insectaries for augmentative releases, and then discontinued them as they saw no reduction in *N. viridula* damage levels. However, there are no quantitative data published on release densities and frequency or the effectiveness of *T. basalis* following augmentative release in macadamia orchards. An understanding of dispersal, host location effectiveness and mortality caused to the pest population are all aspects of the ecology of an augmentative biological control agent that need to be considered before implementing an augmentative program commercially or abandoning an agent (Wright et al. 2005). Such assessment should be continuous through a biological control project so as to avoid wasted effort in attempting releases that have little effect, as Jones (1995) emphasizes.

Augmentative releases of egg parasitoids have proven to very effective in some cases where classical biological control has failed (e.g., Wright et al. 2002), particularly where the parasitoid employed has pronounced dispersal and host location ability, and causes high indispensable mortality to the pest populations (Wright et al. 2005). Considering the reported effectiveness of *T. basalis* in augmentative release programs in tomatoes and soybean, and lack of effectiveness as a classical biological control agent in macadamia nuts in at least some areas in Hawaii, the question arises whether this parasitoid might effectively be used in augmentative programs in macadamia orchards. We examined the ability of *T. basalis* to disperse following simulated augmentative release, and to locate egg masses within experimental plots comprised of plants with varied architecture, and in macadamia nut orchards (on macadamia nut trees and on weeds within the orchards) and in weedy habitats not within orchards (pasture fields with weeds adjacent to orchards).

## Materials and Methods

*Trissolcus basalis* (originally collected from the field on Oahu by placing *N. viridula* sentinel eggs on leaves of spiny amaranth and rattlepod) were reared in the laboratory on eggs laid by *N. viridula* (fed on green beans, peanuts and cabbage). *Trissolcus basalis* were allowed to parasitize fresh or frozen eggs in Plexiglas cages (24 cm<sup>3</sup>) for 24-hour periods. Fresh or frozen eggs were supplied to wasps. Eggs were frozen for up to 100 days before use in some cases, but most were frozen for less than 30 days before use. Parasitism effectiveness on frozen eggs was compared with fresh eggs (by measuring percentage of eggs parasitized, emergence rate, sex ratio and longevity of emerging wasps) to ensure that the wasp colonies were not compromised as a result of using frozen eggs. For mass releases, mixtures of eggs frozen for different periods and fresh were combined to reduce the potential effects of egg storage.

**Habitat selection experiments.** To compare host-location in a monocot habitat, atypical broad leaf shrub habitats for *T. basalis*, and a habitat known to be preferred by *N. viridula* (weeds dominated by rattlepod), we planted common plots of corn (*Zea mays* L.), chili (broad leaf upright shrub, *Capsicum annum* L., Solanaceae), broccoli (*Brassica oleracea*, Brassicaceae) and mixed weed (primarily rattlepod (*Crotalaria mucronata*, Fabaceae), which were sown from field collected seed). Plots of 20 x 20 m were planted each with a quarter

sub-plot (10 x 10 m) planted with one of the plants ("habitats") listed above. The arrangement of the different habitats in each plot was randomized, with four replicates (CRBD). Once the plants within the plot had grown to approximately 50 cm tall, five sentinel egg masses (lab-reared *N. viridula* eggs, 10–20 eggs per egg mass) were placed into each quadrant, pinned onto leaves of the five plants nearest the middle of the plot, and 500 female *T. basalis* were released in the center of each plot (equivalent to 12,500 wasps ha<sup>-1</sup>). The egg masses were replaced every five days over a four to seven week period. Exposed and retrieved egg masses were held in the laboratory until parasitoids or *N. viridula* nymphs emerged. Two of these experiments were conducted during July – August, periods during which *T. basalis* parasitism is typically minimal in Hawaii (Nishida 1966). The experiment was repeated three times in total. The first experiment was at the Waimanalo Experiment Station (Oahu island, 21°20'07"N, 157°42'41"W) in June–July 2005, midsummer. Two releases were made at the Poamoho Experimental Farm (Oahu, 21°32'17"N, 158°05'20"W), one in July 2006 and another in December 2006.

**Dispersal.** A series of five dispersal experiments were conducted. The experiments were carried out in different habitats and modified as we obtained results, in an attempt to seek the best approach for monitoring the dispersal of the wasps in different habitats. Experiment 1 included orchard and weed habitats adjacent to each other; 2–4 were within macadamia orchards, and experiment 5 was in a weedy pasture area.

**Experiment 1.** A 6 x 6 grid of 36 stakes was laid out (spaced 25 m apart; the most distant point was 80 m from the release point), and a yellow sticky card placed on each stake, as well as a sentinel egg mass (from captive rearing) approximately 10 cm away from the card. Half of the grid was within a macadamia orchard at south Kona (MacFarms, August 2004, 19°18'49"N, 155°50'56"W) and the other half in border vegetation consisting of a mix of woody shrubs and weed species (*C. mucronata*, *Bidens pilosa*), at 390 m asl. Approximately 10,000 female *T. basalis* were released in the center of the grid (equivalent to ~15,000 wasps ha<sup>-1</sup>). The wasps were released as eclosing adults (within one day of commencement of emergence) or as advanced-development pupae about to emerge, in paper cups covered with mesh to protect the wasps from predatory insects. TangleFoot® grease was smeared around the base of the stakes bearing the egg masses to prevent predatory ants from climbing the stakes and feeding on the eggs. The sticky traps and egg masses were replaced weekly for four weeks. Sticky traps were examined microscopically for the presence of *T. basalis*, and eggs were held in the laboratory at ambient conditions until either wasps or *N. viridula* nymphs emerged.

A set of ten control egg masses were placed approximately 150 m from the release grids to assess whether *T. basalis* were active in the area of each dispersal experiment. Losses of sentinel egg masses to predatory insects not excluded by the TangleFoot were recorded during each experiment.

**Experiment 2.** The same grid configuration (25 m spacing between monitoring points), as experiment 1 was used at Island Harvest (Hawi, 170 m asl, 20°14'19"N, 155°46'53"W) in April 2004, with 10,000 *T. basalis* released as described above. Sentinel eggs and sticky cards were pinned onto leaves of trees within the orchard, and monitored as above (exp. 1). No presence of *N. viridula* was observed in the orchard.

**Experiment 3.** A smaller 6x6 monitoring grid (12.5 m spacing owing to low dispersal and parasitism observed in previous experiments with 25 m spacing) was laid out at Mauna Loa (Pahala/Kau, 275 m asl, 19°11'38"N, 155°28'34"W) in June 2004, and 10,000 *T. basalis* released (equivalent to 25,000 wasps ha<sup>-1</sup>). Sentinel eggs were placed on leaves in trees in the orchard, and monitored as above. No presence of *N. viridula* was observed in the orchards during this experiment.

**Experiment 4.** A 6 x 6 (12.5 m spacing) grid was laid out at MacFarms (at ~400m asl) in November 2004, with 10,000 *T. basalis*. Sentinel eggs and sticky cards were placed on

weeds within the orchard (between the rows of trees), and monitored as above. *Nezara viridula* were observed in the orchard at this time.

**Experiment 5.** This experiment was again a 6 x 6, 12.5 m spacing grid, at Pahala/Kau (287 m asl) in October 2005. *Trissolcus basalis* were released as above, and sentinel eggs and sticky cards were placed on weeds (primarily spiny amaranth, *Amaranthus spinosus*) in a pasture adjacent to a macadamia orchard. *Nezara viridula* were observed in the pasture during the course of the experiment. Data were collected as above. Although three attempts over a period of three months were made to conduct this experiment, most sentinel eggs were destroyed in each of the first two trials by Orthoptera feeding on the leaves (Diez pers. obs.).

**Statistical analysis.** Percentage of egg masses and eggs parasitized in the fresh/frozen egg comparison and habitat selection experiments were analyzed using a Generalized Linear Model (PROC GLM, SAS 2002), and pairwise comparisons of parasitism levels in the different habitats were done using Tukey comparisons. Although the dispersal experiments were not conducted simultaneously, mean parasitism by experiment was compared using a mixed model ANOVA (PROC MIXED, SAS 2002), with repeated measurements of parasitism as replicates, and each experiment treated as a different treatment. If ANOVA was found to be significant, Tukey's test was used for pairwise comparisons of means.

## Results

Using *N. viridula* eggs frozen for up to 30 days had no significant effect on percent of eggs parasitized, emergence, female: male ratio and longevity of the wasps emerging ( $P > 0.050$ ). Longer frozen storage periods did impact these variables significantly, but the reduction in performance on the frozen eggs was small (Table 1). We are thus confident that the quality of the wasps we were releasing was not compromised by using frozen *N. viridula* eggs as the rearing substrate.

*Trissolcus basalis* parasitism efficiency was significantly higher in mixed weeds than other habitats (Waimanalo:  $F_{3,99} = 5.39$ ,  $P = 0.0018$ ; Poamoho July 2006:  $F_{3,187} = 5.74$ ;  $P < 0.0006$ ; Poamoho December 2006:  $F_{3,99} = 4.13$ ;  $P = 0.0076$ ) (Fig. 1a, b). There was no significant difference between the two Poamoho trials, so these data are combined in Figure 3b. Sixty to seventy percent of sentinel eggs were parasitized in the mixed weed plots, and corn and chili approximately 30–40%. In broccoli (low broadleaf) only 16–25% of eggs were parasitized (Fig. 1).

Wasps dispersed up to 75 m from the release point within two weeks in experiments 1 and 2. In experiment 3, the wasps moved 44 m and parasitized eggs, while in experiment 4, the maximum distance recorded was 24 m from release, two weeks after release. Parasitism levels in these experiments (experiments 1–4) were low, with only 2–7% of egg masses parasitized (Table 2). No control parasitism was recorded during any of the release experiments. Seven to 19 percent of egg masses was lost to predators in spite of the use of TangleFoot on monitoring stakes and branches (Table 2). Sticky cards caught minimal numbers of *T. basalis* throughout the experiments (data not reported).

In experiment 5 (weed-dominated pasture adjacent to macadamia orchard), *T. basalis* dispersed and located *N. viridula* sentinel egg masses more effectively than in the other experiments (done in macadamia orchards) (Table 2;  $F_{4,512} = 18.39$ ;  $P < 0.001$ ). Forty percent of egg masses were parasitized throughout the experiment, and an overall mean of 40.12% of individual eggs were parasitized (Table 2). Parasitism was recorded at 53 m from the release point after one week (Fig. 2). Proportion of egg masses parasitized in experiment 5 was consistently higher over the four-week monitoring period than in any of the other releases (Fig. 2).

**Table 1.** Mean overall effect of freezing *Nezara viridula* eggs used for mass rearing, for up to 100 days (mean 62 days), on *Trissolcus basalis* performance ( $\pm$  SEM)

| Variable         | Fresh Control    | Frozen           | F <sub>1,171</sub> | P       |
|------------------|------------------|------------------|--------------------|---------|
| % Parasitism     | 95.03 $\pm$ 0.13 | 93.83 $\pm$ 0.24 | 20.69              | <0.0001 |
| % Emergence      | 94.94 $\pm$ 0.14 | 91.18 $\pm$ 0.38 | 358.80             | <0.0001 |
| Longevity (days) | 24.71 $\pm$ 0.13 | 21.19 $\pm$ 0.23 | 280.00             | <0.0001 |
| ♀ : ♂ Ratio      | 4.57 $\pm$ 0.05  | 4.18 $\pm$ 0.06  | 20.05              | <0.0001 |

**Table 2.** Percentage<sup>a</sup> of eggs killed by parasitism (apparent mortality) per egg mass ( $\pm$  SEM) in five dispersal releases with a single, central release point. One hundred and forty four egg masses were used in each experiment, with eggs replaced and exposed four times per experiment.

|  | Exp. 1 Mix          | Exp. 2 Trees        | Exp. 3 Trees        | Exp. 4 Weeds in orchard | Exp. 5 Weeds in pasture |
|--|---------------------|---------------------|---------------------|-------------------------|-------------------------|
| Release density <sup>1</sup>                           | 15,000              | 15,000              | 25,000              | 25,000                  | 25,000                  |
| % egg masses found and eaten by predators <sup>2</sup> | 8                   | 13                  | 7                   | 19                      | 42                      |
| % egg masses parasitized                               | 2.00                | 6.00                | 7.00                | 5.00                    | 40.00                   |
| % Eggs parasitized <sup>3</sup>                        | 2.70a<br>$\pm$ 1.24 | 5.18a<br>$\pm$ 1.91 | 6.92a<br>$\pm$ 2.11 | 3.20a<br>$\pm$ 1.58     | 40.12b<br>$\pm$ 5.09    |

(<sup>a</sup> Estimates of apparent mortality as average proportion of *N. viridula* egg masses eaten by predators (number lost / 144), proportion parasitized by *T. basalis* (parasitized / 144 – number lost to predators).

<sup>1</sup> Female *T. basalis* ha<sup>-1</sup>.

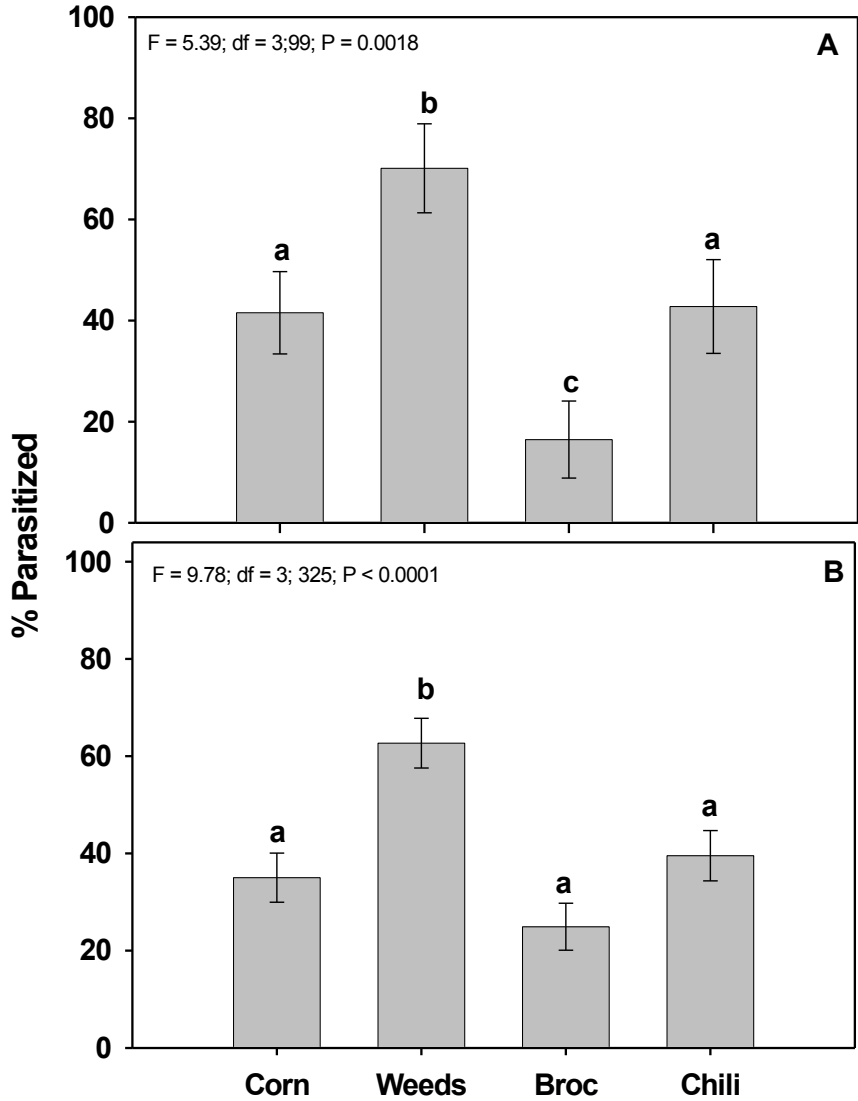
<sup>2</sup> Predators destroyed all eggs in egg masses they attacked.

<sup>3</sup> Means followed by different letters significantly different, P < 0.05, Tukey comparisons adjusted for repeated measures).

Discussion

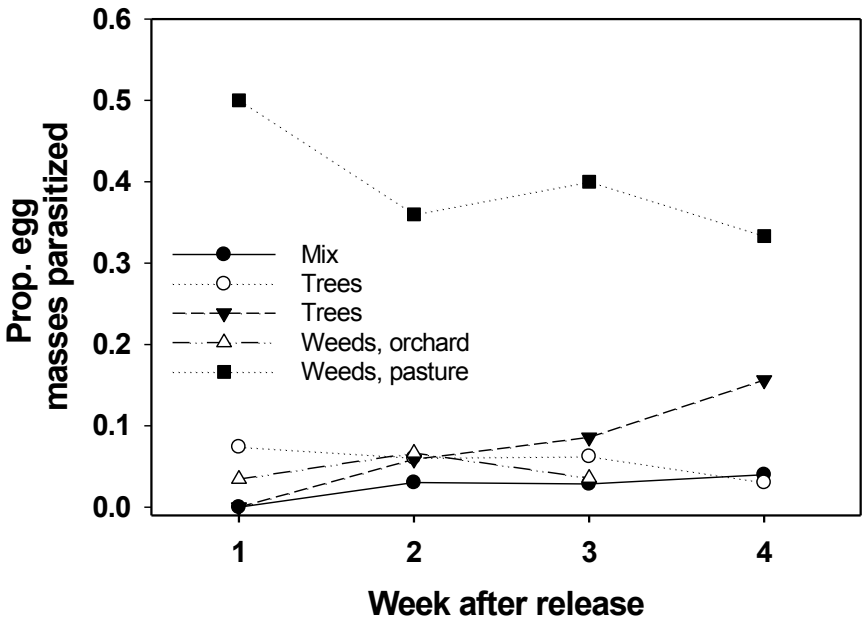
The egg parasitism levels and dispersal patterns following *T. basalis* augmentation in macadamia nut orchards and in weedy areas are consistent with Jones (1995) work, in that there was substantially higher parasitism of *N. viridula* eggs masses by *T. basalis* in weedy habitat. In orchards, parasitism of sentinel egg masses was very low. Jones (1995) recorded zero to 7.3 percent of eggs parasitized by wild *T. basalis* in a sentinel egg mass study on Hawaii island.

These results suggest that *T. basalis* may search for *N. viridula* eggs most effectively in a weedy habitat, rather than within orchards at all, even on weeds within orchards. It is possible that natural enemies were simply more active at the location and time experiment 5 was conducted. The destruction of the first two attempts at conducting the experiment 5 releases



**Figure 1.** Mean percentage ( $\pm$  SEM) of *Nezara viridula* sentinel eggs parasitized by *Trissolcus basalidis* in different habitats (A = Waimanalo release, B = Poamoho releases, data combined for both trials). Bars with the same letter were not significantly different, Tukey test,  $P < 0.05$ .

precludes making any conclusive statements about these results, but the trends observed in this experiment are consistent with what could be expected based on the differential habitat searching observed (Fig. 1). Although statistical analysis of results of the various trials is rather tenuous (owing to logistic considerations related to release sites and production of parasitoids precluded simultaneous replicates of experiments, and only one replicate of the weeds-only area was conducted), the differences between the orchard releases and the single



**Figure 2.** Overall proportion of *Nezara viridula* egg masses parasitized over four-week periods, in five releases of *Trissolcus basalis* in different habitats (trees in macadamia orchards, weeds in orchard or pasture).

weedy area release were substantial (about 6–15 times higher parasitism of egg masses in weeds). Parasitism of sentinel eggs placed on weeds within orchards was also very low. These results show that any effort to use *T. basalis* in augmentative release programs against *N. viridula* should employ releases in weedy habitats adjacent to orchards, otherwise the efforts will be wasted as the impact on the target pest is likely to be negligible. With a single release point of the equivalent density of 25,000 female *T. basalis* per hectare (approx. 10,000 per release in these experiments), about 40% parasitism was obtained over a four-week period in weedy habitat.

The habitat selection experiments were conducted during summer months (July–August) and also in winter (December). It has been suggested that *T. basalis* is scarce during the summer in Australia and probably also in Hawaii (Clarke and Walter 1993, Jones 1995). Our results demonstrate that augmentative releases in weedy patches can increase parasitism levels to as high as 70% during the summer, suggesting that augmentative releases do have potential value, even during the time of year when *T. basalis* is typically not considered to be active or effective. The parasitism levels observed in the habitat selection experiments resulted from equivalent release rates of 12,500 female *T. basalis* per hectare. Releases would have to be repeated approximately every four weeks to maintain maximal parasitism levels, based on observed declines in parasitism levels at three–four weeks after release in weedy habitats (Fig. 2; Wright unpublished data).

The extent of increase in parasitism following by augmentative release is only one of many aspects to be considered when planning management of *N. viridula*. The actual impact of any increased *T. basalis* parasitism on *N. viridula* populations and subsequent changes

in damage to macadamia nut kernels will determine the true usefulness of an augmentative release program. The importance of *N. viridula* egg predation by ants and other predators may outweigh parasitism by *T. basalis* significantly. The economics of making such releases need to be carefully considered before any serious plans are laid to commence mass rearing and dispensing of *T. basalis* in macadamia nut growing areas.

A detailed study of the level of indispensable mortality (Bellows et al. 1992) inflicted by *T. basalis* would provide a quantitative estimate of the true impact the parasitoids have on to *N. viridula* populations. While 40% apparent mortality may seem low in terms of achieving effective biological control, the indispensable mortality contributed may be significant, as has been shown in other studies (Kuhar et al. 2002, Vejar-Cota et al. 2005). Conversely, *T. basalis* may contribute negligible indispensable mortality where predators are highly active. For example, if 42% of egg masses that were parasitized in experiment 5 were subsequently destroyed by predators, the total percentage killed by predators and parasitoids combined would be only 55.2%. Predators such as ants, which were excluded from these experiments, may destroy larger numbers of eggs than predation levels recorded in this study, if present.

A release density of 25,000 females per hectare is relatively low compared to augmentative releases of other parasitoids, and it is possible that inundatively releasing larger number of wasps could increase parasitism and overall mortality of *N. viridula*. However, the economics of implementing an inundative release program for *T. basalis* in macadamia nuts need to be examined with care. With current rearing costs and unpredictable efficacy of the parasitoid, this approach is unlikely to be economically viable in Hawaii macadamia nut production.

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