

**Rearing Lacewings, *Chrysoperla carnea* and
Chrysopa oculata
(Neuroptera: Chrysopidae), on prepupae of
Alfalfa Leafcutting Bee,
Megachile rotundata (Hymenoptera:
Megachilidae)**

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ABSTRACT

Lacewings are an important group of insect biological control agents. Protocols for rearing lacewings often require rearing of an additional insect species to be used as a diet, and this can be costly. Prepupae of alfalfa leafcutting bee, *Megachile rotundata* (F.), which are commercially available, inexpensive and can be stored, were evaluated as a larval diet of the lacewings, *Chrysoperla carnea* (Stephens) and *Chrysopa oculata* Say. Leafcutting bee prepupae were suitable for rearing lacewings: 90% of *Chrysoperla carnea* eggs hatched and the larvae grew to reach adulthood. For *Chrysopa oculata* eggs, survival to adulthood was 66%, but this increased to 91% when pupating larvae had access to empty cocoons of leafcutting bee prepupae. The diet allowed us to establish colonies of the lacewings in the laboratory.

INTRODUCTION

The green lacewings (Neuroptera: Chrysopidae) are an important group of insect predators (Dean and Satasook 1983). Natural populations of chrysopids can be augmented by inoculative or inundative releases (Ridgway and Jones 1969, Nordlund *et al.* 2001). While small numbers are often useful for laboratory studies and inoculative releases, large numbers of cultured chrysopids are required for inundative releases (Nordlund

et al. 2001). Protocols for culturing of chrysopids using natural, subnatural-artificial and artificial diets are available (Finney, 1948; 1950; Ridgway *et al.* 1970; Morrison *et al.* 1975; Morrison 1985; Nordlund *et al.* 2001; Yazlovetsky 2001).

Suitable larval diets include eggs of the lepidopterans, *Phthorimaea operculella* (Zeller) (Finney 1948, 1950) and *Sitotroga cerealella* (Olivier) (Ridgway *et al.* 1970, Morrison *et al.* 1975, Morrison 1977, 1985) (Gelechiidae), and *Anagasta kuehniella* (Zeller) (Pyralidae) (Zheng *et al.* 1993a, 1993b), larvae of *P. operculella* (Finney 1948, 1950) and honey bees (Ferran *et al.* 1981, cited in Yazlovetsky 2001; Matsuka and Nijjima 1985), aphids (Tauber and Tauber 1974) and artificial diets (Hagen and Tassan 1965, 1966, Vanderzant 1969). Finney (1948; 1950) reared *Chrysoperla* larvae on paper sheets covered with *P. operculella* (Zeller) eggs and honey. Ridgway *et al.* (1970) used *S. cerealella* eggs to feed larvae in Hexcel[®] (Hexcel Products Inc., Dublin CA), which has honey-comb like chambers that separate individual larva, whereas, Morrison *et al.* (1975) dispensed the eggs in sheets of ornamental Masonite[®] separated by organdy cloth. Masonite consisted of 0.64 cm² cells removed from solid material in a regular pattern leaving a 0.32 cm² border between cells. When Masonite was discontinued, Morrison (1977) suggested Verticel[®] (Hexacomb, University Park IL), which contains triangular cells. Several other materials including plastic light diffusing grids and shredded paper have also been suggested.

Finney (1948) processed *P. operculella* larvae with sodium hypochloride, hot water and paraffin, and fed them to the chrysopid larvae being reared. Ferran *et al.* (1981, cited in Yazlovetsky 2001) used a mixture of larval worker honey bee powder and honey, whereas Matsuka and Nijjima (1985) used larval honey bee drone powder and water. However, due to its hygroscopic nature, diet made from larval honey bee powder turns into a syrup, and develops mould rapidly. Similarly, several other natural insect materials including powdered *S. cerealella* moths, aphids, and crickets have been used, though these diets failed to give any advantage (Yazlovetsky 2001).

To reduce costs, searches for artificial larval diets have been made. Hagen and Tassan (1965, 1966, 1970), and Vanderzant (1969) developed liquid diets made primarily from enzymatic hydrolysate of yeast and casein, sugar, vitamins and water, but they were not satisfactory: an artificial diet for *Chrysoperla carnea* (Stephens) which contained wax-coated yeast hydrolysate droplets required costly preparation and also resulted in high mortality (Hagen and Tassan 1965; Vanderzant 1969). However, Vanderzant (1969) had better success by adding vitamins and minerals. Today, several diets improved from Vanderzant's are available, though not all larvae reared on them reach adulthood (Yazlovetsky 2001). Cohen and Smith (1998) developed a semi-solid larval diet containing protein, lipid, carbohydrate, cholesterol, and water. This diet resembles the inside of insect prey in both texture and composition. Combinations of honey, chicken eggs, dried cow's milk, beef liver, bacto-agar, pig fat and butter have improved performance of artificial diets further (Yazlovetsky 2001). Although, progress has been made with artificial larval diets, chemically defined diets are often more expensive, and to make them more economical, further improvements are required (Nordlund *et al.* 2001).

Most protocols are suitable for culturing chrysopid larvae on a large scale operation. These protocols can often be inconvenient for small-scale operations. Natural diets require rearing of one insect, which is usually complex and expensive, to feed another

(Finney 1950; Morrison 1985). Artificial diets are even less satisfactory. For example, an artificial diet of wax-coated yeast hydrolysate requires costly preparation, it may cause high mortality of lacewings (Hagen and Tassan 1965, Vanderzant 1969), its required ingredients may be expensive (Nordlund *et al.* 2001) and are often difficult to obtain locally. Hence, it is desirable to have alternative protocols that do not require additional rearing of insects and that rely on materials available locally.

We describe here rearing techniques and bionomics of *Chrysoperla carnea* and *Chrysopa oculata* Say fed as larvae on prepupae of the alfalfa leafcutting bee, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae).

MATERIALS AND METHODS

Specimen collection

Using a sweep net, five to eight adult females of *C. carnea* and *C. oculata* were collected from alfalfa fields in Manitoba in early August of 2001. Each female was placed individually in a 118 ml screw-cap specimen container with a small piece of alfalfa shoot and transferred to the laboratory in a picnic cooler. In the laboratory, females were held in the specimen containers overnight at $20 \pm 2^\circ\text{C}$ and 12:12 (L:D) h. The next day, eggs laid in the containers were carefully harvested using forceps and breaking the stalk beneath the egg and transferring it to containers with leafcutting bee prepupae. A colony of each species was established from these eggs using alfalfa leafcutting bee prepupae, and yeast plus sucrose paste as larval and adult diets, respectively. Eggs obtained from these colonies were used in the present study.

Diets

Alfalfa leafcutting bee prepupae were used as the larval diet. The prepupal cocoons, which were in the second year of storage at 5°C , were transferred into a $15\text{--}18^\circ\text{C}$ room one or two days before use. Cocoons were cut open at one end with a scalpel and the prepupae were pulled out of cocoons with forceps. Care was taken to avoid injury to prepupae.

Adult lacewings were fed on a paste of yeast flakes (52% protein) and sucrose prepared following Morrison (1985). The food was prepared once a week and was kept frozen until use.

Rearing methods

F2 and F3 generation eggs were collected from each colony. Sixty to 78 eggs of each species were placed in conspecific batches of 8–10 eggs per larval rearing unit made of a 60×15 mm covered Petri dish. Two leafcutting bee prepupae were added to each unit which was labeled and placed at $25 \pm 2^\circ\text{C}$, 18:6 (L:D) h and $70 \pm 5\%$ relative humidity, the conditions at which all rearing took place. Eggs were checked daily for hatching.

On the day of hatching, newly emerged lacewing larvae were transferred to fresh units, in batches of 5–10 larvae per unit. Two or three alfalfa leafcutting bee prepupae were provided in each unit. Fresh food was provided every second day during the first six days. Little cannibalism occurred during this period, while the larvae were small in comparison to the size of the food items. After six days, the number of lacewing larvae was reduced to one per unit to avoid cannibalism, and from then until pupation fresh food was provided every day. The lacewing pupae were left undisturbed.

Newly emerged adult males and females, were transferred in conspecific batches of 8–10 into a pre-oviposition unit made of a transparent 1 liter plastic container 14 cm high, 11 and 8.5 cm diameter at the top and bottom, respectively. The lid of the container had a 1 mm hole drilled near the edge for aeration. A small piece of distilled-water-saturated cotton wad in a 35 x 10 mm Petri dish was placed on the floor of the container. Food was presented by placing 2–3 drops of the yeast and sucrose paste on a 10.5 x 4 cm strip of brown cardboard, which was placed on the upper rim of the container so that the food faced down. The food and cardboard were changed every day and every third day, respectively, to avoid mold development. On the day eggs were first seen, females were separated and individual females were placed in oviposition units, which were similar to pre-oviposition units. Eggs laid inside oviposition units were collected and reared for colony development.

Performance of culture

The time needed for egg hatching, the number of eggs hatched, the number of larvae and pupae, the duration of larval and pupal periods, the number and sex of emerged adults and the pre-oviposition period were recorded. To develop a fecundity schedule, 15 females of *C. carnea* and 10 of *C. oculata* were followed for 30 days from initial oviposition in oviposition units.

We also examined whether the provision of empty leafcutting bee cocoons for pupating *C. oculata* larvae improved survival. Two treatments, no cocoon (control) and with cocoon, were compared. In the latter treatment, an empty leafcutting bee cocoon was provided in each larval rearing unit on the ninth or 10th day after hatch. All other operations remained as described above. Chi-square tests were used to conduct the statistical analysis (Sokal and Rohlf 1995).

RESULTS AND DISCUSSION

Alfalfa leafcutting bee prepupae permitted rearing of *C. carnea* and *C. oculata* larvae (Table 1). About 2–3% of eggs failed to hatch because they were eaten or damaged by the larvae that hatched earlier. The total time required from oviposition to adult emergence of *C. carnea* averaged 24.7 (23–29) d. The type and amount of food fed to their larvae is one of the factors influencing the growth and development of *C. carnea* (Zheng *et al.* 1993b, Obrycki *et al.* 1989). The time required from oviposition to adult emergence in the present study is similar to previous reports of developmental duration

(Hagen and Tassan 1965, Zheng *et al.* 1993a). The leafcutting bee prepupae allowed 90% of *C. carnea* eggs to hatch and the larvae to grow to adulthood, and this rate is higher than those obtained on natural or artificial diets in previous studies (Hagen and Tassan 1965, Ridgway *et al.* 1970, Morrison *et al.* 1975, Obrycki *et al.* 1989). Zheng *et al.* (1993a) obtained 75–100% and 65–73% survival from egg to adult on high and intermediate amounts of larval food, respectively. In this study about 52% of emerged adults were females. Zheng *et al.* (1993a) obtained a similar sex ratio on low to intermediate food quantities, and 67% females on a high amount of larval diet.

The total time required from oviposition to adult emergence of *C. oculata* averaged 34.7 (31–38) d, which is comparable to the time required on natural diets (Obrycki *et al.* 1989). Twelve per cent of larvae failed to pupate, and 66% of eggs survived to adulthood. Provision of empty leafcutting bee cocoons significantly increased the survival through the pupal stage ($\chi^2 = 4.02$; $df = 1$; $P < 0.05$), probably by providing a better site for anchoring cocoon-webs (Table 2). Egg to adult survival increased to 91% in the presence of leafcutting bee cocoons for pupation. Sixty five percent of emerged adults in this study were females. Obrycki *et al.* (1989) reared up to 81% of larvae to adults on various natural diets, and less than 57% of those adults were females.

The lacewing species differed in pupation sites. *Chrysoperla carnea* pupated at both the floor-sidewall and lid-sidewall junctions. Cues leading to this site selection are unclear as little is known about pupation of *C. carnea* (Canard and Volkovich 2001). *Chrysopa oculata* usually pupated on the floor-sidewall junctions perhaps because this species pupates in the ground (Burke and Martin 1956).

Four individuals of each species had problems in mating or oviposition and were dropped from the fecundity analysis to avoid bias (Hagen and Tassan 1970). The fecundity schedule of the lacewing species differed (Fig. 1). Females of *C. carnea* laid on an average a total of 679 eggs in 30 d, and oviposition was high until 16 d then declined gradually. Females of *C. oculata* oviposited more or less uniformly and females laid an average of 424 eggs during the period (Fig. 1). Longevity of adult lacewings depends on climatic conditions and resources (Canard and Volkovich 2001). McEwen and Kidd (1995) found *C. carnea* females fed sugar solution survived about 33 d, but the oviposition was non-existent. Unfortunately they did not indicate how long the females would survive and oviposit, but it is clearly indicated that they could potentially survive for over 34 days.

The intrinsic rate of natural increase (r_m) of *C. carnea*, calculated following Southwood (1978), in this study was 0.646 per wk. From Zheng *et al.* (1993a) we calculated $r_m = 0.665$ per wk in 1984, and $r_m = 0.781$ per wk in their 1986 trial. The higher value in the 1986 trial was thought to be due to the higher survival of fresh field-collected eggs used that year and the higher fecundity of the first generation adults (Zheng *et al.* 1993a). In this study, the r_m of *C. oculata* was 0.463 per wk and 0.505 per wk in the absence and presence of leafcutting bee cocoons, respectively. Population increase is influenced by insect fecundity, which depends on the protein concentration of adult diets (Morrison 1985). The protein concentration in adult diet (< 52%) used in this study was less than the recommended level of 65% (Morrison 1985), which may have reduced the r_m value.

We found that *C. carnea* is more fecund and develops faster than *C. oculata*, and as a

result the rate of increase is higher for *C. carnea*. Therefore, populations of *C. carnea* are expected to be greater than those of *C. oculata* in field crops. This is indeed the case in alfalfa fields of Manitoba. Chrysopid populations were studied in alfalfa fields of Manitoba, where it was found that *C. carnea* predominated over *C. oculata* (Uddin 2005).

Diets in this study allowed satisfactory rearing of lacewings. Alfalfa leafcutting bee prepupae can be used as a larval diet for *C. carnea* and *C. oculata*. The bee prepupae are commercially available, cheap (the food required for rearing a larva cost less than 1.3 and 1.95 Canadian cents for *C. carnea* and *C. oculata*, respectively) and can be kept alive for at least a year at 5°C. The method described allows small-scale production and maintenance of lacewing colonies at reasonable cost. The possibility of rearing multiple larvae per unit with the same amount of the diet needs to be studied, as it could reduce per capita diet requirement and costs.

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REFERENCES

- Burke, H.R., and D.F. Martin. 1956. The biology of three chrysopid predators of the cotton aphid. *Journal of Economic Entomology* 49: 698–700.
- Canard, M., and T.A. Volkovich. 2001. Outlines of lacewing development. pp. 130–153. In: P. McEwen, T.Z. New and A.E. Whittington (eds.), *Lacewings in the crop environment*. Cambridge University Press, Cambridge.
- Cohen, C.A. and L.K. Smith. 1998. A new concept in artificial diets for *Chrysoperla carnea*: the efficacy of solid diets. *Biological Control* 13: 49–54.
- Dean, G.J., and C. Satasook. 1983. Response of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) to some potential attractants. *Bulletin of Entomological Research* 73: 619–624.
- Finney, G.L. 1948. Culturing *Chrysopa californica* and obtaining eggs for field distribution. *Journal of Economic Entomology* 41: 719–721.
- Finney, G.L. 1950. Mass-culturing *Chrysopa californica* to obtain eggs for field distribution. *Journal of Economic Entomology* 43: 97–100.
- Hagen, K.S., and R.L. Tassan. 1965. A method of providing artificial diets to *Chrysopa* larvae. *Journal of Economic Entomology* 58: 999–1000.
- Hagen, K.S. and R.L. Tassan. 1966. The influence of protein hydrolysates of yeasts and chemically defined diets on the fecundity of *Chrysopa carnea* Stephens (Neuroptera). *Vestnik Československe Spolecnosti Zoologiske* 30: 219–227.
- Hagen, K.S., and R.L. Tassan. 1970. The influence of food wheat[®] and related *Saccharomyces*

- fragilis* yeast products on the fecundity of *Chrysopa carnea* (Neuroptera: Chrysopidae). The Canadian Entomologist 102: 806–811.
- Matsuka, M., and K. Nijijima. 1985. *Harmonia axyridis*. pp. 265–268. In: P. Singh and R.F. Moore, Handbook of insect rearing. Elsevier: Amsterdam.
- McEwen, P.K., and N.A.C. Kidd. 1995. The effects of different components of an artificial food on adult green lacewing (*Chrysoperla carnea*) fecundity and longevity. Entomologia Experimentalis et Applicata 77: 343–346.
- Morrison, R.K. 1977. A simplified larval rearing unit for the common green lacewing. South-western Entomologist 2: 188–190.
- Morrison, R.K. 1985. *Chrysopa carnea*, pp. 419–425. In: P. Singh and R.F. Moore (eds.), Handbook of insect rearing, 2nd ed. Elsevier.
- Morrison, R.K., V.S. House, and R.L. Ridgway. 1975. Improved rearing unit for larvae of a common green lacewing. Journal of Economic Entomology 68: 821–822.
- Nordlund, D.A., A.C. Cohen, and R.A. Smith. 2001. Mass-rearing, release techniques, and augmentation, pp. 301–319. In: P. McEwen, T.Z. New and A.E. Whittington (eds.), Lacewings in the crop environment. Cambridge University Press, Cambridge.
- Obrycki, J.J., M.N. Hamid, A.J. Sajap, and L.C. Lewis. 1989. Suitability of corn insect pests for development and survival of *Chrysoperla carnea* and *Chrysopa oculata* (Neuroptera: Chrysopidae). Environmental Entomology 18: 1126–1130.
- Ridgway, R.L., and S.L. Jones. 1969. Inundative releases of *Chrysopa carnea* for control of *Heliothis* spp. on cotton. Journal of Economic Entomology 61: 892–898.
- Ridgway, R.L., R.K. Morrison, and M.M. Badgley. 1970. Mass rearing a green lacewing. Journal of Economic Entomology 63: 834–836.
- Sokal, R.R., and F.J. Rohlf. 1995. Biometry, 3rd ed. Freeman, New York.
- Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect populations, 2nd ed. Chapman and Hall, London.
- Tauber, M.J., and C.A. Tauber. 1974. Dietary influence on reproduction in both sexes of five predaceous species (Neuroptera). The Canadian Entomologist 106: 921–925.
- Uddin, J. 2005. Insects of Alfalfa in Manitoba with particular reference to *Lygus* spp., *Adelphocoris lineolatus* (Hemiptera: Miridae) and *Acyrtosiphon pisum* (Homoptera: Aphididae) and their natural enemies. PhD Thesis, University of Manitoba, Winnipeg, Canada.
- Vanderzant, E.S. 1969. An artificial diet for larvae and adults of *Chrysopa carnea*, an insect predator of crop pests. Journal of Economic Entomology 62: 256–257.
- Yazlovetsky, I.G. 2001. Features of the nutrition of Chrysopidae larvae and larval artificial diets. pp. 320–337. In: P. McEwen, T.Z. New, A.E. Whittington (eds), Lacewings in the crop environment. Cambridge: Cambridge University Press.
- Zheng, Y., K.M. Danne, K.S. Hagen, and T.E. Mittler. 1993a. Influence of larval food composition on the fecundity of the lacewing, *Chrysoperla carnea*. Entomologia Experimentalis et Applicata 67: 9–14.
- Zheng, Y., K.S. Hagen, K.M. Danne, and T.E. Mittler. 1993b. Influence of larval dietary supply on the food consumption, food utilization efficiency, growth and development of the lacewing, *Chrysoperla carnea*. Entomologia Experimentalis et Applicata 67: 1–7.

Table 1. Development of two lacewing species reared at 25°C and fed alfalfa leafcutting bee prepupae as a larval diet.

Species	<i>Chrysoperla carnea</i>	<i>Chrysopa oculata</i>
Duration (d) (Mean ± SE)		
Egg	4	5.4
Larva	10.9 ± 0.1	13.2 ± 0.1
Pupa	9.8 ± 0.1	16.5 ± 0.2
Survival (%)		
Egg	98 (60)*	97 (78)
Larva	100 (50)	88 (50)
Pupa	92 (50)	77 (44)
Overall	90	66

*Values in parentheses refer to the number of individuals at the beginning of the stage.

Table 2. Influence of empty cocoons of alfalfa leafcutting bees on developmental success of *C. oculata*.

Treatments	Per cent survival (Mean ± SE)		
	Larvae to pupa	Pupa to adult	Overall
Control (65)*	88 ± 4	77 ± 6	68 ± 6
Empty leafcutting bee cocoons (15)**	100 ± 0	93 ± 7	93 ± 7

*Values in parentheses refer to the number of individuals at the beginning.

**Empty leafcutting bee cocoons provided on the 9th or 10th day after hatching.

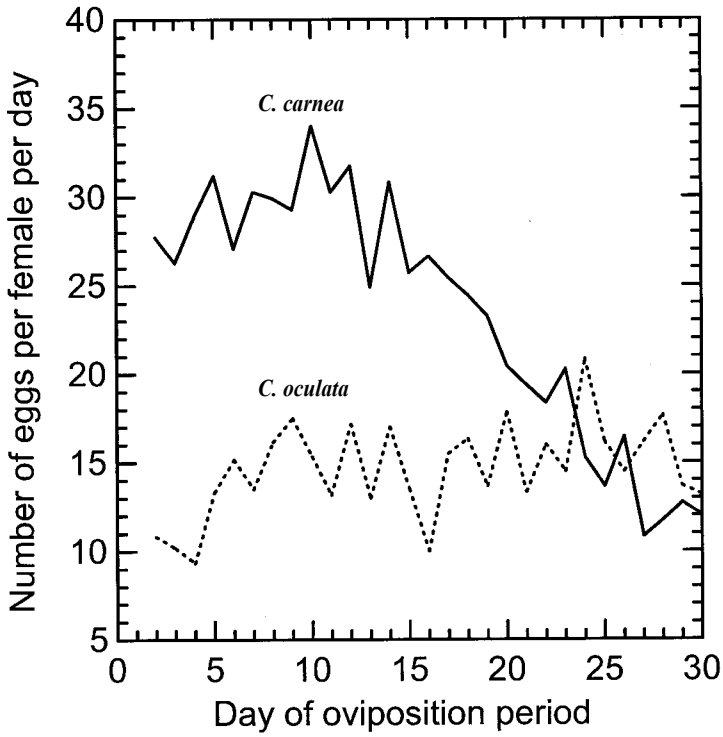


Fig. 1. Mean fecundity of *C. carnea* and *C. oculata* females in the laboratory in the observed 30 days of the oviposition period (results are based on 15 *C. carnea* and 10 *C. oculata* females).