Influence of Different Larval Feeding Regimes and Diet Presentation Methods on *Chrysoperla Rufilabris* (Neuroptera: Chrysopidae) Development[1]

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ABSTRACT Results of a comparative analysis of the effects of seven different larval feeding regimes on the development of individually reared *Chrysoperla rufilabris* (Burmeister) larvae are presented. Also, four methods for presenting liquid artificial diet (capillary tubes, sponge, agarose based jelly, and artificial eggs) are discussed. Development time, pupal weight, fecundity, and reproductive rate were higher for *C. rufilabris* larvae reared on the eggs of either *Helicoverpa zea* (Boddie) or *Sitotroga cerealella* (Olivier) than for those reared on artificial diet. Diet presentation methods that show promise include agarose-based jelly and artificial eggs.

KEY WORDS Biological control, predator, artificial diet, rearing.

Chrysoperla rufilabris (Burmeister) is an important insect predator that is widely distributed in North America (Tauber and Tauber 1983). This insect is predaceous in the larval stage and feeds on a wide variety of pests (Hydorn 1971). *Chrysoperla* spp. are effective biological control agents in field, orchard, and greenhouse crops (Adashkevich and Kuzina 1971, 1974, Ridgway et al. 1977, Miszczak and Niemczyk 1978, Hagley and Miles 1987). The effective use of *Chrysoperla* spp. in biological control programs depends on the availability of a cost effective method for their mass production. An inexpensive artificial diet for *Chrysoperla* larvae would significantly reduce production costs in mass rearing systems.

A number of artificial diets for *Chrysoperla* spp. have been described, beginning with Hagen and Tassan (1965). These diets have been tested primarily on *Chrysoperla carnea* (Stephens). The studies described here involve a comparison of the effect of different larval feeding regimes on the development of individually reared *C. rufilabris* larvae. In addition, several methods for presenting the liquid artificial diet to the larvae were also evaluated.

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[2] Mention of a proprietary product does not constitute an endorsement by the USDA.

Materials and Methods

Experiment 1. Seven different larval feeding regimes were compared: (1) *Sitotroga cerealella* (Olivier) eggs for all larval instars; (2) *Helicoverpa zea* (Boddie) eggs for all larval instars; (3) *H. zea* eggs for the first larval instar and Hassan and Hagen (1978) diet for the second through third instars; (4) Hassan and Hagen (1978) diet for all instars; (5) Vanderzant (1973) diet for all larval instars (6) Hassan and Hagen + liver powder diet, which consisted of the Hassan and Hagen (1978) diet with the addition of 5.00 g of liver powder/100 ml, for all larval instars; and (7) Nepomnyashchaya et al. (1979) diet N4 for all larval instars. The ingredients used in the artificial diets were obtained from SIGMA Chemical Company, St. Louis, MO, with the exception of food yeast flakes and liver powder, which were obtained from General Nutrition Center (GNC), Pittsburg, PA, and bee honey, sucrose, and egg yolk (Table 1).

Each feeding regime was tested with a minimum of 50 *C. rufilabris* larvae, held individually in 15 × 60 mm plastic Petri dishes that were sealed with parafilm. In this experiment the liquid artificial diets were provided via 250-280 μm inside diameter capillary tubes (6-8 mm long) (Microbore tubing of teflon PTFE, #G-06417-11, Cole Parmer, Niles, IL) (Keiser et al. 1985).

The effects of the feeding regimes on larval development were evaluated by measuring development time (from egg hatch to adult emergence) and the weight of the recently formed cocoons. We also determined the amount of food eaten by each larva by weighing the material before and after feeding. Because evaporation from capillaries should be minimal, no effort was made to account for it.

In addition, at least 30 pairs of adult *C. rufilabris* that had been reared on each of the larval feeding regimes described above (except for the *S. cerealella* eggs for all larval instars regime) were used to determine reproductive rates. Adult pairs were maintained in cylindrical cardboard containers (90 × 90 mm). Nylon net was used to cover the tops and bottoms of these containers and they were lined with kraft paper. Adults were provided a moist mixture of Wheast (Dadant & Sons, Inc., Hamilton, IL) and sucrose (1:1) diet (Hagen and Tassan 1970), which was supplied on a plastic strip placed inside each container. Diet strips were changed daily. To change the diet strips and to remove the eggs, the adults were held on the bottom of the container by a vacuum device.

The daily age of females (x), day of oviposition, number of live females in the interval x (1_x), eggs per female, and number of eggs hatching. The number of females produced (m_x) was based on an assumed 50:50 sex ratio. The net reproductive rate (R_o) was calculated using the formula:

$$R_o = \Sigma\, 1_x\, m_x$$

and the intrinsic rate of increase (r_m) was calculated using the formula:

$$r_m = 1n\, R_o / T$$

where T is the mean age of reproduction. The limiting frequency of reproduction ($\lambda = e^{r_m}$) was also calculated (Andrewartha and Birch 1954).

Table 1. Composition of artificial diets used to feed *Chrysoperla rufilabris* larvae.

Hassan and Hagen 1978		
Bee honey	5.00	g
Sucrose	5.00	g
Food Yeast Flakes	5.00	g
Yeast Hydrolysate	6.00	g
Casein Hydrolysate	1.00	g
Egg Yolk (from fresh chicken eggs)	10.00	g
Water to	100	ml
Vanderzant 1973		
Casein Hydrolysate, Enzymatic	5.00	g
Soy Hydrolysate, Enzymatic	5.00	g
Yeast Hydrolysate	2.00	g
Sucrose	15.00	g
Casien	1.00	g
K_2HPO_4	0.16	g
$NaH_2PO_4 \cdot H_2O$	0.08	g
$MgSO_4 \cdot 7H_2O$	0.05	g
$FeSO_4 \cdot 7H_2O$	0.005	g
Soybean Lecithin and Oil	0.50	g
Cholesterol	0.05	g
B-Vitamins*	2.0	ml
Chlorine	0.50	g
Inositol	0.20	g
Water to	120.00	ml
Nepomnyashchaya et al. 1979, N4		
Casein Hydrolysate, Enzymatic	5.00	g
Soy Hydrolysate, Enzymatic	5.00	g
Sucrose	15.00	g
Vitamins, Mix of Lipids	0.56	g
Choline - Chloride	0.050	g
Vitamin C	0.100	g
Vitamin B_{12}	0.00004	g
Nicotinic Acid	0.0020	g
Pantothenate Ca	0.0020	g
Vitamin B_1	0.00050	g
Vitamin B_6	0.00050	g
Vitamin B_{12}	0.0010	g
Folic Acid	0.00050	g
Biotin	0.000040	g
Inositol	0.020	g
Phosphate Na	0.08	g
Sulfate Mg	0.05	g
Sulfate Fe	0.005	g
Phosphate K	0.16	g
Dye Neutral Red	0.024	g
Emulsifier tween-80	0.12	g
Water to	120	ml

* Amounts in mg per ml: Nicotinamide 1.0, Calcium Pantothenate 1.0, Thiamine-HCl 0.25, Riboflavin 0.5, Pyridoxine-HCl 0.25, Folic Acid 0.25, Biotin 0.02, Vitamine B_{12} 0.002.

Experiment 2: The influence of different methods for presenting the Hassan and Hagen (1978) diet to *C. rufilabris* larvae also was evaluated. Four presentation methods were tested: (1). Capillary tubes, as described in Experiment 1. The capillaries were changed every two days; (2) Small cubes (ca 0.75 cm^3) of cellulose sponge saturated with diet, which were changed daily; (3) Agarose based jelly (J.1 = 1 part diet and 1 part 5% agarose; J. 2 = 2 parts diet and 1 part 4% agarose; J.3. = 2 parts diet and 1 part 5% agarose; J.4 = 2 parts diet and 1 part 6% agarose), and; (4) Artificial eggs (Hagen and Tassan 1965).

The jelly was prepared by mixing the appropriate amount of agarose in water at 95-100°C. The agarose solution was allowed to cool to 40-45°C, at which time the appropriate amount of liquid diet was mixed. The jelly preparations were provided on 22 × 22 mm cover slips, which were changed every 2 or 3 days.

Artificial eggs were prepared by mixing the liquid diet with a small amount of a 3:1 mixture of paraffin and vaseline and heating the solution to 53-56°C in a water bath. Droplets were formed by dipping a glass capillary (4 mm in the heated solution and touching the capillary to a piece of microscope slide (25 × 25 mm) (20 eggs per slide) and allowed to cool. (Hagen and Tassan 1956). Larvae were held individually as described in Experiment 1.

The number of eggs hatching, number of larvae developing to the pupal stage, and number of pupae emerging to adult were recorded. The death rate (K) (Varley et al. 1973) was calculated using the formula:

$$K = (1n\ NE - 1n\ NL) + (1n\ NL - 1n\ NP) + (1n\ NP - 1n\ NA)$$

where NE = number of eggs, NL = number of larvae, NP = number of pupae, and NA = number of adults.

The *C. rufilabris* culture was maintained at 26 ± 0.5° C, 80 ± 5% RH, and a 14 h photophase, using methods described by Nordlund and Morrison (1992).

The statistical analyses of the means for development time, pupal weight, fecundity, net reproductive rate, intrinsic rate of increase, and death rate were conducted using the analysis of variance (ANOVA) and Tukey's studentized range test (SAS Institute 1988).

Results and Discussion

The effect of seven larval feeding regimes on larval development were compared in Experiment 1. The results (Table 2) show that the mean development time was significantly shorter and mean pupal weights were significantly higher when either *H. zea* or *S. cerealella* eggs were provided for all instars than when *H. zea* eggs were provided for the first instar only. The means for these parameters were also significantly longer and lower, respectively, when only artificial diet was provided than when *H. zea* eggs were provided for only the first instar. There were no significant differences among the four artificial diet-only feeding regimes. Larval development required 31.5 ± 4.1 mg (x ± SD) of *S. cerealella* eggs or 50.0 ± 4.5 mg of the Hassan and Hagen diet.

Table 2. Comparative analysis of different larval diets for *Chrysoperla rufilabris*.

Feeding Regime	DEVELOPMENT TIME (DAYS)			Pupal Weight* (mg)
	Larvae*	Pupae*	Total*	
H. zea eggs all instars	7.9 ± 0.2 a	7.9 ± 0.2 a	15.8 ± 0.2 a	11.2 ± 0.4 a
S. cerealella eggs all instars	8.3 ± 0.5 a	8.2 ± 0.3 a	16.5 ± 0.6 a	11.0 ± 0.4 a
H. zea eggs 1st instar Hassan & Hagen Diet 2-3 Instars	9.6 ± 0.2 b	8.2 ± 0.2 a	17.8 ± 0.3 b	9.2 ± 0.3 b
Hassan & Hagen Diet all instars	13.4 ± 0.4 c	8.9 ± 0.2 b	22.3 ± 0.4 c	8.0 ± 0.4 c
Hassan & Hagen + Liver Powder Diet all instars	14.0 ± 0.4 c	9.1 ± 0.3 b	23.1 ± 0.4 c	8.4 ± 0.5 c
Vanderzant Diet all instars	14.8 ± 0.3 c	9.1 ± 0.3 b	23.9 ± 04 c	8.4 ± 0.5 c
Nepomnyashchaya et al. Diet all instars	14.1 ± 0.3 c	9.3 ± 0.3 b	23.4 ± 0.5 c	8.5 ± 0.4 c

* Means (± SD) in each column, followed by different letters are significantly different at the 5% level, as determined by Tukey's studentized range test (SAS Institute 1988).

The effect of different larval feeding regimes on reproduction by adults also was evaluated in Experiment 1. The results (Table 3) show that larval feeding regimes significantly affect reproduction in adult *C. rufilabris*. Fecundity, the reproductive rate (Ro), and the intrinsic rate of increase (r_m) were all significantly higher and death rate was significantly lower when the larvae were provided *H. zea* eggs for all instars than when *H. zea* eggs were provided for only the first instar. Yet, there were no significant differences in the means for these parameters when only one of the artificial diets was provided to the larvae, though these means were significantly different from those when *H. zea* eggs were provided for only the first larval instar.

Experiment 2 involved the evaluation of several possible presentation methods for artificial diet to *C. rufilabris* larvae. The results (Table 4) show that there were no significant differences in the development time or pupal weight for larvae fed the Hassan and Hagen diet via any of the presentation methods evaluated. However, the death rate (K) was significantly higher for larvae fed via capillaries or sponge.

The cost of materials for the Hassan and Hagen (1978) diet, to feed 1,000 *C. rufilabris* larvae, was approximately $0.58. Morrison (1985) reported that the cost of *S. cerealella* eggs was $0.00655/1,000 eggs. At that rate, the cost of eggs necessary to feed 1,000 *C. rufilabris* larvae is ca. $10.31, over seventeen times the cost of the diet. Our data indicate that use of an artificial diet based production system would result in a substantial reduction in production costs for these predators.

Artificial diet for *C. rufilabris* larvae needs improvement. An improved diet would result in shorter larval development time, increased pupal weights, and increased fecundity of adult females and significantly improve production efficiencies. The data also indicate that the agarose-based jelly diet formulation or the artificial eggs are diet presentation methods that may prove useful in mass rearing programs. In mass rearing systems, ease of use and cost will be important criteria for selecting a method of diet presentation.

Table 3. Influence of different larval diets on the reproductive rate of *Chrysoperla rufilabris*.

Feeding Regime	Fecundity* (eggs per female)	R_o*	T (Days)	r_m*	Death Rate (K)*	λ
H. zea eggs all instars	802.6 ± 16.6 a	306.2 ± 22.5 a	15	0.358 ± 0.005 a	0.310 ± 0.06 a	1.431
H. zea eggs 1st instar	594.2 ± 15.9 b	222.3 ± 10.9 b	16	0.338 ± 0.003 b	0.514 ± 0.04 b	1.402
Hassan & Hagen Diet 2-3 instars						
Hassan & Hagen Diet all instars	440.6 ± 8.1 c	147.5 ± 6.1 c	17	0.294 ± 0.003 c	1.055 ± 0.07 c	1.342
Hassan & Hagen + Liver Powder Diet all instars	448.1 ± 18.8 c	149.2 ± 13.2 c	17	0.294 ± 0.003 c	1.122 ± 0.09 c	1.342
Vanderzant Diet all instars	452.9 ± 13.8c	148.1 ± 6.7 c	17	0.294 ± 0.002 c	1.122 ± 0.09 c	1.342
Nepomnyashchaya et al. Diet all instars	453.7 ± 11.7 c	152.0 ± 6.8 c	17	0.296 ± 0.003 c	1.161 ± 0.15 c	1.345

* Means (± SD) in each column, followed by different letters are significantly different at the 5% level, as determined by Tukey's studentized range test (SAS Institute 1988).

Table 4. Influence of different methods of presenting liquid artificial diet to larval *Chrysoperla rufilabris*.

Presentation Form	Developmental Time (days)			Pupal Weight, (mg)*	% Development to			Death Rate (K)*
	Larvae*	Pupae*	Total*		Larvae	Pupae	Adult	
Capillary	13.4 ± 0.4 a	8.9 ± 0.2 a	22.3 ± 0.4 a	8.0 ± 0.4 a	91.3	41.1	89.9	1.087 ± 0.06 a
Sponge	13.9 ± 0.9 a	9.6 ± 0.8 a	23.5 ± 1.0 a	8.4 ± 0.7 a	83.3	60.0	80.0	0.938 ± 0.11 a
Jelly J.1	11.6 ± 0.9 a	9.5 ± 0.8 a	21.1 ± 1.0 a	8.0 ± 1.1 a	85.7	70.0	90.5	0.611 ± 0.09 b
J.2	12.4 ± 0.9 a	10.2 ± 0.8 a	22.6 ± 1.4 a	8.3 ± 1.0 a	84.4	71.1	85.2	0.685 ± 0.11 b
J.3	12.0 ± 0.7 a	9.4 ± 0.5 a	21.4 ± 0.9 a	8.3 ± 1.2 a	86.7	73.1	84.2	0.634 ± 0.10 b
J.4	11.8 ± 0.6 a	9.5 ± 0.5 a	21.3 ± 0.8 a	8.2 ± 1.1 a	86.7	73.1	89.5	0.572 ± 0.10 b
Artificial eggs	11.0 ± 1.1 a	9.6 ± 0.8 a	20.6 ± 1.2 a	8.6 ± 0.8 a	82.2	81.1	86.7	0.554 ± 0.07 b

* Means (± SD) in each column, followed by different letters are significantly different at the 5% level, as determined by Tukey's studentized range test (SAS Institute 1988).

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