Evaluation of factors influencing augmentative releases of *Chrysoperla carnea* for control of *Scirtothrips perseae* in California avocado orchards

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**Abstract**

Weekly releases of *Chrysoperla carnea* for control of *Scirtothrips perseae* were evaluated in replicated field plots in two commercial avocado orchards in southern California, USA. Two release techniques and rates commonly employed by commercial pest control advisors who routinely use this generalist predator for *S. perseae* control were assessed. Release technique one utilized *C. carnea* eggs glued to paper squares that were stapled to leaves of experimental trees at a rate of 41,000 eggs per ha. Release technique two used a motorized backpack sprayer to apply a dry mixture of lacewing eggs and larvae to trees at a rate of 514,501 per ha. Pest populations were monitored by making bi-weekly population counts of *S. perseae* larvae and adults on leaves, and adult densities were simultaneously monitored in each experimental plot with yellow sticky cards. In the laboratory, degree-day accumulation until death of immature *C. carnea* was determined at temperatures representative of field conditions when predators were provisioned with varying amounts of food or different food types. Preference for *S. perseae* instars by first, second, and third instar *C. carnea* was assessed in the laboratory, and intraguild predation towards larvae and adult females of a co-occurring generalist predatory thrips, *Franklinothrips orizabensis*, was investigated along with intraspecific predation rates. Both release strategies failed to significantly reduce *S. perseae* populations in comparison to non-treated control plots. Approximately 35–96% of *C. carnea* eggs and larvae applied with the motorized sprayer landed on the ground. *C. carnea* larvae lived for approximately 1–2 days when provisioned with either no food, an avocado leaf or avocado pollen. Longevity was extended to 14–15 days when prey was provided. *C. carnea* larvae showed no preference for first or second instar *S. perseae*, all predator instars attacked first instar *F. orizabensis*, but only second and third instar *C. carnea* managed attacks on second instar *F. orizabensis* larvae. No adult female *F. orizabensis* were attacked and no attacks by *F. orizabensis* on *C. carnea* were recorded. Second instar *C. carnea* engaged in the highest levels of intraspecific predation.

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1. **Introduction**

Avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), is native to Mexico and Guatemala and is an exotic pest that has been present in California (USA) avocado (*Persea americana* Miller) orchards since 1996 (Hoddle et al., 2002a). Economic analyses have indicated that *S. perseae* costs the California avocado industry millions of dollars annually due to reduced market value of fruit that results from feeding damage caused by larvae and adult thrips, and associated management expenses to reduce damage to the harvestable commodity (Hoddle et al., 2003). Consequently, research sponsored by the California Avocado
Commission has focused on identifying control options for *S. perseae* and evaluating the efficacy and impact optimization of either new or currently employed pest suppression tactics. Three management strategies have been subjected to extensive research and include: (1) pesticide evaluations (Yee et al., 1999, 2001a,b,c), (2) biological control (foreign exploration for natural enemies [Hoddle et al., 2002a] and evaluation of augmentative releases of commercially available natural enemies [Hoddle et al., 2004; Silvers, 2000]), and (3) cultural control (composted organic yardwaste to suppress thrips pupation in soil under trees [Hoddle et al., 2002b]).

Biological control of *S. perseae* has been attempted previously, and releases of commercially available green lacewings (*Chrysoperla carnea* [Stephens] and *Chrysoperla rufilabris* [Burmeister] [Neuroptera: Chrysopidae]) and predatory thrips (*Frankliniella orizabensis* Johansen [Thysanoptera: Aeolothripidae]) have been used extensively by commercial pest control advisors (PCAs) who scout and monitor pest densities in orchards and make management recommendations to growers. Some research has been done with augmentative release of natural enemies against two *Scirtothrips* species when 50–100 larvae or 50 larvae + 500 eggs were applied to individual citrus trees.

Hoddle et al. (2004) have demonstrated that augmentative releases of the predatory thrips *F. orizabensis* are ineffective at controlling *S. perseae*. Silvers (2000) released *C. rufilabris* against *S. perseae* on top-worked avocado trees and failed to observe any significant impact against *S. perseae*. Silvers’ work was not considered representative of industry practices with lacewings because predator release timings, release rates, and tree size differed from conditions under which PCAs reportedly achieved control of *S. perseae* with augmentative releases of lacewings. Consequently, the purpose of work reported here was to revisit the issue of the effectiveness of releases of *Chrysoperla* sp. for *S. perseae* control. The efficacy of accepted industry practices pertaining to release timings, rates, and deployment strategies in commercial orchards treated with lacewings for *S. perseae* suppression by PCAs was evaluated. Concurrent work in the laboratory sought to determine the length of time lacewing larvae could survive under field temperature conditions with varying amounts of food, whether lacewing larvae exhibited preferences for either first or second instar *S. perseae* larvae, and if larval and adult *F. orizabensis* were at risk of predation from lacewing larvae.

### 2. Materials and methods

#### 2.1. Study sites and experimental setup

Two study sites were used for evaluating the efficacy of *C. carnea* releases for controlling *S. perseae*. Insecticides for control of *S. perseae* were not applied at either study site during the course of these evaluations. Both sites consisted of ‘Hass’ avocados and were subjected to commercial watering, fertilizing, and soil amendment practices. Site 1 was a 6.5 ha orchard in San Marcos, San Diego County, California, and consisted of 15-year-old trees that had been top-worked (i.e., cut back to the trunk and allowed to re-grow) 2 years before the trial began. Trees that were on average 5.05 ± 0.13 m tall (range 2.62–7.01 m; *n* = 60) and flushing heavily with new leaves in a 3 ha block of the orchard were selected. Experimental blocks were separated by a minimum of two buffer rows. The trial at Site 1 commenced 18 March 2003 and ceased 9 July 2003. Site 2 was in Irvine, Orange County, California, and consisted of large mature trees that were on average 8.30 ± 0.15 m in height (range 6.10–10.89 m; *n* = 60) in a 6 ha block. Experimental blocks were separated by a minimum of three buffer rows. The trial at Site 2 commenced 25 March 2003 and ended 17 July 2003.

At each site, 12 experimental blocks consisting of 15 trees each were selected following pre-release inspections of *S. perseae* numbers. Trees in blocks were selected to create experimental blocks with similar mean starting thrips densities across treatments. Three treatments (four randomly selected blocks each consisting of 15 trees were assigned per treatment) were assessed at each site for *S. perseae* control. Treatment 1 consisted of lacewing eggs attached to paper squares that were stapled to leaves. Treatment 2 applied lacewing larvae and eggs to experimental trees with a motorized blower. Treatment 3 was the control and blocks did not receive any lacewing applications. More details on lacewing releases are provided in Section 2.3.

Temperature and humidity at both study sites were recorded every 30 min with Hobo dataloggers (Onset Computer, Pocasset Massachusetts, USA) attached to stakes 1.5 m above the ground and positioned in the center of experimental area in each orchard.

#### 2.2. Population density monitoring and analysis

Every 2 weeks, 10 trees were randomly selected in each experimental block of 15 trees and ten 3/4 expanded leaves were examined on selected trees and numbers of avocado thrips larvae and adults per leaf were recorded. In the center of each experimental block a yellow sticky card was deployed on a stake 1.5 m above the ground to monitor thrips movement between blocks. Every 2 weeks the card was replaced and the exposed card was examined for *S. perseae* adults. Thrips-days
for *S. perseae* in release and non-release plots on leaves or sticky cards were calculated using geometric summation for each experimental block to estimate the area under population curves. Thrips-days were calculated as the sum of products of the number of individuals at each sampling date and the time interval between sampling dates (Carey, 1993). Total thrips-days per experimental block were calculated, averaged across blocks for each treatment, and compared using ANOVA at the 0.05 level of significance (SAS, 1990) to determine if treatment effects existed for thrips counts on leaves. Two-way ANOVA with treatment and cardinal direction as major factors was used to determine if significant differences in densities of winged adult *S. perseae* caught on sticky cards showed directional movement into or out of experimental blocks.

2.3. Source of *C. carnea*, release rates and methodologies, and quality control estimates

*Chrysoperla carnea* eggs were purchased from a commercial insectary in Santa Paula, Ventura County, California. Lacewing eggs were deployed onto experimental trees in one of two ways: (1) eggs glued to squares of paper (29 mm x 25 mm; ~166 eggs glued per square) were stapled individually to one leaf on each experimental tree. Interior canopy leaves near leaf flush with *S. perseae* larvae were preferentially selected for releases. (2) Lacewing eggs were held at room temperature until 75% egg hatch had occurred. Unhatched lacewing eggs and larvae (~125,000) were then mixed with corn grits to fill a clear 2 L plastic bottle attached to a Stihl SR400 backpack mistblower that was modified to deliver corn grits and predators in a dry stream of air into tree canopies (Takano-Lee and Hoddle, 2001). PCAs released lacewing eggs on paper squares and eggs/larvae mixed in corn grits at the rates of approximately 41,000 or 514,501 per ha, respectively, for each release method. Releases were initiated at both field sites when PCAs first detected *S. perseae* larvae and adults co-occurring with developing fruit vulnerable to feeding damage (fruit length ≤10 mm). Lacewing releases were made every other week until PCAs determined that immature fruit were no longer vulnerable to feeding damage (fruit length >55 mm) irrespective of thrips densities in the orchard.

Emergence rates and mean numbers of lacewing eggs deployed per paper square were estimated by randomly selecting five squares from leaves after 2 weeks exposure on two different release dates (Site 1: 20 March 2003 and 3 April 2003; Site 2: 27 March 2003 and 10 April 2003) and returning them to the laboratory where numbers of hatched and unhatched eggs were recorded.

Quality and quantity of material applied with the blower were assessed in three different ways on the same two application dates quality control data were collected for the paper squares at both release sites: (1) five 20 ml aliquots of corn grits with eggs/larvae were removed from 2 L bottles and returned to the laboratory and the number of emerged, unemerged eggs, and larvae were recorded. (2) Four plastic cups (300 ml) were attached to branches with binder clips in each cardinal quadrant of five randomly selected trees in each experimental plot being treated with the blower. After applications, cups were sealed, returned to the laboratory, and the numbers of lacewing eggs and larvae in cups were recorded. (3) The number of lacewing eggs and larvae that fell through the canopy of trees being treated with the blower were determined by placing two wooden boxes (30 cm wide, 35 cm long, and 13 cm deep) under each of six randomly selected trees in plots being treated with the blower. Plastic panels attached to the tops of boxes were treated with Tanglefoot, which trapped corn grits, lacewing eggs, and larvae. Plastic panels were removed after applications were completed, placed in clear plastic bags, and returned to the laboratory where numbers of lacewing eggs and larvae were recorded. The area under each experimental tree in each plot treated with the blower was measured from the trunk to the edge of the canopy and used to calculate the average area under experimental trees. The average volume of corn grits with lacewing eggs applied to each tree was calculated and the amount of material falling onto the ground was estimated from the sticky panels. Material not accounted for on sticky panels was assumed to have stayed on treated trees and not blown through the canopy and out of the experimental plots (estimates of product blow-through were not made though it is known to occur [see Takano-Lee and Hoddle, 2001]).

2.4. Survivorship estimates for lacewing larvae under varying temperature and food regimens

Laboratory survivorship rates of first instar lacewings were estimated by providing larvae with varying amounts of food. For each of six food treatments, 15 first instar larvae (<24 h of age) were randomly assigned to sealed plastic vials with either: (1) no food; (2) an immature avocado leaf; (3) avocado pollen; (4) 15 irradiated *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs; (5) 150 *E. kuehniella* eggs; and (6) 300 *E. kuehniella* eggs. Lacewing larvae in vials were kept in a temperature controlled cabinet under long days (L:D 16:8). To approximate field temperatures, average day and night temperatures in cabinets were calculated from Hobo data logger recordings from both field sites. Nighttime temperatures were set to 13°C ± 0.10 and daytime temperatures were 21.5°C ± 0.07. Survival of larvae for each food regimen was recorded daily and cabinet temperatures were recorded every 30 min with a Hobo data logger.

Data for larval lacewing developmental times from Butler and Ritchie (1970) were used to determine the lower temperature threshold (9.03°C) used in calculating de-
gree-day accumulations from cabinet temperature studies using the single sine method (UC-IPM, 2003). No upper temperature thresholds were specified for degree-day accumulation because these data are unavailable. The relationship between degree-day accumulation and proportion surviving within a treatment was described using a Weibull function of the form $y = 100e^{-(x/a)^b}$. This function was fitted using non-linear regression in SAS (1990) and used to estimate the degree-day accumulation for median survival times (50% survivorship) of lacewing larvae across treatments. Mean survivorship times in days across diets were compared using ANOVA and significant differences at the 0.05 level were determined using Duncan’s Multiple Range Test (SAS, 1990).

2.5. Interactions between C. carnea larvae with S. perseae larvae and F. orizabensis larvae and adults

Prey utilization by first, second, and third instar lacewing larvae was determined by placing individual lacewing larvae on 5cm × 5cm avocado leaf arenas with five first and second S. perseae larvae as prey. Each leaf square was placed on a water-saturated foam pad and replicated 15 times. Predation of S. perseae larvae was determined visually at 1, 3, and 24h intervals post-setup. Prey preferences and consumption rate comparisons were made within and across predator instars using Friedman’s $\chi^2$ (SAS, 1990). Pairwise comparisons using methods detailed by Critchlow and Fligner (1991) were used to determine where significant differences existed in the change in the mean number of thrips larvae consumed between each observation interval.

To investigate potential intraguild predation, the above setup was repeated with a single first, second or third instar lacewing larvae presented with either a single first, or second instar F. orizabensis larva or adult female. Intraspecific predation by cohorts of similar aged lacewing larvae in the presence of S. perseae larvae was evaluated by pairing lacewing larvae of the same instar together with five first and second instar S. perseae larvae on excised avocado leaf arenas. Mortality of each predator and prey lifestage was recorded at 1, 3, and 24h post-setup.

3. Results

3.1. Population density trends and sticky card monitoring

Releases of lacewings deployed either as eggs glued to release cards or deployed as a mixture of eggs and larvae applied with a mechanical blower to experimental trees failed to alter pest population growth trajectories at either field site (Figs. 1A and B) and C. carnea releases did not significantly reduce densities of avocado thrips at Site 1 ($F = 0.11, \ df = 2.9, \ P = 0.90$) or Site 2 ($F = 0.41, \ df = 2.9, \ P = 0.67$) when mean cumulative thrips-days were compared across treatments (Figs. 2A and B).

At Site 1, significant differences in densities of adult avocado thrips caught on yellow sticky cards were not detected across treatments ($F = 0.46, \ df = 2, \ P = 0.64$), cardinal direction ($F = 4.25, \ df = 1, \ P = 0.053$), or the interaction between these two factors ($F = 2.39, \ df = 2, \ P = 0.12$) (Fig. 3A). Similar results were observed for Site 2. Treatments ($F = 0.11, \ df = 2, \ P = 0.90$), cardinal direction ($F = 0.94, \ df = 1, \ P = 0.35$) and the interactions between these two factors ($F = 0.59, \ df = 2, \ P = 0.56$) were not significant (Fig. 3B).

3.2. Quality control of deployed C. carnea eggs

Egg hatch rates for both release methods were high, ranging from 69 to 76% when all samples across all sampling dates were combined (Table 1). On average at Site 1, the collection boxes used to trap material falling to the ground from spray applications covered 0.94% ± 0.06 of the ground under experimental tree canopies and trapped on average 6.03 ± 0.74 live eggs and larvae. At Site 2, the collection boxes covered 0.22% ± 0.01 of the...
ground under experimental tree canopies and trapped on average 4.01 ± 1.14 live eggs and larvae. Consequently, approximately 35% ± 1.62 and 96.31% ± 3.86 of lacewing eggs and larvae sprayed onto trees at Sites 1 and 2, respectively, fell to the ground and the remaining material either adhered to leaves or was blown through the canopy to land outside the study area. Too few *C. carnea* eggs and larvae were caught in plastic cups at each study site to calculate survivorship rates of material blown and retained in canopies.

3.3. Survivorship estimates for *C. carnea* larvae provisioned with varying food regimens

Non-linear regression analyses fitting the Weibull function to survivorship rates as a function of degree-day accumulation were significant for all evaluated diets (Table 2). Food availability had significant effects on average daily lacewing larval longevity (*F* = 19.98, *df* = 5.84, *P* < 0.005) and degree-day accumulation to median survivorship (Table 2; Fig. 4). *C. carnea* larvae with access to irradiated *E. kuehniella* eggs lived longer than larvae provisioned with nothing, an avocado leaf, or avocado pollen (Table 2; Fig. 4).

3.4. Interactions between *C. carnea* larvae and *S. perseae* and *F. orizabensis*

First, second or third instar *C. carnea* failed to exhibit significant differences in instar utilization of *S. perseae* larvae at observation intervals 1, 3, and 24h (*χ² P* values comparing attack rates all >0.2). *C. carnea* larvae attacked *S. perseae* larvae as predators randomly encountered prey. No significant differences in consumption rates of first instar *S. perseae* larvae by first, second, and third instar lacewing larvae were observed for 1, 3, and 24h intervals (Fig. 5A). A significant difference in consumption rates of second instar avocado thrips larvae were observed at the 1h observation interval (*χ² = 9.53, df = 2, P = 0.009), but not at the 3 or 24h observation intervals (Fig. 5B). Significant differences in total consumption rates of first and second instar avocado thrips larvae combined were observed across predator instars at the 1h (*χ² = 6.95, df = 2, P = 0.03), and
24h ($\chi^2 = 8.97, df = 2, P = 0.01$) observation intervals. No differences were observed at the 3h interval ($\chi^2 = 1.74, df = 2, P = 0.42$) (Fig. 5C). Third instar lacewing larvae tended to consume the most first and second instar avocado thrips, with second instar predators exhibiting intermediate consumption rates and first instar lacewing larvae exhibiting the lowest overall consumption rates.

All C. carnea larval instars successfully attacked and consumed 100% of first instar F. orizabensis larvae within 24h. First instar C. carnea larvae did not consume second instar F. orizabensis larvae. Second and third instar C. carnea consumed 50 and 70% of second instar F. orizabensis larvae, respectively, within the 24h exposure period. Adult F. orizabensis females were not attacked by any larval stage of C. carnea after 24h, and this result did not change after the observation interval was increased to 48h.

Intraspecific predation was observed for C. carnea when the same larval instars were paired in the presence of S. perseae larvae. For first instar C. carnea, 8% of larvae consumed a conspecific, while 75 and 9% of second and third instars, respectively, consumed a same-aged conspecific within 24h when S. perseae larvae were available for attack.

4. Discussion

Augmentative releases of C. carnea applied to avocado trees either as eggs glued to paper squares or sprayed onto trees at 75% egg hatch with a motorized blower failed to significantly reduce densities of S. perseae larvae and adults when compared to control blocks that received no lacewing treatments. Sticky card captures of adult S. perseae failed to show any reduction in adult flight activity or densities across experimental blocks further verifying a lack of treatment effect. No significant differences in yellow sticky card capture rates across cardinal directions were observed. This supports earlier observations and suggests that adult S. perseae fly only when flight can be controlled and not influenced by breezes (Hoddle et al., 2002c). Furthermore, application
of lacewing eggs and larvae with a motorized blower results in at least 35–96% of mechanically applied product falling to the ground without adhering to leaves in the canopy of treated trees. The amount of material blown through the canopy was not measured. When taken together, these data suggest that releases of *C. carnea* eggs and larvae applied at field rates and using methodologies recommended by professional Pest Control Advisors are ineffective at controlling *S. perseae* in commercial California avocado orchards.

*Chrysoperla carnea* larvae die within 1–2 days (~25–30 day-degrees) at temperatures representative of field conditions if prey is not procured. Consequently, preemptive releases of lacewing larvae in anticipation of *S. perseae* outbreaks may be unwarranted as predators will die of starvation if abundant alternative prey are either unavailable or not located quickly. Similarly, if prey are present at either low densities or highly aggregated, and predators are not released in close proximity to prey, the majority of first instar larvae can be expected to quickly die of starvation because they are unable to use immature avocado leaves or pollen for sustenance in the absence of prey. At times when lacewings would be released for *S. perseae* control, other arthropod prey, such as *persea mite, Oligonychus perseae* Tuttle, Baker and Abatiello (Acari: Tetranychidae), or avocado brown mite, *Oligonychus punicae* (Hirst), that could sustain lacewing larvae in the absence of the target pest are typically absent or present at very low densities.

In the laboratory, *C. carnea* larvae fed voraciously on immature *S. perseae* and did not show preferences for particular instars. Third instar predators consumed more prey than younger instars, and all three instars attacked first instar *F. orizabensis* larvae, a generalist predator commonly found in California avocado orchards. Second instar *F. orizabensis* were eaten only by second and third instar lacewing larvae, and immature *C. carnea* were unable to consume adult female *F. orizabensis*. No predation by *F. orizabensis* larvae or adults of *C. carnea* larvae was observed. Intraguild predation in this system, should it occur naturally in avocado orchards, would potentially be asymmetric and biased in favor of *C. carnea* and large scale lacewing releases could potentially affect *F. orizabensis* densities. Intraspecific predation in the presence of prey was most pronounced between second instar *C. carnea* larvae (75% of second instars consumed a conspecific of the same life stage) and <10% of first and third instars engaged in this activity over a 24h period when *S. perseae* larvae were present as prey.

Augmentative biological control of *S. perseae* with commercially available mass-reared generalist natural enemies such as *F. orizabensis* (Hoddle et al., 2004) or green lacewings does not appear to be an effective control option for managing this pest using currently employed release techniques. Several additional factors inherent in the biology and outbreak ecology of *S. perseae* diminish the likelihood of successful augmentative biological control. First, *S. perseae* populations typically increase at times of the year when temperatures do not favor the reproductive biology of key natural enemies (Hoddle, 2002; Hoddle et al., 2000). Second, natural enemy surveys in California avocado orchards and in the area of origin for *S. perseae* have failed to identify specialized natural enemy species that respond rapidly in a positive density dependent manner to pest outbreaks (Hoddle et al., 2002a). Third, the lifecycle of thripids (typically, eggs are oviposited within plant

Fig. 5. Predation rates of first (A), and second (B) instar *Scirtothrips perseae* larvae and total larval thrips consumption (C) by first, second, and third instar *Chrysoperla carnea* larvae.
tissues, there are two exposed feeding larval instars, two pupal stages that pupate within protective cracks on branches or in soil beneath the host plant, and winged vagile adults) minimizes life stage exposure to any single generalist natural enemy species. Under such conditions, a guild of generalist natural enemies would need to be simultaneously available in several distinct habitats (e.g., arboreal to attack thrips larvae and adults exposed on leaves, and subterranean to attack pupal stages in the soil) to minimize the number of life stages benefiting from refuge in natural enemy free space. Fourth, *S. perseae* populations usually drop to almost undetectable levels over summer in California and natural enemies responding to pest outbreaks late in the season subsequently decline quickly to very low levels. This boom and bust ecology, exploitation of temperature regimens unfavorable for natural enemy population growth, patchy distributions of high density *S. perseae* populations within and among avocado orchards, and life stages occupying widely varied niches, makes this pest an unstable resource as population apparencty vascillates immensely making temporal and spatial occurrence unpredictable.

There have been no documented cases with sufficient long-term field data to indicate classical biological control of a thrips pest has been achieved. Additionally, successful and cost effective augmentative control programs against thrips pests in perennial crops have not been achieved either (Parrella and Lewis, 1997). Sustainable control of *S. perseae* in California avocado orchards is going to depend on judicial pesticide use, development of techniques to conserve or enhance the impact of naturally occurring generalist natural enemies, and cultural strategies that disadvantage the pest while simultaneously enhancing orchard health. A concurrent combination of management strategies will be needed to form the basis of an integrated pest management plan that should offer the best potential for long-term cost effective control of *S. perseae* in California (Hoddle et al., 2002b).

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