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QUALITY ASSESSMENT OF *CHRYSOPERLA RUFILABRIS* (NEUROPTERA: CHRYSOPIDAE) PRODUCERS IN CALIFORNIA

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ABSTRACT

Chrysoperla rufilabris (Burmeister) egg shipments from three commercial California insectaries were evaluated during a nine-month period. All three insectaries shipped similar numbers of eggs per unit weight (range 301.0 ± 10.3 to 315.4 ± 7.8 eggs/25 mg). Estimated total number of eggs per shipment for all three insectaries was between 1.80 and 3.30 times the number ordered (1,000). The estimated number of dead eggs per shipment ranged from 76.0 to 418.0 and the estimated number of larvae per shipment ranged from 0 to 9.9. Final hatch rates for all three insectaries were between 70.9% and 73.9%. Hatch began on the third day after shipment receipt and 70% of total hatch had occurred by the fourth day. Implications for timing of egg and larval releases are discussed.

Key Words: *Chrysoperla rufilabris*, lacewing, quality control, insectary rearing

RESUMEN

Los envios de huevos de *Chrysoperla rufilabris* (Burmeister) de tres insectarios comerciales de California fueron evaluados durante un periodo de nueve meses. Todos los tres insectarios enviaron cantidades similares de huevos por unidad de peso (de 301.0 ± 10.3 hasta 315.4 ± 7.8 huevos/25 mg). El número total estimado de huevos por cada envio de los tres insectarios fué entre 1.83 y 3.31 veces mayor del número solicitado (1,000). El número estimado de huevos muertos por envio fué de 75.98 hasta 418 y el número de larvas estimadas por envio fué de 0 hasta 9.9. La taza de eclosión final para los tres insectarios fué entre 70.9% y 73.9%. La eclosión empezó en el tercer día después de recibir el envio y 70% de la eclosión total ocurrió en el cuarto dia. Se discuten las implicaciones para el tiempo de liberar los huevos y las larvas.

Inundative and augmentative releases of natural enemies are widely used as non-disruptive alternatives to chemical control of arthropod pests. *Chrysoperla* (Neuroptera: Chrysopidae) species, commonly known as green lacewings, are among the most commonly marketed generalist insect predators (Tauber et al. 2000). Lacewings are applied in home gardens, row crops, orchards, and greenhouses on a variety of crops (Ridgway & Murphy 1984, Daane et al. 1998). Although *Chrysoperla* adults are not predaceous, all three larval stages are voracious eaters of soft-bodied arthropods and therefore are the desired stages for release (Tauber et al. 2000). High production costs, however, often make high volume larval purchases prohibitive. Alternatively, lacewing eggs can be as much as 17 times less expensive than larvae (Cranshaw et al. 1996). Eggs may be released upon receipt, or held until hatch and released as larvae. Substantial work has been done recently to develop, evaluate, and improve lacewing egg and larva field application methods (Morisawa & Giles 1995, Gardner & Giles 1996, Daane & Yokota 1997, Giles & Wunderlich 1998, Wunderlich & Giles 1999). Few studies, however, have evaluated the quality of commercial insectary egg shipments. The performance of natural enemy releases is only as high as the quality of organisms shipped by suppliers. In a self-regulated industry such as natural enemy mass-production, external product evaluations are important to maintain quality, which, in turn, positively promotes natural enemies as a potential tool for pest management.

O'Neil et al. (1998) evaluated post-shipment quality of four natural enemy species, including *Chrysoperla carnea* (Stephens). They found differences among *C. carnea* suppliers in the ratio of ordered eggs/received eggs, the number of larvae in egg shipments, survivorship of starved first instar larvae, and the sex ratio of reared adults. In addition, lacewing larvae reared to adulthood were identified as *Chrysoperla rufilabris* (Burmeister), not *C. carnea*. For the consumer planning lacewing releases, another important aspect of egg shipments, which O'Neil's group did not test, is

when and how many eggs hatch into larvae. If consumers know what to expect in terms of shipment hatch, then they may be better able to coordinate release rates and timings to optimally target pest phenology and environmental conditions.

In this study, we evaluated *C. rufilabris* egg shipments from three California producers. Number of eggs per unit weight, estimated total number of eggs per shipment, and developmental stages of eggs upon receipt were measured and compared across the three insectaries. To compare egg quality among producers, timing and final percentage of eggs hatched were also determined. Among possible chrysopids, *C. rufilabris* was chosen for study because it was the only species produced by all three insectaries.

Materials and Methods

Three insectaries in California were identified as producers of *C. rufilabris*: Beneficial Insectary (Oak Glen), Buena Biosystems (Ventura), and Rincon Vitova (Ventura). Throughout the experiment, larvae reared from egg shipments were identified as *C. rufilabris* by markings on head capsules and by setal patterns as described in Tauber (1974). For the sake of anonymity, each insectary was randomly assigned a unique number between one and three. Between March and November, 1999, ten shipments each of 1,000 eggs each were ordered and shipped overnight to our laboratory at the University of California, Riverside from Insectary 1 and Insectary 2. Four overnight shipments of 1,000 eggs each were received from Insectary 3 between July and November, 1999. Insectaries were aware that shipments were to be used in experimental evaluations of *C. rufilabris*, but were not told specifically that they were being evaluated on shipment quality.

Upon arrival, egg shipments were opened and the method of packaging noted. Total weight of each 1,000-egg shipment was recorded (Sartorius 1212 MP digital scale, Brinkman Instrument, Inc., Westbury, NY). A 25 mg sample was taken from each shipment, with the following exceptions: two samples were taken from the second shipment from Insectary 1, four from the ninth shipment from Insectary 2, and six and two from the third and fourth shipments, respectively, from Insectary 3. Using a dissecting microscope, the number of eggs per 25 mg sample was counted and eggs were categorized as either dead (ruptured or desiccated), green (indicating recent oviposition), or partially to completely brown with abdominal striping of the developing embryo visible (Gepp 1984). The number of hatched larvae, if any, was also recorded. Data for each category were analyzed for differences among insectaries using an unbalanced, nested ANOVA with unequal numbers of shipments per source and unequal numbers of samples per shipment. The specified model treated source as a fixed effect and shipments within source as a random effect (Sokal & Rohlf 1995, SAS Institute 1999). Tukey-Kramer's test was used for comparison of least square means. The level of significance for all tests was $p = 0.05$.

From each of the 25 mg samples taken from each egg shipment upon arrival, between one and four subsamples of 40 randomly selected eggs each were used in hatch rate determinations. Eggs were placed, one egg per well, in uncoated, plastic assay plates with rounded bottoms (96-well Assay Plates, Corning, Inc., Science Products Division, Acton, MA). Strips of clear adhesive tape were placed over the wells containing a single egg. To maintain relative humidity at a level (≥70%) conducive to *C. rufilabris* development (Tauber 1974), plates were placed on wet sponges and loosely enclosed in plastic boxes. Plastic boxes were kept under ambient laboratory temperature and light conditions, resulting in a temperature of 24 ± 1.5°C inside the boxes. Temperature and humidity were measured by HOBO Temp and HOBO RH, respectively (Onset Computer Corporation, Pocasset, MA). The number of emerged larvae, i.e. those completely separated from the chorion, were counted daily until the number of hatched larvae remained unchanged for two consecutive days. Cumulative percentage of hatched larvae was calculated daily. Thirty-six 40-egg subsample replicates were hatched out for Insectary 1, 33 replicates for Insectary 2, and eight replicates for Insectary 3. Arcsine (square-root) transformation was applied to daily cumulative hatch rates. Transformed data were analyzed for differences in daily cumulative percentage hatch among insectaries using a one-way ANOVA. Significant differences were further separated with Tukey's test for comparison of means. The level of significance for all tests was $p = 0.05$.

Results

All egg shipments arrived on time and were packaged in small plastic cups with tight-fitting lids, wrapped in paper. Both Insectaries 1 and 2 placed each paper-wrapped plastic cup into a styrofoam cooler with artificial ice packs, and the cooler was in turn packaged in a cardboard box for shipping. Insectary 3 shipped each paper-wrapped plastic cup in a cardboard box without styrofoam insulation or ice packs.

The mean weight of shipments from Insectary 2 (145.4 ± 9.2 mg) was significantly ($F = 5.20$; $df = 2, 21$; $p = 0.015$) less than the mean shipment weights from Insectaries 1 (247.5 ± 37.7 mg) and 3 (274.8 ± 47.6 mg). Variation in shipment weight for Insectary 2 (range 100 mg to 203 mg) was also less than that for either Insectary 1 (range 130 mg to 451 mg) or Insectary 3 (range 155 mg to 369 mg).

The mean number of eggs within a 25 mg sample was similar for all three insectaries, at just over 300 eggs (Table 1). Based on these counts, the estimated mean number of eggs per shipment was 3,046 for Insectary 1, 1,834 for Insectary 2, and 3,309 for Insectary 3.

Table 1 shows the composition of the 25 mg samples taken from each insectary's egg shipments. For all insectaries, the majority of eggs (65%) in each sample had reached the striped stage, indicating imminent hatching. The estimated number of larvae per shipment ranged from 0 to 9.9 and the estimated number of dead eggs per shipment ranged from 76.0 to 418.0.

Timing of egg hatch was similar for all three insectaries (Fig. 1). There was little to no hatch on the first and second days. On day three, however, hatch for eggs from Insectary 1 and 3 were 13.2% and 21.6%, respectively, whereas a significantly lower percentage of eggs from Insectary 2 had hatched (3.7%). On days four through seven, percentage hatch for all three insectaries did not differ statistically. Final mean percentages of eggs hatched ranged from 70.9 to 73.9.

Discussion

The similarity in hatch rates observed for all three insectaries suggests similar quality of eggs received from each. The approximately 30% of eggs from which larvae did not emerge was compensated for by the fact that shipments, on average, exceeded the ordered amount by between 83% and 231%. However, implications for shipments to the average consumer are not clear because the university shipping address may have biased insectaries to include extra eggs as a courtesy. This is underscored by comparing our results to those of O'Neil et al. (1998), who used a "blind ordering" system and found fewer lacewings than ordered in a majority of shipments received.

In addition to some level of egg mortality expected during the shipping and handling processes, the risk of egg mortality in lacewing shipments is higher due to the cannibalistic nature of their larvae (New 1975). Eggs held until release tend to be confined at high densities, increasing exposure to predation by newly emerging larvae as holding time increases (Daane & Yokota 1997, O'Neil et al. 1998). Without alternative prey provided, O'Neil et al. (1998) suggested immediate release of lacewing eggs to prevent cannibalism. Our findings suggest that losses to cannibalism may be minimized by releasing lacewing eggs on or before the third day after receipt.

Even with the development of efficient egg release technologies (Gardner & Giles 1996, Wunderlich & Giles 1999), high post-release egg mortality due to environmental conditions (Daane & Yokota 1997) and intraguild predation (Tauber et al. 2000) reduce efficacy of egg releases as compared with larval releases. Our results indicate that for larval releases, holding eggs for four days allows a majority (approximately 70%) of total hatch to occur while limiting larval holding time to 24 hours. Waiting an additional day

Table 1. Composition of 25 mg samples taken from *Chrysoperla rufilabris* egg shipments.

	Mean (±SEM)[1] in each stage			
	Insectary 1: 10 shipments, total of 11 samples	Insectary 2: 10 shipments, total of 13 samples	Insectary 3: 4 shipments, total of 10 samples	ANOVA (num. df = 2)
Green Egg	63.5 a (±15.4)	15.8 a (±15.4)	52.3 a (±24.3)	F = 2.52 p = 0.105 den. df = 20.9
Striped Egg	204.7 a (±20.1)	288.3 b (±20.1)	209.9 ab (±31.4)	F = 4.90 p = 0.018 den. df = 20.6
Emerged Larvae	1.0 b (±0.2)	0.0 a (±0.2)	0.7 ab (±0.3)	F = 4.92 p = 0.025 den. df = 13.8
Dead Egg	36.6 b (±5.3)	13.1 a (±4.9)	38.0 b (±5.6)	F = 760 p = 0.002 den. df = 31
Total	307.7 a (±8.0)	315.4 a (±7.8)	301.0 a (±10.3)	F = 0.65 p = 0.536 den. df = 13.3

[1]Means (±SEM) listed are estimated least square means based on a nested ANOVA model with unequal sample size. Means within a row followed by the same letter did not differ statistically (Tukey-Kramer's test, $p < 0.05$).

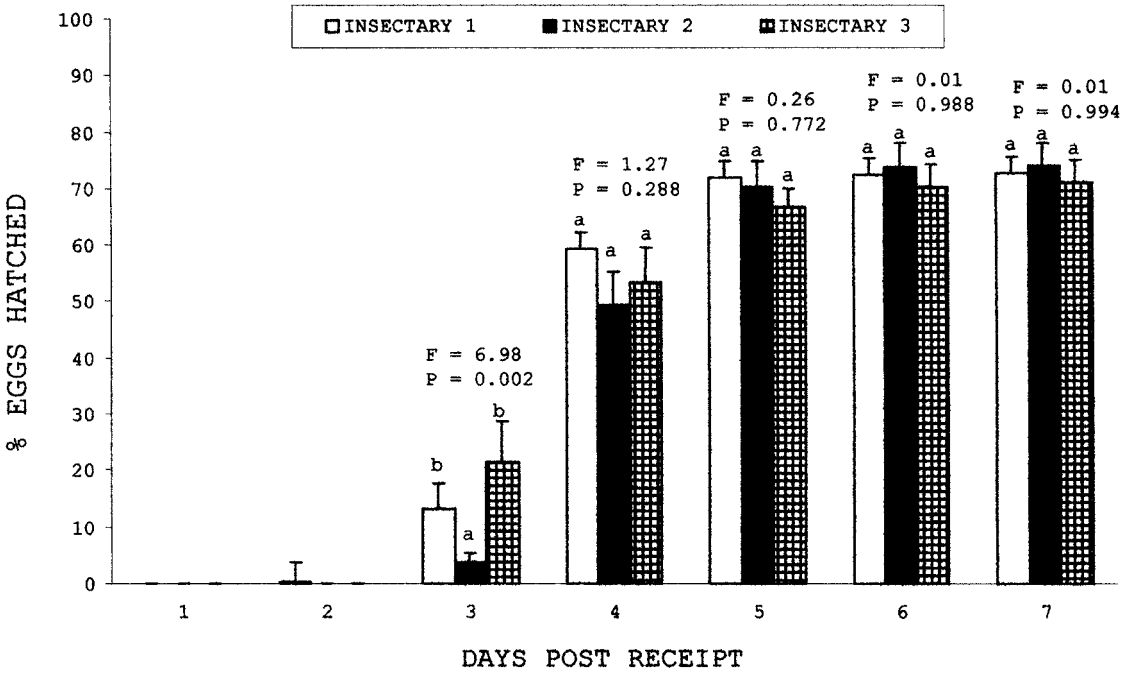

Fig. 1. Cumulative mean percentage (±SEM) of *Chrysoperla rufilabris* eggs hatched each day after shipment receipt from three insectaries. Within days, means labeled with the same letter are not statistically different (Tukey's test, df = 2,74, $p < 0.05$).

may increase hatch, but that may be countered by an accompanying increase in cannibalism (O'Neil et al. 1998).

The few reports of *C. rufilabris* egg hatch rates found in the literature are for untreated controls within studies of egg release methodologies. Our observed hatch rates of 70.9 to 73.9% after seven days fall within the 64.1% after five days reported by Gardner & Giles (1996) and the 91.2% after seven days reported by Daane & Yokota (1997), but direct comparison of these rates is difficult because specific holding conditions were not reported for each. The question should be addressed in future egg hatch studies in which effects of temperature, relative humidity and light regime are compared and related to conditions an average consumer may be able to replicate.

Whereas the development stages present in egg shipments differed slightly among the three insectaries, all contained mostly eggs that were close to emergence and few contained larvae. This is an improvement on the average percentage larvae in shipments ranging up to 52.6% reported by O'Neil et al. (1998). Although sources were not specified in that study, perhaps the wider range of larval emergence they observed was a result of including both producers and distributors. Eggs shipped through distributors may be older and therefore more likely to hatch before arrival than eggs shipped directly from the producer.

Shipments from all three insectaries contained a similar number of eggs per unit weight, indicating little difference in contamination levels. One of the insectaries, however, had less variation in shipment weight, significantly fewer damaged and desiccated eggs, and slightly more uniformity in egg developmental stage as indicated by shipment composition and hatch data. Uniform age structure could contribute to a more synchronous and predictable larval hatch, equally useful for timing of egg releases as for larval releases. These observations suggest slightly better handling techniques and precision in egg collection that perhaps could be employed in the other two insectaries.

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